PROCEEDINGS OF THE

International Scientific Congress *"Life sciences, a challenge for the future"*

(17th-18th October 2019, Iasi, Romania)

Editor
Prof. Liviu-Dan MIRON

filo diritto editore DAL 2008

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FILODIRITTO
INTERNATIONAL PROCEEDINGS

ISBN 978-88-85813-63-2

First Edition December 2019

filodirittoeditore.com
inFOROmatica srl, Via Castiglione, 81, 40124 Bologna (Italy)
inforomatica.it
tel. 051 9843125 - Fax 051 9843529 - commerciale@filodiritto.com

Volume Publication

International Scientific Congress *"Life sciences, a challenge for the future"*
17th-18th October 2019, Iasi, Romania

Published by	"Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iaşi 3, Mihail Sadoveanu Alley 700490, Iaşi, Romania www.uaiasi.ro/congres
Publishing house	Filodiritto Editore Proceedings & Journals Division
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FOREWORD

Dear Colleagues,
Dear readers,

The city of Iași, with a documented existence of over 600 years, is rightly considered the cultural capital of the country and, by the indisputable power of the arguments, has become, 100 years after the Great Union, the historical capital of Romania.

The University of Agricultural Sciences and Veterinary Medicine of Iași has already a European opening by fulfilling its mission of training specialists in the context of the increasingly mobile demands of a society in continuous transformation.

As a center of excellence in agronomic and medical-veterinary research, our university celebrates annually the stages it has proposed to follow, with outstanding results obtained both in the educational field and in the field of scientific research.

The Congress "Life sciences, a challenge for the future" is one of the occasions through which, together with us, researchers from different areas of the world may identify solutions that could contribute to the sustainable development.

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Rector of U.S.A.M.V. Iaşi

Prof. Univ. Dr. Gerard JITĂREANU
President of the Senate, U.S.A.M.V. Iaşi

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Section 1
AGRICULTURE AND FOOD ENGINEERING

Photosynthesis Rate, Transpiration, Stomatal Conductance in Varieties of Eggplant and Sweet Pepper in Different Technological Systems

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Abstract

The research was carried out on the protected crops, greenhouses and solariums investigating the variations of photosynthesis, transpiration, stomatal conductance and also, sugars leaf fractions in different technological systems (organic and conventional) on the varieties of *Capsicum annum* L. (peppers) and *Solanum melongena* L. (eggplant). In May-June, photosynthesis, transpiration and stomatal conductance showed higher values for varieties from the conventional system, while in July these parameters are higher for organically grown varieties, especially at the basis and middle part of plant showing an increase in the latter fruiting phenophase. Wue, the A/E ratio, showed in flowering stage high and close values in classic and in organic systems. In ripening stage, Wue have a quite higher value in organic systems and where plants showed a slower growth than in classic. Higher values of substomatal CO_2, Ci have been observed in organic systems which involved a slower rate of absorbtion of atmospheric CO_2 in photosynthesis process than was observed in classic variants. In ripening phenophasis, in classic technology system, disaccharides fraction is increased because are involved in growth and sugars accumulation in fruit whilst the monosaccharides and polysaccharides decrease but varieties organically grown showed higher values of disaccharides and also of polysaccharides.

Keywords: photosynthesis, transpiration, protected system, organic, classic, eggplants, sweet peppers

Introduction

In last decade agriculture is experiencing the transition from conventional technology with large areas and chemical treatment to the organic or ecologic technology of crop cultivated in protected spaces. Managing growth and development of an entire crop for optimum production involves the manipulation of environmental condition to obtain not only the maximum rate of photosynthesis under the given light conditions, but also the optimum balance of vegetative and generative growth of plants for sustained production and high yields [6]. Modern crop modelling combined the plant physiological processes in their interdependency with environmental condition for providing the information and such that management optimization in protected spaces. Among cultivated crop, Solanaceae occupied over half of them in protected spaces. From that, different types of tomato varieties were intensively researched whilst pepper and eggplant are less investigated.

Besides photosynthesis, some factors, which alter the resource availability, can be anticipated to influence carbohydrate partitioning and fruit growth. The dynamics of the carbohydrates in tomato plant was observed had greatest values after flowering in conventional greenhouse, meanwhile in ecologic solariums the accumulation increasing until maturity. The dependency with the type of technology observed in tomatoes crop was linked to rate of

decomposition of the nutrient from soil such as the synthetic fertilizers are available quickly while the organic nutrients are released slowly during vegetation stage [1], [2], [3]. The aim of this study is to investigate the variations of photosynthesis, transpiration, stomatal conductance and also, glucose metabolism indices in different technological systems (organic and conventional) on the varieties of *Capsicum annum* L. (peppers) and *Solanum melongena* L. (eggplant) in order to assess the environmental influence and the differences among them.

Methodology

The biological material used consisted of fresh leaves in the plant varieties grown in the protected areas (greenhouses and solariums). The studied varieties of peppers and eggplants under ecological conditions (S.C.D.L. Bacău) in comparison with those under conventional technological conditions (AGROSIV Bârlad S.A.) were studied. The varieties taking into account were: – sweet pepper such as Baltasar (S.C.D.L. Bacău), Vergasa (AGROSIV Bârlad S. A.) and also, eggplants: Aragon (S.C.D.L. Bacău) and Black Pearl (S.C.D.L. Bacău).

The vegetables cultivated in these protected systems are selected hybrids. Varieties cultivated in Bârlad greenhouse have the conventional technology (chemical treatment and fertilizers), but varieties from Bacău are cultivated in ecological systems for 12 years before (regarding the time of investigation). The organic crop varieties were transplanted two weeks later than these in classic system. During winter the protected spaces were not supplementary heated. Photosynthesis, transpiration and stomatal conductance were determined with LCi analysing portable system (ADC Bioscientific, U.K) at base, middle and top of plant. The leaf carbohydrates (mono-, di- and polysaccharides) were analysed by using 3,5-dinitrosalicylic acid (DNS) reagent for reducing sugars (Bertrand method combined with method Borel 1953) in dried plant material. Results were expressed as g % of dry matter.

Results and Discussions

Gas-exchange parameters (photosynthesis rate, transpiration, stomatal conductance and internal concentration of CO_2) in flowering phenophase showed an increasing variation from basis to top of plant in both investigated technological condition, conventional (chemical treatment) and organic (Table 1).

Table 1. Variation of photosynthesis, transpiration and stomatal conductivity in studied varieties after 6 weeks of transplantation (May) (flowering stage) cultivated in different type of protected spaces in 2008

Station	Variety	Part of plants	Ci (µmol mol^{-1})	A (µmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	Gs (mmol m^{-2} s^{-1})	Wue (µml/mmol)
Barlad (classic)		Basis	286 ± 0.01	4.45± 0.12	1.36± 0.01	0.24± 0.01	3.27± 0.21
Sweet pepper	*Vergasa*	Middle	288± 0.01	7.41± 0.14	2.40± 0.01	0.34± 0.01	3.09± 0.1
		Top	237± 0.01	15.26± 0.13	3.64± 0.01	0.45± 0.02	4.19± 0.1
Eggplant	*Aragon*	Basis	296± 0.01	5.95± 0.1	2.36± 0.01	0.58± 0.11	2.52± 0.02
		Middle	269± 0.01	11.89± 0.1	2.76± 0.01	0.64± 0.07	4.31± 0.02
		Top	261± 0.01	13.97± 0.01	3± 0.01	0.65± 0.11	4.66± 0.01
Bacau (organic)	*Baltasar*	Basis	151± 0.01	9.36± 0.1	2.21± 0.01	0.2± 0.1	4.24± 0.1
Sweet pepper		Middle	210± 0.01	9.53± 0.1	2.8± 0.01	0.24± 0.1	3.40± 0.1
		Top	159± 0.01	13.16± 0.1	3.09± 0.01	0.27± 0.1	4.26± 0.13
Eggplant	*Black Pearl*	Basis	251± 0.01	8± 0.13	2.8± 0.01	0.27± 0.1	2.86± 0.1
		Middle	205± 0.01	8.77± 0.1	3.01±± 0.01	0.3± 0.1	2.91± 0.1
		Top	143± 0.01	10.03± 0.16	3.56± 0.01	0.32± 0.11	2.82± 0.1

Legend: Ci – Substomatal cavity CO_2 concentration, A – photosynthesis rate, E – transpiration rate, gs – stomatal conductance, Wue – water use efficiency, Mean ± standard error

Higher values of photosynthesis were recorded in sweet pepper from organic system because earlier transplanted than eggplant. Concentration of internal CO_2 registered a close value among different part of plant in conventional system than organic ones which have a variation indirect proportionally with photosynthesis rate (Table 1).

In sweet pepper, transpiration obtained a value between 1.36 mmol m^{-2} s^{-1} until 3.64 mmol m^{-2} s^{-1} in classic crop technology and between 2.8 mmol m^{-2} s^{-1} until 3.09 mmol m^{-2} s^{-1} in organic crop. In eggplant, transpiration registered almost close values between 2.5 mmol m^{-2} s^{-1} until 3 mmol m^{-2} s^{-1}, in both technological systems (Table 1).

In flowering stage, stomatal conductance had almost close values in analysed organic crops with an interval between 0.2 mmol m^{-2} s^{-1} until 0.27 mmol m^{-2} s^{-1} (sweet pepper) and between 0.27 mmol m^{-2} s^{-1} until 0.32 mmol mmol m^{-2} s^{-1} (eggplant). In this stage, the stomatal conductance increased from basis to top in relation with increasing of the photosynthesis and also transpiration process. In flowering stage, Wue (ratio of A/E) is increasing from basis to the top of plant in conventional system but in organic crop is higher at basis and top (Table 1).

Photosynthesis in ripening stage registered higher value at basis and top of the plant in conventional system as a well as in organic system, in all studied variants (Table 2). Highest values around 15 µmol m^{-2} s^{-1} was recorded in Vergasa variety of sweet pepper and 20 µmol m^{-2} s^{-1} in Aragon eggplant variety cultivated in classic technology system. In organic crop, at ripening stage (July), highest values were obtained at the basis plant, of 9.49 µmol m^{-2} s^{-1} in Baltasar sweet pepper and of 11.38 µmol m^{-2} s^{-1} in Black Pearl eggplant variety. Transpiration was higher at basis and top of plant in classic system with values between 4.64 mmol m^{-2} s^{-1} until 7.08 mmol m^{-2} s^{-1} in sweet pepper and between 4.8 mmol m^{-2} s^{-1} until over 7.5 mmol m^{-2} s^{-1} at eggplant.

Table 2. Variation of photosynthesis, transpiration and stomatal conductivity in studied varieties in ripening (July) stage cultivated in different type of protected spaces in 2008

Station	Variety	Part of plants	Ci (µmol mol^{-1})	A (µmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	Gs (mmol m^{-2} s^{-1})	Wue (µml/mmol)
Barlad (classic)		Basis	287±0.01	14.43± 0.1	5.31± 0.1	0.48± 0.1	2.72± 0.1
Sweet pepper	*Vergasa*	Middle	333±0.01	1.06±0.1	4.64±0.1	0.34±0.1	0.23±0.1
		Top	248±0.01	15.88±0.1	7.08±0.17	0.57±0.1	2.24±0.4
Eggplant	*Aragon*	Basis	203±0.01	20.95±0.12	7.73±0.14	0.63±0.1	2.71±0.2
		Middle	268±0.01	6.54±0.1	4.8±0.1	0.32±0.2	1.36±0.1
		Top	206±0.01	20.15±0.2	7.54±0.1	0.67±0.4	2.67±0.1
Bacau (organic)	*Baltasar*	Basis	793±0.01	9.49±0.23	1.99±0.11	0.13±0.1	4.77±0.1
Sweet pepper		Middle	735±0.01	2.35±0.13	2.13±0.14	0.21±0.1	1.10±0.5
		Top	954±0.01	7.14±0.11	3.51±0.17	0.24±0.2	2.03±0.7
Eggplant	*Black Pearl*	Basis	769±0.01	11.38±0.1	1.93±0.1	0.16±0.1	5.90±0.9
		Middle	945±0.01	3.03±0.1	2.04±0.14	0.13±0.3	1.11±0.5
		Top	929±0.01	5.95±0.11	3.01±0.15	0.27±0.1	1.98±0.8

Legend: Ci – Substomatal cavity CO2 concentration, A – photosynthesis rate, E – transpiration rate, Gs – stomatal conductance, Wue – water use efficiency. Mean ± standard error

In organic crop, transpiration was lower than in classic system, values obtained were 1.99 mmol m^{-2} s^{-1} until 3.5 mmol m^{-2} s^{-1} (sweet pepper) and 1.93 mmol m^{-2} s^{-1} until 3.01 mmol m^{-2} s^{-1} (eggplant) but increasing from basis to top of plant (Table 2). Higher values of substomatal CO_2, Ci have been observed in organic systems (Table 2). In Baltasar variety of sweet pepper between 735 µmol mol^{-1} (middle part) until 954 µmol mol^{-1} (top) and in Black Pearl between 769 µmol mol^{-1} (basis) until almost 900 µmol mol^{-1} (top and middle) which involved a slower rate of absorbtion of CO_2 in photosynthesis process than was observed in classic variants.

In organic solarium oftenly it is used for humidity maintaining and plant disease control the polyethylene plastic mulch. In such case, the increasing of CO_2 from plant respiration and deficitary air circulation were led to photorespiration which are observed in tomatoes leaves, especially in the middle part of plant where density of leaves in mature plant are increased [1], [3]. On the other hand, it was reported that prolonged photoperiod showed a carbon loss of metabolism competence due of starch accumulation in leaves [5]. In ripening stage, in classic system, Wue is lower at the middle part and higher at basis and top of plant being in concordance with photsynthesis activity of the plants, the middle part is shaded by the superior foliage (Table 2).

In organic, the higher values of Wue are obtained at the basis of plant (almost 5 for sweet pepper and 6 for eggplant), the middle and top of plant obtained also higher values than obtained at classic system. The difference is due of lower transpiration registered in organic system.

Some data reports a higher gs, greater assimilation rates which lead to the growth fast under optimal conditions, but the plants have lower WUE. Also, it was observed that photosynthetic rate can adjust rapidly irradiance changes, but the latency in stomatal responses will constrain photosynthesis by limiting CO_2 uptake [7]. Our results are confirming that due to closure stomata mechanism that photosynthesis rate could have rather lower even when Ci registered a high value, fact observed at Baltasar and Black Pearl varieties from organic crop at ripening stage.

Analysis of sugar leaf variation showed that in 2008 year and 2009 year in conventional technology the all investigated fractions of sugars are well represented in flowering phase (May) whilst in the ripening phase (July) the lower values of total monosaccharides are corelated with the photosynthesis decreasing which occurred that stage.

In ripening phenophasis, in classic technology system, disaccharides fraction is increased because are involved in growth and sugars accumulation in fruit whilst the monosaccharides and polysaccharides decrease (Figure 1, Figure 2). In conventional system in 2009 year are observed an increasing the disaccharides content in sweet pepper because of the climatic condition of that year (warmer in April), the sweet pepper was earlier transplanted than in 2008.

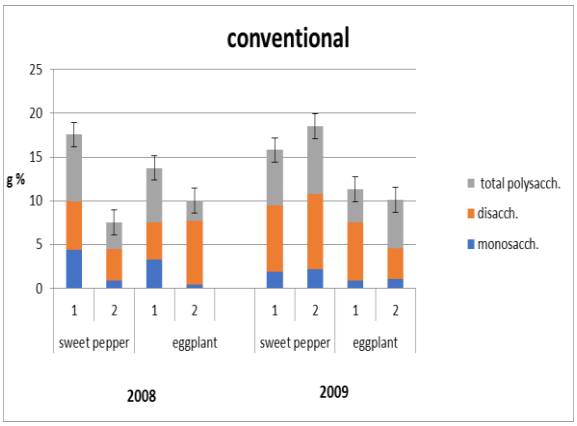

Fig. 1. Graphic representation of sugar leaf fractions in investigated varieties in classic technology system in flowering (1) and ripening stage (2) (bars are error standard)

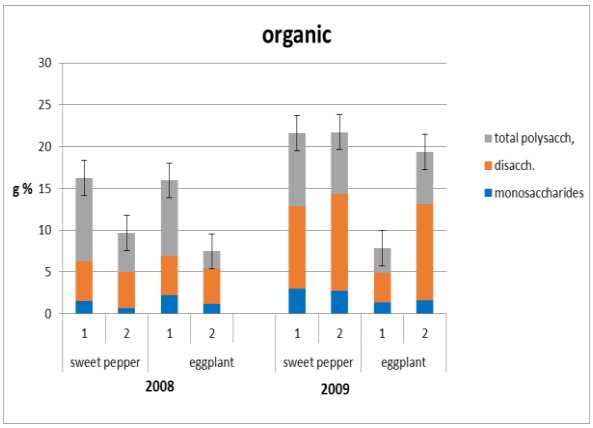

Fig. 2. Graphic representation of sugar leaf fractions in investigated varieties in organic technology system in flowering (1) and ripening stage (2) (bars are error standard)

In 2008, in organic crop, at sweet pepper, the total of polysaccharides showed a value of 10g (around 60%) of total sugars leaf compounds in flowering stage and decreasing until 4 g (40%) in ripening stage. Thus, the fruit in organic crop were with strong consistency (personal observation) because of accumulation of polysaccharides for a long period (Figure 2). Sucrose as disaccharides compound is relatively a stable molecule (it is transported between different plant organs and even stored for long periods) when compared with monosaccharides, which

are promptly metabolized and are seldom transported between cells or accumulated [4]. In protected spaces are observed the influence of environmental condition such as temperature oscillation in sugar partition of leaves. Thus, in unheated protected spaces, total sugars leaf compounds were higher in 2009, warmer year than in 2008. In organic as well as in classic system in ripening stage the monosaccharides are strongly decreasing and the disaccharides is increasing during fruit sugar accumulation. For both analysed system, total content of sugars leaves is increased in 2009 than 2008, especially at sweet pepper in ripening stage. In classic system, that accumulation is around 18% and in organic is almost over 22%. In ripening stage of 2009, the sugar leaf fractions showed that higher values are obtained in organic crop system almost with 10% higher than in classic system at eggplant and sweet pepper analysed varieties.

In other studies, it was observed that organic fertilization has a positive effect on the accumulation of reducing sugar in tomato varieties [2], phenolic compounds and mineral accumulation in eggplant [8]. The leaves transform reducing sugar into sucrose rapidly during the early phenophase and transport it to the stems and fruit for starch synthesis. As a result, the transformation from reduced sugar (monosaccharides) into sucrose (disaccharides) in the leaves increased, as well as the reducing the photosynthesis rate. Through that, an increasing of the polysaccharides amount during the later stage (ripening stage) took place.

Conclusions

In flowering stage, the photosynthesis rate was almost close values for varieties in classic and organic technology of protected spaces. Photosynthesis in ripening stage registered higher value at basis and top of the plant in conventional system as a well as in organic system, in all studied variants.

Higher values of substomatal CO_2, Ci have been observed in organic systems which involved a slower rate of absorbtion of atmospheric CO_2 in photosynthesis process than was observed in classic variants. In flowering stage, Wue obtained higher and close values in classic and organic systems. In ripening stage, Wue have a quite higher values in organic systems and where plants showed a slower growth than in classic.

Analysis of sugar leaf variation showed that in 2008 and 2009 in conventional technology the all investigated fractions of sugars are well represented in flowering phase (May) whilst in the ripening phase (July) the lower values of total monosaccharides are corelated with the decreasing photosynthesis which occurred that stage. The sugars leaf fractions showed that higher values are obtained in organic crop system especially in 2009 year, almost with 10% higher than classic system for eggplant and sweet pepper in ripening stage.

Acknowledgments

This work was supported by the Romanian Ministry of Research and Innovation through the NUCLEU program (Project No. 18180301 and 19-270301) and Program 1 – Development of the National R & D System, Subprogram 1.2 – Institutional Performance – Projects for Excellence Financing in RDI (Contract no. 22PFE/2018).

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GIS Project on the Influence of Morphometric Factors on Soil Erosion in a Hydrographic Basin

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Abstract

This paper proposes a GIS (Geographic Information Systems) methodology on the quantification of soil loss due to hydric erosion. The method is based on GIS techniques, and the novelty consists of the implementation of a mathematical model, through the "overlay" technique at pixel level, which ensures higher accuracy of the results. The database created within the GIS project can be considered a fundament for future forecasts or simulations of hydric erosion in the hydrographic water catchment area.

The variability of natural factors requires the use of GIS for the spatial-temporal monitoring of land erosion-related degradation, and thus the GIS use is justified in this paper by means of an example concerning a hydrographic water catchment area of approx. 4000 ha. The MNT of the water catchment area is developed (basic information layer for further analysis), where the information layers concerning the factor with impact on hydric erosion are developed. The simulation programs (in Fortran language) were imported under the Geo-Graph software under which the entire project was performed. The finality of erosion modelling shows the actual and potential erosion at a certain moment. The morphometric factors may change their values in time and spatial, but as long as the database was set up, the simulation under the new circumstances is extremely easy.

The main objective of the research: highlighting the influence of morphometric factors on erosion for the purpose of quantifying the areas of manifestation, assessing the erosion rate and predicting the erosion manifestation under conditions of environmental changes.

Keywords: hydric erosion, Geographic Information Systems (GIS), land degradation, morphometric factors, layer

Introduction

Erosion – a real problem. The soil is humankind's main natural wealth (resource). Erosion is one of the most serious environmental problems both at national and world level [1]. Along with the natural factors that trigger and maintain the erosion process on the slopes, soil erosion is also aggravated by human anthropic actions, thoughtless and negative actions, which hasten the erosion process [2].

Methodology

Study area

The water catchment area under study is called Antohesti. It belongs to the upper water catchment area of the river Berheci, in the Unit called Colinele Tutovei, and it is located in the Eastern part of Romania, in Bacau county (Fig. 1). The reception area is of 3963 ha, with a highly fragmented relief, hilly type and average slopes of more than 15%. Absolute altitudes ranging between 549.5 m (Hill Dorosanu) and 200 m in the dam accumulation, resulting an

energy relief 349.5 m. The slopes are affected by surface erosion, deep and. The sloping land was affected by sheet erosion, gully erosion and by active landslides. Dominant soils are chernozems and brown soils, and the most extended uses are: arable land – 47.2%, pasture land – 26.78% and forest – 16.8%, [3].

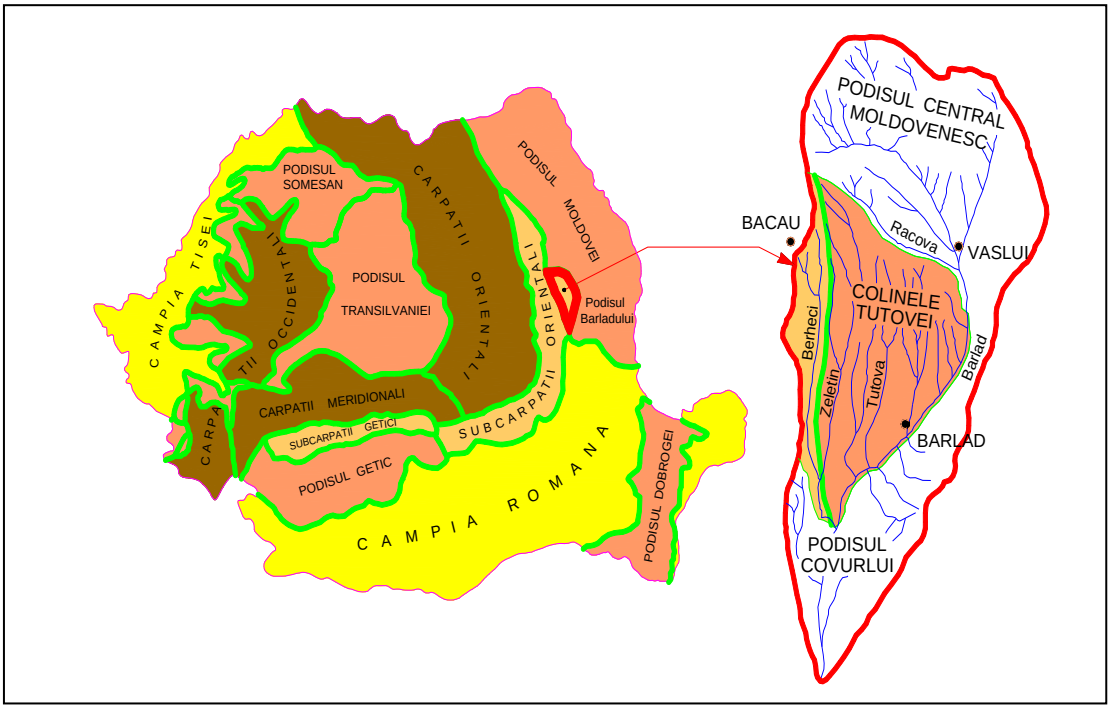

Fig. 1. Location of the study area (Antohesti catchment)

Method of research and input data

During the first stage, the information layers that characterize the landscape were created (because landscape-related information is critical for the modelling of erosion processes on gradient land).

Based on the map with level curves, the obtained Numerical Land Model (MNT) provided fundamental layers for the GIS project, such as: *Layer 1* – Hypsometric map; *Layer 2* – Flowing direction map; *Layer 3* – Gradient map; *Layer 4* – Flowing length map.

By integrating the above mentioned seven layers in USLE equation, under Geo-Graph software, we obtained the information layer of the erosion risk (*Layer 8*), in two simulation versions, namely the *actual risk* (determined by the combined action of all parameters of USLE equation) and the *potential risk* (where the factors that can be controlled in the intake basin – layer 5 and layer 7 – were disregarded).

A Land Numeric Model (MNT) was developed based on an accurate cartographic basis. Due to the fact that the landscape-related information is critical for modelling the erosion processes for gradient land, the expression thereof in a numeric form has significant benefits determined by the fast processing and compatibility with numeric mapping and remote detection techniques.

The numerical model of the land

Land modelling is an important component of GIS applications in the study of erosional, geomorphological and hydrological processes. Moreover, it has been gaining increasing importance lately in other fields of activity as well (land-use planning, buildings, etc.).

Usually, the relief is represented in the form of a field of continuous altitudes, known in each of the nodes of a regular grid, known as the Land Altitude Numerical Model (MNAT), represented in the form of pixel of different dimensions.

The Land Numerical Model for the area studied was drawn up by interpolating altitudinal information from stereo-orthophotoplanes. The punctiform data set was interpolated for validation. The errors were highlighted by directly visualising the model obtained or by deriving products such as: slopes, exposition, profile curvature, plane curvature and shading. After making the corrections, the data set was rasterised, using several interpolation methods. The Land Numerical Models obtained by raster procedure was used in order to develop the geo-referential database included in the above-mentioned equation, and this enabled the overlay of a rectangular grid of square cells of 25 x 25 m (cell size) on the cartographic documentation.

Thus, the computation of water erosion in the reviewed basin was performed on 63,408 cells.

Results and Discussions

Relief altitude decreases progressively from east and northeast to west and southwest, according to the general surface geological inclined strata (Fig. 2). The presence of harder rock horizons in the southwest, resistant to disaggregation and erosion, favoured maintaining interfluvial peaks in this area at altitudes greater than 500-450 m. The greatest heights correspond to the interfluves that separate the main hydrographic basins of the tributaries.

Through regressive erosion, the interfluves were fragmented, which led to the greatest heights being currently associated with hills with an accentuated profile, laid out in a perpendicular direction to the direction of the current river's drainage.

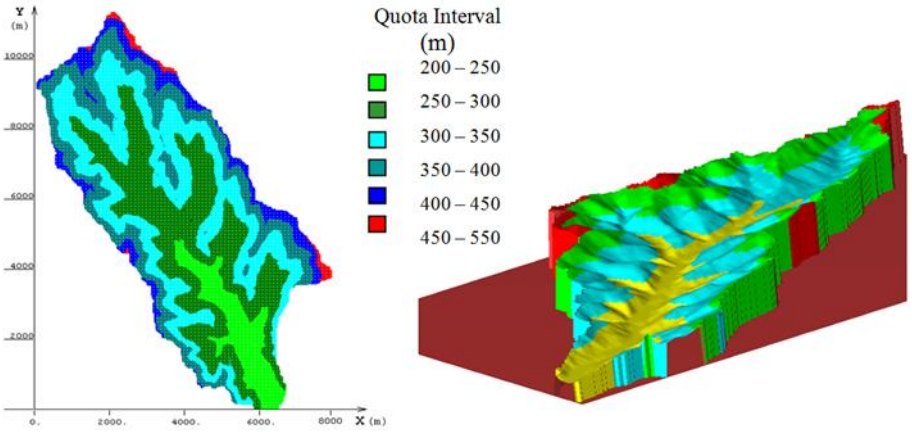

Fig. 2. Hypsometric map: "*layer 1*" and DEM in 3D format

The average relative relief height – is of 298.4 m. The maximum altitude of 494.7m and the altitudinal deviation is equal to 372.4 m.

The modal class is of 100-250 m, the 150-160 m class (representing meadows) also registering a high frequency, most of the values ranging between 150 m and 375 m.

Slope a very important factor that conditions the dynamics and frequency of erosion processes. Values under 5% characterize meadows, deluvial cones, colluvial-proluvial areas.

The land slope was automatically determined based on the numerical model of the altitude, a classification being subsequently applied, which resulted in 9 classes of values with geomorphological relevance (Fig. 3). The maximum slope is equal to 47%, the average slope of the region studied is of 18.9%.

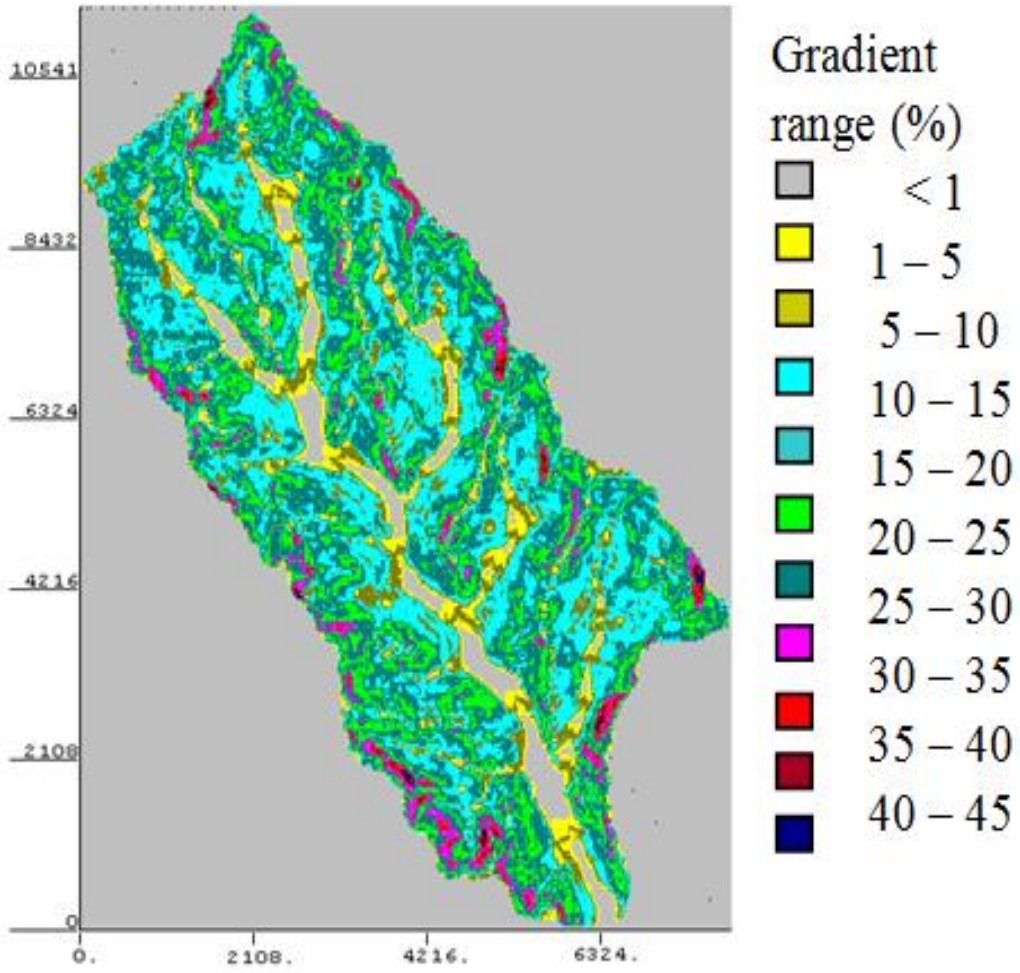

Fig. 3. Relief declivity map: "*layer 3*"

The quasi-horizontal and very gently sloping lands, with slopes under 5, are represented by approximately 11% of the lands (170 ha). The moderately steep lands (5-15) represent 34% of the surface of the region, namely approximately 790 ha. They correspond to the majority of the slopes, but especially to those conforming to the surface geological structure, consequently having a southern or southeaster general orientation. Furthermore, these slopes can also be found in the lower third of the slopes, as well as the majority of the interfluves. Erosion occurs with moderate intensity on these surfaces, the processes mainly consisting in surface erosion.

The steep (15-25) and very steep (>25) lands cover approximately 56% of the surface of the region (1700 ha) and correspond mainly to *cuesta* slopes. The landforms are destroyed here with greater intensity, through a set of processes, which includes surface erosion, landslides and gullying. In the basin subjected to study, the highest share is represented by the lands located on the slopes with steep slopes, while the lowest share is represented by the quasi-horizontal or gently sloping lands.

Relief fragmentation depth – has unevenly distributed values in the space analysed. The highest interfluves are characterized by energy values exceeding 300 m, and the majority of the slopes by values of 250-300 m. The average value of the relief fragmentation depth in the region studied is 261.5 m, the values ranging from 50m to 550 m. The modal class of frequency distribution is that of 50-100 m (62.44%), the value class under 50 m (30.96%) registering halved values, followed by the much less represented classes, the >100 m (together 6.61%).

The raster representation considered that the current pixel can be drained based on one of the eight possible directions (Fig. 4), depending on the positioning of the lowest altitude adjacent pixel.

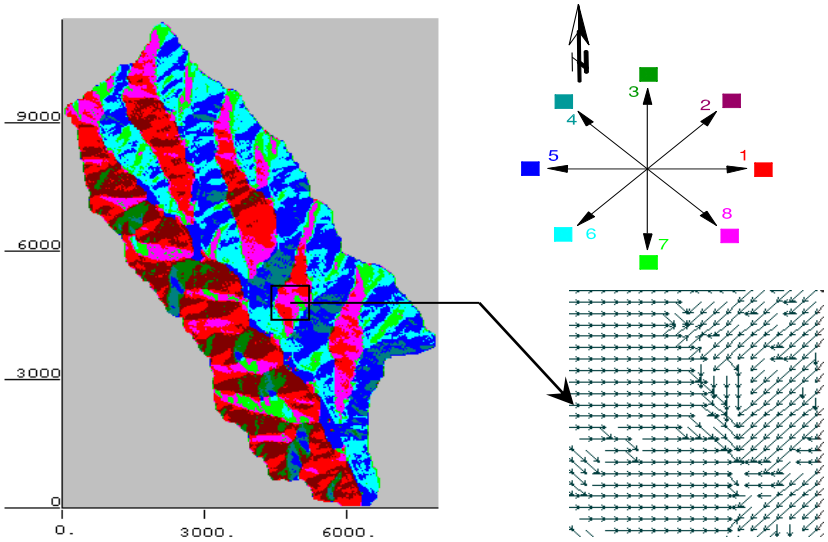

Fig. 4. Drain directions "*layer 2*"

Relief fragmentation density – represents the ratio of the total lengths of the hydrographic systems, including the dry valleys, to the surface unit (km/km^2). It is a parameter that provides a clear image of the degree of relief fragmentation, thus quantifying the nature of the variation in the surface. The differentiations are influenced by lithology, the nature of the current geomorphological processes.

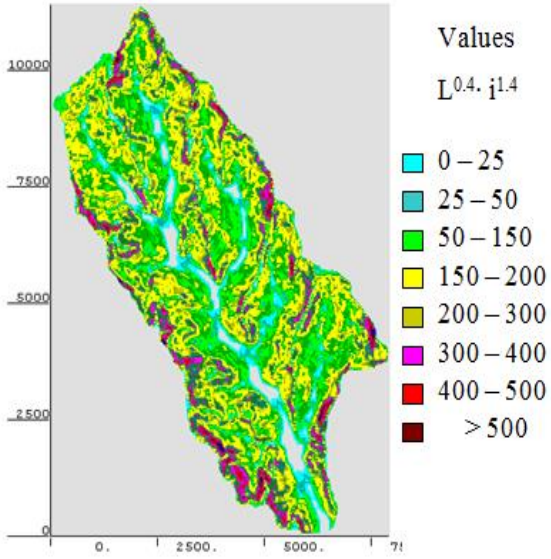

Fig. 5. Topographical factor map: "*layer 4*"

To conclude, the overall morphometry of the hydrographic basin studied is the effect of the morphological evolution of the monoclinally structured lithological deposits. The estimation of the relief factor (the product between the maximum length of drainage and the slope: $L \cdot i_v$) is of major importance to determine soil losses. The method of estimation used has a major role as regards the result. Thus, selecting different erosion flow modelling algorithms determines both the amplitude of the resulting values, as well as the spatial distribution model of the relief factor values. Moreover, it can be concluded that should the precise quantitative determination of soil loss be requested, it is absolutely necessary to calibrate the model used.

Conclusions

1. This project develops the information flow of geo-spatial data, from acquisition to graphic and non-graphic information (alphanumeric) concerning the erosion risk of a water catchment area. The aim of the research is elaborating, validating and implementing computerized modelling techniques of erosion processes, within the area studied, by applying GIS.
2. Modelling soil hydric erosion by surface runoff is a laborious process, which requires a considerable volume of input data, referring to: morphometric characteristics of the relief, intensity and amount of rainfall, coverage/use of lands and anti-erosion practices.
3. The kinetic energy of rainfall determines the initial erosion of rainfall, and the length and degree of slope inclination determine the energy of the running water – as main erosive forces. These give erosions the character of a hazard. Within the USLE model, the energy available to displace sediments during rainfall is equal to the product between the total amount of kinetic energy (E) and the intensity of rainfall (I).
4. The capacity of the soil to resist erosion (erodability), as well as the type and degree of land coverage and the agricultural techniques used are the conditions (forces) that are activated against the erosion phenomenon. All these put together represent the state of vulnerability of the soil.
5. The applicative value of this paper consists in elaborating mathematical models to simulate the process of hydric erosion, creating a database (maps) of the spatial distribution of rainfall erosion, soil erosion, hazard and risk of soil erosion by water.

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Aspects Regarding the Clogging of the Accumulation Lakes Due to the Erosion Processes on the Surface of the Reception Basin

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Abstract

Lake clogging is an extremely complex natural process, which starts from the moment the lake emerges, by collecting water in a natural pool and holding until the clogging fills the lake basin. The rate and intensity of the alluvial accumulation lakes are determined, in general, by several factors, including the intensity and extent of the erosion and sediment transport processes, the physico-geographical features of the receiving basin, the accumulation coefficient, the way the land is exploited on the surface. the basin, the process of abrasion of the banks, the design concept of the accumulation, the regime of its exploitation. Any type of soil is vulnerable to the degradation of its structure. Lack of activity planning or overwork can result in soil compacting or compacting. These processes can prevent the emergence of plants, by degrading the porous space, the conditions of aeration and water supply, necessary for the development of the root mass. Also, soil compaction favours surface drainage processes, by reducing soil permeability to water, infiltrating water into the soil. The intensification of surface runoff causes soil erosion, and the transport of the eroded solid material through the hydrographic network in the accumulation lakes, eventually producing their clogging.

Destruction is the reduction or loss of stability of soil structural aggregates to the action of water and agricultural machinery, being one of the most important physical processes of soil degradation. In turn, the destruction is in fact the cause that generates many other negative processes or the intensification of the existing ones. Thus, the deterioration of the quality of the structural aggregates, that is: of the shape, of their porosity, of the hydraulic and mechanical stability, especially on the soils with arable use, is of the most important because, they influence crucial the hydrological characteristics, the permeability of the soil for water and air, stability and configuration of the porous space. The origin of the solid material driven by water (solid flow) is largely due to the training of the fine material from the river basin, favoured by landslides, collapses and torrential erosions. Measurement-based assessments show that in ordinary large waters (annual maximums), 68% of alluvial material comes from the basin and only 32% comes from the translation of particles from the bed of the riverbed. In the forested regions of Romania, the average turbidity of the rivers is below 100 g/mc, compared to up to 5000 g/mc as they reach the rivers flowing through heavily forested areas.

Keywords: Clogging, erosion, lake, solid flow, landslides

Introduction

The accumulations in the hilly areas constitute the kind of hydrotechnical works that have a complex role in their economic and social activity. The criteria underlying the choice of sites for these hydrotechnical works are rigorous, and for this reason, their number is limited, constituting a resource that, like any resource, must be rationally exploited. Clogging

accumulation is a phenomenon that cannot be stopped but only diminished and has negative consequences that disrupts their normal operation.

The concept of "Alluvium system" is defined [1] in general terms as a subsystem of the river geomorphological system in which the main inputs are the control factors, the transfer and storage of deposits is ensured by the morphodynamic triad: erosion – transport – sedimentation, and the output of the system is the production of sediments which can be assimilated by the ratio of the effluent and silt.

From the point of view of the protection of the accumulation lakes, in the decisions to reduce the transit of alluviums, interest is given to the "alluvium processes" and "sediment storage" which in the alluvium system must be looked at separately but, on a well-defined time and space scale and in connection [2].

In alluvium processes, the analysis of alluvium sources should take into account the area of origin in relation to the basin (slope, riverbed or other geomorphological formations), land use (agriculture, forestry, construction, etc.), and the generating processes of alluviums (erosion, landslides, shore erosion, etc.). A study by R.A. Romanian Waters point out that in the whole country, in an average period of 15 years, in the lakes on the inland rivers, there were deposited about 200 million m^3 alluviums with an annual rate of 13.4 million m^3, which represents 27% of total multiannual alluvial transport. The fastest rates of clogging were recorded at accumulations located in areas with high alluvial transport and which were executed before carrying out some anti-erosion works in the reception basins [3].

Central Moldovan Plateau and Tutovei Hills, as subdivisions of the Moldovan Plateau, are strongly affected by the erosion phenomenon which leads to high alluvial effluent coefficients, respectively a high alluvial effluent, which affects by sedimentation the accumulations located in this area [2].

Methodology

The research method addressed was that of inventorying the predominant forms of erosion in the area of excessive influence on the alluvium transport, of accumulations located in the area of the Central Moldavian Plateau and the Tutovei Hills, of their mode of erosion management and through determinations and observations. the effect of these forms of erosion, arranged or not arranged, on the filling of the respective accumulations [5]. At the same time, the degree, mode and average annual rate of clogging of the respective accumulations with different situations of the area of excessive influence in terms of alluvium transport were established. These objectives were pursued in the idea of highlighting the differentiated contribution of erosion processes to the accumulation of accumulations, differentiation given both by the forms of erosion and by the way of arrangement or the lack of anti-erosion arrangements [6].

For the presented case study, determinations were made on a number of six accumulations regarding the clogging process and, in the area of excessive influence, observations and determinations regarding the anti-erosion arrangements and their behaviour regarding the influence on the clogging of the lakes. The accumulations are located as follows: an accumulation from the Central Moldavian Plateau (the Puscași accumulation located on the Racova river) and five accumulations from the Tutovei Hills: Cuibul Vulturilor located on the Tutova river; Rapa Albastra located on the Simila river; Fichitești located on the Pereschiv river; Antohești located on the Berheci river and Găiceana located on the Ghilăvești river [5].

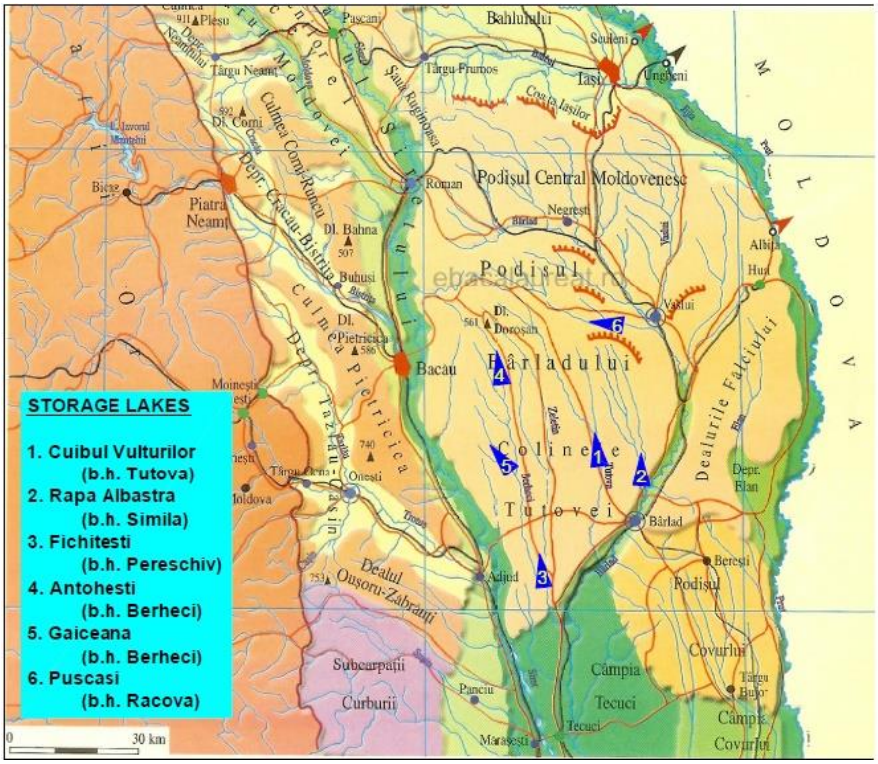

Fig. 1. Locate areas of research and observation in the Moldavian Central Plateau and the Tutovei Hills

From a geomorphological and geological point of view, the surface geological formations belong to the upper sarmatian and pliocene, the former being present at the base of the northern slopes of the Tutovei Hills, in marine, clay-sand to moderate and deltaic facies, predominantly sandy, with sandstone interlayers. and clays in the north and northwest. The largest development is the meotean sediments, with a thickness of over 300 m, formed by a complex of sands, clay sands, clays and marls that start from the base through a horizon of andesite cinerites consisting of banks whose thickness can it reaches 10-20 m in the eastern part of the Tutovei Hills and 70-80 m in their western part towards the Siret valley [4].

Precipitation has a variable distribution both in time and in space. In Fig. 2 is presented the precipitation regime at the main weather stations in the Central Moldavian Plateau and the Tutovei Hills.

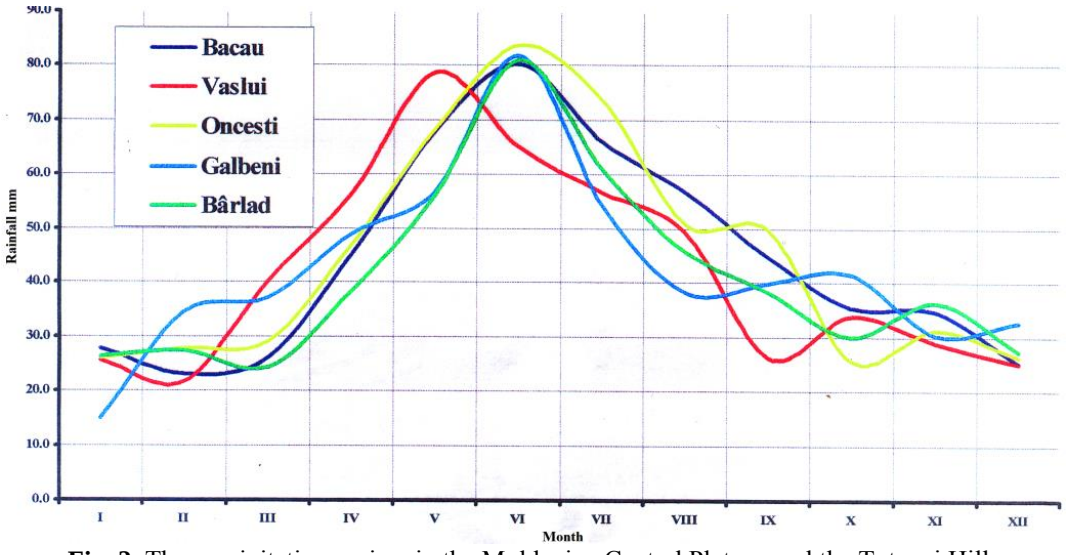

Fig. 2. The precipitation regime in the Moldavian Central Plateau and the Tutovei Hills

The hydrographic network that drains this area is entirely tributary to the Bârlad river through its most important tributaries coming from this area: Tutova, Pereschiv, Berheci, Zeletin and Simila in the Tutovei Hills respectively: Racova, Vasluet and Crasna in the Central Moldavian Plateau.

The feeding of the hydrographic network is predominantly pluvial, with an extremely capricious regime, but which generally has similar variations during the year. Debts below the annual average (30-35% of the multiannual average) start from August to February and during March-June the throughput rates are 1.5-2.0 times higher than the multiannual averages with the maximum reached in the month June, aspect reproduced in the liquid flow module (Fig. 3) on the main rivers within the Central Moldavian Plateau and the Tutovei Hills.

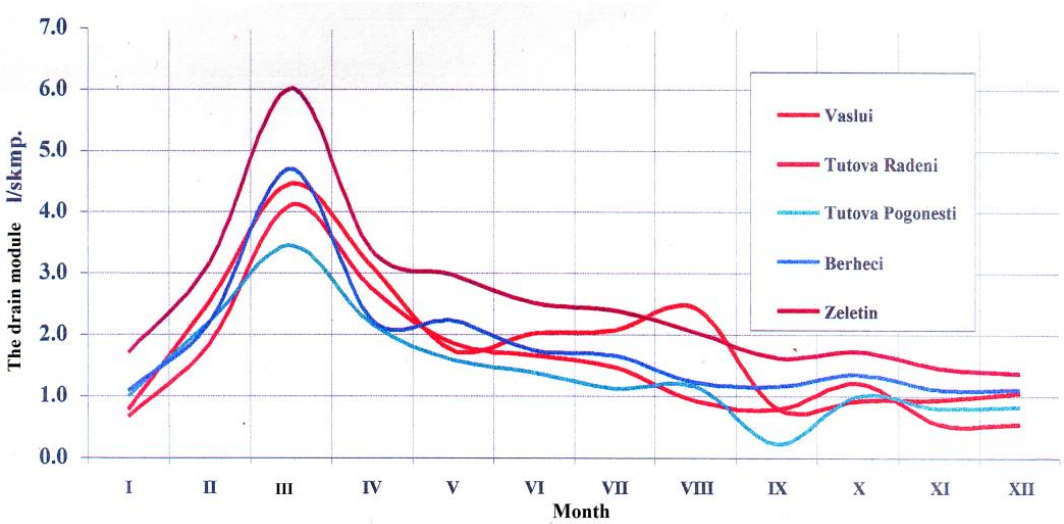

Fig. 3. Module of liquid flow on the main rivers in the Moldavian Central Plateau and the Tutovei Hills

From the point of view of turbidity, for the rivers in the Central Moldovan Plateau area and the Tutovei Hills, they fall as a multiannual average value around 6.0 g/l but the highest degree of alluvium loading is during March-July 5.2-14.2 g/l.

Anthropically, in terms of the use of agricultural land in this area the main share is the arable land 302950 ha (73.6%). Of the total of 411540 ha agricultural land in the area 289610 ha (70.4%) are located on slopes greater than 5%. From the area of arable land, 209450 ha (69%) are on slopes greater than 5%. The pastures and grasslands, which represent 19.6% (80800 ha) of agricultural land, a percentage of 66.8% (54000 ha) are located on slopes greater than 5% [5].

Results and Discussions

The degree and average annual rate of clogging of the analysed accumulations, determined at the NNR (normal retention level) is different. The results obtained, for the accumulations in the area of the Tutovei Hills, highlight differentiated values of the annual degree and average rate of clogging according to the hydrographic basin where they are executed and their location within it. Thus, the accumulations of Antohești and Găiceana, located in the upper and middle part of the Berheci river basin have a filling level of 40.91% respectively 41.46% of the initial volume at the NNR, returning them with an average annual filling rate of 4, 09% respectively 4.15% of the same volume. The accumulation Cuibul Vulturilor located in the lower part of the Tutova river basin is clogged with 32.63%, achieving an average annual clogging rate of 2.33%.

Râpa Albastră accumulation in the lower area of the river basin The similarity has a clogging degree of 21.13% with an annual average rate of 2.33%, while the Fichitești accumulation in

the lower area of the Perschiv river basin is the most strongly affected by clogging, the degree their clogging being 52.6% the average annual clogging rate being 3.3%.

In the case of Puşcaşi accumulation, the degree of clogging is 62.3% and the annual rate of clogging that is the accumulation is 0.436 million m3, that is 2.7% of the volume of useful water initially accumulated at the NNR [5].

Analysing the tendency of clogging of the accumulations in the Tutova Hills according to certain morphological parameters, it can be said that the average annual rate of clogging decreases with the increase of the surface of the receiving basin to a certain limit and then it increases again, a tendency that is observed and depending on the initial water gloss, which is explained by the fact that the small reception basins coincide with the surface of the area of excessive influence.

As a result of the measurements made on these six accumulations regarding the degree, the average annual rhythm and the way of filling, the alluvial effluence from the area of accumulations could be appreciated. At large accumulations, through the exploitation regime (minimum levels before forecasted floods), the degree of alluvium retention can be considered as 95%-98% leading to values (indicative for the location of accumulations) of alluvial effluence of 14, 16 m^3/ha year in the Racova basin, 4.08 m^3/ha year in the Tutova river basin, 6.23 m^3/ha year in the Simila river basin, 11.08 m^3/ha year in the Perschiv river basin.

Conclusions

The average annual rate of clogging, of the accumulations in the studied area, decreases with the increase of the surface of the reception basin to a certain limit and then it increases again, a tendency that is observed also according to the initial water glow at the NNR, which is explained by that that the small reception basins coincide with the surface of the area of excessive influence.

Depending on the surface of the area of excessive influence on the transport of alluviums and the initial volume of water at the NNR, the tendency of the average annual rate of clogging is decreasing with the increase of these two parameters. In the large accumulations, where the area of excessive influence on the transport of alluviums is 2.0%-10.0% of the surface of the entire reception basin but being of the order of thousands of hectares, a differentiation of the rate of deposit of the alluviums is observed. In the area of direct entry into the accumulations of the microbazines it is 4 to 6 times greater than the area of directly adjacent slopes.

The lowest value of the average annual rate of clogging is achieved when the surface of the receiving basin is between 30000 and 40000 ha, the surface of the area of excessive influence on the transport of alluviums is less than 4000 ha, the initial volume of water at the NNR is more high of 6000000 m^3, the surface of the water gloss at the NNR has values between 200 and 260 ha.

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Assessment of Biochar Derived Agri-Waste on the Sorption of Metribuzin Pesticide

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Abstract

Rapid industrialization and modern agricultural practices raise soil and water contamination by imposing toxic effects to humans and ecosystems. Based on principle of adsorption, application of amendments is often considered as a cost-effective alternative for remediation of pesticide polluted soils. Biochar has arisen as promising material which is eco-friendly, renewable, cheaper, and easily available, that will solve the problem of soil remediation.

Moreover, due to highly porous structure and the presence of carboxylic and phenolic groups in their structure, biochar has the ability to sorb and retain organic pollutants including pesticides and heavy metals. Therefore, the objective of this study was to assess the pesticides adsorption capacity of biochar-based wheat, in order to understand the impact of biochar amendment on the fate of metribuzin in soil. Initially, the efficiency of the biochar derived agri-waste was evaluated after pyrolysis and then SEM analysis were carried for morphological analysis. Kinetics and isotherms studies were performed in order to characterize the biochar sorption capacity. The results of this work indicate that biochar's can be used as an alternative adsorbent to remove metribuzin pesticide from aqueous solutions.

Keywords: Adsorption biochar, pesticide, metribuzin

Introduction

Soil polluted by pesticides is a global concern, with concentrations of residues in soils that often exceed the self-purification capacity of the soil. High concentration of pesticides poses harmful side effects, different types of endocrine malfunction, interaction with estrogen and androgen receptors and thyroid. Therefore, the efficient removal of pesticides from aqueous solutions have become a significant issue from economical and health perspective [1]. The methods for pesticide removal from aqueous solutions, are adsorption, reverse osmosis and ultrafiltration, precipitation and ion exchange. Among all these methods, adsorption has gained importance due its efficiency, cost effectiveness, versatility and easy handling.

Biochar is a carbon rich material, which can be produced from various agri-waste biomass pyrolysis in an oxygen-limited conditions which can be used as adsorbent to remove pesticide molecules from aqueous solutions [2]. As a soil amendment, biochar helps to improve soil quality due to its high porosity and large surface area that bind the nutrients and prevent leaching loss. All these effects provide suitable conditions for microbial growth associated with the degradation of organic matter and pesticides. Biochar as a soil amendment can improve soil quality, increase crop yield, reduce irrigation and fertilizer demands and mitigate greenhouse carbon emissions [3], [4], [5].

Herein, biochar derived wheat straw was applied to the adsorption of metribuzin from water.

The performance of biochar for attracting metribuzin molecules from aqueous solutions was extensively tested at various batch adsorption variables, such as initial concentrations, contact

time and solution pH. The experimental data were analysed by Langmuir and Freundlich models while pseudo-first order and pseudo-second order models were adopted for the analysis of kinetic data. The chemical composition and surface characteristics and morphology of prepared biochar's were also analysed.

Methodology

Materials

Metribuzin with a purity of 95%, chemical formula of $C_8H_{14}N_4OS$ and molecular weight of 214.29 g mol^{-1} was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The soil and wheat straw material were collected from Ezăreni, – the Experimental Farm of the Agricultural University Iasi Romania. Pyrolysis of wheat straw was conducted under different temperatures (600°C, 700°C and 800°C) under oxygen limited environment for 6 h. The obtained biochar's were referred as WS-600°C, WS-700°C and WS-800°C.

Characterization of biochar's

The surface morphology and structural images of each biochar was examined using a Scanning Electron microscope (SEM) (FEI – Field Electron and Ion Company).

Adsorption performance

The performance of the biochar's for retaining metribuzin molecules was evaluated under initial concentrations, contact time and solution pH of 7. 2 g of soil and 0.02 g of biochar was added to a set of 100 mL Erlenmeyer flasks containing 20 mL of solution with the required initial concentration. The influence of contact time (0-24 h) was explored at various initial concentrations (1 – 100 mg L^{-1}). The removal efficiency and the adsorbed amount q (mg g^{-1}) were evaluated by: $q = \frac{(C_o - C)}{W} V$, where: C (mg L^{-1}) and C_o (mg L^{-1}) are the residual and initial concentration of metribuzin, respectively. The soil and agri-waste mass and the volume solution are W (g) and V (L), respectively.

Adsorption studies

The adsorption studies were performed by adding 2 g of soil and 0.02 g of biochar to 20 mL of metribuzin solution at different concentrations (1-100 mg L^{-1}) at pH 7.0 for 120 min. The experimental data were analysed by Langmuir and Freundlich models as follow: Langmuir isotherm: $q_e = q_{max} \frac{K_L C_e}{1+K_L C_e}$ and Freundlich isotherm: $q_e = K_F C_e^{1/n}$, where: q_{max} is the maximum adsorption capacity (mg g^{-1}); C_e is the concentration of metribuzin at equilibrium (mg L^{-1}); K_L (mg g^{-1}), K_F (mg g^{-1}) and n are the Langmuir and Freundlich constants.

The kinetic study was conducted for the time range of 0 – 24 h; using the residual concentration at time t, C_t (mg L^{-1}), the adsorbed amount at time t, q_t (mg g^{-1}) was also evaluated from Eq (1). Pseudo-first order (Langergren, 1898) and pseudo-second order (HO and McKay, 1999) models were adopted for the analysis of kinetic data, as follows: Pseudo – first order: $q_t = q_e (1-e^{-k_1 t})$, Pseudo-second order: $q_t = \frac{q_e^2 k_2}{1+K2qet}$, where: q_e (mg g^{-1}) and q_t (mg g^{-1}) are the metribuzin adsorbed at equilibrium and time t, respectively; k_1 and k_2 (mg g^{-1} min^{-1}) are the rate parameters of both models.

Results and Discussion

Characterization of the adsorbents

In Table 1 are presented the elemental compositions of WS, WS-600°C, WS-700°C and WS-800°C. The results indicate that the C content increased with increasing temperature which

implies an increase of surface area of biochar's. Both carbon content and aromatic structure are relevant items that contribute to pesticide molecules adsorption [6], [7], [8], [9]. Furthermore, the higher C content in WS-800℃ facilitated the formation of structures which can improve the adsorption of metribuzin and another aqueous contaminant [10]. These results are in agreement with the results described by [11] where reported that the carbon content of biochar increased with increasing temperature.

The atomic H/C ratio is a reflection of a more intensive aromatic structure which establish different pore size while surface area is more stable against decay, difficult to be decomposed and mineralized [12] [13]. The ratio of O/C was lower with increasing temperature, suggesting the loss of oxygen content and its close relationship with composition of functional groups.

Table 1. The main composition of WS, WS-600℃, WS-700℃ and WS-800℃

Material			**Elemental composition (wt %)**				**Atomic ratios (wt %)**		
	Ash	**Moisture**	**C**	**H**	**O**	**N**	**H/C**	**O/C**	**(O+N)/C**
WS	8.72	3.15	48.29	7.13	41.75	2.83	0.147	0.864	0.923
WS-600℃	14.45	4.58	54.71	3.58	17.54	1.15	0.065	0.320	0.341
WS-700℃	12.11	4.43	66.93	2.86	14.25	0.89	0.042	0.212	0.226
WS-800℃	7.19	4.97	74.12	2.15	10.17	0.35	0.029	0.137	0.141

Similar results were found by [1] who utilized barley straw as precursor to prepare biochar adsorbent through thermal pyrolysis.

The morphological features of agri-waste before and after pyrolysis were characterized by scanning electron microscopy (SEM). SEM images of the WS, WS-600℃, WS-700℃ and WS-800℃ are presented in Fig. 1. Prior to pyrolysis treatment, were numerous well-ordered parallel textures on the surface of wheat straw. After that, the parallel structures were broken and the biochars had on surface different dimensions in the form of spherical agglomerates that are intersecting and interconnecting. [1] prepared activated carbon from barley straw by thermal pyrolysis. They found that the porosity surface with a honeycomb structure is due to carbonaceous skeleton from the biological capillary structure of the raw material.

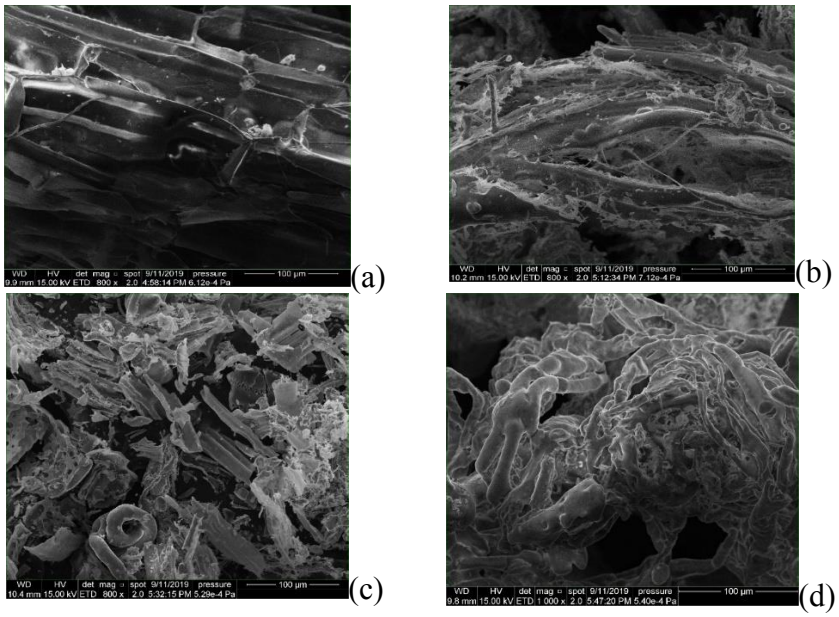

Fig. 1. SEM images for (a) – wheat straw (WS), (b) – WS-600℃, (c) – WS-700℃, (d) – WS-800℃

Adsorption isotherms and kinetics

Metribuzin adsorption onto soil and biochar's varied with time and was influenced by the pyrolytic temperature. Metribuzin sorption rate was fast during the first 1 h and then slowly reached equilibrium after 5 hours.

To describe both the adsorption mechanism (surface properties) and the maximum adsorption extent, the Langmuir and Freundlich models were used. The results showed good fitting of data by the Langmuir model with R^2 values (0.977-0.998) relative to the R^2 values (0.714-0.960) for the Freundlich model (Table 2). The results of experimental equilibrium data, reveals the monolayer coverage of metribuzin molecules may occur onto the homogeneous surface of biochar. The values of K_L increased with pyrolysis temperature, which indicate that the new material obtained was more effective in metribuzin adsorption.

Table 2. Adsorption isotherm parameters for metribuzin on soil and biochar's

Isotherm	Constants	Soil	WS-600℃	WS-700℃	WS-800℃
Langmuir	R^2	0.996	0.998	0.984	0.977
	q_L	126.4	256.1	294.7	347.3
	K_L	0.31	0.42	0.67	0.74
Freundlich	R^2	0.960	0.848	0.714	0.890
	n	0.96	0.61	0.78	0.67
	K_F	1.84	141.25	135.1	126.18

The different K_L values of WS, WS-600℃, WS-700℃, WS-800℃, suggest different adsorption mechanism could be involved in metribuzin adsorption capacity. Similar results were found on various biochar's derived from rice straw, corn straw and wheat straw [14].

The pseudo-first-order kinetics model of Lagergren (1898) and the pseudo-second-order model of Ho and McKay (1999) were used to describe the kinetics data. In terms of correlation coefficient (R^2), the results obtained for the pseudo-second-order type showed to be higher relative to the R^2 values of the pseudo-first-order kinetics model (Table 3). The results also showed good agreement between the experimental q_e values and calculated q_e values of the pseudo-second order model.

Table 3. Kinetic model parameters for metribuzin and soil-treated biochar's system

	Pseudo-first-order				Pseudo-second-order		
	$q_{e\ exp}$ (mg g^{-1})	$q_{e\ est}$ (mg g^{-1})	K_1 (mg g^{-1} min^{-1})	R^2	$q_{e\ est}$ (mg g^{-1})	K_2 (mg g^{-1} min^{-1})	R^2
Soil	1.2159	2.9485	0.02	0.756	1.193	1.212	0.996
WS-600℃	1.4112	5.1321	0.001	0.723	1.392	0.798	0.978
WS-700℃	1.4638	5.8031	0.001	0.831	1.445	0.821	0.993
WS-800℃	1.5109	5.7091	0.001	0.892	1.489	0.834	0.992

The pseudo-second-order type suggests that the adsorption rate is controlled by the active sites, then the concentration of metribuzin, identifying adsorption under chemisorption type.

Additionally, the second model, implies ion exchange or superficial complexation between the superficial groups of the biochar's and metribuzin molecules from aqueous solution [2], [10].

Conclusions

The capacity of biochar's to remove metribuzin was investigated. Biochar derived agri-waste at three pyrolysis temperatures showed highly reactive surfaces, as confirmed by SEM. The adsorption equilibrium data of metribuzin onto the biochar's were best described by the Langmuir model, while the adsorption kinetics follow the pseudo second order model. The adsorption process was found to be complex depending on both time and concentration.

Acknowledgements

Authors acknowledge the logistic support from Competitiveness Operational Programme (COP) 2014-2020, under the project number 4/AXA1/1.2.3. G/05.06.2018, SMIS2014+ code 119611, with the title "*Establishing and implementing knowledge transfer partnerships between the Institute of Research for Agriculture and Environment – IAȘI and agricultural economic environment*".

We would like to thank all anonymous reviewers for reading the paper carefully and providing thoughtful comments, which greatly valued this version of the manuscript.

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Study on Innovation Potential of Romanian Agriculture

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Abstract

Technical progress, innovation, and knowledge are the neo-factors of production that determine over 70% of the competitiveness of the economic sectors including agriculture.

Increasing the competitiveness of the agricultural sector and the overall performance of the agricultural business in Romania depends on the farmers' access to innovation, information, and knowledge. Through the research conducted, it was sought to find an answer to the question: What innovation potential has the Romanian agriculture? From the methodological point of view, the following methods were applied to answer the research problem: the documentary analysis; statistical analysis; method of comparison. The results of the study highlighted the following aspects related to the innovation potential in agriculture: *i*) the expenses for research and development activities in agriculture represent less than 1% of the gross agricultural added value, 80% less than the European Union average of 5.5%; *ii*) Romanian agriculture has a growing human resource of research, represented in 2016 by 76,500 thousand persons with agricultural studies or employed in the field of agricultural research and technology; *iii*) the 105 agricultural research infrastructures in Romania, which provide more than 700 specialized services, as well as the 14 active clusters in the field of agriculture, bio-economy and the agri-food industry, are another benchmark for the innovative potential of the sector.

Keywords: Potential; innovation; agriculture

Introduction

The agricultural sector is subject to significant challenges in terms of increasing economic competitiveness at the same time protecting the environment and increasing food security (Leaver, 2010). Facilitating farmers' access to innovative technologies is a must for the success of the economic entities in the field on the world market. Most of the innovative technologies in agriculture are produced by the private sector and target agricultural inputs and equipment [1]. Most technological innovations have been created to help farmers increase the productivity and quality of agricultural production. The latest challenges of innovation are to reduce the impact on the environment [2]. The study of Dogliotti *et al*., [3] highlights the importance of innovation in agriculture for increasing productivity in this sector.

Economic actors in the field are directed to innovate and integrate into national and international innovation networks to increase their economic performance and market competitiveness. For example, at European Union level, through the programs of the Common Agricultural Policy 2014-2020, farmers and other actors in the field (processors, research organizations, consultants etc.) can access non-reimbursable financing for investing in innovative technologies and receive compensatory financing for practicing technologies that are environmentally friendly.

The development of agriculture through innovation implies the adoption of a multidisciplinary strategy which will lead to the cohesion between technological, economic, social, cultural and environmental problems [4]. One of the main features of agriculture compared to the other sectors of the economy is the increased dependence on natural factors, such as climate and biological factors. In this regard, we can say that the statement of Brian Brett: "the profession of the farmer is a profession of hope" is fully true. An essential role of innovation in agriculture is to reduce dependence on natural factors that are difficult to control [5].

The agricultural sector in Romania is having difficulties in the process of exploiting its potential, under the influence of technical, economic, social and political challenges. Thus, according to the SWOT analysis presented in the National Program for Rural Development 2014-2020, the following factors were identified which press on the potential for the development of the Romanian agriculture: *i*) low agricultural yields, especially at the level of small and family farms; *ii*) high weights of farmers without agricultural training at the level of small farms; *iii*) high levels of aging of farmers; *iv*) low degree of integration of agricultural production at the farm level through the processing and marketing of processed products; *v*) low degree of technical endowment of the farms etc. These unfavourable situations specific to non-commercial, family farms, bring down the overall performance of the Romanian agricultural sector and, moreover, contributes to a distorted image of the real potential and performance of this economic sector, with a strategic importance for Romania's development. The aim of the research is to answer the following question: What innovation potential has the Romanian agriculture?

Material and Methods

To answer the research problem, the following research methods were used in the paper: documentary analysis; statistical analysis; method of comparison.

The documentary analysis was performed on the research studies, reports and statistical publications. The statistical analysis involved the collection and processing of the statistical data provided through the Statistical Yearbooks of Romania and the Eurostat Database, by 2006-2016. From the qualitative analysis, the indicators of appreciation of the resources and the innovative potential of the Romanian agriculture were compared with the situation at European Union level – EU 28, from 2016.

Results and Discussions

For a competitive Romanian agriculture, on the European and international markets, investments in research – development – innovation and active measures to increase farmers' access to information and knowledge are needed. The innovative process in agriculture involves the mobilization of financial, human, technical, organizational and information resources, to obtain new or significantly improved products or processes.

Research and development expenditure in agriculture

The evaluation of the innovative potential of agriculture in Romania had as a starting point the analysis of the financial resources allocated to investments and research-development activities (R&D). The R&D activities of the Romanian agriculture requested in 2016 financial resources worth 42.43 million euros, representing 0.73% of the gross added value of agriculture (5.8 billion euros) (Fig. 1).

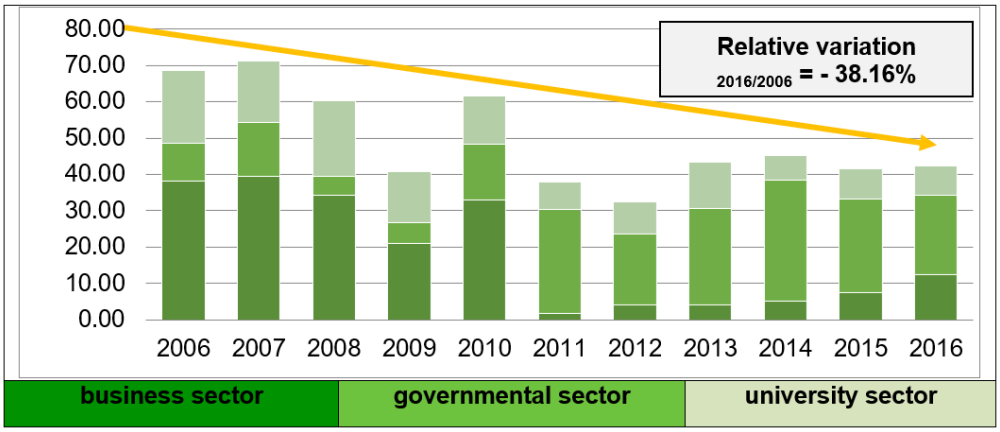

Fig. 1. Dynamics of research and development expenditures in the field of agricultural sciences in Romania, between 2006 and 2016 (mil. euro) (Eurostat data)

Between 2006 and 2016, the value of research and development expenses (R&D) decreased by 38.16%, and their structure by source of origin has undergone considerable changes. Thus, by 2010, the business sector contributed over 50% to the value of R&D expenditure, and since 2011, the largest contribution has been the government sector (over 60% of the total R&D expenditure), which can influence negatively the performance of innovative activities in agriculture, according to specialized studies [6], [7]. According to Eurostat, at the European Union level, 5.50% of the agricultural gross value added (GVA) is invested for activities in the field of agricultural research, development and innovation (RDI) of new processes and products. The highest investment rates in RDI activities for agriculture, over 10% of GVA, are registered in the Scandinavian countries (Finalnda, Sweden and Denmark). The policy in the field of research at European Union level is to boost the activities of the R&D within the economic entities and to increase their contribution to the development of the economy through innovation.

The human potential for innovation in Romanian agriculture

The human resource is the active element that mobilizes all other resources that determine the innovation potential of the agricultural sector.

A first indicator of qualitative evaluation of the human resource in agriculture is the degree of qualification in the field of the head of the commercial agricultural holding, with an exploited area of more than 100 hectares (Fig. 2). In 2016, 55.12% of the commercial holdings in Romania are headed by managers without any theoretical training in the field, twice as much as the European average (28.15%) (Fig. 2). We also note that about a quarter of the managers in Romania (22.26%) have completed specialized university studies, representing 83.65% of the European Union average (26.61%). Accordingly, at both European and Romanian level, approximately one in four farm managers with more than 100 hectares has complete agronomic studies, which shows a good potential for development and innovation in agriculture. This conclusion is supported by research in the field, which reflects the strong direct link between the degree of education in the field and the manager's ability to change, integrate science and innovation into agricultural production processes [8].

According to the specialized literature, a relevant indicator for the evaluation of the human resources of R&D is represented by the weight of the research personnel in the employed population in the analysed activity sector [9]. In our study, the percentage of persons with higher education or who are employed in the agricultural scientific and technological field was calculated, in the total number of remunerated persons employed in agriculture. In Romania, in 2016, there were 76,500 thousand persons with agricultural higher education or employed in the field of agricultural research and technology (Fig. 3), representing 38 persons to every 100

persons economically employed in agriculture, with only 2% less than the average. European Union (39 persons/100 persons economically employed in agriculture).

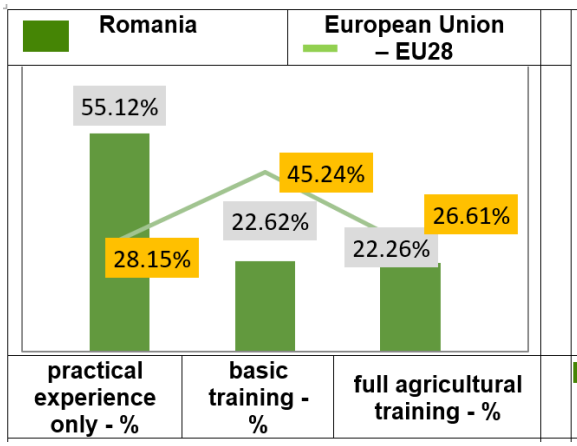

Fig. 2. Structure of commercial farms according to the agricultural qualification of the head of the farm in Romania and the EU (2016) (processing after Eurostat)

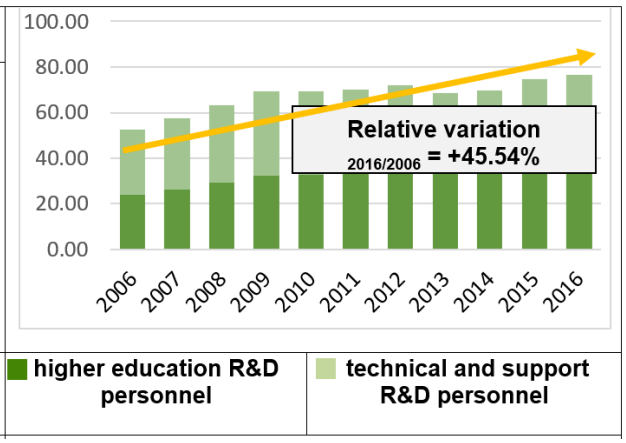

Fig. 3. Dynamics and structure of the personnel employed in the field of agricultural science and technology in Romania, between 2006-2016 (thousands) (processing after Eurostat)

The personnel with higher education who is employed in the field of agricultural science and technology, was composed of 38.20 thousand people in 2016, increasing by 60.50% compared to 2006 (23.8 thousand people). From the analysis of the human resource, it has emerged that there is a high human potential that can contribute to the development of the Romanian agriculture, by carrying out research and innovation activities in public or private organizations.

R&D organisations and infrastructures

Research institutes and institutions of higher education in the field of agriculture are the main economic actors that invest in agronomic research in Romania.

According to National Register of Research and Development Infrastructures ERRIS, at the end of 2018, in Romania, 105 public and private research infrastructures were registered in the field of agronomy and plant breeding. The research infrastructures are located mainly in the Bucharest-Ilfov Region and the Central Region (80% of the total), these being the most economically developed regions in the country. At the research infrastructures level, were identified more than 700 research services are provided, such as: *i*) testing of varieties and hybrids; *ii*) soil analysis; *iii*) physico-chemical analyses of agricultural products; *iv*) physico-chemical analyses of fertilizers and pesticides; *v*) testing the agrobiological potential of plants, etc.

The agriculture of the future is dependent on the information flows regarding the new production technologies, the pedo-climatic conditions, the market of agricultural inputs, markets, financial markets, etc., and the agricultural entrepreneurs must be connected in real-time to this information.

Numerous studies have confirmed the positive impact of cooperation on increasing innovation performance, as measured by the share of innovative products and processes launched on the market [9], [10]. However, at the level of the Romanian economy, the relations between the economic environment and the research organizations are still very weak.

An important organizational resource for the innovation process in Romania's agriculture is the cluster innovation partnerships. The cluster represents a social community specialized in the creation and transfer of knowledge, respectively, constitutes a network of independent economic actors that share the same geographical area and market segment in order to meet common strategic objectives, including innovation [10]. The clusters accumulate three

economic actors and their interests: *i*) enterprises – with the interest of having access to innovation, information, and technology; *ii*) research organizations – with the interest of capitalizing on the research results; *iii*) the government – with the interest of increasing employment and community wellbeing. In 2018, at national level, 12 clusters were active in the field of agriculture, bioeconomy and the agri-food industry, according to the European Cluster Collaboration Platform (ECCP).

Enterprises adhere to innovation clusters out of desire to improve economic performance, to change and innovate, through an attitude of openness and cooperation [6]. The advantages of the economic entities involved in these partnerships consist in the access to high quality RDI services (for example, the elaboration of soil studies with state-of-the-art equipment, by the research institute partner), the transfer of scientific information from the research environment, access to networks of suppliers of agricultural inputs and new markets. Farmers specialized in various branches of agriculture have common interests in the fields of production, market, and innovation.

Increasing the efficiency of agricultural activities is based on farmers' interaction with new technologies and their ability to position themselves in an information flow. Access to information and integrated cooperation networks (such as innovation clusters, technology parks) determines a better anchorage of farmers to the current economic reality, having an impact on the growth of economic performances.

Conclusions

Thus, the main conclusions of the study are: *i*) the weak involvement of the private business environment in RDI activities can be influenced by the lack of confidence in public research organizations, by a high bureaucracy in the public system, by the conservatism of farmers and by the lack of specialized education for 55% of agricultural managers; *ii*) the growing human resource can ensure the revitalization of agronomic research, by initiating and actively involving in public or private research projects, funded by non-reimbursable grants; *iii*) the existence of over 100 research and innovation infrastructures in agricultural sciences, which provide about 700 types of specialized services, is a pillar of agricultural research development; *iv*) the development of a cooperation network between the research environment and the business environment in the Romanian agriculture, through partnerships such as innovation clusters, is the key element of increasing the competitiveness of the agricultural sector in Romania.

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Ecological Problems in the Development of Greenhouse Gas Emissions Directly at Sectoral Level and Soil Resources

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Abstract

The research object of this study is the agricultural soils of the Republic of Moldova. From an economic point of view, they are attributed to the field of phytotechnics and soil resources in the agricultural economic sector. Greenhouse gas (GHG) emissions from agriculture have three major sources of origin: enteric fermentation, manure management (both in the livestock sector) and agricultural soils (in the sector of plant and soil resources). The 2010 year was determined as the reference year for projections of CO_2 emissions/seizures from agricultural soils in the Republic of Moldova. For modelling future emissions, the results of national inventory of greenhouse gas emissions for 1990-2012 were used as a basis. The projections of CO_2 emissions from groundwater were developed for 2015, 2020, 2025 and 2030 after some scenarios. The Table 3 presents the prospecting on the areas to be applied in the Republic of Moldova to the agricultural conservative systems in the period until 2030. Changing the use of agricultural land and soil management practices can greatly influence the organic carbon reserves in the soil. Carbon of organic origin and nitrogen are closely related to the organic matter (humus) content of the soil. In the case of soil carbon losses, mineralized nitrogen is considered as an additional source of nitrogen available to convert to direct greenhouse gas emissions.

Keywords: crop rotation, greenhouse gas emissions, soil carbon leakage, Republic of Moldova

Introduction

The carbon cycle has a decisive role in the global changes in the environment with which the rest of the cycles are closely linked, as well as the climate-dependent climate change. The carbon cycle in terrestrial systems is determined by the balance between the carbon dioxide stored in the vegetal carbon and the amount of CO_2 emitted mainly through the respiration process of the soils. Soil respiration is the most important source of CO_2 and other greenhouse gases. This can be illustrated by the fact that only 10% of total CO_2 emissions are responsible for industrial CO_2 emissions, while the rest of the bio-systems, with the predominance of soils, is 90% [16]. Organic matter, stored in humus and dead biomass in the planet's soils, contains three times as much carbon as all terrestrial vegetation [8]. Each year soil releases 4-5% of its carbon in the atmosphere by transforming organic matter into CO_2 and other compounds due to biochemical mineralization processes.

This phenomenon is characteristic for other countries as well as for the Republic of Moldova.

Research carried out by various authors has shown that chernozems of Moldova 100 years after recovery have lost as much as 25% of the CO_2 – accumulated carbon dioxide [11, 12, 17] as well as Canada's soils. Multiple researches carried out abroad and in the Republic of Moldova found that the exploitation and exploitation of soils in agriculture necessarily lead to the gradual decrease of the microbiological activity of the soils and consequently to the

reduction of the CO_2 emissions from the agricultural lands [4, 9, 13, 15, 16]. Namely, the value of the CO_2 balance in arable soils considered their contribution to the increase in the atmosphere of this gas and its contribution to the phenomenon called greenhouse effect.

Methodology

Until now, no valid method has been developed to determine greenhouse-gas emissions from arable land that could be applied on large areas. Research carried out in the Republic of Moldova and other countries determined that the CO_2 emissions in the soils used are 2.0-2.5 times lower than those covered with natural vegetation. Hence, agricultural land, unlike land with natural vegetation, is characterized by a negative carbon footprint, which is why it can be seen as a source of CO_2 with the contribution to greenhouse effect and climate change.

Measuring CO_2 emissions from agricultural soils that lead to anthropogenic interference in the atmosphere is of particular importance for predicting climate change in view of the enormous amount of carbon stored in the organic matter of the soils.

From a methodological point of view, this is very difficult because the carbon circuit in agrofitocenoses is influenced by multiple natural and anthropogenic factors, often very variable in space and time. In the country and abroad literature, a large amount of data on soil respiration has accumulated according to the most diverse natural and anthropogenic factors, but there is virtually no research that would highlight carbon emissions that remain uncompensated. Here we have to mention that a universal method in this sense usable for all possible cases cannot be elaborated because the factors that influence the carbon circuit are multiples and quite frequently have a regional or even local character.

It is also worth mentioning that changes in humus in the ground where carbon is deposited are slow, that the changes produced can be measured significantly with a long period of time (5-10 years). The method is similarly appreciated by Canadian researchers, although this country has a network of 15 000 polygons that carries out carbon monitoring in soils [5].

The possibility of using the nitrogen exported from the soil to the agricultural plants for the appreciation of the humus consumed was based on the academic I.V. Tiurin [14], then the idea was concretized by A.M. Lâcov [13]. In the soils of Moldova, the ratio of carbon and nitrogen in humus is equal to 10.7, ranging from 10.1 to 11.3 [11, 12]. This ratio is characteristic of the upper soil layer and slightly decreases to greater depths.

The elaborated methodology follows the purpose of assessing the greenhouse gas emissions from agrofitocenoses taking into account the agricultural lands of the Republic of Moldova.

Data from the universal and local scientific literature, including recent information [7, 9, 15], were used in the development. The field works were carried out according to the methodology of pedological field research. Laboratory analyses were performed according to classic methods and standard.

The carbon balance is determined for the area occupied by each crop:

$$B \pm = (V-C) * S \qquad (1)$$

B – carbon balance; V – carbon entering the soil by humification of vegetable residues and organic fertilizers; C – carbon released from the soil through CO_2 emissions as a result of humus mineralization; S – the area occupied by the crop, ha.

The amount of carbon entering the soil (V) is determined according to the equation:

$$V = V1 + V2 \qquad (2)$$

V1 – carbon entering the soil with vegetal remains; V2 – carbon entering the soil with organic fertilizers.

The amount of carbon entering the soil with vegetal debris (V1) is equal to the result obtained from the multiplication of the basic crop with the accumulation and humification coefficients of the vegetable residues divided by the coefficient of 1.724 for the transition from humus to carbon. For this purpose, the data in the annex is used. The amount of carbon entering the soil with the applied organic fertilizers (V2) is equal to the result obtained from the multiplication of the dose with the respective humification coefficient (appendix) and divided by the coefficient 1.724 for humus to carbon. The sum of the results (V1 + V2) considered your carbon bound (entrained) by the soil into humus (V).

The amount of carbon released from the soil is estimated by the equation:

$$C = [Er - (Em+Eo+ Ev+ Es)]\ r_1 \cdot r_2 \cdot 10.7 \qquad (3)$$

Er – the amount of nitrogen exported with the crop production (main and secondary) is determined by multiplying the main crop of the crop by that coefficient in the Annex; Em – the amount of nitrogen exported from the chemical fertilizer account; Eo – the amount of nitrogen exported from organic fertilizers; Ev – the amount of nitrogen used in plant debris; Es – the amount of symbiotic nitrogen exported from the soil; r_1 – the coefficient expressing the dependence of humus mineralization on the soil granulometric composition; r_2 – the coefficient expressing the dependence of the cultivation of humus mineralization; 10.7 – the nitrogen passage in carbon.

Results and Discussions

The imbalance in agrofitocenoses between soil mineralization processes and those responsible for the synthesis of humus is well demonstrated by the data presented in the fundamental work "The Basics of Soil Science", the author of which is V.A. Covda [10]. The negative (carbon footprint) in the measured soil considered the contribution of the soil to greenhouse emissions. In Tables 1 and 2, prospects for nitrogen, organic and green chemical fertilizers (sidereal crops) are submitted to 2030.

Table 1. Prospects regarding the application of natural nitrogen and organic chemical fertilizers in the Republic of Moldova in the period 1990-2030, thousand tones N

Name	1990	1995	2000	2005	2010	2011	2012	2015	2020	2025	2030
	SLB (baseline scenario)										
Chemical fertilizers, nitrated, FSN	92.10	10.51	10.24	16.10	20.63	24.99	34.05	55.00	59.40	90.00	99.90
Natural organic fertilizers, FON	54.54	9.96	0.47	0.25	0.10	0.18	0.13	0.28	0.84	2.52	4.20
	SM (scenario with measures)										
Chemical nitrate fertilizers, FSN	92.10	10.51	10.24	16.10	20.63	24.99	34.05	45.00	49.50	77.50	86.00
Natural organic fertilizers, FON	54.54	9.96	0.47	0.25	0.10	0.18	0.13	0.49	1.68	5.04	10.08
	SMA (scenario with additional measures)										
Chemical nitrate fertilizers, FSN	92.10	10.51	10.24	16.10	20.63	24.99	34.05	37.50	42.50	70.00	80.00
Natural organic fertilizers, FON	54.54	9.96	0.47	0.25	0.10	0.18	0.13	0.56	2.52	6.72	11.76

In natural steppe phytocoenoses annual phytomasium production accumulates 10 t/ha of carbon, of which 1.04 t/ha is stored in soil humus as a result of humification processes.

In agrofitocenoses, the amount of carbon in the crop production reaches only 2 t/ha, of which 0.16 t ha is stored in humus. The annual amount of carbon stored in the humus "input" in natural phytocoenoses is 6.5 times higher than agrofitocenoses.

Table 2. Prospecting for Green Fertilizer Applications in the Republic of Moldova 1990-2030, thousand tones N

Name	2012	2015	2020	2025	2030
	SLB (baseline scenario)				
Areas where green fertilizers will be applied – autumn vetch, thousands of ha	0	0	25	50	75
Ground green vetch embedded in the soil, thousands of tons	0	0	500	1000	1500
Green fertilizers transferred to equivalent organic fertilizers, thousands of tons	0	0	700	1400	2100
Green fertilizers – $F_{SIDERAL}$ thousands of tons N	0	0	3.92	7.84	11.76
	SM (scenario with measures)				
Areas where green fertilizers will be applied – autumn vetch, thousands of ha	0	25	50	75	100
Ground green vetch embedded in the soil, thousands of tons	0	500	1000	1500	2000
Green fertilizers transferred to equivalent organic fertilizers, thousands of tons	0	700	1400	2100	2800
Green fertilizers – $F_{SIDERAL}$ thousands of tons N	0	3.92	7.84	11.76	15.68
	SMA (scenario with additional measures)				
Areas where green fertilizers will be applied – autumn vetch, thousands of ha	0	50	75	100	150
Ground green vetch embedded in the soil, thousands of tons	0	1000	1500	2000	3000
Green fertilizers transferred to equivalent organic fertilizers, thousands of tons	0	1400	2100	2800	4200
Green fertilizers – $F_{SIDERAL}$ thousands of tons N	0	7.84	11.76	15.68	23.52

In the case of baseline scenario (SLB), the prospecting was carried out on the basis of the information available in the New Land Recovery and Soil Fertilization Program [1, 4].

For scenario with measures (SM), the prospecting was based on the Soil Fertility Conservation and Enhancement Program for 2011-2020, the National Development Strategy "Moldova 2020" and the National Strategy for Agricultural and Rural Development for the years 2014-2020.

With reference to scenario with additional measures (SMA), the recommendations of good practices on sustainable development of the agricultural sector as well as the draft version of Moldova's low-emission development strategy were taken into account until 2020 [2, 3, 4].

With respect to green fertilizers (autumn vetch as an intermediate crop), the following basic parameters were taken into account: average green weight - 80%; average nitrogen content in the green mass – 0.8%; average productivity – 20 t/ha; 1.4 (or, in other words, 1 tonne of green lemon meal equivalent to 1.4 tonnes of manure).

It is planned to sow autumn meadows as an intermediate crop used as a green fertilizer, and crop rotation will be as follows: autumn wheat or autumn barley – vetch as intermediate crop – corn or sunflower.

The introduction of intermediate crops as a green fertilizer will be carried out in parallel with the implementation of the farming conservative system ("No-Till" and "Mini-Till").

Table 3 presents the prospecting on the areas where the agricultural conservative systems will be applied in the Republic until the year 2030 in Moldova [2, 4, 6].

Table 3. Prospects for the application of nitrogen fertilizers in the Republic of Moldova in the period 1990-2030, thousand tones N

Name	2012	2015	2020	2025	2030
	SLB (baseline scenario)				
Areas where agricultural conservative systems will be applied, thou ha, including:	0	0	50	100	200
wheat autumn	0	0	20	40	70
autumn barley	0	0	5	10	30
maize	0	0	20	40	70
sunflower	0	0	5	10	30
	SM (scenario with measures)				
Areas where agricultural conservative systems will be applied, thou ha, including:	0	50	100	200	300
wheat autumn	0	20	40	70	90
autumn barley	0	5	10	30	60
maize	0	20	40	70	90
sunflower	0	5	10	30	60
	SMA (scenario with additional measures)				
Areas where agricultural conservative systems will be applied, thou ha, including:	0	100	200	300	400
wheat autumn	0	40	70	90	120
autumn barley	0	10	30	60	80
maize	0	40	70	90	120
sunflower	0	10	30	60	80

In case of extension of the project during the next years, the research polygon will ensure the planned crop rotation: bushy vines → maize → autumn wheat → autumn barley → sunflower. Under these systems all plant residues from the basic crop are to remain in the field for mulching [2]. In the Republic of Moldova, 3 rounds of agrochemical cartoons were carried out, covering virtually all arable land with a period of 5 years. However, the data obtained cannot be used to determine carbon balance in soils, and is in many cases contradictory [1].

A network of long-lasting field experiences has been established in the Republic of Moldova for the development of advanced cultivation technologies for field crops, which studies the evolution of soil fertility according to various fertilization systems. Experiences are 35-55 years old and cover all the country's pedoclimatic zones. The data obtained in this way are qualified as very approximate and must be compared with direct estimates of the carbon circuit parameters.

Of those from the total nitrogen exhaust, the nitrogen bound by the leguminous crops, that is used by plants from industrial and organic fertilizers, vegetable debris is reduced.

An insignificant amount of nitrogen enters the soil with atmospheric precipitations (7 kg/ha), by nesting (5 kg/ha).

Conclusions

Soils with natural vegetal carpet (natural phytocoenoses) cannot contribute essentially to the greenhouse effect due to a balanced carbon balance. It should be noted that for the conditions of the Republic of Moldova the methodology developed can provide satisfactory results.

So, the remediation of the quality and the increase of the production capacity of the studied soil is possible only by increasing the flow of organic matter into the arable layer. The use of the vetch as a green fertilizer is an effective way of achieving this genre.

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Effect of Growth Regulators on Some Physiological Processes of Bean Plants Under Salt Stress

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Abstract

This research was conducted to determine the effects of two biostimulants on some physiological processes such as photosynthesis and transpiration of three bean cultivars under salt stress. This study was carried in 2018 at the USAMV Iasi Romania, under greenhouse condition and the biological material was represented by three bean populations. The application of biostimulants was done every ten days by foliar spraying throughout the vegetation period and one week before flowering it began the application of saline treatments for a 20-day period. We used a saline solution in a concentration of 200 mM. We followed the pods number, the total chlorophyll content using the CCM plus 200 and the stomatal conductance using SC1 leaf porometer. The treated plants with biostimulants were adapted easier to saline stress conditions compared to untreated plants with growth stimulators.

Determinations performed have shown that the treatment with biostimulants has proven to be very effective to improve the growth and yields of bean plants by attenuating the soil salinity stress inhibiting effects.

Keywords: salt stress, bean, growth regulators

Introduction

Beans are an important source of protein in developing countries. Dried beans play a prominent role in the diets of many vegetarians and may contribute to some of the health benefits associated with this eating pattern. Beans contain a number of polyphenolic compounds (tannins, phenolic acids, and flavonoids) that may confer a variety of health benefits [1].

Salinity is one of the most significant environmental challenges limiting plant productivity, particularly in arid and semi-arid climates [2]. Salinity in irrigation water and in soils is one of the major abiotic constraints on agriculture worldwide, and the situation has worsened over the last 20 years due to the increase in irrigation requirements in arid and semi-arid regions [3].

Many physiological and agronomic practices have been performed to improve salt stress tolerance in different crops including traditional breeding programs. However, commercial success has been limited. As alternatives, plant growth regulators and antioxidants, plant biostimulants/extract and humic substances have been widely applied for agricultural crops to mitigate the detrimental effects of environmental stresses including salt stress [4].

Biostimulants contribute to plant nutrition, they have positive effects on plant growth, but also on abiotic and biotic stress tolerance [5]. Biostimulators aim at minimizing the influence of unfavourable stressors on crops, stimulating their growth, development, and enhancing the size and quality of the yield ([6], [7]). This particularly concerns abiotic factor sensitive plants such as bean [8]. In plants, salt stress is a factor that affects plant growth and metabolism [9], that why we need to find the solutions to create plants that are tolerant to salt stress.

Methodology

This study was carried in 2018 at the USAMV Iasi Romania, under greenhouse condition and the biological material was represented by three bean populations collected from the central-eastern region of Romania, the locality Luncani. For easier use the genotypes were noted with L1, L2 and L3, by the name of the area where they come from. The bifactorial experience was conducted in a pots experiment in randomized blocks with three repetitions.

The application of biostimulants (Cropmax and Atonik) was done every ten days by foliar spraying throughout the vegetation period and one week before flowering it began the application of saline treatments for a 20-days period. We used a saline solution in a concentration of 200 mM. For easier use the treatments were noted with 200 mM (plants watered with saline concentration 200 mM), ATx200 (plants sprayed with Atonik and irrigated with saline concentration 200 mM), CRx200 (plants sprayed with Cropmax and irrigated with saline concentration 200 mM).

Atonik is the oldest biostimulator of growth and fructification in the world, being intensively used by farmers in over 70 countries on 5 continents. Its unique polyphenol composition has, among many others, a role in the proliferation and growth of livers, photosynthesis, flower fertility and fruit formation, even in conditions of biotic and abiotic stress.

Cropmax is a growth stimulator for all types of crops. Its activity is based on the combination of microelements, amino acids, vitamins and polysaccharides. Cropmax is highly concentrated, 100% organic and is suitable for application to all agricultural and horticultural crops. We used 0.3% solutions of Atonik and Cropmax, that we applied in double spraying of plants.

Research was focused on the influence of saline treatments on bean plants which was sprayed with growth regulators by foliar way. We followed the pods number, also we determined the total chlorophyll content using the CCM plus 200 and the stomatal conductance using SC1 leaf porometer. All the results compared to control, where plants were treated with water. The results were analysed with Anova correlations, and the interpretation was done after Colton (1974) [3].

Results and Discussions

The application of saline solutions started one week before flowering and continued until the emergence pods. We observed the influence of saline stress on photosynthesis and fruiting processes, but at the same time the interaction between biostimulants and the saline solution of 200 mM concentration.

To observe the influence of saline stress on plants sprayed with biostimulants on the photosynthesis process, we determined the chlorophyll content index (CCI) and the stomatal conductance. Regarding the total chlorophyll content, is noted the genotype L3 from the variant ATx200 with the highest value (15.87 CCI). A similar behaviour was also observed in the L2 cultivar variant CRx200, in which also the chlorophyll content was higher (fig.1) than in the other comparative groups, which demonstrates the positive interaction between genotype and the active substances from the biostimulants. These substances contribute to the manifestation of a strong reaction of adaptation to the conditions of saline stress for these local populations.

Compared with the values of the control group, the plants watered with saline solution of 200 mM had lower values, the differences being between 3.63 and 4.24 CCI. Also, compared to the ATx200 and CRx200 variants, the plants of the 200 mM variant had lower values. The results show us that the local populations taken into the study had low values of the chlorophyll index. This indicates a low degree of tolerance to salinity, also the plants had in a short time ionic toxicity phenomenon that correspond to the occurrence of chlorosis and foliar necrosis

specific to salinity sensitive plants ([10] [11]), but by the prior spray with biostimulators they show an increase of the tolerance to salinity.

In this situation the biostimulators by their composition contribute to maintaining a state of homeostasis at the level of chloroplasts. In this situation, the variants ATx200 and CR x200 had values of total chlorophyll content close to or greater than the control group, which indicates that the plants created a high tolerance mechanism for the action shown by the increased salinity. These results highlight the importance of biostimulatory application under saline stress conditions, which contribute to increasing tolerance to this type of abiotic stress.

The foliar stomatal conductance was determined using the porometer. This device is used to measure the water vapor flow between the leaf stomata and the outside, which is a direct indication of the aperture and therefore the stomata conductance. Thus, by direct measurements on the leaves can be found very important information regarding the hydric stress of the plants, the capacity of photosynthesis, or the exchange of gases with the atmosphere (O_2/CO_2) [12].

The plants from the 200 mM group had lower stomatal conductance values than the control group (Fig. 2), that indicating the movements of the stomata are affected by the osmotic effect of saline stress [13].

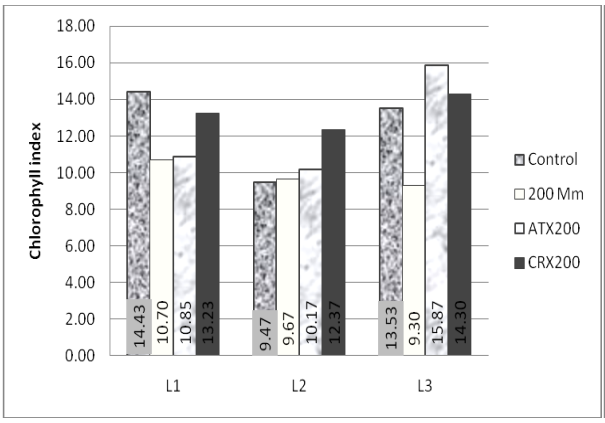

Fig. 1. Effect of growth regulators on chlorophyll index of bean plants under salt stress

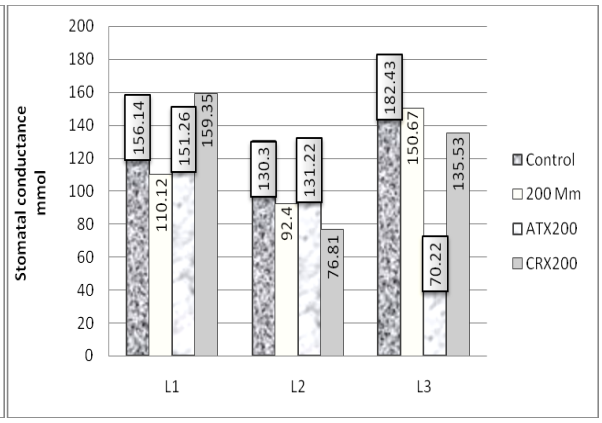

Fig. 2. Effect of biostimulants on stomatal conductance of bean plants under salt stress

To reduce transpiration, the stomata are partially closed and the conductance values decrease. Significant reduction in transpiration rate was observed in salt stress conditions. The stomatal conductance also had lower values for variants that were prior sprayed with biostimulators, but the differences from the control group are smaller, which shows a higher adaptation capacity to the saline concentrations. The L1 genotype of the CRx200 variant and the L2 genotype of the ATx200 variant are noted to have the highest values of stomatal conductance, fact that configures a positive interaction between these genotypes and the biostimulators used, generating a higher adaptation mechanism to saline stress. Application of growt regulators, might improve the salt tolerance of bean variety by reducing the uptake of Na^+ ion and improve the capacity to absorb water by increasing in osmotic component of soil water potential [14]. The foliar exchange of gases is achieved through the stomatal movements.

This is crucial for the process of acquiring carbon dioxide, which is directly linked to the photosynthetic production of biomass, as well as to the control of sweating in order to maintain the water balance [15].

As can be seen from the Fig. 3 according to the correlation coefficient described by Colton (1974) between the number of pods/plant and the stomatal conductance there is a correlation coefficient (r=0.36) with an acceptable degree of association. This correlation coefficient corresponds to a coefficient of determination $R_2 = 0.13$, which shows that 13% of the data demonstrate a linear dependence between the number of pods and the stomatal conductance.

The results showed that the number of pods per plant was higher in the plants treated with growth regulators, and the most affected were the plants in the group treated only with 200 mM saline. The saline treatments applied before flowering differently influenced the number of pods per plant depending on genotype but also on the interaction with the biostimulators. It is noted that the plants that were first treated with biostimulators (Fig. 4) behaved much better under the influence of saline stress compared to the local populations that were not prior sprayed with biostimulators, which indicates that the active substances in biostimulators improved the tolerance of the local populations to saline stress.

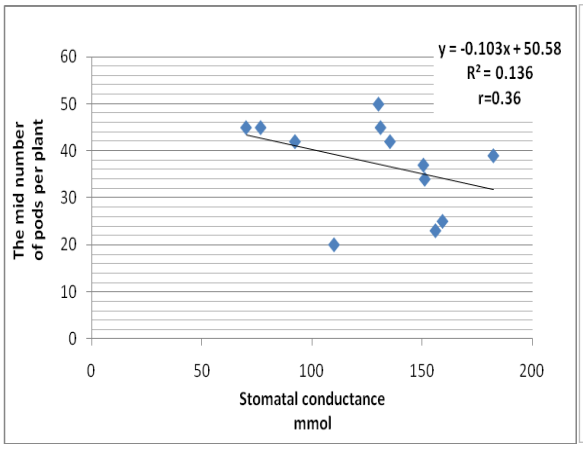

Fig. 3. Correlation between the pods number of plants and the stomatal conductance

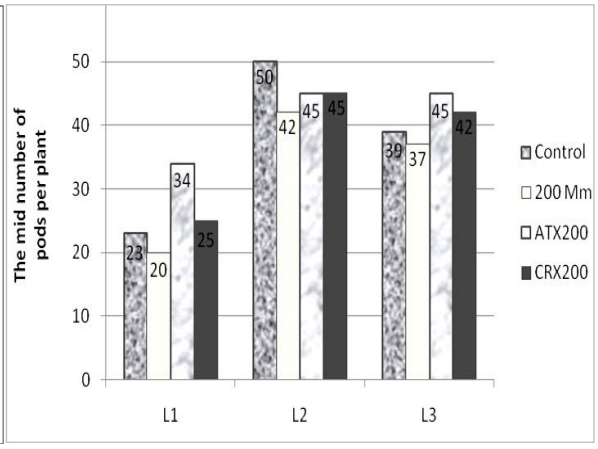

Fig. 4. Effect of growth regulators on the number of pods per bean plant under salt stress

Comparing the results, we can see that the values recorded are higher in the group ATx200 mM except for the local population L2, in which the witness recorded the highest number of pods per plant. The fact that the plants in the ATx200 group showed these high values indicates that the polyphenol composition in Atonik determined the concentration of vacuolar juice for better water absorption. At the same time, we can consider that the active substances in the composition of this biostimulator have increased the capacity of vacuolar storage of excess sodium and chlorine ions, so that the beans plants treated with biostimulators they were better adapted to the saline stress conditions, maintaining their water status at normal parameters. In this case the physiological processes were not disturbed, and the production of pods per plant was not negatively influenced by the high concentration of NaCl used in the experiment.

The variant untreated with biostimulators, but subjected to saline stress, registered a significant decrease of production both in comparison with the control variant and in comparison, with the plants in the variants ATx200 and CRx200, this behaviour is explained by the fact that the sodium and chloride ion have exceeded the maximum allowed level storage in the vacuoles. These ions migrated to the other cells and affected them. At these plants could also be observed the phenomenon of abortion of flowers. We noticed that besides the diminution of the production in the plants subjected to the saline stress there was observed a qualitative depreciation of it. The values we obtained indicate that biostimulators caused a high degree of salinity tolerance printing.

Conclusion

Biostimulators by their composition contribute to maintaining a state of homeostasis at the level of chloroplasts. Atonik and Cropmax biostimulators maintained a balance of photosynthetic pigment content.

The stomatal conductance had lower values for variants that were prior sprayed with biostimulators, but the differences from the control group are smaller, which shows a higher adaptation capacity to the saline concentrations.

Results showed that the number of pods per plant was higher in the plants treated with growth regulators, and the most affected were the plants in the group treated only with 200 mM saline.

The biostimulants used increased the tolerance to salt stress for all 3 local populations of beans studied.

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Soil Properties Analysed Under Different Tillage Systems at Ezareni Research Station

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Abstract

Soil tillage represent important measures to ensure water content, fertility, air control, soil temperature and improve the physical and chemical properties of the soil. The experiment was conducted at the Didactic Station of the University of Agricultural Sciences, Iasi-Ezareni Farm, between November 2016 and July 2018. Four crops were cultivated, under conventional and conservative tillage systems: winter wheat, rapeseed, maize and sunflower, in a four years rotation. Two variants of soil tillage systems: no-till and conventional tillage were investigated.

Bulk density and soil aggregates water stability for winter wheat crop were analysed. In order to determine the main physical properties such as bulk density, soil samples were collected in 10 cm increments, from 0 to 40 cm depth: 0-10, 10-20, 20-30 and 30-40 cm, at sowing and right after harvesting. To determine the influence of soil tillage systems on water stable aggregates with diameter >0.25 mm, referring only to aggregates from the 1-2 mm fraction, samples from seeding and harvesting were analysed, from 0-10, 10-20, 20-30, 30-40 cm depths. The determinations during the two experimental years showed that the values of the studied indices have increased from a year to another, in both variants of soil tillage. The purpose of this study was the to quantify the effect of tillage systems on physical properties of the soils, for winter wheat crop.

Keywords: no-tillage, bulk density, water stable aggregates

Introduction

Bulk density has a direct effect on soil properties, such as porosity, available water content, organic matter content and hydraulic conductivity, and has an indirect effect on other properties, such as root growth and agricultural production [1].

Soil tillage represent one of the fundamental practices of agricultural management, being the procedure by which man disturbs, overturns and rearranges the soil to create favourable physical conditions for the growth and development of crops. Most soil tillage operations involve altering the bulk density, pore size distribution, water holding capacity, infiltration rate, and soil erosion [2].

Soil physical chemical properties are critical indicators for soil health assessment. By changing the physical conditions of the soil, including water content, soil temperature, soil structure can be also changed [3].

Conventional tillage involves subsoiling, harrowing and plowing operations and causes severe soil disturbance down to a 40 cm depth. This intensive tillage results in faster oxidation of organic matter [4], reducing soil stability and adversely affecting soil porosity matrix [5].

The tillage system employed also affects soil moisture, and conservationist systems are more beneficial to soil water retention than conventional tillage systems, by promoting higher water infiltration in the soil [6].

Intense tillage can increase surface soil compaction, reduce aggregate stability, disrupt surface vented pores, decrease retention and transmission of water and solutes, and exacerbate losses due to runoff and erosion. Intense tillage may also deplete SOM as a result of the increased rate of organic carbon mineralization following tillage as well as contribute to erosion loss and reduced cycling of organic matter through crop removal. Soils under no tillage (NT) management systems tend to become more porous with time due to the creation of a more stable soil structure, an increase in the SOM pool and an increase in the number of biopores directly connected to the soil surface. Higher infiltration rate measured in NT compared with conventional tillage (CT) may be attributed to macropore flow and reduced surface sealing under the mulch [7].

Methodology

The experiment was established in 2014 at the Didactic Station of the University of Agricultural Sciences, Iasi-Ezareni Farm in the NE part of Romania (47º07'36" N, 27º30'45" E), 125 m altitude on a clay-loam Cambic chernozem, 6.8 pH, humus content of 2.7% and average level of fertilization, no irrigation. The present study focuses mainly on the agricultural years 2016-2017 and 2017-2018.

In order to determine the influence of tillage on bulk density (Bd) and water stable aggregates (WSA) for winter wheat (*Triticum aestivum* L.), we investigated two tillage treatments: no-till (NT) and conventional tillage (CT), registered at the sowing and harvest, between november 2016 and July 2018.

In CT, the topsoil (0-20 cm) was ploughed immediately after harvesting the previous crop.

Next, the disc harrow GD 3.4 was used two times on the ploughed soil and the seedbed was prepared on the same day as the sowing, on 7th of November 2016 in the first year of experience and at 28th September 2017 in the second year of experience, using the Kompactor cultivator + Valtra tractor (200 HP). In CT the SUP 15 sowing machine was used + Goldoni tractor (50 HP).

The winter wheat variety was Izvor, at a rate of 280 kg/ha. In NT, the sowing was made in the same day, using FG 150-FABIMAG seed drill, at 17.5 cm between rows, using the same sowing rate as in CT.

Bd was determined on four depth intervals: 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm, from undisturbed soil samples, using steel rings (5 cm in diameter and 5.1 cm in height, with a total volume of 100 cm^3 per sample). The soil samples were collected from three points, diagonally from each plot and three replications for every depth interval.

To determine water stability aggregates (WSA), on samples collected from 4 layers as follows: 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm, an average soil sample weighing 1 kg, from each horizon studied, was air-dried for 48 hours, it was screened to extract the aggregates with a diameter between 1-2 mm, then 4 grams of soil were taken which were saturated capillary by spraying, and then the sample was subjected for three minutes to a wet sieving process in distilled water, during which the aggregates that did not have water stability dissociated. The soil that remained on the site was washed for 10 min. in a solution of 2% NaOH (in the situation of acid pH soils). The stainless-steel cans with soil resulting from the two washing processes were introduced in the oven at 110ºC for 8 hours, finding the amount of soil completely dry, with and without water stability.

The data were correlated with the rainfall and temperature registered at the Ezăreni Weather Station belonging to USAMV Iasi (www.fieldclimate.com).

Table 1. Temperature and rainfall regime, Iasi (2016-2018)

Month	Rainfall (mm)					Air temperature (ºC)				
	Iași		Multi-annual average of 50 years	Deviation		Iași		Multi-annual average of 50 years	Deviation	
	2016/ 2017	2017/ 2018		2016/ 2017	2017/ 2018	2016/ 2017	2017/ 2018		2016/ 2017	2017/ 2018
September	10.2	23.2	27.2	-17.0	-4.0	18.3	17.2	15.9	2.4	1.3
October	212.0	62.4	24.6	187.4	37.8	8.1	10.9	10.2	-2.1	0.7
November	69.8	34.2	29.2	40.6	5.0	4.0	5,9	4.9	-0.9	1.0
December	20.6	48.2	47.4	-26.8	0.8	0.3	3.0	-0.6	0.3	1.9
January	6.7	18.8	58.0	-51.5	-39.2	-4.9	-0.8	-2.7	-2.2	2.8
February	13.8	24.8	82.7	-68.9	-57.9	-0.8	-1.8	-1.2	-0.4	-0.6
March	107.0	56.8	76.5	30.5	-19.7	8.0	1.2	3.7	4.3	-2.5
April	140.4	18.0	52.2	88.2	-34.2	10.1	15.4	10.6	-0.5	4.8
May	72.8	16.8	45.6	27.2	-28.8	16.1	18.7	16.5	-0.4	2.2
June	71.6	216.0	32.1	39.5	183.9	21.1	20.8	19.9	1.2	0.9
July	84.4	136.6	33.5	50.9	103.1	21.6	21.3	21.6	0.0	-0.3
August	61.8	1.2	27.4	34.4	-26.2	21.9	22.6	20.9	1.0	1.7
Total	871.8	657	536.5	334.6	120.5	10.31	11.20	9.96	0.35	1.61

The agricultural year 2016-2017 can be considered a rainy year, registering a surplus rainfall regime with an amount of 334.6 mm above the multi-annual average recorded in Iasi, thus creating good conditions for plant growth and development.

Analysing the total quantities of precipitation recorded during the agricultural year 2017-2018, we observe that during the whole year there were 657.0 mm, with a deviation of 120.5 mm higher than the multi-annual average in the area.

Regarding the temperature registered in the agricultural year 2016-2017 we observe that the average annual temperature did not differ much from the multiannual average, the deviation being +0.35 ^{0}C. In terms of temperatures, the agricultural year 2017-2018 was warm, with an annual average of 11.2 ^{0}C compared to the multiannual average, which is 9.96 ^{0}C, the average deviation is + 1.6 ^{0}C higher compared to the one recorded in the previous year.

The ANOVA test was used to evaluate the significance for a randomized complete block design with three replicates. Treatment means were separated by the least significance difference (LSD) test and all significant differences were reported at 5%, 1% and 0.1%.

Results and Discussions

Regarding the Bd variation for the winter wheat crop (Table 1), it had the lowest value, as an average of the 4 analysed depths, registered at the sowing in the no-tillage variant (1.31 g/cm^3) and the highest value at harvest for the conventional tillage (1.54 g/cm^3).

Analysing the Bd values for the wheat crop, they slightly increased during vegetation for both soil tillage systems. The average data obtained at the sowing for the 0-40 cm soil layer, showed a higher value of Bd for the no-tillage (1.36 g/cm^3) compared to conventional tillage where the average was 1.31 g/cm^3.

At wheat harvesting, it is noted that Bd recorded the highest value, as an average of the 4 depth intervals, for the conventional soil tillage system (1.55 g/cm^3) while in the no-till variant, the average on the four soil layers was 1.45 g/cm^3. In the agricultural year 2017-2018 (Table 2) the results obtained at sowing as averages over the range 0-40 cm in the two experimental variants, showed that the values of the Bd increased with the depth and it is observed that the

value Bd as average over those four depths are higher in the CT (1.26 g/cm^3) compared to the NT variant (1.19 g/cm^3).

Following the evolution of the average values at harvest, it is observed that the NT variant has a higher value of Bd of 1.41 g/cm^3, and the CT variant provides a value of 1.39 g/cm^3.

Table 2. Tillage system influence on soil bulk density, winter wheat crop

Bulk density (g/cm^3)								
Depth (cm)	Time of sampling 2016-2017				Time of sampling 2017-2018			
	Sowing		**Harvesting**		**Sowing**		**Harvesting**	
	CT	**NT**	**CT**	**NT**	**CT**	**NT**	**CT**	**NT**
0-10 cm	1.24	1.26	1.55	1.49	1.21	1.12	1.27	1.28
10-20 cm	1.28	1.36	1.59	1.45	1.24	1.18	1.39	1.43
20-30 cm	1.33	1.40	1.52	1.40	1.27	1.22	1.43	1.46
30-40 cm	1.40	1.42	1.53	1.44	1.34	1.25	1.47	1.48
Significance	**control**	**o**	**control**	**oo**	**control**	**oo**	**control**	
Mean	**1.312**	**1.360**	**1.548**	**1.445**	**1.265**	**1.193**	**1.390**	**1.413**
Differences	-	**-0.048**	-	**0.103**	-	**0.072**	-	**0.023**
LSD 5%	0.051 g/cm^3		0.056 g/cm^3		0.033 g/cm^3		0.024 g/cm^3	
LSD 1%	0.093 g/cm^3		0.102 g/cm^3		0.06 g/cm^3		0.044 g/cm^3	
LSD 0.1%	0.207 g/cm^3		0.226 g/cm^3		0.133 g/cm^3		0.097 g/cm^3	

(*), (o) – indicate significant at 5% level of probability and (oo) at 1% level / ns = not significant (NT = no-tillage, CT = conventional, plough at 30 cm)

The analysis of the evidence for water stable aggregates showed that the determinations carried out in the agricultural year 2017-2018 (Table 3) the average values of the HS registered both at the sowing and at the harvesting of the winter wheat crop, were superior in the NT variant in comparison with values obtained in CT.

In an analysis of the average values from the harvesting we see clear differences between the CT and the NT variant, the NT variant having a higher value of HS (77.85%) compared to the CT (70.97%).

Table 3. The influence of tillage system in WSA (%)

Depth (cm)	**WSA (%)**			
	Time of sampling 2017-2018			
	Sowing		**Harvesting**	
	CT	**NT**	**CT**	**NT**
0-10 cm	62.16	65.99	76.45	70.71
10-20 cm	67.35	66.60	66.58	77.53
20-30 cm	70.44	73.89	63.38	78.07
30-40 cm	71.24	79.23	77.48	85.10
Significance	control		control	
Mean	**67.80**	**71.43**	**70.97**	**77.85**
Differences	-	**3.63**	-	**6.88**
LSD 5%	5.7 %		14.1 %	
LSD 1%	10.4 %		26.0 %	
LSD 0.1%	23.1 %		57.6 %	

Conclusions

From the analysis of the amplitude with which the values of the bulk density increase on the profile 0-40 cm in the two experimental variants, from sowing and until harvesting, it is observed that they recorded lower values in the NT variant and visibly higher values in the variant CT. This fact indicates the presence of a compacted soil layer found in the CT, which formed below the working depth of the plow, due to the execution of the basic tillage of the soil several years in a row at the same depth. The lower value was found in the no-tillage variant, because in this system, all soil tillage process is performed in a single pass, using a complex machine that achieves all at once soil loosening, fertilization and the seeding.

The highest value of the WSA on average between 0-40 cm, at sowing, was obtained in the NT (71.43%). Until harvesting, natural phenomena have improved WSA in both soil tillage variants, with the maximum value being recorded in the NT variant (77.85%).

Acknowledgements

This work was co-financed from Competitiveness Operational Programme (COP) 2014 – 2020, under the project number 4/AXA1/1.2.3. G/05.06.2018, SMIS2014+ code 119611, with the title "*Establishing and implementing knowledge transfer partnerships between the Institute of Research for Agriculture and Environment - IAŞI and agricultural economic environment*".

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Sodisation and Alkalinisation of Soils Developed on Saline Deposits from Slope Land of Bejeneasa Farm-Cotnari

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Abstract

The study was focused on the intensification of sensitive land degradation by soil sodification (*i*) and alkalinisation (*ii*) in the studied area in order to establish regional sustainable management. The studied site is located on slight slope (faces west) from Bejeneasa farm (Conari vineyard). The soil characteristics developed on the saline deposit are the ideal conditions for iodization and alkalinisation processes. After these processes result sodic and alkaline soils. The sodic soils are the most troublesome of salt affected soils, characterized by a disproportionately high concentration of sodium (Na) in their cation exchange complex. When sodium makes up more than about 5% of all cations bound to clay particles, structural problems begin to occur, and the soil is said to be sodic. The amount of sodium as a proportion of all cations in a soil is the main measure of sodicity used, and is termed the Exchangeable Sodium Percentage (ESP). The field observations showed that after leaching of soluble salts the process of sodisation takes place. Sodisation occurs starting with depth interval of 30-50 cm. The analytical data showed a high content of exchangeable sodium (over 15%) and a high content of clay. High sodicity causes clay to swell excessively when wet. For this reason, water and air movement through sodic soils is severely restricted. Sodic layers in the soil prevent adequate water penetration, significantly diminish internal drainage and therefore waterlogging is common. The small depth of salt-bearing clay deposits (50-80 cm) associated with high susceptibility to sodisation of soil horizons make difficult land melioration. The use of gypsum will help to suppress soil dispersion and to improve internal drainage.

Keywords: soil sodisation, alkalinisation, dispersion, waterlogging

Introduction

Soil degradation by salinization, sodisation and alkalinisation represent the major environmental threat to soil fertility and agricultural productivity. Soil salinization can be achieved with neutral or alkaline salts. Alkaline salt accumulation in soil is also known as alkalinisation.

Soil alkalinity is a measure of the amount of base in a solution and express as concentration of hydroxyl ions in that solution [1]. After Elsevier`s Dictionary of Soil Science alkalinity has a more specific sense in soil science and represent a capacity parameter in system contain carbonates (HCO_3^-, CO_3^{2-}).

Sodicity is a measure of the amount of exchangeable sodium in soil. Increasing of the exchangeable sodium in the cation exchange complex of soil is known as sodisation or alkalization processes. Sodic soils are characterized by a disproportionately high concentration of sodium (Na) in their cation exchange complex. They are usually defined as containing an exchangeable sodium percentage greater than 15% [2].

Sodic soils contain large amounts of exchangeable sodium in their cation exchange complex and frequently, have low content of soluble salts.

According to the Romanian Soil Taxonomy System (SRTS, 2003) sodic soils consist of hiposodic horizon with upper limit on 0-50 cm or natric horizon on the 50-100 cm depth.

Solonetz has, within the upper 50 cm of the soil profile, a so-called natric horizon that contains more than 15% exchangeable sodium [3].

After Reference Soil Group of the World Reference Base for Soil Resources [4], solonetz has, natric horizon within the upper 100 cm.

Sodic or natric soil horizon has physical restrictions to plant growth and makes tillage difficult. High level of sodium (>15%) disperse soil particles and become very sticky when wet, nearly impermeable to water, and very hard in dry state.

Plant growth is adversely affected by excess sodium level in sodic soils and by both with excess salts and excess sodium levels in saline-sodic or salsodic soil.

Sodic soils can be reclaimed, but it may be slow and expensive due to the lack of a stable soil structure, which slows water drainage [5]. Saline-sodic soils are degraded simultaneous by salinization and sodisation processes. In these conditions it is necessary to remove both the soluble salts and the exchangeable sodium after the replacement with calcium ions in soil solution [6]. In order to achieve the saline – sodic soil reclamation, it is necessary that the leaching of excess soluble salts be preceded by the replacement of exchangeable sodium (Na+) by calcium (Ca2+) from soil solution. If the excess salts are leached and calcium does not replace the exchangeable sodium, the soil will become sodic.

Methodology

The study site was located at Bejeneasa Farm, Cotnari vineyard from North-East part of Romania. The studied area is about 6 hectares. It is situated on the upper part of slope. The absolute altitude ranges between 152m and 172.5 m and the average annual precipitation and annual temperature values are 524.9 mm and 8.9^{o}C.

The selection of sampling sites was based on the slope category of the land and the properties of the taxonomic soil units. One soil profile was done in each selected location. Following the clearing of the vine plantation 5 soil profiles were developed, studied and designated (Figure 1).

In this paper we present the sodization and alkalinisation intensity of the representative soil profiles located on different slopes. Characterization of soil formation factors and profiles was done following the instructions from guidelines for soil and land descriptions [7].

Soil samples were taken from each paedogenetic horizon in order to conduct laboratory analyses: granulometry, pH, contents of calcium carbonate, soluble salts, exchangeable sodium and alkalinity (HCO_3^-, CO_3^{2-}), according to the current methodology [8, 2].

Following the processing and analysis of the data obtained in the field and laboratory, several reclamation measures have been recommended.

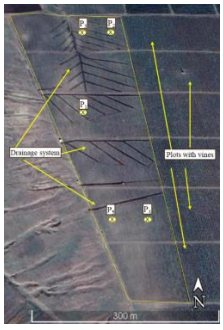

Fig. 1. Location of soil profiles on the slope land after the clearing of the vine plantation

Results and Discussion

The representative soil of the studied area is a Salsodic Chernozem (According SRTS, 2003).

The content of clay ranged between 35.1 and 55%. The maximum clay value is recorded in the salsodic horizon on the depth 65-75cm.

The suggestive analytical data regarding soil sodisation are shown in the Table 1.

The soil is very strongly alkalized over the depth range 50-85 cm. Strong sodification is highlighted by the Na^+ content higher than 15% and the high content of CO_3^{2-} (>10 mg/100g soil) within the BCsc-na (salsodic BC) horizon located in the depth range 65-75 cm.

The maximum value of the saturation degree of exchangeable sodium of 18.36% was recorded over the depth range of 50-65 cm. We mention that the intensity of the sodification (alkalization) took place after leaching of the main mass of soluble salts. This aspect is highlighted by the massive structure, the state of pronounced compactness of the soil and by strong dispersion of the elementary particles.

Table 1. The values of some chemical properties of salsodic Chernozem

Depth	$CaCO_3$	pH	ESP %	ss mg/100g	CO_3^{-2} mg/100g	HCO_3^- mg/100g
0-10	2.1	7.74	2.52	90	-	23.9
20-30	2.7	7.97	4.51	82.4	-	17.9
35-50	2.6	8.11	5.28	88.1	-	23.9
55-63	2.6	8.83	18.36	143.9	-	19.9
65-75	3.1	9.03	16.52	183	13.2	47.82
76-86	25.4	8.6	17.68	931.6	-	89.7
90-110	23.65	8.6	9.53	1162	-	107.6

ESP – exchangeable sodium percentage; ss soluble salts

The pH values between 7.74 and 9.03 place the soil in the weak alkaline to strong alkaline reaction classes (pH>9).

The slight alkaline reaction of the soil (pH = 7,74-8,11) is due to the presence of calcium carbonate in the soil and the incipient alkalization of the soil in the hiposodic horizon AB slight alkalized (ac). The strong alkalization (sodisation) of the soil is also evidenced by the CO_3^{-2} anion content of over 10 mg/100 g soil. The HCO_3^- content- greater than 60 mg/100gsol is characteristic of sodium soils.

Conclusions

Reclamation of sodic soils with high content of exchangeable sodium can be achieved by replacing with Ca^{2+} cations from a soluble source such as gypsum.

In order to achieve the saline – sodic soil reclamation, it is necessary that the leaching of excess soluble salts be preceded by the replacement of exchangeable sodium (Na+) by calcium ($Ca^{2+)}$ from soil solution.

Reclamation of compacted natric horizon could be done by deep loosening works without mixing the soil layers. This work will be carried out only after the gypsum administration.

Acknowledgements

Authors acknowledge the logistic support from Competitiveness Operational Programme (COP) 2014-2020, under the project number 4/AXA1/1.2.3. G/05.06.2018, SMIS2014+ code 119611, with the title "*Establishing and implementing knowledge transfer partnerships between the Institute of Research for Agriculture and Environment – IAȘI and agricultural economic environment*".

We will be also grateful to all anonymous reviewers for their constructive comments, which greatly it will improve this version of the manuscript.

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Are there Alternatives at Maize Seed Treatment for Controlling of the Maize Leaf Weevil (*Tanymecus Dilaticollis* Gyll)?

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Abstract

Romania has more than 2.5 million hectares as maize cultivation which represents the highest area within the EU. However, the maize monoculture is favourable for pest's attack.

Tanymecus dilaticollis (*Coleoptera: Curculionidae*) is the main pest of the maize crops mainly in south and south-east part of the country. The insect is dangerous when maize plants are in the early vegetation stages (BBCH 10-BBCH 14). Each year, around one-million-hectare area is attacked by this pest with different level of attack intensities. In case of high *T. dilaticollis* weevil's invasion, the maize seedlings could not survive and the farmers have to sow again their fields, causing unexpected costs. Spring drought and higher temperatures conditions are even more favourable circumstances for weevil's attack. Also, maize monoculture has an increasing effect on pest density associate with higher impact of attack.

Studies from the last decades pointed out that chemical treatment of the maize seeds with systemic insecticides was the most effective method to reduce the loss. However, the use of neonicotinoid insecticides as seed treatment of the spring crops was restricted from 2014, according to the EU directive 485/2013. Later on, as result of European Commission regulations 218/783, 218/784 and 218/785, the use of imidacloprid, clothianidin and thiamethoxam active ingredients for all field crops, both like seed treatment and foliar application will be total banned in UE, starting from 2019. Because of these regulations, no insecticides will remain available for maize seed treatment against T. dilaticollis in Romania.

For this reason, researches are facing with the challenge to find some alternative treatments instead of neonicotinoids seeds treatment. This paper presents some results about the effectiveness of the single foliar application with acetamiprid, thiacloprid and delthametrin active ingredients comparative with single granules application, at 7 days from plant emergence, with cypermrthrin active ingredient for T. dilaticollis control. The experiment was carried out in south-east of Romania, in conditions of commercial farm, between 2018 and 2019. The single foliar spraying insecticide on plants of BBCH 10-14 vegetation stage did not provide enough protection against weevil's attack under high pest density. Similar results were obtained in case of granules applications, at 7 days from plants emergence. In this experiment no alternative was found at banned active ingredients used for seed treatment in controlling of maize leaf weevil.

Keywords: maize, weevils, alternative, insecticides, farm

Introduction

According MADR data, maize is one of the most important crops in Romania [1]. In the last years, this country has more than 2.5 million hectares as maize cultivation which represents the

highest area within the EU. In same time, Romania occupy second place in EU, after France, with a maize grains production higher than 10 million tonnes [2].

Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is one of the most dangerous pests for maize crops in Romania ([3], [4], [5], [6]). Weevils attack is very dangerous when the maize plants are in early vegetation stages, from emergence until four leaf's [3]. In this stage, in case of high weevil's attack, seedlings can be total destroyed and farmers must sow again [7]. After four leaf's stage (BBCH 14) the attack is less economically important, the weevils consume only the margins of leaf's or weeds and maize plants survive [8]. High air temperatures registered in spring period (April-May) and draught represents the most favourable conditions for weevil's activity [9].

Data from the literature suggest that, every year, in favourable areas of this pest, located in south and south-east of Romania there were attacked around one million hectares cultivated with maize ([7], [10]). Researches made at NARDI Fundulea make in evidence that in case of high pest density, ranged between 25 and 30 weevils/m^2, average maize yield losses can arrive at 34% [3].

However, in Romania, it has recorded higher densities of this pest (15-80 weevils/m^2), especially in south-east ([7], [11]). In some favourable years there where extreme cases when it has recorded a pest density of 160 weevils/m^2, in Dobrogea area [8].

Researches made in Romania make in evidence that chemical seed treatment with systemic insecticides was the most effective method to protect maize young plants from weevil's attack ([6], [7], [12], [13], [14], [15], [16]). After European Commission Regulations, 218/783, 218/784 and 218/785, the use of imidacloprid, clothianidin and thiamethoxam active ingredients for all field crops, both like seed treatment and foliar application will be total banned in UE, from 2019 ([17], [18], [19]).

As result no insecticides will remain available for maize seed treatment against *T. dilaticollis* in Romania. In this paper there were presented results of the first study effectuated in this country, in conditions of commercial farm system, for researching if there are possible alternatives for replacing banned active ingredients used at seed treatment for controlling of the maize leaf weevil.

Methodology

The experience was carried out in 2018 and 2019, at commercial farm Sopema SRL, located at Mihail Kogalniceanu, Ialomita County, Romania (latitude: 44°42'N, longitude: 27°40', altitude: 18 m). In conditions of the commercial farm, the area of each experimental plot was of 8000 m2. In 2018 maize was sowed in 11 April and one year later plants were sowed on 13 April. In both years previous crop was soybean. In 2018 plants emergence was recorded in 22 April.

Because of low soil humidity registered in March and April, 2019, maize plants emergence was recorded with delay, comparative with previous year, on 30 April. In both years it has used MAS 47P maize hybrid (FAO 440). Experimental variants are presented in Table 1.

It has tested single foliar spray with acetamipird (20%), thiacloprid (480 g/l) and deltamethrin (100 g/l) active ingredients (variants 2-4). Also, it has tested granules application with cypermethrin (8.0 g/kg) at 7 days after sowing (variant 5).

When maize plants arrive in first vegetation stages (BBCH 11-12 and BBCH 14-16) it has assessed plant densities. On each variant it has established four assessment points. At each assessment point it has counted emerged maize plants from 20 row meters (80 row meters/variant).

Table 1. Active ingredients used for controlling of the *Tanymecus dilaticollis* Gyll in commercial farm conditions, from south-east of the Romania, between 2018 and 2019

Variant	Active ingredients	Rate	Rate type	Application type
1	—	—	—	—
2	acetamiprid (20 %)	0.1	Kg/ha	A
3	thiacloprid (480 g/l)	0.09	L/ha	A
4	deltamethrin (100 g/l)	0.075	L/ha	A
5	cypermethrin (8.0 g/kg)	12.0	Kg/ha	B

A-Foliar applications (BBCH 11-12);
B-Granules application at 7 days after sowing (BBCH 09-10)

Attack intensity was evaluated when maize plants arrive in four leaf's stage (BBCH 14), according a scale from 1 to 9, elaborated and improved by Paulian ([3]), as follows: note 1-plant not attacked; note 2-plant with 2-3 simple bites on the leaf edge; note 3-plants with bites or clips on all leaf's edge; note 4-plants with leaf's chafed in proportion of 25%; note 5-plants with leaf's chafed in proportion of 50%; note 6-plants with leaf's chafed in proportion of 75%; note 7-plants with leaf's chafed almost at the level of the stem; note 8-plants with leaf's completely chafed and beginning of the stem destroyed; note 9-plants destroyed, with stem chafed close to soil level. At each variant, it has established four assessment points. At each assessment point it has evaluated 50 maize plants, from five rows (10 plants/row). Before assessment plants were marked with sticks, in stair system.

Meteorological data was provided by meteorological station of the Sopema farm, located at 1 km from experimental site. It has monitoring air temperature and rainfalls amount occurred in April and May. This period is the most important for both, weevil's activity and maize plants emergence and first development stages. Data from the field assessments was statistical analyzed using Student-Newman-Keuls test ([20], [21], [22]).

Result and Discussions

During assessments period, at experimental site, weather conditions from spring were atypically. In 2018, average air temperature registered in April and May was higher compared with multiyear average (Fig. 1). However, in 2019, average air temperature registered in April and May were lower comparative with multiyear average. Also, in both years, in April, it has registered higher differences between minimum and maximum daily temperature (more then 15-20 °C). In 2018, rainfalls amount registered at Sopema farm, both, in April and May, were bellow multiyear average (Fig. 2).

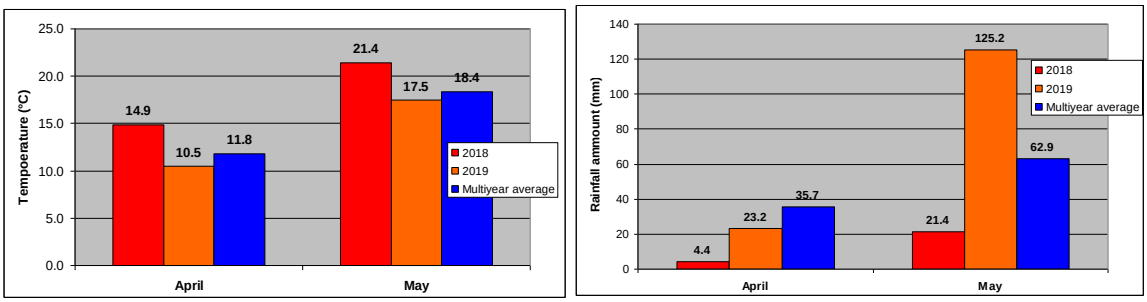

Fig. 1. and 2. Average air temperatures and rainfalls amount registered at Sopema farm, in April and May, 2018 and 2019

Rainfalls amount registered in April, 2019, were below then multiyear average while in May, rainfalls amount was higher comparative with multiyear average. In 2019 it has registered a delay of emergence of the maize plants because unfavourable weather conditions. In both years

it has registered a slowly development of the maize plants in early vegetation stages, both because of low soil humidity and high air temperature differences between day and night.

However, weather conditions from 2018 were favourable for *T. dilaticollis* weevil's activity.

Because of heavy rains occurred in May, 2019, combined with lower air temperatures, weevil's activity at the soil surface were lower in this year comparative with previous one.

In 2018, first assessment made after the emergence of the plants (BBCH 12) and second assessment, when maize was in four leaf stage (BBCH 14), make in evidence high pest population level at the experimental site, with a density ranged from 25 to 30 weevils/m^2.

In the climatic conditions of the year 2018, weevils attack intensity at the maize plants, on a scale from 1 to 9, was higher than **8.1** at both untreated and treated variants. In all experimental plots, the majority of the maize plants were destroyed by the weevils.

According Student-Newman-Keuls (SNK) test there weren't significant statistical differences between attack intensity registered at control (untreated) variant and variants with single foliar spray (without seed treatment) or variant with granules application at 7 days after sowing.

Regard as maize plants density, data from table 2 ascertained that in spring of 2018, at experimental location, this parameter ranged from 4.51 to 5.29 plants/row meter.

At second assessment effectuated on 24 May, when most of the maize plants were in four leaf stage (BBCH 14), it has ascertained a dramatically decreasing of the plant's density. At all variants from this experiment, maize plants density was below 0,70 plants/row meter.

Table 2. Results of foliar and granules application for controlling of the *Tanymecus dilaticollis*, in commercial farm conditions, from south-east of the Romania, in 2018

Active ingredients	Plants (no/Rm) 4.05.2018	Phytoxicity (%) 4.05.2018	Incidence (%) 4.05.2018	Attack (I:1-9) 4.05.2018	Plants (no/Rm) 24.05.2018
control (untreated)	4.55a	**0a**	**100a**	8.40a	0.45a
acetamiprid (20%)	4.51a	**0a**	**100a**	8.32a	0.63a
thiacloprid (480 g/l)	5.06a	**0a**	**100a**	8.41a	0.36a
deltamethrin (100 g/l)	5.29a	**0a**	**100a**	8.28a	0.65a
cypermethrin (8.0 g/kg)	4.96a	**0a**	**100a**	8.20a	0.34a
LSD (P=.05)	0.583	0	0	0.324	0.609
Standard deviation (SD)	0.379	0	0	0.210	0.395
Coefficient of variation (CV)	7.770	0	0	2.530	81.500

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls test)

Table 3. Results of foliar and granules application for controlling of the *Tanymecus dilaticollis*, in commercial farm conditions, from south-east of the Romania in 2019

Active ingredients	Plants (no/Rm) 9.05.2019	Phytoxicity (%) 13.05.2019	Incidence (%) 13.05.2019	Attack (I:1-9) 13.05.2019	Plants (no/Rm) 13.05.2019
control (untreated)	5.44a	**0a**	**100a**	6.16a	4.70a
acetamiprid (20 %)	5.63a	**0a**	**100a**	6.06a	4.39a
thiacloprid (480 g/l)	5,75a	**0a**	**100a**	6.09a	4.99a
deltamethrin (100 g/l)	5.54a	**0a**	**100a**	6.02a	4.56a
cypermethrin (8.0 g/kg)	5.56a	**0a**	**100a**	6.14a	4.50a
LSD (P=.05)	0.339	0	0	0.125	0.526
Standard deviation (SD)	0.220	0	0	0.081	0.341
Coefficient of variation (CV)	3.940	0	0	1.340	7.380

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls test)

Weather conditions registered in April and May, 2018, such as drought and high air temperatures were favourable for weevil's attack. As result of both, high pest density and high attack intensity most of the maize plants from all experimental plots were destroyed (Table 2).

In 2019, observations made when maize plants were in BBCH 12 stage and when maize plants were in BBCH 14 stage make in evidence high pest level at the experimental site, with a weevil's density ranged from 15 to 20 adults/m^2. In the climatic conditions of the year 2019, weevils attack intensity at maize plants, on a scale from 1 to 9, was higher than 6.0 at both untreated and treated variants. Most of the maize plants from this experiment have leaf chaffed in proportion of 75% as result of weevils feeding process. Some plants have leafs completely chafed and beginning of the stem destroyed. However, the majority of the plants from experimental site survived. Possible explication for lower attack in 2019 comparative with 2018 is because of the weather conditions from period when plants were in early vegetation stages (BBCH 10-14). In that period, it has registered high rainfalls amount comparative with multiyear average and lower air temperatures. This condition wasn't favourable for weevil's activity at the soil surface. Insects can't feed with maize leafs in period with higher rainfalls amount. Weevils activity was higher in second decade of May. However, maize arrives at four leaf stage (BBCH 14) that represents the end of the most sensitive period of the plants for weevil's attack. Date from *table* 3 ascertained that in 2019 it has registered a decreasing of the plant's density between 9 and 13 May with more than 10%. This fact can be related with increasing of the weevil's attack. Low pest activity as result of unfavourable weather condition from period when maize was in early vegetation stages have result in lower damages at the maize plants in 2019, comparative with 2018. However, according Student-Newman-Keuls (SNK) test, there weren't significant statistical differences between plants density registered at untreated variant comparative with single foliar spray variants (without seed treatment) or granules application at 7 days after sowing in weather conditions of the 2019 (Table 3).

Further studies are necessary concerning possible finding of the new alternatives at banned chemical seed treatment with systemic insecticides for controlling of maize leaf weevil. Lack of the effective chemical control measures for controlling this pest can have negative consequence for Romanian maize growers in next years.

Acknowledgements

This research work was carried out with the financial support of the Romanian Maize Growers Association (APPR).

Conclusions

Climatic conditions from spring period (April-May) at experimental site (Sopema farm, Ialomita County) were favourable for maize leaf weevil (*Tanymecus dilaticollis* Gyll) attack in 2018 and less favourable in 2019.

In 2018, in conditions of high pest pressure (25-30 weevils/m^2) from experimental site, the attack intensity of the weevils was higher and most of the maize plants didn't survive of the attack.

In 2019, in conditions of high pest pressure (15-20 weevils/m^2) from experimental site, the attack intensity of the weevils was moderate, as result of unfavourable weather conditions from period when maize plants were most sensitive for weevils' attack. Most of the maize plants survive of the attack.

In both years, there weren't registered significant statistical differences between untreated variant and treated variants. All active ingredients tested in this experiment, didn't have effectiveness in controlling of the maize leaf weevil attack and couldn't be an alternative for chemical seed treatment with banned active ingredients.

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Influence of the Harvesting Phenophase on the Quality of Forage Obtained from A *Festuca Valesiaca* Schleich. *Ex*. Gaudin Grassland from Moldova Forest Steppe

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Abstract

The objectives of this study were to determine the influence of the harvesting phenophase and of the organo-mineral fertilization on the quality of forage obtained from a *Festuca valesiaca* Schleich. *ex*. Gaudin meadow. The researches were conducted at the University of Agricultural Sciences and Veterinary Medicine Iasi, Ezareni farm (47°05'-47°10' North latitude and 27°28'-27°33' Eastern longitude). The experimental factors were represented by the harvesting stage, with three graduations: a_1 – harvesting at plants height of 15-18 cm, a_2 – harvesting at the ear formation (control), a_3 – harvesting to full flowering and fertilization with seven graduations: b_1 – unfertilized (control), b_2 – $N_{50}P_{50}$ kg·ha^{-1} annually, b_3 – $N_{75}P_{75}$ kg·ha^{-1} annually, b_4 – $N_{100}P_{100}$ kg·ha^{-1} annually, b_5 – 10 Mg·ha^{-1} sheep manure annually, b_6 – 20 Mg·ha^{-1} annually and b_7 – 30 Mg·ha^{-1} annually sheep manure applied at two years. The obtained results showed that the quality of the feed, the chemical composition of the obtained feed was influenced by the harvesting phenophase as well as by the fertilization system.

Keywords: organic and mineral fertilization, CP, NDF, ADF, RFQ

Introduction

The nutritional characteristics of the feed are defined by the nutritional value given by the concentration in crude protein, the metabolized energy and the content of the feed in ADF and NDF [1]. The quality of the feed is positively correlated with the harvesting phenophase, the fiber content and its digestibility during the vegetation [2]. The crude protein content of plants decreases with the maturity of the plant [3].

The quality of the feed can be improved by the application of fertilizers and the management of the pasture. Some studies have investigated the long-term effects of different fertilization systems on the quality of forage on permanent grassland [4], [5], [6].

The application of nitrogen positively influences the crude protein content of the feed, with the amount of protein increasing as the nitrogen application rate increases [7], [8]. It has been shown that with the advancing of vegetation, the plant accumulates more lignin in the cells, which decreases the digestibility and protein component of the feed; also, as the level of maturity increases, the fiber fraction concentrations have increased [9], [10].

In general, the digestibility of the feed decreases with the maturity stage of the plant [11].

The stage of plant maturity at harvesting time is the main factor responsible for decreasing the nutritional value of the feed [12]. Achieving optimum nutritional value of the feed requires special attention, given the maturity of the feed species [13], [14].

The aim of the study was to analyse the influence of the harvesting phenophase under the influence of organic and mineral fertilization at different doses on the quality of the feed obtained from a meadow of *Festuca valesiaca*, and the objectives were the determination quality indicators: plant crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and calculate forage quality relative (RFQ).

Methodology

The experience was organized on a *Festuca valesiaca* Schleich. *ex*. Gaudin type plant association situated in Moldavian Forest Steppe, between the geographical coordinates 47°05'-47°10'N-27°28'-27°33' E, on a slightly inclined ground, with NE exposition.

The experience was bi-factorial, arranged in randomized plots, in three replicates.

The experimental factors were represented by the harvesting stage, with three graduations: a_1 – harvesting at plants height of 15-18 cm, a_2 – harvesting at the ear formation (control) and a_3 – harvesting to full flowering and fertilization with seven graduations: b_1 – unfertilized (control), b_2 – $N_{50}P_{50}$ kg·ha^{-1} annually, b_3 – $N_{75}P_{75}$ kg·ha^{-1} annually, b_4 – $N_{100}P_{100}$ kg·ha^{-1} annually, b_5 – 10 Mg·ha^{-1} sheep manure applied annually, b_6 – 20 Mg·ha^{-1} sheep manure applied annually and b_7 – 30 Mg·ha^{-1} sheep manure applied at two years.

Fertilization was done with two types of fertilizer: organic represented by well fermented sheep manure (older than two years) and mineral represented by complex fertilizer with nitrogen and phosphorus ($N_{20}P_{20}$).

The manure and mineral fertilizers were manually applied in the spring, at the beginning of plant growth.

Nitrogen content was determined by Kjeldahl method, and NDF and ADF content were determined by Van Soest method [15]. RFQ (Relative Forage Quality) was calculated using the Equation 1 [16], [17].

The results were statistically analysed by the analyses of variance and limit differences. We also determined the correlation equations and the significance of the square regression between the type of fertilization, harvesting phenophase and the feed content in CP and RFQ.

$$RFQ = \frac{(4{,}898 + 89{,}796 \cdot (1{,}085 + 0.0124 \cdot ADF)) \cdot \frac{120}{NDF}}{1.23}$$

Equation 1.
Relative Forage Quality

Results and Discussions

Improving the quality of permanent grasslands, regardless of the area in which they are located represented and represents the main objective of pratological studies.

Most previous studies have shown that the quality of the forage is significantly influenced by the harvesting phenophase [18], [19], [20], the changes that take place in the quality of the feed being evaluated at the plant level or the plant community level [21], [22].

Analysing the influence of the studied factors on the crude protein content (*table 1*), it is found that the values of the feed content in CP are different, depending on the fertilization variants used, and at the same doses, depending on the harvesting phenophases.

Thus, harvesting at plants height of 15-18 cm caused very significant increases of the crude protein content in relation to the unfertilized variant, the highest crude protein content, of 13.43 g·100 g^{-1} DM being registered in the fertilized variant with $N_{100}P_{100}$ kg·ha^{-1} annually. As can be seen (*table 1*).

Table 1. The influence of the interaction between harvesting phenophases and fertilization on the feed quality

Variant			Quality parameters			
			CP	NDF	ADF	RFQ
			(g·100 g^{-1} DM)			
a_1 – harvesting at plants height of 15-18 cm	b_1-unfertilized		10.51**	49.53^{ooo}	30.53^{ooo}	134.58***
	b_2-$N_{50}P_{50}$		11.79***	48.10^{ooo}	30.09^{ooo}	139.58***
	b_3-$N_{75}P_{75}$		12.22***	47.90^{ooo}	28.17^{ooo}	144.53***
	b_4-$N_{100}P_{100}$		13.43***	47.05^{ooo}	26.37^{ooo}	151.29***
	b_5-10 Mg·ha^{-1} manure		10.77***	56.43	31.02^{ooo}	117.20*
	b_6-20 Mg·ha^{-1} manure		11.43***	54.20^{ooo}	30.96^{ooo}	122.14***
	b_7-30 Mg·ha^{-1} manure		12.27***	53.55^{ooo}	30.46^{ooo}	124.63***
a_2 – harvesting at the ear formation (C)	b_1-unfertilized (C)		8.17^{c}	56.77^{c}	34.84^{c}	109.20^{c}
	b_2-$N_{50}P_{50}$		10.65**	56.40	34.18^{oo}	111.17
	b_3-$N_{75}P_{75}$		11.71***	55.78^{o}	33.34^{ooo}	114.04
	b_4-$N_{100}P_{100}$		12.36***	54.43^{ooo}	32.29^{ooo}	118.97**
	b_5-10 Mg·ha^{-1} manure		8.86	48.92^{ooo}	34.99	126.38***
	b_6-20 Mg·ha^{-1} manure		10.12*	57.77*	34.64	107.67
	b_7-30 Mg·ha^{-1} manure		11.11***	54.18^{ooo}	35.25	113.58
a_3 – harvesting to full flowering	b_1-unfertilized		7.57	57.62	37.59**	102.39
	b_2-$N_{50}P_{50}$		9.46	55.71^{o}	35.51**	109.95
	b_3-$N_{75}P_{75}$		9.53	54.19^{ooo}	35.40*	113.26
	b_4-$N_{100}P_{100}$		10.25**	52.67^{ooo}	34.12^{oo}	119.18**
	b_5-10 Mg·ha^{-1} manure		8.26	52.30^{ooo}	35.50**	117.15*
	b_6-20 Mg·ha^{-1} manure		9.41	51.71^{ooo}	34.96	119.62
	b_7-30 Mg·ha^{-1} manure		10.20**	49.73^{ooo}	34.01^{ooo}	126.45***
		LSD 0.01	2.60	1.52	0.80	11.93
		LSD 0.1	1.97	1.15	0.61	9.04
		LSD 0.5	1.47	0.86	0.45	6.75

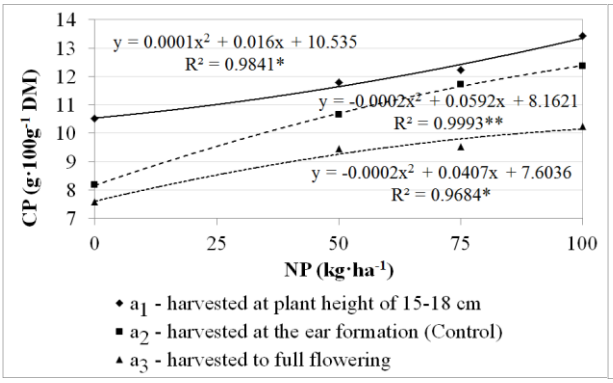

Fig. 1. Regression curve between NP dose (kg·ha^{-1}) and CP content

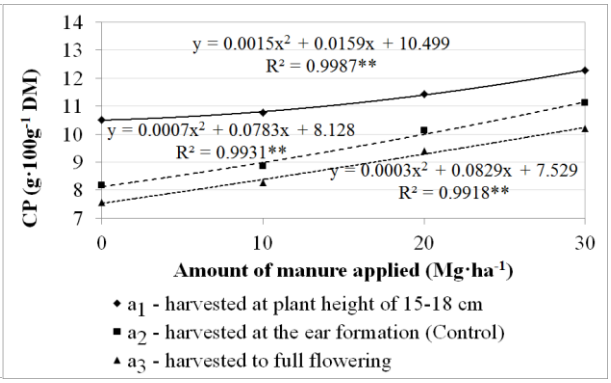

Fig. 2. Regression curve between the amount of manure sheep applied (Mg·ha^{-1}) and the CP content

The variants that have the highest protein content are those fertilized with $N_{100}P_{100}$ kg·ha^{-1} annually and 30 Mg·ha^{-1} sheep manure applied at two years with a value of 10.25 g·100 g^{-1} DM and 10.20 g·100 g^{-1} DM respectively. In this phenophases on the same variants fertilized with $N_{100}P_{100}$ kg·ha^{-1} annually, the crude protein content registered a decrease of 38.9% from the first phenophases and 25.8% from the ear formation phenophases taken as control (*table 1*).

Between the doses of applied NP and the forage content in CP, were obtained positive correlations for all variants (*figure 1*).

Distinctly significant values of the regression coefficient were also obtained in the case of the correlation between the applied manure doses and the feed content in CP at all three harvesting phenophases studied (*figure 2*).

Harvesting in different phenophases, as well as the administration of organic and mineral fertilizers had produced important changes regarding the feed content in ADF and NDF obtained from the *Festuca valesiaca* grassland (*table 1*). Analysing the influence of the studied factors on the content of the forage in the NDF (*table 1*), we observe that the harvesting at plants height of 15-18 cm caused a decrease of the NDF values in most experimental variants.

From the results obtained on the ADF content of the feed, following the application of organic and mineral fertilization, a decrease of the values of this parameter is observed in most of the fertilized variants studied compared to the control variant, the differences being statistically ensured, but there is a tendency to increase the content values in ADF, due to the accumulation of cell walls (lignin and cellulose) (*table 1*).

The results regarding the relative qualitative value of the feed (RFQ) (*table 1*), show us that the RFQ values of the feed obtained from the permanent grassland were influenced by the applied fertilization, but especially by the harvesting phenophases, with significant, distinctly significant differences and very significant in relation to the control variant.

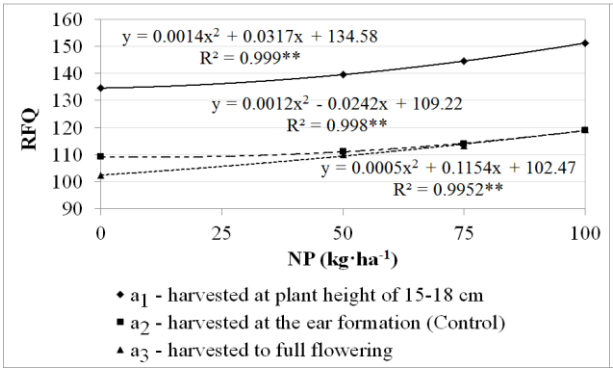

Fig. 3. Regression curve between NP dose (kg·ha^{-1}) and RFQ

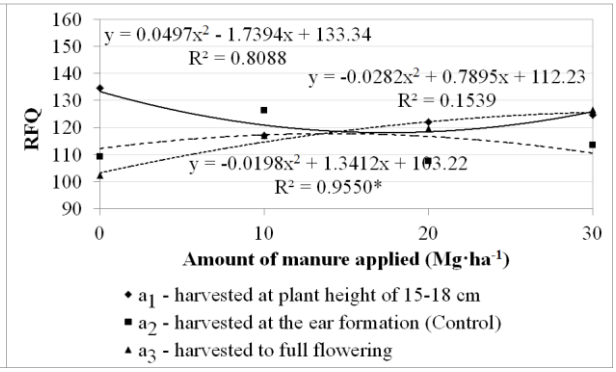

Fig. 4. Regression curve between the amount of sheep manure applied (Mg·ha^{-1}) and RFQ

The highest value of the RFQ, of 151.29 was obtained in the variant harvested at plants height of 15-18 cm fertilized with $N_{100}P_{100}$ kg·ha^{-1} annually, with an increase compared to the unfertilized variant taken as a control of 38.5%. The relative qualitative value of the feed (RFQ) showed a general tendency of increase with the increase of the applied NP doses, correlating significantly with this value (*figure 3*). In the case of manure due to the adverse climate conditions that negatively influenced the use of manure by plants, the content of the feed in cell walls, respectively RFQ did not correlate in the incipient vegetation phenophases, the relative qualitative value of the feed (RFQ) being significantly correlated with full flowering phenophases on the applied fertilization (*figure 4*).

Conclusions

The obtained results show that the stage of development is an essential factor that determines changes in the quality of the feed, the chemical composition of the feed obtained being influenced by the harvesting phenophases, type of fertilizer and the applied doses.

The qualitative value of the forage proved to be the highest during the vegetative growth, when the harvest was done at the plant's height of 15-18 cm, the plants having a high protein content and a low cell wall.

The results of the study highlight that as the plants age increases, the proportion of protein decreases, instead increasing the proportion of cellulose, hemicellulose and lignin (ADF and NDF), thus reducing the digestibility of the feed. Both organic and mineral fertilizers have

influenced the quality of the feed obtained from the *Festuca valesiaca* meadow. The crude protein content increased significantly compared to the unfertilized variant.

Also, the two factors studied positively influenced the relative qualitative value of the feed (RFQ) with significant, distinct and very significant increases, compared to the control variants, the quality of the forage being a very good one (quality class 1) in the phenophases in which its harvesting was done at plants height of 15-18 cm.

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Behaviour of some Maize Hybrids Under Cojocna Conditions

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Abstract

In Romania, more and more consideration is being given to cultivating maize hybrids suitable for the area. Our objective was to determining the influence of the genetic potential of the maize hybrids used on the response to the complex application of all agricultural inputs specific to modern technology and the study of the behaviour of the genetic material (maize hybrids from different sources) under the particular conditions of the agricultural year 2018.

Elaboration of specific recommendations for maize cultivation technology depending on the biological material, the inputs used and the timing and harvesting methodology.

Keywords: Cojocna, inputs, maize hybrids

Introduction

Corn (*Zea mays*), also called *Indian corn* or *maize*, cereal plant of the grass family (*Poaceae*) and its edible grain. The domesticated crop originated in the Americas and is one of the most widely distributed of the world's food crops [1]. Last year, in the project AGRIM-Integrated management of agricultural inputs [2], the objective was to establish the main technological elements specific for maize cultivation under the eco-geological conditions in the Transylvanian Plain. As expected, results have been considered: elaboration of specific recommendations for maize cultivation technology depending on the biological material, the inputs used and the timing and harvesting methodology and also organization of meetings and visits of the AGRIM Polygon by farmers, input producers' associations, AGRIM partners, students etc.

Methodology

The experience was located in Cojocna, Cluj County, on a type of phaeoziom soil in 2018.

The provenance of the seeds is from Romanian Corn Producers Association (APPRP) – Pioneer Hybrids and Agricultural Research and Development Station Turda (ARDS Turda). Biological material: 8 Pioneer and 2 Turda hybrids. The hybrids used were from Pioneer: H3 – P 0216 (X03A115), H4 – P 0933 (X08B335), H5 – P 0023 (X00C208), H6 – P 0412 (X8M193), H7 – P 9415 (X95F646), H8 – P 0268 (X00K446), H9 – P 9757 (X95K979), H10 – Variety X00K449, H11-Turda 201 and H12 – Turda 332.

The land was pickled and labelled according to the experimental protocol (each hybrid was put in 7 rows on a surface of 2784 square meter).

The works of the soil according to the settlement (previous plant-alfalfa), were carried out in compliance with the technological parameters monitoring by professor from Mechanization section of UASMV Cluj.

The density of seeding was 63.500 plants/ha, distance between rows = 75 cm and distance between plants/row = 21 cm. the date of sowing was 02 May 2018 and harvesting was in 26 October 2018. Basic fertilization was made with 20: 20: 0 + Zn and fertilization at sowing: 27: 13.5: 0 with Azomures products. The herbicides were from Alcedo Company and were used Astral 40 Sc and Ceredin Forte 464 Sl.

The climate of the area is of continental type according to the Koppen system. In 2018, the hottest month was August, with an average monthly temperature of 22.3°C, and the coldest, February, with an average monthly temperature of -0.3°C.

The average annual rainfall was 540 mm, of which 68% fall during the vegetation period, the rainiest being June, when they fall on average 84.8 mm, and in 2018 they fell by +13.5 deviation more than the average on the last 60 years. In this paper, the F-test Test were used to analyses whether the changes, observed at the waist of the maize plants in the experimental variants, are due to the treatments applied or have occurred by chance. For the phytosanitary condition, the Duncan Test or the multiple comparisons method was used to determine the significance of the differences for the 5% probability of transgression.

The higher the F value than 1.00, the higher the effect of the treatments; when the F value is close to 1.00 the transformations have accidentally occurred [3].

Results and Discussions

Regarding to the determined character of the weight of the corn cob, it was found that the lowest average had the hybrid H12 followed by the hybrid H5, at the opposite pole being H10 followed by the H8 hybrid (Table 1).

Regarding the character of the weight of grains on cob, the smallest value registered in this case was hybrid H11 followed also by the hybrid H5. The highest value of grain weight per cob was recorded in hybrid H8 followed by hybrid H4.

Regarding the character of cob length, the H10 hybrid has registered in this case.

The highest value of the cob length was recorded in the H4 hybrid followed by the H8 hybrid.

As for the character, the number of rows on cob the smallest values were recorded in this case the H6 hybrid.

The highest value of the number of rows per cob was recorded in the H4 hybrid followed by the H8 hybrid. As for the character, the number of grains/cob the lowest value registered in this case the hybrid H6. The highest value of the number of grains per row was recorded in the H1 hybrid.

Regarding the character of the cob diameter the H12 hybrid has registered in this case.

The highest value of the cob diameter was recorded in the H8 hybrid, with a difference of 11.02 cm.

As for the character of the diameter of the rachis, the smallest value was recorded in this case the H12 hybrid.

The highest value of the diameter of the rachis was recorded in the H8 hybrid, with a difference of 3.99 cm. Regarding the evaluation of the corn cultivation before harvesting on 30.10.2018 from the experimental polygon Cojocna it was found that the two herbicides that were applied are able to solve the weed problem in the corn crop under the pedoclimatic conditions of Cojocna in experimental year 2018.

Table 1. LSD comparisons of the maize hybrids

	Hybrid	Weight of corn cob	Weight of grains on cob	Cob length	No. of row/cob	No. of grains/cob	Cob diameter	Rachis diameter
Hybrid		-0.279*	-0.233*	0.001	0.079	-0.264*	-0.139	0.059
Weight of corn cob	-0.279*		0.967*	0.644*	0.209*	0.487*	0.817*	0.412*
Weight of grains on cob	-0.233*	0.967*		0.639*	0.201*	0.496*	0.820*	0.409*
Cob length	0.001	0.644*	0.639*		0.281*	0.647*	0.361*	0.077
No. of row/cob	0.079	0.209*	0.201*	0.281*		0.136	0.286*	0.309*
No. of grains/cob	-0.264*	0.487*	0.496*	0.647*	0.136		0.206*	-0.003
Cob diameter	-0.139	0.817*	0.820*	0.361*	0.286*	0.206*		0.595*
Rachis diameter	0.059	0.412*	0.409*	0.077	0.309*	-0.003	0.595*	

Note: Marqued values (*) indicate significative correlation. Breakdown table of descriptive statistics N=100.

Multiple comparisons to the determinations made in the AGRIM Polygon have resulted in results that fall within the parameters of the descriptive statistics performed.

Observations regarding the phytosanitary status of maize plants concerned the frequency of attack of *Fusarium sp.* and *Ostrinia nubilalis* on cob, but also the total number of broken plants and the frequency of attack of *Ostrinia nubilalis* on the stem (Table 2). Of the tested hybrids, H8 and H1 were positively highlighted with the lowest values of the attack, at all the indicators followed. Also noteworthy are the H6 and H7 hybrids with low values of the frequency of the spike attack.

Table 2. Phytosanitary status of the studied maize hybrids

Tested hybrid	Frequence of *Fusarium sp.* on cob	Frequence of *Ostrinia nubilaris* on cob	Total fallen plants	Frequences of *Ostrinia nubilaris* on stem
P9911	6.94 ^{A}	4.51 ^{A}	19.28 ^{ABC}	15.20 ^{ABC}
P9903	15.96 ^{AB}	13.28 ^{AB}	20.39 ^{ABC}	15.96 ^{ABCD}
P0261	8.42 ^{A}	2.46 ^{A}	17.82 ^{AB}	12.49 ^{AB}
P0933	33.98 ^{BCD}	17.61 ^{AB}	25.61 ^{BC}	17.68 ^{ABCD}
P0023	12.03 ^{AB}	10.82 ^{AB}	39.07 ^{D}	31.52 ^{CD}
P0412	3.18 ^{A}	7.88 ^{A}	24.55 ^{BC}	15.45 ^{ABC}
P9415	6.10 ^{A}	4.25 ^{A}	55.56 ^{E}	32.54 ^{D}
P0268	3.03 ^{A}	6.28 ^{A}	11.13 ^{A}	6.28 ^{A}
P9757	15.78 ^{AB}	14.19 ^{AB}	21.91 ^{ABC}	18.79 ^{ABCD}
x00k449	23.22 ^{ABC}	26.17 ^{B}	31.92 ^{CD}	28.95 ^{BCD}
T 332	46.31 ^{D}	18.14 ^{AB}	23.40 ^{ABC}	17.55 ^{ABCD}
T 201	39.99 ^{CD}	14.44 ^{AB}	32.27 ^{CD}	22.83 ^{ABCD}
	DS 20.94- 23.95	DS 13.95- 16.31	DS 11.45- 13.39	DS 14.68- 17.15

The classification was mentioned by the letters of the alphabet (A, B, C, D), the distance in the alphabet being given by the most significant impact of the variant.

The production obtained and related to the standard humidity of 14% in the studied hybrids is shown in Table 3. The highest production was recorded in hybrid 10 (11079 kg/ha) and the smallest in Turda 201 hybrid (6021.33 kg/ha). Between the H3 and H6 hybrids the harvest differences do not have significant increases between them, being close enough in value.

Table 3. The production of maize hybrid in Cojocna in 2018

Hybrid	FAO Group	Humidity at harvesting (%)	Grain production at the harvest (kg/ha)	Grain production at humidity 14% (kg/ha)
H3 (P0216)	450	16.5	10714.8	9091.34
H4 (P0933)	500	16.4	11246.41	9600.59
H5 (P0023)	400	15.6	10571.12	9486.90
H6 (P0412)	480	17.1	11806.75	9666.34
H7 (P9415)	340	15.9	10104.17	7644.31
H8 (P0268)	420	16	10025.14	8841.14
H9 (P9757)	370	15.9	12345.55	8827.17
H10 (00K449)		15.6	12345.55	11079.34
H11 (Turda 332)	380	15.8	8624.28	7641.76
H12 (Turda 201)	340	15.3	6580.46	6021.33

FAO = Food and Agriculture Organization of the United Nations

Conclusions

Based on particularities of the biological material, the specific consumption are basic elements in choosing the type of fertilizer, including setting the recommended doses, together with soil analysis, climatic conditions and other factors that determine agricultural production.

The experiment shows that the type of fertilizer recommended must take into account the productive potential and the genetic particularities of the cultivated hybrids, which differentially value the nutrients made available, given their different consumption.

Acknowledgment

Thanks to our partners from AGRIM.

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Implementing the Analytic Hierarchy Process to Select the Most Promising Wild Berries from Botoşani County

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Abstract

This paper implements the Analytic Hierarchy Process (AHP), a multi-criteria decision-making methodology, in the selection of the most promising wild berries in Botosani County.

The selected wild berries were: *Rubus idaeus*, *Cornus mas*, *Sambucus nigra*, *Prunus spinosa*, *Crataegus monogyna*, *Sorbus torminalis*, *Prunus avium* and *Pyrus pyraster*. First, based on the criteria taken into consideration, it was established a hierarchical structure of the eight selected wild berries and subsequently, it was tested using Expert Choice software. The ranked list indicates that the higher scores were obtained by wild cherry and red raspberry, while the lower ones were obtained by blackthorn and European cornel. In Botoşani County the wild berries sector is characterized by great potential and diversity, but this sector is not capitalized on the real potential. The analysis of the potential of Botoşani County with regard to the most important wild berries for collection and capitalization can help improve in the future the management strategies of these resources.

Keywords: analytic hierarchy process, Botoşani County, wild berries, wild cherry

Introduction

There is an increasing trend at a global level regarding the consumption and merchandising of forest fruits due to their nutritive values and active principles [1]. These aspects offer them the status of sanogen nutriment [2], being considered biological, natural and ecological products [3]. Together with the increase of population and implicitly with the increase of fruit consumption, many species have become the object of culture expansions. The main advantage is represented by the production's high productivity. However, wild berries are superior as a qualitative ratio [4].

In Romania, data regarding the quantities harvested from forest ecosystems mark values gathered between 3000-4000 tons of forest fruits, with the most significant quantities recorded by *Rosa canina* L., *Rubus idaeus* L., *Rubus hirtus* W. et K., *Vaccinium myrtillus* L. and *Hippophaë rhamnoides* L. [5].

At a national level, Botoşani County has a varied assortment of forest trees and shrubs that generate edible fruits that can be capitalized. Even though the forest fund's surface is situated much under the national average [6], the area has the possibility of collecting considerable quantities of forest fruits.

According to the last annual report [7], the only forest fruits that were capitalized from Botoşani Forest Directorate were rosehip. The diversity of forest fruits can offer the possibility of using these resources to obtain new and varied consumption products and an alternative source of income for this sector.

Therefore, it is recommended to take into consideration the economic value of these products when developing forest management plans [8].

Methodology

Botoşani County is part of the North-East development region and occupies a surface of 4986 km^2 (Fig. 1).

Fig. 1. Location of Botoşani County

The relief is not very varied, being mainly hilly, while the climate is strongly influenced by East air masses. This leads to a lower average annual temperature lower (8-9°C), with precipitations irregularly distributed during the year.

The fields covered with forests amount to 55,869 ha [9], from which 60% are mainly owned by the state [7] and are managed by Botoşani Forest District through its six subunits (Botoşani, Darabani, Dorohoi, Flămânzi, Mihai Eminescu and Truşeşti). The vegetation is predominantly composed of broad-leaved forests formed of pedunculated oak and holm, mixed with common beech, hornbeam, ash, linden, silver linden, bird cherry, shadberry, maple etc. In addition, shrubs frequently appear in forest clearings or near them such as hawthorn, hazel, cornel-tree, dogwood, brier or blackthorn.

Information regarding the type and frequency of wild berries present in the forests from Botoşani Forest Directorate were obtained by consulting the following documents: forest management plans, annual reports from ROMSILVA – the National Forest Registry, as well as reports from the National Institute of Statistics.

The study was conducted in two phases: the first phase included defining the criteria and the preliminary list of wild berries types common for Botoşani County. Thus, a set of nineteen criteria and eight types of wild berries were taken into consideration. The same set of criteria was applied in several recent investigations focused on the potential of non-wood forest products conducted in Maramureş [10], Prahova [11], Bihor [12] and Gorj [13].

The second phase of the study involved an AHP (analytical hierarchical process) analysis in order to establish criteria importance. This method was designed by Saaty T.L. [14] and is a popular approach used in prioritizing alternatives and making choices. Therefore, the limited number of criteria and alternatives make this methodology appropriated for use in this type of study. The criteria were evaluated in pairs and quantified by experts according to a scale from 0 to 8. Subsequently, the expert's weights were computed using the Expert Choice software.

Results and Discussion

The eight main wild berries considered in the case of Botoşani County were: red raspberry (*Rubus idaeus* L.), European cornel (*Cornus mas* L.), European black elderberry (*Sambucus nigra* L.), blackthorn (*Prunus spinosa* L.), common hawthorn (*Crataegus monogyna* Jacq.), chequers (*Sorbus torminalis* (L.) Crantz), wild cherry (*Prunus avium* L.) and European wild

pear (*Pyrus pyraster* (L.) Burgsd). The AHP alternative ranking along with the scores of the criteria is provided in Tab. 1.

Table 1. AHP alternative ranking

Criterion		Berries							
		Rubus idaus	*Cornus mas*	*Sambucus nigra*	*Prunus spinosa*	*Crataegus monogyna*	*Sorbus torminalis*	*Prunus avium*	*Pyrus piraster*
		1	2	3	4	5	6	7	8
1	Harvesting period	1	4	3	5	8	6	2	7
2	Harvested quantity/worker /8 hours	2	3	6	4	1	5	7	8
3	Harvesting cost	2	1	3	4	8	5	6	7
4	Knowledge for harvesting	1	5	2	6	7	8	3	4
5	Tools needed for harvesting	1	2	3	4	6	5	8	7
6	Complexity of harvesting process	1	2	3	4	6	5	8	7
7	Development of harvesting process	1	2	3	4	5	6	8	7
8	Knowledge for recognition	1	5	6	7	2	8	3	4
9	Distribution range	3	8	7	4	5	6	2	1
10	Biotic threats	3	7	2	8	5	4	1	6
11	Abiotic threats	7	4	6	1	2	3	8	5
12	Perishability	8	3	6	1	2	5	7	4
13	Market potential	8	3	2	1	6	4	7	5
14	Market demand	8	4	6	1	3	5	7	2
15	“Celebrity” of the product on market	8	2	4	1	6	3	7	5
16	The price of raw product	8	4	3	1	2	6	7	5
17	The price of the derived products	8	3	5	1	4	6	7	2
18	Portfolio of derived products	8	3	2	1	4	5	7	6
19	Transport (harvesting - storage centre)	2	4	4	5	1	7	8	6

As shown in Fig. 2, the best results were obtained by *Prunus avium* and *Rubus idaeus*, while the lowest results were obtained by *Prunus spinosa*, closely followed by *Cornus mas*.

It is clear from the ‘Performance Sensitivity’ graphic that *Prunus avium* L. outperforms the rest of the alternatives, mainly due to the low number of tools needed for harvesting (criterion 5), the low the harvesting process complexity (criterion 6), the highly developed harvesting process (criterion 7), the low number of abiotic threats (criterion 11) and an easy transportation from the point of harvest to the storage centre (criterion 19). The main drawbacks of sweet cheery are the high number of biotic threats, the limited distribution range and the short harvesting period.

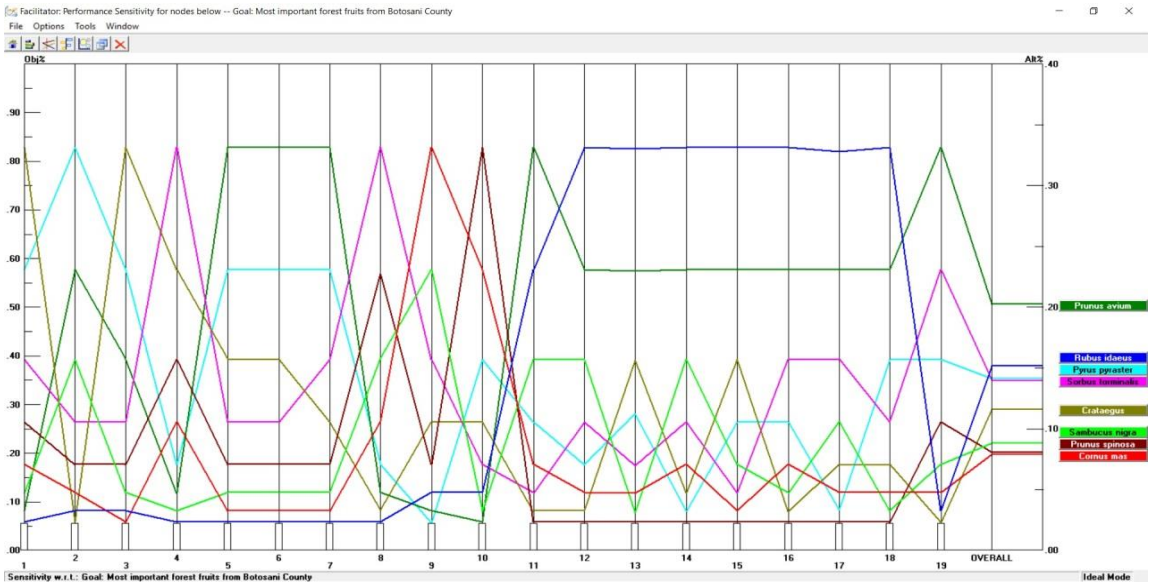

Fig. 2. Ranking of the selected NWFPs

In our country, wild strawberry grows in hill regions wherein it enters as a mixture species in broad-leaved forests under a disseminated form, covering a surface of approximately 7600 ha [15]. The species has an annual and abundant fructification, with a national annual fruit production of approximately 42000 tons [16].

The fruits are rich in sugars, organic acids, vitamins, pectin and also contain significant amounts of mineral substances and colorant agents [17].

From an economic point of view, sweet cherry is considered a high-value fruit being valorised as fresh fruit or as various processed products (frozen cherries, canned and jar cherries, brined cherries, juice, wine, liqueur, distilled spirits, etc.) [18].

Red raspberry (*Rubus idaeus* L.) is the second most promising berry for the studied county.

Compared to sweet cherry, the red raspberry performs better in distribution range, biotic threats, perishability, market potential, market demand, "celebrity" of the product on market, the price of raw product, the price of the derived products and the portfolio of derived products.

The species is common in our country, being present in the entire forest area situated between hills and the superior forest limit, preponderantly in thin forests and clearings where it vegetates well on trophic soils [19].

It is considered to be one of the most valuable and commercially important forest fruit shrubs, with quantities obtained from one hectare varying between 70 and 520 kg [4]. It is also one of the most popular berries worldwide because of its flavour and nutritional content [20].

The red raspberry's popularity on the market is also a consequence of the fact that the fruit has multiple uses. As such, it can be consumed fresh or processed into various products (juice, syrup, sherbet, jam, marmalade, vermouth, liqueur, etc.). In addition, this fruit contains a high concentration of bioactive compounds with antioxidant, tonic, depurative, diuretic and laxative properties being recommended in traditional and alternative medicine to treat several affections [21].

According to numerous experts, blackthorn (*Prunus spinosa* L.) was placed near the bottom of the ranking, very close behind European cornel (*Cornus mas* L.). A similar result was obtained by Vechiu E. and Dincă M. [22] in Neamţ County.

This result is explained by the high number of criteria that have obtained a score of 1. Amongst all berries, blackthorn is the most unpopular product, with the lowest potential and demand on the market, although it is known to have a lot of positive properties [23]. Fruits are reported to possess significant levels of nutritional compounds (sugars, organic acids, polyphenols, anthocyanins, vitamin C, tannins) [24]. Moreover, these compounds are

recognized to have pharmacological effects, including anti-inflammatory, anti-septic, anti-diarrheic and spasmolytic effects [25].

On the other hand, blackthorn fruits are not commonly appreciated and consumed fresh because of their astringent taste and high acidity, which is usually used in combination with other fruits to make jams, juices or syrups.

Conclusions

The wild berry sector from Botoşani County is characterized by great potential, an aspect highlighted by the diversity of edible fruit species. As shown by official data, these resources are not managed and capitalized at their real potential. The most promising forest fruits from this area are sweet cherry, red raspberry and European wild pear. In addition to dog rose, these wild berries could be included in forest management programs as they possess a high economic potential.

In conclusion, these types of analyses can help contribute to a better knowledge of the harvesting, benefits and utilization of these species, as well as to improve future management strategies for these resources.

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Physico-Geographical Conditions of the Hydrographic Basin of the Moldova River

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Abstract

In the national economy of a country, of a region, the rational use of surface and ground water resources for the purpose of ensuring water equilibrium as a continuous balance between resources and demands, as well as the protection of water quality are a vital requirement for all economic and social activities. The surface and ground water flow regime is influenced by the hydrogeomorphological and hydrogeological conditions within the hydrographic basin. This paper presents the physico-geographical conditions within the Moldova hydrographic basin, which drains a surface of approximately 4300 km^2. The hydrographic basin of the Moldova River is located in the northeaster part of the Eastern Carpathians and the north-western part of the Moldavian Plateau. The altitudes are ranging between 180 m (Roman) and 1856 m (Gimalau peak): approximately 80% of the basin surface has altitudes between 250-1000 m and 16% has elevations above 1,000 m. When it comes to the incline of the land, about 72% of the surface has a slope smaller than 15%, 23% has a slope between 15-25% and over 5% of the basin surface has a slope larger than 25%. The mean annual temperatures increase from the value of 2.00ºC in the upper part of the hydrographic basin, to the value of 8.7ºC in the lower part. The average amount of rainfall decreases gradually from springs, where 925 mm of rainfall is recorded annually, to the value of 514 mm, towards the confluence with the Siret River, in Roman. The dense hydrographic network (0.55 km/km^2) with its sinuous route, the presence of a great number of old runways and abandoned meanders from the extra-Carpathian sector of the hydrographic basin, the relatively flat lands with many micro-depressions in the meadow and the terrace areas, as well as the presence in the soil profile of the Bt horizon, with low permeability, has led to the appearance and maintenance of excess water both on soil surface and profile. In order to regulate the hydric regime on the surface and profile of the soils from the extra-Carpathian sector of the hydrographic basin of the Moldova river, drainage works have been carried out over time totalling a surface of 8761 ha of which 3059 ha have been underground drainage works.

Keywords: hydrographic basin, land slope, drainage

Introduction

The hydrographic basin may be defined as a physiographic unit bounded by topographic divides that limit the areas of land drained by a main river, its affluent and sub-affluent [1].

Romania has a 78 905 km long hydrographic network, which drains an area of 238 391 km^2.

The water resources in the inland rivers amount to 40 billion m^3, which represent 20% of the water resources of the Danube River. Romania has a specific resource from the inland rivers of 1840 m3/inhabitant/year and it ranks 13th in Europe [2].

Environmental issues regarding the protection of water resources are a real challenge to sustainable development and require protection policies and measures. Vulnerable situations in water resources, especially surface ones, are the subject of debates at both international and national levels [3].

Through their different activities, men intervened and modified the geomorphological landscape both indirectly, influencing the dynamics of the modelling processes, and directly, by erecting certain buildings [4]. Anthropic activities carried out for the complex development of river basins for primary economic goals, such as: drinking water supply, industrial and irrigation water supply, flood prevention and control, soil erosion control, drying-drainage works, etc. significantly impact the environment [5].

Slope waters, which occur in cases of sudden slope changes (mountain areas, transition areas from hill to plateau), where liquid flows may take with them suspensions and coarse material, have unpredictable negative effects when they are associated with areas of massive deforestation [6], [7], [8].

The quantitative knowledge of hydrological parameters (rainfall and flow) and their spatial and temporal variability on the regions or basins should be understood as essential to the efficient planning and management of water resources [9].

Methodology

The Moldova river basin is located in the NE part of the Eastern Carpathians and in the NW of the Plateau of Moldova (Fig. 1). The basin is bounded by $25^0$08'37″-$26^0$58'35″ meridians east longitude and $46^0$55'37″-$47^0$43'38″ parallels north latitude.

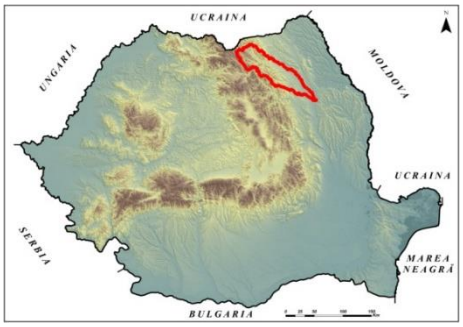

Fig. 1. Localization of the Moldova river basin in Romania

Many geographers [10], [11], include this area in the Subcarpathia region, as an external hilly piedmont plateau unit. In 1980 it was included in the Moldova Plateau under the name Piedmont Plateau [12]. This Piedmont Plateau also comprises the wide Moldova floodplain between Păltinoasa and up to Roman, called the Baia-Moldova-Roman piedmont plain [13].

The SRTM 90 data re-projected in Stereo 70 were used and processed using the QGIS software in order to draw the thematic maps of the Moldova river basin (hypsometric map, land inclination map).

River basin data extracted from a worldwide climate database, covering the period 1950-2000 [14] available on the website http://www.worldclim.org/ were used to determine the monthly and yearly average thermal and rainfall conditions.

Results and Discussions

River basins are geomorphological river systems that may be described by a large number of variables. Hydrogeomorphological and hydrogeological conditions influence the surface and underground runoff within the river basin. The Moldova River has its source (springs) at an

altitude of 1116 m in Obcina Feredeului, Lucina Peak. The river is 213 km long, 110 km of which are in the extra-Carpathian area, downstream from the town of Gura Humorului.

The Moldova River basin drains an area of about 4300 km2, about 2410 km2 of which in the extra-Carpathian region. The hydrographic network has a winding route, with numerous old river beds and deserted meanders, with an average density of 0.55 km/km2, which exceeds the country average of 0.49 km/km2.

The altitudes, within the basin, range between 180 m, at Roman, and 1856 m, Giumalău peak (Fig. 2). In the floodplain, the altitude of the Moldova river decreases from 480 m, at Gura Humorului, to 285 m, at Drăguşeni, reaching the level of 236 m at Tupilaţi and 180 m at the confluence with the Siret river.

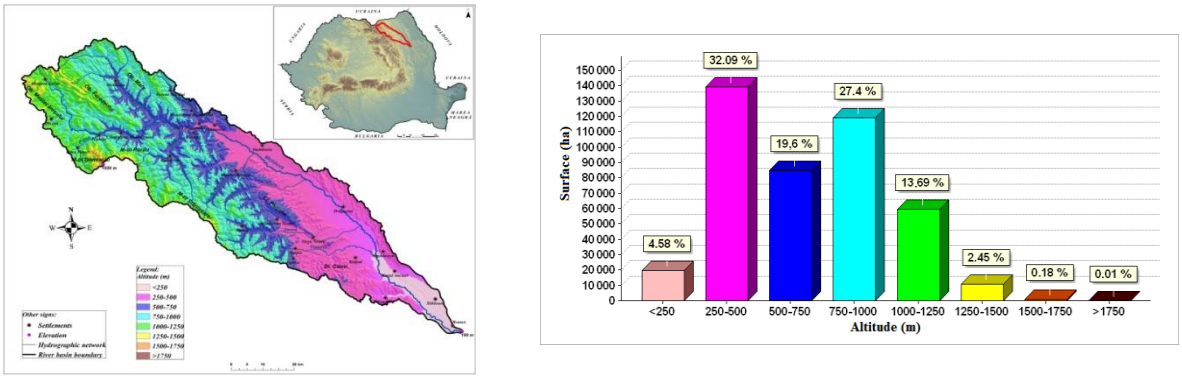

Fig. 2. Altitude map and their share

About 16% (702 km2) of the river basin area has altitudes greater than 1000 m, 27% (1178 km2) between 750 and 1000 m and 20% (843 km2) between 500 and 750 m. The altitude of the largest area of 1380 km2 (32%) ranges between 250 and 500 m and only about 197 km2 (5%) have altitudes between 180 and 250 m, these areas being located in the extra-Carpathian area, in the SE part of the river basin.

Within the Moldova river basin, the variety of the relief influences the territorial distribution of the water flow. This influence is exerted by the fragmentation and slopes of the relief on which the shallow flow occurs. The Moldova river basin has a fragmented and rugged relief represented by mountains, hills and plateaus.

As concerns land slope intervals, the slope of the largest area in the river basin, about 1419 km2 (33%), is less than 5%, the slope of other 870 km2 (20%) ranges between 5 and 10%, 792 km2 (19%) between 10 and 15%, 620 km2 (14%) between 15-20%, 372 km2 (9%) between 20-25% and approximately 227 km2 (5%) have a slope greater than 25% (Fig. 3).

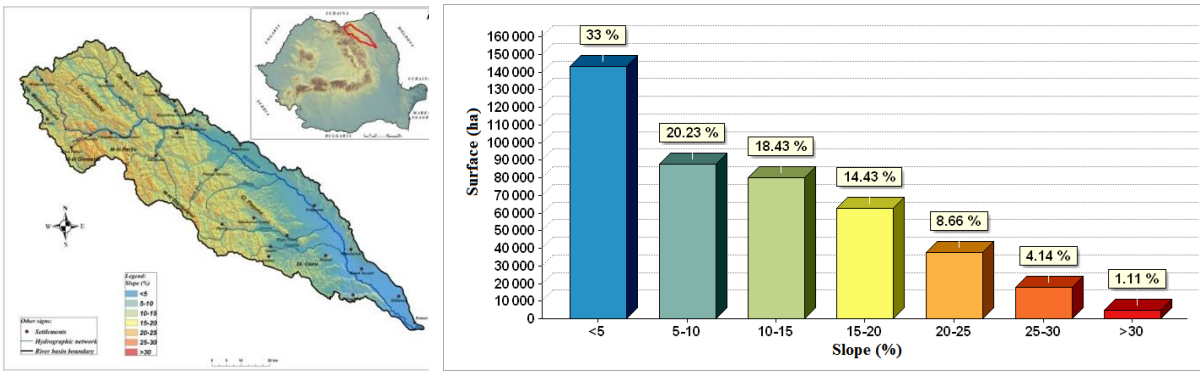

Fig. 3. Map of variations in relative altitude and of the share of slopes

Climate, through solar radiation, thermal and precipitation regime, air humidity and wind speed, is the main genetic factor of flow and flow variations, with great implications in the morphogenesis of the relief. From a climatic point of view, the Moldova river basin is thought to belong to the temperate continental eastern European region, which also presents some transition characteristics from wet, oceanic and sub-Baltic components in the upper course, to more excessive ones in the lower course, which are accompanied by the föehnization of air masses descending on the eastern slopes of the Eastern Carpathians. The NV-SE direction of the Moldova valley and its couloir shape allow air masses to be channelled in this direction and especially in the cold season they facilitate frequent thermal inversions [15].

The relief determines a vertical progression of climatic processes and phenomena so that temperature decreases with altitude. Within the basin, the air temperature increases from 1.99ºC in the upper part to an annual mean temperature of 8.7ºC in its lower part, at the confluence with the Siret river. Given the various locations of the weather stations within the basin, one may rely on the spatial diversity of this parameter. Within the Moldova river basin, the 2.3ºC isotherm is located in the Eastern Carpathians area, the Rarău Mountain, then it increases to 6.5ºC in the Câmpulung-Păltinoasa sector, to 8.2ºC in Fălticeni and Târgu Neamţ and 8.6ºC in Roman (Fig. 4).

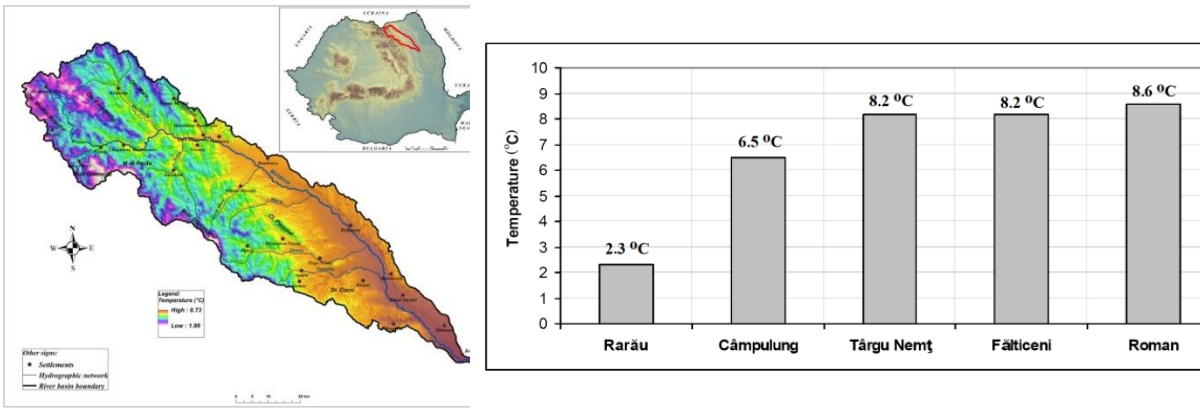

Fig. 4. Map of temperatures and average values recorded at the main weather stations within the Moldova river basin

The amounts of precipitation gradually decrease from the springs (925 mm) to the confluence with the Siret river (514 mm). In Roman, the amount of precipitation decreases considerably compared to the upper part of the basin and approaches values specific to the forest steppe climate (Fig. 5).

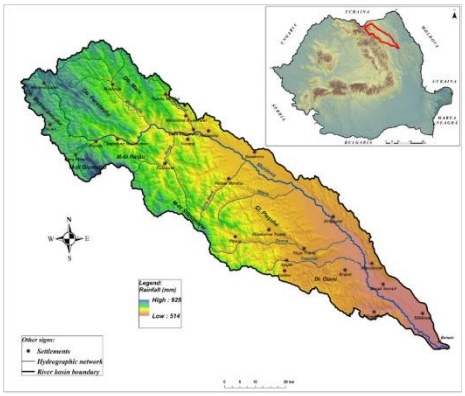

Fig. 5. Map of precipitation distribution

The Moldova river basin being located on different types and forms of relief, presents a complex soil cover. The distribution and characteristics of the pedogenetic factors favoured the appearance of a diverse range of zonal soils, but also the presence of hydromorphic and/or halomorphic intrazonal soils, which results in the mosaic appearance of the soil cover.

The relatively flat land in the Moldova river floodplain and terraces, with many micro-depressions, the presence in the soil profile of the Bt low permeability horizon, the large amounts of precipitation fallen in 1-5 consecutive days and/or the melting of snow led to the occurrence and maintenance of excessive water both on the surface of the soil and in its depth.

In order to regulate the surface and in-depth water regime in the extra-Carpathian soils of the Moldova River basin, surface and subsurface drainage work shave been developed over time on a total area of 8761 ha, 3059 ha of which also included underground drainage works.

Conclusions

Given the rocks, altitude, fragmentation, slopes and obvious altitudinal zoning, the Moldova river basin relief is a very important element on which depends the flow regime.

The altitudinal position is of great importance in terms of water inflows and outflows and alluviums moving in the river basin. The altitude of the relief in the Moldova river basin determines a vertical zoning of different climate conditions. The annual average temperature in the upper part of the river basin is 2ºC, whereas the rainfall amounts are as high as 925 mm, whereas in the lower part the average temperature is 8.7ºC and the annual rainfall mean is 514 mm.

Considering the temporal and spatial variation of rainfall and implicitly of the Moldova River flows, in conjunction with the demand, it is essential to properly estimate the available supply of water for the efficient implementation of all its management and sustainable use tools.

The physico-geographical conditions within the river basin have supported the occurrence and maintenance of excessive moisture, in the soil and on the surface of the land, in the Moldova river floodplain and terraces. The Moldova river floodplain and terraces in the form of strips, which are almost parallel to the river bed, with small slopes, with flat areas and many micro-depressions, facilitate water stagnation. The excess of rainwater and/or groundwater, as well as the excess of water from hydrographic network outflows materialized in different forms and intensities, both on flat and sloping land.

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The Stages in the Introducing the Systematic Cadastre to the Territorial Administrative Unit of Gâdinţi, Neamţ County

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Abstract

The cadastre and the land register are a unitary and mandatory information system for keeping technical, economic and legal records of all immovable properties, regardless of their destination and owner. Such properties can be registered in the land register either by sporadic or systematic cadastre. Currently, the National Cadastre and Land Register Program 2015-2023 is running, the purpose of which is the free registration of immovable property into the integrated cadastre and land register system, the drawing up of the cadastral plan and the opening of land registers at the level of all territorial administrative units. This paper presents the cadastral documentation stages necessary in order to introduce the systematic cadastre in cadastral sector 2, with a surface of 525,942 m^2, located outside the built-up area of Gâdinţi commune, Neamţ county. The systematic registration of immovable property is carried out within a complex process that includes: conducting information and public awareness campaigns for the owners or possessors; identifying TAU and cadastral sector boundaries; identifying the immovable properties; the holders of real rights and their owners; taking cadastral measurements and collecting legal documents; notifying the chamber of public notaries in case of un debated inheritance rights; updating information collected from the field with information from sporadic records; receiving cadastral technical documents; publishing and displaying cadastral technical documents; recording and solving appeals; updating cadastre technical documents; issuing certificates by the notary public for the registration in the land register of the acquirers as owners; closing systematic cadastre works, in order to be registered in the land register; opening new land registers; closing old records; communicating certificates of completion of registration, land register extracts and the extract from the new cadastral plan; archiving documents. One hundred and four real estate were identified within the sector as being positioned according to the documentary evidence of the property, without any errors related to positioning or surface. The registration in the land register was carried out without the financial contribution of the owners, the money acquired from ANCPI's own funds.

Keywords: systematic cadastre, land register, immovable property, owner

Introduction

The inventory and systematic registration of real estate could be considered as one of the largest actions of systematization performed at national level, having significant economic, legal and social implications. The complexity of this process involves the use of working techniques and methods that would generate a high degree of precision and accuracy during each stage, given the fact that the potential errors that could occur for various reasons could exercise negative repercussions on the people and institutions involved [1], [2], [3].

The issue regarding the systematic management of properties and the renting of lands and properties is a characteristic of every developed society, the increase in land value entailing higher quality demands. One of the historical reasons for creating the real estate cadastre was to collect taxes and, naturally, to define property rights over real estate. Currently, the real estate cadastre has an irreplaceable function in market economy [4].

The onset of the Greek financial crisis has directly brought to the fore the lack of integrated digital data on both public and private properties. Thus, three consecutive programs were established in 2010-2012, 2012-2014 and 2015-2018 which prioritized the completion of the Greek cadastre in order to facilitate taxation, to exploit public property, and to develop the economy and investments [5].

Methodology

The Romanian National Cadastre and Land Book Program (PNCCF) 2015-2023 aims at registering real estate into the integrated cadastre and land book system, drawing up the cadastral plan and opening land books at the level of all territorial administrative units, free of charge [6].

The systematic registration of properties into the integrated cadastre and land book system, based on the reality in the land, is done by default and free of charge. This program is financed from the Romanian National Agency for Cadastre and Land Registration (ANCPI)'s own funds, external non-reimbursable funds (EU), or allocations from the budgets of territorial administrative units.

The cadastral works are done at the level of the territorial administrative units (TAU), based on cadastral sectors. Thus, a number of 3,181 TAUs are involved, of which 103 are municipalities, 217 are cities and 2,861 are communes [7], which estimate approximately 40,000,000 real estate.

The territorial administrative unit Gâdinţi, subjected to study, is located in the southeast of Neamţ county, 6 km from Roman municipality, on the left bank of the Siret River, at 46º56'00'', northern latitude and 27º01'00'' eastern longitude (Fig. 1).

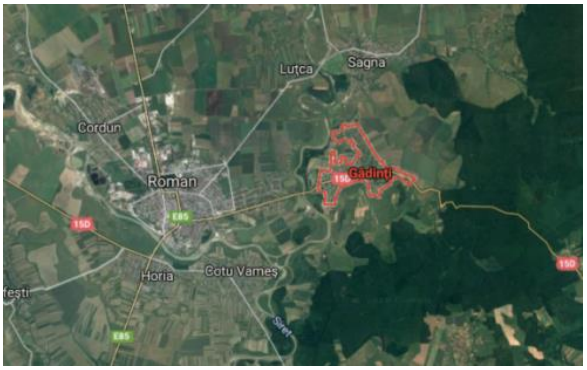

Fig. 1. Location of Gâdinţi commune

The total surface of Gâdinţi territorial administrative unit is of 4,329 ha, of which 1,649 ha are an agricultural area and 2,680 ha a non-agricultural area. The structure of the useful area is the following: 1,274 ha arable land, 241 ha pasture, 89 ha meadow, 13 ha vineyard, 32 ha orchards, 2,284 ha forests, 91 ha water, 70 ha roads, 209 ha yards and buildings and 26 ha non-productive land. The built-up areas of the commune of 396 ha comprises 40 cadastral sectors.

Moreover, the 3,933-ha area of unincorporated area comprise 28 cadastral sectors.

The systematic cadastral works within the cadastral sector no. 2, having an area of 525,942 m2, from the unincorporated area of Gâdinţi TAU were carried out and completed in the 2016-2017 financing phase from ANCPI's own funds, through the Romanian National Cadastre and

Land Book Program. The cadastral sector no. 2, with the category of use of arable land, comprises the land parcels "Coşere" and "Cemetery Plan" (fig. 2).

Fig. 2. Cadastral sector no. 2 of the unincorporated area of Gâdinţi TAU

In order to take the topographic measurements necessary to draw up the systematic cadastre works in the cadastral sector no. 2, the ROVER STONEX S9 GPS was used, while the following programs were used for data processing: AutoCAD Map 2012; TopoLT; Raster Design; CG v3 Generation application; Eterra3 National Program; Microssoft Office 2010 package.

Results and Discussions

For the systematic registration of real estate from the cadastral sector no. 2 and the unincorporated area of Gâdinţi TAU, through PNCCF 2015-2023, the steps provided in the Technical Specifications for carrying out the systematic cadastre works on cadastral sectors in order to register the real estate in the land book financed by the Romanian National Agency for Cadastre and Land Registration, Annex to Order no. 979/05.08.2016 [8] have been taken.

In order to notify the owners and any other holders, respectively, who have properties in the cadastral sector no. 2, about the beginning of the systematic registration works, as well as of the procedure, benefits, rights and obligations entailed during these works, the provider has distributed leaflets to these residents of Gâdinţi TAU and posted 5 posters. The posters were displayed at the Town Hall, the community centre, the church and in two bus stations.

In the pre-preliminary stage of the systematic cadastre works, the provider has analysed analogue and digital data related to the cadastral sector no. 2, in the possession of the Gâdinţi Town Hall and the Neamţ Office for Cadastre and Land Registration (OCPI): the limits of Gâdinţi TAU and of the built-up area; the limits of the cadastral sector; the orthophoto map; plot plans approved by the local commission and received by OCPI; pdf files extracted from the database of property deeds and textual data extracted from the DDAPT platform for the plots of land in the established sector; copies of the land books and of the location and delimitation plans of the real estate taken from sporadic registration. The limit of cadastral sector no. 2 was established based on the built-up/unincorporated area limit, on the orthophoto map and on the topographic plans at a scale of 1:10 000.

The provider identified the locations of the real estate that were not included in the land book with the help of the sporadic cadastre, based on the property deeds and limits indicated by the owners and town hall representatives. The topographic measurements used to determine the cadastral sector no. 2 and the component real estate were taken with the help of ROVER STONEX S9 GPS. Moreover, the data processing and the determination of the flat rectangular coordinates were carried out in the national system STEREO 70.

The holders of real estate in the cadastral sector no. 2 were identified based on the documents presented by the owners and provided by the Gâdinţi Town Hall and OCPI Neamţ. Furthermore,

the provider has collected, in the form of legalized copies, the legal instruments attesting to the property right or other real rights of the real estate holders (property deed, civil judgment, heir certificate, etc.), with the exception of those existing in the archive of Gâdinți Town Hall or OCPI Neamț. In order to maintain a proper evidence and an efficient organization, data files were drawn up of the 104 real estate within the cadastral sector no. 2, which included the names of the owners and/or holders, their identification data, the surface, the number of the cadastral sector, the position of the property in the land plot, the instrument making proof of the property right, etc.

After updating the information collected from the field with those from the sporadic registration, the provider has drawn up the technical documents specific to the systematic cadastre: the real estate cadastral register; the alphabetical index of the holders of real property rights, of the owners and other holders; the overall cadastral plan (Fig. 3); the basic cadastral plan. These documents highlight the state of fact determined on the land and constitute the grounds for the default registration of the real estate into the cadastre and land book integrated system.

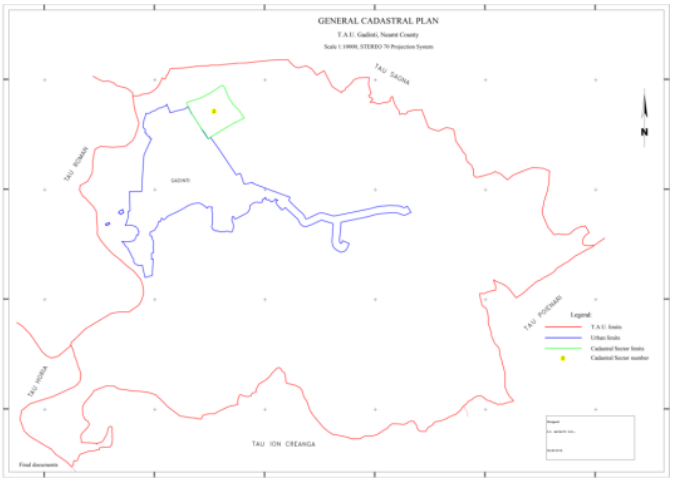

Fig. 3. Overall cadastral map

The technical documents of the cadastral sector no. 2, countersigned by the mayor of Gâdinţi commune, were submitted with OCPI Neamţ in view of reception, respecting the two stages: reception for publication and the final reception. After the technical documents were accepted for publication by OCPI Neamţ, the territorial office together with the Gâdinţi Town Hall established the date of publication, once it was communicated to ANCPI and to the provider.

The previous announcement about the public display of the cadastral technical documents at the Town Hall was published on the official website of the Gâdinţi Town Hall and in the local newspaper. Furthermore, the holders of the real estate were notified within a public meeting with regard to the date and the period when the technical documents were on display and their obligation to check the information related to their real estate in order to submit potential requests for rectification or appeals about their capacity as holders. In the case of the cadastral sector no. 2 from the unincorporated area of Gâdinţi commune, no requests for rectification were made and no sporadic cadastre works were done in parallel, therefore the technical documents were submitted for the final reception.

In the aftermath of the final reception, the systematic cadastral works for cadastral sector no. 2 were closed, and the new land books for the 104 real estate was opened by default (Fig. 4).

The Romanian National Agency for Cadastre and Land Registration has sent to Gâdinţi territorial administrative unit the land book extracts for information and the cadastral plan extracts to be communicated to those concerned.

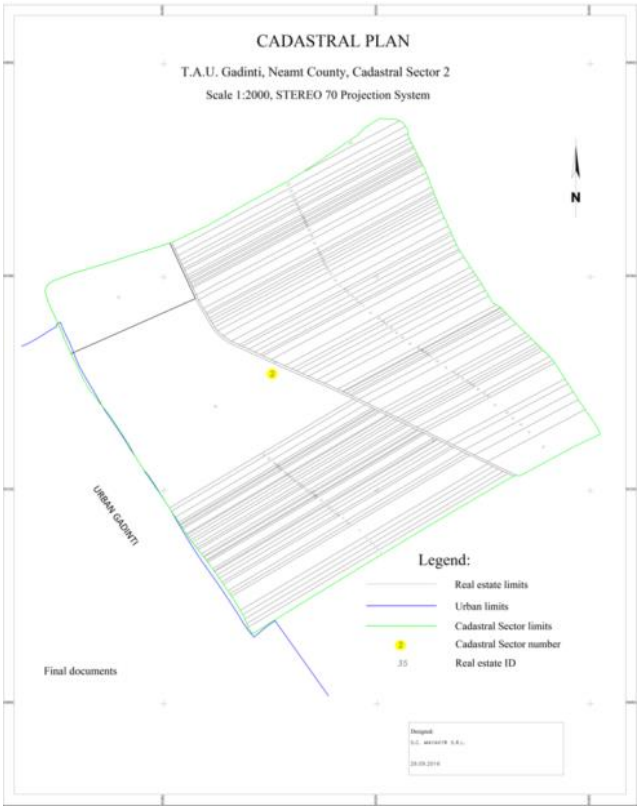

Fig. 4. Basic cadastral plan of Sector 2

Of the 104 real estate delimited within the cadastral sector, 24 real estates with a total surface of 99,887 m^2 are the subject of the estate division and 14 real estates with a surface of 26,505 m^2 are in the possession of Gâdinţi commune. Based on the protocol entered into between ANCPI and the National Union of Notaries Public of Romania, the territorial office notified the chamber of notaries public, in case of undivided estate, in order to distribute the successional cause to the notary public competent to issue the heir certificates as well as, under the law, the certificates for registering the holders as owners in the land book.

Within the cadastral sector no. 2, the largest real estate has a surface of 32,500 m^2 and the smallest 500 m^2, however, there is the possibility of reducing the surface of the real estate following the estate division and severance of the joint tenancy, given the fact that there are up to 10 heirs to a single real estate.

Conclusions

The cadastre and the land book as a unitary and mandatory information system for keeping technical, economic and legal records of real estate facilitates the fair collection of taxes and fees, the development of the mortgage credit market and the increase in the safety of the civil circuit of properties.

The systematic cadastre ensures the inventory of land at an alert pace, and highlights the mistakes, shortcomings as well as the additions of land held with or without documentation.

The cadastral works are more correctly and more accurately done than in the case of the sporadic cadastre, offering a high accuracy of data and a sure location of the real estate.

The free registration of all properties, through the Romanian National Cadastre and Land Book Program, contributes to completing the process of restoring real property at TAU-level, to registering real estate held without documentation (holders) and to issuing heir certificates.

Moreover, it creates the premises of land reparcelling, contributes to the rapid development of infrastructure projects and accelerates economic growth.

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Aspects on the Size Optimization of the Exploited Areas in Some Vegetable Farms

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Abstract

For this paper, the authors prepared a research based on the data of 8 different farms. The 8 farms operate in the vegetable production sector and are located in different areas of Iasi and Suceava counties. The main analysed elements in the research are the areas allocated to each crop for the agricultural year 2018-2019. Starting from these values, the main economic-financial indicators of the companies were analysed, such as turnover, profit and employees' number. Within these farms, the area allocated annually to each crop is established on the basis of crop rotation principles. In addition to the scientific arguments related to the nature of the production, the studied farms also use economic arguments as will be explained in the paper.

The eight studied farms total 22 crops or other types of land use. They sum a total area of 3,589.20 ha, with an average of 448.65 ha per farm. The most important crops from the area allocation point of view are wheat (787.34 ha – 21.94%), corn (668.30 ha – 18.62%), and seeds (577.22 ha – 16.08%). The analyzed farms have a size from 100.00 ha (2,79% of the total) and 1,097.18 ha (30,57% of the total). The farms selected for the research were not necessarily similar in terms of size, in order for the authors to obtain a clearer comparison of the unit's different economic-financial indicators, as it will be seen in the paper.

Keywords: financial competitivity, efficiency, farm optimization

Introduction

The paper presents a series of considerations regarding the optimization of the areas exploited within the primary production vegetable farms. The topic of the agricultural size of the exploitations in Romania and in the European Union represents a particular interest for the specialized institutions for several reasons.

There are numerous strategies, policies and tools for improving working conditions in all types of farms, especially those of subsistence and semi-subsistence. Based on the statistics compiled by the relevant institutions of the Member States, the decision makers issue Recommendations which are transposed into the national legislation of each state.

Alternatively, a number of instruments, in particular financial ones, are being promoted to improve the current discrepancies between Member States. All these initiatives are implemented on the principles of the Common Agricultural Policy.

The Common Agricultural Policy is the agricultural policy of the European Union which aims on the one hand at the sustainable growth of agricultural productivity in order to ensure food security for Union citizens and on the other hand at ensuring a reasonable standard of living for agricultural producers and rural residents [1].

All these initiatives are based on a series of findings, among which the most important are: a. in 2016, there were 10.5 million agricultural units in the European Union, and of these, two thirds had an area of less than 5 Ha; b. in 2016, agricultural units in the territory of the E.U. exploited 173 million hectares of agricultural land of production, this area representing 39% of the total area of the E.U.; c.

The number of agricultural exploitations is in a sharp decline, while de exploited area has remained constant [2].

In the same year, one third, respectively 32.7% of agricultural units in the territory of the U.E. were in Romania. This share is a very high one, by far the highest of all the E.U. 28 states.

At the same time, the number of agricultural units is comparable to those of Poland (13.5% of units), Italy (10.9% of units) and Spain (9% of units) combined, these three states being the next in terms of share, after Romania [2].

The Romanian rural area has numerous shortcomings, these representing also the reason of disparities between urban and rural areas with all their components: rural economy, demographic potential, health, school, culture, etc. [3].

Since the date of the Romania's accession to the E.U., a number of functional financial mechanisms and instruments within the E.U. have been made available to Romania. They operate on the basis of the rules of the Common Agricultural Policy and aim to reduce disparities regarding the size of the farms.

These instruments include: 6.1 Branch from E.A.F.R.D. – Young farmers, and is meant to decrease the average age of the employed population in agriculture [4]; 6.5 Branch – scheme for small farmers, aims to increase the average area of agricultural units at national level by assigning, in exchange for a non-reimbursable support, the areas of small agricultural units in favour of larger farms, and others.

Methodology

The necessary studies for the elaboration of this paper have been carried out during the years 2018-2019, using as sources of information especially the primary data. In this regard, a series of interviews were carried out at the headquarters of the agricultural unit studied, in the counties of Iasi and Suceava.

The documents of the unit regarding the evolution of the areas used for crops in the agricultural year 2018-2019, the financial accounting documents regarding the evolution of the economic indicators, the evolution of the technical capacity through the investments made and other aspects were analysed.

On the other hand, data from secondary sources, respectively the specific literature in Romania and abroad was used, including the statistical directories available at national level.

Both the data obtained in the analysis of the documents of the agricultural units as well as those of the specialized literature were processed and interpreted in order to highlight the size of each unit analysed separately.

Results and Discussions

The authors carried out an analysis regarding the main economic efficiency indicators on a number of 8 farms.

The 8 agricultural units have areas with a total size between 100.00 ha and 1,097.18 ha, the average area exploitation of the 8 units is 448.65 ha.

By the interviews conducted with the farmers regarding their intentions to optimize the exploited areas, the main ideas are the following:

- The agricultural units analysed are part of the category of large, industrialized and performing farms.
- The management within the interviewed farms have as permanent goal the extension of the exploited areas, by acquiring land or leasing it from the owners;
- The development of the areas exploited by acquisition of land has an insignificant contribution. This is due to several factors, the most important being: 1. The population in rural areas, owners of agricultural land are mostly old and has a conservative mentality, refusing to sale the land areas, even for competitive prices. 2. The small share of the population from the rural area willing to sell the agricultural land has difficulties due to bureaucratic reasons. Specifically, the vast majority of owners do not have updated documents regarding the land, as they do not carry on the inheritance debate due to ignorance or displacement. For these main reasons, the situations in which the private agricultural units complete a transaction regarding the acquisition of land area are very seldom.
- The increase of the areas exploited by the lease from the landowners has a higher share. Thus, the land owners are rather willing to offer the land for lease rather than to sell it permanently to the industrial farmers.
- Regarding the increase of the exploited area through the lease of land, from the research carried out and the discussions with all the 8 farmers, the following were found: 1. There is competition between the agricultural units that lease arable areas from the owners, meaning that they offer higher and higher amounts per hectare of land or equivalent in agricultural products; 2. Because of the modernization of irrigation infrastructure in recent years, due to farmers with initiative many assets and large areas, the landowners from the perimeter of the modernized irrigation infrastructures demand, without any of their contribution, increasing leasing values. They motivate these increases on the land productivity increase in the irrigated areas, an advantage due to the farmers, and not to the landowners.

By analysing the APIA area centralizations for the agricultural year 2019-2020, we could centralize the areas of all the 8 studied agricultural units. It is thus noted that agricultural units use the land for a very wide variety, totalling 20 agricultural crops and other forms of land use.

Due to the positioning of the 8 studied units in different regions of Iași and Suceava counties, the specific pedoclimatic conditions do not allow for all a very large variety of the cultivated crops. Tab. 1 presents the share of the 20 crops areas grown by the 8 agricultural units analysed.

The table shows both the size of the areas expressed in ha and the share of each crop in the total area, respectively of each of the 8 analysed units.

It can be seen that the most important share has the crops of corn, wheat and sunflower.

Also, an important weight has the area allocated for seed lots, of 16.08% of the total area analysed.

Seed lots have benefited, especially in recent years, from increasingly allocated areas.

The cultivation of seed lots is an activity with a very high economic efficiency. It requires special precision in the soil and crop works, sufficient and high-performance machinery, and a very precise timing in terms of start and finish of the crop works.

Instead, the prices offered for the production are significantly higher than for conventional products.

In addition, prices are generally agreed before the establishment of the crop, based on a contract, so that the farmer has a very accurate forecast of the financial flow of the agricultural unit.

Table 1. Area centralization of the 20 crops of the 8 analysed agricultural units – ha and percent

Crop name	Crop share %	Total area – ha
Corn	18.62	668.30
Wheat	21.94	787.34
Sunflower	10.10	362.49
Rape	8.19	293.78
Soybean	6.16	221.22
Seed lots	16.08	577.22
Beekeeping	0.72	25.75
Alfalfa	3.47	124.96
Feed crops	0.10	3.70
Permanent lawns	2.07	74.31
Temporary lawns	0.49	17.47
Barley	6.15	220.64
Oat	0.23	8.35
Potatoes	1.78	63.95
Meadows	2.54	91.05
Nurseries	0.06	1.98
Vineyards	0.33	11.70
Triticale	0.22	7.77
Chickpeas	0.30	10.75
Green peas	0.46	16.47
TOTAL	**100.00**	**3,589.20**

Customers of these products are, generally, multinational companies. They negotiate the seed production with the large and efficient farms. They aim for these farms to own, at least partially, irrigated crops, modern and sufficient technical resources and other assets and key features. Fig. 1 shows the main categories of the land use for all the eight analysed farms. As can be seen, the most important areas are allocated to cereal crops, followed by oil crops and seed lots. Another analysis undertaken by the authors was a correlation between the exploited area together with the number of employees, on the one hand and the turnover of the unit together with the profit, on the other. Thus, Tab. 2 was developed, presented in the following.

Despite the fact that such an analysis is more relevant when several agricultural/calendar years are subjected to analysis, even a one-year situation, 2018, reveals a series of data.

It can be observed, for example, that the highest rate of profit per employee is registered within the units with a number of 7-9 employees of the eight studied units.

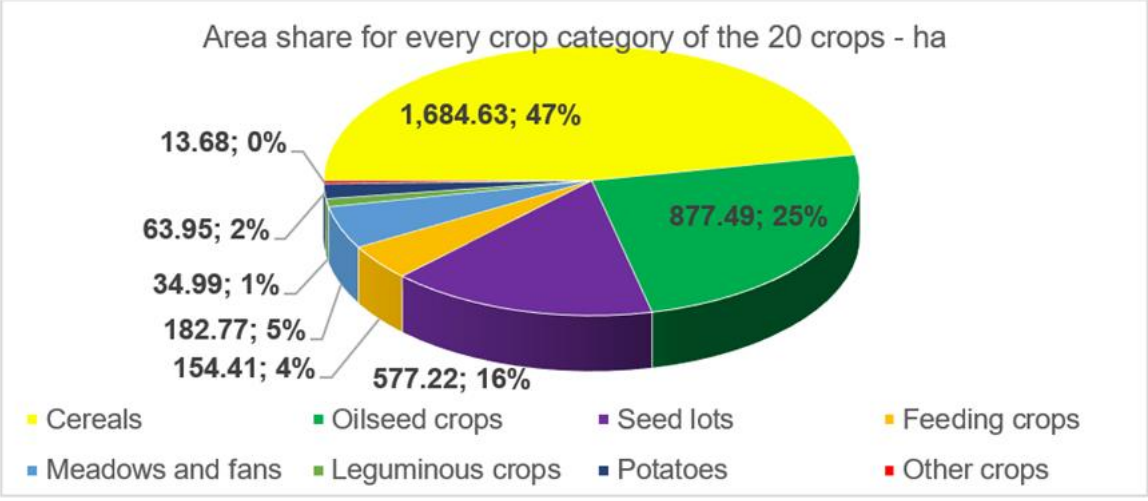

Fig. 1. Area share for every crop category of the 20 crops of all the 8 producers – ha and percent

Also, it can be noted that the highest turnover per ha is registered in the case of units with larger areas, of minimum 400 ha, up to 1,097 ha.

Table 2. Analysis of financial indicators in correlation with the exploited area (ha) and number of employees, in 2018

Criterion	1st unit	2nd unit	3rd unit	4th unit	5th unit	6th unit	7th unit	8th unit
Exploited area 2019 – ha [5]	1,097.02	150.98	446.24	171.43	100.00	286.30	351.01	986.22
Employees number [6]	21	2	9	2	3	9	1	7
Turnover – lei [6], [7]	5,719,801	385,520	3,124,818	425,719	780,544	1,430,054	819,018	3,556,616
Profit – lei [6]	255,239	42,590	292,635	44,625	68,875	544,786	135,349	967,565
Turnover per employee	272,371	192,760	347,202	212,860	260,181	158,895	819,018	508,088
Turnover per used ha – lei	5,214	2,553	7,003	2,483	7,805	4,995	2,333	3,606
Profit per employee – lei	12,154	21,295	32,515	22,313	22,958	60,532	135,349	138,224
Profit per used ha – lei	233	282	656	260	689	1,903	386	981

The most relevant information is given by the turnover and the number of employees that is in close correlation with the allocated area. It can be seen as, as expected, the turnover increases progressively with the number of exploited ha.

A number of other relevant information could be observed by studying the internal documents of the companies. For example, a high degree of profitability could be observed in terms of seed lot crops and maize crop, which justifies the allocation of large areas of these crops.

Conclusions

- The eight agricultural units on the basis of which the analysis was carried out are growing a total number of 20 types of crops, on a total area of over 3,000 ha;
- A permanent concern of the management of the unit is to expand the exploited area, either through land acquisition or land lease;
- From the research carried out, it seems that the acquisition of land has a negligible contribution on the development of the exploited areas;
- The main modality of area development is currently the lease, but in this situation the agricultural units face some other certain problems;
- The emergence on the market of the opportunities regarding the cultivation of seed lots raised the rent value perceived by the landowners because of the competing agricultural units high demand;
- The development of the irrigation system is a major advantage for all industrial producers, but it also implies the increase of the value of the rent perceived by the owners who own land areas in the irrigated perimeters;

- ❖ Starting from the number of employees, correlated with the exploited area, it was found that within the 8 units studied the profit per employee has the highest rate at the units that have 7-9 employees.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018 within PNCDI III.

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The Packaging Design as a Marketing Strategy: A Case Study on a Local Tea Producer

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Abstract

In recent years, packaging design has been increasingly perceived as an important branding and rebranding element and as an important tool in a brand's marketing strategy.

The present paper analyses how packaging design is part of a local agricultural producer's marketing strategy and how it can help change the target audience's perception of the agricultural product and the brand itself.

The study was carried out through a comparative analysis between the old packaging of a series of agricultural products existing on the market for several years and the new packaging design, part of the manufacturer's rebranding. The reasons for the change and the main meanings behind it were also studied, together with the target audience' perceptions, attitudes and purchase decision regarding the old vs. new package.

The analysis was carried out within the "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iași, the agricultural producer being the Research Center for Medicinal and Aromatic Plants within the Faculty of Agriculture.

The market research data conducted among 160 people located in Iasi, Romania, revealed that the new packaging design succeeded to communicate a more actual brand image and better-quality perceptions, through a modern and complex packaging with multiple functionalities.

Keywords: Packaging design, agricultural marketing, marketing strategy, agricultural product, tea

Introduction

Marketing, through the complexity of its aggregate phenomena, has as main objective the study of the product, price, placement and promotion and the way in which the interdependence of these factors contributes to the success of a product or service in the current economy [1].

Being considered an integral part of the product-environment system, the packaging should perform a series of functions, such as physical and barrier protection – product preservation, handling, storage and transport, marketing – informing the consumers and promoting the product as a "silent seller" [5].

In the rebranding strategy a variety of aspects regarding the packaging are considered, such as: the ability to transmit the desired message and to reposition the brand in a few seconds in the viewer's mind correctly – depending on the desired message to be transmitted, the packaging must have a pleasant appearance, the graphic design must support and strengthen the other elements of identity, be easily noticed amongst a variety of products, to be functional (to make the product easy to use and manipulate, to be easy to open, to resist pressure, not to increase the volume of the product very much etc.) [3].

The packaging design has the function of communicating certain product information, transmitting certain ideas and perceptions that help to form an opinion regarding the positioning

of the product from several points of view: price segment (premium product/low-cost product), characteristics (composition, quality), targeted niche of consumers, advantages of consuming the product [2].

Methodology

The studies necessary for the elaboration of the present paper were carried out during 2019, using mainly primary data gathered by means of a market research. Using a quantitative data research method – a market survey carried out on 160 people located in Iasi County, Romania, a comparison analysis between the existing package design of an agricultural product, used in the last couple of years, and the new package design was performed. Items such as attitudes toward packages, expected product quality, and purchase intention were measured. Categorical items captured respondents' gender, age, household income. Also, secondary data sources were used such as statistical analyses, papers and books in the field of packaging design, marketing, and consumer buying behaviour.

Results and Discussions

The present study was conducted within the „Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iași and aimed at the implementation of a gradual rebranding process for the teas produced by the Research Center for Medicinal and Aromatic plants, Faculty of Agriculture. Since 1977, research activities have been carried out within this center in the interest of production teas for therapeutic purposes, these being marketed to the general public in the form of 37 assortments, adjuvants in more than 200 affections, such as: diabetes, obesity, cardiovascular, lung, liver and kidney disorders etc.

Starting with 2019, it was desired to develop and implement a rebranding strategy with the objective of repositioning of the existing teas on the profile market and increasing sales by attracting more categories of audience. In the development process of a new packaging design were considered some aspects aimed to be communicated about the produced teas:

- the teas assortments are 100% natural, containing plants carefully selected from spontaneous flora by the "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iași specialists;
- depending on their composition, the teas assortments are adjuvants in different conditions;
- the packaging contains high quality products;

In order to occupy limited storage space, the new package is compact, volume-packed, easy to store, handle and distribute. Therefore, in comparison with the old packaging, the new one presents a series of changes regarding functionality, design and used materials (Fig. 1).

The new tea package was made of waterproof materials, with a high degree of protection against moisture, air and sunlight, the opening system has a resealing option, with watertight materials which do not allow the degradation of plants through contact with external factors during storage and use (Fig. 1).

In order to differentiate the product from other tea packages in online and offline stores, it was desired to choose vivid colours that attract attention and are not usually used in this area.

Thus, purple tones were chosen for the colour of the package, a shade rarely encountered in this area where colours such as green and cream predominate (Fig. 1).

Fig. 1. The initial tea package (left) vs. the new tea package (right)

The new package includes also a transparent window, shaped as a stylized leaf inspired by nature which allows the content of the package to be observed and helps the consumer to form an opinion on the quality of the contained plants and composition, which can represent a competitive advantage when analysing the product before purchase (Fig. 2).

Fig. 2. The new tea packages (different assortments)

To convey the idea that the studied teas are adjuvants in various diseases, with remedies offered by nature, the labels were made through graphic illustration technique, the central element being a metaphorical character "*Mother nature*" which offers healthful plants targeting various internal organs (Fig. 3).

Fig. 3. Elements of design illustration for the new tea packages

This idea has been graphically transposed through illustrations of the character in various situations, with emphasis on certain key organs, depending on the remedy and the adjuvant role of the product (Fig. 3). The packaging label has two parts, one for each side of the package and include important information, such as the product name, composition, manufacturer, method of use and other data according to the regulations in force regarding the packaging of herbs and teas (Fig. 1). Also, representations of the plants in the composition of each assortment were illustrated, such as: basil, lavender, mint etc. The new packaging has been created with the purpose of offering a higher protection to the product contained, increasing the functionality during the consumption, but also a for generating positive perceptions and expectations.

Through the new package it was aimed at communicating three main messages about the product: the product has a superior quality and is natural; the packaging is functional. In order to determine the consumers' perceptions regarding the new packaging design, a quantitative market research was conducted among 160 respondents aged between 18 and 82 years, women and men living in Iasi County, Romania, in which attitudes, perceived quality, associated price level and preference for the initial vs. new package were analysed comparatively. The respondents answered a questionnaire consisting of 13 questions regarding the two packages.

In order to analyse the first perception created by the two packages, the respondents were asked to answer freely on two open-ended questions regarding the first impression the two packages generated them. No choices were offered, and most of the answers regarding the new package (Question 1) were represented by positive perceptions, respondents using terms such as: interesting, modern, attractive, elegant, beautiful, beautiful colour, resistant, sophisticated for describing the new package. At the same question, this time regarding the initial package (Question 2) most of the answers referred to negative perceptions, such as: outdated, classic, poverty, pessimistic, not resistant. Question 3 and Question 4 aimed at discovering the attitudes towards the two packages in comparison and the purchase intention. 79% of the respondents answered that they like more the new package while 81% answered that they would buy the new package. Attitudes toward the new package and the initial one were analysed with the help of Question 5 and 6. Using a 4 point likert-type scale questions anchored between "I don't like it at all" (1) and "I like it very much" (4), respondents opined regarding some design aspects, like: colour, shape, label, general look & feel, material and functionality (Fig. 4 & Fig. 5).

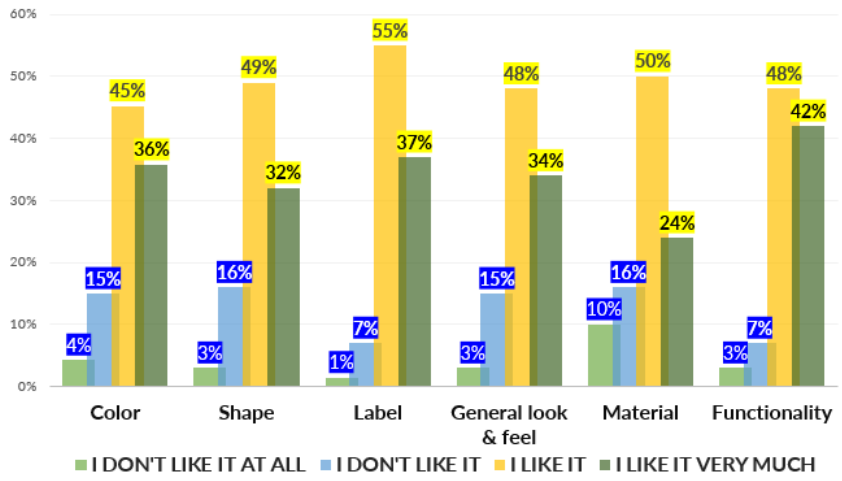
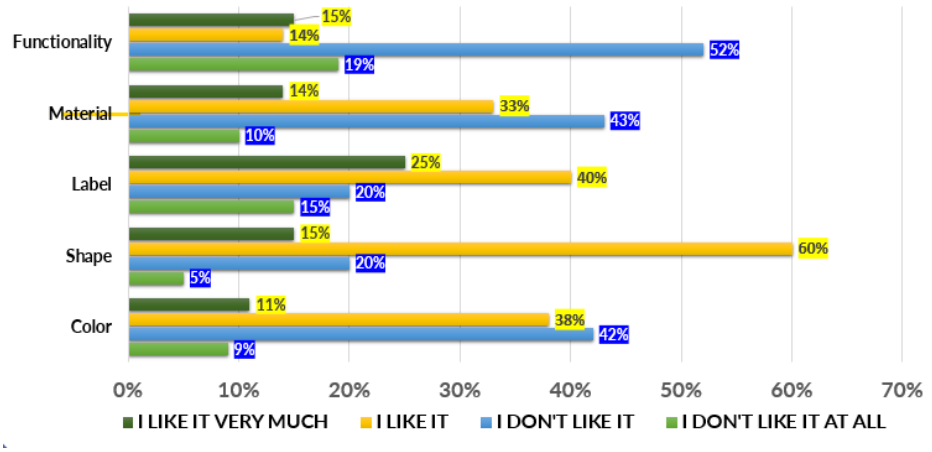


Fig. 4. Answers for Question 5: Attitudes towards the new package design

Using a 4 point likert-type scale questions anchored between "I don't like it at all" (1) and "I like it very much" (4), respondents opined regarding some design aspects, like: colour, shape, label, general look & feel, material and functionality (Fig. 4 & Fig. 5).



As concerns the attitudes regarding the new package, there were observed very positive attitudes regarding the functionality (42% of respondents marking "I like it very much" and 48% "I like it"), the label, colour and general look& feel being also very appreciated. In comparison, the same question was asked regarding the initial package. The attitudes were dissimilar, only the shape being appreciated, while the functionality and colour were the aspects with the most negative attitudes (Fig. 5). As regards a series of important perceptions about the two compared packages, through Questions 7 and 8 it was noticed that the new package is considered easy to use and functional (99%), durable and protective (98%). Judging by the new package, respondents perceived the product as natural (98%) and very qualitative (95%). The only aspect that rises problems is the fact that the new package transmits the idea of expensive product (55%) while only 5% of respondents considered the same about the initial package.

One of the most important parts of the study is represented by the revelation of the most important factors which influence tea purchase in Iasi County, Romania. The answers regarding these aspects will contribute in creating and implementing efficient marketing strategies focused on the most important factors. Respondents were asked to reveal how price, packaging, quality, composition, naturalness and origin determine their purchase decision (Fig. 6).

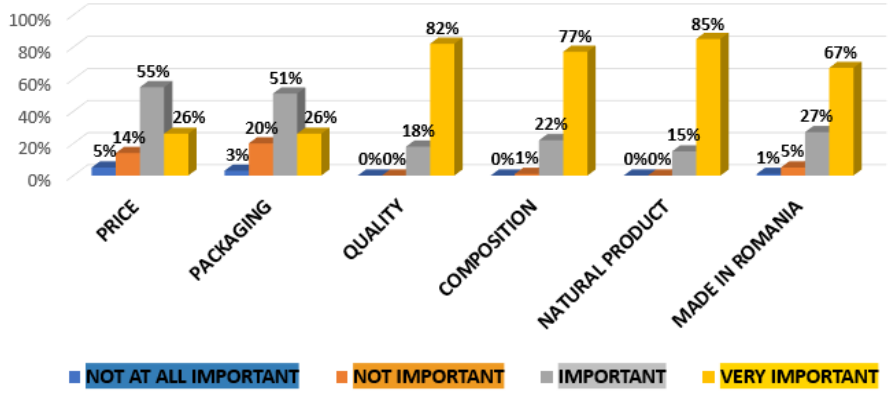


Fig. 6. Answers for Question 9: most important factors which influence tea purchase

The figures show that the most influential factors are, in order: the quality, the degree of naturalness, the composition and the fact that the products is local, made in Romania (Fig. 6).

The packaging is also influential (78%). However, considering the fact that the package is a mirror of quality and naturalness, as it was demonstrated with the help of the previous questions, therefore, as long as it reflects other important perceptions, it can be considered one of the most influential factors which determine the purchase decision. The final questions of the study

revealed interesting correlations between the income and the price as an influential factor – even 66% of respondents have low incomes, the price is not a factor as important as expected in their buying decision.

Conclusions

The development of self-service requires the package to promote the product on the shelves by attracting the consumers' attention and creating positive associations and expectations regarding the contained product. The package must have the ability to transmit the desired messages and to correctly position the brand in a few seconds in the viewer's mind.

The current research findings show that packaging elements such as graphics, colour, label design and functionality are seen important by most of the participants – they impact whether shoppers will notice the package, how consumers interpret the package information and whether they will purchase the product or not. The study revealed that a series of important aspects are communicated through package, such as: the expected quality, the degree of naturalness and the company policies regarding customer care, as long as a functional and easy to use package improves the experience with the product and the brand itself. The packaging design is the component that provides an advantage for the products in a competitive environment, being the first brand component, which establishes a connection with the consumer, through a direct contact with the product. Therefore, when it comes to natural, agri-food products, the package influence is notable as long as in the process of consumption, the first step is opening/reopening the package, thus, the consumer coming repeatedly into direct contact with the package and being influenced by this experience at many important levels.

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Evaluation of the Discharge Coefficient of Diesel Nozzles when Using Biodiesel Fuels

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Abstract

The paper presents the comparative results regarding the discharge coefficient of the injection nozzle of an agricultural when using diesel fuel and biodiesel blend fuel. The material used for the production of vegetable oil methyl ester was waste cooking oil collected from a local branch of the McDonalds' restaurants.

The discharge coefficient was calculated for the injector mounted into the injection rate meter, based on the measured cyclic fuel delivery.

The experimental results showed that lower discharge coefficients and lower Reynolds numbers of the fuel flow were recorded when the biodiesel type fuel was used: for the diesel fuel, the discharge coefficient was comprised between 0.67 and 0.8, while the values of the discharge coefficient were comprised between 0.62 and 0.69 when the biodiesel type fuel was used. The values of the Reynolds number for the diesel fuel were comprised between $18 \cdot 10^3$ and $27 \cdot 10^3$; for the biodiesel blend, the Reynolds number achieved values of $16 \cdot 10^3$ …$23 \cdot 10^3$.

Keywords: Discharge coefficient, biodiesel, diesel engine

Introduction

The fuel injection system of a compression ignition engine has a major effect over the fuel consumption and emissions performances. One of the most important characteristics of an injector nozzle is the discharge coefficient, defined as the ration between the actual fuel flow passing through the orifice and the flow that could theoretically pass through the same orifice, at the same pressure drop, considering the flow frictionless and without any contraction or phase changes [1].

The discharge coefficient depends on the nozzle geometry, the characteristics of flow and fluid properties. Several authors [2] show that the discharge coefficient has a significant effect over the emissions of the diesel engine, high values of the discharge parameters leading to their reduction. Caprotti R. *et al*., [2] also noticed that particulate emissions achieve higher values for the nozzle having a lower discharge coefficient.

Important operating disadvantages of biodiesel in comparison with petrodiesel (cold start problems, lower energy content, fuel pumping difficulty due to higher viscosity) [3] imposed the use of biodiesel-diesel fuel mixtures in order to fuel compression ignition engines; it is agreed that a proportion of 2…5% methyl ester in diesel fuel does not affect the engine's performance and efficiency and does not involve any changes in the construction of the engine's fuelling system. This type of biodiesel blends can be burned directly in unmodified diesel engines [4].

Cooking oils, used for frying food, have a limited life in food production due to their contamination with material from food and due to fatty acids formation; waste cooking oil can

be seen as a "near to waste" by-product of food production industry. As a result, the use of waste cooking oil instead of virgin oil in order to produce biodiesel is an effective way to reduce the raw material cost and helps to solve the problem of waste oil disposal. These vegetable oils contain some degradation products of vegetable oils and foreign material. However, analyses of used vegetable oils claimed that the differences between used and unused fats are not very great and, in most cases, simple heating and removal by filtration of solid particles makes the oil appropriate for subsequent transesterification [5, 6].

Methodology

Because blends containing only small amounts of biodiesel are less susceptible to lead to a significant reduction of the overall consumption of diesel fuel, in this study blends containing large amounts of biodiesel (B50: 50% biodiesel+ 50% diesel oil) were considered for testing of the injection equipment; waste vegetable oil was used in order to produce the methyl ester.

The material used for the production of the vegetable oil methyl ester was waste cooking oil collected from a local branch of the McDonalds' restaurants. The base catalysed method was used for producing the methyl ester. Some physical properties of the tested fuels are shown in Table 1.

Table 1. Physical characteristics of the fuels

Item	Diesel fuel	Waste cooking oil	B100[a]	B50[b]
Density at 15°C [kg/m^3]	839.3	891	857	851
Dynamic viscosity at 40°C [x10^{-2} Pa·s]	0.411	3.029	0.488	0.442
Kinematic viscosity at 40°C [x10^{-6} m^2/s]	4.9	34.0	5.7	5.2
Acid value [mg KOH/g]	0.089	2.67	0.92	0.42
Ash content [%]	0.085	0.075	0.038	0.016
Flash point [°C]	69	115	110	82
Cu strip corrosion	1b	2e	2a	1b
Surface tension [N/m]	0.0281	0.0336	0.0296	0.0290

[a]B100 – pure methyl ester; [b]B50 – 50% methylester+50% Diesel fuel

The tested injection equipment is the one used for the D-110 direct injection diesel engine:

- RO-PES4A90D410RS2240 type A in-line injection pump;
- RO-KBL103S15 injectors, opening at 17.7 MPa;
- RO-DLLA 150S720 nozzles, with four 0.275 mm discharge orifices.

The injection equipment was tested on a MIRKOZ (Hungary) test rig, using a BOSCH injection rate meter [7]. The tests were developed at different pump speeds (500, 700 and 900 rev/min) and displacements of the injection pump control rack (1, 2, 3, 4, 5, 6 and 7 mm) on a fuel injection test rig [8].

The fuel mass flow was calculated based on the cyclic fuel delivery and injection duration.

The following relation was used in order to calculate the discharge coefficient [9, 10]:

$$c_d = \frac{\dot{m}_{actual}}{A_0\sqrt{2\rho_l \Delta p}} \qquad (1)$$

where $\dot{m}_{actual}$ is the measured fuel mass flow through the orifice of the injector nozzle, A_0 is the geometrical outlet area of the orifice, ρ_l is the fuel density and Δp is the pressure drop across the orifice; the backpressure of the injection rate meter p_b was set to 3 MPa. Because the pressure at the nozzle orifice is difficult to measure, the injection pressure p_{inj} was taken into account in order to calculate the pressure drop [11].

The Reynolds number at the nozzle outlet was also calculated using the equation [9]:

$$Re = \frac{D_0 \cdot \dot{m}_{actual}}{\eta \cdot A_0} \quad (2)$$

where D_0 is the diameter of the nozzle outlet orifice and η is dynamic viscosity of the fuel.

In all the calculations the maximum mass fuel flow and maximum injection pressure were considered, in order to avoid the transient phenomena related to the opening and the closing phases of the injector [12].

Results and Discussion

Table 2 summarizes some of the experimental results concerning the mass fuel flow through the orifice of the injector nozzle; Table 3 presents the results concerning the Reynolds number.

Table 2. Mass fuel flow [g/s]

Position of the control rack	Diesel fuel			B50 fuel		
	500 rev/min	700 rev/min	900 rev/min	500 rev/min	700 rev/min	900 rev/min
2	9.18	10.68	10.84	8.43	9.73	11.12
4	9.55	11.23	11.47	8.74	10.83	11.24
6	10.20	11.52	12.47	9.42	11.65	12.03

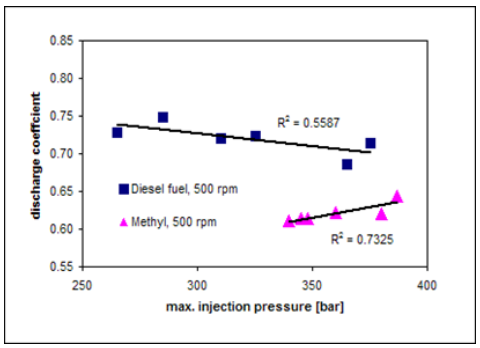
Table 3. Reynolds number

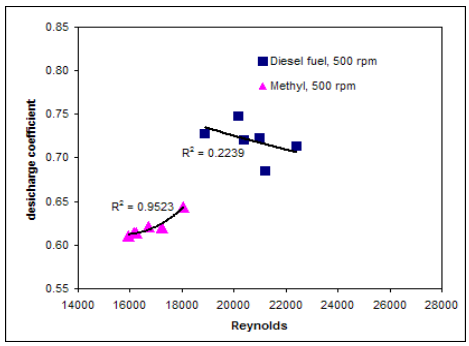
Position of the control rack	Diesel fuel			B50 fuel		
	500 rev/min	700 rev/min	900 rev/min	500 rev/min	700 rev/min	900 rev/min
2	20162	23447	23810	16148	18629	21289
4	20968	24664	25188	16734	20727	21517
6	22400	25303	27639	18046	22300	23022

The results presented in Table 2 show that fuel flow increased with the pump speed and position of the control rack, as expected. In the meantime, lower values of the maximum fuel flow were achieved when the B50 fuel blend was used, compared to diesel fuel.

As far as the Reynolds number is concerned, the results presented in Table 3 show that lower values were recorded for the B50 fuel, due to the higher viscosity and lower mass fuel flow.

Fig. 1 presents the effect of the maximum injection pressure over the discharge coefficient, for the two types of fuels. The use of the biodiesel type fuel led to the achievement of higher injection pressures, due to its higher viscosity [11]; in the meantime, higher values of the discharge coefficient were recorded for the diesel fuel than for the biodiesel blend, for all the tested regimes. Lower values of the discharge coefficient when biodiesel fuels were used were also reported by other authors [11].





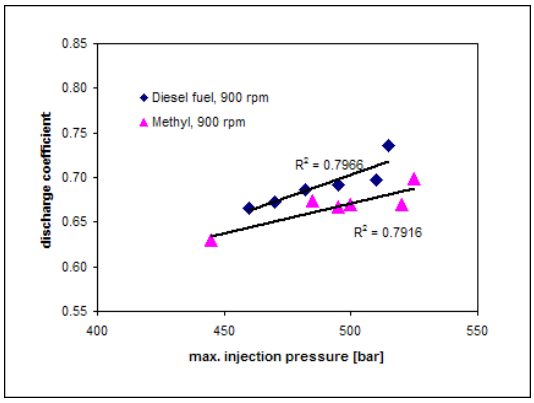


Fig. 1. Effect of injection pressure on the discharge coefficient

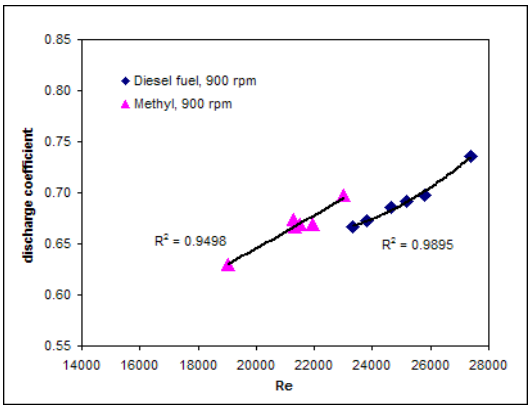


Fig. 2. Discharge coefficient vs. Reynolds number

It is interesting to notice that, for the biodiesel fuel, the discharge coefficient increases with the injection pressure and this result is in accordance with the findings of other authors [12], while for the diesel fuel, at 500 rev/min and 700 rev/min, the discharge coefficient decreases when the injection pressure increases, while at 900 rev/min, the discharge coefficient increases with the injection pressure. This behaviour could be related to the lower injection pressures recorded for the diesel fuel, compared to biodiesel, especially at lower pump speeds. The decrease of the discharge coefficient was also remarked by other authors [13], but in this case it was attributed to non-conformity in the construction of the nozzle.

Fig. 2 presents the relationship between the value of the Reynolds number and the discharge coefficient. The results show that higher Reynolds numbers were recorded for the diesel fuel than for the biodiesel fuel (as presented in *table 3*). These charts also show that, for the biodiesel type fuel, the discharge coefficient has a clear increasing tendency with the value of the Reynolds number; when diesel fuel was used, at lower pump speeds (500 and 700 rev/min), the discharge coefficient decreased when the Reynolds number increased. According to Payri R. *et al.*, [9], for the cylindrical nozzle orifices, the decrease of the discharge coefficient from a certain Reynolds number value indicates the onset of cavitation; further researches should be performed in order to establish why cavitation occurred at lower pump speeds and not at 900 rev/min, but the correspondingly lower values of cyclic fuel delivery and mass flow (at 500 and 700 rev/min) could be an explanation. The higher viscosity (inducing greater nozzle wall friction) and the higher values of vapor pressure of the biodiesel type fuel could be the cause of the different cavitation flow pattern [14].

Simulations and tests performed by other authors [10, 15]; Ishak M.H.H. *et al.*, [15] confirmed the increase of the discharge coefficient with the injection pressure and Reynolds number. However, it should be noted that these authors have used injection pressures up to 220 MPa and 180 MPa, respectively; the Reynolds numbers of the flow have reached values up to 33000 [10]. The above-mentioned authors present mathematical equations for the discharge coefficient, based on the Reynolds number of the flow.

In our tests, which were performed at lower injection pressures and Reynolds numbers due to the nature of the injection system, the results were consistent with these types of equations only at 900 rev/min, for both diesel fuel and biodiesel type fuel; the results are summarized in Table 4. At lower pump speeds (corresponding to lower injection pressures and Reynolds numbers) the equations were significantly different for diesel fuel and B50.

Table 4. Equations for the discharge coefficient, at 900 rev/min

Fuel type	Equations	
Diesel	$c_d = 7.644 + \frac{2.075 \cdot 10^3}{\sqrt{Re}} + \frac{1.541 \cdot 10^5}{Re}$	$c_d = 2.738 - \frac{92.49 \cdot 10^4}{Re} + \frac{1.031 \cdot 10^9}{Re^2}$
B50	$c_d = 2.739 + \frac{0.502 \cdot 10^3}{\sqrt{Re}} + \frac{0.291 \cdot 10^5}{Re}$	$c_d = 1.434 - \frac{2.512 \cdot 10^4}{Re} + \frac{0.187 \cdot 10^9}{Re^2}$

Conclusions

The experimental results showed that lower discharge coefficients and lower Reynolds numbers of the fuel flow were recorded when the biodiesel type fuel was used: for the diesel fuel, the discharge coefficient was comprised between 0.67 and 0.8, while the values of the discharge coefficient were comprised between 0.62 and 0.69 when the biodiesel type fuel was used.

When diesel fuel was used, the discharge coefficient decreased with the increase of the Reynolds number for low and medium speeds of the injection pump. When biodiesel was used, the discharge coefficient increased continuously with the increase of Reynolds number.

At high pump speeds (900 rev/min) the results regarding the dependence between the discharge coefficient and Reynolds number are consistent with the findings of other authors, indicating lower discharge coefficients for the biodiesel type fuel.

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Seed Germination and Seedling Growth of *Triticum Aestivum* L. And *Hordeum Vulgare* L. Under Allelopathic Effects of *Brassica Napus* L. – Aqueous Extract

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Abstract

The expansion of energy crop acreage, such as oilseed rape, and the increasing demand for cereals have led to a reduction in the biodiversity of agricultural crops. The cultivation of many crop plants is abandoned, which leads to the shortening of a plant's return time in the same field.

Agricultural practice has shown that the replacement of a 5-6-year crop rotation with a 2-3-year rotation causes at least a reduction in crop productivity in very favourable years. In recent years, the importance of this phenomenon has been increasing – despite significant countermeasures such as the use of chemical fertilizers, pesticides, mechanization or irrigation.

Many researches have attributed these crop losses to allelopathic phenomena in agroecosystems but the problem needs to be addressed more thoroughly. More than 10,000 secondary metabolites are known from the plant kingdom which displays important more or less plant-specific allelopathic effects. They can lead to alterations in water and nutrient uptake, stomatal conductivity and photosynthesis. This paper presents some aspects regarding the allelopathic effect of oilseed rape on seed germination and seedling growth of wheat cv. Boema1, Dropia, Izvor and barley cv. Flavia, Gabriela, Laura. We have observed that the physiological reactions of the plants exposed to the treatments with aqueous rapeseed extract are strongly dependent on the cultivar investigated. The inhibitory allelopathic effect was expressed as a change in germination phenology in the range of 05 to 09, according to the BBCH scale. Notably a reduction in seedling growth and effects on the biosynthesis of chlorophyll have been recorded, which could contribute to the reduction of crop production.

Keywords: allelopathy, Triticum aestivum, Hordeum vulgare, Brassica napus

Introduction

Currently is unanimously accepted that the communication between plants is done at chemical way. This biochemical communication between plants was defined since 1837, by Molisch at allelopathy. The first definition, of allelopathy indicate all of the effects that directly and indirectly result from biochemical substances transferred from one plant to another [1].

Subsequent further studies have strengthened the fact that allelopathy is a common biological phenomenon by which one organism produces biochemicals that influence the growth, survival, development, and reproduction of other organisms [2]. The influence can be positive or negative. Understanding this biological phenomenon could help to understand the environmental changes caused by allelochemicals the mechanisms of action of these compounds [3]. At the agroecosystem level, the plant communities are not immune from allelopathic interferences [4]. Many researches have attributed these crop losses to allelopathic

phenomena [5]. In a crop rotation, the succeeding plants may be influenced by the phytotoxins released by the preceding plants [6]. More than 10,000 secondary metabolites are known from the plant kingdom which displays important more or less plant-specific allelopathic effects [7].

They can lead to alterations in water and nutrient uptake, stomatal conductivity and photosynthesis. Allelochemicals can inhibit the cell division and elongation [2]. In the last years, due to the constantly higher demand for cereals, but also for oilseed crops, in many countries, only a few crops (wheat, oilseed rape, maize, barley) have been cultivated. The winter wheat we more often after rapeseed cultivated. In this case, even under favourable culture conditions, a decrease in plant production was observed. It has been demonstrated that this decline can be caused by the allopathic action of rapeseed plants [8]. Detrimental effects of toxins from Brassica spp. on the next year's wheat, barley, or flax crops were reported [9]. At 10% oil seed rape extract in concentration, the shoot lengths of wheat were significantly reduced. [8]. Sunflower after Brassica species, in the crop rotation, may reduce sunflower germination and growth [5]. Studies on allopathic phenomena must be performed under the field conditions, but in these cases, the interferences are more difficult to detect. Several researches about allelopathy give the reason to conclude that, the standardized laboratory assay is a rapid and an inexpensive procedure for screening the allelopathic potential of large numbers of crop genotypes against weed species [10], [11]. This method has also been used in the study of allelopathic phenomena of crop plants.

The aims of the present study were to investigate whether the winter wheat varieties react differently to the allelopathic effect of oilseed rape and whether this effect manifests itself in the winter barley plants.

Methodology

The allelopathic effect of oilseed rape on seed germination and seedling growth of wheat cv. Boema1, Dropia, Izvor and barley cv. Flavia, Gabriela, Laura was performed in a bifactorial experiment on the Petri dishes.

Experimental design: the germination experiment was conducted as factorial based on a completely randomized design with four replications.

Extracts preparation: The aqueous extract of Brassica was prepared according to a method described by Ebrahimi *et al*., [8]: plant residues of oil seed were ground, and 100 g. of residue powder were extracted with 1000 ml distilled water in a shaker for 24 h at room temperature.

The mixture was filtered and then the aqueous extract was diluted to 5% and 10% concentrations.

Triticum aestivum L. and *Hordeum vulgare* L. seeds were immersed in a 10% sodium hypochlorite solution for 10 min and then rinsed three times with DDW to ensure surface sterility. Each of 100 seeds was placed on the Petri dishes and watered separately by different concentrations (5% and 10%) of extracts or distilled water as control treatment. All Petri dishes were maintained at 24±2°C and 16 h photoperiod. Measurements and observations: seeds germination (%), plants stages (according to BBCH), root and shoot length, seedling dry mater content and, chlorophyll concentration. The determination of the dry mater of the plant material was carried according to a VDLUFA method [12]. The plant material was weighed in aluminium dishes, dried for 24 hours at 105°C and weighed again, after cooling in the dryer.

Chlorophyll determination was carried out as described by Zörb *et al*., [13]. After extraction in acetone 80% the absorbance was read at UV-1800 SIMATZU spectrophotometer, and the chlorophyll concentration was calculated using the following formula: Total chlorophyll (μg/ml) = 20,2 *A (645) + 7,52*A (663).

Statistical analysis: The experimental data were analysed by the analysis of variance (ANOVA). For multiple comparisons between means of different treatments was used

Duncan's test. Means with different letters indicate significant differences according to Duncan's multiple range test – p,0.05

Results and Discussions

Four days after the beginning of the trial it was observed that the germination of seeds exposed to rapeseed extract was negatively affected compared to the control (seeds germinated in distilled water). The largest decrease in germination was recorded for cultivar Boema1 in the case of wheat (Fig. 1) as well as for barley cultivars Gabriela and Flavia (Fig. 2). Twelve days after the start of the experiment, seed germination in all test variants was higher than 97.5%, which confirms that rapeseed extract affects the germination energy and not the germination capacity.

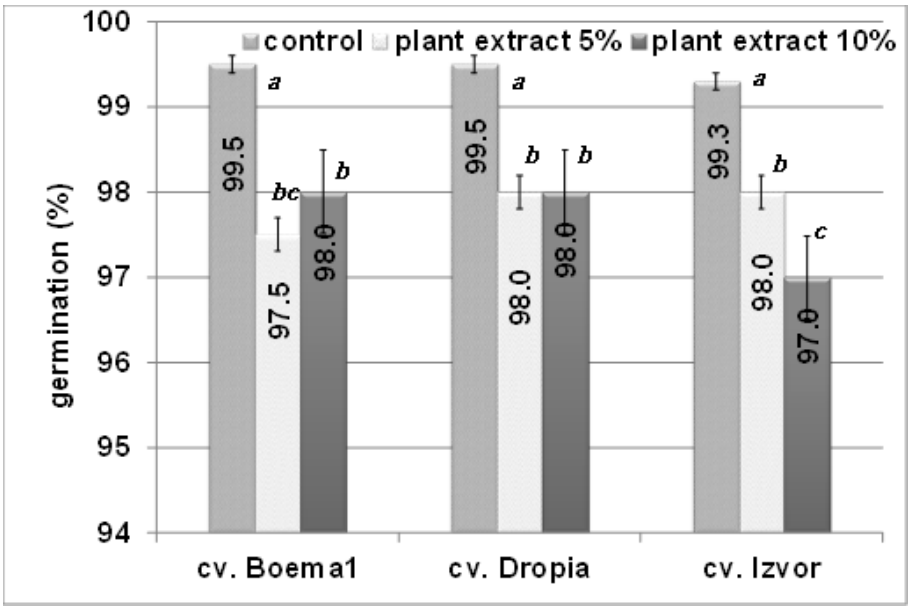

Fig. 1. Effect of oil seed rape aqueous extracts of winter wheat seed germination
(means with different letters indicate significant differences according to Duncan's multiple range test – p,0.05)

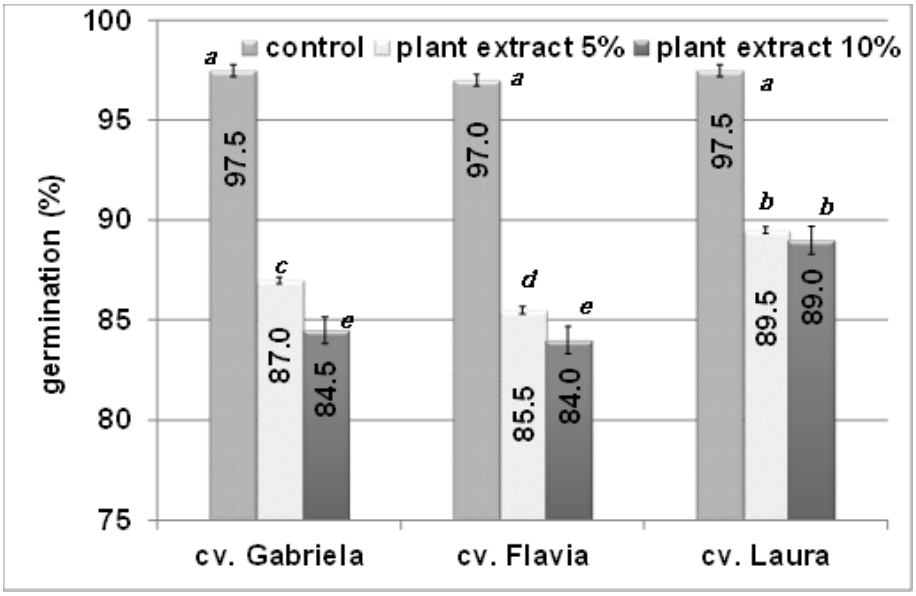

Fig. 2. Effect of oil seed rape aqueous extracts of winter barley seed germination
(means with different letters indicate significant differences according to Duncan's multiple range test – p,0.05)

The inhibitory allelopathic effect was expressed as a change in germination phenology in the range of 05 to 09, according to the BBCH scale. The largest differences in growth were recorded for cv Boema 1, in the case of wheat, and for barley in cultivars Gabriela und Flavia, respectively (Fig. 3 and 4).

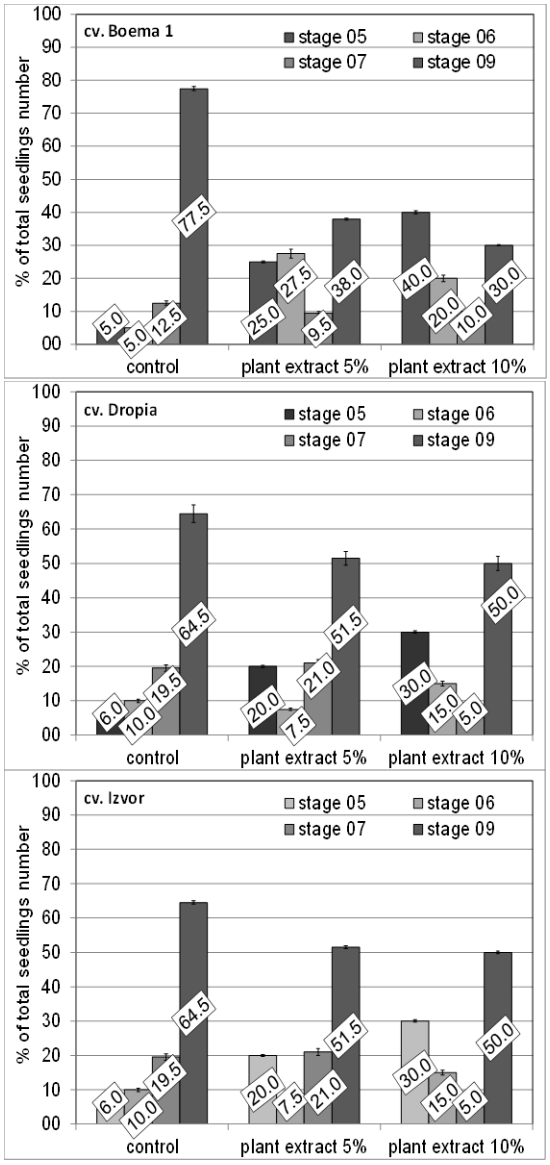

Fig. 3. Effect of oil seed rape aqueous extracts on growth of winter wheat seedlings (plants stages, according to BBCH, after 12 days from the trial beginning)

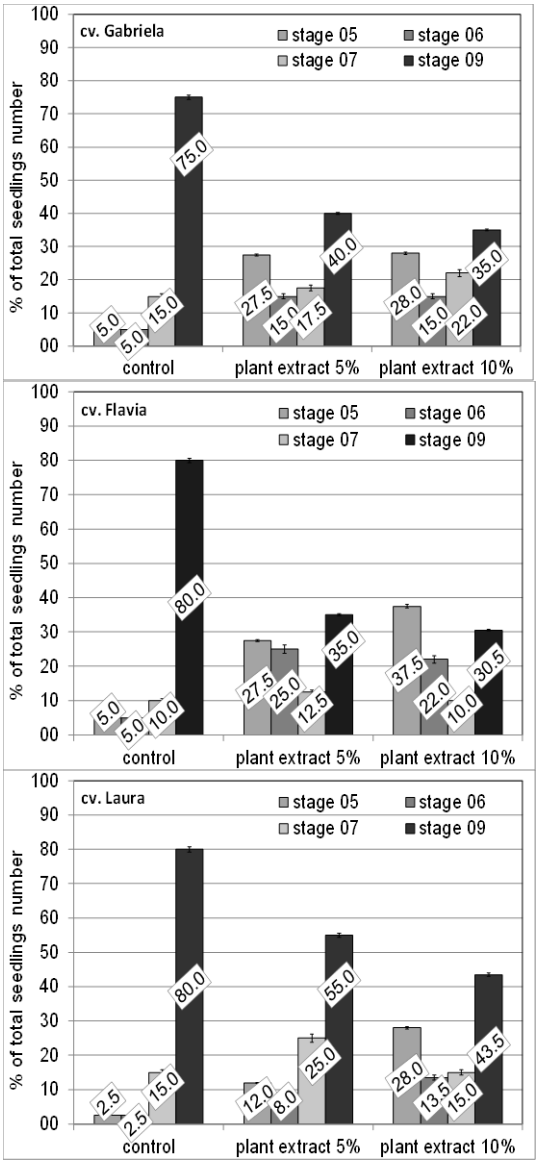

Fig. 4. Effect of oil seed rape aqueous extracts on growth of winter barley seedlings (plants stages, according to BBCH, after 12 days from the trial beginning)

The aqueous rapeseed extract treatments by the two species affected the growth of both the roots and the shoot. Sprout growth was less affected by the treatments than root growth (Table. 1. and 2).

The dry matter content was also influenced under the effect from Brassica.

After treatments, in both wheat and barley varieties the dry matter content of seedlings exposed to different extract concentrations was significantly lower than that of the control seedlings, watered with distillate water (Tab. 1. and 2)

Table 1. Effect of different *Brassica napus* L – aqueous extract on seedling growth,

total chlorophyll and dry matter content of winter wheat

Cultivar	Plant extract concentration (%)	Shoot length (cm)	Root length (cm)	Total chlorophyll (mg/g FW)	Dry matter (g/plant)
Boema1	0	7.80 a	8.55 a	2.97 a	1.00 b
	5	5.00 b	5.35 b	2.80 ab	0.95 b
	10	3.50 c	3.50 c	2.45 c	0.50 c
Dropia	0	8.05 a	8.00 a	2.95 a	1.20 a
	5	6.80 a	6.50 b	2.65 b	0.90 b
	10	5.50 b	5.50 b	2.55 c	0.87 b
Izvor	0	8.50 a	7.50 a	2.95 a	1.10 a
	5	6.55 b	6.00 b	2.70 b	0.95 b
	10	6.00 b	5.00 b	2.77 b	0.90 b

Table 2. Effect of different *Brassica napus* L – aqueous extract on seedling growth, total chlorophyll and dry matter content of winter wheat

Cultivar	Plant extract concentration (%)	Schoot length (cm)	Root length (cm)	Total chlorophyll (mg/g FW)	Dry matter (g/plant)
Gabriela	0	7.55 a	7.00 b	1.97 a	0.80 c
	5	6.50 b	5.50 c	1.80 b	0.75 cd
	10	5.30 c	5.30 d	1.45 cd	0.50 e
Flavia	0	7.50 a	8.50 a	1.95 a	0.85 a
	5	5.50 b	5.25 cd	1.65 c	0.65 b
	10	3.00 d	3.50 e	1.55 d	0.50 e
Laura	0	7.80 a	7.50 b	1.95 a	1.00 a
	5	6.50 b	6.00 c	1.85 b	0.90 b
	10	6.00 b	5.00 d	1.75 b	0.85 bc

Conclusion

The treatments of wheat and barley with rape extracts clearly demonstrated its allelopathic effects. The latter are characterized by a change of germination dynamics (according to the BBCH scale), a reduction in seedling growth and dry matter content of the seedlings, as well a decrease of the chlorophyll concentration.

The three wheat cultivars react differently to the allelopathic effect of oilseed rape, with cv. Boema1 being most sensitive. In contrast, the other two varieties, Dropia and Izvor, respectively, can be grown after an oilseed rape harvest.

The same different behaviour was observed in winter barley. In this case, cv. Laura was found to be most resistant to the allelopathic effect of an aqueous Brassica extracts.

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Variation of Saponins Content in Alfalfa (*Medicago Sativa* L.)

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Abstract

Alfalfa (*Medicago sativa* L.) is one of the forage species with a high-quality potential.

The research conducted during May 2014-October 2015, on the Ezăreni farm, Iasi (47°05'-47°10' North latitude and 27°28'-27°33' East longitude), followed, in alfalfa (*Medicago sativa* L.), in the fourth year of vegetation, the influence of alfalfa growth stage at harvest (early bud, mid bud, late bud; early bloom, 10% bloom and full bloom) on the plants saponins content. The content of the plant in saponins was determined by the carbon-pyridine method; the principle of the method consists in the extraction with ethanol of the total saponins, the isolation with the help of the chromatographic paper and their recovery [1].

The leaves/stems ratio is an important quality indicator because quality alfalfa depends on it. The influence of harvest time on saponins content in the entire alfalfa plant, leaves or stems showed that as plants are aging, the concentration decreased very significant.

Keywords: growth stage at harvest, leaves/stems ratio, quality

Introduction

The productivity and quality of the alfalfa depends on a number of factors, including pedoclimatic conditions, the genetic potential of the variety used and the cultivation technology used, seed inoculation, fertilization and harvesting time, which are of great importance.

Like any other agricultural culture, the ability to control the impact factors on the quality of the alfalfa will be reflected in the volume and quality of the production obtained.

Of all the factors involved in determining the productivity and quality of the alfalfa, the moment at which it is harvested is the decisive factor. The level of production and their quality depend on the harvesting phenophases [2], [3].

The leaves/stems ratio, depending on the harvesting phenophases, represents a very important quality indicator because of this depends on the quality of the alfalfa. The percentage of leaves is desirable to be as high as possible, the reason being that in the leaves there is a protein content at least double to the stems, as many authors show [4], [5].

Purpose of the research was the determination the saponin content of alfalfa harvested in different phenological phases (*Medicago sativa* L.), under the conditions of Moldavian Forest Steppe.

Many different plant species, examples include crop plants such as legumes, synthesise saponins as part of their normal programme of growth and development and their likely role as determinants of plant disease resistance. Saponozides (saponins) are glycosides that have the characteristic of foaming. Saponins taste bitter and reduce palatability of forage. Saponins are not, in general, a problem for fodder legumes, lucerne (*Medicago sativa* L.), being one of these species, with a content ranging from 0.07 to 1.35% from DM [6], [7].

Although alfalfa contains several types of saponins (medicagenic acid, soyasapogenol A, soyasapogenol B, lucernic acid), the medicagenic acid seems to be responsible for its

antinutritional effects. The saponin content of the alfalfa leaves is low in spring and fall and high in the middle of summer. To limit these shortcomings, breeders are continually trying to obtain cultivars with the least saponozide content [8], [9], [10], [11], [12].

Methodology

The research was conducted between May 2014 - October 2015, at Ezăreni Farm, Iaşi (47°05'-47°10' North latitude and 27°28'-27°33' East longitude). The soil is a cambic chernozem, characterized by pH 6.73, 40.3% clay, humus 2.32%, 0.164% total nitrogen, P-Al 18 ppm, 210 ppm K-Al.

The research followed, in alfalfa (*Medicago sativa* L.), in the fourth year of vegetation, the influence of alfalfa growth stage at harvest on the leaves, stems and plants saponins content.

The experiment was laid by randomized block method, with a harvested area of 10 m^2 (2 m x 5 m) in three replicates. Graduations of the studied factor were represented by the development phenophases: early bud (v_1), mid bud (v_2), late bud (v_3); early bloom (v_4), 10% bloom (v_5) and full bloom (v_6), phenological phases described by numerous authors [13], [14], [15], [16].

Production was determined by weighing the yield obtained from a harvested surface of 10 m^2, which was afterwards transformed per hectare. The dry matter was determined by drying in an oven at 103ºC for 3 hours.

The leaves and stems productions were calculated based on the leaves/stem's ratio.

The leaves/stems ratio was determined by separating the petiole, leaflets, buds and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems).

The content of the plant in saponins was determined by the carbon-pyridine method; the principle of the method consists in the extraction with ethanol of the total saponins, the isolation with the help of the chromatographic paper and their recovery [1].

The biological material used was the alfalfa variety Sandra (F 660-94), registered in 2003 at National Agricultural Research and Development Institute (NARDI) Fundulea, Bucharest [17].

The data were interpreted statistically by means of variance analysis and limit differences calculation. Also, equation correlations were calculated (quadratic regression significance).

Results and Discussion

From the analysis of the obtained results, it was observed that with the advancement of the vegetation, the production of dry matter to the whole plant and stems was constantly increasing.

The production of dry matter on the leaves increased until the end of the bud – the beginning of flowering, after which it decreased.

The phenomenon that directly influences the quality of the obtained production is the etiolation, drying and falling of the leaves from the lower nodes of the stems, with the appearance of the first flowers. Thus, as the plants are harvested later than the beginning of flowering, the production of leaves will be lower, and the quality of the feed obtained will be also lower (*table 1*).

The results obtained in the current experiment were similar to those existing in the literature [18], [19], [20], [21].

Table 1. The influence of growth stage at harvest on the content of alfalfa in saponins

Experimental variant	Dry matter production*			Leaves/ stems ratio	Saponins content		
	Whole plants	Leaves	Stems		Whole plants	Leaves	Stems
	(Mg·ha^{-1} DM)				(mg·kg^{-1} DM)		
V_1-early bud (C)	2.65^C	1.15^C	1.50^C	0.76^C	1030^C	1341^C	790^C
V_2-mid bud	3.21***	1.30**	1.92***	0.68^{ooo}	992^{ooo}	1302^{ooo}	785^o
V_3-late bud	3.67***	1.38***	2.29***	0.60^{ooo}	927^{ooo}	1256^{ooo}	729^{ooo}
V_4-early bloom	3.93***	1.39***	2.54***	0.54^{ooo}	791^{ooo}	1166^{ooo}	587^{ooo}
V_5-10% bloom	4.08***	1.30**	2.79***	0.46^{ooo}	675^{ooo}	1107^{ooo}	475^{ooo}
V_6-full bloom	4.12***	1.23*	2.88***	0.43^{ooo}	598^{ooo}	1033^{ooo}	412^{ooo}
LSD 0.5	*0.23*	*0.08*	*0.17*	*0.03*	*6*	*10*	*4*
LSD 0.1	*0.33*	*0.12*	*0.24*	*0.04*	*8*	*15*	*6*
LSD 0.01	*0.48*	*0.17*	*0.35*	*0.05*	*11*	*21*	*9*

C – control variant; * – first cut in the fourth year of vegetation

Harvesting phenophase was the factor that caused the largest differences in leaf/stem ratio.

The difference between the control variant, harvested at the early bud stage (v_1) and the variant with the lowest leaf/stem ratio (v_6) harvested at full flowering, was negative, 0.33, and all the others differences obtained were negative, very significant. The harvesting phenophase had a significant and very significant influence on the content of leaves, stems and entire plants of alfalfa in saponins. Thus, the saponin content of the alfalfa leaves ranged between 1033-1341 mg·kg^{-1} DM and of the alfalfa strains varied between 412-790 mg·kg^{-1} DM. Even if the saponin content has a higher value in the alfalfa leaves, 69-163% higher than in the stems, finally, the saponin content of the plants will be influenced by the leaf/stem ratio (*table 1*).

There was a negative correlation between the number of days required to reach each phenological phase in alfalfa plants and the saponin content of leaves, stems and whole plants (*figure 1*). The results obtained from the study were close to the results obtained by other researchers [10], [11], [12], [22], [23], [24].

Conclusions

The content of plants, leaves and alfalfa stems in saponins was firstly influenced by the leaf stem ratio and then by the harvesting phenophase; with the succession of the phenological phases the lucerne content in saponins has been steadily decreasing, the differences obtained being statistically ensured. As the phenophase succeed, the amount of saponins exported together with the production of dry matter has been decreasing continuously, the values of this indicator being correlated with the phenophase in which the lucerne was harvested.

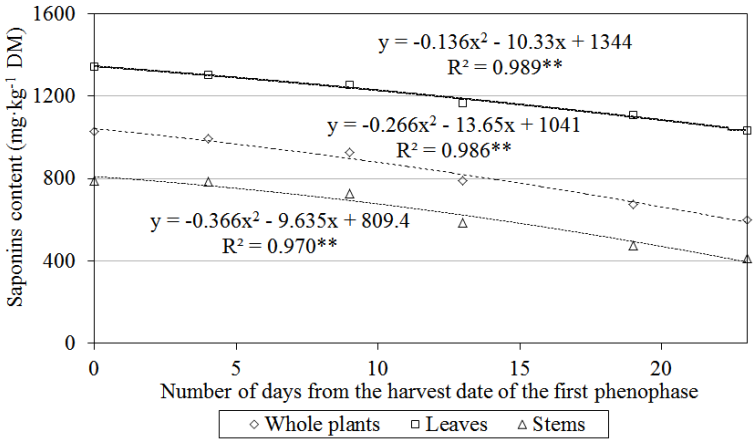

Fig. 1. Correlation between growth stage at harvest and the saponin content of leaves, stems and the whole plant

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Studies Regarding Technologies of Valorisation as Biomass of Vine Pruning Residues Resulted from Dormant Pruning

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Abstract

The problem of replacing fossil fuels in energy production is becoming increasingly stringent, both in terms of reducing CO2 emissions and as an alternative to the obvious decrease in fossil fuel reserves. Biomass from the dormant pruning of vines meets these needs. Vine pruning residues harvesting technologies as biomass are based on specific methods and use special machines for each stage. The chopped vine pruning residues represent a mixture with the particle size between 10 and 50 mm, which uses compacting agglomeration technologies in order to be transformed into a biofuel for direct combustion. Pelleting and briquetting are the most used biomass agglomeration operations. Different machinery were developed worldwide in order to put into practice the technologies for the use of the residues resulted from the dormant pruning of vines as solid biofuel: equipment's for operating the shears of cutting residues; equipment for carrying out the work of pruning the vines; equipment for the gathering of vine residues in the middle of the interval between rows; equipment and aggregates for gathering vine residues through chopping; baling machines for vine residues; machines for chopping and chopping vine residues for the manufacture of pellets and briquettes; equipment for chopped dried residues; machines for the pelletizing or briquetting chopped and dried chords; automated technological lines for the valorisation as a solid biofuel of pruning residues resulting from the dry cutting of the vines in the form of pellets or briquettes.

Keywords: technologies for solid fuels, pellets, vine pruning residues

Introduction

At the global level, biomass means the biodegradable part of agricultural products, waste and residues, including plant and animal substances, forestry and related industries, as well as industrial and urban waste.

Biomass is one of the most widespread renewable resources on Earth, representing approx. 15% of the primary energy sources, and does not contribute to the increase of CO_2 concentration in the atmosphere, reduces the greenhouse effect and does not produce acid rain. This is due to a lower sulphur content than the one existing in the structure of fossil fuels [1].

The vines residues resulting from the fruition pruning of the vineyards can represent an important resource of biomass, which at present, is very little used. For these reasons, new technologies are required to allow the gathering, primary processing and higher capitalization of these plant residues, while ensuring an economically, technologically and environmentally sustainable technological process.

The research carried out in this paper is based on the fact that at the European Union level vineyards are cultivated with more than 3.2 million ha and that about 1 ha of biomass can be recovered from each ha [2; 3] it turns out that from this area, from an economic point of view, over 3.2 million tonnes of biofuel can be made available as an energy source every year. On the other hand, the recovery of these wastes is also important for the protection of the environment, as it eliminates the possibility of burning in the field, avoiding the pollution of the atmosphere with smoke, dust and unpleasant odors.

Thus, in the context of increasing energy efficiency, harnessing the vines residues resulting from the fruiting cuts has a major contribution to achieving sustainable energy development, competitiveness in saving primary energy resources and reducing greenhouse gas emissions [4].

Methodology

In order to use as a biomass, the vines residues resulting from the dry cutting, in the world, specific and diverse machinery and equipment are used, which allow the accomplishment of the working processes in conditions of minimum effort. Technologies for harvesting vines residues as biomass include cutting vines, gathering, chopping or picking them up and drying them. The dried and shredded vines residues can be harnessed as solid biofuel in the form of chips, pellets or briquettes.

Tools and tools for the dormant pruning of vines. Pruning of vine plantations can be performed manually or mechanically. For the manual pruning manually operated scissors are used, or, in order to reduce the physical effort, the tools are electrically or pneumatically operated. The mechanical pruning is performed as a preliminary operation, for removing the tendrils, after which it is performed manually, the actual cutting. A wide range of equipment for vines residues preliminary cutting has been developed worldwide. These equipment's can complete the cut in a row [5], being mounted on the tractor on the side, front or rear, on the hydraulic lift, and are operated hydrostatically. Other equipment is built to excite the cutting in two halves [6], or in a half row [7].

According to the construction of the cutting devices, the machines can be: with cutting devices with disk knives arranged horizontally [5], when the sectioning of the vine is performed, at the desired height, without being restricted by the system plantation support; cutting machines with knives and plywood, with rectilinear-alternative movement [6] and cutting machines with blades-knives or disc-knives with vertical arrangement [7]. In the last periods have been made complex machines for pretending vines residues and even intelligent robots for selective cutting, depending on the technological requirements.

For example, researchers at Vision Robotics Corporation in San Diego, USA, have designed and built a smart robot for cutting the vine, *"Intelligent Robotic Vineyard Pruner"*. The robot works day and night, with remote monitoring [8].

The machines used to gather the vine residues, resulting from the dry cutting of the vines, make the rakes and leave them in the middle of the interval between rows. Such equipment, for raking and bringing the residues in the middle of the interval between rows, is mounted in the front of the tractor on wheels, has as working members two rotors provided with 4-6 elastic arms made of steel wire.

The machines for gathering, chopping and transporting the vines residues can be with the unloading of the chop in the trailer [9] or in their own bin, after which the material is transferred to a means of transport [9] that is stationed at the end of the plot. Another type of machine is the self-propelled type, for harvesting and chopping vines [10].

Cording machines. The strings resulting from the fruiting cuts and gathered in the middle of the interval between rows are rounded, in cylindrical or parallelepiped form.

The machines for picking and pressing residues in cylindrical shaped bales are of the type with fixed pressing chamber, with chains and cylindrical rollers engaged in rotational movement, with the diameter of the ball of 0,6-1,2 m [11] and [10]. The bales formed and pressed, with a diameter of 1.2 m, with presses with a fixed pressing chamber are unloaded on the interval between the rows of vines [11]. The machines for collecting and pressing vines residues, with a diameter of 0.6 m, are provided with a storage trough, having the possibility to store up to 8 bales, which are then unloaded at the end of the plot [10].

The machines for the valorisation as a biomass of the vine residues resulting from the cutting in the dry make their preparation and densification in the form of pellets or briquettes.

The preparation of the biomass from the vine's residues, for the purpose of densification, includes technological operations of shredding and drying. The chopping of the residues is done with the help of the mills with hammers, of large capacity, of general use. The drying of the residues is done with dryers, of general use for the dehydration of agricultural raw materials of vegetable origin, with continuous flow operation.

The most used dryers are with conveyor belt, rotary drum, aerodynamic fluidized layer etc.

Densification of biomass, resulting from the cutting in vines, is done in the form of pellets or briquettes.

Pelleting is done by extrusion; crushed and dried residues, which pass continuously and continuously through holes with diameter of the pellet size (6 ... 25 mm and length 3,15 ... 40 mm) [12].

Briquetting machines are used for densification of biomass pressing chambers equipped with piston (6, Fig. 6.a), mechanically or hydraulically operated, with discontinuous operation, or with cylindrical-truncated snail, at which the working regime is continuous [12].

Results and Discussions

Technologies for biomass recovery of vineyards include technological processes for harvesting and manufacturing solid biofuel.

When establishing the technologies for harvesting the residues of vineyards, the system of machines developed worldwide, the conditions of densification, as well as the following aspects must be taken into account:

- the fruition pruning can be performed manually, semi-mechanized or using automated robots (Fig. 1);
- the assembly of the strings cut in the middle of the interval between rows is done with various machines (Fig. 1), which work as independent aggregates or in the form of complex aggregates (raking and chopping or raking and baling);
- the harvesting of the vines residues, resulting from the dry cutting of the vines, for the biomass is carried out with specific machines, in which the raw material can minced, after which it is dried by different processes, or is packaged and stored for natural drying (Fig. 1);

Based on these aspects and taking into account the system of machines made worldwide, 41 technologies were developed for harvesting ropes for use as densified biomass (TRC1 ... TRC41), of which: 2 technologies are manual (TRC1 and TRC2); 10 – semi mechanized technologies for chopping residues (TRC3 .... TRC12); 10 – partially mechanized technologies for harvesting vines residues by chopping (TRC13 .... TRC22); 10 – fully mechanized technologies for chopping residues harvesting (TRC23 .... TRC32); 3 – semi-mechanized technologies for harvesting residues through baling (TRC33 .... TRC35); 3 – Partially mechanized technologies for harvesting residues by baling (TRC36 .... TRC38) and 3 – Totally mechanized technologies for harvesting residues by baling (TRC39 .... TRC41) [13]. As an example, Table 1 shows the harvesting technology for each group.

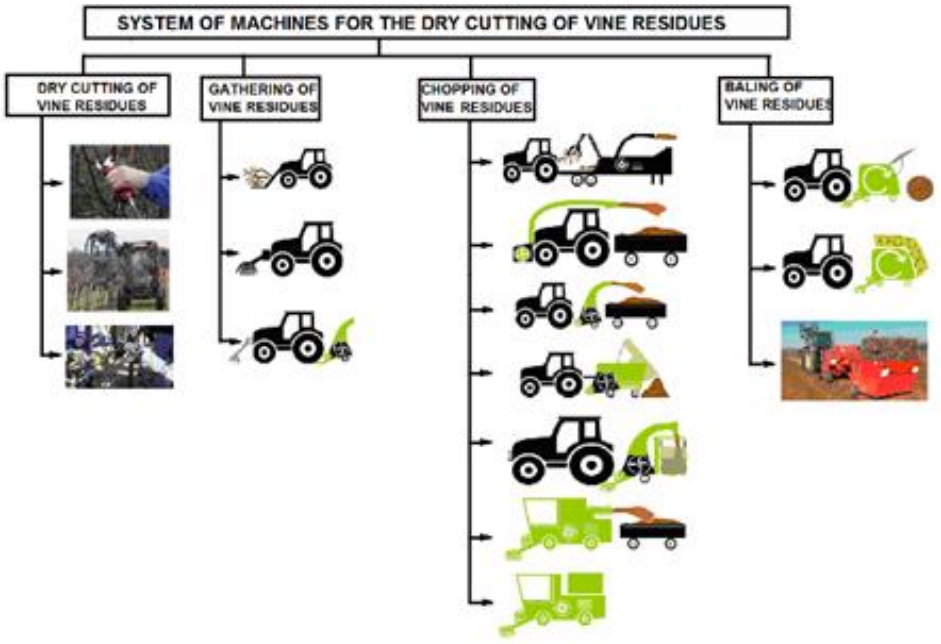


Fig. 1. System of machines for the dry cutting of vines and harvesting of vines residues for biomass

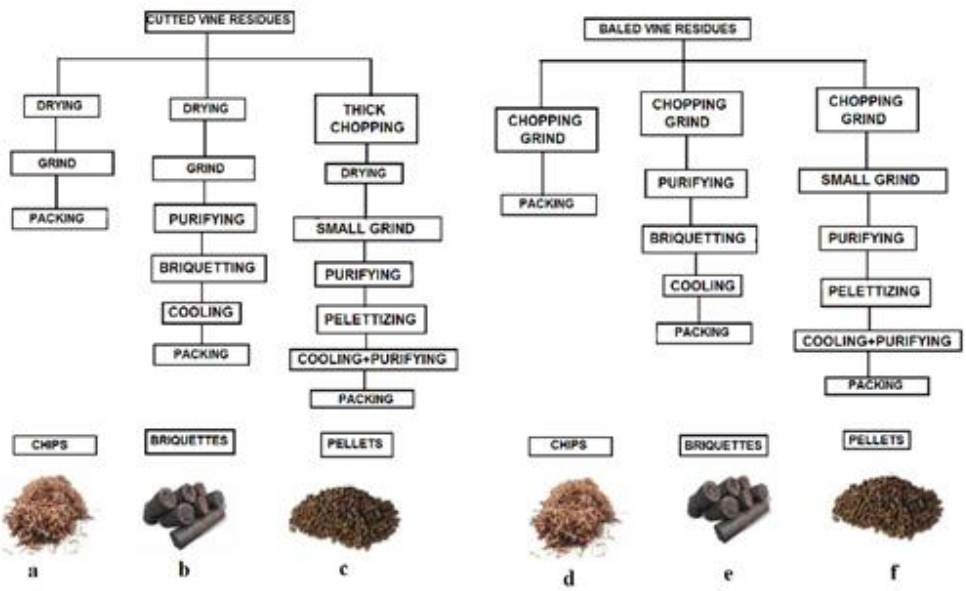
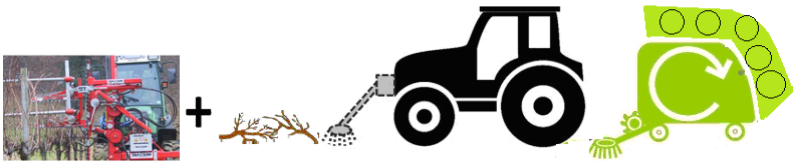
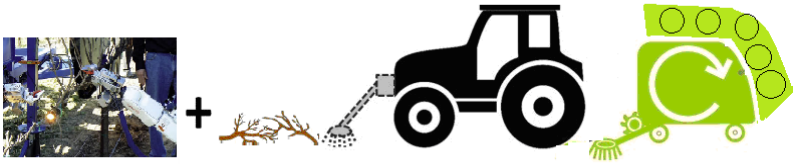


Fig. 2. Technologies for the valorisation of vine residues as solid biofuel

For manual technologies, specific to small surfaces, cutting, gathering and transport work are done manually and the cutting is done with a chopping machine. The works for the semi-mechanized technologies involve the manual cutting of the strings, and the other operations are mechanized. Within the partially mechanized technologies, a chord pre-treatment is performed, after which the other works are mechanized. For the fully mechanized technologies of vines residues harvesting, all the works are mechanically carried out, including the fructification cuts, which are made with remote controlled robots, working continuously for a day.

Technologies for the use of vineyards as a solid biofuel. In order to establish the technologies of harnessing the vines residues as solid biomass, the following aspects are considered: the way of harvesting the vine residues, chopped or baled; solid biofuel to be produced (pellets, briquettes or chips).

Table 1. Mechanization technologies for harvesting vines (extract)

No.	Harvesting technology	Technology development scheme
Semi-mechanized technology for chopping vines residues		
1	The manual cutting of the vine residues, the gathering and the chopping are done mechanically – T_{RC5}	
Partially machined technology for chopping vine residues		
2	Preliminary cutting of the vine residues is done mechanically, the gathering and chopping are done mechanically, the mince is carried with jumbo bags – T_{RC17}	
Fully mechanized technology for vine residues		
3	The cutting of the vine residues is done with robots, the gathering and the chopping of the vine residues is done mechanically, the chopping is unloaded in the trailer – T_{RC31}	
Semi-mechanized technology for harvesting vine residues by baling		
4	The cutting of the vine residues is done manually, the gathering, the baling of the vine residues and the transport of the bales are done mechanically – T_{RC34}	
Partially mechanized technology for harvesting vine residues by baling		
5	Preliminary cutting of the vine residues is performed mechanically, the gathering, the baling of the vine residues and the transport of the bales are performed mechanically – T_{RC38}	
Totally mechanized technology for harvesting vine residues by baling		
6	The cutting of the vine residues is done with robots, the gathering, the baling of the vine residues and the transport of the bales is done mechanically – T_{RC41}	

As shown above, the chopped chords resulting from the dry cutting of the vines are in the form of a mixture of minced particles, with the size between 10 and 50 mm and with humidity of 38-48%. To be transformed into solid biofuels were generated three technologies, depending on the finished product, namely chips (Fig. 2.a), briquettes (Fig.2.b) or pellets (Fig. 2.c).
For the baled residues were generated three technologies depending on the finished product, namely chips (Fig. 2.d), briquettes (Fig. 2.e) or pellets (Fig. 2.f).

Conclusions

At present, globally, biomass is a primary carbon source alongside other renewable energy sources. It can be used as a raw material to produce energy, high energy value solid biofuels or biochemical fuels required for economic activities.

Several technologies are used to capitalize on vine residues as a solid biofuel, depending on the following factors: dry cutting method, manual cutting, preliminary cutting with machines equipped with cutting machines and cutting with robots; of the way of collecting the vine residues, chopped or baled; according to the way of harnessing it as a solid biofuel of the vine residues, the technologies can be for: the production of chips; manufacture of pellets; processing as briquettes, etc.

Acknowledgement

This works was supported by a grant of the Romanian Ministery of Research and Innovation, CCCDIUEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0251/4PCCDI/2018 (PNCD III framework program).

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The Evaluation of the Quality of Dry Phytomass and Briquettes from *Miscanthus Giganteus*, *Phragmites Australis* and *Zea Mays* Grown in the Republic of Moldova

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Abstract

Plant biomass – phytomass from energy crops to agricultural or forestry residues – is clean and environmentally safe. *Poaceae* phytomass is the most commonly used raw material for the production heat energy, by direct combustion, in Moldova. The densification of phytomass in briquettes improves fuel efficiency, due to its high energy content, which makes it suitable for use by small households and by industrial consumers. The main objective of this research was to evaluate some physical and mechanical properties of dry phytomass and briquettes form *Poaceae* species: *Miscanthus giganteus, Phragmites australis* and corn stalks, *Zea mays*, collected from the NBGI, Chişinău. The physical and mechanical properties were determined according to the European standards accepted in the Republic of Moldova; the equipment used to produce briquettes was a piston type briquetting press. It has been established that *Miscanthus giganteus* stems defoliated and dehydrated faster than *Phragmites australis.* The bulk density of the milled chaffs by 10 mm sieve, of tested *Poaceae* species, varied from 100 to167 kg/m^3. The specific density of briquettes reached 831-923 kg/m^3, but the bulk density of briquettes 430-501 kg/m^3, the net calorific value varied from 14.0 to 15.8 MJ/kg and the ash content 2.51-4.71%. *Miscanthus giganteus* was distinguished by high density of milled chaffs and net calorific value and low ash content; *Phragmites australis* briquettes were characterized by moderate net calorific value and optimal density, and high ash content; *Zea mays* briquettes – high ash content, bulk and specific density.

Keywords: biomass, briquettes, Miscanthus giganteus, Phragmites australis, physical and mechanical properties, Zea mays stalks

Introduction

Plant biomass – phytomass from energy crops, agricultural and forestry residues is a promising renewable resource to achieve a low carbon bio-economy with the production of biofuels and energy for heat and power, is clean and environmentally safe. One of the most important tasks in the production of energy crops is the selection of suitable plant species that could thrive in specific soil and environmental conditions of the changing climate, be used in many areas and produce a large amount of biomass that is easily converted into energy [1].

Giant miscanthus, *Miscanthus giganteus* Greef et Deu, family *Poaceae,* is natural hybrid between *M. sinensis* x *M. sacchariflorus,* was firstly developed in Japan, C_4 metabolism perennial plant, with thick and stout rhizome; stem 2.5-3.5 m long; leaf blades linear 50.0 × 3.0 cm; inflorescence 30-55 cm long, rachis of panicle 15-21 cm long; flowering September – November; no seed, is propagated asexually, usually by dividing the rhizomes and by tissue culture. The exceptionally vigorous growth, high photosynthetic capacity and remarkable adaptability to different environments make this natural hybrid suitable for cultivation in

Europe and North America, it is a crop with great potential, providing up to 40 t/ha/year, are considered important substrates for bio refining industry and energy production [2, 3].

Common reed, *Phragmites australis* (Cav.) Trin. *ex* Steud, native to Eurasia and Africa and one of the most widely distributed vascular plant species in the world. It is a typical wetland perennial species and can cover vast areas almost in monoculture. Is a robust, erect, aquatic or subaquatic, C_3 metabolism grass, strongly tufted, with vertical and horizontal creeping rhizome, often also with stolons, stem up to 6 m tall and 15 mm in diameter; leaves alternate up to 60 cm long and 20 to 60 mm wide; inflorescence – a feathery panicle15-50 cm long, flowering July-October; fruit 1-5 mm long dark brown. Common reed is very important for biodiversity, provides food and habitat for some organisms, improves water quality and serves to stabilize soils against erosion. It has been used for many purposes around the world: as building material for houses and rafts, as thatching, to make mats, as fodder and bedding for cattle, as cellulose source in the paper and textile industries, to provide energy. The biomass productivity of *Phragmites australis* widely, from 5 to 15 t/ha in natural areas, to 40-50 t/ha dry matter when cultivated using fertilizers [4, 5, 6, 7].

Agricultural residue products remain the major primary source of energy for appreciable domestic and industrial application such as household cooking and heating. *Zea mays* L., maize or corn, is an annual plant with C_4 metabolism, adapted to a wide range of conditions, one of the most commonly cultivated crops, with recent worldwide production of around 1000 million tons per year [8]. In the Republic of Moldova, the annual area sown with *Zea mays* hybrids is about 433 thousand ha or 29% of the total area. It has been estimated that approximately 100.000 dry tons of corn Stover are available annually to support the biomass industry, but at present it is usually burnt in the field, resulting in environmental pollution. Therefore, the recycling of corn stalks in densified solid biofuel is urgent and necessary.

The main objective of this research was to evaluate some physical and mechanical properties of dry phytomass and briquettes form *Poaceae* species: *Miscanthus giganteus, Phragmites australis* and corn stalks *Zea mays.*

Methodology

The *Poaceae* species: giant miscanthus *Miscanthus giganteus* cv. *Titan*, common reed *Phragmites australis* and corn stalks, *Zea mays* collected from the Botanical Garden (Institute), Chişinău, served as subjects of this study. The collected phytomass was chopped into chaff with the use of stationary forage chopping unit. The chopped phytomass was milled in a beater mill equipped with a sieve with diameter of openings of 10 mm using equipment SM 100. The physical and mechanical properties of dry biomass were determined according to the European Standards in the State Agrarian University of Moldova: the moisture content of the plant material was determined by SM EN ISO 18134 in an automatic hot air oven MEMMERT100-800; the content of ash was determined at 550ºC in a muffle furnace HT40AL according to SM EN ISO 18122; automatic calorimeter LAGET MS-10A with accessories was used for the determination of the calorific value, according to SM EN ISO 18125; the particle size distribution was determined according to SM EN ISO 17827 using standard sieves, the collected particles in each sieve were weighed; the cylindrical containers were used for the determination of the bulk density, calculated by dividing the mass over the container volume according to SM EN ISO 17828, SM EN ISO 18847. The briquetting was carried out by hydraulic piston briquetting press BrikStar model 50-12 (Briklis). The mean compressed (specific) density of the briquettes was determined immediately after removal from the mould as a ratio of measured mass over calculated volume.

Results and Discussions

It is known that moisture, stem and leaf share in harvested phytomass influence the costs of transport, storage, drying and processing, and the physical and mechanical properties of solid biofuel reduce the final usable energy and thus the efficiency of the energy system, contributing at the same time to the increased emission of pollutants. The results of moisture and leaf contents in harvested phytomass of *Poaceae* species are shown in Tab. 1. It was found that, at the end of the growing season, the stems of *Poaceae* species contained a lot of moisture 52.1-72.1%, but the leaf and panicle share in the phytomass varied significantly from 26.3% (*Phragmites australis)* to 47.0% (*Zea mays*). After the establishment of negatives temperatures below 7-12 °C, the studied species differed in the pace of dehydration of tissues, *Miscanthus giganteus* in the field dehydrated faster than *Phragmites australis* and *Zea mays*. In March, the amount of moisture in the collected phyitomass was 12.1-12.8% in *Miscanthus giganteus* and *Zea mays*, but 21.8% in *Phragmites australis.* Similar results were presented by other authors: for example, the moisture content of *Miscanthus giganteus* plants decreased from 58.36% to 23.23% [9] and *Phragmites australis* plants – from 46.5% to 14.8% [10].

Table 1. Biomass moisture and leaf contents of the studied *Poaceae* species

Harvesting period	*Zea mays*		*Miscanthus giganteus*		*Phragmites australis*	
	Moisture content, %	**leaf+ panicle +husk content, %**	**moisture content, %**	**leaf + panicle content, %**	**moisture content, %**	**leaf + panicle content, %**
October	53.0	47.0	52.1	39.0	72.1	26.3
November	46.3	40.0	48.0	37.7	65.2	25.0
December	42.3	35.0	43.0	21.3	44.5	22.4
January	31.5	29.9	31.6	16.4	37.6	19.9
February	28.8	25.4	23.0	12.2	33.0	17.6
March	12.1	18.3	12.8	8.9	21.8	16.0

The particle size distribution affects the flow ability, heating, diffusion and rate of reaction.

Analysing distribution on particle size in milled chaffs, Tab. 2, it can be stated that the highest content of particles larger than 5 mm was in common reed and corn chaffs, the lowest – in giant miscanthus chaffs. The fine particle fractions, below 3 mm, were optimal in common reed chaffs (43.1%) and very high in the milled giant miscanthus (53.4%). In the case of corn milled chaffs, we obtained the highest percentage of particles larger than 3 mm (60.4%), and the lowest values for the particles of 1 mm (3.9%). This is probably an effect of the morphological nature of corn stalks, the high level of pith microstructures, influences the passage of particles through the sieve meshes.

Table 2. Particle size distribution of milled chaffs of the studied *Poaceae* species, %

Particle size	*Zea mays*	*Miscanthus giganteus*	*Phragmites australis*
<5mm	25.7	17.0	27.6
4-5mm	19.5	14.7	16.7
3-4mm	14.9	14.9	17.5
2-3mm	18.0	18.5	15.5
1-2mm	17.5	21.1	17.3
1mm	3.9	13.8	10.3

Physical and mechanical properties of biomass and prepared briquettes form studied species are presented in Tab. 3. The bulk density of chopped plant material varied from 87 kg/m^3, in corn chaffs, to 146 kg/m^3, in giant miscanthus. The milled and particle distribution in samples

with *Phragmites australis* and *Miscanthus giganteus* influenced positively the increase in bulk density of milled chaffs up to 153-167 kg/m^3, versus corn chaffs – 100 kg/m^3. Other authors, noted that the bulk density of chopped *Miscanthus giganteus* plants was 111 kg/m^3 [11], corn residues – 81.61 kg/m^3 leaves, 127.32 kg/m^3 stalks and 282.38 kg/m^3 cobs [8], reed stems – 60 kg/m^3 [5], which creates significant transportation costs.

Ash, as a solid residue of combustion, plays an important role in conversion efficiency and corrosion processes of combustion equipment and its auxiliary devices. Comparing the obtained results on ash content (Tab. 3), one can add that the highest average amount is contained by *Zea mays* and *Phragmites australis* phytomass (4.40% and 4.71%), the lowest average ash content was found in *Miscanthus giganteus* biomass (2.51%). The greatest ash level reported in research studies conducted by other authors, 8.8% of miscanthus biomass [12], 6.0% corn stalk [13], 6.5-8.0% in reed stems and 8.0-13.2% in reed leaves [14].

We could mention that the gross calorific value of studied biomass varied significantly (17.8-19.3 MJ/kg), *Miscanthus giganteus* and *Phragmites australis* have high gross caloric values.

The briquettes produced from tested *Poaceae* species were very solid and not cracking, their specific density reaching values of 831-923 kg/m3 and bulk density 430-501 kg/m3. The specific density of briquettes from *Phragmites australis* was 831 kg/m3, *Miscanthus giganteus* – 882 kg/m3 and corn stalks – 923 kg/m3. The estimated net calorific value of prepared briquettes form *Poaceae* species ranged from 14 MJ/kg to 15.8 MJ/kg.

There are different results reported in research studies conducted by other authors. The greatest gross calorific value of *Miscanthus giganteus* biomass 20.3 MJ/kg was obtained in Czech [15]. The yield of Estonian reed beds were 8.1-9.1 t/ha, moisture proportion 20.5-26.4%, 2.1-4.4% ash, 18.6-19.2 MJ/kg gross calorific value, 17.5-18.0 MJ/kg net calorific value and energy density at 20% moisture content reached 13.7-14.9 MJ/kg or 3.8-4.1 MWh/t [10], in Latvia the reed biomass reached 19.0 MJ/kg gross calorific value and 15.94 MJ/kg net calorific value, the average ash content was 2.76% [16], in Poltava, Ukraine, in November-March, the ash content of *Phragmites australis* plants varied from 9.77% to 3.91%, the gross calorific value – from 18.9 to 19.7 MJ/kg and the net calorific value – from 15.2 to 16.5 MJ/kg [7]. The calorific energy distribution in *Phragmites australis* plant organs was 17.933 MJ/kg in the main stem, 18.274 MJ/kg in leaves, 18.482 MJ/kg in dry glumes collected in Greece [17]. The fuel analysis for biomass showed that the low heating value of miscanthus biomass was 17.8 MJ/kg, common reed biomass – 17.7 MJ/kg and maize straw 16.8 MJ/kg, ash proportion – 2.7%, 8.8% and 5.3%, respectively [4]. The briquettes from *Miscanthus sinesis giganteus* reached specific density of 850 kg/m^3, durability 91%, ash content 3.2% and calorific value 16-18 MJ/kg [18].

In dependence of particle size the specific density of reed briquettes varied significantly, the minimum of density 0.87 g/cm^3 have briquettes with particle size 12-13 mm, but maximum density 1.03-1.04 g/cm^3 two particle sizes <0.5 mm and 32-33 mm [19]. The quality of briquettes made from corn stalks were: 20.8 MJ/kg gross calorific value and 20.2 MJ/kg net calorific value with 811 kg/m^3 specific density [13], the bulk density ranged from 300 to 500 kg/m^3 [20].

Table 3. Some physical and mechanical properties of biomass and briquettes from studied species

Indices	***Zea mays***	***Miscanthus giganteus***	***Phragmites australis***
Bulk density of chopped chaffs 7-35 mm, kg/m^3	87	146	107
Bulk density of milled chaffs 10 mm, kg/m^3	100	167	153
Ash content of biomass, %	4.40	2.51	4.71
Gross calorific value of biomass, MJ/kg	17.8	19.3	18.9
Specific density of briquettes, kg/m^3	923	882	831
Bulk density of briquettes, kg/m^3	501	488	430
Net calorific value of briquettes, MJ/kg	14.0	15.8	15.5

Conclusions

The studied *Poaceae* species differed significantly in the rate of stem defoliation and dehydration, ash content and calorific value of biomass. *Miscanthus giganteus* stems defoliated and dehydrated more rapidly than *Phragmites australis*.

The bulk density of the milled chaffs of tested species varied from 100 kg/m^3 (*Zea mays*) to 167 kg/m^3 (*Miscanthus giganteus*).

The specific density of briquettes reached 831-923 kg/m^3, but the bulk density of briquettes 430-501 kg/m^3, the net calorific value varied from 14.0 to 15.8 MJ/kg, the ash content from 2.51 to 4.71%.

Miscanthus giganteus was characterized by high density of milled chaffs, high calorific value and low ash content; *Phragmites australis* briquettes – by moderate calorific value, optimal density and high ash content; *Zea mays* briquettes – high ash content, bulk and specific density.

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Effects of Conservative Tillage on Soil Quality and Crop Productivity in Moldavian Plateau

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Abstract

The objective of this study is to assess the impact of conventional and no-tillage systems on physical properties and winter wheat crop production. Aggregate stability was measured by the wet-sieving method. For soil analysis, three soil depths were sampled: 0-7 cm, 10-20 cm and 20-30 cm, but for microcomputed tomography only samples from 20-30 cm depth were analysed. In spring and after harvesting, disturbed composite soil samples from each of these three depths were taken using an auger. To collect a composite sample, soil samples were taken at three randomly selected points in each treatment plot and mixed. The tillage treatments include: (1) No-till: direct drilling in untilled soil with a disc drill without previous removal of residues, using a FABIMAG FG01 seeder (NT); (2) a reduced system using a chisel after harvest at 22-25 cm depth, without overturning the furrow (RT); (3) mouldboard ploughing after harvest to a soil depth of 28-30 cm and disking twice (CT). NT conservation tillage practices consistently increased soil bulk density, 1.47 g cm^{-3} in the spring of 2018 and 2019, on 0-30 cm depth, but the values remained below the critical limit that may compromise crop growth. In 2017 the mean of winter wheat (*Triticum aestivum* L.) yield was significantly higher (LSD 5%) where wheat was planted in NT system (5430 kg ha^{-1}) compared with control treatment, plough at 30 cm depth (4366 kg ha^{-1}).

Keywords: no-tillage, soil porosity, microcomputed tomography, water stable aggregates

Introduction

Conservation tillage, which is generally defined as minimal soil disturbance resulting in a residue retention of at least 30% and which in Romania is becoming an economical viable option for the farmers but also conserving energy and providing favourable soil conditions is an important issue for sustainable crop production. Introduction of water-conserving tillage practices are considered to be an important adaptation measure.

The biggest and the most important negative consequences of modern agricultural is probably the soil physical degradation with consequences in compaction and soil erosion, which is attributed to deep and intensive tillage practices [1].

Soils simultaneously provide multiple ecosystem functions which are of critical importance in terms of climate regulation and fertility maintenance [2]. Conservation tillage practices are recognized for their advantages in reducing input costs, enhancing water use efficiency, and preserving soil carbon [3].

After several successive years applied of these reduced or no-tillage systems, subsoil compaction at 15-30 cm depths appear to be higher due to the increase of bulk density (BD) resulted by water infiltration and seeding and combine harvester machineries [4].

The stability of soil aggregates represents a key indicator of soil quality, heaving a great significance in both agricultural and ecological soils. The methods for determining soil aggregate stability seems to be hindered by a lack of instrumentation and standardized procedures.

Soil compaction involves changes in bulk density and porosity. Decrease in soil porosity has been widely reported in mechanically compacted soils [5].

Methodology

The study site was located at Ezareni Farm, Iasi County (47º07'N, 27º30'E). Site annual average precipitation and annual temperature were 517.8 mm and 9.4 ºC, respectively, during the experiment. The experimental site was cropped with a rotation of soybean (*Glycine max* L. Merr.), winter wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.*)* but in this work we will approach only the wheat plots. Each year, the wheat was sown at the beginning of October and was harvested at the in July of the following year. Weed control was achieved by using recommended rates and timing of appropriate herbicides.

In no-tillage variant all biomass, except harvested seeds, was evenly distributed across the plot to cover the surface.

The experiment was a "complete block design" with three replicates.

Meteorological data to calculate monthly rainfall and meant temperature was obtained from a local meteo station (iMetos). The experimental site received a total rainfall between 587.2 mm and 751.8 mm during 2018 and 2017, respectively.

The soil under investigation is classified as Cambic Chernozem (WRB) with a clay-loamy texture at the surface. Initial physical characteristics of the soil on 0-20 cm depth were: bulk density 1.33 $g.cm^{-3}$, pH (1:2.5) 6.8, clay 364.2 g.kg-1, silt 263.2 g.kg-1, sand 372.6 g.kg-1, humus 2.7%.

Soil sampling was carried out in spring between 2017-2019, when undisturbed and disturbed soil samples were collected from the winter wheat plots. Soil aggregate samples were collected from a depth of 0-10, 10-20 and 20-30 cm under each treatment in each plot. The three random point's aggregate samples of the same depth in each plot were deposited into one box. Initially the aggregate samples were air-dried and crushed along the natural cracks. Water stable aggregates (WSA) were measured in disturbed samples, whereas undisturbed samples were used for BD.

Soil BD was determined on an oven-dry basis by the core method. To determine bulk density, undisturbed 100 cm^3 core samples of 5 cm diameter were taken from all the three variants in March 2019, in increments of 10 cm.

The samples for determining were previously prepared by air-drying at room temperature for six weeks. For the wet sieving 4.0 g of air-dried soil aggregates having a predetermined value of mean weight diameter equal to 1.5 mm (an average value of the selected soil aggregates between 2- and 1-mm sieve openings) were used. The aggregates were then placed on a sieve with mesh size of 0.25 mm, directly immersed without pre-wetting and sieved in a stainless steel can containing distilled water for 3 min using a device commercially available as wet sieving apparatus (08.13, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The soil retained in the sieve was immersed again in a sodium hexametaphospate solution (2 g L^{-1}) for 15 min and 35 cycles min-1 and the stainless-steel pan was also dried in order to calculate the WSA percent [6]. All analyses were replicated four times for each sample.

Soil porosity was quantified using a micro CT scanner (SkyScan 1172 Bruker, Kontich – Belgium), at 80 kV, 100 mA and a voxel size of 6.79 μm. In order to achieve better image quality, the samples were scanned over 360 degrees with a rotation step of 0.4 degree and the random movement parameter was set with amplitude (in number of detector lines) of 10. We

used clods of 2-3 cm diameter, from 20-30 cm depth from NT, RD and CT. Soil clods were sampled in March 2019. For the reconstruction of the cross-section images, the NRecon® (v.1.6.10.1) program package was used, and the image processing analysis was performed using CTAn® (v.1.15.4.0) software.

Wheat variety Fundulea 4 was grown at a sowing rate of 280 kg ha^{-1} and 12.5 m cm row width along the long axis of each plot in CT and RT and 17.5 cm between rows in NT, in rainfed systems. Winter wheat grain yield was harvested with Wintersteiger Delta Plot Combine (Austria) for small plots and fitted with a moisture sensor and weigh bucket. Yield was adjusted to a water content of 14%.

The ANOVA procedure was used to evaluate the significance for the split plot design in three replicates. Treatment means were separated by the least significance difference (LSD) test and all significant differences were reported at 5%, 1% and 0.1% levels to find out differences among individual treatments. For WSA Duncan test was used.

Results and Discussion

Regarding soil physical properties, in this work, we present data from the spring period of 2017-2019. Key soil physical property indices include soil bulk density, aggregate stability, and soil porosity.

Soil porosity

Soil porosity and pore-size distribution changes in response to compaction are important for heat, water, and air flow in soils. The response of total porosity to tillage systems was less consistent than the responses of other soil physical properties (Table 1). Soil total porosity ranged from 54.31 to 56%.

Table 1. The influence of tillage systems on Total porosity and Pores categories

Variant	Total porosity (%)	Pores <50 µm	Pores 50-250 µm	Pores 250-750 µm	Pores >750 µm
NT	54.31	42.73	50.70	5.80	0.78
RT	56.00	38.18	43.46	12.29	6.07
CT	55.25	30.26	39.71	15.47	14.56

Analysing the pores with diameter smaller than 50 µm, characterized as storage pores, we can observe that in NT increased this category with 12.46% and RT with 7.92%, compared to the control (CT). Also, the volume of pores with the diameter between 50-250 µm, responsible for water transmission, are higher in both conservative tillage variants compared with CT. The tendency was contrary in the for the fissures (pores >750 µm). In conclusion, pores of different sizes are not reduced proportionally during the soil compaction process and large pores are preferentially reduced due to compaction.

Soil Bulk Density

Soil bulk density is probably the most frequently measured soil quality parameter in tillage experiments. BD of soil as a major indicator of soil physical state and effect of all investigated factors on it was estimated at the end of the growing season.

In the spring of 2017, the lowest BD value was registered at the soil surface (0-10 cm) on RT variant (1.26 g cm^{-3}). As average on 0-30 cm the also the RT had the lowest value (Table 2).

NT conservation tillage practices consistently increased soil bulk density, 1.47 g cm^{-3} in the spring of 2018 and 2019, on 0-30 cm depth, but the values remained below the critical limit

that may compromise crop growth. Analysing the 20-30 cm layers we observed that the biggest values have been provided also by NT (1.59 g cm^{-3}) in the spring of 2018.

Table 2. The influence of tillage system on BD

System	Depth (cm)	Bulk density (g cm^{-3})		
		2017	**2018**	**2019**
NT	0-10	1.33	1.35	1.32
	10-20	1.45	1.48	1.53
	20-30	1.58	1.59	1.56
Mean		**1.45 (ns)**	**1.47 (*)**	**1.47 (*)**
RT	0-10	1.26	1.19	1.25
	10-20	1.30	1.36	1.33
	20-30	1.39	1.39	1.41
Mean		**1.32 (o)**	**1.31 (oo)**	**1.33 (ns)**
CT (control)	0-10	1.27	1.29	1.28
	10-20	1.41	1.41	1.39
	20-30	1.52	1.53	1.49
Mean		**1.40**	**1.41**	**1.39**
LSD 5% =		0.083 g cm^{-3}	0.056 g cm^{-3}	0.081 g cm^{-3}
LSD 1% =		0.137 g cm^{-3}	0.093 g cm^{-3}	0.134 g cm^{-3}
LSD 0.1% =		0.257 g cm^{-3}	0.174 g cm^{-3}	0.250 g cm^{-3}

(*), (o) – indicate significant at 5% level of probability and (oo) at 1% level / ns = not significant (NT = no-tillage, RT = chisel tillage and CT = conventional, plough at 30 cm)

Water stable aggregates

Overall, conservation tillage practices are expected to improve soil structure due to reduced soil disturbance and residue retention [7].

Soil structure is a fundamental property of agricultural soils and aggregated soil structure plays a crucial role in increasing resistance to soil erosion, water conservation and crop growth.

A well-structured soil contributes to soil productivity and to agricultural sustainability. Good soil structure relies on the presence of stable aggregates.

The wet sieving and previously fast wetting techniques of soil aggregates seems to be one of the most used, but it has several disadvantages: very fast wetting of sample causes the disruption of soil aggregates due to pressure build-up of entrapped air inside of the aggregates.

The basic statistical results indicate that WSA is synergetically affected by the soil tillage system (Table 3).

Table 3. The influence of tillage system in WSA (%) – 2019

System	0-10 cm	10-20 cm	20-30 cm	Mean
NT	70.37	81.13	83.17	78.22**a**
RT	77.98	80.38	78.17	78.84**a**
CT	73.65	66.05	68.43	69.38**b**

Means for groups in homogeneous subsets are displayed – Subset for alpha = 0.05 Means off all treatments are separated by different letters when interaction effect was significant – Duncan

Aggregate stability is the most sensitive indicator of soil structure. Both conservation tillage practices increased WSA, regardless tillage intensity compared with CT. Analysing the average

values on 0-30 cm horizon, the largest effect size took place under chisel (78.84%), followed closely by direct seeding treatment (78.22%) as compared to the control (CT) – 69.38%.

Similarly, a study on silty soils in Argentina found that aggregates were 30% more stable with NT, compared to the control (CT) [8].

Winter wheat yield

Extreme climatic events like drought are becoming common and have more impact in rain-fed agriculture. In 2017 the mean of winter wheat (*Triticum aestivum* L.) yield was significantly higher (LSD 5%) where wheat was planted in NT system (5430 kg ha^{-1}) compared with control treatment, plough at 30 cm depth (4366 kg ha^{-1}). In 2018 there were no significant difference between those three treatments, NT, RT and CT, production ranged from 5350 kg ha^{-1} to 6250 kg ha^{-1}. In the last year the winter wheat yield ranged from 3245 kg ha^{-1} in chisel treatment to 3600 kg ha^{-1} in plough at 30 cm treatment. Unfortunately, the lower yield from 2018/2019 is due to the extreme drought from the autumn of 2018 and also inappropriate conditions from the spring of 2019.

Conclusions

Generally, our results firmly established the hypothesis that reduced tillage would improve physical properties.

In some situations, decreases in crop yield in response to long-term no-tillage adoption may be possibly caused by reduced seed germination and emergence, not optimal soil temperatures, below-optimal plant populations, deficient weed control, delayed plant maturity, and high kernels moisture content.

When we must choose the right tillage system, not only the arguments regarding the yield should be considered, but also the long-term view in order to ensure the conservation of soil and water resources and also the economic benefits for reduced and no-till systems and reported higher energy efficiency.

The effect size of water stable aggregates was significantly greater under no-till. In our study, NT and RT had little effect on total porosity, which may be due to improved aggregate stability from conservation tillage practices

Acknowledgements

Authors acknowledge the logistic support from Competitiveness Operational Programme (COP) 2014-2020, under the project number 4/AXA1/1.2.3. G/05.06.2018, SMIS2014+ code 119611, with the title "*Establishing and implementing knowledge transfer partnerships between the Institute of Research for Agriculture and Environment – IAȘI and agricultural economic environment*".

We would like to thank all anonymous reviewers for reading the paper carefully and providing thoughtful comments, which greatly valued this version of the manuscript.

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Research Regarding the Influence of Drying Agent's Velocity and Temperature on the Work Process of Sunflower Seed Dehydration

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Abstract

For the proper progression of the work process for cereal drying it is necessary that the distribution of the thermic agent should be uniform and steady through the product layer, so that the variation of the product's humidity and temperature could be similar in all the layer's spots.

The purpose of this paper is to obtain a uniform distribution of heat in the product along with a close variation of its humidity on the layer's thickness.

In order to achieve those proposed, sunflower seeds with different humidity's were subjected successively to be dried, in three adjoining cells with a total thickness of 150 mm. To fulfil the objective, there was an installation designed and built for dehydration of cereal seeds in laboratory conditions. During the research, was monitored the influence of the structural and functional parameters of the installation for dehydration on the variation of humidity.

The results of the experimental researches highlight the conversely proportional variation of moisture in the three layers while increasing the drying agent's speed and temperature. Values of layers humidity's have varied evenly for temperatures up to 60 ^{o}C.

In the present research work, we demonstrated that with decreasing humidity the porosity increases in the product layer, the drying agent speed increases, the drying agent temperature decreases, and the drying agent humidity increases.

Keywords: sunflower, dehydration parameters, seed drying

Introduction

The cereal and technical plant seeds are basic elements in nutrition, due to their content rich in proteins, lipids, carbohydrates, minerals, their consumption being beneficial to health. [1]

Because of the humidity that the agricultural seeds have after the harvest, their perishability is very big or medium, so, they may be used only after the dehydration, an advantageous method of conservation of agricultural seeds for longer periods of time. [2]

The drying objective is the humidity decrease of the seed mass, up to a balanced or critical one, at which the storage may be possible for long time, without losses. [3]

The phenomena that take place due to the physiological processes running during the storage, with different intensities, mostly unwanted and with particularly serious consequences, are caused by the too high humidity of the stored seeds. [4]

There are many systems of seed storing, but the storage in dried state is actually the most largely used one, regardless the destination, because in these conditions the physiological processes are running at an extremely little intensity and the microorganisms do not find growing conditions, the system thus being efficient and economical. [5]

The working process for the controlled drying of the agricultural seeds proved to be beneficial for the possibility of storing and using them for long periods of time, as long as there are solutions for technical installations for seed drying, with superior output and which can preserve the nutritive and sensory properties. [6]

One can prove that by using relatively simple equipment's, the drying process may be managed so that the finite products should be of best quality and the costs as low as possible. [7]

Many authors of different researches have stated that the drying agent temperature, its velocity and humidity have the greatest influence on the drying process. [8]

The purpose of this paper is to obtain a uniform distribution of heat in the product layers along with a close variation of its humidity on the layer's thickness.

For the experiment sunflower seed were submitted to dehydration in three adjoining cells (50 mm thickness each) with four initial moisture contents (21, 19, 17, 15 %) through five temperatures (40, 50, 60, 70, 80 °C) and four fan speeds (1, 1.5, 2, 2.5 m/s).

Methodology

The heat is brought into the product layer by means of hot air (convection). Water vapor produced are taken out of the air, which is the mass transfer medium.

Once the heat penetrates the grain mass, the mass transfer (water) starts inside the product to its surface.

The water can easily reach the surface of the product or product easily occurs the phenomenon of evaporation. [9]

The water then moves under the influence of capillary forces and due to shrinkage of the product during dehydration.

The experiences of drying products were made in the Department of Agricultural Mechanization of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Iaşi, Romania, using laboratory facilities for drying agricultural products.

The laboratory dryer allows control and monitoring of the drying process parameters that can be chosen by the user before or during the drying process. The technical characteristics of the dryer are shown in table 1.

Humidity of the drying agent is monitored using moisture sensors mounted in two positions: before the cereal layer and after.

The speed of the drying agent is controlled by the automation system, by varying fan speed.

The control of speed of the drying agent is made by using the hot wire anemometer TROTEC TA 300. During the research, we observed the influence of the structural and functional parameters of the installation for drying, the variation of humidity in the three layers of seeds up to the humidity of 8%.

Table 1. Dryer technical features

No.	Technical features	Values
1	Reaching drying temperature	110ºC in max 20 min.
2	Thermostatic temperature programming	+5ºC...+120ºC
3	Programming the thermostat time	0...999 min.
4	Thermostatic accuracy	+/-2ºC
5	Temperature sensor input and output	PT100
6	Humidity Sensor input and output	SHT 25+/-2%.
7	Power consumption up to the programmed temperature	3500W
8	Power consumption for temperature maintenance scheduled	max.550W
9	Air flow resistance before heating	min. 900 m3/h
10	Dryer inlet air pressure	min. 980Pa
11	Engine power drive centrifugal fan	2,2 kW

Results and Discussions

In order to achieve those proposed were subjected successively sunflower seeds to be dried with 21, 19, 17, 15 % humidity in the three adjoining cells with a total thickness of 150 mm.

By varying the speed and temperature of the drying agent between 1 and 2.5 m/s and between 40 and 80 °C were studied a total of 20 experimental variations for each initial moisture content.

In the case of the sunflower seeds the drying time gradually increased at the same time with the air velocity and temperature decrease.

Generally, the drying time for sunflower seeds was much longer than the time values obtained for cereal seeds drying, regardless the initial humidity of the seeds.

This is due to the low conservation humidity for the sunflower seeds, that is to the STAS.

The variations of the final humidity values in the seed layers I and II followed mostly the same patterns as in the case of the other studied seeds.

Unlike the cereal seeds, the variant with uniform drying, in which the humidity values of seed layers I and II didn't drop very much in comparison to the third one, was obtained for the air velocity of 1 m/s, at a temperature of 40ºC.

The lowest humidity in the first seed layer, of 2.86%, was reached at the drying agent velocity of 1.5 m/s at a temperature of 80ºC. For the 1.5 m/s velocity and the 80ºC temperature the humidity of the second seed layer recorded the value of 3.71%.

The energy consumption per mass unit was calculated turning the active power into kWh and reporting the consumption to the seed mass.

The energy consumption varied according to a very well-defined pattern, in the following way: its values decreased as the air temperature increased and they increased as the velocity of the thermal agent got higher. For the both boxes the minimum energy consumption was recorded at the thermal agent velocity of 2.5 m/s and the temperature of 80ºC.

It is evident the fact that unlike the studied cereal seeds, the maximum of energy consumption for sunflower seed drying, especially for those with an initial humidity of 19%, was also obtained for the same thermal agent temperature of 40ºC, but at a velocity of 2 m/s.

The protein content of the sunflower seeds varied mostly in the same way as in the case of the corn seeds.

It was drastically affected during the drying process at the air velocity of 1 m/s and at temperatures of 60ºC, 70ºC and 80ºC. For temperatures of 40ºC and 50ºC there were not changes recorded.

The protein content recorded lower values starting with temperatures of 55ºC ... 60ºC. Once the drying agent velocity increases, the protein content is no more so much affected.

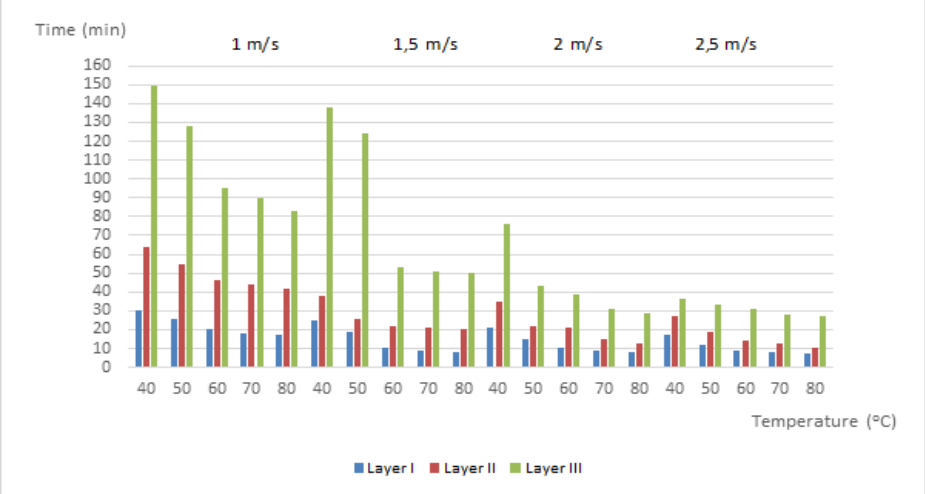

Fig. 1. Drying time variation in layers for the initial moisture content of 21%

The drastically decrease of the protein content was recorded at the drying agent velocity of 1 m/s, at the temperature of 80ºC.

In Fig. 1 are presented the variations of the drying time in all layers for the four initial moisture contents. In Fig. 2 final humidities are plotted for the three seed cells for each initial moisture content.

At the exit, the humidity follows the same downward trend in the first seven minutes due to the elimination of water. After the half of the drying time, it decreases with increasing the temperature.

The lowest humidity of the first layer was achieved at the speed of the drying agent of 2,5 m/s at 80 °C.

High values for the duration of drying were recorded at the speeds of 1 and 1,5 m/s. The decreasing trend of the drying time stands out with increasing the speed and temperature.

It was found that the most uniform drying occurred at the speed of 2,5 m/s at 40 ºC.

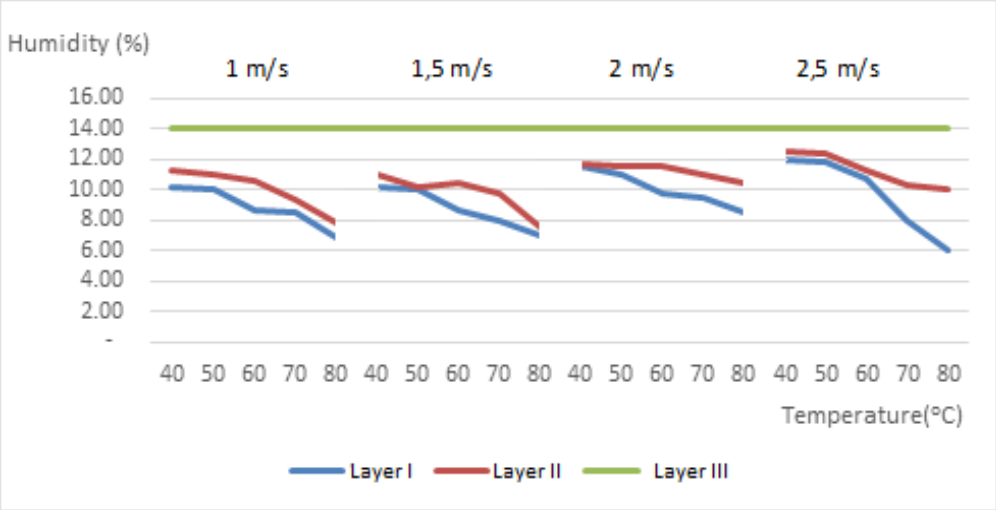

Fig. 2. Variation of final layers humidities for the initial moisture content of 21%

Conclusions

Due to the advantages, in the recent years, the use of artificial drying of grain with both hot and cold air has grown up.

During the research, it was observed the influence of the structural and functional parameters of the installation for drying, the variation of humidity in the three layers of seeds.

The conclusions concerning the optimization of the drying process for sunflower seeds by using the cylindrical box are the following:

- both the maximum and the minimum value of the drying time were obtained for sunflower seeds with the initial humidity of 15%;
- the energy consumption was reduced with 5.28% up to 10.15%;
- for the air velocity of 1.5 m/s and the temperature of 70ºC, the protein content of the sunflower seeds with initial humidity of 15% was increased with 1.81%;
- the germination power of the seeds recorded bigger values with 1.04% up to 38.46%.

Despite the fact that for the temperatures of 60ºC, 70ºC and 80ºC, the protein content and the germination power of the sunflower seeds were increased, the optimal drying variant was chosen for the drying agent temperatures of 50ºC, as there were not recorded low values reported to the witness.

After the statistical analysis, significant differences for the protein content and the germination capacity of dried sunflower seeds, in both drying boxes, were obtained for the air temperature variation.

For the air velocity variation there were no significant differences obtained neither for the protein content nor for the germination capacity of dried sunflower seeds.

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Studies Regarding CFD Simulation of the Clearing Process for the Grape Raw Juice in a Hydrocyclon

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Abstract

The separating unit operation of solid particles from suspensions by utilization the hydrocyclon is increasingly used in the food industry. The hydrocyclone is widely used in industry, especially for the separation of minerals and chemicals, due to its constructive simplicity, small dimensions, large flow rate and reduced maintenance costs. Due to these advantages, hydrocyclones are used in the food industry for various separations. For the design of new constructive types of hydrocycles, was used CFD (Computerizing Fluid Dynamics) simulation. The paper includes a series of researches regarding the CFD simulation of the process of clearing the raw grape juice. Also, the studies can be used to optimize the geometric shape of the hydrocyclone used to clear the raw grape juice, respectively to increase the efficiency of separation of solid particles.

Keywords: grape juice, clearing, hydrocyclone

Introduction

The hydrocyclone is a device for clarifying or separating solid particles, which are suspended in a liquid, based on the difference in volume mass. This device is widely used in industry, especially for the separation of minerals and chemicals, due to its constructive simplicity, small size, large working capacity and reduced maintenance costs [1, 2].

The centrifugal force developed in the hydrocyclone can be hundreds of times greater than the gravitational force. The quantification of the separation degree of the solid particles in a hydrocyclone is achieved by using the distribution curve. This curve shows the material fraction with a specified size when feeding the hydrocyclone, relative to the particles collected at its base. In general, the distribution curve decreases monotonously with the particle size and tends asymptotically to a certain value called "bypass" [3].

Up to now, many studies have focused on the analysis of fluid flow in hydrocycles and few researchers have studied the motion of solid particles [4, 5, 6, 7].

Several authors [8, 9] published an experimental investigation using a high-speed motion analysis system (HSMA) to track the trajectories of solid particles, and more recently they analysed the dynamics of the particles using the Lagrange stochastic model.

Studies on the motion of particles of organic nature in a hydrocyclone have been carried out by Medronho R.A. *et al*., [10] which aimed at the separation of microorganisms and mammalian cells, and Hsu C.Y. *et al*., [11] CFD simulated the trajectory of potato starch particles of different sizes, at different working pressures.

Most of the papers addressed the CFD simulation for determining the flow and trajectory of particles in hydrocycles with conventional geometry of various dimensions.

Methodology

In order to optimize the operating process of the hydrocyclone, experimental studies were performed, through CFD simulation, of the discrete phases (DPM – "Discrete Phase Model"), by which the trajectory of the separated particles inside it is determined.

Figure 1 represents the geometry and components of the hydrocyclone used in CFD simulation are shown in

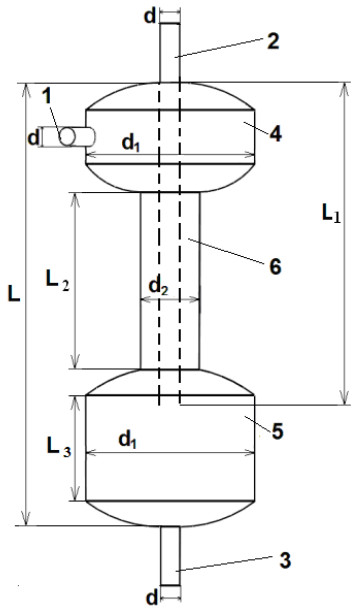


Fig. 1. Geometry of hydrocyclone
1 – suspension supply pipe; 2 – partially clarified liquid evacuation pipe; 3 – pipe for purging solid particles; 4 – superior body of centrifugation; 5 – inferior body of sedimentation; 6 – intermediate body

The dispersion of solid particles due to turbulence can be described using the Lagrange model of probabilistic (stochastic-random) tracking, which includes the effect of instantaneous turbulent velocity variations on the particle trajectory. In the FLUENT software, the mathematical model by which the trajectory of a particle can be simulated as a discrete phase in a liquid plus solid mixture medium, is realized by integrating the balance of forces on a particle. The buoyancy force and sedimentation force are calculated in a Lagrangian reference frame, and the balance of these forces can be written (in the vertical direction) as follows:

$$\frac{d\vec{u}_p}{dt} + F_D\left(\vec{u} - \vec{u}_p\right) + \frac{\vec{g}\left(\rho_p - \rho\right)}{\rho_p} \qquad (1)$$

where: $F_D(u - u_p)$ is the "sedimentation" force per unit mass of the particle, u – is the velocity of the fluid phase, u_p – particle velocity, ρ – fluid density, ρ_p – particle density.

The coupling between the discrete phase (the particles) and the continuous phase (the grape raw juice) in the FLUENT is made with a model in which the continuous phase has an effect on the discrete phase, being not valid and reciprocal, and as steps the continuous phase flow is resolved until the solution stability is reached, then the discrete phase model is solved.

Table 1 presents the conditions imposed in the simulation of the hydrocyclone that are necessary to be able to process the system of equations formed.

Table 1. Contour conditions for CFD simulation

Work sections		**Medium**	
		Fluid	***Particles***
Inlet	tangentially	u = constant	u_P = constant
Upper outlet	open	p = 0	collection
Lower outlet	open	p = 0	collection
	closed	$\frac{\partial u}{\partial n} = 0$	

(n = surface normal)

The contour condition on the inlet section of the fluid and particle mixture in the hydrocyclone is introduced as a constant velocity assumed according to the experimentally obtained data. Also, for the simulation the velocity of the juice and the particles in the grape juice are equal.

In the section of the outlet connection from the upper part of the hydrocyclone, a free-flow contour condition is imposed in the atmosphere (outflow type), where we have only atmospheric pressure (101325 Pa = 1 atm.), and the over-pressure is considered null (p = 0).

In the section of the exhaust connection at the bottom of the hydrocyclone, two boundary conditions are imposed. In the first stage of the simulation the contour condition is of solid wall, that is to say closed (the normal velocity at the wall is zero), and in the second stage, for a very short period of time, it is necessary to purge the solid particles sedimented at the base of the body is of free discharge type. in the atmosphere, where the overpressure is zero.

Table 2 presents the contour conditions resulting from the experiment and calculation are required as numerical values for the simulation.

Table 2. Experimental conditions contour

Pump revolution **n** [rev/min]	**Velocity**		**Flow**	
	u [m/s]	$\mathbf{u_P}$ [m/s]	$\mathbf{Q_\ell}$ [kg/s]	$\mathbf{Q_P}$ [kg/s]
1200	2.62	2.62	0.358	0.0372
1500	3.10	3.10	0.423	0.0441
1800	3.63	3.63	0.488	0.0508
2100	3.93	3.93	0.531	0.0553

(u – juice velocity, u_p – particle velocity, Q_ℓ – juice flow, Q_p – particle flow)

The physical parameters of the grape juice and the particles introduced into the hydrocyclone have the following values: juice viscosity η = 0,0018 Pa/s, juice density ρ_l = 1085 kg/m^3, particle density ρ_p = 1130 kg/m^3.

The flow rate of particles introduced into the hydrocyclone through the inlet connection represents 10 % from the juice flow. This flow of solid particles should not exceed 10…12%, falling within the recommendation of the FLUENT manual [12].

Experimentally, several tests were performed by varying the revolution *n* of the hydrocyclone feed pump, resulting in more flow rates and inlet velocity.

The presentation of the simulated trajectories is realized starting from the average size of a grape seed (2 mm) to smaller and smaller dimensions, reaching up to 10 micrometres.

The simulation of the displacement of particles in the hydrocyclone within a reasonable time was performed with the workstation TYAN (2XCPU-Intel Xeon 3,33GHz; RAM-16Gb DDR3).

Results and Discussion

The processing results are presented in the form of the particle trajectory, for several dimensions.

The ultimate goal is to identify the size of the separated particles in the hydrocyclone and how the evolution of these particles correlates with the turbulence of the juice when the hydrocyclone travels from the entrance to the exit.

Fig. 2 presents the trajectory of a single particle for the three simulated flow variants, shows a longer path length of the particle when the flow is higher, compared to the path length for the smallest flow.

Fig. 3 presents the trajectory of a single particle with a diameter of 500 μm for the three simulated flow variants, shows a longer path length of the particle when the flow is higher (Q_{P3}) compared to the path length for the Q_{P2} flow.

In the inferior body of sedimentation, the path length of the particle is minimal for the Q_{P1} flow, and at the Q_{P3} flow, where the motion is more sinuous, the path length is larger. Finally, for all three flows, the particles become collected in the purge pipe.

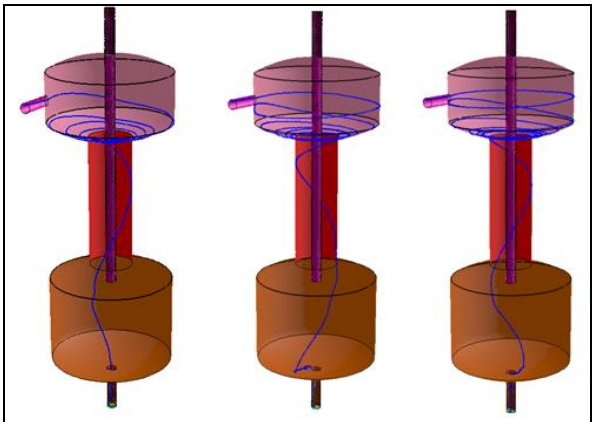


Fig. 2. Representation of a particle trajectory in hydrocyclone with d_p= 2 mm for three different juice supply flow rates

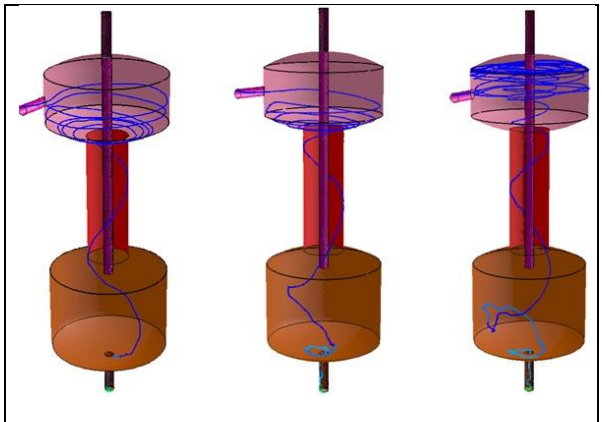


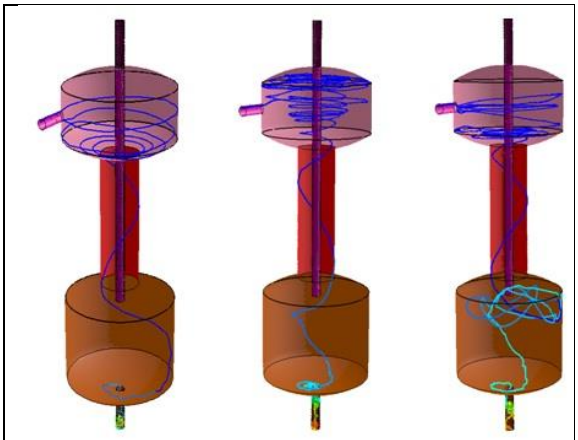
Fig. 3. Representation of a particle trajectory in hydrocyclone with d_p= 500 μm that reaches in the purging pipe area, for three different juice supply flow rates

In figure 4 it can be observed that the trajectory of a single particle for the three simulated flow variants, shows an increase of the particle circulation in the superior body, and the length of the trajectory in the inferior body is minimum for the Q_{P2} flow, average for the Q_{P1} flow and maximum for the Q_{P3} flow. Finally, for all three flows, the particles become collected in the purge pipe.

Fig. 5 represents the analysis of the trajectory of a 100 μm particle and it is observed an entrainment of the particle by the must on the outlet pipe, for all three flow rates.

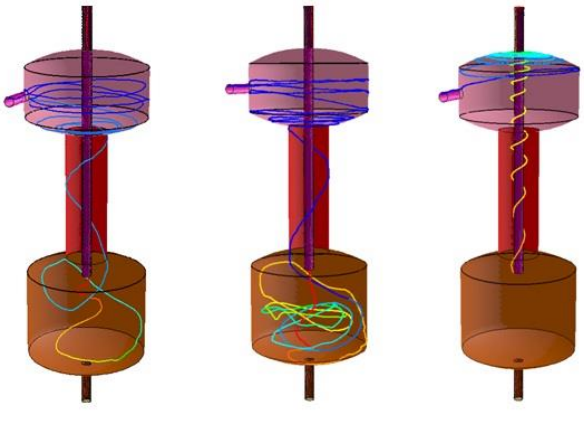
The probability of the particle being collected at the bottom is higher at the Q_{P2} flow rate compared to the other flow rates, because it reaches the lower wall, and by rubbing it loses the energy needed to move. At the Q_{P1} must flow rate, the particle has a sinuous trajectory and is very close to reaching the bottom wall of the sedimentation body, and at Q_{P3} flow, the particle being small compared to the 200 and 500 μm particles, it has a very trajectory. near the outside of the central evacuation pipeline being practically drawn inside the pipeline once it enters the lower sedimentation body.

Also, from this figure it can be concluded that the efficiency of separation for the particle diameter of 100 μm is achieved at the Q_{P2} must flow rate, fact confirmed numerically by the FLUENT software.



Q_{P1} 0.0372 kg/s | Q_{P2} 0.0508 kg/s | Q_{P3} 0.0553 kg/s

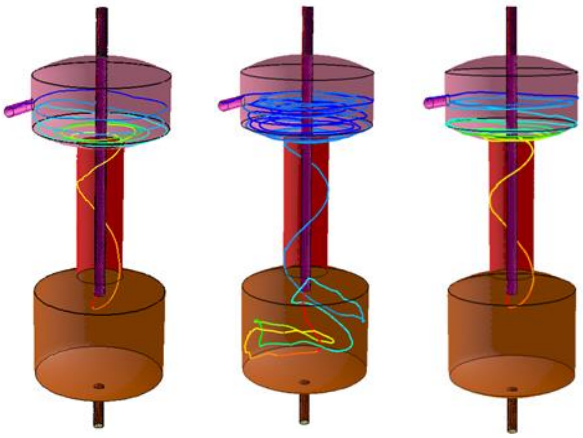
Fig. 4. Representation of a particle trajectory in hydrocyclone with d_p=200 μm that reaches in the area purging pipe for three different juice supply flow rates



Q_{P1} 0.0372 kg/s | Q_{P2} 0.0508 kg/s | Q_{P3} 0.0553 kg/s

Fig. 5. Representation of a particle trajectory in hydrocyclone with d_p=100 μm, for three different juice supply flow rates

Fig. 6 presents the trajectory of a 10 μm particle and it can be observed that the particle is entrainment by the juice on the outlet pipe for all three feed rates.



Q_{P1} 0.0372 kg/s | Q_{P2} 0.0508 kg/s | Q_{P3} 0.0553 kg/s

Fig. 6. Representation of a particle trajectory in hydrocyclone with d_p= 10 μm, for three different juice supply flow rates

At the Q_{P2} flow, compared to the other flow rates, the trajectory of the particle in the lower sedimentation body is more sinuous, but it does not reach the lower wall of this body, therefore the kinetic energy loss is lower, and finally the particle is driven by must on the outlet pipe. At Q_{P1} and Q_{P3} flow rates the particle trajectories are similar although the flow rates vary, and a possible explanation is given by the small size of the approaching particle.

Conclusions

CFD simulation was useful in operating process optimization of the hydrocyclone. As a result of these simulations it was found that at the hydrocyclone flow rate $Q_2 = 0.488$ kg/s, the best separation of the solid particles from the grape juice was achieved, compared to the other two simulated flow rates, respectively $Q_1 = 0.358$ kg/s and $Q_3 = 0.531$ kg/s.

At the same time, it has been established that separation of solid particles smaller than 100 μm is not possible, so it is demonstrated that this hydrocyclone is suitable only for removing coarse solid particles from the juice.

The use of CFD simulation for the study of the working processes of the machines having as main advantages the time saving and the reduced material resources, when using efficient hardware programs and equipment (FLUENT, TYAN workstation);

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Agro-Morphological Studies Carried Out at Some New Genotypes of Pea Garden Obtained at V.R.D.S. Buzău

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Abstract

In Romania, garden peas have been cultivated for a long time, but most of the local varieties have been lost due to genetic erosion. Over time, new varieties have been imported, with high resistance to biotic and abiotic stress and have replaced the local varieties. Fortunately, many varieties have been kept by farmers or in Gene banks. Over the years, VRDS Buzău had managed to collect genetic resources from local farmers and has a valuable germplasm collection. In this study, eight accessions were selected, from the germplasm collection, among Getica variety, control variant. The aim of this study was to evaluate part of germplasm collection from an agro-morphological point of view. Accession 1 it was characterized with the shortest flowering period, with over 65 days, being a mid-early variety; followed by A3, A5, A6 and Getica. Mid-late varieties were represented by A2, A4 and A7. The highest plant was recorded by genotype Getica with a mean of 80.40 cm, followed very closed by A5 (80.0 cm) while the lowest plant height was recorded by genotype A1 (47.85 cm). As regards pea pod length, accession A7 had the longest size (11.0 cm) and the smallest was recorded by A4 with a size of 6.7 cm. Number of ovules per pod varied from a number of 6.5 of grain (A6 and Getica) to 8.5 grain (A1, A2 and A7). Regarding weight pods per 1 m^2, the highest values was reported by 731.3 g by A3 and the lowest was also registered by A7 with 610 g. During the agro-morphological characterization, it can be concluded that there was a great variability within studied genotypes of pea.

Keywords: diversity, Pisum sativum L., accessions, germplasm, Romania

Introduction

Garden pea (*Pisum sativum* L.) is a member of the *Fabaceae (Papilionaceae)* family and is a diploid species with chromosome number 2n=14. Archaeological evidence found in Syria attest that pea was among the first domesticated species in the world [1]. Veteläinen *et al*., (2009) [2] states that the existing pea populations in Europe came from Russia, more precisely migrated from the northern Ural Mountains and Komi area. In Volga region occurs a variety of peas that grow like a weed. The pea crop is linked to a few main purposes: first, a vegetable grown for pods and seeds consumed either for fresh consumption or for canning [3]. Second, because of the high levels of carbohydrates and total digestible nutrients it is an excellent livestock feed [4]. Another good reason is like Janzen *et al*., (2014) [5] states through symbiosis, garden pea can fix atmospheric nitrogen and therefor does not need fertilizer especially since it provides nitrogen for the crop following it. From nutritionally point of view, Sepehya *et al*., (2015) [6] states that garden pea has a significant content in proteins 7.2 g, fats 0.1 g, minerals 0.8 g, carbohydrates 15.8 g, calcium 20 mg, magnesium 34 mg, phosphorus 139 mg, cooper 0.23 mg, iron 1.5 mg, sulphur 95 mg, riboflavin 0.01 mg, nicotinic acid 0.8 mg and vitamin C,

9.0 mg/100g of edible portion. Garden pea is the second grown vegetable crop from Fabaceae family, after the bean [7]. From 2010 to 2017, the crop area cultivated with garden peas has been growing steadily from 2.184.662 ha to 2.669.305 ha, with a yield of 15.985.461 t to 20.699.736 t. Main cultivated countries were China, India, USA, France and United Kingdom.

In Romania, the garden pea suffered a considerable decrease from 5.803 ha in 2010 to 3.695 ha in 2017, the yield also had a decrease from 23.313 t to 16.043 t, between the same years taken into study [8].

In Romania, garden peas have been cultivated for a long time, but most of the local varieties have been lost due to genetic erosion. Over time, new varieties have been imported, with high resistance to biotic and abiotic stress and have replaced the local varieties. Fortunately, many varieties have been kept by farmers or in Gene banks. Over the years, VRDS Buzău had managed to collect genetic resources from local farmers and has valuable germplasm collection.

In this study, eight accessions were selected, from the germplasm collection, among Getica variety, control variant.

It is essential for a breeding laboratory to own and improve constantly the germplasm collection. If the germplasm collection is not well documented, characterized, evaluated by an agro-morphological and/or biochemical point of view, then its use is limited. Also, not using the germplasm collection for breeding is a waste of resources [9]. The present study aims to evaluate part of the germplasm collection from an agro-morphological point of view. In order to preserve and improve the environmental sustainability, in the experimental plot no synthetic chemicals were used and no substance for control of diseases and pests. Thus, the behaviour of genotypes to stress and pest resistance was also evaluated.

Methodology

The experiments were carried out in the research sector of VRDS Buzau for a period of several years, and the research conducted on the accession in the study were completed in July 2019. The study aimed the evaluation of seven accessions and one control variant, Getica variety. Throghout the vegetation period, phenological determinations were made as can be seen in Table 1.

Table 1. Information of garden pea crop phenology

Accession Name	Sowing date	Germination date	Flowering period	Emergence of the first flat pod	Harvest fresh pods	Harvest dry pods
A1	21.02	18-22.03	01-06.05	13.05	30.05	17.06
A2	21.02	20-25.03	14-20.05	27.05	13.06	1.07
A3	21.02	18-22.03	07-13.05	17.05	07.05	20.06
A4	21.02	19-22.03	13-17.05	20-27.05	10.06	26.06
A5	21.02	19-22.03	06-10.05	14-21.05	07.06	21.06
A6	08.03	04.09.04	15-20.05	27.05-03.06	17.06	1.07
A7	21.02	25-31.03	12-20.05	24-31.05	20.06	8.07
Getica	21.02	20-28.03	06-10.05	14-22.05	07.06	22.06

During vegetation period, observations were made for 22 agro-morphological characters described by UPOV Guidelines [10]. In tables 2 are presented the qualitative characters targeted in the study.

The quantitative traits used for phenological character was appearance of the first flower (AFP); for morphological characters were used: plant length (PL), number of nodes including first fertile node (NN), stipule length (SL), stipule width (SW), leaflet length (LL), leaflet width (LW), petiole length measure from axil to first leaflet or tendril (PLA), pod length (PL), pod

width (PW); and for yield characters were used: number of ovules per pod (PNO), weight of 10 seeds (W), number of pods per $1m^2$ (NP) and weight of pods per $1m^2$ (WP).

Table 2. Qualitative trait

Descriptors	Polymorphism
Anthocyanin coloration of axil (ACA)	1.absent; 2. single ring; 3. double ring
Leaflet: dentation (LD)	1.absent; 3. weak; 5. medium; 7. strong; 9. very strong
Stipule: flecking (SF)	1.absent; 9. present
Stipule: density of flecking (SDF)	1.very sparse; 3. sparse; 5. medium; 7. dense; 9. very dense
Flower: colour of the wing (FCW)	1.white with pink blush; 2. pink; 3. reddish purple
Flower: colour of standard (FCS)	1.white; 2. whitish cream; 3. cream
Seed: shape (SS)	1.ellipsoid; 2. cylindrical; 3. rhomboid; 4. irregular
Seed: marbling of testa (SMT)	1.absent; 9. present
Seed: colour of testa (SCT)	1.reddish brown; 2. brown; 3. brownish green

Results and Discussions

Qualitative traits

Soldberg *et al*., (2015) [11] states that a combination of morphological and genetical characterization can identify if the material is unique or just duplicates of gene bank material. Contrariwise, Yirga and Tsegay (2013) [12] have been using only qualitative traits related to colour and flower and seed shapes to characterize pea genotypes.

Table 3. Qualitative characters of the studied genotypes

Accessions	ACA	LD	SF	SDF	FCW	FCS	SS	SMT	SCT
A1	1	1	9	3	-	2	2	-	-
A2	1	1	9	3	-	2	2	-	-
A3	1	1	9	5	-	2	1	-	-
A4	1	1	9	5	-	2	1	-	-
A5	1	1	9	3	-	2	2	-	-
A6	1	1	9	5	2	-	2	2	1
A7	2	1	9	5	3	-	1	1	9
Getica	1	1	9	3	-	1	2	-	-

The result of qualitative traits used can be found in Table 3. The presence of anthocyanin coloration of axil on stem was evident in 14.28% genotypes. Four genotypes have sparse flecking in stipule and the other four have medium sparse. The colour of the wing was present in only two varieties: one was pink and the other reddish purple. The standard colour of the flower was mainly whitish cream, one genotype was cream. Seed shape was 50% cylindrical and the other half ellipsoid. The marbling of tesla on seed was noticed only on one genotype.

Also, the colour of tesla was present in two genotypes, one was reddish brown and one was reddish green.

Phenological characterization

Phenology was represented by sowing date, germination, flowering, emergence of the first flat pod, harvest of the fresh pods and harvest of the dry pods. The most representative character is the appearance of the first flower, but this character is dependent on the environment.

Flowering is considered early when the number of days between sowing and appearance of the first flower is until 50 days, mid-early varieties fits between 65-75 days, mid-late varieties over 75 days and late varieties exceed 90 days [13]. Accession 1 it was characterized with the

shortest flowering period, with over 65 days, being a mid-early variety; followed by A3, A5, A6 and Getica. Mid-late varieties were represented by A2, A4 and A7.

Morphological and yield characterization

The highest plant was recorded by genotype Getica with a mean of 80.40 cm, followed very closed by A5 (80.0 cm) while the lowest plant height was recorded by genotype A1 (47.85 cm) as can been seen in Table 5. The difference between plant height might be due to genetic diversity and adaptability to a particular environment [14], especially because this character is dependent on environment conditions [11]. As regards pea pod length, accession A7 had the longest size (11.0 cm) and the smallest was recorded by A4 with a size of 6.7 cm. Khan *et al*., (2013) [14] states that the pod size is a genotype character and its affected by plant vigour.

Concern yield characters, it was measured the number of ovules per pods, weight of 10 seeds, number of pods per 1 m^2 and weight of $1m^2$. Number of ovules per pod varied from a number of 6.5 of grain (A6 and Getica) to 8.5 grain (A1, A2 and A7). In Fig. 1 can be seen crop details and different types of flowers.

Fig. 1. Crop details and different type of pea garden flower

The weight of 10 seeds had the highest record with Getica variety, with 4.9 g and the lowest was reported by A4 with 3.8 g. A3 had registered the highest number of pods per square meter, 247.5 pods and the lowest was recorded by A7 with a number of 120.5 pods. Regarding weight pods per 1 m^2, the highest values was reported by 731.3 g by A3 and the lowest was also registered by A7 with 610.8 g. More values can be found in table 4. During the vegetation period, the economic damage threshold was low, the A1 and A3 were the most resistance. In table 4, statistical analysis revealed a great variability within studied genotypes. The diversity is vital among breeding programs.

Table 4. Means of studied accessions and statistical analysis

Code	A1	A2	A3	A4	A5	A6	A7	Getica	Mean	SD	CV	p value
PL	47.9	62.2	72.4	77.9	80.0	67.8	58.8	80.4	68.4	10.82	15.82	< 0.001***
NN	6.5	11.0	8.0	6.3	8.3	7.9	5.3	8.0	7.65	1.61	21.01	< 0.001***
SL	8.0	9.0	8.9	8.8	9.3	7.4	7.3	9.8	8.56	0.84	9.794	ns
SW	4.4	4.5	4.8	3.6	5.0	7.9	5.7	5.6	5.17	1.19	22.96	ns
LL	5.3	6.7	5.3	6.0	6.3	5.0	4.6	6.0	5.63	0.65	11.64	< 0.002***
LW	3.0	3.5	3.0	2.9	3.5	3.7	3.3	3.6	3.30	0.29	8.91	0.1 ns
PLA	5.4	5.5	6.4	5.9	6.2	6.8	6.8	5.3	6.03	0.57	9.386	> 0.05 ns
PL	8.7	9.4	7.0	6.7	8.3	8.1	11.0	7.4	8.32	1.31	15.76	> 0.05 ns
PW	1.4	1.6	1.6	1.4	1.5	1.8	1.8	1.5	1.55	0.14	9.276	> 0.05 ns
PNO	8.5	8.5	8.0	7.0	8.0	6.5	8.5	6.5	7.69	0.83	10.75	> 0.05
W	4.8	4.8	4.3	3.8	4.6	4.0	5.8	4.9	4.63	0.58	12.49	> 0.05
NP	191.7	138	247	210	169	131	120.5	132.3	167.5	42.5	25.35	< 0.001***
WP	691.7	621	731	712	648	614	610.8	630.5	657.4	44.6	6.780	> 0.05

Conclusions

V.R.D.S. Buzau has valuable accessions in the germplasm collection, A1 and A3 had the lowest percentage of economic damage thresholds. Accession 1 it was characterized with the shortest flowering period, with over 65 days, being a mid-early variety; followed by A3, A5, A6 and Getica. As regards pea pod length, accession A7 had the longest size (11.0 cm) and the smallest was recorded by A4 with a size of 6.7 cm. Number of ovules per pod varied from a number of 6.5 of grain (A6 and Getica) to 8.5 grain (A1, A2 and A7). Regarding weight pods per 1 m^2, the highest values was reported by 731.3 g by A3 and the lowest was also registered by A7 with 610.8 g. The diversity is vital among breeding programs and the statistical analysis revealed a great variability within studied genotypes.

Acknowledgments

The work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI/2018, within PNCDI III.

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Biological Crust as Ecological Indicator of Moist Soils from Jijia Rolling Plain

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Abstract

Biological crust crusts are relatively thin, dense, more compact, hard and brittle (when dry), somewhat continuous surface layers, composed of non-aggregated soil particles on the surface of tilled and exposed soils. Considering the manner of formation, soil crust could be physical, chemical and biological. The biological soil crusts, also known as microbiotic or cryptobiotic soil crusts, are formed by living organisms and them by-products, creating a crust of soil particles bound together by organic materials. In our investigations on the soils from Jijia Rolling Hills were noted, frequently, biological crusts on the surface of clay soils, moist and even saline or sodic soils. Frequently, in biological crusts, prevail prokaryotic organisms represented by *Cyanobacteria*, especially by *Nostoc commune*, known as blue-green alga. In the field we established the representative locations for soil profiles in the rivers plain area of Jijia Rolling Plain. Diagnosis and name of the soil was done according to World References Base of Soil Resources (WRB, 2006, 2014). In the rivers field area of Jijia Rolling Plain prevail Aluviosols, Fluvic Cenozems, Mollic Gleysols), Solonet, Solonceac (Solonchaks). In our investigation, we found frequently the biological crust on the moist surface of undisturbed area by excessive grazing. Usually, biological crust has been present in the small depressions of gilgai micro-relief. The dark colour of algal crusts is due higher abundance blue green algae and to the dark soil surface layer. From ecological point of view, the biological crusts help to the increasing of soil aggregate stability reduce albedo and determine greater absorption of solar energy. One negative effect could be hampering seedling germination of vascular plants. Algal crusts are a useful indicator for highlighting soil variability in floodplain and for pointing the heterogeneity of agro-ecosystems.

Keywords: soil biological crust waterlogging

Introduction

Soil crusts are relatively thin, dense, more compact, hard and brittle (when dry), somewhat continuous surface layers, composed of non-aggregated soil particles on the surface of tilled and exposed soils [1]. Considering the manner of formation, soil crust could be physical, chemical and biological. The formation of chemical and physical soil crusts occurs by the impact of raindrops, compressional forces, evaporative processes and trapped gas bubbles [2].

Biological soil crusts or *biocrusts* are formed by diverse living communities such as cyanobacteria, algae, lichens, mosses, fungi, and other bacteria and which could be find on the soil surface. The soil *biocrust* highlight that living microorganisms and their products are the main constituents [3].

Such crusts occur on a variety of substrata ranging from exposed rock to hot deserts, arid areas, forest soils, moist meadows soils, rice fields [4].

In the soil research methodologies in the field are not presented the biological crusts, although it is a useful ecological indicator [5].

In the guide for the description in the field of the soil profile and of the specific environment conditions [6] the categories of crust that can be predicted at the surface of the soil are mentioned, but there is no classification of the biological crusts.

In our investigations on the Moldavian Plateau soils were noted, frequently, biological crusts on the surface of clay soils, moist and even saline or sodic soils.

Methodology

Our investigation was done in the rivers field area of Jijia Rolling Plain from Moldavian Plateau. The choice of locations for soil profiles was made in areas with a micro- relief, characterized by micro ridges and micro-depressions.

In our investigations were identified biological crusts, consisting of blue-green algae with gelatinous appearance. These crusts are also called the glue of the earth.

In the areas with biological crust some soil profiles have been made. Every soil profile was morphologically described according to the Methodology of soil survey [5, 7]. In the field, we studied the characteristics of micro relief, vegetation cover (plants, species, plants density) and we also took into account the presence of discontinues biological soil crusts. Disturbed and undisturbed soil samples were used to determine some soil properties such as size particles, bulk density, soluble salts, content of the exchangeable Natrium, organic carbon, pH, total nitrogen, contents of calcium carbonate, according to the current methodology [8, 9].

After the processing and analysis of the obtained data in the field and laboratory we recommend the use of the biological crust as a useful indicator for soil characterization.

Results and Discussion

In the rivers field area of Moldavian Plateau prevail a Fluvisols, Fluvic Cenozems, Mollic Gleysols, Solonetz (A and B from figure 1), Solonchaks (C from Fig. 1).

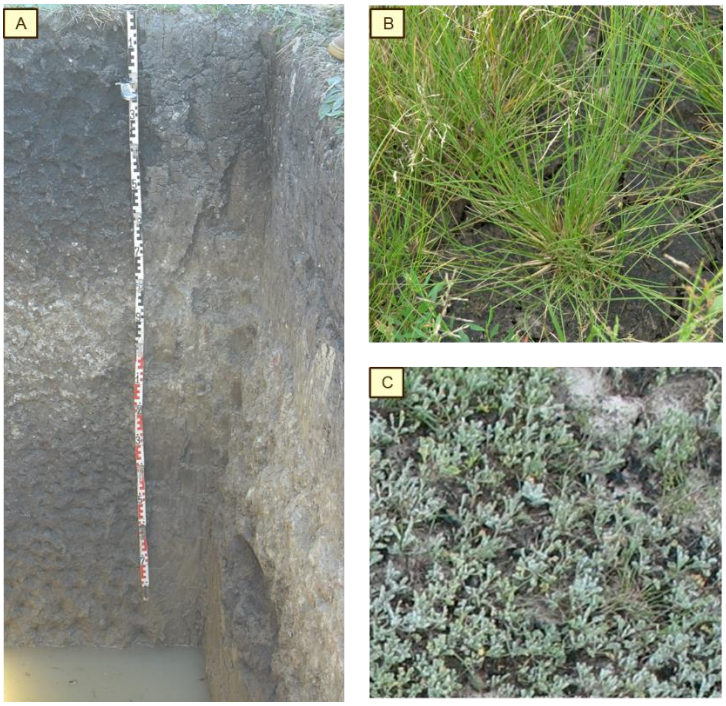

Fig. 1. Solonetz from Jijia Rolling plain (A). Biocrust on the surface of sodic (B) and saline soils (C)

Soils from middle and third part of rivers field are clayey, (frequently, clay content >40%).

The analytical data regarding solonetz are shown in the table 1.

The soil is slight alkaline up to strong alkaline, pH values are between 8.1-9.36. The alkaline reaction of the soil is due to the high content of exchangeable sodium which is between 9.3 and 28.9%.

The content of clay is higher than 50% on the depth interval 6-60cm. The organic carbon distribution gradually decreases at depth of 0 37 cm from 3.8 to 1.05%.

Calcium carbonate is present starting with depth of 61cm. We mention that the intensity of the sodification begins with dept of 12 cm. This aspect is highlighted by the massive structure, the state of pronounced compactness of the soil and by strong dispersion of the elementary particles.

Table 1. The values of some chemical properties of Fluvic Pelic Solonetz

Depth	**Clay <2µ**	**BD g/cm^3**	**ESP %**	**ss mg/100g**	**pH**	**$CaCO_3$ %**	**OC %**	**N %**
0-6	47.3	1.15	9.3	77	8.1	-	3.8	0.34
6-12	54.1	1.14	12.9	77	8.4	-	2.68	0.25
12-27	53.5	1.24	15.8	108	8.7	-	2.27	0.22
27-37	55.6	1.27	12.9	462	8.4	-	1.05	0.24
37-61	50.4	1.56	5.7	1068	8.3	-	0.31	0.12
61-80	44.0	1.56	18.8	509	8.8	3.4	-	-
80-106	36.0	1.54	25.1	378	9.27	9.0	-	-
106-119	56.6	1.49	28.9	428	9.36	9.2	-	-

ESP – exchangeable sodium percentage; ss soluble salts, BD – bulk density, OC – organic Carbon, N – Nitrogen

In our investigation, we found frequently the biological crust (A and B from fig. 2) on the moist surface of undisturbed area by excessive grazing and on the surface of weak and moderately salinized or alkalized soils.

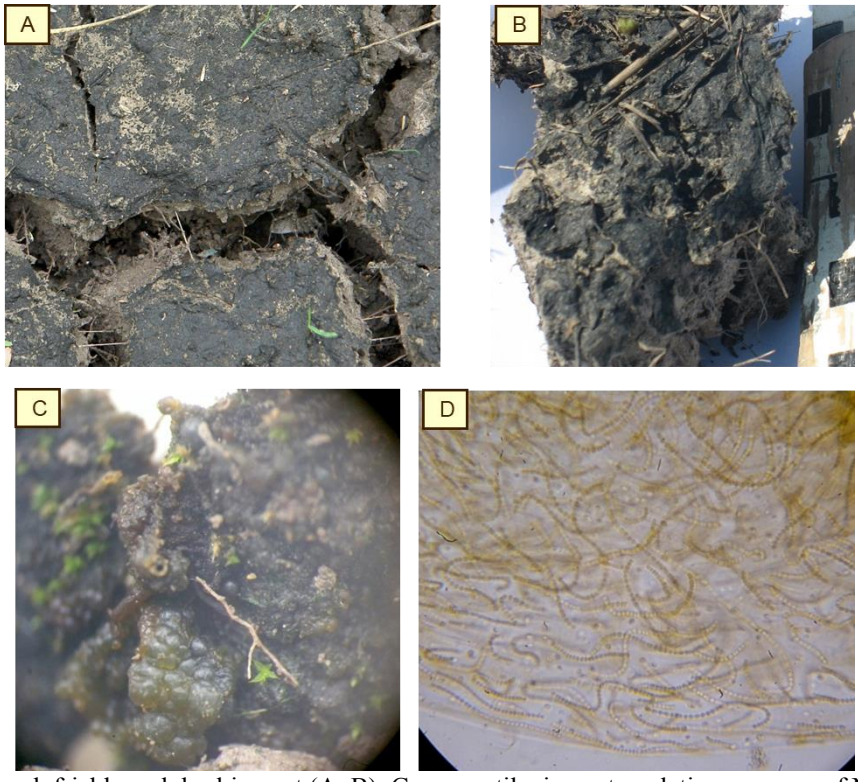

Fig. 2. Blackened, friable and dry biocrust (A, B). Green cartilaginous to gelatinous masse of Nostok sp. (C) and colonies of green-blue algae (D)

Biological crust is dark in colour. Colonies of *N*ostoc sp. (*N. commune, N gelatinosum*) are cosmopolitan and occur as free-living, blackened, friable crusts when dry and as green masses with a cartilaginous to gelatinous texture (A and B from figure 2) when rehydrated. The dark colour of algal crusts is due higher abundance blue green algae and to the dark soil surface layer.

The microscopic study showed dominance of blue – *Nostoc sp*. (C and D from figure 2).

Biological crust with *Nostoc sp.* we find on surface of slight and moderately saline soils, but more frequent in less saline wet places. It is alkaline – tolerant, with maximum development after rainfall. Biological crust within from rivers field area of Jijia Rolling Plain indicates high moisture, which may occur temporarily several times at the surface of the soil during a year.

Cyanobacteria, represented by genus *Nostoc* are free-living nitrogen-fixing prokaryotic microorganism. Another ecological function of algal crusts are contributions to the increasing of soil aggregate stability reduce albedo and determine greater absorption of solar energy. One negative effect could be hampering seedling germination of vascular plants. Algal crusts are a useful indicator for highlighting soil variability in floodplain and for pointing agro-ecosystem heterogeneity.

Conclusions

Biological crusts identified within the surfaces of the rivers field area from Jijia Rolling Plain consist mainly of cyanobacterial colonies of *N*ostoc sp. It's easy to recognize by cartilaginous to gelatinous texture and by black colour when dry or green masses when rehydrated.

Frequently biocrust with *Nostoc sp.* we find on surface of slight and moderately saline soils, but more frequent in less saline wet soils. It is alkaline - tolerant, with maximum development after rainfall.

Biological crust within from rivers field area of Jijia Rolling Plain indicates high moisture, which may occur temporarily, several times on the year, at the surface of the soil during a year.

In soil survey, biological crust may be an additional indicator high moisture, which may occur frequent on the soil surface.

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Evaluation of Odor Activity Values and Aromatic Series in Red Wines Aged with American and French Oak Chips

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Abstract

The use of oak wood during the process of wine ageing is an ancient and common practice in most of the world's wine producing regions. In this study, the contribution of a chemical compound to the aroma of a wine was evaluated by determine the odour activity value (OAV).

OAV was calculated as the ratio between the concentration of an individual compound and the perception threshold reported in the literature. The analytical aroma profile was established by using the OAVs of each odorant compounds exhibiting similar odor descriptor grouped in an aroma series. Major aroma contributors in the Feteasc㠠neagrã wine aged with American and French oak chips were the fruity, chemistry, fatty and floral series followed by the woody series.

Red wines analysed at 1.5 and 3 months present similar behaviour, however wines aged with 5 g/L of French oak chips distinguished clearly in PCA.

Keywords: Aromatic series, OAVs, American and French oak chips, red wines

Introduction

Red wine produced from Feteasc㠠neagrã (*V. vinifera*) is a popular alcoholic drink consumed in Romania known to have potential health benefits related to its phytochemical composition.

Ageing is a fundamental technological process that leads to a harmonious development of aromatic, gustatory and chromatic attributes of wine [1], thus reflecting the global quality of wine. Therefore, during wine ageing, groups of subtle reactions occur, which tend to improve the taste and flavour of wine over time. The wine chemical composition is very complex and changes continuously during ageing. Volatile compounds related to aroma have a significant impact on the quality of wine and, hence, on the consumer acceptance. Various chemical classes of compounds are responsible for the aroma wines, such as: esters and terpenes are well-known to confer to floral or floral characters [2], alcohols and aldehydes own green leafy aroma characters [3]; methoxypyrazines are strongly linked to green capsicum descriptors.

Meanwhile, C13-norisoprenoids generally contribute too many flavours [4] in fruits and wines, such as berry, tobacco, honey, balsamic and violet aromas. Ageing can modify these compounds and give wines their distinct fragrances and typicity.

The use of oak pieces aims to accelerate the chemical reaction rates (esterification, polymerization, condensation and oxidation, spontaneous clarification), which take place within wine [5]. Oak chips use in ageing has been reported previously as a method to improve the sensory attributes of young wine in a short period of time [6].

Part of the aroma in wines, is acquired from the wood during the ageing process. The role of oak wood is fundamental since it releases important compounds that have great influence on the final wine characteristics, reducing the astringency, and improving important flavour characteristics, taste, colour, phenolics and aroma [7]. The number and quantity of components

released by the wood during the ageing process will depend on the species of wood, and the individual oak chips (including seasoning, manufacture, toasting, dosage and contact time), very important factors that will affect the quality of red wines. Volatile compounds from oak wood require a powerful separation technique for their determination, such as gas chromatography, preferably coupled to mass spectrometry (GC–MS) the main technique used to obtain chemical information [8]. The extraction step previous to the chromatographic analysis is the most difficult task of the analytical methodology.

Wine aroma is the result of the volatile compounds that constitute it. Not every volatile contributes with the same intensity to aroma. The concentration-odour threshold ratio, well-known as the "odour activity value" (OAV), must be considered as the only norm to estimate the contribution of each compound to aroma, although interactions (antagonistic and additive effects) among different aroma components occur in the matrix [9]. Because an individual compound generally has several flavours, it is difficult to establish or evaluate global aroma profiles only using the odour activity values (OAVs) of volatiles. The organoleptic profiles of red wines are comprised by arranging the OAVs of the aroma compounds with identical descriptors into aromatic series. This activity reports quantitative information obtained by chemical analysis to sensory perceptions. This methodology might simply and effectively examine and compare the aroma characters.

Nonetheless, there is lack of information about the effect of oak chips during ageing on the aromatic profile of wine. Hence, further in-depth researches have to be conducted to understand the mechanisms that impact on the organoleptic features of the aged wine. Therefore, the aim of this study was to assess the impact of oak chips on ageing techniques and their influence over the OAV of red wines.

Methodology

Fetească neagră variety grapes (*V. vinifera*) were grown in North-East Romania winemaking region and harvested in 2013. The maceration-fermentation process was made at 10-12 ºC for 7 days. Afterwards, the grape skins were pressed in order to extract the remaining juice. The pressed wine was blended with the must and the mixture was pumped off into stainless steel tanks for alcoholic and malolactic fermentations. The wine obtained was divided in 8 batches with 5 L each and placed in independent glass vessels. The batches were aged with different types of oak wood chips (American and French) and different dosages (3 and 5 g/L) for 1.5 months and 3 months. The dimensions chips in centimetres were of 0.5 x 1.5 x 0.2 (width x length x thickness).

The contribution of a chemical compound to the wine aroma was evaluated by determining the odour activity value (OAV). OAV was calculated as the ratio between the concentration of an individual compound and the perception threshold reported in the literature [10].

Furthermore, the analytical aroma profile was established by using the OAVs of each odorant compounds exhibiting similar odour descriptor grouped in an aroma series.

Statistical data analyses were performed using Statgraphics Centurion XVI of StatPoint Technologies Inc. (Warrenton, Virginia). Principal component analysis (PCA) was performed using R package "ggbiplot.

Results and Discussion

Volatile compounds are essential for wine high quality, determining their aroma and varietal properties. Aroma compounds are present in wines, and flavour intensity depends on both concentration and threshold. Furthermore, just a restricted number of volatiles can be found at

concentrations high enough to be perceived (OAV ≥1) and considered as major flavour contributors or active odorants [9].

In order to evaluate the wine overall aroma, the aroma compounds were grouped into different aromatic series according to their odour descriptor and each compound was assigned to one or more aromatic series based on their similar odour character. The series used in this study contain compounds in groups with similar odour descriptors and these represent the main constituents for the aroma of Fetească neagră wines, namely chemistry, fruity, fatty, buttery, floral, green, citric fruit, toasty, spice and woody odours. The total intensities for each aromatic series were calculated as the sum of the OAVs for each of the compounds assigned to a given series. The results are represented in Fig. 1. One of the most evident differences between 1.5- and 3-months ageing was the increase in all series, especially in the fruity and chemistry series, due to the high quantity of esters formed in the alcoholic fermentation. Other differences were that, in general, French oak chips presented the highest series in comparison with series of American oak chips.

The red wines aged with American oak chips of Am3-3M (>400) and French oak chips of Fr5-3M (>650) showed relatively high levels of aromatic series, which suggested that these lastly oak types provided more powerful aroma than the other type. Meanwhile, the American oak chips of Am3-1.5M (<180) and the French oak chip of Fr3-1.5M (<210) displayed weak aroma.

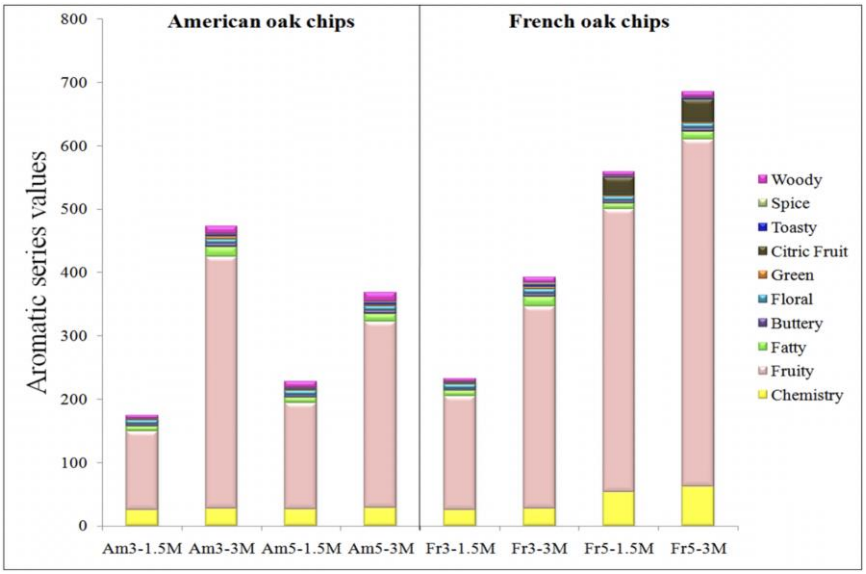

Fig. 1. Aromatic series values for American and French oak chips of aged wines. These results are shown as the mean values

The chemistry series showed a higher intensity in wines aged with American oak chips (>27) from Am5-3M and French oak chips (>60) from Fr5-3M; the fruity series showed a higher level in American oak chips (>350) from Am3-3M and French oak chips (>500) from Fr5-3M; fatty aroma was rich (>15) in American oak chips from Am3-3M and (>14) in French oak chips from Fr3-3M.

Regarding the buttery series, the maximum value was found in Am3-3M (5.44) and Fr5-3M (5.08). In wines aged with American oak chips (Am5-3M) showed the highest floral flavour (7.33), followed by French oak chips (Fr5-3M) (7.54). The wines aged with French oak chips presented the maximum values for citric fruit series in Fr5-3M (>30) and American oak chips showed values much lower (<0.7), which suggested that these types of flavour cannot be perceived by humans. The other series such as green, toasty and spice presented slight flavour

(<4.4). In addition, the woody series presented a higher intensity in wines aged with American oak chips (>14) from Am5-3M and French oak chips (>9) from Fr3-3M.

The principal component analysis (PCA) was used to evaluate the profiles of aromatic series were useful to discriminate and group the different types of wine selected in this research (Fig. 2). The first principal component (PC1) accounted for 53.8% of the total variation, while PC2 explained another 29.4%. Plots of the PCA revealed that the 10 aromatic series were scattered in quadrants I and IV, showing their positive correlations with PC1.

The wines aged with American oak chips (Am3-3M and Am5-3M) and French oak chips (Fr3-3M) for 3 months were located on the positive side of PC1 and the negative side of PC2.

Then, the wines aged with American oak chips (Am3-1.5M and Am5-1.5M) and French oak chips (Fr3-1.5M) for 1.5 months were located on the negative region of PC1 and PC2 (Fig. 2).

The other wines aged with French oak chips (Fr5-1.5M) for 1.5 months were positioned on the positive side of PC2 and negative side of PC1 and the same type of wine aged for 3 months were located on the positive side of PC1 and PC2.

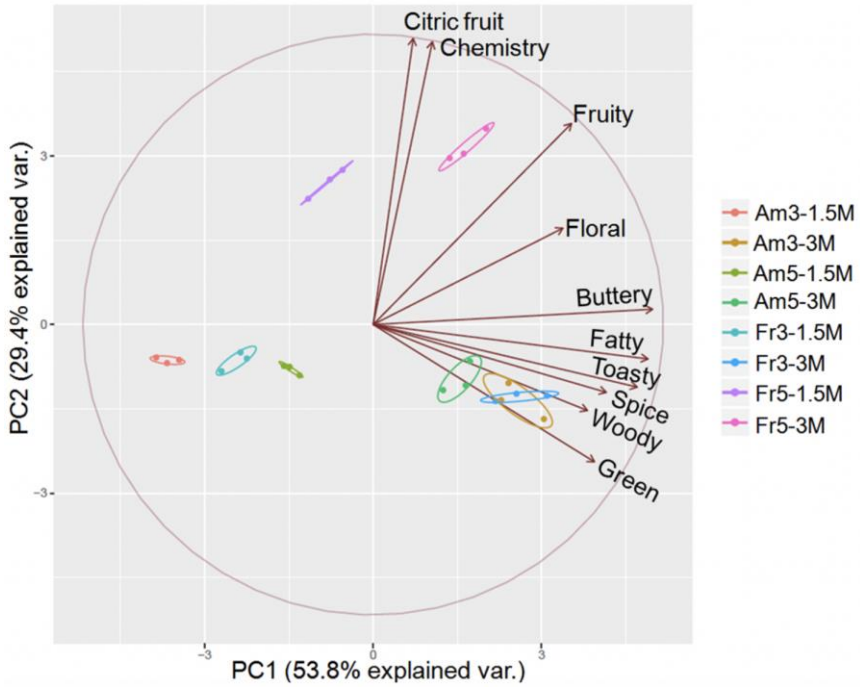

Fig. 2. Principal components analysis using as classifying variables the compounds analysed in *Feteasc*ă *neagr*ă wines

Wines produced with Am3-3M, Am5-3M and Fr3-3M were marked differently from the Fr5-3M samples and were characterized by concentrations of woody, spice, toasty aromatic series. In contrast, a wine aged with Fr5-3M was described with fruity, chemistry and citric fruit aromatic series.

Conclusions

These results confirmed the important role, during ageing, of oak wood geographical origin in the aromatic series of wines. Each wood type transmits specific aromatic components to wines, with various quantities, according to its characteristics. The wood-wine exchange generates differences in OAVs and aromatic series of aged wines. Therefore, during winemaking, producers could make wines with different characteristics. The application of alternative techniques stimulates an increase of odour activity values at 3 months, when compared to samples aged for 1.5 months.

PC1 differentiated among wines aged for 1.5 months and 3 months with two type of oak chips. PC2 separated French oak chips with 5 g/L from all other samples. However, samples aged with 3g/L French oak chips have a similar profile with American oak chips and cannot be differentiated.

Acknowledgements

This work was supported by a grant of Ministry of Research and Innovation, CNCS – UEFISCDI, project number PN-III-P1-1.1-PD-2016-0325, within PNCDI III. Additional funding was provided by the University of Córdoba Research Plan, call 2018, modality synergy (Spain).

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Genomic Selection for Disease Resistance in *Brassica napus*

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Abstract

Comparative analysis of structural organization and allelic diversity associated with resistance factors to important fungal oilseed rape diseases was performed using Genome-Wide Association Studies (GWAS) and Genomic Selection (GS). Resistance screening to major fungal pathogens was performed in greenhouse and field experiments using a *B. napus* Nested Association Mapping (NAM) panel. Phenotyping for resistance to two quantitative diseases (D1 and D2) was done in different locations across Europe.

GWAS identified a large number of significant marker-trait associations including in new genomic regions for resistance. GWAS also identified overlapping QTL for multiple disease resistance. GS including stretches of single nucleotide polymorphism (SNP) markers from 60k SNP chip genotyping increased the prediction accuracies of disease resistance suggesting the involvement of short-, medium- and long-range structural variation in the genome.

These findings will improve future breeding efforts on *Brassica napus* and other closely related species by using the new alleles identified and speeding up the overall genetic gain.

Keywords: genome wide association studies, genomic selection, quantitative traits, resistance, oilseed rape

Introduction

Since the advance of human civilization, agriculture has continued to be the backbone of the economic welfare around the globe, especially as most humans depend upon the agriculture sector for necessary goods as food, feed, shelter and clothes. However, recent reports suggest that the world population is expected be more than 10 billion people by the year 2050. This strong increase (more than 45%) in the world population dynamics will create a higher demand for food and other raw materials. Nowadays the supply of fossil fuel, fertilizers, water and chemicals such as insecticides, pesticides and fungicides are at their peak; but this situation is expected to change in the future. Modern agriculture is essentially based on varieties breeding for high performance under high-input systems which generally do not perform well under low-input conditions. Global warming will lead to a significant yield decreases of important food, feed and fiber crops. Thus, now agriculture scientists and plant breeders face the challenge to improve constantly the genetic architecture of modern cultivars with increased performance against threats and stresses; this will require innovative approaches and interdisciplinary collaborations. Today's crop breeders have access to huge genomic and phenotypic datasets derived from revolutionary new genome sequencing and phenotyping methods. However, crop genomes are often many times larger, more complex and considerably more gene-rich that those of humans, and crop performance is determined by extremely complex interactions between the gene makeup (genotype) and the highly variable environments in which the crops are planted.

This makes it challenging to transfer knowledge into breeding processes.

Genomic selection (GS) is revolutionizing the way breeders select material and design future crosses to achieve maximum of genetic gain. GS offers perspective of selecting the best

individuals out of the available population pool, in contrast to trying to create the ideal individual based on an ideotype. Genome based selection removes the constraint for selecting the best alleles at each locus in a trade-off for selecting the best combination of alleles across the genome. In the plan breeding field, the efficacy of GS has been demonstrated for wheat (*Triticum aestivum* L.) [1] maize (*Zea mays* L.) [2], oilseed rape (*Brassica napus* L.) [3, 4].

Having an accurate prediction of important alleles present in material as earlier as possible will allow breeders and farmers to adjust their management practices in order to achieve the target market requirements [4, 5]. However, traditional prediction methods may not be powerful enough to capture complex interactions while avoiding overfitting.

The aim of this study is to investigate whether modern GS methods can be used to predict the performance of plant breeding genotypes, make selection decisions and estimate genotypes adaptability under potential future pathogen-crop interactions.

Methodology

Phenotypic multi-environment data from a multi-parental population (i.e., a *Brassica napus* Nested Associated Mapping – BnNAM population) where be used to train and validation GS models. Accuracy and algorithm performance were evaluated. Disease resistance scoring was done for 200 genotypes in controlled condition. For the first disease (D1) the area of necrosis was evaluated for 30 plants per genotypes at crop maturity as previously described in [6]. The second disease (D2) was evaluated in field experiment in Germany. Plants in pots were inoculated after flowering and the lengths of necrotic surfaces were measured at 7, 14, 21 days after inoculation (dai). For each genotype approximately 25 plants were scored and an area under the disease progress curve (AUDPC) was calculated as described by Gabur *et al*., [6].

Data resulted from disease scoring were included as mean values in Genomic Selection models.

Genotyping data was produced using the 60K Illumina Infinium Brassica SNP array which contains a number of 52,158 SNP probes. All markers were anchored to the Darmor-*bzh* reference genome [7]. As previously described by Qian *et al*., [8], a total of 28, 073 SNP marker were retained for further analysis.

Genomic Selection for the traits were conducted via the following mixed linear model:

$$y = Xb + Zu + e$$

in which y is a $n \times 1$ vector of phenotypic observations and n being the number of genotypes. X is an incidence matrix relating fixed effects to genotypes; b is the corresponding vector containing the respective effects. Best linear unbiased estimators of the fixed effects and BLUPs of random effects were calculated using a ridge regression BLUP implemented in the R package *rrBLUP* [9], assuming that all marker and residual effects were normally distributed. Prediction accuracies for every marker subset within a specific trait were assessed by running 100 cross-validations. In each cycle, the diversity set was randomly subdivided into 80% training population (TP) and 20% validation population (VP). The average prediction accuracies were determined as the mean Pearson correlation coefficient (r) between observed and predicted values.

Results and Discussion

Phenotypic analysis confirmed that within the BnNAM panel we have mainly assessed quantitative resistance for Disease1. Most genotypes presented typical lesions in the autumn, showing a high genetic variability in disease resistance with disease incidence (DI) ranging

from resistant (0.07) to susceptible (5.67) with an average of 1.61. Therefore, the high variance and a normal distribution of results are indicators that qualitative resistance (or monogenic resistance) conferred by major genes is missing. Genotypic variation within the NAM panel was estimated at 0.58 and presented a significant p-value (P<0.001). For GS the mean DI values have been used (Table 1). For Disease 2 (D2) phenotypic analysis confirmed that within the NAM panel mainly quantitative resistance is present. For all genotypes scored AUDPC values ranged from resistant (15.05) to susceptible (204.96). Genotypic variation within the NAM panel was estimated at 32% for AUDPC and presented a significant p-value (P<0.001). For GS the mean values of necrotic surface scored on 25 plants and the AUDPC values have been used (Table 1).

Table 1. Summary of disease scoring for D1 and D2 on the 200 NAM lines

Disease	Min	1st Qu.	Median	Mean	3rd Qu.	Max.	NA's	CV*
D1	0.07	0.91	1.525	1.645	2.12	5.67	0	58.01
D2_7dai	0	1.855	3.14	3.135	4.41	7.47	1	50.81
D2_14dai	0.68	4.95	6.69	6.547	8.075	14.3	1	35.50
D2_21dai	1.94	9.93	13.19	12.56	15.24	20.46	7	29.28
D2_AUDPC	15.05	87.08	116.94	114.72	142.91	204.96	7	32.25

**Coefficient of variation = (Standard Deviation / Mean) * 100.*

From the total of 52,158 SNP calls produced by using the 60K Illumina Infinium Brassica SNP array for genotyping only SNP calls were included in the analyses where their corresponding 50 bp SNP probe sequences could be unambiguously anchored by BLASTN alignment to the Darmor-*bzh* v4.1 *B. napus* reference genome [7]. A total of 24,085 SNP probes that were not mapped or presented multiple hits in the reference genome so they have been excluded from further analysis. The remaining 28,073 single-locus SNPs were thus implemented in downstream analysis. Additional quality control filtering was performed using the following criteria: minor allele frequencies (MAF) <0.05, failed call frequencies per SNP <10% and missing data per line <10%. 10,005 SNPs and 4 BnNAM lines did not meet the quality control parameters and were removed from analyses. Thus finally, 18,068 polymorphic SNPs have been selected for GS.

The mean genomic physical distance between SNPs was approximately 37.5 kb on the whole genome, with 27.7 kb average on the A chromosomes and a 47.3 kb average on the C chromosomes. In general, the number of SNPs per chromosome is consistent with the physical length of that specific one (C03 with 1 599 SNPs and a physical length of 60.5Mb, C04 with 1 474 SNPs and a physical length of 48.8 Mb). Results are similar to a panel that contains 203 Chinese semi-winter *B. napus* accessions [8] and another *B napus* doubled-haploid (DH) population that contained 124 lines.

For all traits, predictions of genomic selection models based on the SNP markers were relatively stable when using as few as 1000 markers. Results showed average prediction accuracies fluctuating only slightly from those obtained with the whole marker set. Similarly, the use of randomly selected markers led to comparable accuracies, although these generally remained below those of the whole set.

Methods as ridge regression best linear unbiased predictions (rrBLUP) are currently available within the toolkit of genomic selection showed a relative high prediction accuracy for some traits, but are limited or with quite low accuracy of prediction for quantitative traits (i.e., yield). Moreover, including the whole matrix of SNP markers in rrBLUP increased prediction accuracy with 2% to 5% for D1 and D2 in oilseed rape. Similarly, we observed an increase in QTL detection power in this dataset when we performed GWAS using the phenotype and

genotype data sets. Similar results were also reported by Werner *et al*., 2017 [3] and Werner *et al*., 2018 [3] for qualitative and quantitative traits as oil content, glucosinolate content (GSL) and plant height (PHT) in oilseed rape.

Conclusions

In this study, we demonstrated that low-density subsets of single nucleotide polymorphic markers comprising several thousand may represent viable selection criteria from a commercially available high-density genotyping platform. This strategy can be sufficient for successful application of genomic selection in polygenic traits. Moreover, reduced marker densities used in GS allow a major reduction in costs, enabling extensive field phenotyping to be done on preselected breeding material. Even with relatively lower selection accuracy, breeders are still likely to improve available lines from their collection and accelerate breeding progress.

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Ensuring Nutrition Security and Sustainability of Food Systems as Basis of Human Healthy Life

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Abstract

This study is based on the evaluation of some vegetable products safety before being marketed in terms of the risk caused by the presence of pesticide residues on human health.

High performance gas chromatography *GC MS – MS* with *ECD* detector was used to determine the organochlorine, organophosphorus, carbamate and pyretroid pesticide residues from six common vegetable products (apple, lettuce, potato, rice, tomato and wheat).

Pesticide residues ranged from below detectable limit (<0,01) to 0,59 mg/kg. *Chlorpyriphos-methyl, bifenthrin, malathion, chlorthalonil, cypermethrin, lindan, boscalid, endosulfan, deltamethrin and folpet* were identified over detectable limit on some wheat and its derived products samples of which two substances (*lindan and folpet*) exceeded than MRL (maximum residue limit). Over detectable value was identified *pyrimethanil* in rice, *bifenthrin* on apple, *folpet and iprodione* on tomatoes samples of which *bifenthrin* exceeded than MRL value. Apart of this residual substance, all another pesticide residue shows lower values than detectable limits.

Hazard risk index (HRI) for lindan (0,034 mg/kg) from wheat sample and bifenthrin (0,228 mg/kg) from apple indicate a risk for child consumers with average weight under 25 kg. Rest of the pesticide residues were evaluated as not with a health risk.

A continuous monitoring and strict regulation with the application rules of pesticide treatments and control of pesticide residues is necessary for products that is intended to be marked for food consumption.

Keywords: monitoring, healthy life, pesticide residues, vegetable products

Introduction

The use of pesticides has become very common in our days, which is a real concern for each of us because they are extremely toxic to the human body.

A pesticide is any substance or mixture of substances used to destroy, suppress or modify the behaviour or life cycle of any disease or pest [15].

For over half a century, the global agricultural system has largely based on a large – scale application of millions of tons and hundreds of types of synthetic pesticides to reduce the damage of agricultural and horticultural crops. As a result of global dependence on pesticide substances and their persistence and ubiquity, almost every ecosystem in the world has already been adversely affected by these harmful chemical compounds and implicitly the health of all living creatures [8, 17].

Pesticide residues are a source of toxic risk due to the remanence in soil, plants and bodies.

Because there are very toxic to the human body, must be taken measures to prevent accidental exposure, in the case of people who come into direct contact with pesticide

substances by the nature of profession and, moreover, in the case who consume them, without knowing, through food products [12].

Human can be exposed to pesticides by different ways: by inspiring the air, by food ingesting that contain pesticide residues or by consuming contaminated water [7], by soil, fauna and flora contact that can be contaminated.

The main purpose of these study is to assess the exposure of the Romanian population through the identifying and monitoring pesticide residues from some vegetables and fruits before being accepted on the market in 2014 years taking into account different types of diet according to the recommendation of the *World Health Organization* and identifying the risks by assessing consumers exposure to pesticide residues taking into account acute and chronic risks.

The growing concern about human health and the environment has led many researchers to identify the balance between the benefit of pesticides and the costs of the environment in terms of pollution, the way they are used, especially by the farmers.

At worldwide level, the history of pesticides use in agriculture and horticulture reveals a narrative of the beneficial improvements in the living standards for most people.

In response to the recognition of potential humans' hazards generated by pesticides use, developed countries throw monitoring authorities have developed a number of requirements that must be fulfil before new pesticides can be released for general use.

Modern agriculture and horticulture involve combating pests by applying substances as efficient as possible to increase both production and profit. Ideally, they should present as small a field of action as possible without exceeding the remaining time in the applied area.

The human exposure to pesticide residues is closely linked with the appearance of various diseases, such us: cancer, asthma, hypersensitivity, allergies as well as congenital defects, death of the fetus or his weight loss [1].

Cereals, fruits and vegetables play an essential role in human nutrition [14, 16], respectively in maintaining health and preventing diseases. The presence of nutrients, antioxidants, vitamins, mineral substances and fibers in fresh fruits and vegetables or in cereals is the main source of human health. Increasing the consumption of fruits and vegetables is the safest way to achieve a nutritional balance and the health of human body.

Nutrition education is the central component of intervention strategy in consumer behaviour and in monitoring eating habits.

The habit of eating cereals, fruits and vegetables is formed at early age, a period in which the eating habits and preferences that tend to be maintained in the adulthood are also established [2].

Methodology

For the estimation of pesticide residues belonging to different types of compounds, from raw materials it was used different methods of extraction and quantification. 56 samples were analysed, at the request of the beneficiary for different raw materials and products of vegetable origin. The analysed samples consisted of wheat, wheat flour, rice, potatoes, tomato, apple and lettuce.

By residues is meant any substance that has a pharmacological function and other substances including its derivatives and metabolites which are not naturally found in raw materials and products of plant origin, but which can be recovered as a result of consciously or accidentally incorporating these products and which, by exceeding permitted limits, may constitute a risk factor for human health, including the health of performance athletes.

For the determination of pesticide residues, the gas chromatographic method on GS – MSMS with ECD detector. The pesticide residues were extracted with organic solvents (acetone, methylene chloride + hexane), followed by homogenization, centrifugation and concentration.

The extract was dissolved in a specific organic solvent and then injected into gas chromatograph using MS-MS and ECD detectors.

The ECD detectors was used for the following categories of pesticides: *hexacorcyclohexane (HCH) isomers, dichlorodiphenyl trichloroethane isomers and its metabolites (DDT), aldrin, endosulfan, alpha cypermethrin, bifenthrin, boscalid, captan, chlorothalonil, cyfluthrin, mixt, deltamethrin, dildrin, endrin, fenvalerate, folpet, hepthachlor, iprodione, cyhalothrin lamda, methoxychlor* and *mixed permethrin*.

The presence of the following pesticide categories has been evaluated through the MS MS detector procedure: *dichlorvos, phosdrin, acephate, omethoate, diphenylamine, phorate, dimethoate, carbofuran, atrazine, diazinon, disulfoton, pirimicarb, chlorpyriphos – methyl, metribuzin, vinclozolin, parathion – methyl, carbaryl, pirimiphos – methyl, fenitrothion, dichlofluanid, malathion, chlorpyriphos, fenthion, parathion* and *phenthoat*.

Standard solution and internal standards (Triphenylphosphate – TPP and Mirex) were prepared by weighing 100 mg of active substance with toluene to the mark in a 100 cm^3 flask.

It was prepared individual calibration solution and internal standard containing 10 μg/ml in isooctane – toluene and the pesticide compound standard solution containing 10 μg/ml, 20 μg/ml and 50 μg/ml in isooctane – toluene and standard compound solution of 1 μg/ml of pesticide in isooctane – toluene.

The calibration curve was determined using intermediate solutions, respectively standard compound solutions of 0,01 μg/ml; 0,02 μg/ml; 0,04 μg/ml; 0,06 μg/ml; 0,08 μg/ml and 0,1 μg/ ml. Also, it was run a blank sample for the purity test of the reagents.

Working conditions for the GC/ ECD separation: injection temperature: 250^0C; detector temperature: 300^0C, carrier gas: helium purity 99,99% at a flow rate of 2 ml/ min and make-up; nitrogen purity 99,99% with a flow rate of 25 ml/ min and injection volume: 1 μl [10, 11].

Working condition for GC/MS: injection temperature: 250^0C; detector temperature: 250^0C, carries gas: helium, injection volume: 1 μl.

The pesticide content was expressed in ppm (mg/kg).

Based on food consumption rate for fruits in Europe, the estimated lifetime exposure dose (mg/kg/day) was obtained by multiplying the residual pesticide concentration (mg/kg) in the samples with the food consumption rate (kg/person/day), and dividing the product by the body weight (kg) [5] (*Table 1*).

In order to assess a more accurately human health risk estimation of pesticide residues from samples, the hazard indices (HI) for adults (70 kg) and children (25 kg) were evaluated as the ratio between estimated pesticide exposure doses and the corresponding RfDs (reference doses).

The food involved is considered either a risk to consumers if HI >1, either acceptable if HI <1 (Figs. 2).

Results and Discussion

The result obtained revealed that from 26 wheat samples, 9 showed the following residues (*chlorpyriphos-methyl, bifenthrin, malathion, chlortholonil, α-cypermethrin, lindan* (0.034 ppm), *boscalid, endosulfan, folpet deltamethrin and α-cypermethrin*).

The calculation methodology applied in this model developed by EFSA (*European Food Safety Autority*) is in agreement with that developed by the WHO (*World Health Organization*) [6].

It should also be underlined that the analysed imported sample of provenance from U.E. presented 6 residues of pesticides (lindane, boscalid, endosulfan, chlorothalonil, cypermethrin

and deltamethrin; the boscalid showing superiority of 0,263 mg/kg being surpassed by cypermethrin that reached a rate of 0,593 mg/kg.

From 26 samples (represented by wheat products) it was revealed that 17 samples don't have any pesticide residues, occupying a percentage of 65,4%. Also, it is highlighted that potatoes, represented by 5 analysed samples, doesn't presents any pesticide residues.

Regarding the 14 apple samples, the residues were presented at 2 samples (bifenthrin = 0.228 ppm and iprodione = 0.025 ppm); for 5 samples of tomatoes it were found two residues (folpet = 0.035 ppm and iprodione = 0.079ppm) and for the 5 samples of salad it was detected one residual substance (pyrimetaryl = 0.293ppm). For rice and potato no pesticide residues were identified.

Table 1. Lifetime exposure dose of adults and children to pesticides residues founded in wheat and its derivate products for autochthon and imported products analysed in 2014

Pesticides residues (mg/kg)	Reference dose (mg/kg)	Concentration of pesticides residues (mg/kg)		Adults (with 70 kg average weight)		Children (with 25 kg average weight)	
		Wheat and it's derivate products	Imported wheat and its derivate products	Life time exposure (mg/kg/day)	Hazard indices	Life time exposure (mg/kg/day)	Hazard indices
Chlorpyriphos-methyl	3	0.013	-	0.0027	0.0009	0.0075	0.002
Bifenthrin	0.5	0.033	-	0.0068	0.013	0.019	0.04
Malathion	8	0.018	-	0.0037	0.0005	0.01	0.0013
Chlorthalonil	0.1	-	0.017	0.0035	0.035	0.0098	0.098
Cypermethrin	2	-	0.593	0.122	0.06	0.343	0.17
Lindane	0.01	-	0.034	0.007	0.7	0.0196	1.96
Boscalid	0.8	-	0.263	0.054	0.07	0.152	0.19
Endosulfan	0.05	-	0.038	0.0078	0.157	0.021	0.44
Deltamethrin	1	-	0.037	0.007	0.008	0.021	0.021
Folpet	0.4	0.56	-	0.116	0.29	0.323	0.8

Table 2. Lifetime exposure dose of adults and children to pesticides residues founded in apple, tomatoes and salad analysed in 2014 year

Pesticides residues (mg/kg)	Reference dose (mg/kg)	Concentration of pesticides residues (mg/kg)				Adults (with 70 kg average weight)		Children (with 25 kg average weight)	
		Apple	Tomatoes	Lettuce	Rice	Life time exposure (mg/kg/day)	Hazard indices	Life time exposure (mg/kg/day)	Hazard indices
Bifenthrin	0,05	0,228	-		-	0,04	0,74	0,19	3,77
Folpet	5	-	0,035		-	0,003	0,00065	0,014	0,002
Iprodione				0,29	-	0,031	0,063	0,15	0,3
	5	-	0,079		-	0,007	0,0015	0,032	0,006
Pyrimethanil	0,5	-	-		0,293	0,031	0,063	0,15	0,3

Analysing the highest frequency of the identifications number of the active substances in the analysed samples, folpet is first on position, followed by deltamethrin.

Table 3. The properties of pesticides identified in vegetable raw materials

Active substance	Formula	Molecular mass [g/mol]	Solubility in water [mg/l]
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	422.9	<1 µg/L
Chlorpyriphos-methyl	$C_7H_7Cl_3NO_3PS$	322.5	3
Malathion	$C_{10}H_{19}O_6PS_2$	330,35	145
Chlortholonil	$C_8Cl_4N_2$	265.9	0.81
α-cypermethrin	$C_{22}H_{19}C_{l2}NO_3$	416.3	4.54
Lindan	$C_6H_6Cl_6$	290,83	7,5
Boscalid	$C_{18}H_{12}Cl_2N_2O$	343,2	4,6
Endosulfan	$C_9H_6Cl_6O_3S$	406,9	0,33
Folpet	$C_9H_4Cl_3NO_2$ S	296.6	0.8
Deltamethrin	$C_{22}H_{19}Br_2$ NO_3	505.2	0.0002
Iprodione	$C_{13}H_{13}Cl_2N_3O_3$	330,164	12,2
Pyrimethanil	$C_{12}H_{13}N_3$	199,26	6,07

It is also highlighted that all most of identified residues showed values (mg/kg) below the MRL limit with the exception of one apple sample exceeding the maximum permissible limit (0.05 mg/kg) for bifentrin with a value of 0.228 mg/kg and one wheat sample exceeding the maximum permissible limit (0.4 mg/kg) for folpet recording a value of 0.56 mg/kg.

The active substance – folpet was identified as being present in different proportions in 4 of the wheat samples analysed and two samples of wheat flour.

By the gas chromatographic analysis carried out in the researches presented for the identification of pesticide residues from the vegetable raw materials and its derived, the presence of 12 compounds whose physicochemical properties have been described in *Table 3*.

Conclusion

The results of health risk analysis based on consumption data in EU-27 revealed that *lindane* from imported wheat and *bifenthrin* from apple can pose a health risk to children with body weight below 25 kg, after those products consumption.

For all the others residues identified in wheat, apple, tomatoes and lettuce, HI is <1 for all analysed pesticides indicating that those products consumption doesn't have a risk to human health.

On the basis of the above findings, the results obtained in our study suggest the necessity for surveillance and monitoring programs for pesticide residues in all food commodities in order to defend the final consumers from exposure to this kind of substances.

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The Influence of Natural Sweeteners on the Innovated Fruit Paste

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Abstract

The natural sweeteners can successfully replace refined sugar, a highly controversial product, due to the negative impact on consumers' health. The adding of the natural products preserved in the manufacture represents an important factor for their use in the consumption of natural products, nutritionally beneficial. The experimental research follows the behavioural of natural sweeteners while preserving native fruit paste. The experiment was performed using modern methods of expertise, regarding the authentication of natural sweeteners. Thus, the natural sweeteners added to the fruit paste were: agave syrup, stevia syrup, which bring a beneficial nutritious contribution beneficial to the health, especially due to the fact that they are not chemically processed. Following the research study, organoleptic, physical and chemical characteristics were performed, as well as spectrophotometric analyses for measuring the quality characteristics of the innovated products. The evaluation of the sensory characteristics of fruit paste, as well as the measurement of their quality characteristics, by describing and the qualitatively results recording. Regarding the physical and chemical characteristics, these were highlighted quantitatively and the results obtained were used in comparative analyses aiming the indicator of ratio sugar/acidity. This is the level of technological maturity of the fruits that represent the basic raw material used for obtaining the samples. Presently research comes to outline a quality indicate the ratio sugar/acidity that defines the preservation of fruit paste innovated without the addition of crystalline sugar.

Keywords: natural sweeteners, fruit paste

Introduction

The attractive commercial image of natural food makes the consumer often have a paradoxical behavioural because he wants a non-industrial food, while demanding absolute safety, often obtained through technological treatments. Natural food would rather be a dream, if not a myth, before the impossibility of defining and associating it with strict regulation [3].

Natural sweeteners are a series of substances that are found in pure form in food and which, by synthesis, replace the refined sugar found in an exaggerated amount in different food. This has led to the replacement of sugar with other types of substances that can give the same sweet taste as: honey, maple syrup, agave syrup, coconut sugar, stevia sweetener.

Being a controversy for human health and producing diseases such as diabetes, obesity and other disorders of the human body, worldwide efforts are being made to eliminate sugar from nutrition and to replace it with various substances that have recently started to emerge in the domestic market. The use of natural sweeteners is a very important and necessary aspect in choosing a suitable diet and promoting a healthy lifestyle, in the context in which sugar is found in the highest proportion in food, its consumption being a precursor of different diseases that affect human metabolism. The biochemical composition of natural sweeteners is a particular

nutritional importance. That is why the use of natural sweeteners can be a solution to replace the sugar used in preserving fruit paste.

Agave syrup (*Agave american*). Agave syrup native in America and is extracted from agave, a species of cactus, which has a sweet syrup in the composition. From the point of view of nutritional benefits, agave syrup has a high fructose content. It is source of vitamins (B, C, D, E) and mineral substances such as: Fe, Ca, Mg, K and Si. So that, the agave syrup used in the diet fights different diseases such as: it prevents stress and anxiety, plays an important role in the prevention of osteoporosis, helps the transport of oxygen in the blood. It has a neutral taste, so it can be easily used in the preparation of a large number of foods. Agave syrup has the same sensory characteristics as honey bees. [2]

Stevia (*Stevia rebaudiana).* Stevia is a natural sweetener that is obtained from the Stevia rebaudiana plant. This plant is native to Arizona, New Mexico, Texas and Brazil.

Stevia is known as the sweet leaves of Paraguay, honey leaves, candy leaves. This plant is 10 times sweeter than refined sugar, but does not contain carbohydrates or calories. Steviol presents in stevia, a sweet diterpenoid glycoside is 300 times sweeter than sucrose. It is recommended in the diets of diabetic and obese people.

Sugar obtained from stevia has antibacterial, antiseptic, anti-inflammatory, anti-fertility, hypo-tensive, diuretic and cardiotonic properties. This has shown good results in skin cleansing problems such as acne, dermatitis, eczema. Steviol regulates blood glucose levels and can also be used as a digestive tonic [5]. For hundreds of years, several people have used stevia leaves for different herbal treatments and like sweetener. They also used this plant to sweetener teas and food and as a medicine, cardio tonic for obesity, high blood pressure and heartburn and also to help lower uric acid. In addition to being a sweetener, stevia is considered to be hypoglycemic, diuretic, cardio tonic. The leaf is used to combat diabetes, obesity, high blood pressure, fatigue, depression, cravings and infections [5]. Stevia presents a complex called steviol glycosides which classifies this compound among sweeteners thus eliminating the claim that the stevia sweetener is a food additive [7]. Being considered a natural sweetener, stevia also brings certain benefits to the metabolism of consumers by regulating the blood sugar level, helps to maintain the glycaemic index constant and it is an important source of natural antioxidants.

The nutritional value and quality of the natural sweeteners are that they are rich in: carbohydrates (glucose, fructose, cellulose), vitamins (A, C, B1, B2, B6, PP, K), organic acids and mineral substances (magnesium, zinc, iron, calcium, potassium, phosphorus).

The nutritional value of the natural sweeteners corresponds of the raw material from which they are extracted. The quality of the natural sweeteners is given by all their organoleptic properties: the clear appearance of syrups, the colour close of the raw materials from which they are obtained, an easy taste of the additive substance is admitted and the pleasant smell, close to the natural one. The natural sweeteners must be without oxygen, to prevent their oxidation, thus maintaining the highest nutritional and therapeutic value. The packaging of these sweeteners must ensure their stability over time and the food safety of the finished product [6]. The sweetener in stevia contains a significant amount of Ca, P, K and Fe, essential for maintaining health and preventing diseases [7].

The taste of sweet has been a very important basic taste for humans, although sweeteners are always linked to either weight gain or tooth loss. Sweeteners entered the food industry in the 1800s. Despite their long relationship with food, sweeteners have been in the spotlight for many reasons. Because it is the perfect choice for diabetics, for the dangers of toxicity, cancer and other health problems associated with their consumption, sweeteners have come a long way.

The contradictory results for the same sweeteners and the divergent regulations are grounds for a wide-ranging debate on the impact of sweeteners on industry, health and the lifestyle of mankind [4].

Methodology

In this experiment we studied the pulp from four types of fruit: grapes, plums, nectarines and peaches. In this research we obtained results related to the raw material that was subsequently used to obtain the innovated fruit paste. Then, the dosing of the sweetening syrup in fruit pulp was performed in different concentrations by: 3% (1-grape sample), 5% (2-plum sample), 10% (3-nectarine sample), 15% (4-peach sample). Thus, the innovated samples were examined both from the sensory characteristics and from the physical-chemical characteristics.

Regarding the carbohydrate/inverted substances sugar content, the acidity, the pH and the soluble dry substances we studied in the sometimes the fruit paste with concentrations of sugar solutions by: 3% (1-grape sample), 5% (2-plum test), 10% (3-nectarine test), 15% (4-peach test).

In the laboratory experiments we obtained taste variations regarding the sweet taste and we followed the behavioural of the innovated paste by comparative analysis in time, the storage period.

The experimental methods used a number of samples, coupled with the witness sample to be analysed for each type of fruit taken in the experimental research. Two types of sweeteners were used in the sample: agave syrup, stevia syrup. We have started with determination of carbohydrates from natural syrups through High performance capillary syrup.

High performance anion exchange chromatography (HPAE) coupled with pulsed amperometric detection (PAD) is a well-established technique for identifying and quantifying carbohydrates in the studied samples. This technique is important for nutritional quality control, monitoring and production process, testing authenticity because it offers key product matrices quality and related properties, contamination or falsification. HPAE-PAD allows the direct quantification of non-derivative carbohydrates with a minimally prepared sample and solves most sugars and organic acids. With HPAE, carbohydrates are ionized to the strong base, the eluent which is potassium hydroxide and separated by ion exchange chromatography. The Dionex CarboPac resin is introduced into a polymer body of the column formed inert hydroxide, thus reducing the column and avoiding clogging of the electrode by metal contamination. Using carbohydrate – the optimized waveform, PAD is sensitive and specific for carbohydrates by detecting most compounds containing hydroxyl functional groups. As a result, the sensitivity of the carbohydrate column is higher than for the other analyte classes by the order of size.

Column: Dionex CarboPac PA 20, 0.4 x 150mm, temperature: 30°C, eluent: 50 mm potassium hydroxide (EG), flow rate: 10 µl/min, injected volume: 0.40 ml, detection: PAD, 4 – potential carbohydrate, Au, reference electrode: PdH, gasket thickness: 25µm (Fig. 1).

The chromatogram indicates the degree of the stevia syrup sweetening, as well as the agave syrup. The level of fructose 1, glucose 2 and sucrose 3 shows the authenticity and properties of the sweeteners which it will be use for the innovated fruit paste (Fig. 1)

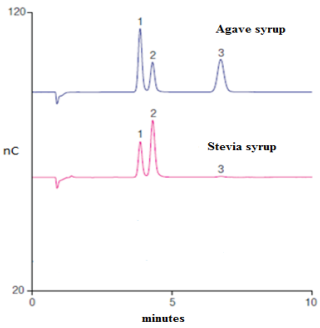

Fig. 1. Capillary analysis HPAE – PAD for carbohydrates from sweeteners (1-fructose, 2-glucose, 3-sucrose)

Results and Discussion

The experimental study followed the analysis of the quality parameters for the fruit paste obtained from: grapes, plums, nectarines, peaches both in the control sample and in the samples where natural sweeteners were used. The samples of fruit paste were with stevia syrup/agave syrup. The fruit pulp showed decreasing values from 24% soluble dry substances in Augusta Grapes, 12% in Moldova plums, 10% in nectarines and only 6% in peach pulp. The inverted sugar determined in the fruit pulp studied indicates an upward trend from 6.3 mg/100g in grapes, 9.4 mg 100g in plums, 12.6 mg/100 g in nectarines and 19.2 mg/100g in peaches (Fig. 2).

The acidity of the fruit pulp also varied from 4 g/100g to grapes, to 4.25g/100g to plums, 5.5 g/100 g to nectarines and 6.5 g/100g to peaches. It was observed that grapes and plums are belong to the acid fruit category, while nectarines and peaches are less acidic, tending to a pH=5. (Fig. 2) Regarding the pH, it observed that the values recorded are in the range 3.28-3.76 grapes, plums, nectarines and peaches.

The analysis of the quality characteristics for the raw material of the fruit pulp led us experimentally to choose appropriate concentrations for the sampling.

Thus, we obtained the first series of samples (samples 1, 2, 3, 4) consisting of four types of fruit paste to which was added agave syrup with concentrations by: 3%, 5%, 10%, 15%. We performed two series of samples (samples 5, 6, 7, 8) using 3%, 5%, 10%, 15% stevia syrup (Fig. 2).

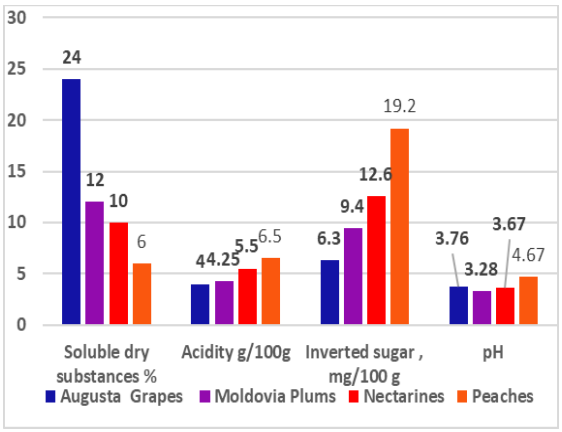

Fig. 2. Dynamics of quality characteristics

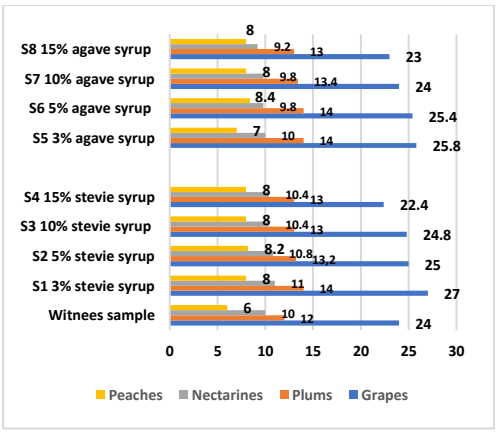

Fig. 3. The dynamic of the soluble dry substances at the fruit paste innovated with stevia syrup and agave syrup

The evolution of the soluble dry substances from the fruits paste innovated to peas were added natural sweeteners of stevia and agave syrup indicates that the grape paste recorded the highest growth from 24% to 25.8%, followed by plum paste from 12% to 14% both for the use of stevia syrup and for the use of agave syrup (Fig. 3).

Nectarine and peach paste had values of 6-10% and the reduction of inverted sugar was 9-10%. It can be concluded that the addition of natural sweeteners leads to a moderate increase in glucose in the finished products (Fig. 3).

The greatest increase in acidity was in nectarines pasta of 6 g/100 g at the addition of agave syrup, as well as of 6.25 g/100 g at the stevia syrup added. Lower acidity was recorded in the plums, grapes and peaches paste (1-5.5 g/100 g) (Fig. 4).

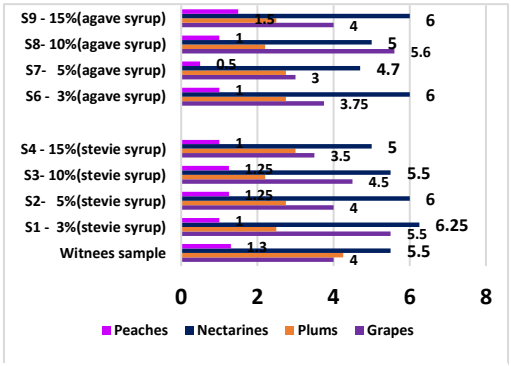

Fig. 4. The dynamic of the acidity at the fruit paste innovated with stevia syrup and agave syrup

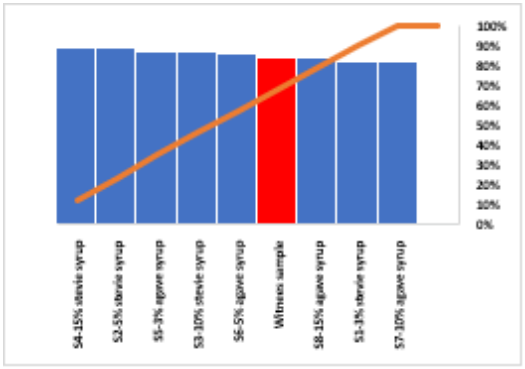

Fig. 5. pH dynamic of fruits pasta innovated with stevia syrup and agave syrup

In terms of the dynamic of pH, it has a slightly increasing, almost constant, linear tendency.

From the Pareto diagram it is observed that the pH values of the innovated fruit paste are similar to the pH of the control sample. It follows that by adding natural sweeteners they do not significantly change the pH index of the innovated fruit paste (Fig. 5).

In the case of innovated pasta for obtaining the first samples was used concentrations of 3%, 5%, 10%, 15% stevia syrup by 8% in glucose and in the second samples 3%, 5%, 10%, 15% agave syrup in 10% concentration (Fig. 6). When it was used stevia syrup glucose increased it was from 6.3 mg/100 g paste (witness sample) to 28.9-35.7 mg/100 g (samples 1,2,3,4). When it was used agave syrup glucose increased it was from 9.4 mg/100 g to 37.32-47.43 mg/100 g samples 5, 6, 7, 8. (Fig. 7).

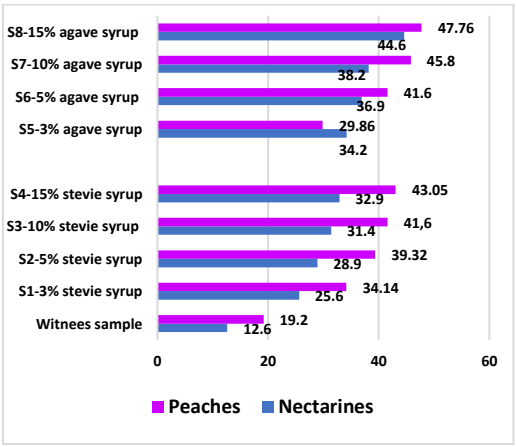

Fig. 6. The dynamic of the glucose at the nectarines and peaches pasta innovated with stevia syrup and agave syrup

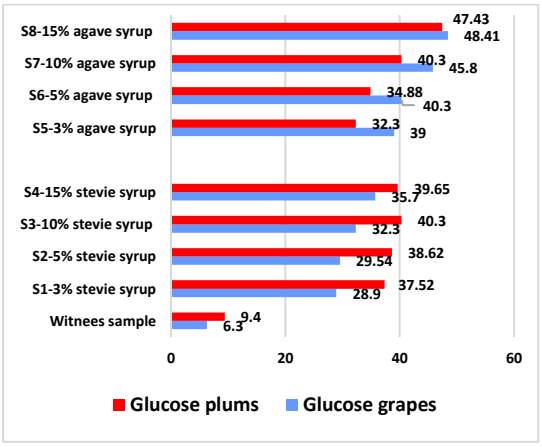

Fig. 7. The dynamic of the glucose at the plums and grapes pasta innovated with stevia syrup and agave syrup

Conclusions

1. Glucose, fructose and sucrose from syrups were separated using a Dionex CarboPac PA20 capillary column and an electrolytic eluent generator with potassium hydroxide. RFIC (Reagent Free Ion Chromatography) capillary systems have been extended to the application of ion chromatography for the analysis of carbohydrates in food, respectively of sweeteners, by bringing an increased sensitivity, as well as the easy use, reducibility with repetition in the determination of carbohydrates glucose 1, fructose 2 and sucrose 3.

2. The addition of natural sweeteners stevia syrup and agave syrup causes the growth of soluble dry substances, as well as inverted sugar-glucose.
3. The acidity and pH indicators remain within relatively constant limits, because the acidity of the stevia syrup or the agave syrup did not change the acidity of the innovated fruit paste.
4. Innovated fruit paste that have been used natural sweeteners can be successfully recommended to consumers with the following diseases diabetes, cholesterol, obesity. They can also be indicated for healthy eating of children to prevent tooth decay.

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Engineering Measures for The Control of Soil Erosion on the Pastures from the Perimeter Izlaz Bacu, Ipatele Commune, Iasi County

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Abstract

The lands constituted in the improvement perimeter Izlaz Bâcu, Ipatele commune, Iasi county, with a total area of 89 ha, are located in the outskirts of the locality, namely 41 ha is highly degraded pasture and 48 ha unproductive. According to the current evidence from the Ipatele Local Council, these lands are totally unproductive, as a result of very strong and excessive surface erosion, depth erosion, landslides but also the excess moisture.

As a working methodology, the inventory of agricultural land in the studied area was used, as recorded in the cadastral registers of Iasi County, on administrative units, on the latest topographic maps, with the help of Google Earth site and field visit to identify the problems existing.

The improvement is necessary for the enhancement of the soil protection, the water regime but also the improvement of the environmental conditions, at present with a desolating aspect.

The investment is opportune because the realization of the complex of hydrotechnical and agrotechnical works proposed, it leads to the introduction of the agricultural productive circuit of 53 ha, and the provision of such forest use on 35 ha and agricultural technological roads 1 ha, non-agricultural (non-productive) lands within the improvement perimeter, as well and the improvement of ecological and environmental conditions, with a positive socio-economic impact on the community in the area. Also, the works proposed in the work are necessary in order to ensure that the expansion of the lands removed from the productive agricultural circuit and at the same time the accentuation of the degradation due to the surface erosion, the extension of the formation of the depth erosion and the excess moisture from the slope base.

Keywords: Ipatele commune, unproductive land, excessive erosion, antierosion works

Introduction

From an administrative point of view, the lands related to the development perimeter are located in the southern part of Iasi county, on the territory of Ipatele commune. The access to the area is made from the county road DJ248 Iaşi-Grajduri, DC60 Grajduri-Cioca Boca, DC70 Cioca Boca-Bâcu and then on exploatation roads.

The lands established in the improvement perimeter Izlaz Bâcu, extend on a total area of 89.65 ha and belong to the Local Council of Ipatele commune and are located in the area bounded: to the east by Cioca-Boca, to the south by Ipatele, west of the forest.

From the economic point of view, the categories of current land use are heavily degraded pasture 41.10 ha and non-productive 48.55 ha but according to the current evidence obtained from the Ipatele Local Council, the entire surface has become totally unproductive, due to the very strong surface erosion, of landslides, depth erosion and ex The relief is represented by high

plains in contact with the slope and uniform slopes, as well as affected by active and stabilized landslides in which there are no ravines. cess of moisture.

From a geological point of view, the researched area represents a small part of the large unit of the Moldavian Plateau, where deposits of Sarmatian age (Bessarabian and Kersonian) and Quaternary are developed, the rest of the formations being known only from boreholes.

On the slopes the water from the rainfall is removed by leakage to the surface and on certain alignments so that the danger of erosion and the occurrence of ravines is high. The very unevenness of the slopes affected by the sliding causes the surface runoff to be reduced. On the high plateau the groundwater is at a relatively shallow depth, which imprints a certain degree of gleeization to the soil (Fig. 1) [1].

From a climatic point of view, the area has a prominent continental character, falling within the humid steppe. According to the data from Iasi, the average annual temperature is 9.4°C, the average for January is -3.7°C and for June 20.4°C.

The main characteristic of the area is the uneven distribution of precipitations and their torrentiality. Thus, the average annual rainfall value is 537.5 mm, with maximum rainfall values in 24 hours during vegetation period between 50 and 110 mm.

The rainfall regime indicates a low level of rainfall in the cold season, with a minimum in February. In the hot season, precipitations sometimes have a pronounced torrential character, especially in summer, when there are heavy showers. The hail occurs very rarely and is local.

The climatic, lithological, geomorphological and vegetation conditions have determined the formation of different soil types. The predominant soils are pheeosome, erodosol, preluvosol, alluviosol. The texture is differentiated in profile, the clay content rising from the surface to the depth, the reaction is weak-moderately acidic and the humus content is low [2], [3], [4], [5].

Fig. 1. Degraded pasture from Izlaz Bacu

Methodology

The field studies consist of topographic studies prepared by SC EXPERCO-ISPIF SRL Bucharest, comprising the updating of the situation plan scale 1: 5000 of the use categories and of the new boundaries between the land holders and pedological studies at scale 1: 5000 with elements of lithology and hydrogeology prepared by the Office for Pedological and Agrochemical Studies Iasi.

As a working methodology, was used too, the inventory of agricultural lands in the studied area, as they were recorded in the cadastral registers of Iasi County, on administrative units, on the most recent topographic maps and even with the help of the Google Earth site.

In order to know the complex problems of the quality of the ground-land units in terms of the sustainable use of land resources, a study was carried out on the current state of the land

and land improvement works in the Izlaz Bacu area; at APIA Iasi, North-East Region ANIF, Agricultural Chamber from Ipatele County. Soil maps were also used at stairs 1: 200 000 and 1:100 000 [6], [7].

Results and Discussion

The improvement of the Izlaz-Bâcu improvement perimeter includes a set of agrotechnical and hydrotechnical works, elaborated according to the natural conditions, the intensity of the land degradation processes and the requirements of the prospective development of the studied area, proposed for the improvement of the lands affected by erosion, landslides and excess moisture.

1) Hydrotechnical works. Specific works were provided for the area of 69.65 ha, namely:
   - Cleaning the land of spontaneous woody vegetation on an effective area of 22.50 ha;
   - Cleaning the land of spontaneous vegetation -14.80 ha;
   - Modelling works on slips – it was proposed for surfaces affected by landslides, with a total volume of earthworks of 33 800 cubic meters, in order to correlate the slope of the land with the attenuation of the ground kneading , the construction of the embankment on the unstable exits, the elimination of the micro-depressions in which water persists om the surface and the clogging of cracks and the gullies, by doing this a continuous slope of the land and a system of gullies that allow to regularisation the superficial leaks, avoiding the rapid infiltration of the water into the depths and create conditions for the realization of the works for catchment the springs and the valorisation of the land by categories of agricultural use.
   - Redevelopment of agricultural technological roads. In order to avoid the accentuation of the phenomenon of the formation of ravines and to ensure the access for the agricultural exploitation, as well as the maintenance of the development works, it was planned to redevelop1.60 km of agricultural technological roads, by strengthening the platform with a width of 4 m, with the levelling of the streams and gullies formed on their route, with a total volume of earthworks of 1760 mc.
   - Spring catchment, it was planned to catch 3 coastal springs that appear up-to-date in the slope, consisting of: spring catchment chambers in number of 3 pieces, which will be constructed with circular section Dn 1000 mm, from simple concrete with the height 2.7 m average. On the front wall of the chamber there are barbecans, made of pipes PVC type M , with Dn 90 mm and the length of 0.5 m, in the upstream achieving a three-layer filter prism: rough stone with a thickness of 0.6 m, sand and crushed stone in a thickness of 0.2 m and a layer of ballast of 0.2 m. The water is collected by the capture chamber and through the interception-capture drains placed on both sides of the chamber in the direction of the level curves, being taken over from the drains of the collectors and discharged in emissary.
   - Interception-capture drains, with a length of 0.99 km, with a dual purpose: to intercept and taking over groundwater, but also eliminating excess moisture from the soil profile affected by the phenomenon of gleization.

Constructively, the interception-capture drains will be made of PE reflate tubes, Dn 83 mm, with a filter element of GEOTESS PP.HT400 geotextile wrapped around it, laid in a trapezoidal trench having a bottom width of 0.5 m, slope of slopes 1: 0.5 and average depth of 1.4 m. Above this, the high filter of gravel-sand is placed on a height of 40 cm, with a granulometric

composition depending on that of the ground at the depth of laying, which prevents clogging of the filter-drain assembly.[8], [9].

- Collector drains, with a length of 0.9 km, which ensure the take-up by means of the capture chambers or manholes of the flows captured and carried by the interception-capture drains and their discharge into the emissary, through reinforced exhaust vents.
- Manholes, in number of 7 pieces, provided on the collector drains at the connection with the interception-capture drains and the changes of directions or slopes, which are composed of the precast concrete tube Dn 1000 made according to STAS 2448/82, adapted for works drainage, laid on a simple concrete foundation with dimensions of 1.5x1.5x0.4 m. The connection of the interception-capture drains to the capture chambers and the manholes will be made through a PVC tube Dn 90 mm, with the length of 1 m.
- Exhaust mouth, for discharging collector drains into emissary, which will be made of concrete slabs 0.5x0.5x0.06 m, grouted with bituminous mortar.
- Addition of forest protection plantations. It will be realized on about 40% of the total area of the improvement perimeter, on unproductive lands, that is on 35.95 ha by filling the gaps, with a planting scheme consisting of species that are approved by animals, namely: willow (*Eleagnus angustifolia*- 30%), acacia (*Robinia pseudoacacia* -50%) and wicker (*Salix purpurea*-20%).
- Strips of shrubs, on the meadows affected by landslides in order to fix them, were provided shrub strips on a length of 2.6 km. These will be made from 3 to 3 rows of willow planted in individual pits at distances of 0.5 m in a row and between rows [8],[9].

2) Agrotechnical works. In order to improve the unproductive and degraded agricultural lands in order to be transformed into a higher category of use, namely grassland and to increase the production capacity of agricultural lands as a result of the hydrotechnical works, the following agrotechnical works and measures were provided:

- A gipsic amendment for the regulation of the saline soil regime on 32.50 ha, with 3t / ha amendments.
- Ameliorative fertilization with chemical fertilizers on 53.06 ha: nitrogen 75 kg s.a./ha, superphosphate 72 kg s.a./ha.
- Sowing and overseeding with perennial grasses on 53.06 ha. It is recommended to use in the first two years the grassland established and improved only as meadow, until a well-grassed carpet is made and only after this period its use as a pasture.
- Disking on 53.06 ha, work that will be carried out in two directions [10], [11], [12].

The proposed works for the development of the perimeter of improvement are with the role of protection and restoration of the degraded lands that will contribute to the establishment of a zonal natural balance, currently degraded.

The development of the slopes affected by surface erosion and landslides, including depth erosion formations, with improvement works for drainage control, and consolidation through drainage, local afforestation, grassland establishment and improvement of degraded lands, contributes to the stabilization and creation of evolution of soils, vegetation, biocenoses from soil. These can be transformed into new biotopes, balanced and stable, able to build ecosystems with their own flora and vegetation, only if the arrangements made in accordance with all the existing ecological conditions and factors, will be maintained permanently and periodically supplemented with specific works. Thus, through the planned development works, ecological changes are expected in large areas, including climate, flora and fauna. [13], [14], [15]

The forest plantations from the deep erosion formations will have multiple functionality, in addition to the role of soil fixation and environmental protection, and can later be used later be used as natural resources. These plantations, together with the meadows with a rich grassy carpet, also contribute to the breaking of leaks, maintaining the fertile soil in the perimeter and preventing landslides. The plantations vegetative consolidated will lead to the diminution of the negative influence of the climatic factors (radiation, temperature, precipitation, atmospheric humidity, evapotranspiration, wind), will allow the ozonation of the air, the gradual restoration of the flora and the fauna specific to the area [16], [17], [18].

Conclusions

By realizing the complex of works exposed in the work, the following is ensured:

- Highlighting from the point of view of soil protection, water regime and improvement of environmental conditions on 89.65 ha, currently non-agricultural land due to very strong surface erosion, depth erosion, landslides but also due to excess soil moisture and salinization;
- It is recovered and capitalized by a forest use 35.95 ha, land currently unproductive;
- The introduction of 53.06 ha in productive agricultural set-aside will be carried out, at present non-agricultural;
- Achieving a favourable impact on the environmental conditions and ecology of the area, by increasing the area covered with forest protection plantations and restoring the grassland carpet on the meadows and thereby improving the general appearance of the area, which is currently desolate.

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Section 2
HORTICULTURE AND ENVIRONMENTAL ENGINEERING

Assessment of the Agrobiological and Ameliorative Potential of some Resistant Grape's Varieties

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Abstract

Grape varieties with high biological resistance are the crossing result of two or more *Vitis* species. Due to the lack of recent and complete data regarding grape quality, breeding potential and ornamental value of the hybrid grapevine genotypes created in the last decades, the purpose of this study was to evaluate the agrobiological and technological value of resistant varieties: Purpuriu, Radames and Moldova (having variety Villard blanc as common genitor) and the variety Mara (Ozana × Seyve Villard 12-303), frequently cultivated in the temperate climate vineyards, recreational areas and private gardens. Grape maturity of consumption was achieved in the last decade of September, their colour varying from red to black-purple. Grape yield ranged from 17 to 22 t/ha, with a balanced sugars/acidity ratio and good antioxidant activity.

The obtained results contribute to a better understanding of the ameliorative value of the resistant varieties studied.

Keywords: nutritional value, quality characteristics, resistant grape varieties

Introduction

Assessment of the agrobiological and productive potential of grape varieties is essential for the selection and maintaining in culture of genotypes with superior qualitative and productive characteristics, as well as for the spreading in culture of the varieties well adapted to the climatic conditions, and also to the consumer requirements. Biological resistance to stress factors, regardless of their nature, can be decisive in the selection of resistant grapevine varieties, which has as destination small vineyards or gardens, mainly for ornamental and recreational purposes.

Due to the lack of data regarding the quality characteristics, the ameliorative and ornamental potential of new grape cultivars, the aim of this study was the assessment of agrobiologycal and technologycal caracteristics of some resistant cultivars (Purpuriu, Radames and Moldova), which have as common genitor the variety Villard blanc (Seyve Villard 12-375) and new variety Mara (Ozana × Seyve Villard 12-303), which are frequently found in vineyards in our country, but also in private gardens.

Materials and Methods

Biological material

The study was carried out in the years 2016 and 2017, plant material being represented by four hybrid grapevine varieties with high biological resistance and good adaptability to the environmental conditions of Iasi vineyard, two for wine: Radames, obtained at Research-Development Station for Viticulture and Winemaking Blaj- Romania, by the crossing of varieties Traminer roz × (Seyve Villard 12.376 × Regina Viilor) and Purpuriu, obtained at Valea Călugărească Institute by hybridization of Ceauș blanc × Seyve-Villard 12.375 varieties, and

two varieties for table grapes: Mara (Ozana × Seyve Villard 12.303), obtained at Research-Development Station for Viticulture and Winemaking Iasi, Romania and Moldova (Guzali kara × Seyve-Villard.12.375), created in the Republic of Moldova (Fig. 1).

Radames Purpuriu Mara Moldova

Fig. 1. Analysed grape varieties

The plantations are located on a chernozem soil, with planting distances of 2.2/1.2 m in the semi-high culture system, protected over winter. To highlight the yield and quality potential of the resistant varieties, researches were focused on observations and determinations regarding the vegetation phenophases, yield and quality of the plants, in direct relation with the ecological factors.

Physico-chemical determinations

Total acidity (g/L tartaric acid), sugars (g/L), total phenolic compounds (g gallic acid equivalent/100 g f.w.) were determined according to the Compendium of international methods of wine and must analysis [1]. Antioxidant activity of grape extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method [2], using a 1700 Pharmaspec® UV spectrophotometer (Shimadzu, Japan).

Results and Discussions

Climatic condition

In the period 2016-2017 the average temperatures were higher than the multiannual value (9.8 °C), reaching 11.0 °C in 2016 and 10.8 °C in 2017; absolute minimum temperatures were below the frost limit of vine, respectively -18,7 °C in 2017. The year 2017 was considered normal from the point of view of rainfall, the amounts of rain during the vegetation period showing values very closed to the multiannual value in the wine center Copou-Iași, respectively 293.4 mm in 2017 and 338.8 mm in 2016. The high number of days with maximum temperatures over 30 °C (39 days in 2017 and 53 days in 2016) as well as absolute maximum temperatures varying from 34.9 °C (2016) to 37.3 °C (2017) favoured the harmonious development of the foliar apparatus, having a positive influence on grape ripening and sugars accumulation.

Phenology

On the background of the presented climatic conditions, the resistant varieties studied covered all the phenophases of the active vegetation period, starting with budburst, occurred between 12 and 25 April, followed by flowering, between 2-10 June and the grapes veraison, in 5-16 August. In the Copou-Iasi wine ecosystem, the technological maturity of grapes occurred in the last two decades of September, respectively the first decade of October (Moldova variety), the time of harvest being determined according to the sugars/acidity ratio.

The number of days of the active vegetation period was between 182-195, ending with the normal cessation of metabolic processes (leaf fall), between 28th and 30th October (Table 1).

Table 1. Phenological spectrum of resistant grapevine varieties (2016-2017)

Variety	Budburst	Blooming	Veraison	Grapematuration	Leaf fall
Radames	12 – 19 IV	2 – 3 VI	6 – 8 VIII	12 – 15 IX	28 – 30 X
Purpuriu	12 – 21 IV	2 – 3 VI	5 – 10 VIII	12 – 18 IX	28 – 30 X
Mara	13 – 18 IV	3 – 4 VI	5 – 16 VIII	16 – 26 IX	28 – 30 X
Moldova	15 – 25 IV	7 – 10 VI	15 – 16 VIII	1 – 3 X	28 – 30 X

Physico – structural determination

The hybrid variety Moldova, for fresh consumption, recorded the highest values of berry weight (4.9 g) and grape weight (296 g), and the value of the berry index, respectively the number of berries per 100 g of grape, was 20.41, confirming the classification of this genotype in the category of table grape varieties. The weight of Mara grapes was medium, reaching up to 220 g, with an average berry weight of 3.1 g. The structure index, representing the ratio between the weight of the berries and the weight of the rachis, was 42.12, specifically for table grapes varieties (Table 2).

Grape composition index, represented by the ratio pulp weight/skin and seeds weight, showed values between 1.58 and 2.16 for wine varieties and mixed properties (for wine and fresh consumption) and 3.54 for table grape variety Moldova.

Table 2. The main physico-mechanical characteristics of the studied varieties

Features	Radames	Purpuriu	Mara	Moldova
Grape weight (g)	197	196	220	296
Rachis weight (g)	4.2	4.8	5.1	9.6
Berries weight (g)	192.8	191.2	214.9	286.4
Berry weight (g)	3.14	2.97	3.1	4.9
Weight of 100 berries(g)	315	298	310	496
Skin weight (g)	0.35	0.42	0.45	0.56
Pulp weight (g)	2.65	2.45	2.34	4.22
Seeds weight (g)	0.14	0.10	0.31	0.12
Number of seeds	2.00	2.57	2.48	2.00
Structure index	45.90	39.83	42.14	29.83
Composition index	2.16	1.93	1.58	3.54
Berry index	31.85	33.67	32.26	20.41

In the climatic conditions of the Copou-Iasi wine center, the average of calculated production per hectare ranged between 17.00 t/ha (Purpuriu variety) and 22.07 t/ha (Moldova variety).

Regarding the potential of sugar accumulation in grapes at full maturity, were distinguished varieties Radames and Mara, which accumulated sugar concentrations of 193 and 194 g /L respectively (Table 3).

Chemical determination

Moldova variety showed the highest grape yield per hectare, with a sugar concentration at full maturity of 160 g/L, the value of the glucoacidimetric index (sugar/acidity ratio) falling within the specific range for table grapes. According to OIV, black table grapes with a soluble dry matter content between 12.5 and 16 Bx must have a sugar/acidity ratio of at least 20/1 to be considered ripened [3].

Table 3. Quantitative and qualitative production of the studied varieties (2016-2017)

Variety	Yield		Weight of grapes (g)	Sugars (g/L)	Total acidity (g/L tartric acid)	Sugar/ acidity ratio
	Actual (kg/vine stock)	Calculated (t/ha)				
Radames	5.17	19.57	197.00	193.00	6.36	30.34
Purpuriu	4.48	17.00	196.00	174.00	6.80	25.58
Mara	4.90	18.50	220.00	194.00	6.20	31.29
Moldova	5.83	22.07	296.00	160.00	7.88	20.30

Phenolic compounds contribute to the colour, taste and aroma of grapes and are involved in enzymatic browning reactions, being, after sugars and acids, the most abundant constituents of grapes [4]. The highest phenolic content was recorded in seeds (4.21-4.36 g gallic acidequivalent/100 g) and skins (1.09-1.84 g GAE/100 g). Grapes of resistant variety Moldova showed the highest concentration of phenolic compounds (Fig. 2).

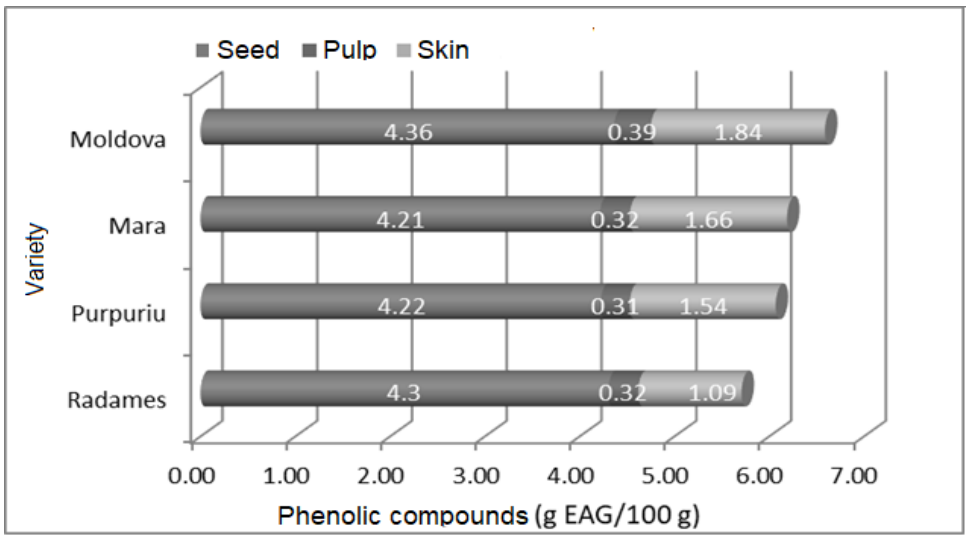

Fig. 2. Total phenolic content of resistant varieties grapes

Antioxidant activity of the grape ethanolic extracts (ethanol 96%, overnight, at 20 °C), was evaluated by testing their ability to annihilate the free radical DPPH. The antioxidant activity of the grapes was estimated as high, varying between 60.02% (Radames) and 71.13% (Moldova) (Fig. 3).

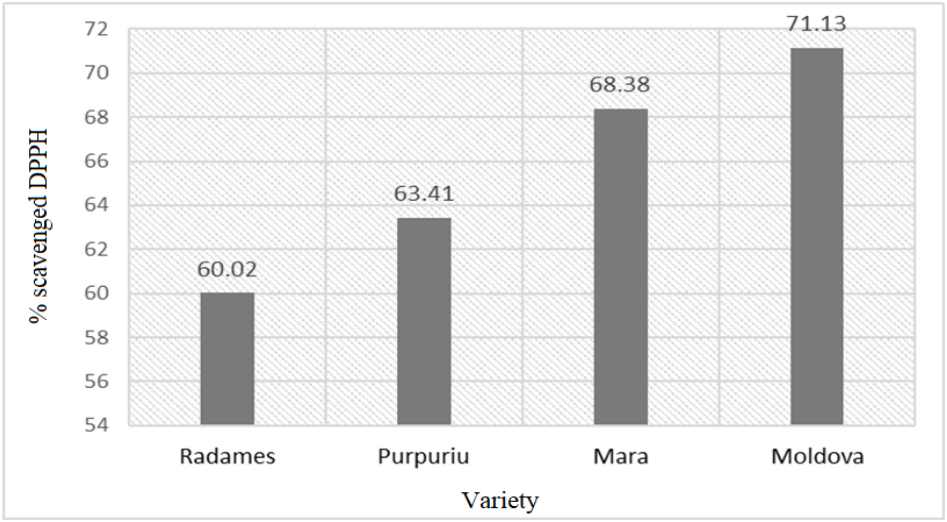

Fig. 3. Antioxidant activity of grape extracts

At the analysed resistant varieties, was found a positive correlation between the antioxidant activity and the content of phenolic compounds in seeds (r = 0.8988) and skins (r = 0.8538), similar to that recorded in the grapes of some *Vitis vinifera* L. varieties [5].

Conclusions

1. The climatic conditions of the reference years positively influenced the development of the vegetation phenophases, high temperatures during summer and the uniform distribution of the rainfall favouring grape ripening and the accumulation of sugars in grapes of hybrid varieties analysed.
2. In the climatic conditions of the Copou-Iasi wine-growing center, Moldova variety was noted for its high yields, however grapes retaining a slightly higher total acidity until maturity, but accumulating significant concentrations of phenolic compounds with antioxidant potential.
3. Mara resistant variety was characterized by earlier grape maturation, compared to Moldova variety, and high yields, grapes with black-blue medium sized berries and good crunchiness, feature that can be correlated with a high resistance to handling, transport and storage.
4. The experimental results obtained indicate that the studied interspecies grapevine varieties can successfully replace direct producer hybrids, the grapes being destined for fresh consumption (Moldova, Mara) or wines (Purpuriu, Radames), as well as for ornamental purposes due to the appearance of grapes and leaves, as well as their high vigour.

Acknowledgments

This paper was published under the frame of 2181/19.09.2018 project "Colectarea, conservarea şi monitorizarea resurselor genetice valoroase în noua colecţie ampelografică a SCDVV Iaşi", financed from Research-Development Station for Viticulture and Winemaking Iasi-Romania own revenues.

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Preliminary Selection of Malolactic Bacteria Strains Isolated from Indigenous Microbiota

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Abstract

Malolactic fermentation (MLF) is defined as the enzymatic bioconversion of malic acid in lactic acid, a process performed by lactic acid bacteria. The procedures for the isolation of lactic acid bacteria (LAB) strains from red wines in spontaneous MLF, obtained in Copou-Iasi wine center, resulted in the obtaining of 12 strains with a high potential for conversion of malic acid, in synthetic wine. Catalase-negative and Gram-positive isolates have been tested for their ability to produce biogenic amines by decarboxylation of amino acids and to use citrate as the sole source of carbon and energy (Simmon's medium). Also, the ability of LAB isolates to produce acetoin from both citrate and glucose has been tested. The tested malolactic strains showed low citrate utilization capacity, eight of them being able to decarboxylate arginine.

Although they did not produce acetoin from citrate, three of the bacterial isolates produced acetoin by glucose metabolism.

Keywords: acetoin, citric acid, indigenous microbiota, lactic acid bacteria, malolactic fermentation

Introduction

In winemaking practice, it is generally accepted that wine is the result of two biological processes of fermentation, alcoholic and malolactic, determined by the microorganisms that develop on the grapes during their ripening period. Malolactic fermentation (MLF) of wine is defined as the enzymatic bioconversion of malic acid into lactic acid, a process carried out by lactic acid bacteria (LAB), and which usually precedes the alcoholic fermentation of wine. Run under optimum conditions, MLF has important effects on wine quality: it reduces acidity and slightly increases the pH, increases the biological stability of the wine, changes the aroma and taste of the wine, increasing its complexity.

LAB is Gram-positive, unsporulated, immobile, catalase-negative microorganisms, belonging to the genera: *Lactobacillus* (fam. *Lactobacillaceae*), *Pediococcus* (fam. *Lactobacillaceae*) and *Leuconostoc* (fam. *Leuconostocaceae*), bacteria that assimilate carbohydrates both in the homofermentative and heterofermentative pathways [1, 2]. Isolation and selection of LAB strains for starter cultures are complex activities, which involve various screening procedures, including testing the ability of bacteria to produce undesirable or even toxic fermentation by-products (biogenic amines, ethyl carbamate, diacetyl). Given the importance of the biological factors involved in the MLF process, isolation and selection of LAB strains from different vineyards is required as a necessity, being attested that the most appreciated wines, characterized by a higher typicity, are obtained when the microorganisms used in the wine biotechnological processes are isolated and selected from the indigenous microbiota of the respective vineyard.

Materials and Methods

Lactic Acid Bacteria Isolation

Isolation of LAB strains was performed from red wines undergoing spontaneous MLF, obtained in 2018 at the Research Development Station for Viticulture and Winemaking Iasi, Romania, wine center Copou-Iasi, from varieties: Merlot, Cabernet Sauvignon, Cabernet Sauvignon clone 4 Is, Cabernet Sauvignon clonal elite 16.6.9, Arcaş, Feteascăneagră and Pinot noir. Monitoring of the MLF process was performed by thin layer chromatography (TLC), using cellulose plates 20 x 20 cm (Merck, Germany) and a mixture of development: n-butanol/distilled water/acetic acid/bromophenol blue, in ratio 100/20/20/0.1. Culture media used to isolate, test and conserve LAB were based on De Man-Rogosa-Sharpe (MRS) medium [3]. For the preliminary selection of LAB isolates, was performed the catalase test by the rapid technique (with hydrogen peroxide 3%) and Gram staining. The physico-chemical characterization of wines was carried out according to the methodology presented in the Compendium of international methods for the analysis of musts and wines of the International Organization of Vine and Wine [4].

Selection of Malolactic Bacteria Strains

For testing the ability of LAB to use citrate as the sole carbon source, Simmon's citrate agar medium was used [5].In order to highlight the production of acetoin (Voges-Proskauer reaction) was used Clark-Lubs medium (5 g/L glucose) and for testing the production of biogenic amines by the LAB strains, the amino acid-decarboxylase test was performed, on MDA medium (Modified Decarboxylating Agar) [6]. Thus, after the initial reactivation of the bacterial culture in MRS medium with 3 g/L malic acid and incubation for 4 days at 28 ℃, the strains were plated on Petri dishes with MDA agar medium with 4 g/L of each tested amino acid (histidine, arginine, lysine, ornithine and tyrosine), incubated in anaerobiosis, at 28 ℃, 48 hours, after prior adaptation on MRS medium with 1 g/L malic acid and 2 g/L of each amino acid, at a cell density of 10^9 CFU/mL.

Results and Discussions

Lactic Acid Bacteria Isolation

Due to the increasing concentrations of ethanol produced by yeasts during alcoholic fermentation, presence of molecular SO_2, low pH, poor nutritional status and competitive interactions with the yeast population, the bacterial population gradually decreases, most species not being able to multiply until the end of alcoholic fermentation [7, 8]. For these reasons, the protocol used to isolate LAB requires that strains to be obtained from wines undergoing spontaneous MLF, when the bacterial populations have high cell densities.

Initiated spontaneously, after the alcoholic fermentation of grape must, the MLF of wines lasted between 25 and 28 days, at a temperature of 20±2 ℃. At the time of sampling, the wines showed an alcoholic concentration between 11.50 and 13.50% vol., pH 3.36-3.62, a total acidity that varied from 5.25 to 6.12 g/L tartaric acid and residual sugars below 1 g/L (dry wines).Wine samples were collected aseptically, 1 mL of wine being used to make decimal dilutions (10^{-1} ... 10^{-4}) in sterile distilled water. The dilutions obtained were inoculated on MRS medium, supplemented with 5 g/L malic acid and 1% $CaCO_3$ (pH 4.5) and incubated at 30 °C, 5 days, in anaerobiosis (GENbag anaerobic®; BioMérieux, France). The number of LAB varied from 16×10^3 (Pinot noir) to 58×10^3 (Arcaş) (Table 1).These values are slightly lower in comparison to data obtained for red wines produced in the Miniş-Măderat vineyard (Arad, Romania), where in the similar phase of MLF the density of indigenous lactic bacterial populations was between $5{,}0\times10^5$ (Pinot noir) and 16×10^6 (Cabernet Sauvignon) [9], concluding that the diversity and

density of LABare influenced by both the grape variety and the geographical region from which they originate.

Table 1. Lactic acid bacterial load of the wine samples

No.	Wine sample	Dilution	Number of colonies	Number of lactic acid bacteria /mL
1	Merlot	1:1000	35	35×10^3
2	Cabernet sauvignon	1:1000	44	44×10^3
3	Arcaş	1:1000	58	58×10^3
4	Cabernet sauvignon cl. 4 Is	1:1000	29	29×10^3
5	Cabernet sauvignon cl. 16.6.9	1:1000	30	30×10^3
6	Fetească neagră	1:1000	27	27×10^3
7	Pinot noir	1:1000	16	16×10^3

Individualized colonies, which showed transparent halo (Fig. 1), were passed in liquid MRS medium with 5 g/L malic acid (pH 4.5) and incubated in anaerobiosis at 30 °C for 5-7 days.

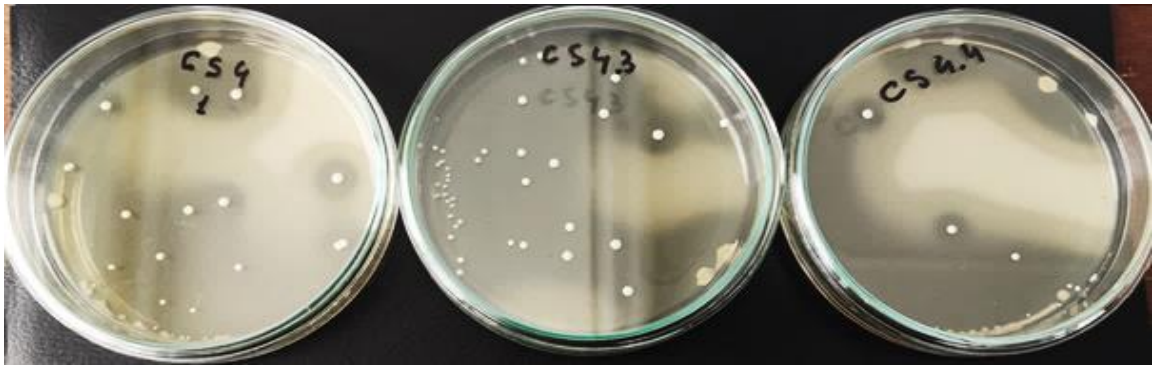

Fig. 1. Development of lactic acid bacteria colonies on MRS agar medium

For purification, Gram-positive bacterial strains, which were not able to synthesize the exoenzyme catalase and showed morphophysiological characteristics specific to LAB, were retained and cultured repeatedly on liquid and solidified MRS medium (4-5 steps) (Fig. 2).

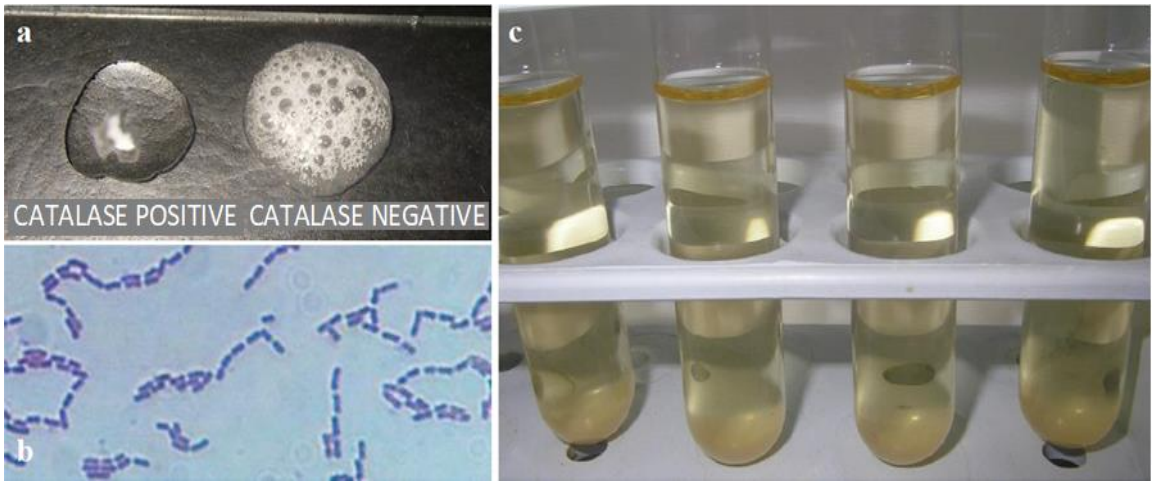

Fig. 2. Catalase test (a) and Gram staining (b) in purified lactic acid bacterial isolates by repeated cultivation (c)

Thus, 67 LAB strains were isolated and purified, 58 of them belonging to genus *Lactobacillus* (bacilli) and nine to the genus *Oenococcus* (circular-ellipsoidal cell chains; coco-bacilli). After microscopic examination of strain purity, isolates were preserved in liquid MRS medium supplemented with 20% glycerol, at a temperature of -20 °C [10].

After prior adaptation on MRS medium with 10% ethanol (v/v) at 28 °C for 48 hours, strains were inoculated at a cell density of 10^8 CFU/mL (0.5 McFarland) in 100 mL synthetic wine with 12% ethanol (v/v), 20 days, for performing MLF, according to specific protocol [11]. The completion of MLF in synthetic wine was evidenced by TLC, only 12 LAB strains showing high potential for malolactic bioconversion (Fig. 3).

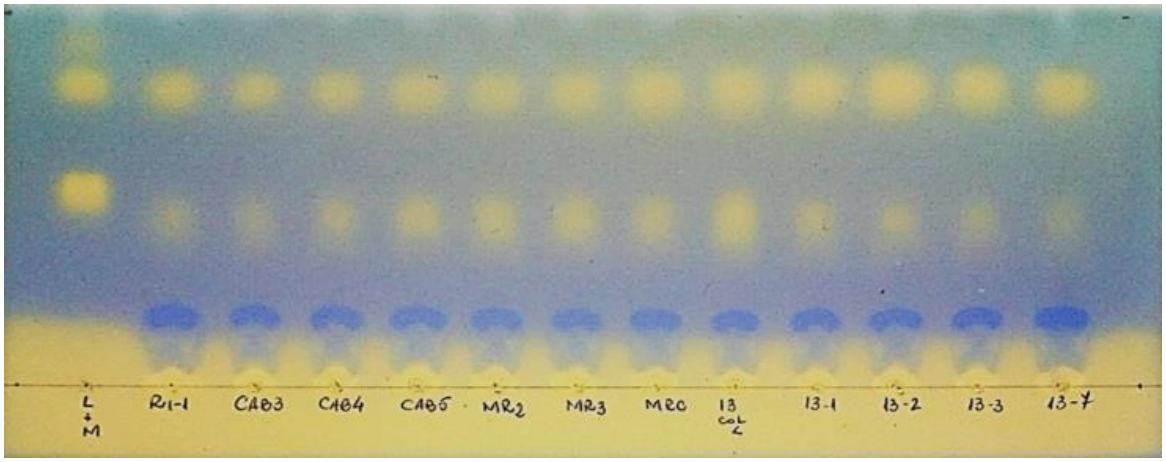

Fig. 3. Screening of bacterial isolates in the malolactic fermentative process
Legend: L + M - malic acid and lactic acid standards. R1-1 ... 13-7: bacterial isolates.

The ability to produce biogenic amines in wine is an essential criterion in the selection of LAB starter cultures. Some lactic bacteria convert amino acids into biogenic amines that can give to the wine an unpleasant taste and smell (cadaverine, putrescine) or cause pathological conditions (histamine, tyramine) [12]. Of the 12 LAB isolates tested, eight strains of the *Lactobacillus* genus were found to be weakly producing biogenic amines by decarboxylation of arginine, fact evidenced by the pale-purple staining of the culture medium (Fig. 4).

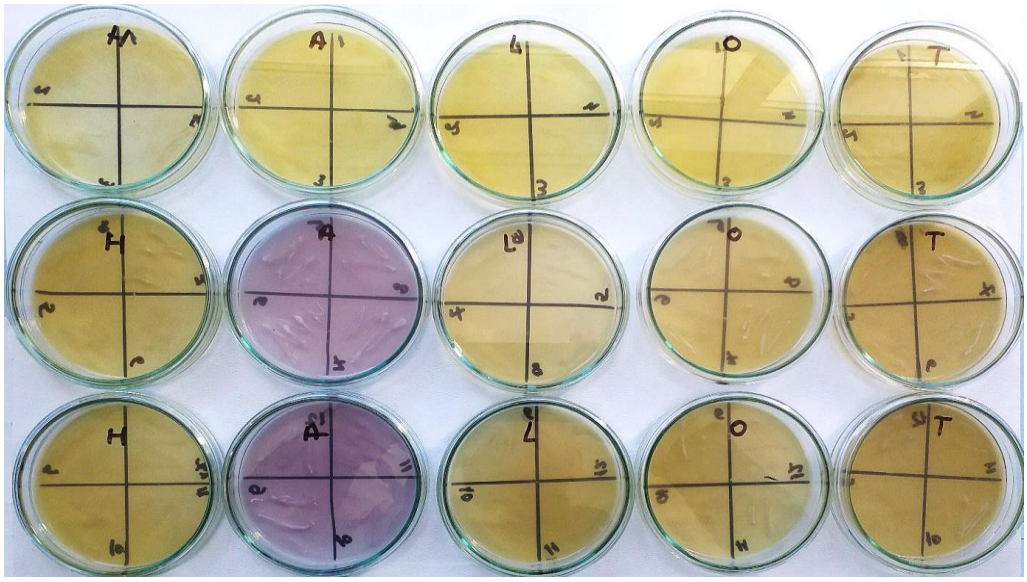

Fig. 4. Amino acid decarboxylase test – purple appearance indicates positive reaction

Citrate-positive LAB species are capable of degrading citrate during MLF. These bacteria possess citrate-permease and citrate-lyase enzymes, responsible for citrate degradation.

Citrate metabolism results in the production of compounds such as acetate, diacetyl, acetoin and 2,3-butanediol, which can negatively affect the quality of the wine. Bacterial isolates were tested for their ability to use citrate as the sole source of carbon and energy. Inoculated on Simmon's citrate agar medium, with sodium citrate as the sole source of carbon and bromtimol blue as pH indicator, the bacterial strains weakly alkalized the culture medium, proving a reduced ability to metabolize citric acid (Fig. 5). For exemplification, in Fig. 5 was added a plate with anundesirablecitrate-consuming LAB strain, the environment being coloured in blue.

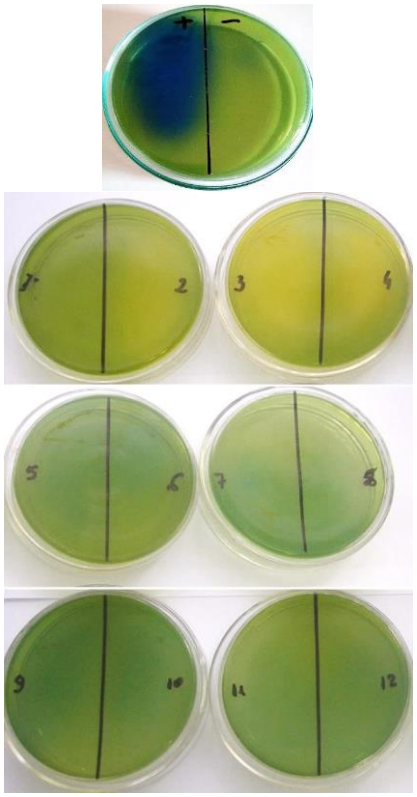

Fig. 5. Citrate utilization test

The production of acetoin from citrate and glucose (Fig. 6) was tested by centrifugation of 1 mL of cell suspension of liquid Simmon's medium or Clark-Lubs medium (5 g/L glucose) and adding to the supernatant two drops of alcoholic solution of α-naphthol 5% and two drops of 40% KOH solution (Voges-Proskauer reaction). The appearance of the red colour indicated the presence of acetoin. Tested LAB strains were not able to produce acetoin from citrate (Fig. 6, a). Two of the strains belonging to the *Lactobacillus* genus, as well as one strain belonging to the *Oenococcus* genus, metabolized glucose producing low concentrations of diacetyl, transformed by oxidation into acetoin, colouring the culture medium into light red, the intensity of the reaction being very low (Fig. 6, b).

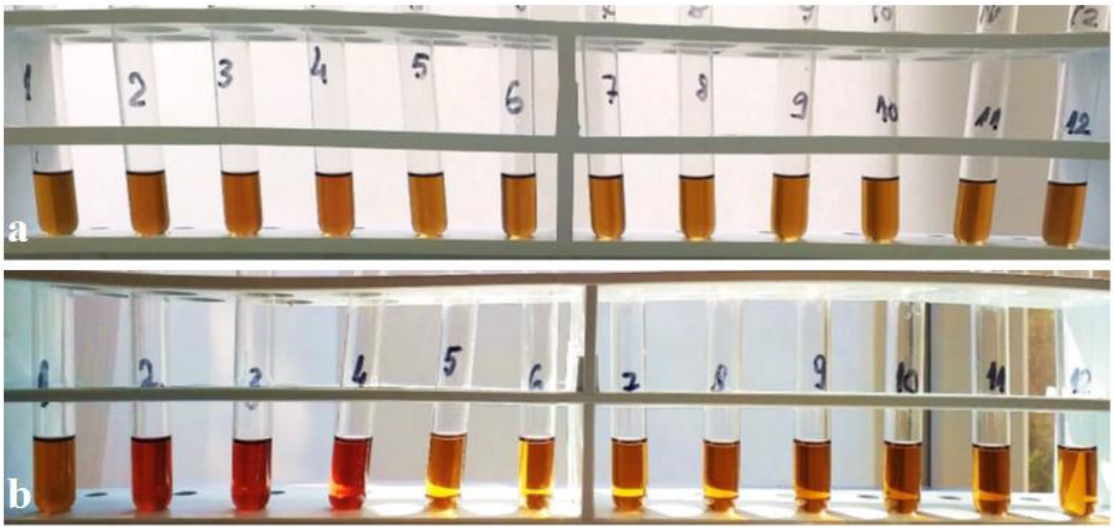

Fig. 6. Voges-Proskauer test. Red colourindicates positive test, respectively the synthesis of acetoin from citrate (a) or from glucose (b); Legend: 1-12 – bacterial isolates.

After all screening procedures were applied, two LAB strains potentially effective in performing wine MLF, belonging to the *Lactobacillus* (R1-1) and *Oenococcus* (13-7) genera, were retained for further characterization.

Conclusions

1. From red wines produced at the Research Development Station for Viticulture and Winemaking Iasi -Romania, found in spontaneous malolactic fermentation, were isolated, purified and retained, after testing in the fermentative process, 12 Gram-positive and catalase-negative lactic acid bacterial strains, potentially effective in conducting MLF of wines.
2. Amongst bacterial isolates, eight strains belonging to the genus *Lactobacillus* showed poor ability of arginine decarboxylation and low capacity of citrate metabolization.
3. Two strains belonging to the genus *Lactobacillus* and one strain belonging to the genus *Oenococcus*, were able to metabolize glucose producing low concentrations of diacetyl, transformed by oxidation into acetoin.
4. After the application of screening procedures, two malolactic bacterial strains belonging to *Lactobacillus* and *Oenococcus* genera were selected, their further characterization and testing in microvinification trials being necessary.

Acknowledgments

This paper was published under the frame of the project no. 2180/19.09.2018, financed from the state budget.

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Evaluation of the Phenolic Potential of some Varieties for Red Wine Cultivated Vineyards in the Wine Center of Iaşi Copou

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Abstract

The tendency to increase the thermal regime from Copou Iasi viticulture center, observed in last years, determine the increase of the favourability and extension of the cultivation area of grape varieties for red wines. So, to evaluate phenological potential of red wine grape varieties growth in Copou Iasi viticulture center, were taken into the study during two years the varieties: Arcaş and Cabernet Sauvignon. The results confirm the possibility of obtaining through an appropriate technology of some red varieties of quality wines.

Keywords: red wines, chemical composition, phenolic profile, anthocyanins

Introduction

The varieties for red wines are cultivated in vineyards with high heliothermal resources, where grapes accumulate big amount of plyphenols and sugars [1, 2, 3]. The content of the grapes in phenolic compounds (anthocyanins, tannins), represents a basic technological condition for the quality of red wines [4, 5]. The values of synthetic ecological indicators from the last decades from Copou Iasi viticulture center, indicates a good favourability of variety culture for quality red wines [6]. In this context, to evaluate the phenological potential of red varieties of vine cultivated in Copou Iasi viticulture center where been taken into the study Araş variety obtained at Viticulture and Oenology Research and Development Station in Iasi and Cabernet Sauvignon variety.

Material and Method

Researches were carried out between 2016 and 2017. In order to optimize the extraction of phenolic compounds from grape skin they have experimented two technological variants for maceration and fermentation on the broth, 8 days (V1) and 16 days (V2). The resulting wines were physically and chemically characterized according to the OIV standards, and in order to carry out the phenolic profile of the wines, the HPLC analysis was carried out, whereby a series of phenolic acids, stilbeni (trans-resveratrol), some non-hydrolyzable tannins (catechin and epicatechin), acetylated and coumarylatedanthocyanins. Their identification was based on chromatograms.

Results and Discussions

The technological potential of varieties Arcaş and Cabernet Sauvignon in the two years of study, it was influenced by both the level of the climatic factors and the fruit loads attributed to

the cutting. The grapes presented at harvest a sugar content between 179 and 211 g/L, a total acidity between 3,3 and 4,9 g/L H_2SO_4 and they have accumulated in the skin of the grain's sufficient quantities of polyphenols, which will ensure, through appropriate technology, a successful colour of wines. The Arcaş variety was highlighted with a higher technological potential than the Cabernet Sauvignon parent variety [7]. The main composition characteristics of Cabernet Sauvignon and Arcaş wines are presented in table 1.

Table 1. Physical-chemical characteristics of red wines obtained in 2016 and 2017

Determined parameters/Variant	Cabernet Sauvignon				Arcaş			
	2016		2017		2016		2017	
	V1	V2	V1	V2	V1	V2	V1	V2
Alcoholic concentration (% vol)	11.2	10.4	10.6	10.4	11.8	11.7	10.5	10.5
Reducing Sugars (g/L)	0.8	1.0	1.0	1.2	2.2	1.4	0.8	1.1
Total acidity (g/L $C_4H_6O_6$)	6.0	5.3	5.9	6.0	5.1	5.2	6.0	5.8
Volatile acidity (g/LCH_3COOH)	0.5	0.4	0.6	0.5	0.5	0.6	0.5	0.6
Total dry extract (g/L)	22.3	22.4	23.4	22.2	24.8	23.7	22.4	21.9
Non-reducing extract (g/L)	21.5	21.4	22.4	21.0	22.6	22.3	21.6	20.8
Colour intensity (Ic)	3.8	2.0	1.8	1.5	5.8	5.2	2.7	2.9
Tent (T)	0.6	0.9	1.0	1.0	0.6	0.7	0.8	0.9
Anthocyanins (520 nm)	409.1	332.4	403.1	405.8	574.1	594.2	560.7	570.0
Totals polyphenols	1.2	1.0	1.5	1.4	1.7	1.9	1.6	1.8
dA%	61.4	48.2	41.5	41.7	57.8	56.9	49.2	45.5
d 420%	34.9	42.1	45.9	45.0	34.3	35.8	40.0	41.8
d 520%	56.5	49.1	46.1	46.2	54.2	52.8	49.6	47.9
d 620%	8.6	8.8	8.0	8.9	11.4	11.5	10.4	10.4

The average values of the alcoholic concentration of the wines obtained vary from a minimum of 10.40% volume (Cabernet Sauvignon 16 days maceration) to a maximum of 11.80% volume (Arcaş – 8 days maceration). Although the wines were fermented "dry", the content of the wine in ethyl alcohol differs between the studied variants by 0.1-0.8% vol. This difference may appear due to the losses in the yeast respiration process, as well as the alcohol losses which are released during the alcoholic fermentation together with CO_2. The wines fermented until the total exhaustion of the sugars, becoming dry wines with contents less than 4 g/L, the maximum limit being 2.2 g/L at Arcaş and 1.2 g/L at Cabernet Sauvignon.

The average values of the total acidity, vary in Cabernet Sauvignon wines from 5.3 g/L to 6.0 g/L, and in the Arcaş variety between 5.1 g/L and 6.0 g/L, being within limits normal, thus giving the wines a balanced and fruity taste. The volatile acidity shows average values between 0.4-0.6 g/L CH_3COOH. The Arcaş variety shows higher values of volatile acidity in the 16-day maceration variant. In both varieties the non-reducing extract has close values, their average being 21.6 g/L for wines obtained from the Cabernet Sauvignon variety and 21.8 g/L for wines obtained from the Arcaş variety. The obtained results show that under the climatic conditions of the studied period, wines with an extract content of less than 23 g/L were obtained, which cannot be classified in the DOC category.

The colour intensity (CI) shows super unitary values, specific for red wines, ranging from 1.5 to 3.8 in the Cabernet Sauvignon variety, respectively between 2.7 and 5.8 in the Arcaş variety. In both varieties, higher values are observed in the fermentation maceration variant for 8 days. Tent values (T) are between 0.6 and 1.0, being characteristic of young wines. In the fermentation maceration variant for 16 days, slightly higher values of the hint are observed, which indicates a beginning of anthocyanin copolymerization with the wine tannin, the absorbance A520 nm corresponding to the red colour decreases, and the wine gets a reddish tint.

The identification and quantification of the phenolic compounds was performed on the basis of the chromatograms, and the obtained results are presented in table 2.

Table 2. The phenolic profile of wine obtained from Cabernet Sauvignon and Arcaş variety

Phenolic Compounds	Cabernet Sauvignon				Arcaş			
	2016		2017		2016		2017	
	V1	V2	V1	V2	V1	V2	V1	V2
gallic acid, mg/L	5.12	1.36	22.28	33.63	9.33	15.58	21.93	39.36
procatechinic acid, mg/L	0.95	0.91	1.20	1.34	0.74	0.73	0.91	1.41
p-hydroxybenzoic acid, mg/L	0.55	1.70	0.25	0.49	5.35	5.38	0.14	0.26
chlorogenic acid, mg/L	0.60	0.67	-	-	1.38	1.68	-	-
p-coumaric acid, mg/L	0.52	0.93	1.01	0.69	2.04	2.58	1.00	0.80
ferulic acid, mg/L	0.65	1.00	0.08	0.07	0.92	1.07	0.16	0.12
caffeic acid, mg/L	-	-	0.57	0.52	-	-	0.55	0.52
syringic acid, mg/L	-	-	35.05	63.89	-	-	31.43	66.90
catechin, mg/L	0.92	3.78	8,90	16,45	5.61	8.18	5,17	9,04
epicatechin, mg/L	1.05	1.95	8,62	19,84	1.29	1.72	5,68	11,92
resveratrol, mg/L	0.49	0.76	2.11	1.45	0.98	1.41	3.01	2.68
naringin, mg/L	1.61	1.59	-	-	3.55	4.12	-	-
myricetin, mg/L	-	-	0.89	0.65	-	-	2.97	2.75
quercitin, mg/L	-	0.44	0.08	0.02	-	-	0.88	0.17

Analysing the data obtained it can be notice that gallic acid present at Cabernet Sauvignon variety, medium values between 5,12 mg/L at 8 days maceration variant and 33,63 mg/L at 16 days maceration variant. Similar situation was observed at Arcaş variety respectively lower average values for the maceration variant for 8 days (9,33 mg/L) comparative with wine obtained after 16 days maceration (39,36 g/L). In relatively large quantities have been identified and other hydroxybenzoic acids, respectively procatechnic acid and p- hydroxybenzoic acid with limits of variation between 0,73 mg/L and 1,41 mg/L, respectively 0,14 mg/L și 5,38 mg/L.

The source of those last acids can be seeds and clusters.

The HPLC analysis also identified a series of hydroxycinnamic acids, namely chlorogenic, p-coumaric, ferulic, caffeic and syringic acid. Chlorogenic acid is higher at Arcaş wines, compared to the famous Cabernet Sauvignon. Thus, the minimum values can be recorded in Cabernet Sauvignon, variant of 8 days (0.60 mg/L), and the maximum in Arcaş at 16 days (1.68 mg/L).

The p-coumaric acid content is predominantly compared to the ferulic one. The values are between 0.52 mg/L and 1.01 mg/L Cabernet Sauvignon wines, respectively 0.80 mg/L and 2.58 mg/L Arcaş wines. The amount of p-coumaric acid decreases slightly with increasing fermentation maceration time, which could denote that the acid was released by hydrolysis of the ester compounds with coumaric acid.

Caffeic acid can have values very close to the two varieties. The amount of syringic acid can have a maximum value of 63.89 mg/L in the Cabernet Sauvignon variety and 66.90 mg/L in Arcaş. Compared with other phenolic acids which have a tendency to decrease with the prolongation of the maceration-fermentation period, the amount of syringic acid has a significant increase.

The content of wines in catechin and epicatechin, as well as the relationship between the two monomers is dependent on the variety, but also on the technology of vinification. The prolongation of the maceration time allows the advanced extraction of these compounds from the seeds, which has as a result an increase of the catechin content and a decrease of the ratio between them. Regarding the content of catechin, we note the wide range of variation, from 0.92 mg/L to 16.45 mg/L for Cabernet Sauvignon wines and from 5.17 mg/L to 9.04 mg/L at Arcaş.

In addition to phenolic acids in the analysed wines, trans-resveratrol was also identified. In 2017, there is a higher concentration of trans-resveratrol in Arcaş wines with 8 days of maceration, respectively 3.01 mg/L and 2.68 mg/L respectively with a 16-day maceration.

Analysing the anthocyanic profile of the wines (Table 3), it is found that for all the maceration variants used, malvidin is found in the highest proportion, followed by petunidine and peonidine, the percentage of cyanidine being the lowest.

The majority participant in the composition of the wines is malvidina, with an average value of 62.54% in the Cabernet Sauvignon variety and 57.87% in the Arcaş variety. Petunidine and peonidine, which transmits the red wines colour, are found in 1.7-4.89% and respectively 0.38-1.79%. Delphinidine represents on average 0.79-3.06% of total anthocyanins, its presence in red wines being very important due to its antioxidant and anti-inflammatory properties.

Cyanidine, which transmits purple red to wines, is found only in the proportion of 0.03 and 0.20%.

Table 3. Anthocyanin profile of Cabernet Sauvignon and Arcaş wine (participation %)

Free anthocyanins (%)	Cabernet Sauvignon				Arcaş			
	2016		2017		2016		2017	
	V1	V2	V1	V2	V1	V2	V1	V2
delphinidin-3-monoglycoside	1.37	0.79	1.12	3.06	2.97	2.44	2.41	2.67
cyanidin-3-monoglycoside	0.04	0.06	0.03	0.04	0.20	0.03	0.03	0.03
petunidin-3-monoglycoside	2.44	1.70	1.96	3.86	4.84	4.76	4.82	4.89
peonidin-3-monoglycoside	0.87	0.38	0.46	1.79	1.56	1.61	1.47	1.58
malvidin-3-monoglycoside	63.06	65.48	64.68	56.95	59.90	57.91	59.06	54.62

For the complete establishment of the anthocyanin profile, their acetylated and coumarylated derivatives were also determined (Table 4). Acetylated forms are found in varying amounts and are a particular feature of each variety. The obtained results show that the studied varieties are characterized by a higher number of acetylated anthocyanins, which makes the acetylated/couarylated ratio super unitary. In the Cabernet Sauvignon variety, the acetylate of peonidine ranges from 0.83 to 1.50%, and that of malvidin between 25.63 and 27.29%. Also, the coupling of peonidine is between 0.20 and 0.50%, and that of malvidin between 3.38 and 4.34%. The acetylated derivatives of malvidin have the highest values, determining the anthocyanin imprint specific to Cabernet Sauvignon wines.

Table 4. The acylated derivatives of peonidine and malvidin from Cabernet Sauvignon and Arcaş wines (participation %)

Free anthocyanins (%)	Cabernet Sauvignon				Arcaş			
	2016		2017		2016		2017	
	V1	V2	V1	V2	V1	V2	V1	V2
peonidin-3-acetyl-glycoside	1.50	0.83	1.05	0.89	1.96	2.87	1.65	1.47
malvidin-3-acetyl-glycoside	25.88	26.98	25.63	27.29	21.99	24.26	27.63	27.44
peonidin-3-coumaryl-glycoside	0.50	0.22	0.23	0.20	1.19	1.20	0.99	0.67
malvidin-3-coumaryl-glycoside	4.34	3.56	3.66	3.38	5.40	4.92	5.94	4.13
Σ (%acet +%cum)	**32.22**	**31.59**	**30.58**	**31.75**	**30.53**	**33.25**	**36.21**	**33.71**
Σ %acet / Σ%cum	**5.66**	**7.36**	**6.85**	**7.88**	**3.64**	**4.44**	**4.22**	**6.02**

The wines of the Arcaş variety are noted by higher values of acetylated derivatives compared to the wines obtained from Cabernet Sauvignon. Thus, the acetylate of peonidine ranges from 1.47 to 2.87%, and of malvidin between 21.99 and 27.63%. Also, the couarylate of peonidine is between 0.67 and 1.20% and that of malvidin between 4.13 and 5.40%. In both varieties, in the variant of maceration fermentation on the broth for 16 days there is a tendency to decrease the values of acetylated and coupled anthocyanins. In order to know the percentage in which acetylated derivatives contribute to the anthocyanic profile of the wines, their sum was calculated, which presents values between 30.58% and 32.22% in the Cabernet Sauvignon variety and between 30.53 and 36.21% in the variety Arcaş.

Conclusions

In the climatic conditions of the wine center Copou Iaşi, the wines obtained from the Cabernet Sauvignon and Arcaş varieties, depending on the physical-chemical parameters of composition and the colour parameters, were classified in the category of wines with geographical indication (GI).

The phenolic profile of Cabernet Sauvignon and Arcaş wines, made by HPLC analysis (high performance liquid chromatography), highlights the presence of important quantities of phenolic acids (gallic acid, procatechinic acid, p-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid ferulic), stilbeni (trans-resveratrol), non-hydrolyzable tannins (catechin and epicatechin), as well as some flavones (quercitin and naringin).

The analysis of the anthocyanic footprint shows that the wines from the Cabernet Sauvignon variety have a higher percentage of acylatedanthocyanins, compared to the Arcaş variety, which gives them a better resistance and colour stability during maturation and aging.

Acknowledgments

The work was elaborated within the project within the Sector Plan ADER 2020, PS 3.3.10 entitled "Researches regarding the identification and definition of the typical elements of Romanian wines. Harnessing the sanogenic potential of wines by increasing the phenolic content".

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Technological Sequences for Recovery of Vineyard Plants Affected by Extreme Climate Phenomenes

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Abstract

In the Copou Iaşi wine center, in recent years, there is a frequent extreme climatic fenomena, frozen and drought, that affected the vegetative potential and the productivity of the vine plants.

To highlight the levels at which temperature drops are possible and to assess the degree to which they have a destructive influence on vines, were analysed the lowest values of the temperatures, established the number of days with character of climatic accident, the frequency and periodicity of the years in which these temperatures occur. Also, the analysis of the rainfall regime, in recent years, shows us an increase in the frequency of drought. In the drought years, the high values of the temperatures corroborated with the water deficit of the soil have led to the accentuation of the atmospheric and pedological drought with unfavourable effects on the state of vegetation of the buds and on the productions of grapes.

Keywords: minimum temperatures, drought, restoration cuts

Introduction

Vineyards in the northeaster part of the country, located on the northern edge of the vineyard culture, are increasingly affected by climate change that has occurred in recent decades.

Changing the environmental conditions causes changes in metabolism, in the development and growth processes, with positive or negative influences on the quality and vitality of the plants [1]

The evolution of the climatic factors in the Copou-Iasi wine ecosystem, determined by the global warming of the climate through the increase of the average annual temperature, the decrease of the precipitation regime, the increase of the frequency of the drought years, induced a modification of the phenology of the varieties, of the chemical composition, with negative consequences on their quality and the quantity of grape productions [2, 3].

Material and Method

For the analysis of the climatic factors, the data recorded at the Agroexpert station of SCDVV Iaşi (minimum air temperatures, minimum surface temperatures, precipitation), as well as those of the Regional Meteorological Center Moldova Iaşi were used. The multiannual (normal) values were calculated for a period of 30 years, 1981-2010.

Results and Discussions

The main risk factors for the culture of vines, in the last years, have been *the winter frost* appreciated by the critical value of the absolute minimum temperature, *the drought* due to the

small amounts of precipitation, the maximum absolute temperatures higher than 30°C as well as other climatic factors with accidental character (hail).

The analysis of the absolute minimum temperatures of the winter months showed that the lowest value was recorded on January 26, 2010, respectively – 27.0°C in air and – 35.0°C on the soil surface (Table 1).

Table 1. The absolute minimum temperatures recorded in the wine center Copou Iasi (1998-2018)

Year	In air			At surface of soil		
	Month	Day	t ºC	Month	Day	t ºC
1998	XII	03	-19,0	XII	24	- 24,0
1999	XII	24	-13,0	II	20	-19,3
2000	I	25	-15,9	I	26	- 22,2
2001	XII	18	-20,4	XII	18	- 24,5
2002	XII	26	-19,8	XII	26	- 21,0
2003	I	13	-21,6	I	13	-30,6
2004	I	31	-17,0	I	31	-19,0
2005	II	08	-19,4	II	06	-27,6
2006	I	23	-25,1	I	25	- 29,0
2007	II	24	-19,6	II	24	- 25,0
2008	I	05	-19,5	I	05	-24,2
2009	XII	19	-17,0	XII	21	-29,0
2010	**I**	**26**	**-27,0**	**I**	**26**	**-35,0**
2011	I	05	-14,8	I	25	-20,5
2012	II	12	-26,7	II	08	-33,0
2013	I	09	-14,3	I	29	-20,5
2014	I	31	-20,6	I	31	-22,5
2015	I	01	-21,0	I	01	-27,4
2016	I	04	-17,5	I	03	-18,2
2017	I	20	-18,7	II	11	-27,1
2018	I	24	-19,7	I	24	-25,5
media			**-19,4**	**media**		**-25,0**

Also, the average of the lowest absolute minimum temperatures in the air was – 19.4°C, and on the soil surface of – 25.0°C. These values confirm once again that the Copou-Iaşi wine center is in the semi-protected area of vines.

To highlight the levels at which temperature drops are possible and to assess the degree to which they have a destructive influence on vines, the lowest values of absolute temperatures were analysed, establishing the number of days with lower negative temperatures. or equal to – 15°C, – 20°C and – 25°C, which have the character of climatic accident in the vine, the frequency of the years in which these temperatures appear, as well as their periodicity (Table 2).

So, in air, *temperatures considered dangerous for vines (≤ - 15 °C),* those at which the fruit buds are affected, were recorded during the analysed period in 65 % of years in January, in 35 % of years in February and only in 20 % of the years in December.

Table 2. Frequency and periodicity of negative temperatures recorded between 1998-2018

The analysed elements	*Negative temperature grouping*					
	≤ - 15 °C		*≤ - 20 °C*		*≤ - 25 °C*	
	Air	*Soil*	*Air*	*Soil*	*Air*	*Soil*
January						
Number of days	***26***	***67***	***9***	***23***	***3***	***12***
Number of years	***13***	***14***	***5***	***14***	***2***	***4***
Frequency of the years	***65,0***	***70,0***	***25,0***	***70,0***	***10,0***	***20,0***
Periodicity of the years	***1,5***	***1,4***	***4,0***	***1,4***	***10,0***	***5,0***
February						
Number of days	***17***	***32***	***6***	***17***	***1***	***14***
Number of years	***7***	***12***	***1***	***7***	***1***	***6***
Frequency of the years	***35,0***	***60,0***	***5,0***	***35,0***	***5,0***	***30,0***
Periodicity of the years	***2,9***	***1,7***	***20,0***	***2,9***	***20,0***	***3,3***
December						
Number of days	***12***	***43***	***1***	***15***	***-***	***2***
Number of years	***4***	***8***	***1***	***6***	***-***	***2***
Frequency of the years	***20,0***	***40,0***	***5,0***	***30,0***	***-***	***10,0***
Periodicity of the years	***5,0***	***2,5***	***20,0***	***3,3***	***-***	***10,0***

The very dangerous temperatures (≤ - 20 °C), those affecting the buds and the annual wood, were registered in 25% of years in January, in 5.0% of years in February and in 5.0% of years in December. The periodicity of the years with temperatures ≤ - 20°C was 4 years in January, 20 years in February and 20 years in December.

The temperatures that are considered extremely dangerous for the vine (≤ - 25 °C), which affects all organs, are recorded with a frequency of 10% of years in January and 5% of years in February. In December, during the analysis period, temperatures are recorded ≤ - 25 °C. The periodicity of the years with temperatures ≤ - 25 °C was 10 years in January and 20 years in February.

On the surface of the soil, there was a much higher frequency of very low temperatures compared to those in the air. These temperatures have led to large losses of the main eyes, affecting the annual and multi-annual wood and implicitly to obtain some productions well below the average of the thermally normal years.

In the Copou wine center, in recent years, we are witnessing a decrease in the rainfall regime, compared to the multiannual average, that is 579.6 mm, and during the vegetation period 398.1 mm (Table 3). During the analysis period of the most drought years were: 2000, 2003, 2007, 2009, 2012, 2015, 2016 and 2017.

In general, there is an uneven distribution of them throughout the year. The precipitation deficit appears especially during the winter and the beginning of the vegetation period (April-May) with repercussions on the beginning of the vegetation phenophases in the vines and in the months of July-August. The lack of moisture in the soil has led to a decrease in the growth of shoots, a reduction in the foliar surface, a reduction in photosynthesis and consequently a decrease in the growth of the grains.

Table 3. Precipitation regulation and characterization of the years between 1998-2018 in the Copou wine center (according to the system used by N. Topor, 1964)

Year	Rainfall, l/m^2		The way of the year	Year	Rainfall, l/m^2		The way of the year
	Total	IV-IX			Total	IV-IX	
1998	***653,5***	***358,8***	***A Bit Rainier***	*2009*	*493,7*	*214,0*	*Excessive Droughts*
1999	***518,8***	***334,1***	***Normal***	*2010*	***674,3***	***419,9***	***Slightly Rainier***
2000	*399,7*	*269,2*	*Very Dry*	*2011*	***493,1***	***390,8***	***Slightly Drier***
2001	***748,0***	***533,2***	***Rainy***	*2012*	*535,9*	*287,1*	*Dry*
2002	***602,3***	***432,0***	***Normal***	*2013*	***656,1***	***501,1***	***Very Rainy***
2003	*485,4*	*293,5*	*Dry*	*2014*	***618,0***	***377,1***	***Slightly Rainier***
2004	***593,5***	***386,1***	***Normal***	*2015*	*365,5*	*180,6*	*Excessive Droughts*
2005	***646,1***	***433,9***	***A Bit Rainier***	*2016*	***646,8***	***333,8***	***A Little Drier***
2006	***500,2***	***341,5***	***A Little Drier***	*2017*	***546,6***	***293,4***	***A Little Drier***
2007	*523,5*	*283,6*	*Dry*	*2018*	***727,8***	***460,0***	***Rainy***
2008	***707,3***	***532,5***	***Very Rainy***				

The main technological sequences for the restoration of the vineyards affected by frost, drought and other climatic factors of accidental character are: the cuts applied to the logs and the maintenance works of the period of vegetation.

Pruning and maintenance cuts of the vineyards affected by frost

Cuts to restore the vegetative and productive potential of the buds with eye losses of over 50%. In this case, cuts will be made to compensate for the fruit load, taking into account the losses of eyes registered in each variety, by leaving, in addition, 1-2 fruit rings on cords, 1-2 clearing strings from safety plugs from the base of the hub. Also, it is taken into account that the large loads of fruit cause the cord to break. They should not exceed 60 eyes/bud in the varieties of small vigor (Muscat Ottonel, Sauvignon blanc, Chardonnay) and 80-100 eyes/bud in the varieties of medium and large vigor (Aligoté, Fetească regală, Fetească albă), in the case plantations with planting distance of 2.2 m between rows.

Recovery of the cords from the strings formed on the safety pins with the green design of the cords. From the strings formed from the safety straps, one will be chosen to form the new strain, and 1-2 strings to compensate for the grape production. The rope chosen for the formation of the strain will shorten at the level of the supporting wire and will be linked to the guardian, or to the old strain.

During the vegetation, the shoots will be removed from the stem, minus the last two shoots from the top, which will be used for green formation of the cords. By the time they reach half the distance between the stumps in a row, the tips will be punctured, favouring the issuance of children who will provide the fruit for next year.

Recovering the buds from the shoots started from their base. From the shoots started from the dormant eyes from the base of the buds are kept 3-4 more vigorous, and the others bend.

One of the shoots will be used to form the stem, and the others, with the role of balancing the vegetation, will pinch at 3-4 leaves. The children started from these are closely linked to the tutors, to be used the next year to provide 1-2 ropes of fruit to compensate for the production.

The stem chosen for the green formation of the stem is paled by the guardian and pinched under the supporting wire. From the children emitted at the top of the new strain, in the following year, half of the length of the cords will form in dry, and the rest will be completed in green.

Pruning and maintenance cuts of vineyards affected by drought

The drought mainly affects the plantations of young vines (1st and 2nd year), the old ones, the less vigorous buds and those with big loads of eyes left to the cutting. The stems and cords are dehydrated, deep longitudinal cracks appear, which cause the buds to dry.

In the case of vineyards affected by drought, differentiated cuts are applied depending on the variety, the driving form (low, semi-high and high). As a general rule, a lower fruit load is allowed, by reducing the number of fruit elements and their dimensions, in proportions of 25-30%, depending on the effects of the drought. Through these cuts a minimum balance is achieved, between the water consumption of the soil and the losses through the aerial organs of the hub. Also, in the year following the drought, moderate fruit loads will be assigned to cutting, in order to restore the vegetative and productive potential of the logs.

Special works during the vegetation period.

During the vegetation period, green works will be carried out, which will lead both to the restoration of the cuttings and to obtaining some grape productions that will allow partially to cover the expenses and to start the production cycle again. These are: the pinching of the sterile shoots started from the secondary buds, in order to stimulate the appearance of fruit bearing children; sprouting shoots, directing and linking them, in order to maintain at the base of the logs a sufficient number of shoots (3-5), which will be used both for the green restoration of the forms of management and for compensating the fruit load in the following year; the administration of foliar fertilizers with macro and microelements, that favour the growth of shoots and the restoration of the vegetative apparatus; special attention will be paid to combating diseases and pests; performing on time and under optimal conditions the soil maintenance work and combating weed; reducing the number of grapes on the stump, especially on table varieties or on small areas within the households.

Conclusions

1. From the analysis of the absolute minimum temperatures recorded in the winter months, it was found that in all the years the lowest temperatures, with a climate accident character, were in January, with a frequency of 25%.
2. In the wine-growing center Copou-Iasi, during the last two decades, there has been a decrease of the rainfall regime and a very uneven distribution throughout the year. Also, it has been found that after very rainy periods of 1-2 years, excessive 1-2 years of droughts will follow.
3. Particular situations were recorded in 2006, 2008, 2010, 2012, 2013, 2014, 2015 and 2018, when during the same year, the vineyards were affected by several extreme phenomena: frost, drought, rain abundant and hail.
4. In this context, the permanent restoration of the logs, as a result of their drought, frost and hail damage, should be an important link in the technology of growing vines.

Acknowledgments

This work was carried out within the Project 2179/19.09.2018 “Improvement of the technologies of cultivation of the vines, in order to ensure the sustainability of the vineyards in the vineyards of the northeast of the country, in the context of climate change”, financed from revenues from the Budget of state.

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Observations Regarding the Abundance, Dynamics and Damage Caused by the *Cydia Pomonella* L. and *Adoxophyes Reticulana* Hb. In Apple Tree Orchards

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Abstract

Dynamics of the flight adult of Cydia (Laspeyresia) pomonella and Adoxophyes reticulana were tracked in two stationary of apple tree orchards, these belonging to the didactic farm Vasile Adamachi from Iaşi. The observations were made in the two fields (ecological and conventional), with readings being recorded at intervals of 3-5 days, inventing catches at each reading, and the captured butterflies were removed from the trap. Depending on the results achieved, the dynamics of the butterfly flight was established for each generation: the beginning of the flight; maximum flight; the end of flight. Finally, according to this data, the time of application of the treatments for each generation was established, and depending on the number of catches and the opportunity of their application.

Keywords: dynamics, biology, phytosanitary control

Introduction

The importance of cultivating fruit trees and shrues results primarily in the food and therapeutic value of fruits, but the presence of these crop plants is at the same time a comforting, aesthetic and very useful element for people's lives by diminishing air pollution [1].

Through a given strategy, it is considered the location of culture, the choice of suitable rootstocks and assortment, fertilization, soil work, training and fruition cuts and the pest protection program what affects the culture species.

Widespread use in the apple tree culture of pesticides for phytosanitary protection has also caused many undesirable effects by accumulating them in soil, water and plants, as well as the emergence of numerous ecological imbalances.

One of the ways of a clean battle against these pests is the use of their natural enemies represented by predators and parasitides and based on the principle that each species has a place and a well-defined function within the ecosystem of which it is a part and constitutes a link to the trophic chains, sometimes very complicated.

Material and Methods

The experimental stationary of organic and chemically treated apple orchards from the didactic farm "V. Adamachi" are located in the fruit sector being framed in the geographical coordinates of 47°10' and 47°15' north latitude and 27°30' east longitude respectively, with a southern exhibition

On the area occupied with the old pomological collection of apple varieties (established in the year 1978), the new measures to combat specific pests have been applied, being eliminated pesticides, being ecologically treated.

This collection has a variety of local apple varieties, run in the classic system, with a distance of planting trees of 4x3 m.

The area occupied by the new pomological collection of apple varieties (established in 1996), where the conventional phytosanitary protection measures were applied, being chemically treated.

The abundance and dynamics of the adult flight, was made with the help of synthetic sex pheromones of the type ATRAPOM (Fig. 1) produced at the Institute of Chemistry Research "Raluca Ripan" from Cluj [3], [11].

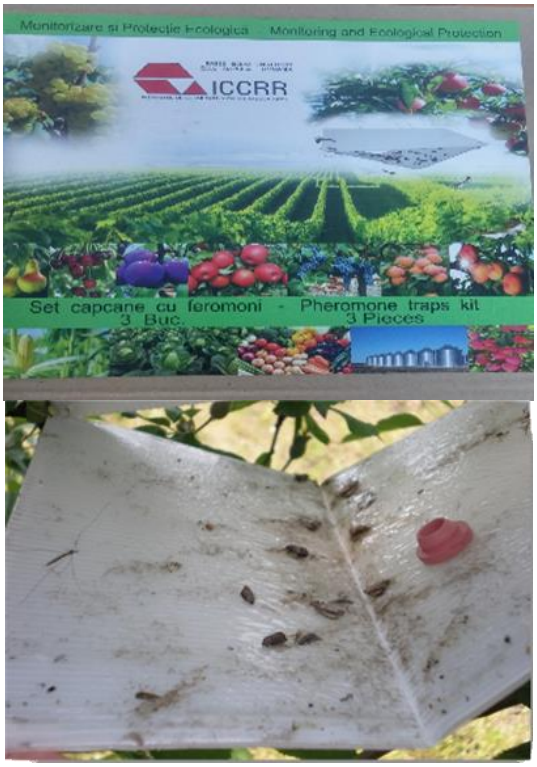

Fig. 1. Fermonal traps set

The key pests of this pomicole species, registered on experimental batches, requiring the application of measures to combat are: *Cydia pomonella* L., *Adoxophyes reticulana* Hb., *Aphis pomi* DE GEER, *Eriosoma lanigerum*HAUSM., *Panonychus ulmi*KOCH, *Psylla* sp., and *Quadraspidiotus perniciosus* COMST and the main phytopatogens agents are *Venturia inaequalis* (CKE.) WINT. şi *Podosphaera leucotricha* (ELL. et EV.) SALM. [2], [4], [7]

Results and Discussions

In the climatic conditions of the Central Moldovian plateau, the fruit worm is the most important pest of apple plantations, because through the exclusive carpofag diet of larvae where the fruit production is directly affected [3], [9], [11].

Cydia pomonella (L, 1758) – Apple worm

In the climatic conditions of Iasi, with an average multiyear temperature of 9.6°C, the biological cycle of apples worm presents an interesting particularity [3], [4].

Thus, the hibernating larvae result in a curve with two well-separated flight peaks over time (G-1). From the adults of the first flight peak, the next generation (G-2) flight results from the same year, and from this, the 2nd flight peak of G-1 of the following year. Of the adults of the 2nd flight peak of G-1 results in the first flight peak of G-1 of the following year [6], [11].

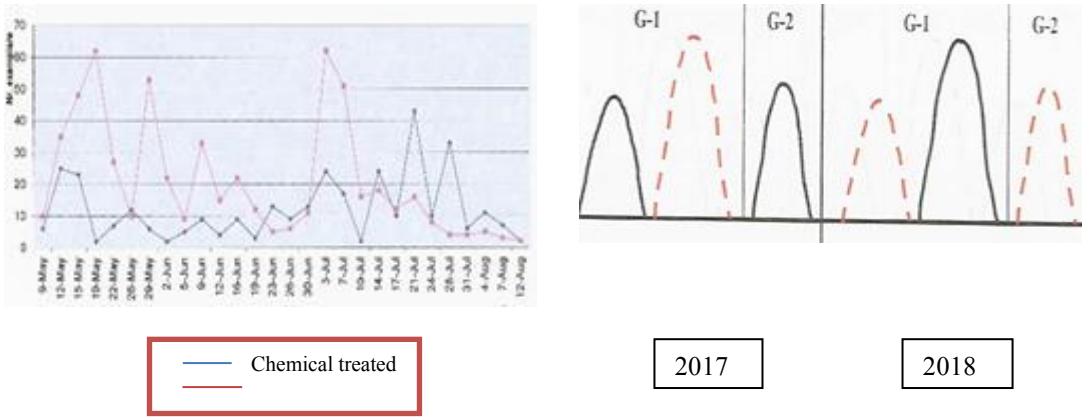

Fig. 2. Abundance and adult flight dynamics of *Cydia pomonella* under normal climatic conditions for Iasi

Adoxophyes reticulana (Hв, 1834) – summer fruit tortrix moth

Unlike the previous species, for the Iaşi, the biological cycle of this pest has two complete annual generations, and in warmer years it can have even 3 generations [3], [4]. The dynamics of the adult flight (Fig. 3) recorded with the help of the synthetic sex pheromone traps AtraRET, mounted in the ecological group of the Adamachi Farm, highlights the two flight curves of the two generations the normal situation for this area.

According to the genus *Cydia* species, this tortricid is the most important pest of the apple orchards (Fig. 4).

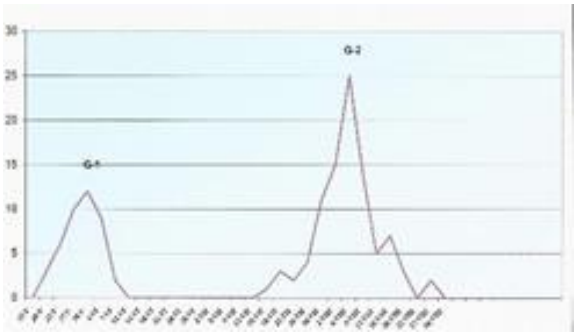

Fig. 3. Abundance and dynamics of adult flight of *Adoxophyes reticulana* in normal climatic conditions in Iaşi

Fig. 4. Attack *of Adoxophyes reticulana* in shoots and apple fruit

Normally, the caterers record two periods of attack, spring – when starting in vegetation, on the buds by the hibernating larvae and towards the end of summer – before harvesting, on mature fruits by young larvae, before the withdrawal or hibernation.

Most of the time, the damage caused in the first case goes unnoticed, or lies below the economic threshold of harm, leading at most to a slowdown in the growth of shoots. Instead the chafing caused to mature fruits no longer have time to scar, so they constitute open gates for deposit diseases.

The combat treatments were carried out at the warning, with several factors being considered: the biology of the pest; The evolution of local climatic conditions; Evolution of the phenology of fruit trees; Products used and their control period.

Determining the optimal moments of application of the measures to combat, in the two key pests (fruit worms, mining moth), for which synthetic sex pheromones are available, the dynamics of adult flight was recorded using pheromonal traps, Atra-POM, Atra-RET [3].

From the analysis of the presented data, it is observed that during the two years (Table 1) of observation for pest control on the ecological variant, the level of attack of this key pest

remained below the economic threshold of damage, compared to the conventional variant, with a significant decrease from one year to another.

Table 1. The level of the fruit worm attack (*Cydia pomonella* L.) when harvesting on the conventional and ecological variants of the experimental lot from the Adamachi farm

Year	Variants			
	Ecological		Cz=onventional	
	% Fruit attacked	Attack level	% Fruit attacked	Attack level
APPLE				
2017	8,9 %	weak	15,1 %	middle
2018	7.5 %	weak	10,5 %	middle

The analysis of the situation of the populations of the mining microlepidopterans (*Adoxophyes reticulana*) and the parasitoid complex represented a very good indicator of the application of the new measures to control the specific pests of this fruit species [2].

Thus: – in 2017 – the degree of destruction of the mines by the parasitoids was similar in both groups, with values that sometimes reached 75-80%.

Each variant:

- in 2018 – on ecological variants, there was virtually no attack of mining insects, which was particularly weak. It turns out that the beginning of the attack or the weak attack of 2017 was naturally broken by the parasitoid complex. As for the percentage of parasitic in the organic group it had an average of 28.57%
- the most efficient parasites have proven to be: S*ympiesis sericeicornis, Holcothorax testaceipes* şi *Apanteles circumscriptus.*

Conclusions

The apple orchards is attacked by a large number of damages, most important being the species:*Cydia pommonella* L. (worms of apples), *Quadraspidiotus perniciosus* Comst. (San Jose scale), *Adoxophyes reticulana* (fruit moth).

In the conditions of the Vasile Adamachi Iasi Teaching Farm, the apple worm (*Cydia pommonella* L.) is a frequent damage in apple orchards. This is how data are presented to ecology, dynamics and the application of measures to combat.

In connection with the biology of the species *Cydia pommonella* and *Adoxophyes reticulana* we followed the evolution of the damage in the ecologic and conventional apple plantations at the Vasile Adamachi Iaşi Farm, during the period of 2017-2018, establishing the date of the occurrence of the first adult, the stage time (adult, egg, larvae and pupae), fly dynamics at these 2 species of lepidoptera.

The chemical method of combat is the most widely used method with significant results that are applied in the specific treatment for each of the two species. The treatments are carried out only on warning and follow the use of products from group III and IV of toxicity, as part of the protection of useful fauna from the plantation area.

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Influence of Different Types of Sodium Chloride on Green Tomatoes – *Solanum Lycopersicum* L. Preserved by Lactic Fermentation

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Abstract

When green tomatoes are immersed in water and salt solution, an anaerobic environment is created for acidifying bacteria – lactobacilli – that are present at the surface of all living organisms, abundant above the leaves and roots that grow near the soil. As soon as the vegetables are immersed in water, fermentation begins. Lactobacilli begin to consume sugars from vegetables and fruits and produce, among other things, lactic acid. The lactic acid produced by these bacteria is a natural preservative that inhibits the growth of putrefaction bacteria and other pathogenic aerobic microorganisms, such as mould. This paper presents the evolution and the influence of lactic fermentation on green tomatoes (*Solanum lycopersicum* L.) using different sources of water and salt. The highest amount of lactic acid (0.092%) was obtained with the use of non-iodate, recrystallized salt and spring water.

Keywords: lactic fermentation, tomatoes, salt, water

Introduction

Processed vegetables, prepared by traditional methods are of increasing interest to consumers worldwide. Traditional products, without preservatives and food additives, obtained from quality raw materials, also have a unique bouquet of flavours, for which producers having a high interest in obtaining new products, especially those obtained by some traditional methods, thus increasing the diversity of offered products to consumers [1].

One of the oldest methods of preserving vegetables is lactic fermentation, which also presents a means of making attractive assortments of foodstuffs, given that over 20 species of vegetables are consumed in fermented, whole, chopped or in the form of juices. For a time, fermented vegetables were underestimated in more developed countries, but given the recommendations presented by physicians and dietitians due to their health promotion properties, these products have become increasingly appreciated [2, 3].

Vegetables processed by lactic fermentation, have a low level of calories, their sugar content being much lower than the fresh vegetables, the lactic fermentation process offering an added value to the organoleptic properties of the vegetables [2, 4].

Lactic acid gives fermented vegetables a pleasant, invigorating taste, also reducing intestinal pH and inhibiting the development of rot bacteria [5]. Lactic fermented vegetables have a high content of B and C vitamins, being a rich source of dietary fiber, which prevents the assimilation of fats, being considered foods with probiotic properties in certain diets [2, 6].

Microorganisms present in vegetables used as raw materials, greatly influence the quality of naturally fermented vegetables, in the presence of lactic acid [7, 8]. In some cases, the quality

of fermented products may be impaired because, under adverse soil and climate conditions, vegetables can be highly contaminated with bacteria, which form spores or contaminate with pathogens such as Shigella, Listeria, Salmonella or Escherichia, yeasts and molds, which can influence the activity of lactic bacteria [9].

By lactic fermentation, carbohydrates are broken down into simpler or more complex forms of lactic acid. Lactic fermented vegetables also present new bioactive compounds, which represent a new area of research, due to their exceptional functional and nutritional properties, regardless of whether they have been fermented by traditional or modern techniques [10].

Lactic fermentation is a simple and valuable biotechnology, with which it is possible to maintain and improve the nutritional and sensory characteristics of vegetables and increase their shelf life [11, 12, 13, 14].

As can be seen from the literature, by combining modern biotechnologies with this ancient method of bio conservation through lactic fermentation, controlled fermentation processes can be carried out, as well as the use of certain starter cultures of fermenters, to increase the value of the obtained products [15].

Lactic fermentation is also the oldest method of preserving vegetables, but also the healthiest, the lactic acid being the one that preserves all the natural ingredients of unaltered vegetables and improves their taste, quality and aroma, but at the same time, bringing a beneficial bacterial flora to our intestines [16]. Probiotic bacteria (*Lactobacillus plantarum*, *L. acidophilus*, *L. rhamnosus*, *Bifidobacterium bifidum*, *B. longum*, *Saccharomyces boulardii* and *Pediococcusacidilactici*), assign on obtained products by lactic fermentation probiotic properties, being very useful in the treatment of certain disorders such as lactose sensibility, diarrhoea, cholesterol, high blood pressure, colon cancer, irritable bowel syndrome and colitis [17].

Vegetables are known to be fundamental sources of vitamins, provitamins, minerals, dietary fibres and phytosterols, that are very important for a normal diet [18], scientific evidence encouraging the consumption of vegetables to prevent certain chronic diseases, such as coronary heart disease risk [19], high blood pressure [20], the Food and Agriculture Organization (FAO) (www.fao.org) and the Organization World Health (WHO) (www.who.int), recommending daily consumption of 400 g vegetables and fruits per day.

Usually vegetables are consumed fresh or processed. If vegetables are applied with minimal processing, like preservation, drying, etc., they will have a short shelf life, if they are not subjected to other processes such as boiling and pasteurizing or adding chemical preservatives, but they bring a series of changes in the physical and organoleptic characteristics of vegetables, but also in their chemical composition. [21, 22]. By applying modern technologies, such as new packaging systems and the use of natural antimicrobial preservatives [23], ionizing radiation and electric fields with pulses [23, 24, 25], or their preservation by lactic fermentation, these shortcomings may be partially eliminated.

Physical and nutritional conditions greatly influence the epiphytic microbial population of plants [26], each species offering a unique niche in terms of competitive biota, natural antagonistic compounds, chemical composition and buffering ability, all these being influenced by temperature and especially by harvest conditions [11]. According to the data presented by Spurr, [27] the microbial population of raw vegetables varies between 5.0 and 7.0 log cfu g-1, the lactic bacteria being only between 2.0-4.0 log cfu g-1, of these [11].

Material and Method

In the experience were used fresh tomatoes, medium-sized, with a diameter between 5-7 cm.

It was considered that the fruits should not be dehydrated, so that during lactic fermentation no gaps appear inside them.

The saline solutions prepared for preservation by lactic fermentation had a concentration of 40 g/l NaCl, the pH of the initial solutions was between 8.05 and 8.26, for both the drinking water and spring water versions, as well as for the different types of salt used. The iodized gem salt used in the experiment showed an amount of 36.47 mg potassium iodate (KI)/kg salt (NaCl), so the amount of potassium iodate (KI) in the solution was 0.136 mg/100 ml solution.

The solution prepared with iodine recrystallized salt showed 0.2125 mg potassium iodate/100 ml solution.

From the combination of the experimental factors, 8 variants resulted, which are presented in the table no. 1.

Table 1. Experimental factors

Experimental variants	The type of salt used for preservation	The water source used for the filling liquid
V1	Iodized gem salt	Drinking water
V2	Iodized gem salt	Spring water
V3	Non-iodized gem salt	Drinking water
V4	Non-iodized gem salt	Spring water
V5	Recrystallized iodized salt	Drinking water
V6	Recrystallized iodized salt	Spring water
V7	Recrystallized non-iodized salt	Drinking water
V8	Recrystallized non-iodized salt	Spring water

The jars with tomatoes subjected to lactic fermentation were stored in the dark, at the temperature of 17-21°C, lactic fermentation starting after 4 days from the introduction of their tomatoes in solution.

After a period of 7 weeks, during which time the way the fermentation process was followed, some chemical parameters that influence the performance of the lactic fermentation were analysed, respectively the pH of the solution, the total acidity expressed in lactic acid, as well as the salt concentration from the solution.

Results and Discussions

Following the data in table no. 2 it can be observed that there are differences regarding the values obtained for the eight variants taken in the research, the process of lactic fermentation proceeding similarly for both the non-iodized salt and the iodized salt used in brine, but the water influenced the amount of lactic acid that formed. The initial pH level decreased from 8.26, to values between 3.53-3.77, the total acidity expressed in lactic acid, showed values between 75.00-89.67 ml/100 litres solution (0.075-0.089%), and the salt content in brine varied between 1.78-2.04%. The fermentation process proceeded normally for all experimental variants, regardless of the salt used in the preparation of the brine. (Table no. 2)

Table 2. Analyses for brine used in tomatoes

Sample	pH	Total acidity Lactic acid ml /100 l solution	NaCl %
V1	3.69	77.00 ^{f}	2.04 ^{a}
V2	3.78	84.33 ^{d}	1.99 ^{b}
V3	3.73	78.00 ^{e}	1.81 ^{f}
V4	3.69	86.00 ^{b}	1.88 ^{e}
V5	3.79	77.00 ^{f}	1.86 ^{e}
V6	3.65	85.00 ^{c}	1.93 ^{d}
V7	3.64	75.00 ^{g}	1.78 ^{g}
V8	3.52	89.67 ^{a}	1.97 ^{c}

SD Total acidity 4.87-5.52; SD NaCl 0.08-0.09

Analysing the unilateral influence of the experimental factors on the amount of lactic acid that formed during the lactic fermentation, it can be observed that the experimental variant a8 presents statistically assured differences, of 11.67 being very significant compared to the variant a3 considered as a control (table 3).

Table 3. The unilateral influence of acidity in the recipes used on formed lactic acid content

Recipe	*% acid lactic*		*Difference ml acid/100 liters solution* ±D	*Signification of difference*
	Obtained values	%		
A3	78.00	100.0	0.00	Avg.
A1	77.00	98.7	-1.00	-
A2	84.33	108.1	6.33	*
A4	86.00	110.3	8.00	**
A5	77.00	98.7	-1.00	-
A6	85.00	109.0	7.00	**
A7	75.00	96.2	-3.00	-
A8	89.67	115.0	11.67	***
DL (p 5%)			4.89	
DL (p 1%)			6.78	
DL (p 0,1%)			9.41	

The amount of salt in the lactic fermented pickles is of major importance as a preservative not just as a spice. Analysing the unilateral influence of sodium chloride, in table 4 the values obtained at the concentration of NaCl of 1.78-2.04% for the recipes used are in line with those of the specialized literature recommended for lactic fermentation.

The variant V1 and V2 respectively register values with differences of 0.22 and 0.18, being significantly different from the control variant (V3).

Table 4. Unilateral influence of sodium chloride in the recipes used in the experience

Recipe	*Sodium chloride*		*Difference % salt* ±D	*Signification of difference*
	Obtained values	%		
V3	1.81	100.0	0.00	Avg.
V1	2.04	112.3	0.22	***
V2	1.99	109.9	0.18	***
V4	1.88	103.5	0.06	-
V5	1.86	102.8	0.05	-
V6	1.93	106.3	0.11	**
V7	1.78	98.2	-0.03	-
V8	1.97	108.5	0.15	**
DL (p 5%)			0.08	
DL (p 1%)			0.11	
DL (p 0,1%)			0.16	

The amount of lipids, proteins, sugars and carbohydrates decrease, due to the diffusion in brine of the soluble substances and due to their partial decomposition by microorganisms.

The amount of invert sugar decreases greatly, and after a longer time completely disappears.

Also, the sugar content is greatly reduced, in its place forming lactic acid. The values determined by us are in accordance with those found in the specialized literature [15].

Also, in lactic fermented green tomatoes, as in other vegetables, the cellulose content is reduced, due to its partial hydrolysis.

Lactic-fermented green tomatoes, kept in the dark and temperatures of 2-6°C, had a green colour, with an olive-yellow hue, were not turned or with hollows inside, were crispy, with a sour and salty taste, pleasant and with a characteristic aroma to the spices used.

Conclusions

1. From the data presented above it is found that the largest quantity of lactic acid 89.67 ml / 100 litres was formed on lactic fermented green tomatoes, of medium size, using spring water and recrystallized non-iodized salt, the products obtained being of superior quality.
2. In the finished product, the largest amount of salt was found when using iodized gum salt and drinking water.

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An Approach Towards Modelling the Human Health Risks Posed by Pesticides Residues in Lettuce

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Abstract

Pesticides persistence in the environment and their bioaccumulation capacity in living organisms, generate risks, both to the environment and to human health. In this study, we focused on acute and chronic human health risks considering different categories of consumers exposed to pesticides residues in lettuce by dietary exposure. All data were provided by the Romanian monitoring programme of pesticides in fruits, vegetables and cereals for the year 2016. The risk evaluation was done for different age groups, adults and children, and for different types of diets given the recommendations of the World Health Organization. Although we could observe that the MRLs of pesticides in lettuce samples were exceeded by chlorotalonil, iprodione, deltamethrin and tebuconazole, acute risks were posed by cyprodinil, iprodione, propyzamide and fenhexamid, which exceeded 100% of the ARfD, for children exposure. In all scenarios, the intake % of the ADI was lower than 100%, meaning that, on long-term, there are no chronic risks posed by the consumption of lettuce with the specified residual concentrations of pesticides.

Keywords: MRLs, pesticides, PRIMO model, risk assessment, vegetables

Introduction

The negative effects on human health caused by the presence of pesticide residues in plant products such as fruits, vegetables and cereals are based on the severity of the short or long-term exposure and the exposed population category, with adults being the least affected.

Thus, due to the continuous exposure of the population to different pesticide residues in plant products, we need to focus our attention, beside the environmental issues, on human health risks [1], [2], [3], [4], [5].

For a good management of pesticide use, various organizations, such as the European Food Safety Authority (EFSA) or the US Environmental Protection Agency (USEPA) have worked on the development of various instruments which could facilitate risk assessment strategies focused on pesticide residues in plant-based products.

These programs were developed considering different mathematical models and can be implemented by analysing multiple databases, such as IRIS, PPDB, EU database – pesticides, ECHA, IUCLID or US ECOTOX. For modelling pesticides risks to human health, data on the characteristics and concentrations of pesticides, fruits and vegetables consumption and the

effects to human health are essential. Further efforts are needed to understand the behaviour, degradation and transport of pesticides along different environmental compartments, in order to implement robust risk assessment strategies [6], [7], [8], [9], [10]. On the other side, when investigating dietary exposure, there are several approaches focused on consumption patterns, processing information and residue levels. One deterministic approach to modelling risks to human health is by the implementation of Pesticide Residue Intake Model (PRIMo), developed by EFSA [11].

Given this context, we propose to assess short and long-term risks to human health arising from the presence of pesticide residues in lettuce considering different age groups (adults and children) and different types of diet as suggested by the World Health Organization (WHO), using the deterministic approach within the PRIMo model.

Methodology

All data on pesticide residues were identified from the Romanian national plan for monitoring pesticide residues in fruits, vegetables and cereals for the year 2016. Within the collected data from different counties of Romania, 1145 samples were analysed within the national monitoring program, of which 343 are fruit samples, 683 are vegetable samples and 119 are cereal samples [12]. This work proposes the use of the deterministic PRIMo model (Pesticide Residue Intake Model) to identify the risks caused by lettuce consumption given the specified data. PRIMo model, used for the ingestion of pesticide residues, was initially developed by EFSA for risk assessment on temporary MRLs. Currently, it can be applied for the assessment of acute and chronic risks. To assess the risk with this model, data on food consumption and unit weights (data provided by the Member States of the European Union) and the various risk assessment methodologies implemented and agreed at international level are used to evaluate short and long-term exposure for children and adults caused by pesticide residues [11]. PRIMo integrates the option to run short-term dietary exposure assessments with the IESTI (International Estimated Short-Term Intake) methodology, while the long-term dietary exposure is calculated according to the Theoretical Maximum Daily Intake (TMDI).

The results on ingestion exposure indicate the number of residues consumed as a result of using an active substance in accordance with Good Agricultural Practices. These values should not exceed the value of Acute Reference Dose, ARfD (for single exposure) and Acceptable Daily Intake, ADI (for lifetime exposure). A distinction is made between health risks for the general adult population and children. This distinction is made since children have a less varied diet and a higher caloric intake compared to adults. EFSA PRIMo provides also values for the mean body weights for each consumer's population. The EFSA PRIMo model methodology is fully described by EFSA [13]. Processing was not taken into consideration in this work; therefore, an over-estimated exposure could be encountered.

Results and Discussion

Pesticides monitoring in lettuce

According to the report developed within the national plan for monitoring pesticide residues in fruits, vegetables and cereals from the domestic production of the year 2016, a number of 15 pesticides were identified in analysed samples of lettuce provided from different Romanian counties such as Bucharest (B), Ialomița (IL), Bacău (BC) and Iași (IS) (Table 1). A number of 6 pesticides were identified in lettuce samples from Bucharest (B), namely boscalid, chlorothalonil, cyprodinil, fludioxonil, fluopicolide and iprodione, while 4 pesticides were identified insamplesfrom Ialomița (IL), e.g., propyzamide, fenhexamid, thiamethoxam and pendimethalin. Regarding the counties of Bacău (BC) and Iași (IS), the pesticides identified

were deltamethrin, chlorothalonil, metalaxyl, tebuconazole and respectively, iprodione [12]. As indicated in Table 1, we can observe that chlorotalonil, iprodione, deltamethrin and tebuconazole exceed the MRLs. All data included in Table 1 are inputs necessary for the risk assessment using PRIMo model.

Table 1. Data driven from the Romanian National Plan for Monitoring Pesticide Residues in Fruits, Vegetables and Cereals for the year 2016 (residues in lettuce)

County	Active substance	Analysis technique	Sample quantity/ batch	Pesticide residues (mg/kg)	MRL[a] (mg/kg)	ARfD[b] (mg/kg bw)	ADI[c] (mg/kg bw)
Bucharest (B)	Boscalid	GC-MS	5 pieces/100 pieces	1.088	50	considered unnecessary	0.04
	Chlorothalonil	GC-MS	5 pieces/80 pieces	0.019	0.01*	0.5	0.015
	Cyprodinil	GC-MS	-	8.691	15	1.5**	0.03
	Fludioxonil	GC-MS	-	1.074	40	considered unnecessary	0.37
	Fluopicolide	GC-MS	-	0.023	9	0.18	0.08
	Iprodione	GC-MS	5 pieces/100 pieces	5.552	0.01*	0.06	0.02
Ialomița (IL)	Propyzamide	GC-MS	5 pieces/50 kg	0.938	0.6	0.13	0.05
	Fenhexamid	GC-MS	5 pieces/50 kg	0.744	50	considered unnecessary	0.2
	Thiamethoxam	LC-MS	5 pieces/50 kg	0.235	5	0.5	0.026
	Pendimethalin	GC-MS	5 pieces/50 kg	0.065	4	0.3	0.125
Iasi (IS)	Iprodione	GC-MS	1.46 kg/50 kg	0.390	0.01*	0.06	0.02
Bacău (BC)	Deltamethrin	GC-MS	-	0.921	0.5	0.01	0.01
	Chlorothalonil	GC-MS	-	0.020	0.01*	0.5	0.015
	Metalaxyl	GC-MS	-	0.196	3	0.5	0.08
	Tebuconazole	GC-MS	-	0.703	0.5	0.03	0.03

[a]MRL Maximum residue level, EU Pesticides database, https://ec.europa.eu/food/plant/pesticides_en
[b]ARfD Acute Reference Dose, EU Pesticides database, https://ec.europa.eu/food/plant/pesticides_en
[c]ADI Acceptable Daily Intake, EU Pesticides database, https://ec.europa.eu/food/plant/pesticides_en
*Indicates lower limit of analytical determination
**ARfD proposed, but a formal decision has not yet been taken

Short-term (acute) risk assessment

Fig. 1 shows the acute risk assessment for children and adults identified in the case of consumption of lettuce with pesticide residues. The graph shows that pesticides cyprodinil, iprodione, propyzamide, fenhexamid and deltamethrin generate short-term effects in children, while pesticides cyprodinil, iprodione and deltamethrin can produce short-term effects in adults.

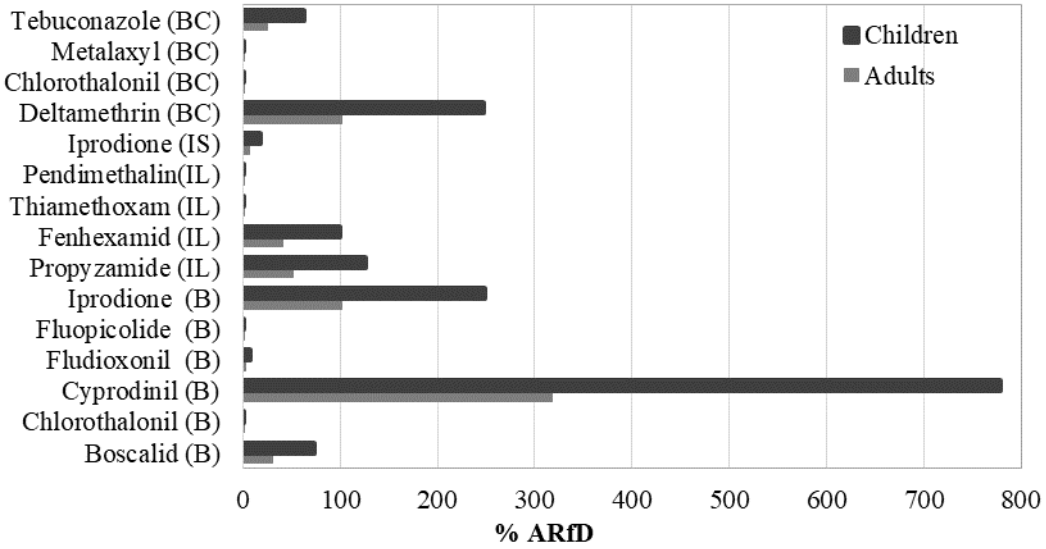


Fig. 1. Acute risk assessment for children and adults generated by lettuce consumption with pesticide residues

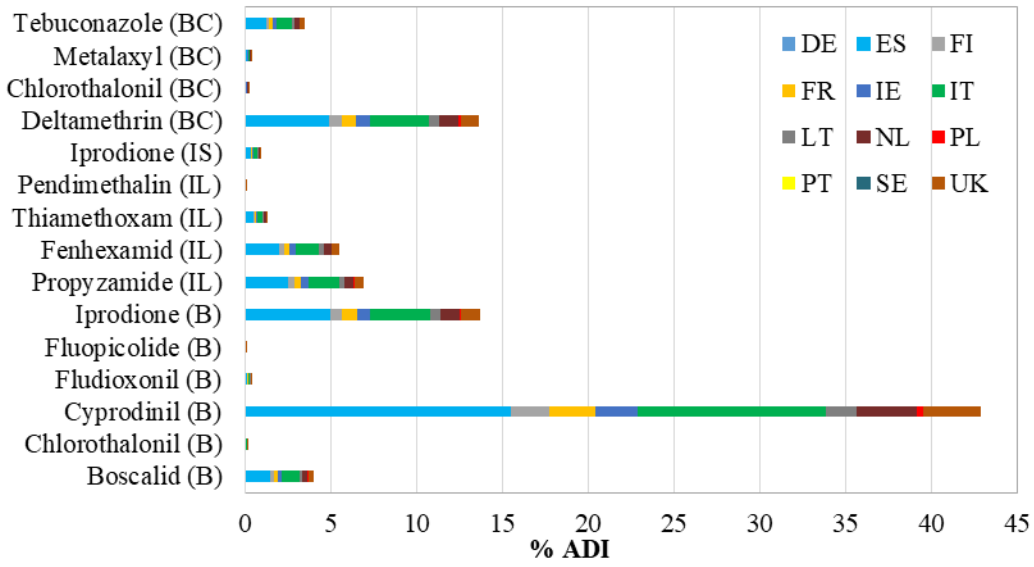
The value of the acute risk for these pesticides is higher than 100%, which can cause concern, especially for children which are a more vulnerable category. It was pointed out that the acute risk is higher in the case of analysed samples taken from Bucharest (B), containing cyprodinil, even if according to the data collection, the concentration of residues does not exceed the MRLs.

In the samples taken from Ialomița (IL), the acute risk indicated an increased concern for children considering the contamination with propyzamide and fenhexamid pesticides, while for samples taken from Bacău (BC), deltamethrin pesticide has the highest acute risk value for both adults and children, 101.2% and 247.8% of the ARfD, respectively. Interesting, for pesticide iprodione encountered in analysed samples from Iasi (IS), data shows that the acute risk is below 17.5% of the ARfD, for all consumers. Similar results were obtained for pesticide cholorothalonil which exceeded the MRL value for lettuce samples in Poland. The authors concluded that the residues which were above the MRL value, exceeded the %ARfD, generating short-term risks for children and adults [14]. Skretteberg *et al*., [15] studied the amount of pesticide residues in fruit and vegetables from Asia. Some of the assessed products were considered to present possible acute risks to consumers since the estimated acute intake was higher than 100% of the ARfD.

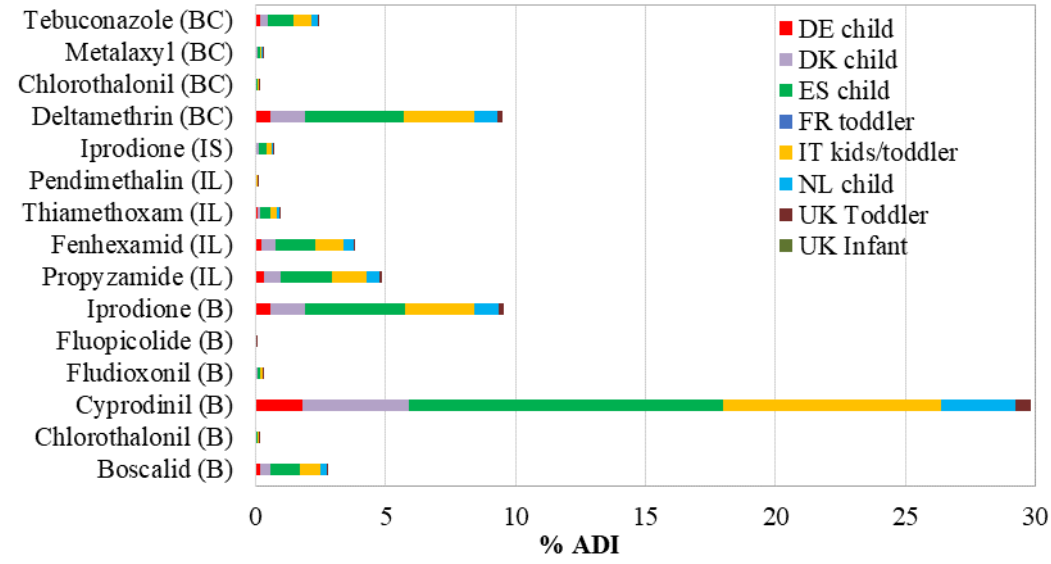
Long-term (chronic) risk assessment

The chronic risk estimation is represented in Fig. 2 for major European countries, where consumption data were available within the PRIMo model. From the graph, we can observe a similar pattern as in the case of acute risk. Pesticides such as cyprodinil, iprodione and deltamethrin have the highest values of the chronic risk, the most exposed countries being Spain (ES) and Italy (IT) (Fig. 2a). However, the values of the chronic risk are below 15%, which implies that in the long term, the risk is acceptable. In a second scenario, we could identify the chronic risk for children and toddlers in several European countries (Fig. 2b). The most exposed group was found to be children living in Spain (ES), followed by Italy (IT) and the Netherlands (NL). The highest intakes are given by pesticide cyprodinil, accounting for ≤12% of the ADI.

However, in all cases the value of the chronic risk is much below 100%, which means that in the long-term the risk is considered acceptable. A similar example was given by Stephenson and Harris [16]. The authors identified an intake of 68.6%, 7.8%, and 2.0% of the ADI for the UK toddler group, exposed to glyphosate in sugar beet (root), wheat, and milk and cream.



(a)



(b)

Fig. 2. Chronic risk posed by letucce consumption with pesticide residues for adults (a) and children (b) for major European countries

Considering the chronic risk assessment for groups of countries with similar diets (Fig. 3), we could identify pesticides such as cyprodinil, iprodione, propyzamide and deltamethrin, having the highest value of the chronic risk, the most affected population being the one following WHO regional European diet (0.01-10.9% of the ADI) followed by WHO cluster B diet (0.01-10.4% of the ADI), which includes countries such as Cyprus, Greece, Israel, Italy, Lebanon, Portugal, Spain, Turkey, United Arab Emirates and WHO cluster F diet (0.009-8.69% of the ADI), which includes countries such as Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Sweden. Romania is part of WHO cluster D diet, with intake estimates between 0 and 0.009% of the ADI. For the UK vegetarian diet consumers, the intakes are between 0.004 and

4.08% of the ADI. In all identified types of diets included within WHO clusters, the chronic risk is below 100% of the ADI, meaning that on long-term, the risk is acceptable.

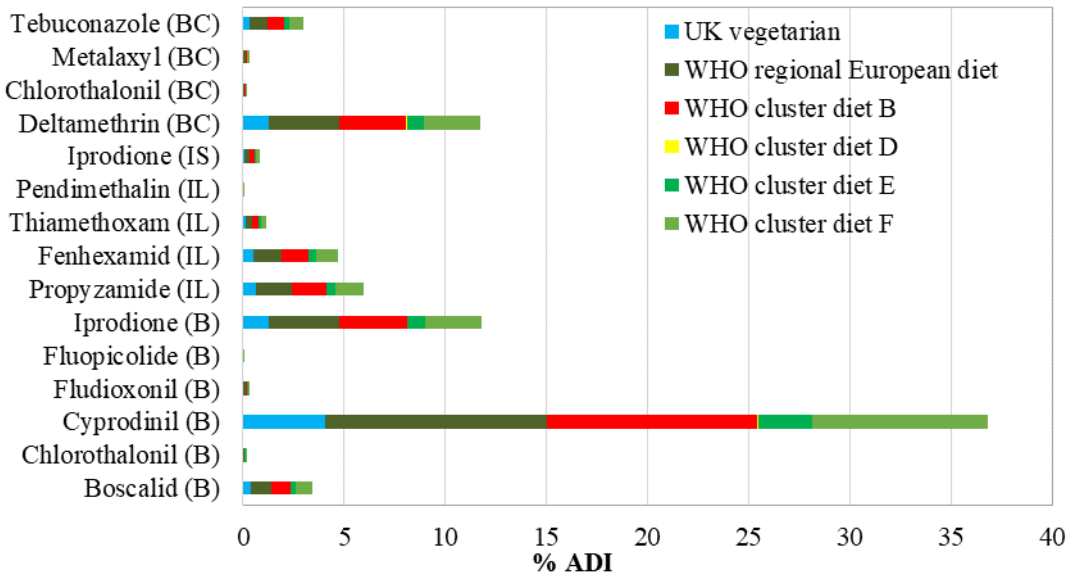


Fig. 3. Chronic risk assessment for groups of countries with similar diets according to WHO clusters

Conclusions

We investigated the short and long-term risks posed by lettuce consumption with pesticide residues given the data provided by the Romanian National Plan for Monitoring Pesticide Residues in Fruits, Vegetables and Cereals for the year 2016. The acute risk assessment for children and adults identified by lettuce consumption with pesticide residues shows that on short-term, the risk is higher for analysed samples from Bucharest (B)containing cyprodinil (779.4% of the ARfD for children and 318.4% of the ARfD for adults) and iprodione (249% of the ARfD for children and 101.7% of the ARfD for adults). A similar pattern was observed in the case of chronic risk, where pesticide cyprodinil had the highest values for all scenarios considered, the most exposed countries being Spain (ES) and Italy (IT), when both adults and children are included in the assessment. In this case, highest intakes are given by pesticide cyprodinil, accounting for ≤12% of the ADI. However, all the intake values were much below the ADI, meaning that the chronic risk is considered negligible.

Acknowledgements

This work was supported by the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-III-P2-2.1-PED-2016-1662, Contract no. 10PED/2017. Partial support of project number PN-III-P4-ID-PCE-2016-0683, Contract no. 65/2017 is also acknowledged.

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Selection of Suitable Support Materials for Adsorptive Immobilization of Rhodococci Cells

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Abstract

Whole cell immobilization technique has such advantages as long-term stability of cells, high biocatalytic activity, and possibility of biocatalyst's regenerating. The aim of this study was to evaluate adsorption properties of natural support materials for immobilization of *Rhodococcus rhodochrous* cells. The immobilization was performed using five natural matrixes with high adsorptive capacity: bentonite clay, kieselgur, granulated diatomite, charcoal from grape seeds and charcoal from walnut shell. The cell adsorption depended on the nature of carriers, and in spite of highly porous structure, charcoals demonstrated low rate of cells immobilization (15-33%). A high level of bacterial immobilization was obtained on kieselgur and crushed granulated diatomite (97% and 94%), which allowed to characterize them as excellent adsorbent materials for preparation of immobilized *Rhodococcus rhodochrous* cells.

Keywords: Immobilization, bioremediation, soil, pesticides, Rhodococcus rhodochrous cells, supports

Introduction

Environmental pollution with anthropogenic organic compounds is the global problem of our planet. Such type of contamination is mainly the results of stopping of application of pesticides, percolation of contaminated surface water, oil and fuel dumping, leaching of wastes from landfills or direct discharge of industrial wastes to the soil. The most common chemicals involved are petroleum hydrocarbons, solvents, pesticides, lead and other heavy metals.

Microorganisms are well known for their ability to break down a huge range of organic compounds and absorb inorganic substances. The genus *Rhodococcus* is an appropriate group of bacteria aimed at biodegradation of recalcitrant contaminants, such as petroleum hydrocarbons, chlorinated, nitrogenated, and other complex organics. The biochemical potential of rhodococci has been increasingly explored because of their broad catabolic versatility and unique enzymatic capabilities [1]. Xenobiotic compounds metabolized by rhodococci cover a wide range of structural groups, including aliphatic and aromatic hydrocarbons, oxygenated and halogenated compounds, nitroaromatics, heterocyclic compounds, nitriles, and various pesticides [2], [3], [4].

The use of immobilized microbial cell is a promising technique, which has recently been applied in various environmental studies. They have commonly been used for various biotechnological applications, for examples: antibiotic production, soil bioremediation, biodegradation and biotransformation of xenobiotics in wastewater treatment plants [5], [6].

The majority of changes observed in immobilized cells result from protection provided by the supports [7]. It is well known that interaction between bacteria and solid phase results in a variety of physiological changes in microbial behaviour. For instance, the thermal and mechanical resistance of inorganic supports is generally higher. Organic materials can also be obtained with strictly controlled porosity, but they are usually very sensitive to pressure or pH,

or in many cases to both. On the contrary, the typical stiffness of the inorganic supports ensures the invariance of pore diameter/pore volume, which guarantees constant volume and shape to the support itself. Inorganic carriers showing various pore diameters are commercially available [8]. Inorganic supports have been selected to immobilize microorganisms because they can survive microbial degradation and are thermostable [9], [10]. Some materials that have been used to immobilize cells for bioremediation include particulate supports made from polymers (e.g., polystyrene), silica (particularly kieselgur or diatomaceous earth), porous and nonporous glass beads, polyacrylamide, polyvinyl alcohol, cryogel, alumina, oxides of various transition metals, graphite, many different membrane compositions, and even sand or soil [3], [11], [12].

In accordance with the above, the aim of the study was to evaluate adsorption properties of natural support materials for immobilization of *Rhodococcus rhodochrous* cells.

Material and Method

Bacterial strain

The object of research was the strain *Rhodococcus rhodochrous* CNMN-Ac-05, deposited in the National Collection of Non-Pathogenic Microorganisms, Institute of Microbiology and Biotechnology, the Republic of Moldova, which is able to grow and develop on a medium containing herbicide trifluralin as the only source of carbon and energy.

Method of Cultivation

The *R. rhodochrous* strain was preserved in aliquot on TS medium, with the following composition g/L: hydrolysed casein – 17.0, soybean meal papaya extract – 3.0, NaCl – 5.0, $K_2HPO_4 \times 3H_2O$ – 2.5, glucose – 2.5, pH (at 25°C) – 7.3±0.2.

For the production of bacterial biomass, the *R. rhodochrous* strain was grown under continuous aeration conditions on a stirrer 180-200 rpm at 28°C for 48 hours on TS medium. *R. rhodochrous* cell mass was separated by centrifugation for 30 minutes at 5000 rpm and washed twice with NaCl solution (0.8%).

The *R. rhodochrous* biomass was determined on the spectrophotometer by the optical density of *R. rhodochrous* cell suspensions, with subsequent recalculation of the dry mass of the cells according to the calibration curve. The dry biomass of *R. rhodochrous* was determined by gravimetric method, by drying at 105°C [13].

Supports Preparation

Seven natural matrixes were selected for rhodococci cells immobilization: charcoal from grape seeds, charcoal from walnut shell, bentonite clay, kieselgur, diatomite, granulated diatomite (INZ-600), and crushed granulated diatomite. Inorganic substrates washed with distilled water in three repeats and passed through deionized water the same in three repeats. The supports were dried in an oven for 2-3 hours at 80-90°C to set up the constant weight. A 1.0 g sample was placed in a 250 ml Erlenmeyer flask, then the supports were sterilized at 1 atm for 15 minutes.

Obtaining of R. rhodochrous Cells Immobilized

To immobilize rhodococci cells, Knapp buffer was used with the following composition g/L: K_2HPO_4 – 1.0; KH_2PO_4 – 1.0; $MgSO_4 \times 7H_2O$ – 0.04; $FeCl_3 \times 6H_2O$ – 0.004, pH – 6.7.

R. rhodochrous whole cells, 150 mg, and 50 ml of Knapp buffer were added to a 250 ml Erlenmeyer flask with 1 g of sterile support. In other flask, only Knapp buffer was added, without the addition of cells. Flasks were placed under continuous aeration conditions on shaking 180-200 rpm for 20 minutes, t = 24°C, then placed in the refrigerator for 16-20 hours [12]. The content of the flasks was filtered through a capron filter. The support on the filter was

washed three times with Knapp buffer, 50 ml for each wash. The filtrate was poured into a volumetric flask, adjusted to 250 ml with Knapp buffer.

The amount of immobilized biomass was estimated on spectrophotometer, by measuring the D_{540} optical density of the cell suspension before and after immobilization.

Results and Discussions

The rhodococci cells immobilization was performed with carrier binding method by physical absorption. The method applied depends on the character of immobilizing matrix used, which included insoluble in water, high ion exchange power and wide interlayer surface which was able to protect the cells. In this work there were used natural matrixes with high adsorptive capacity: bentonite clay, kieselgur, granulated diatomite, charcoal from grape seeds and charcoal from walnut shell. The rhodococci cells were absorbed to the matrix surface via hydrophobic bond, hydrogen bond and Van der Waals force. High adhesive activities of these bacterial cells and their ability to colonize surfaces are essential for adsorptive immobilization [4].

The charcoal from grape seeds and charcoal from walnut shell (with chemical composition: cellulose (26.87%), hemicellulose (22.45%), lignin (47.68%) and ash (0.94%) it was chosen because of their high thermal stability [14]. In spite of highly porous structure, charcoals demonstrated a low rate of cells immobilization – from 15.09% on the charcoal from grape seeds to 32.78% on the charcoal from walnut shell (Fig. 1).

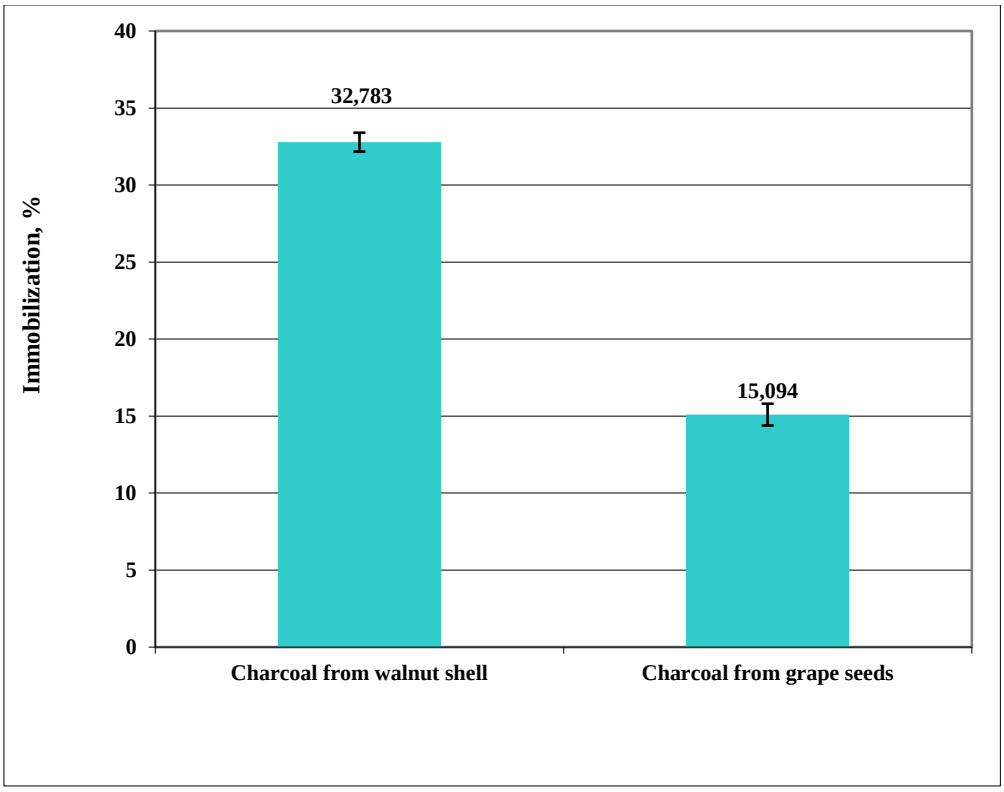

Fig. 1. The immobilization efficiency of the charcoal after the adsorption of *R. rhodochrous* cells

Bentonite was chosen as supporting matrix due to its characteristics, such as insoluble in water, high ion exchange ability, inexpensive, wide particle surface, easily activated, rigid, stable and non-toxic [15]. The rhodococci cells immobilization degree on bentonite made up 69.25% (Fig. 2).

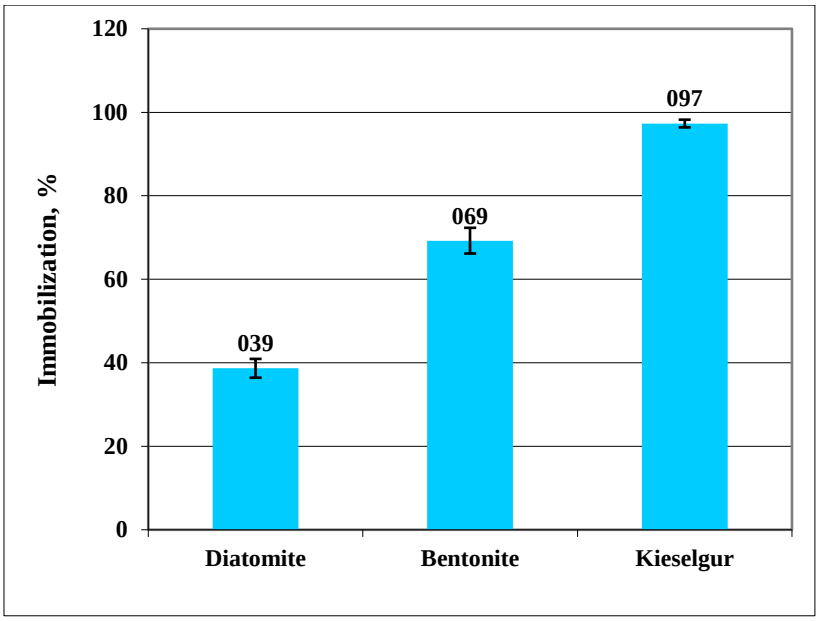

Fig. 2. The immobilization efficiency of the inorganic matrix after the adsorption of *R. rhodochrous* cells

Diatomite is abundant in many areas of the world and has unique physical characteristics, such as high permeability (0.1-10 mD) and porosity (35-65%) [16], small particle size, low thermal conductivity and density [17], and high surface area [18]. Kieselgur also has the same properties as diatomite because are mineral deposits of diatomaceous algae. The properties of diatomite's surface, such as hydrophobia, solubility, charge, acidity, ion exchange and adsorption capabilities, are highly governed by the presence of water, which is partially structurally connected to the crystal mesh of the diatomite, forming active hydroxyl groups on it [19]. The immobilization degree was 38.68% on diatomite matrix and 97.30% on kieselgur, respectively (Fig. 2).

Diatomite's highly porous structure, low density and high surface area resulted in a number of industrial applications as filtration media for various beverages and inorganic and organic chemicals as well as an adsorbent for per liter and oil spills. In the Republic of Moldova, the diatomite is mainly used as filtering material [20].

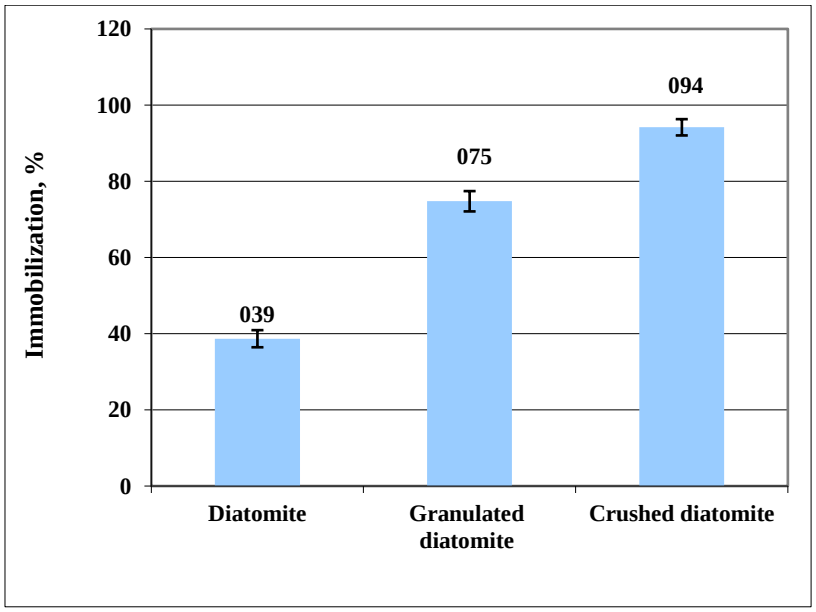

Fig. 3. The immobilization efficiency of the varints of diatomites matrix after the adsorption of *R. rhodochrous* cells

As a result of applying as a carrier for rhodococci cells immobilization the granulated diatomite (INZ-600), fraction 0.25-0.50 mm, the immobilization efficiency increased almost in two times, in compare with previous value (Fig. 3).

This solid diatomaceous carrier was crushed to obtained fraction of 0.05-0.1 mm, and that procedure improved the cells immobilization to 94.18%.

Conclusions

The charcoal from grape seeds and charcoal from walnut shell used as a carrier for immobilization of *Rhodococcus rhodochrous* cells demonstrated low rate of cells immobilization (15-33%).

The kieselgur and crushed granulated diatomite, applied as support matrix for *Rhodococcus rhodochrous* cells immobilization, demonstrated the highest adsorbent qualities, providing the immobilization efficiency of 97.30% and 94.18%, respectively.

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Isolation of Microbial Consortia in the Presence of Herbicide Trifluralin and Iron Nanoparticles in Acidic Conditions

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Abstract

The aim of this study was to isolate microbial consortia in the presence of herbicide trifluralin and to estimate the effect of iron nanoparticles (NPs) (magnetite (Fe_3O_4) and zero-valent iron Fe (0) on its formation in acidic conditions. The gradual increase in the concentration of trifluralin led to a decrease in the number of bacterial and fungal species and their ratio in the consortium. Addition of iron NPs to the culture medium clearly diminished the cytotoxic action of trifluralin on the microbial communities. In acidic conditions, Fe (0) NPs added to the enrichment medium, contributed to restore the bacterial concentration.

Keywords: microbial consortia, trifluralin, iron nanoparticles, acidic conditions

Introduction

One of the major problems faced by the industrialized world is the contamination of soils, groundwater, sediments, surface water and air with hazardous and toxic chemicals. An environmentally friendly option is bioremediation, which offers the possibility to degrade contaminants using the natural biological activity of microorganisms.

In the case of complex pollution, the creation of microorganism consortia has more advantages and chances for total mineralization of pollutants [1], [2]. The advantage of using mixed cultures is the greater degradation capacity, synergistic and co-metabolism relationships and division of labor between microorganisms. It is also preferable that members of the consortium belong to different taxonomic groups as they have developed different adaptation and survival mechanisms [3], [4], [5].

Recently, nanotechnology became a new generation of eco-friendly technology that offers highly effective solutions for cleaning and remediating the environment by using nanoparticles (NPs) of zero-valent iron or iron oxides as a chemical reducing agent [6], [7]. However, numerous researches had demonstrated that iron NPs could have potentially harmful effects on the microbial community, inhibiting some microbial groups, but promoting the domination of others, which in turn may degrade soil fertility [8], [9], [10]. However, there are researches that demonstrated that the use of NPs in the creation of microorganism consortia allows to accelerate growth of the bacterial consortium and to enhance the rate of biodegradation [11], [12].

Taking into consideration both the growing popularity of NPs in technologies for detoxification of contaminated environments, as well as the proven efficacy of iron NPs in persistent organic pollutants (POPs) remediation, the aim of our study was to isolate microbial consortia in the presence of herbicide trifluralin and to estimate the effect of iron NPs (magnetite (Fe_3O_4) and zero-valent iron Fe (0) on their formation in acidic conditions.

Material and method

Materials

In our experiment we used *magnetite* (Fe_3O_4) NPs with size 20-25 nm and *zero-valent iron* (Fe(0)) NPs – size 4-4.7 nm in the form of a colloidal aqueous solution. The poly-N-vinylpyrrolidone was used as a stabilizer for NPs. *Trifluralin* (α, α, α-trifluoro-2-6-dinitro-N-N-dipropyl-p-toluidine), a pre-emergent herbicide belonging to the dinitroaniline chemical family, was used as a solution in acetone at stock concentration of 100 mg/mL.

Soil sampling

Soil was collected near the former storage of persistent organic pesticides located in the central part of Republic of Moldova, Chişinau municipality, Sîngera village. Soil sample was cleaned of roots and other impurities, homogenized, sieved (mesh No. 2) and air-dried at 22-23°C. The rate of DDTs (DDT, DDE, DDD) was 1.82 mg/kg dry soil, but the major component of contamination was trifluralin – 19.67 mg/kg dry soil [13].

Media

A mineral salt medium PAS was used for the isolation of microbial consortia. The composition of the media is (per liter): 4.35 g K_2HPO_4, 1.7 g KH_2PO_4, 2.1 g NH_4Cl, 0.2 g $MgSO_4$, 0.05 g $MnSO_4$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.03 g $CaCl_2 \cdot 2H_2O$ [14]. The pH of the media was 5.0. The density and diversity of the microorganism's population was determined on the Nutrient Agar medium (Oxoid, England) and Czapek-Dox agar (per liter: 2.0 g $NaNO_3$, 1.0 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4$, 30.0 g Glucose, 30.0 g Agar-agar, pH = 4.5-5.0).

Research Methods

The consortia of microorganisms were obtained by the enrichment cultures method, on PAS medium containing trifluralin and colloidal solutions of NPs (Fe_3O_4 or Fe (0)) in different concentrations. Thus, three experimental variants were formed: 1) PAS + trifluralin (100-400 mg/L); 2) PAS + trifluralin (100-400 mg/L) + NPs Fe_3O_4 (25-100 mg/L); 3) PAS + trifluralin (100-400 mg/L) + NPs Fe(0) (25-100 mg/L).

Totally 12 passages were performed. Over each 3 passages (1 enrichment cycle) the dozes of trifluralin and NPs in the PAS medium were increased, so that the final concentration of trifluralin was 400 mg/L and concentration of Fe_3O_4 and Fe(0) NPs was 100 mg/L (Fig. 1).

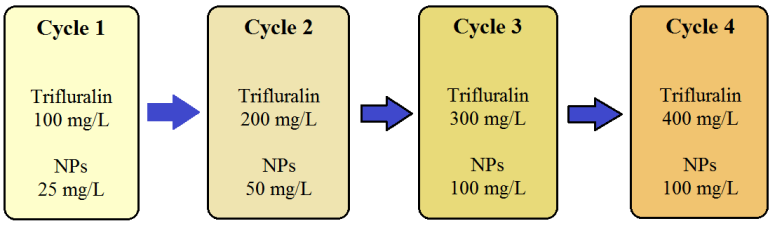

Fig. 1. Experimental design for the isolation of microbial consortia

The density (colony forming units – CFU/mL) and diversity of microorganisms in consortia was determined after each cycle by spread plate method [15]. The trifluralin was added in the culture media in concentrations corresponding to each cycle.

Results and Discussions

The results presented in the Table 1 demonstrated that in the soil had already existed a microorganisms' community, which survived and has adapted to the conditions of complex pollution; both fungi and bacteria were represented by an equal number of species.

Table 1. Density and diversity of microorganisms after the first passage

Density/Diversity	Microorganisms	
	Bacteria, × 10^6	Fungi, × 10^3
CFU/mL	32,24 ± 2,23	21,40 ± 2,72
Number of species	5	5

After the addition of trifluralin to the culture medium the increase of fungal CFU index, especially in the first two cycles (by 8 times) occurred (Fig. 2). The increase of the trifluralin concentration in the following cycles led to a decrease in the CFU index, so by the end of the Cycle 4 fungal growth decreased significantly, but still exceeded the initial values (~ 4 times).

Starting with the Cycle 2, the bacterial concentration decreased dramatically, and at the end of the experiment it became 100 times less than original value. With each cycle, it was observed the reduction of microorganism's diversity in all experimental variants. So, at the end of the Cycle 4, the consortium consisted of one fungal and one bacterial strain.

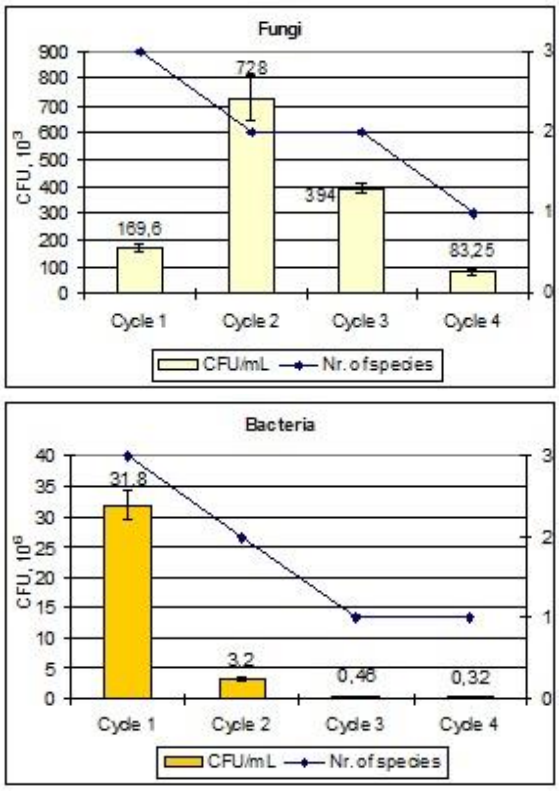

Fig. 2. Modification of the density and diversity of fungi and bacteria from enrichment culture, cultivated in the presence of trifluralin

Trifluralin and magnetite NPs added to PAS medium also stimulated the increase in the fungal concentration in the first two cycles (by 42 times), followed by the CFU decrease in the next cycles (Fig. 3). However, at the end of the Cycle 4, fungal concentration was by 2.7 times higher than initial. The bacterial concentration decreased throughout the experiment, so at the

end of the Cycle 4 the bacterial CFU index became by 17.6 times lower than initial values. At the end of the Cycle 4 the obtained consortium was formed of one fungal and one bacterial strain.

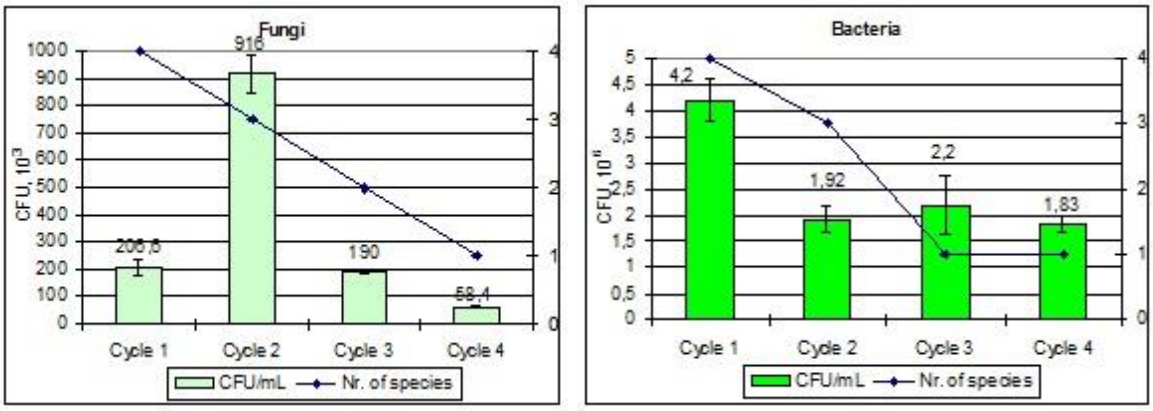

Fig. 3. Modification of the density and diversity of bacteria and fungi from enrichment culture, cultivated in the presence of trifluralin and magnetite NPs

The CFU index of fungi in experimental variants with trifluralin and Fe(0) NPs (Fig. 4) also had maximum after the Cycle 2 (by 38 times) and minimal after the Cycle 4 (~ 3.4 times).

Bacterial concentration decreased, but not sharply. By the end of the Cycle 4, the density of bacterial population was restored and varied within the limits of initial values. As in the other experimental variants, the number of species has reduced. At the end of the Cycle 4 the consortium of 1 fungal and 2 bacterial strains was obtained.

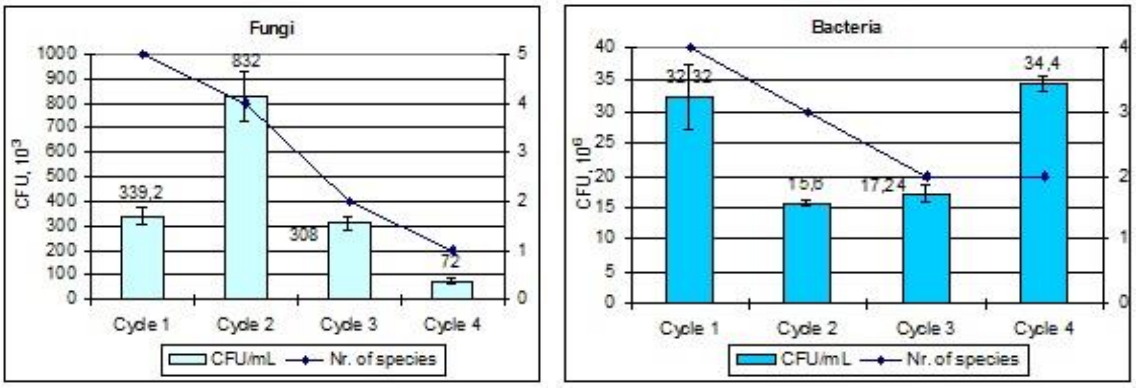

Fig. 4. Modification of the density and diversity of bacteria and fungi from enrichment culture, cultivated in the presence of trifluralin and Fe(0) NPs

When comparing the action of iron NPs on the enrichment cultures, it was observed that the magnetite NPs are more toxic than NPs of Fe(0), both for fungi and for bacteria. The toxic effect of magnetite NPs on bacteria from enrichment cultures was conditioned by several factors. It was described several mechanisms and factors that determine the toxicity of iron NPs to living organisms: size, surface characteristics and magnetic properties of NPs; oxidative stress induced by NPs; cell wall structure of bacteria [10], [16]. In our experiments another unfavourable factor for bacterial strains was acidic conditions.

It is known that pH strongly influences the redox reactions occurring at the Fe(0) NPs surface by accelerating corrosion at low pH and passivating the iron surface at high pH through the formation of iron oxides/hydroxides. The oxidation of Fe(0) NPs in aqueous systems releases OH^- ions, increasing the pH of the system [6], [7], [17]. In the case of magnetite, reactions of

oxido-reduction are slower, thus the acidic pH maintained in the medium, so more favourable conditions for the development of fungi was created.

Conclusions

A gradual increase in the concentration of trifluralin, as the sole source of carbon and energy for microorganisms in the enrichment culture, led to a restructuring of the species composition of the microbial community, which was reflected in a decrease in the number of bacterial and fungal species and their ratio in the consortium.

Addition of iron NPs to the culture medium clearly reduced the cytotoxic action of trifluralin on the microbial community.

In conclusion, in acidic conditions, Fe(0) NPs added to the enrichment medium contributed to restore the bacterial concentration and more numerous and diversified population, that made them effective in creation of a microbial consortium able to resist, and, perhaps, to degrade high concentrations of trifluralin.

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Possibilities of Geothermal Water Use for the Heating of Greenhouses

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Abstract

One of the most current problems of the contemporary world economy is energy, still dominated by oil. The gradual depletion of fossil fuel resources as a result of increasing energy demand for economic development and the unforeseeable fluctuation of oil prices, but also as a result of increasing greenhouse gas emissions, have triggered and intensified research to find and use other renewable energy resources existing around the globe. In order to produce vegetables and flowers in greenhouses, a significant share in production costs is the cost of fuel.

In this context, the authors of the paper analysed the possibility of reducing the costs for heating greenhouses by using geothermal resources. The studies in this regard confirm that Romania has a high potential for geothermal energy, being the third country in Europe after Greece and Italy with high geothermal potential, but which is currently not sufficiently well known or properly exploited especially in the western area of the country. Romania can increase its energy efficiency by putting particular emphasis on the exploitation of renewable energy resources, which can contribute to reducing energy and food dependence of our country on imports, to limiting the negative effects on the environment and, last but not least, to increasing the supply of the population with food as a result of the use of geothermal waters for heating greenhouses, which are abundantly found in the western part of Romania, particularly in Timiş County.

Keywords: geothermal, greenhouses, energy, economic efficiency

Introduction

As it is itself defined, sustainable development is the process of evolving society "which seeks to meet the needs of the present, without compromising the possibility of future generations to satisfy its own needs". Based on these principles, the protection of the environment from pollution caused by energy production, and the promotion of measures limiting the consumption of non-renewable natural resources, occurs as an obligation of the present society for future generations [1]

Today, classical fuels represent the cheapest and the best available energy resource, but there are major arguments that triggered the economic interest for the sustainable development of modern society and, implicitly, for the promotion of renewable energy resources [2], [3], [4], [5] These arguments refer to:

- the need for sustainable development that protects humans and environment's health;
- depletion in a very short time of the resources of classical fuels;
- security of energy supply;
- ensuring the energy independence, as far as possible, of all countries.

In this respect, the current research themes aim at responding to the measures to be taken, with a view to ensuring energy resources necessary for sustainable development in the situation of the depletion of conventional resources [6], [7]

Energy is regarded as an essential factor in the development of society and it is noted that care is aimed at reducing costs in the context of sustainable development. [8]

Climate change ranks second among the most serious problems faced by the world today, after poverty, famine and lack of drinking water. After 1980, the population is witnessing a new crisis driven by climate change, increasingly more, common worldwide, and causing disasters on economies and social life in all countries of the world [9], [10]

In recent times, more and more extreme (high-risk) natural phenomena have occurred in Romania, which have increasingly serious consequences for the population and activities carried out. Most long-term forecasts announce for our country drastic changes in climate, namely extremely dry summers, sudden changes in temperature from day to night, and torrential rains on extended and long-lasting areas (over 150 litres per square metre), followed by flooding.

Meteorologists forecast that, by 2030, there will be an average annual heating from 0.5 to 1.5 degrees.

This will lead to a sharp increase in precipitation deficit, i.e., drought enhancement, especially in southern and south-eastern Romania, followed by forecasted desertification. The most important areas that will be directly affected by climate change are agriculture, forestry, infrastructure, energy, tourism, population, and, last but not least, biodiversity [10, 11]

Among the sectors of the economy most affected by climate change will be agriculture

Thus, in areas heavily affected by drought, it will be necessary to find new food production solutions to ensure the food security of the population, by refocusing on new production systems and agricultural crops in protected spaces.

A main consequence of the drought will be reduced river flow, which will result in the reduction of total energy production in hydropower plants, as it is expected by the year 2030, the demand for energy during the summer will increase by about 28%, due to higher temperatures. It is appreciated by specialists that Romania will be divided into two distinct areas: the northern part of the country that will be more intensely affected by rains and low temperatures, and the southern part of the country that will record high temperatures, which over time will cause desertification in some areas [11]

The use of alternative sources for energy production has a fairly recent history, after 1973, when the world was confronted with **the first oil shock**. The grim forecasts and scenarios for the **exhaustion of conventional natural resources** after this event led the world and science to search for new sustainable and inexhaustible sources for energy production [3], [12], [6], [7]

In recent years, at EU level, the issue of the use of renewable energy has enjoyed great importance and has known concrete incentive measures through the *"Europe 2020 Strategy"*, through the *"European Climate and Energy"* policy, as well as through the *"Paris Agreement"* in 2015, on limiting the increase in global average temperature at 2°C.

The European Union also assumed the leadership of the GHG emissions reduction mission, with its main objective being 80-95% reduction by 2050, compared to 1990, and secondary targets being 40% by 2030 and 60% by 2040. [13], [14]

In order to sustain the needs of Romanian consumers in the long term, it is necessary to modernize and streamline the Romanian energy sector in terms of technology and environmental protection. As regards Romania, the studies carried out consider that the country's potential for renewable resources is a very important one, thanks to the favourable natural framework in this regard. [13], [14]

The implementation and development of systems for the production of renewable energy became a real and legitimate necessity of Romanian society, Romania being considered one of the most polluted European countries because of the production of conventional energy. [5]

The production of green energy presents a number of advantages for Romania such as creating new jobs, especially in rural areas; attracting substantial investments for the construction of production units; increasing the degree of energy independence; adopting sustainable measures to protect the environment and combat climate change. [3], [12], [13].

However, increasing our country's economy, as well as increasing living standards, also led to increased demand for energy. Thus, the prerequisites for adopting viable measures to encourage the production and uptake of renewable energy in our country have been created.

Romania's electricity production for the years 2016 and 2018 according to the primary energy source is structured according to Table 1.

Table 1. Primary source for electricity production in Romania (%)

No.	Primary energy source (%)	Electricity production in Romania (2016-%)	Electricity production in Romania (2018-%)
Conventional sources		***57.62***	***58.72***
1	Coal	24.47	24,24
2	Nuclear	17.49	17,65
3	Natural gas	14.99	15,02
4	Fuel oil	0.28	0,03
5	Other conventional sources	0.06	1,78
Renewable sources		***42.38***	***41.28***
6	Hydroelectric	28.86	27,87
7	Aeolian	10.13	9,78
8	Biomass	0.75	0,47
9	Solar	2.60	3,15
10	Other renewable sources	0.05	0,01
TOTAL		100.00	100.00

Source: Data published by ANRE, 2016, 2018

Material and Method

The main methods mainly addressed in the present research are the analytical and synthetic method the statistical and mathematical calculation; data processing and interpretation and case study.

Results and Discussions

In Romania, the use of renewable energy becomes an increasingly discussed topic, mainly by mediating European environmental protection policies. In this respect, European funds have been granted in recent years through operational programmes or through national programmes supporting the renewable energies sector (Casa Verde Programme, Photovoltaic Systems Programme) [3], [15]

Our country intends to increase its energy efficiency by putting particular emphasis on the exploitation of renewable energy sources, which can contribute to reducing the energy dependence of our country towards imports, to limiting adverse effects on the environment and, last but not least, to increasing the supply of food to the population as a result of the use of renewable sources in agriculture, such as geothermal waters which are found in abundance in the western area of Romania.

Geothermal or ***geothermal energy*** is a form of energy obtained from the heat inside the earth.

Hot water and steam, captured in areas with volcanic and tectonic activity, are used for heating water, housing, electricity production, heating of greenhouses and solaria, pasteurization of milk etc., most often in rural areas, provided that the distance from the place of extraction of hot water does not exceed 35 km. [16]

The use of geothermal energy where possible reduces the consumption of fossil fuels considerably, and geothermal systems can function without taking into account climate conditions.

Western Romania is an area rich in geothermal waters, with very favourable conditions for the production of geothermal energy, as well as for the development of various forms of tourism that harness thermal waters. Geothermal aquifers from the Romanian Pannonia Depression are the most known in the country. Thus, counties such as Bihor, Satu Mare, Arad or Timiş hold important natural reserves to be exploited. [5]

The average temperatures at a depth of about 2,000 m are around 120^0C, and those operated from the depth of about 3,000 m can reach the values of 150^0C, representing the highest temperatures of geothermal waters of Romania. Another complex of our country rich in geothermal water resources is represented by the area of the Romanian plain, between the Dâmboviţa Valley and the Olt Valley, where geothermal water temperatures can reach values ranging from 100-120^0C at a depth of 3,000 m. Geothermal resources are also found in the central area of Dobrogea, south of the line of Hârşova – Medgidia – Constanţa, where geothermal waters can reach temperatures very similar to those in the Romanian plain.

However, geothermal resources are poorly exploited in Romania, with a substantial potential to increase the use of these resources in the coming decades, as a result of the promotion of European environmental policies in the field of renewable energy use.

Geothermal energy presents the advantage of providing constant energy, without variations from day to night or from summer to winter, compared to other types of alternative energy, such as wind power or solar energy.

Use of geothermal waters for the heating of greenhouses and solaria

Greenhouses are special glass or plastic constructions specially designed for the housing and cultivation of plants, despite adverse weather conditions during the cold periods of the year. The efficiency of the greenhouses is very high, allowing the control of plant growth conditions and having a much higher yield than a traditionally cultivated agricultural land in the field.

Some specialists in the field consider that greenhouses can produce up to five times more than a conventional agricultural land, while also having a number of benefits such as high yield, successful use of renewable energy (geothermal water, solar panels), the release of large areas of land that can acquire a new destination (forests, orchards, grassland) or the exploitation of inappropriate land for conventional agriculture.

With regard to the use of geothermal waters for the heating of greenhouses and solaria for vegetable production, the successful experience of other countries that possess geothermal potential, is certainly the most appropriate example. For example, in Hungary, the locality Szentes has been cultivating organic vegetables for several years in the geothermal water-heated greenhouses.

The organic products market is growing and is an opportunity for sustainable rural development. [17] In our country, we have successful examples of the use of geothermal waters for heating greenhouses in Biled, Timiş County.

Through the action of a private investor and through European funding, the former beach and pool have been transformed into a greenhouse that grows tomatoes after the latest technology. Other examples of success are also in Bihor County, where there are several

greenhouses producing tomatoes heated with geothermal water, which is exploited from a depth of over 2,400 meters. Thus, the energy from the geothermal source has eliminated the use of fossil fuels and also pollution.

Economic efficiency of greenhouse tomato culture

To measure the economic efficiency indicators, the expenses of the tomato crop cultivated in the greenhouse (Table 2) must be known.

Table 2. Spending target for tomato culture. Calculations per hectare (%)

No.	Specification	M.U.	Value/ha- RON
1	**Average production**	**tons**	**279.939**
A	**DIRECT EXPENSES (I+II)**	RON	**579.339**
I	Material expenses (1+2+3+4+5)	RON	**460.089**
1	Expenses with preparation work	RON	**1.739**
2	Material expenses	RON	**235.772**
	Propagating material	RON	65.452
	Chemical fertilizers	RON	44.000
	Natural fertilizer	RON	3.520
	Packaging	RON	65.800
	Insecticides and fungicides	RON	36.000
	Pollination with bumblebees	RON	6.000
	Other materials	RON	15.000
3	Energy expenses	RON	**196.103**
	Thermal	***RON***	***105.600***
	Electrical	RON	90.503
4	Irrigation expenses	RON	13.662
5	Supply expenses 5% of 2	RON	12.813
II	Labour costs (6+7+8+9)	RON	**119.250**
6	Salaries	RON	90.000
7	CAS 22.5% of 6	RON	20.250
8	Unemployment aid 3% of 6	RON	2.700
9	Health aid 7% din 6	RON	6.300
B	**INDIRECT EXPENSES (10+11+12+13+14)**	RON	**219.674**
10	General and common expenses 2% of A	RON	11.587
11	Collaborations and outlets	RON	110.774
12	Transport	RON	90.952
13	Local taxes	RON	1.700
14	Insurance	RON	4.661
C	**TOTAL PRODUCTION EXPENSE A+B**	RON	**799.013**

Source: Own calculations

The technical and economic data necessary for calculating the indicators of economic efficiency in tomato culture are presented in the Tables 3 and 4.

Table 3. Technical and economic data for tomato culture

No.	Specification		M.U.	Tomatoes
1	Cultivated area		ha	**1 hectare**
2	Total production:		t	**279.939**
3	of which cargo		t	**279.939**
4	Total production expenses		RON	**799.013**
5	Average sales price	Main production	RON /kg	**4,006**
		Main production	RON /t	**4006,00**
6	Expenses for the disposal of goods production		RON /t	**39.00**
7	Total work time fund consumed		Hours/labourer	**11.550**

Source: Own calculations

Table 4. Main indicators of economic efficiency in tomato culture

No.	Indicators		M.U.	Tomatoes
1	Production per ha		kg / ha	**279,939,000**
2	Total expenses per ha		RON / ha	**799,013**
3	Value of final production per ha		RON /ha	**1,121,555**
4	Unit cost of production		RON / kg	**2.85**
5	Gross profit	per ha	RON / ha	**312,691**
		per kg delivered	RON / kg	**1.12**
6	Expenses per 1,000 RON value of commodity production		RON	**721**
7	Profit rate		%	**38.66**
8	Labour productivity	in work time units	Hours-labourer / t	**41.3**
		In value units	RON Vpf/ hours-labourer	**97.1**

Source: Own calculations based on data retrieved from the vegetable farm

Conclusions

The economic indicators obtained in the tomato culture highlight a gross profit of 312.691 RON/ha.

Vegetable growers in areas where there are geothermal water sources have the possibility to increase the profit per hectare in crops made in the greenhouse, by eliminating the costs incurred with heat consumption, which, in the present case, have a share of 13.22% of total expenses made on tomato-cultivated hectares according to calculations made.

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Pomological Attributes to New Peach Varieties Cultivated in the Northeast of Romania

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Abstract

The research was carried out at Research Station for Fruit Growing Iasi for three consecutive years. Three peach cultivars as follows 'Raluca', 'Cora' and 'Delta' were studied. The analysis of the results highlights the fact that the phases from the swelling buds to the beginning of the blossoms occurred from a calendar point of view between 25 February and 12 April. The vigor of the tree was expressed through the trunk section surface that ranged between 34.7 cm^2 (Raluca) and 59.3 cm^2 (Cora). In terms of the weight of the fruit (g), Raluca (103.3 g) were noted in the three years that statistically significant differences from the average.

Keywords: peach, cultivars, fruit, quality, assortment

Introduction

Persica vulgaris Mill. is a species demanding light, resistant to frost and drought but sensitive to late spring frosts [14]. Fruits are of great interest due to the presence of natural compounds, such as vitamins, carotenoids and phenolic compounds that can be considered as natural antioxidants [5]. Natural phenols have been reported to have properties as food preservatives, and a good role in protecting against pathological disorders [7]. The rooting system of trees requires well-drained soil to ensure productivity and longevity of orchards [8].

In Europe, the highest production in the last 10 years was recorded in 2017, with 4.5 million tons of peaches and nectarines [15]. Peach and nectarine consumption per capita remain unchanged or decreases in some European countries, that due to some complaining about the lack of aroma or problems with texture. This was the reason to search for new varieties with the high quality of the fruits adapted to the climate and soil conditions of the area [1], [10].

Peach tree culture in Romania ranks 6th after the main species as apple, plum, cherry and apricot. The pomological characteristics of the species are influenced by soil and climate conditions [12], [13], that why Northeast area of Romania is considered the northern limit for the efficiency of peach tree growing. The zoning of peach tree culture is very important to meet the requirements of ecological factors, especially those related to temperature, light and soil.

The existing assortments have a fresh fruit consumption from June to October [6]. The most significant production recorded in the last ten years was 23,000 tons in 2014 [15]. In this paper we analysed the climatic conditions, the vigor of the tree and the mass of the fruit of new peach cultivars in conditions from North-Eastern area of Romania.

Materials and Methods

The research was carried out on the peach varieties *Raluca, Cora* and *Delta*. The varieties studied are located in the peach competition culture within the experimental polygon. The place

of experience is characterized by the appearance of the normal soil, the parent/underlying material is cambic cernoziom, the soil between the rows of trees is grassed and the soil between the trees on the row is maintained with work done with the palpating disc. Irrigation and support systems are not installed on the plots. The trees are in the 11th year of planting, the crown shape being of flattened vessel and planted at a distance of 4m x 4m. The seeding material was grafted on *Prunus cerasifera*. The analysis of the climatic conditions during the observation period was carried out through the AgroExpert weather station. Phenological data were determined by the Fleckinger system [4]; [2]: C – the upper part of the bud has opened and the individual flower buds are visible; E – beginning of flowering: flowers are open for 5%.The force of the trees was performed by measurements with the subler on the trunk, data that were transformed into cm² and the mass of the fruits was determined by weighing using the electronic balance (Radwag, 0.01 sensitivity).

The comparison of the variants was done against the average of the variants and they were interpreted by the method of analysis of the variance [11].

Results and Discussions

The researches were carried out at the Research-Development Station for Iasi fruit growing during three consecutive years 2016, 2017 and 2018. The analysis of the results shows that the phenological phases from the swelling of the buds to the beginning of the flowering occurred from a calendar point of view between February 25 and April 12.

Temperature is a limiting factor for the cultivation of fruit species, but especially for species that prefer heat and hardly adapt to low temperatures and climatic accidents. In the paper we highlighted the temperatures above the biological threshold and the number of days in which they were recorded during the studied period.

Fig. 1. Varieties of peach – (original photo)

Table 1. The number of days and the sum of the temperatures above the biological threshold was exceeded of peach cultivars (RSFG Iaşi, 2016-2018)

Cultivars	Sum of temperatures (°C)			Number days			Average temperatures (°C)	Average number of days
	2016	2017	2018	2016	2017	2018		
Raluca	128.9	198.4	144.7	12	17	11	157.3^{ns}	13.3^{ns}
Cora	128.9	156.1	179.6	12	13	13	154.9^{ns}	12.7^{ns}
Delta	128.9	188.1	144.7	12	16	11	153.9^{ns}	13^{ns}
Average (control)							155.4	13.0
DL 5%							47.7	3.7
DL 1%							78.9	6.1
DL 0,1%							147.7	11.5

The diameter of the trunk is the main parameter for determining the vigor of the tree. It shows differences in species and variety. In this case there were differences between the first and third year, ranging from 34.7cm^2 (*Raluca*) to 59.3cm^2 (*Cora*). Statistically the values of the differences have an insignificant value compared to the average of 48 cm^2.

Table 2. Trunk section surface of peach cultivars (RSFG Iasi, 2016-2018)

Cultivars	Trunk section surface (cm^2)			
	2016	2017	2018	Average (cm^2)
Raluca	22.3	29.2	52.6	34.7^{ns}
Cora	40.1	52.8	84.9	59.3^{ns}
Delta	45.3	51.5	53.5	50.1^{ns}
Average (control)				48.0
DL 5%				22.6
DL 1%				37.3
DL 0,1%				69.9

The mass of fruit specific to the varieties of peach varies between 55g and 140g. Of the varieties taken in the study (Fig. 1), the lowest weight was recorded in 2016 with the weight of 76 g at *Cora* and the highest weight was found in 2017 at *Raluca* (Fig. 2) with a weight of 111 g. The values resulting from the studies were significant in the case of the *Raluca* variety, registering a value of 103.3 g compared to the average and at *Cora* variety the results have a significant negative value, evidencing a value of 82.7 g compared to the average.

Table 3. Fruit mass of peach cultivars (RSFG Iasi, 2016-2018)

Cultivars	Fruit mass (g)			
	2016	2017	2018	Average
Raluca	102	111	97	103.3*
Cora	76	90	82	82.7 ^{00}
Delta	91	101	90	94 ^{ns}
Average (control)				93.3
DL 5%				6.4
DL 1%				10.5
DL 0,1%				19.7

Fig. 2. Peach cultivar Raluca (original photo)

Our results are similar with other studies. However, Cantin *et al*., 2009 don't find any correlations between fruit weight and annual peach tree yield.

Conclusions

During the studies, among the Romanian varieties pursued (*Raluca, Cora* and *Delta*), *Raluca* presented a significant fruit value compared to the average, which concludes that the variety is suitable for spreading in assortment.

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The Assessment of Fruits Technological Features in Some Cherry Cultivars Grown Under the Ecological Conditions from the N-E of Romania

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Abstract

The aim of this paper is to present the valuable characteristics of some cherry cultivars created at SCDP Iasi that improve the autochthonous assortment. Analysing the values of the fertility index during the three years of study, the 4 cultivars show high productivity because the recorded values were between 30,0-36.2%. Regarding fruits' weight (g) and equatorial diameter (mm), the cultivars that got highlighted are Lucia (8,2 g and 25,2 mm) and Cătălina (8,0 g and 24,0 mm). The SUS values were between 17,8% (Iaşirom) and 19,4% (Radu), the titratable acidity (AT) of the fruits varied within large limits with values between 0,44-0,90 mg malic acid/100 ml juice, with a ratio between SUS and AT between 20,36-40,58%. Moreover, the values of the total polyphenols varied between genotypes, this indicator positioning itself between 382,15- 588,33 mg gallic acid/100 ml fresh juice.

Keywords: cultivars, cherry tree, fruit, measurements, features

Introduction

The cherry tree is a fruit-growing tree species of great economic importance, given by the nutritive, technological and commercial traits of the fruits [1]; [6]; [8].

The cherries are the first fresh fruits of the year and due to their high content of vitamins, mineral salts, easily assimilable sugars and lovely taste represent one of the most efficient commercial activity that takes place between the second half of May and July [4]; [9].

The fruits are appropriate both for fresh consumption and industrial processing. The fruits' capitalization has been set based on examinations, fruits' tasting and measurements.

During 2018 (530,5 mm) and 2019 (53,2 mm during the first six months of the year), there have been recorded quantities below the multiannual limit (562,6 mm), resulting a deficit of 32,1 mm for 2018 and 77,8 mm for 2019 (this climatic variability influences the fruit's growth negatively) while in 2017 the multiannual limit was surpassed, resulting 1045,8 mm (a surplus of 483,2 mm). The aim of this paper is to present the valuable features of some cherry cultivars created at SCDP Iaşi that improve the autochthonous assortment.

Material and Method

The research was performed during 2017-2019, with 4 cherry cultivars created at SCDP Iaşi and grafted on mahaleb as research material (Cătălina, Iaşirom, Radu şi Lucia).

The trees are planted at 4x5 m with the shape of free flattened pelmet, while being in years XIV-XVI since they were planted. The land where the plantation is located is situated in the

Jijia-Bahlui depression, where the annual average temperature was 13°C in 2017, 10.7°C in 2018 and 9.2°C in the first six months of 2019 (the multiannual average being 10.2°C).

The meteorological factors (during the three years) have been analysed, measurements (according to the UPOV TG/35/7 questionnaire) concerning the fruit's dimension (equatorial diameter – mm), the fruit's and stone's weight (g), the ratio stone/fruit, shape, colour and fruit's firmness, pulp adherence to stone, soluble dry substance (SUS%), titratable acidity (AT), ratio between SUS and AT, total content of polyphenols, resistance of fruit to cracking and fruit's suitability for fresh consumption and industrial processing have been performed.

The fruit's and stone's weight have been measured through weighing, the equatorial diameter of the fruit has been measured with the electronic callipers, SUS has been measured with the Zeiss type refractometer, AT has been measured using the potentiometric method [5], the total content of polyphenols has been measured using the Folin-Ciocâlteu method [7]. and the resistance of the fruits to cracking has been measured using the Cristensen method, counting the cracked fruits after soaking in distilled water at 20°C for six hours [10].

The productivity was measured by the fertility index that represents % of fruits resulted 25-30 days after the petals fall and based on it, the cultivars are considered of high productivity if they have values above 30-35% [2].

The experimental data was interpreted statistically by analysing the variance.

Results and Discussions

Analysing the values of the fertility index during the three years of study, the 4 cultivars are highly productive because the average of the recorded values were between 30.0-36.2% (Table 1).

Table 1. The fertility index for four cherry cultivars in their XIV-XVI years since planting

Cultivar	Fertility index			
	2017	2018	2019	Average
Cătălina	26,9	24,0	39,5	30,1
Iaşirom	44,1	25,0	21,0	30,0
Radu	39,9	37,6	25,0	34,2
Lucia	35,4	44,2	29,0	36,2

All the studied cherry cultivars have a dark red fruit (Fig. 1, 2), firm pulp excepting Cătălina that has a semi-firm pulp and concerning the pulp adherence to stone, all the cultivars are non-adherent. The fruit's shape for Cătălina and Iaşirom is heart-shaped, while for Radu and Lucia is kidney-shaped. The studied cherry cultivars have a good resistance to the phenomenon of fruit cracking, the recorded values being between 0,7 % (Iaşirom) and 8,3% (Lucia) (Table 2)

CĂTĂLINA IAŞIROM

Fig. 1. The studied cherry cultivars

LUCIA

RADU

Fig. 2. The studied cherry cultivars

Table 2. Physical and quality traits of the fruits for the studied cherry cultivars

Cultivar	Epidermis colour	Pulp firmness	Fruit's shape	Pulp adherence to stone	Fruit's resistance to cracking (%)
Cătălina	dark red	semifirm	heart-shaped	non-adherent	6,5
Iaşirom	dark red	firm	heart-shaped	non-adherent	0,7
Radu	dark red	firm	kidney-shaped	non-adherent	2,7
Lucia	dark red	firm	kidney-shaped	non-adherent	8,3

The fruit's weight is a dimension influenced by the local climatic conditions and by the biological particularities of each cultivar.

Statistically, it can be noticed that the cultivars recorded nonsignificant differences in comparison with the variants' average (7,2 g), therefore the cultivars with the largest values were Lucia (8,2 g) and Cătălina (8,0 g) (Table 3).

Regarding the equatorial diameter (mm), Lucia (25,2 mm) and Cătălina (24,0 mm) got highlighted again (Table 3).

Regarding stone's size, the cultivars recorded a weight between 0,26-0,36 g, classifying them of small to middle size according to the UPOV questionnaire.

The ratio fruit/stone was between 20,29 (Radu) and 28,02 (Lucia), all the cultivars recording statistically, nonsignificant differences in comparison to the variants average (23,34) (table 3).

Table 3. Physical traits recorded during 2017-2019

Cultivar	Fruit's average weight -g-	Stone's average weight -g-	Fruit/stone ratio	Fruit's equatorial diameter -mm-
Cătălina	8,0	0,36	21,91	24,0
Iaşirom	6,1	0,26	23,17	22,8
Radu	6,6	0,33	20,29	22,6
Lucia	8,2	0,29	28,02	25,2
Average (X)	7,2	0,31	23,34	23,6
DL 5% DL 1% DL 0,1%	1,8 2,7 4,4	0,06 0,09 0,14	6,54 9,90 15,90	3,2 4,8 7,7

The content in dry substance is extremely important in cherries, the taste of the fruits highly depending on it and the ratio between the soluble dry substance and the titratable acidity determines the balance between the sweet and sour taste of the [3].

For the studied cultivars, the SUS values were between 17,8% (Iaşirom) and 19,4% (Radu), the titratable acidity (AT) of the fruits varied in large limits with values between 0,44 (Iaşirom) and 0,90 (Cătălina) mg malic acid/100 ml juice, with a ratio between SUS and AT between 20,36-40,58% (table 4).

Table 4. Fruits biochemical traits recorded during 2017-2019

Cultivar	SUS (Brix)[2]	AT (mg Malic acid/100 mL^{-1})[3]	SUS and AT ratio (%)	Total content of polyphenols (mg GAE/100 mL^{-1})
Cătălina	18,3	$0,90^{+++}$	20,36	430,21
Iaşirom	17,8	$0,44^{ooo}$	40,58	$588,33^{+}$
Radu	19,4	$0,49^{oo}$	30,37	382,15
Lucia	18,8	0,68	27,83	391,22
Average (X)	18,5	0,62	29,78	447,97
DL 5% DL 1% DL 0,1%	4,4 6,6 10,7	0,07 0,11 0,17	13,54 20,51 32,95	127,40 192,92 309,92

Moreover, the values of the total polyphenols varied among genotypes, this indicator sitting between 382,15-588,33 mg gallic acid/100 ml of fresh juice (table 4).

A high content of polyphenols is associated with an intense colour of the fruits, with a high content of dry substance, but with a more intense flavour as well.

The four studied cultivars can be capitalized both for fresh consumption and for industrialization.

Conclusions

1. Under the conditions of 2018 and the first six months of 2019 (dry years) as well as 2017 (rainy year), in the Iaşi area, in terms of productivity, the four cultivars got highlighted as highly productive and with a good resistance to the phenomenon of fruit's cracking.
2. Analysing the values of the fruit's size, the cultivars Lucia (8,2 g and 25,2 mm) and Cătălina (8,0 g and 24,0 mm) were highlighted.
3. The fruits of the cultivars Cătălina, Iaşirom, Radu and Lucia can be considered as fruits with a taste that is extremely appreciated by the consumers due to the balance between the sweet taste (SUS content) and sour taste (AT) and they have a high content of polyphenols giving them a more intense flavour.

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Consideration on Some Reclamation Methods of Urban Compacted Soils in Residential Areas

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Abstract

The effects of urbanization on compactness state of soils from urban area can be extensive.

In strong compacted soil infiltration of water through soils is greatly diminished. The method commonly used to valor compacted soil is to cover it with fertile soil from different sources.

Although results can be obtain using some annual plants with shallow root system, but in a short time, most of the cultivated plants are stagnant growing due to waterlogging and asphyxiation roots. Even if the compacted layer is covered with fertile soil constraints of compacted layer are maintained which actsas a barrier to water infiltration and roots penetration. In this work we proposed some reclamation methods in order to prevent the negative effects of water stagnation and improve the soil internal drainage.

Keywords: residential area, compacted soils, reclamation

Introduction

The residential areas in Romania have increased considerably in the last 25 years as a result of rural to urban migration.

The development and improvement of the urban horticulture is one of the current demands of residents and communities from residential area.

It is recognized that gardens are a vital part of our towns and cities. Among the benefits of horticultural gardens in urban areas we mention:

The benefits urban gardens and green spaces are also included control urban temperatures (i), offers favourable conditions for wildlife (ii); improve human health (iii); for communities interested in gardening on a site, it is useful to know the characteristic of the soil at the site.

It the residential area it is very important to test soil characteristics such as pH and nutrient availability, state of compactness etc. In order to create green spaces in residential areas it is also required to take into consideration the site's land use history and test the soil accordingly for potential contamination.

Soil compaction in the residential areas occurs during construction cutting and filling operations, general grading works, and other processes of running heavy equipment over the soil. After construction, soil compaction can occur with site activities such as walking, sports, and even parking heavy vehicles on grassed areas.

The strong compaction of soil during construction and use causes most decreasing causes a considerable decrease in the rate of infiltration of water into the soil.

The remediation of strong compacted soil could be done by levelling and loosening the soil and by adding organic matter such as compost and manure. These additions can increase the amount of water that sandy soils can absorb or hold and can improve the drainage of clay soils.

Slow improvements in soil compaction may occur with time in relatively undisturbed areas by deep rooted plants or by soil fauna such as boring animals.

Methodology

The studied site is located in the residential area of Iasi city (Romania).

Several soil profiles were made within the studied location. Establishing the locations of soil profiles has been done according to the instructions within Soil Survey Methodology. [2] The studied soils have been diagnosed according to the Romanian System of Soil Taxonomy [3] and World Reference Base for Soil Resource [4].

Characterization of soil profiles was done following the instructions from guidelines for soil and land descriptions [5].

Soil samples were taken from each paedogenetic horizon in order to conduct laboratory analyses: granulometry, pH, contents of calcium carbonate, bulk density, according to the current methodology [1, 2,] following the processing and analysis of the data obtained in the field and laboratory, some remediation measures have been recommended.

Results and discussion

The representative soil of the studied area is a Cambic Cernoziom Chernozem [3] or Haplic Chernozems [4]. Within the studied location was also identified Urbic Technosolswith high content of artefacts (Figure 1).

Fig. 1. Haplic Chernozems (A) and Urbic Technosols (B) from studied residential area

On the depth interval of 0-100 cm, the content of clay ranged between 32.4 and 35.7%.

The bulk density values are between 1.34 g/cm^3 and 1.782 g/cm^3. Strong soil compaction is highlighted by shallow distribution of *Elymus repens* rhizomes. (Figure 2).

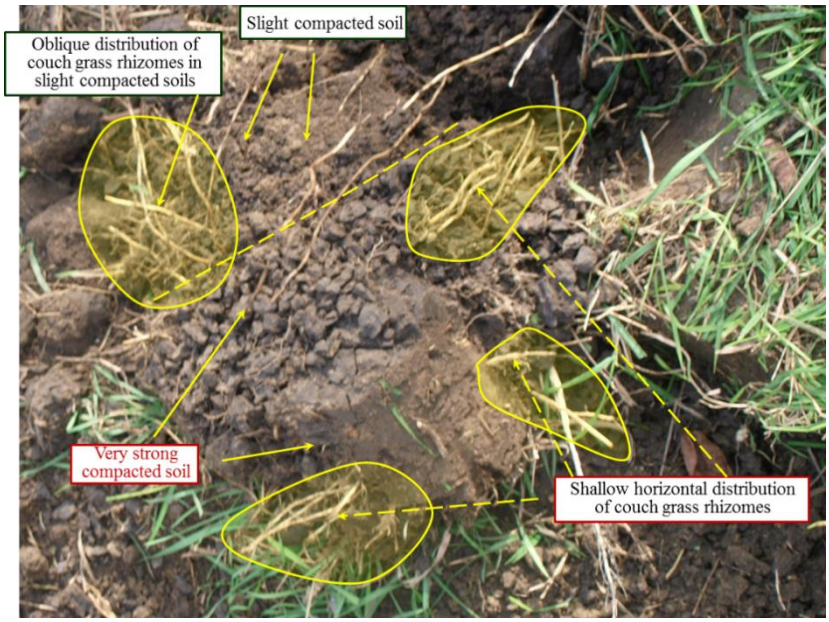

Fig. 2. Distribution of *Elymus repens* rhizomes in slight and strong compacted soils from residential area

In order to ensure water infiltration, roots penetration and the prevention of waterlogging, it is necessary to apply some remediation works on soils with bulk density values higher than 1.5g/cm^3.Soils containing artefacts are weak alkaline, pH values are 7.5-8.1.

The restoration of compacted clayey soils on the depth of 0-20 and 20-40 cm could by the spading works and incorporation of the plants stems fragments (Figure 3).

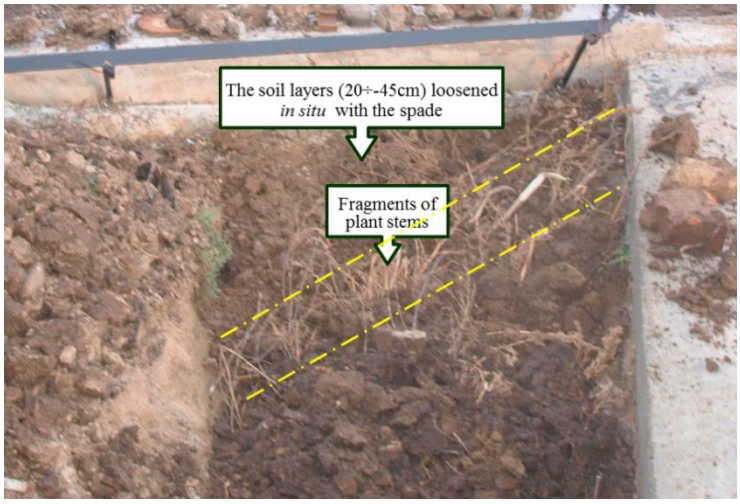

Fig. 3. Fragments of plants incorporated at the same time with the reclamation works of the strong compacted soil on the depth of 20-45cm

The incorporation of plant stems fragments in the soil layer on the depths 20-45 cm will gradually improve the internal drainage of the by macro pores that will result after decomposition of the organic matter.

Soils containing artefacts are weak alkaline, pH values are 7.5-8.1.

The stages of improvement of the highly compacted Urbic Technosols consist of the loosening and removing of the soil of a strip of 1m and depth of 0-20cm (i), the loosening of the soil on the depth of 20-45cm and the incorporation in the vertical or slightly oblique position of the fragments of plant stems (ii), covering the loose soil layer with the soil that was initially removed from the depth 0-20cm (iii), covering with a fertile soil layer of a thickness of 10-15cm.

Fig. 1. The stages of reclamation of the strong compacted soil

Conclusions

The restoration of compacted clayey soils on the depth of 0-40cm by the spading works which are the safest methods for large scale improvements.

The incorporation of plant stems fragments in the soil layer on the depths 20-45 cm will gradually improve the internal drainage of the by macro pores that will result after decomposition of the organic matter.

The use of deep-rooted plants allows deeper soil profile remediation and may be suitable to enhance drainage in problem site and implicitly to prevent water stagnation.

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The Influence of Environmental Factors in Polytunnels on Some Tomatoes Nonparasitic Disorders

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Abstract

Frequently, in polytunnels are found some nonparasitic or physiological disorders on vegetable plants such as tomatoes, peppers, eggplants, and some melons. The growing stagnation of tomato plants cultivated in polytunnels is evidenced by some symptoms that appear during the vegetation season. These symptoms may be due to biotic (e.g., diseases, pests) and environmental factors such as high fluctuations in soil moisture, low or high values of relative humidity of air, atmospheric drought, high temperatures, soil characteristics, etc. One of the soil attributes that favours physiological disorders, are also the substrate reaction, characterized by pH values lower than 5 or higher than 8. High content of some nutrients such as N, K, Mg, Na, Mn, antagonists to Ca, can also induce physiological disorders. The physiological disorders symptoms are manifested especially on the leaves and fruits. Some physiological disorders can often be misidentified as caused by pathogens, for example Blossom End Rot, can often be misidentified as gray rot caused by pathogen *Botrytis cinerea* Pers., which is a fungus (Ascomycota). Both disorders manifests by brown spots on fruit skin surface, both are favoured by high humidity and weak airing in polytunnels. One of the objectives of the paper is the environmental factors characterization which influence tomato plants affected by nonparasitic disorders such Blossom-end rot or leaf curling and leaf distorting. Following our investigations, it was found that frequently, some symptoms on tomatoes were not due to a parasite action. The correct identification and differentiation of parasitic disorders caused by the environmental factors, presents a particular practical importance in order to establish prevention measures and cultural recommendations.

Keywords: polytunnels, physiological disorders, environmental factors

Introduction

The environmental factors that influence the quality of tomatoes are: soil, temperature, light, water. Since the atmospheric humidity is not beneficial for tomato plants, favouring the attack of diseases and pests, it is advisable to choose as method of irrigation, the one by drip or the furrow irrigation. Irrigation by sprinkling or using the hose is excluded. Tomatoes are thermophilic plants with high demands on heat.

At temperatures above 30ºC the plants do not grow longer because the pollen does not germinate, at more than 35ºC they do not grow, and at 40ºC they can die. At lower soil temperatures of 10°C and higher than 37°C the roots do not grow and thus cannot support the plant [7].

The physiological disorders are non-parasitic, being found on tomatoes, peppers, eggplants etc. It represents a symptom of calcium deficiency in fruit. The most obvious symptoms are on

fruits and leaves. The causes of symptoms due to deficiency or excess of nutrients are difficult to identify. Similar symptoms can be induced by different causes.

One of the common disorders on tomatoes is Blossom-end rot, which can be caused by a low calcium level in soil or other cultural factors, especially the fluctuation of soil moisture.

These disorders are usually more severe in extreme soil moisture conditions, too dry or too wet [5].

These conditions lead to a calcium deficiency, that occurs during fruit maturation and the appearance of a lesion in that place. These disorders are more prevalent on tomatoes; however, it may also occur on other cultures, and the symptoms on the fruit are the same [6].

Other symptoms of a calcium deficiency are curled and distorted leaves, which often show a hook at the leaf tip and stunted growth.

From a practical point of view, it is important to note that calcium deficiency in fruit is rarely due to the calcium content of the soil [1]. One of the attributes of the soil that favours physiological disorders, are also the substrate reaction, characterized by pH values lower than 5 or higher than 8.

High content of some nutrients such as N, K, Mg, Na, Mn, antagonists to Ca, can induce physiological disorders. The excess of these elements leads to insufficient uptake of calcium.

Climatic factors like low temperature of air and soil, high humidity or low temperature difference between day and night, can also favour physiological disorders [1].

It is important to mention that some physiological disorders can often be misidentified as caused by pathogens, for example Blossom End Rot, can often be misidentified as gray rot caused by pathogen *Botrytis cinerea* Pers., which is a fungus (Ascomycota).

Methodology

The investigation carried outing several polytunnels from North-Eastern Romania.

Field observations have been made on plants that showed some symptoms of growing stagnation. It was prevailed field samples of biological material, the samples being analysed in the laboratory, using the Olympus stereomicroscope, or a magnifier. In the polytunnels where we found blossom end rot, soil profiles were made in order to establish some soil characteristics which influence tomato plants growing. Characterization of soil profiles was done following the guidelines for soil and land descriptions [3].

Soil samples were taken from each pedogenetic horizon in order to conduct laboratory analyses [2, 4].

Following the processing and analysis of the photos and data obtained in the field and laboratory we designed a diagram showing the symptoms of calcium deficiency and some recommendations.

Results and Discussion

The link between the environmental conditions and physiological disorders present on tomatoes is shown in figure 1. We can see how under conditions of low soil humidity, below 50% of field capacity, on the same plant appears the symptoms of Blossom-end rot but also the distorting of the leaves at the tip. These are symptoms of calcium deficiency in fruit and leaf, caused by low levels of calcium in soil, especially because of the low soil moisture.

Fig. 1. One tomato plant, manifesting both physiological disorders, Blossom-end rot and distorted leaves with a hook at the leaf tip

The disorders can also manifest in moisture excess, water logging limiting the extension of the root system and implicitly diminishing the calcium uptake by the root system. Our research has shown that moisture excess is favoured by the presence of plowpan or a compacted soil layer below the arable layer, which obstruct water infiltration and root development. We mention that the moisture excess is manifested even in the situation where drip irrigation is applied.

Physiological disorders on tomato plants can often favour other pathologies (Figure 2), such as pathogenic infection with gray rot (A) or pest attack (B) by *Helicoverpa armigera* Hübner (Lep., Noctuidae).

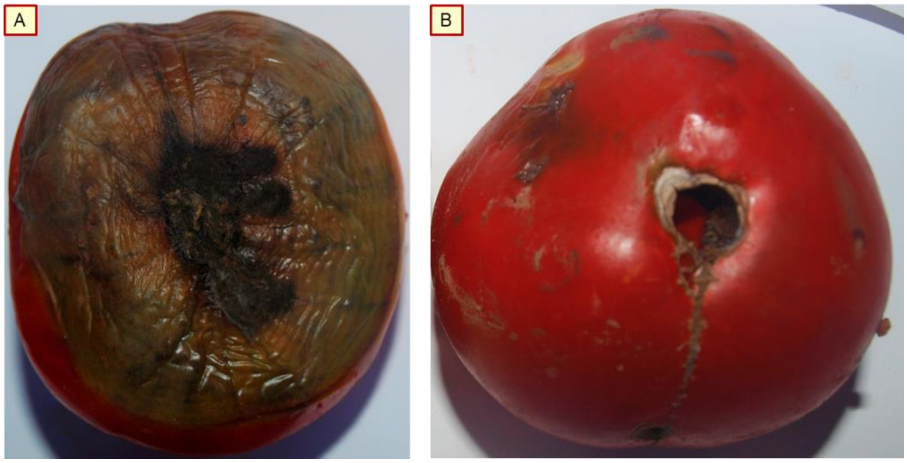

Fig. 2. Blossom end rot as an entry gate for pathogen infection (A) and pest attack (B)

The symptoms of calcium deficiency inducing Blossom-end rot are presented in figure 3.

First two options, mobile elements or sulphur/manganese deficiency are excluded because of browning leaf tissues, leaf distorting and hooked tip, characteristic to calcium deficiency.

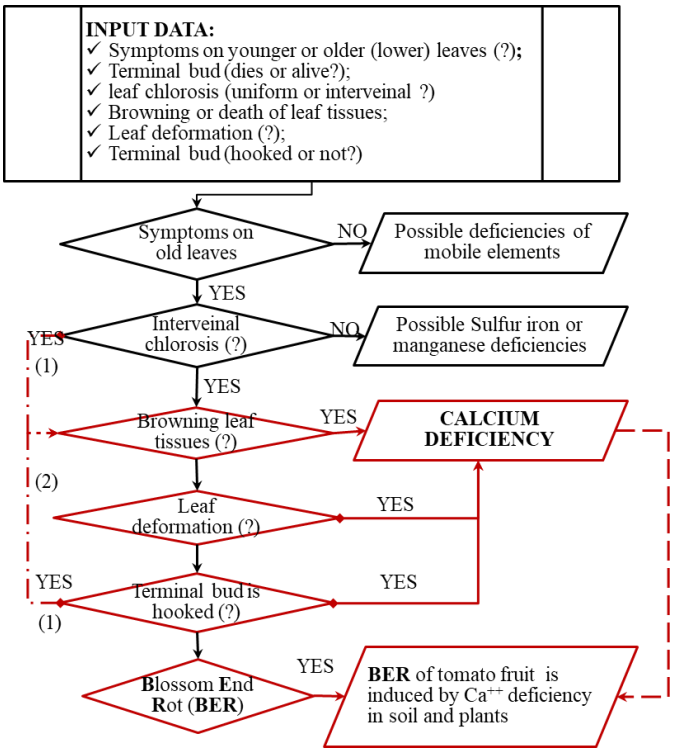

Fig. 3. Diagram showing the symptoms of calcium deficiency in the tomato plants

Conclusions

Under conditions of low soil humidity, below 50% of field capacity, on the same plant appears the symptoms of Blossom-end rot but also the distorting of the leaves at the tip.

The symptoms of calcium deficiency in fruit and leaf are caused by low levels of calcium in soil, especially because of the low soil moisture.

Diagram with symptoms of calcium deficiency could be supplementary criteria to identify Blossom end rot.

Acknowledgements

This work was financed by Romanian Ministry of Research and Innovation (**PN 18180301**)

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Section 3
ANIMAL SCIENCES AND ANIMAL PRODUCTIONS VALORIZATION

The Effect of Diet Containing Mangosteen Peel Extract (*Garcinia Mangostana* L.*)* and Suplemented with Zinc and Cooper on the Quality of Sentul Chicken Carcass

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Abstract

Sentul chicken is a specific local chicken from Ciamis region in West Java and a dual-purpose type that can utilized for eggs and meat production. One of the alternatives to improve performance is by giving the diet added with a mangosteen (*Garcinia mangostana L.*) peel extract. Mangosteen peel extract contains xanthone compounds as antioxidants and antimicrobials. This research aims to determine the optimal level of mangosteen peel extract (*Garcinia mangostana L.*) with supplemented Zn and Cu on carcass quality of Sentul chicken.

The number of chickens used 100-day old Sentul chicken that kept until 12 weeks. The experimental design used was Complete Randomized Design (CRD) with 5 treatment levels of mangosteen peel extract with supplement Zn and Cu with 4 replications respectively with 5 chicks in each replicate. The treatments consisted of P0 (only basal feed), P1 (basal diet + 60 mg/kg extract and Zn-Cu supplements), P2 (basal diet + 120 mg/kg extract and Zn-Cu supplements), P3 (basal diet + 180 mg/kg extract and Zn-Cu supplements) and P4 (basal diet + 240 mg/kg extract and Zn-Cu supplement). The investigated parameters were final body weight, carcass weight, dressing percentage, and the meat cholesterol content of Sentul chicken.

The results showed that using 120-180 mg/kg mangosteen extract with supplemented Zn and Cu significantly increased final body weight, carcass weight, and reduced meat cholesterol content compared to basal diet, but with the addition of 240 mg/kg diet final body weight and carcass weight decreases. The study concludes that the addition of mangosteen peel extract with supplemented Zn and Cu to 180 mg/kg diet resulted in optimal carcass quality.

Keywords: mangosteen peel extract, supplemented Zn and Cu, carcass quality, Sentul chicken

Introduction

Sentul local chicken is a specific one which comes from Ciamis region in West Java with grey feathers as its distinctive feature, with a variation of grey and brown yellows feathers and orderly arranged feathers on its breast like dragon scales [1]. Ciamis people also call Sentul chicken as '*Kulawu* Chickens', *kulawu* means grey since the plumage colors of Sentul chickens are dominated by grey [2]. Sentul chicken is a dual-purpose type, that could be used for eggs and meat production. In another way, this bird is very good to genetically improve chicken meat breeds, because has a compact body and white skin colour [3]. Effort to increase the productivity of Sentul chickens, need other alternatives to improve the quality of carcass Sentul chicken.

Feed additives added to the diet are intended to improve the feed consumption, digestibility, and endurance of chicken livestock. Chickens growth rate and productivity could be increased by using synthetic antibiotics as feed additives, but this leads to residual traces in carcasses therefore to antibiotic resistance of microorganism in human consumers' organisms, that could be harmful. An alternative substitute for antibiotics in chicken nutrition would be the natural feed additive based on mangosteen peel extract.

The nutrient content contained in the mangosteen peel is: 0.63% crude fat, 0.71% protein, ash 1.01%, total sugar 2.10%, and carbohydrates 35.61% [4]. Mangosteen peel also contains xanthone compounds that function as antioxidants, antiviral, antifungal and antimicrobial, and not found in other fruits. Xanthone compounds consist of mangostin, mangostenol A, mangostinone A, mangostinon B, trapezi folixanthone, tovophyllin B, alpha mangostin, beta mangostin, garcinon B, mangostanol, flavonoid epicatechin and gartanin [5]. Inclusion of mangosteen peel meal in diets is problematic because of its antinutrient content in the form of tannins [6]. High tannin content will inhibit feed absorption and chicken growth. To reduce tannin levels in mangosteen peel, an extraction procedure must be carried out. Extraction is a sepa diet process of solid or liquid material with the help of solvent. The solvent used should be able to extract the desired substance without dissolving other materials. The process of extracting the peel of mangosteen fruit to obtain antioxidant substances usually use a macediet process, which is a simple extraction method to extract simplicial containing soluble chemical components in the solvent fluid [7]. Candra stated that giving mangosteen peel extract 120 mg/kg was able to increase the carcass percentage by 68.58 [8]. Xanthone compounds contained in the peel of mangosteen can improve the structures of intestinal villi in the process of nutrient absorption. Antibacterial herbs are able to suppress the growth of pathogenic bacteria in the intestine [9]. Mangosteen peel also has an anti-microbial power against bacteria such as *Staphylococcus aureus* [10]. Antioxidant compounds (xanthones) contained in mangosteen peel can also prevent or neutralize free radicals due to air pollution in the environment. An increase in ambient temperature over a comfortable temperature zone range causes oxidative stress, leading to the occurrence of free radical attack on the cell membrane. Free radicals are an atom, a molecule, or a compound in which it contains one or more unpaired electrons, making it highly reactive [11]. Research [12] on mangosteen peel extract analysed with GCMS (Gas Cromatographic Mass Spectrometry) states that mangosteen peel extract contains organic unsaturated methyl ester compounds which are easily oxidized so that the chain link must be transformed into a chain of bonds with mineral metal catalysts Cu and Zn. The minerals Cu and Zn are cationic minerals that will work on enzymes involved in growth and the immune system, but their biological availability to the animal body is affected by the presence of phytic acid in diets [13]. Copper is a mineral element that is needed in the metabolic process, haemoglobin formation and physiology in the animal body [14]. Although needed in small amounts in the organism, any excess will be able to interfere with health and gastrointestinal disorders [15].

The need for mineral Cu in poultry is 5 ppm and mineral needs of Zn are 40 ppm [16]. The results showed that 120 mg/kg providing of mangosteen peel extract had a significant effect on growth performance in broiler chickens [8]. While the results of Abidin's study [17], the use of mangosteen peel extract 41 to 120 ml/kg did not affect the feed conversion of Sentul chicken.

Mangosteen peel extract is a natural feed additive, used in order to improve the quality of diets in supporting quality of carcass Sentul chicken growth phase. This research aims to determine the optimal level of mangosteen peel extract (*Garcinia Mangostana L.*) with supplemented Zn and Cu on carcass quality of Sentul chicken.

Material and Methods

The study used 100 day- old Sentul chickens with the average body weight of 27.8 grams (coefficient of variation 8.27%). The Sentul chickens were kept in deep litter system until the age of 12 weeks, 20 pens were used, sized 90 cm x 90 cm x 60 cm (length x width x height).

Each pen consisted of 5 chickens. The feed ingredients of the basal diet were yellow corn (56.00%), rice bran (21.50%), fish meal (9.25%), soybean meal (12.00%), bone meal (0.75%), and $CaCO_3$ (0.50%). Diets were prepared based on protein and metabolisable energy requirements for the local chicken growth phase, i.e., 17% protein and 2750 Kcal/kg [18].

Mangosteen peel extract is made in the laboratory by maceration method using ethanol solvent for 2 days, then filtered and mangosteen peel filtrate is evaporated with a Rotary evaporator Bunchi R-300 with a temperature of 60^0C which aims to separate 96% ethanol with mangosteen peel extract then dried in an oven with temperature of 80^0C to get mangosteen peel extract powder. The treatment consisted of the use of mangosteen peel extract with supplement Zn and Cu, Zn and Cu supplement concentrations used in accordance with the needs of chickens are Zn 40 mg/l and Cu 5 mg/l. i.e.,: P0 (Only basal diet), P1 (basal diet + 60 mg/kg extract and Zn-Cu supplements), P2 (basal diet + 120 mg/kg extract and Zn-Cu supplements), P3 (basal diet + 180 mg/kg extract and Zn-Cu supplements), and P4 (basal diet + 240 mg/kg extract and Zn-Cu supplements). Experiments were conducted using Completely Randomized Design, consisting of 5 treatments and 4 replications. Data were analysed using Varian Analysis and differences between treatments using Duncan Multiple Range Test. The measured parameters were Final body weight, carcass weight, dressing presentage and the meat cholesterol content of Sentul chicken. Determination of meat cholesterol levels was carried out by the CHOD-PAP (*Cholesterol Oxidase Phenylperoxidase Amino Phenozonphenol*) method. Meat samples were taken from the chest and thighs [19].

Results and Discussion

The effect of mangosteen peel extract with Zn and Cu supplements on final body weight, carcass weight, dressing percentage, and the meat cholesterol content of Sentul chicken, is shown in Table 1 and Fig. 1.

Table 1. The Final Body Weight, Carcass weight, Dressing Percentage and The Meat Cholesterol Content of Sentul Chicken

Variables	P0	P1	P2	P3	P4
Final Body Weight (g)	723.25^a	838.00^b	876.75^b	808.25^b	729.00^a
Carcass Weight (g)	470.50^a	548.25^b	579.00^b	541.75^b	475.50^a
Dressing Percentage (%)	65.06^a	66.65^b	67.75^b	67.05^b	65.22^a
Meat Cholesterol (mg/100g)	124.71^a	118.36^a	111.65^b	110.12^b	96.00^c

Note: Similar superscripts in the same row show not significant difference (P>0.05)

P0 (Only basal diet)
P1 (basal diet + 60 mg/kg extract and Zn-Cu supplements)
P2 (basal diet + 120 mg/kg extract and Zn-Cu supplements)
P3 (basal diet + 180 mg/kg extract and Zn-Cu supplements)
P4 (basal diet + 240 mg/kg extract and Zn-Cu supplements)

Final Body Weight

The final body weight in Sentul chicken due to the use of mangosteen peel extract with supplement of Zn and Cu, varied between 723.25-876.75 g. The result of variance analysis showed that addition of mangosteen peel extract with supplement Zn and Cu in the basal diet has a significant effect (P<0,05) on final body weight of Sentul chicken. Effect of adding

mangosteen peel extract supplemented with Zn and Cu to the diet from 60 to 180 mg/kg was significantly higher than the control treatment (P0) and adding 240 mg/kg diet. The body weight on treatment P1, P2, and P3 did not show any significant effect. This means the addition of mangosteen peel extract with supplement Zn and Cu until 180 mg/kg gives a positive response to final body weight. It is known that mangosteen peel meal containing xanthone active substances that serves as antioxidants that could reduce cell damage, especially caused by free radicals. It is also reach in antimicrobials that are allegedly able to improve the structure of intestinal villi in the process of absorption of nutrients and are able to suppress the growth of pathogenic bacteria in the intestine, resulting in higher final body weight. Following the opinion of Lannag *et al*., [20], Xanthone compounds are also able to suppress oxidative stress that increases the rate of better chickens. The addition of Cu and Zn supplementation in mangosteen peel extract can control and improve the right environmental conditions and lead the beneficial microbial population in the digestive tract to produce a better final weight on Sentul chickens.

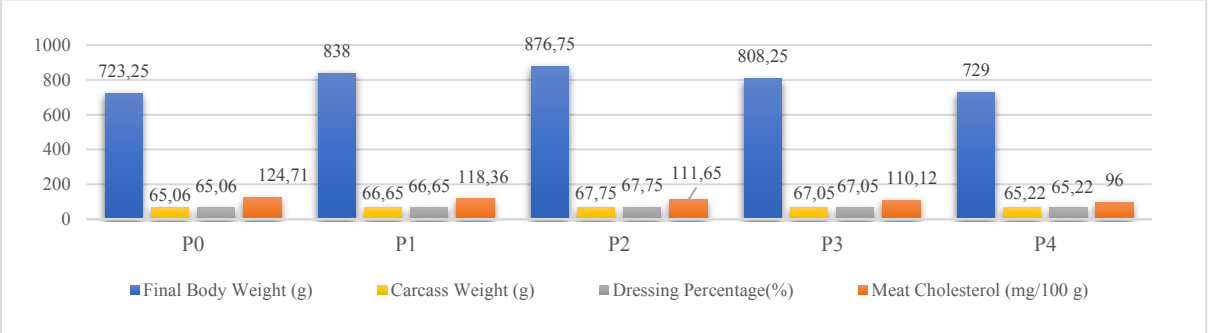

Fig. 1. Sentul Chicken Average of Mangosteen Peel Extract in Final Body Weight, Carcass Weight, Dressing Percentage, Meat Cholesterol

Carcass weight and Dressing percentage

The increased level of mangosteen peel extract with supplemental Zn and Cu in the diet, induced an average carcass weight varying from 470.50-579.00 grams and a dressing percentage within the 65.06-67.75% limits. The usage of the experimental factor generated a significant effect ($P < 0.05$) on carcass weight and dressing weight. By adding a level of 120-180 mg extract/kg diet, carcass weight and dressing percentage experienced a significantly higher increase than P0 and P4 treatment. The extract of mangosteen peels through the antioxidants within also played a role in growth and increased bodyweight of Sentul chicken, supported also by the presence of minerals Zn and Cu. Therefore, carcass weight and dressed percentage increased. This suggests that the xanthone content found in mangosteen skin extract works accordingly to its function as an *antioxidant*, *antiproliferative*, and *antimicrobial*. The xanthones improve the structure of intestinal villi in the process of absorption of nutrients and can suppress the growth of pathogenic bacteria in Sentul chicken intestine. Higher the xanthones uptake by chickens, the better the nutrients absorbed, the better the growth will be, ultimately inducing optimal carcass weight. According to Lannang *et al*., [20], xanthones in mangosteen peel have antioxidants that can suppress oxidative stress due to environmental pollution. Antioxidants convert free radicals into compounds that are relatively stable and stop the chain reaction from free radical damage so that will have an impact on the rate of chicken growth [21].

Meat Cholesterol

The cholesterol content in the meat of Sentul chicken aged 12 weeks ranged from 96.00-124.71 mg/100g. Cholesterol from native chickens according to Zaboli *et al*., [21] ranges from 100 mg to 120 mg/100g meat. The cholesterol content in the blood is considered safe if it does

not exceed 225 mg/dl [23]. The results of the statistical analysis showed that the addition of mangosteen peel extract with Zn and Cu supplements in the basal diet markedly reduced the meat cholesterol content. The basal diet diet without mangosteen peel extract with supplement Zn and Cu (P0) and the diet added with mangosteen peel extract and Zn and Cu at the level of 60 mg/kg (P1) did not have a significant effect on the cholesterol content of Sentul chicken meat. On the contrary, higher the xanthones contents consumed by chickens (treatments P2, P3 and P4), the lower the cholesterol content of meat. The extract of mangosteen peel contains several active compounds including alkaloids, triterpenoids, saponins, flavonoids, tannins, and polyphenols. Polyphenols that have the ability as antioxidants are xanthone compounds.

Flavonoid is one of the phytochemical groups that have the same structure, namely polyphenols, whose mechanism can reduce cholesterol levels due to HMG-CoA (*Hydroxy Methyl Glutatyil-CoA*) reductase activity, reduce the activity of the enzyme *acyl-CoA cholesterol acyltransferase (ACAT)*, and reduce cholesterol absorption in the digestive tract [24]. Apart from xanthone, mangosteen peel contains *α-mangosteen*, a pigment that can improve secretion of pancreatic lipase and α-amylase; enzymes that play a significant role in the antiobesity mechanism [25].

Alpha-mangostin which is thought to increase the activity of the lipoprotein lipase enzyme which will increase the catabolism of VLDL (*Very Low-Density Lipoprotein*) As a result the total cholesterol level decrease [25].

Conclusions

The results showed that using 120-180 mg/kg mangosteen extract with supplemented Zn and Cu significantly increased final body weight, carcass weight, dressing percentage and reduced meat cholesterol content compared to basal diet. The addition of 240 mg extract/kg diet induced decreases of final body weight, carcass weight and dressing percentage. This study concludes that the addition of mangosteen peel extract with supplemented Zn and Cu to 180 mg/kg diet resulted in optimal carcass quality.

Acknowledgments

Scientific studies have been conducted in the Grand Research Academic Leadership Project, Directorate of Research, Community Service and Innovation Padjadjaran University, Indonesia.

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Overview of Milk and Dairy Products Food Fraud on European Union Market

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Abstract

European Union is the world's leading cheese exporter and one of the world's top three suppliers for dairy exports (cheese, skimmed milk powder and packed milk). In milk and milk products industries, the adulteration has been reported almost all the time as a result of various risks associated with food safety hazards. Because of this, vulnerability reduction of dairy foods to adulteration is a high priority to everyone, from processors to consumers. In this context, our study analysed notifications made in E.U. dairy market through the Rapid Alert System for Food and Feed for the past two decades (between January 2000 and September 2019) in order to identify patterns and correlations between types of dairy products and the incidence of various hazards by dairy product category, notification type, origin of dairy products, action taken and distribution status. For the period taken into study, we identified 979 notifications, representing 1.77% from the total E.U. food notifications. The highest number of notifications was registered in 2018 (n=76, representing 2.10%) and the lowest in 2001 (n=15, representing 2.13 %). The findings of this study can be used to prioritize and target the research areas for identification of adulterants and food fraud practices in dairy products, to boost the awareness of the final customer and to promote and enhance the legislation on food safety, with emphasis on the European diary sector.

Keywords: RASFF, E.U., food fraud, dairy products

Introduction

Food fraud and adulteration in milk and dairy industry is a topic that has escalated to research and legislative involvement due to some tragic incidents in the last years [1, 2]. In this industry, the adulteration has been reported almost all the time as a result of various risks associated with food safety hazards [3]. Today, due to the growing and more complexity of modern food supply systems, this has increased the risk of food fraud to the entire global population, usually this subject being linked to high-quality or limited produced quantities of dairy products, this being a real concern to all the parties involved (regulators, food producers, retailers and consumers) [4, 5, 6, 7, 8, 9, 10]. For proper control, in E.U. member states it was implemented the Rapid Alert System for Food and Feed, created since in 1979, with the scope of sharing information between parties in cases where dangerous food or feed is detected on the market or at the boarders [11].

In a context where E.U.28 is the world's leading cheese exporter and one of the world's top three suppliers for dairy exports (cheese, skimmed milk powder and packed milk), the objective

of this present paper is based on the continuous need of being up to date in order to prioritize and target the research milk and dairy products (*MDP*) area for adulterants identification and food fraud practices in dairy products, to boost the awareness of consumer and to promote and enhance the legislation on food safety, with emphasis on the European diary sector [12, 13, 14].

Methodology

Data were retrieved from the RASFF portal [15, 16]. Search criteria for the original *RASFF notifications* with E.U. involvement was considered for *date* [time period: notified between 01/01/2000-01/09/2019], *product* [category: milk and dairy products – *MDP*] and *hazard* [category], the remaining criteria being no filtered *[all] [query from 20/09/2019, Version 1.9].*

Both two data sets were transferred to Microsoft Excel 2010 (Microsoft Corp., Redmond, USA) to create descriptive statistics, including frequency distributions (Pivot tables, with filtering). The principal filter classes for further data interpretation were: *date* (by year), *product type* (food, feed), *notification type*, *notification basis, country's role* (notification, origin, distribution), *action taken* and *risk decision*. All 979 notifications were included in creating the database cases (n=1010) by h*azard category*, some involved notifications containing more than one item, hazard or origin country. The code to generate the chord diagram (Fig. 1E) was performed using the Microsoft Power Platform. Articles in specialized journals found on the Web of Science Group, Scopus, Google Scholar and official EU databases have been used as references.

Results

In milk and milk products industries, the adulteration has been reported almost all the time as a result of various risks associated with food safety hazards. Because of this, vulnerability reduction of dairy foods to adulteration is a high priority to everyone, from processors to consumers. Recent studies regarding food fraud statistics patterns highlighted that dairy products, like milk, cream or different types of cheese and dairy ingredients are the items that are most at risk of food fraud. These trends are not largely changed, since the statistics from 2016 placed milk on the second most common adulterated ingredient, with 14% of global 1980 to 2010 records [17, 18].

Currently, *RASFF notifications* are studied from a statistical point of view and results are provided by *RASFF* through an annual report. Although the information is almost up to date, there is a deficiency of data in the studied time frame for 4 years (2000-2001 & 2015-2016), even if some statistics are available from 1999 to 2014 in relation to the total number of original notifications (n=698).

MDP RASFF notifications

In nearly 20 years' time, a total of 979 original *MDP notifications* were transmitted via *RASFF*, of which 59% were classified as alert (n=578), 21.14% (n=207) as information, 10.21% (n=100) as information for follow-up, 7.46% (n=73) as information for attention and 2.15% (n=21) as border rejection. Overall, in the last decade, MDP notifications have increased with 21.14% (60.57%, n=593) compared to the period 2000-2009 (39.43%, n=386), more than half of them being classified as serious (32.2%, n=315) from risk decision responsibility point of view. Between 2000-2009, all *MDP notifications* were categorized as undecided, with data improved over the last decade (2000-2019), these notifications declining dramatically (46.37%, n=207), while those considered not serious (7.3%, n=71), as a final answer to the decision risk category, increased.

The largest category of *MDP notifications* concerned official controls on the internal market (35.3%, n=346), usually carried out at business operators (manufacturer, wholesaler, storage, retailer), which involved an inspection and possibly also a sample taking for the purpose of analysis. Typically, three special types of *MDP notifications* were identified: company's own checks (34.8%, n=341), consumer complaints (10.8%, n=106) or food poisoning (3.8%, n=37).

In this time frame, only 5.52% (n=54) of *MDP notifications* concerned checks at the outer European Economic Area (EEA) borders at points of entry or border posts. When the consignment was not accepted for import, a border rejection notification was issued, in some cases being taken samples for analysis for final decision (detained or released). From all border controls, statistics indicates more detained dairy products (4.39%, n=43) than released (1.12%, n=11). Only a small number of *MDP notifications* were triggered by the official controls in a non-member country (0.72%, n=7) or through *RASFF* network (0.1%, n=1).

MDP RASFF notifications: origin, products affected and hazards

99% of all *original RASFF MDP notifications* studied referred to a *MDP* food type, with an output of 1009 cases, while the single case was referring to an antibiotic presence (chloramphenicol) in processed milk, being a residue of veterinary medicinal products used at animals. France and Italy were notifying countries in almost half of these notifications (France 32.77%, n=331; Italy 14.06%, n=142), followed by Germany (9.60%, n=97), all three countries accounted for 56.44% of all cases. Belgium, Netherlands, Spain, United Kingdom, Lithuania, Austria and Poland have, as a group, a share quota of 20% (with less than 5% each) of all *MDP* cases, as country of origin, while the remaining 238 cases (23.47%) came from 63 countries worldwide, each with less than 2% share quota.

Table 1 below shows the number of *MDP* cases per product type (roughly similar to what is reported in *RASFF*) between 2000 and 09.2019, while hazards were borrowed from *RASFF*.

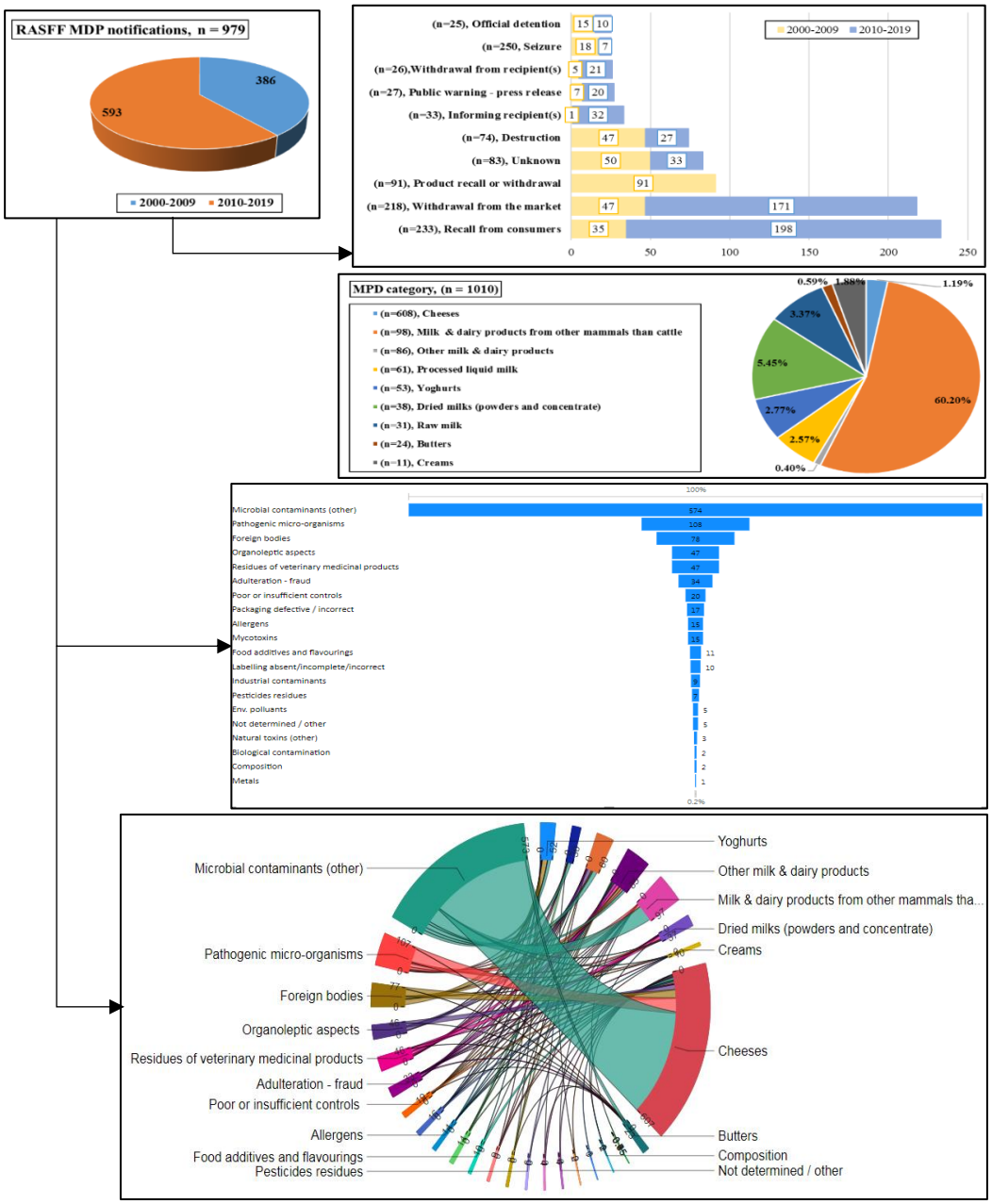
Table 1. MDP product category cases by identified hazards between 2000-09.2019

MDP types	Adulteration - fraud	Allergens	Biological contamination	Composition	Env. pollutants	Food additives and flavorings	Foreign bodies	Industrial contaminants	Labelling absent/incomplete/incorrect	Metals	Microbial contaminants (other)	Mycotoxins	Natural toxins (other)	Not determined / other	Organoleptic aspects	Packaging defective / incorrect	Pathogenic micro-organisms	Pesticides residues	Poor or insufficient controls	Residues of veterinary medicinal products	Sum
Butters	4				2		1	1			12				1		1		1	1	24
Cheeses	3	3	2	1	2	8	36		4		436	3	2	4	22	7	62	3	7	3	608
Creams	2	1					1		1		2						4				11
Dried milks (powders and concentrate)	4	2		1			2				1					1	6		4	17	38
Milk & dairy products from other mammals than cattle	1	1							2	1	68			1		1	16	4	2	1	98
Other milk & dairy products	14	4				1	12	5	1		11	3			3	5	10		3	14	86
Processed liquid milk	2				1		4	2			18	1			18	2	5		2	6	61
Raw milk	1										13	8	1				3		1	4	31
Yoghurts	3	4				2	22	1	2		13				3	1	1			1	53
Sum	34	15	2	2	5	11	78	9	10	1	574	15	3	5	47	17	108	7	20	47	1010

Most of *DMP notifications*, 2000 to 09.2019, concerned various types of cheese (60.20%, n=608), particularly soft cheese, followed by milk &dairy products from other mammals than cattle (9.70%), other milk & dairy products (8.51%), processed liquid milk (6.04%), yoghurts (5.25%), dried milks (3.76%), raw milk (3.07%), butters (2.38%) and creams (1.09%). Within the cheese category, the majority of cases were related to microbiological contamination, Fig. 1 presenting the overall image of studied cases. In the studied time frame, except cheese category, the proportion of *MDP* food categories complained every year varied, shifting the main focus between other milk and dairy products from other mammals than cattle, other milk and dairy, processed liquid milk or yoghurts (Fig. 2). Overall, more than half of products

(59.60%, n=602) remained undecided regarding final risk decision, this contributing active to the increased number of follow ups, from the last decade, to improve the situation.

It is clear from the point of view of the hazard type that microbiological contamination is the main reason, being responsible for 67.52% of all cases (10.69% due pathogenic micro-organisms & 56.83% due to other microbial contaminants). The most common food borne outbreak were cause by *spp.* as follow: *Salmonella, Bacillus cereus, Campylobacter, Clostridium* or *Escherichia coli*. Foreign bodies, such as plastic parts, glass fragments or metal wires were usually identified in the 7.72% affected *MDP* cases. Residues of veterinary medicinal products (4.65%), organoleptic aspects (4.65%) and adulteration – fraud (3.37%) complete top 5 of 20 identified hazards. In case of adulteration – fraud, the most frequent subjects were: illegal imports, absence, improper of fraudulent documents related with *MDP* food safety (health certificates), fraud of shelf life dates or tampering with needles.



From left to right and up to down: **Subfig. A** – Total number of original RASFF milk and dairy products (MDP) notifications in the period 2000-2009 vs. 2010-09.2019; **Subfig. B** –

Top 10 action taken measures for original *MDP RASFF notifications* in the period 2000-2009 vs. 2010-09.2019; **Subfig. C** – Distribution of MDP in relation with category of product (2000-09.2019); **Subfig. D** – Distribution of MDP in relation with the involved type of hazard (2000-09.2019); **Subfig. E** – *MDP RASFF* cases of notifications, by the most frequent hazard types, 2000-09.2019. Arc lengths on the inner circle are proportional to the number of cases of a product or of a hazard type.

Fig. 1. Overview of *RASFF MDP notifications* in nearly last 20 years

Product / Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Sum
Butters			1	1	2	1	3		1	3	2	2	1	1	1	1	2		1	1	24
Cheeses	16	9	19	20	30	37	20	14	29	18	63	27	28	25	45	45	43	35	43	42	608
Creams			2	1			1				1	1			1			2	2		11
Dried milks (powders and concentrate)	2		12	1	5	1	1			4		5	1	1	1	1		2		1	38
Milk & dairy products from other mammals than cattle	3	3		2	1	4	3		8	4	4	3	4	5	10	7	3	14	14	6	98
Other milk & dairy products	1		6	12	7	6	5	1	16	1	2	3	4	4	3	3	3	1	7	1	86
Processed liquid milk	2	1	4	2	1	5	2	4	7	6	4	3	4	4	2		2	4	4		61
Raw milk		1			1	1		1		2	7	1	6	1	5	2	2		1		31
Yoghurts	1	1		2	2	2	2	2	5	2	4	7	5	2		1	4	3	7	1	53
Sum	25	15	44	41	49	57	37	22	66	40	87	52	53	43	68	60	59	61	79	52	1010

Fig. 2. Percentages of *MDP* cases for product category, 2000-09.2019, per year

Remarks

Independent of the country affected, five countries in the EU28 area were the main players in releasing *MDP notifications*: France, Italy and Germany, followed by Netherlands and United Kingdom, first three having the highest gross domestic products at market prices. The connections between trade relations and nation particular differences in awareness and efficiency of own food surveillance system could be the major factors for their top ranking.

Milk and dairy products cases of notifications have been increasing in the last decade.

Listeria spp. caused the most notifications.

Cheese was the product type most notified.

The main issues regarding *MDP* food fraud adulterations are presence of different illegal chemical substances (as pesticides, residues veterinary medicinal products or substances to enhance the quality); illegal imports; absence, improper of fraudulent documents related with *MDP* food safety (health certificates), fraud of shelf life dates or biosecurity (tampering with needles).

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Incidence of Some Classes of Antibiotics in Bee Products. Sources of Contamination: Case Study on Honey and Bee Collected Pollen

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Abstract

Bee products are rich in minerals, antioxidants, and simple sugars. Honey is known to be rich in both enzymatic and non-enzymatic antioxidants. Bee-pollen is a complete natural supplement, with high and good protein and aminoacids content, lipids and fatty acids, as well as simple sugars. Beside the basic quality parameters, determination of bee products contamination is of crucial importance. Environmental contaminants in products of the hive involve heavy metals, organic pollutants, pesticides and genetically modified organisms. Also, other type of contamination may be due to the improper beekeeping practices. Major contaminants associated with beekeeping practices are acaricides and antibiotics used for the control of bee diseases. Monitoring antibiotics residues in honey and honey products helps to assess the potential risk of these products to human health. APHIS Laboratory have implemented chromatographic methods for determination of different classes of antibiotics.

Different samples of honey and bee-pollen were collected from beekeepers and subjected to tetracycline, oxytetracicline and sulphonamide determination. Positive samples were identified, representing 15% from the whole sample numbers.

Keywords: antibiotics, beekeeping practices, bee-pollen, chromatography, honey

Introduction

Bee products are supposed to be natural; man must not interfere in any way in their production, processing, storage and marketing [1]. But specialized laboratories are confronting with the presence of different type of contaminations.
The contamination sources can be divided in apicultural and environmental sources as can be seen in Table 1.

Table 1. Type of bee products contamination

A	**Apicultural contamination**
1	Antibiotics used for bee disease control (chloramphenicol, sulphonamide, streptomycin, tetracycline)
2	Acaricides (synthetic compounds or some non-toxic substances)
3	Paradichlorbenzene (used for wax moth control)
B	**Environmental contamination**
1	Heavy metals
2	Organic pollutants, polychlorinated biphenyls
3	Pesticides (insecticides, fungicides, herbicides)
4	Pathogenic bacteria

According to European Union regulations, honey and the other bee products, must be free of any contaminants, as they are natural products [2].

If environmental contamination is not solely up to human practices, the apicultural contamination is only the consequence of man practice. Beekeepers use antibiotics for fear of losing their beehives, mainly due to the presence of American Foulbrood Disease, but monitoring bee products in respect of antibiotic presence, is, consumer health protection by a better product quality and not lately the commercial competition [3].

Laboratory for Quality Control of Bee Products from USAMV Cluj-Napoca, have implemented analytical methods for determining the presence of 2 classes of antibiotics: tetracycline (tetracycline and oxytetracycline) and sulphonamides (sulphanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfamethazine), using high performance liquid chromatography (HPLC) with photodiode array and fluorescence detection [4, 5]. The importance of monitoring all types of residues in bee products helps the assessing the potential risk of these products to human health and provide data of the incidence in using pesticide treatments on field crops surrounding the hives where honey and pollen are produced.

Material and Methods

Bee Products Samples

Different type of honeys and fresh bee collected pollen, were screened for the presence of antibiotics during 2018 and 2019 in APHIS Laboratory Cluj-Napoca. Samples were collected directly from beekeepers, from profile fairs and local stores. Honey samples were kept in the dark at room temperature, pollen was kept in the freezer at -18^{o}C, and the analysis were performed in maximum one week after the collection. Thirty-eight pollen and fifty-three honey samples (black locust, amorpha, linden, rape, multifloral and honeydew declared by the producers), represented the samples for the present screening.

Equipment and Methods

The HPLC system (Shimadzu VP series, Japan), was equipped with binary LC-10AD pump, DGU-14A degasser, SLC-10A system controller, CTO-10AS column oven, SIL-10AF auto-injector and SPD-10A UV-VIS detector set at 360 nm wavelength.

The separation of tetracyclynes was performed on a Nucleosil 100 RP-18, 5 µm column, 250x4.6 mm ID with a guard column (4.6x7.5 mm ID). For sulfonamide determination the same analytical system was used, but with a fluorescence detector RF-10A XL with excitation wavelength of 405 nm and emission wavelength of 495 nm, and analytical column was a Phenomenex Luna C8 (250x4,6 mm, 5µm). As stated before, the validation was made in the lab, registering the retention time of the pure standards, realizing the equation of the concentration curve, determining the limit of detection (LOD) and limit of quantification (LOQ) and also the recovery % [4, 5].

Chemicals and Reagents

Standards of tetracycline and oxytetracycline were purchased from Sigma-Aldrich. Ultra-pure water (LC-grade), methanol and acetonitrile (LC grade, Merck KGaA Darmstadt, Germany) and absolute ethanol (reagent grade) were used.

The extraction solution was sodium succinate buffer (0.1M succinic acid (Sigma-Aldrich) solution, adjusted to right pH with 5M sodium hydroxide). Also, chelating Sepharose fast Flow resin (GE Healthcare Bio-Sciences Sweden) in 20% ethanol suspension and 10 mM copper sulphate (Chempur, Poland) solution were used for extracting the antibiotics from honey in the sample preparation step.

Reference substances of sulfanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfamethazine, sulfamerazine and sulfametoxazole were purchased from Titol Chimica, Rovigo, Italy. 2M hydrochloric acid, acetonitrile, dichloromethane, 0.1M acetic buffer solution (pH=5), C18 (500mg, 3ml, 45µm) SPE column (Biotage, 0.1M acetic buffer (pH=5), Fluram (Sigma-Aldrich), were used in sample preparation for the sulfonamide determination.

Statistical Analysis

All determinations were made in duplicate. Fortified samples were made, when positive results were obtained, for result confirmation.

Results and Discussions

The major bee diseases for which antibiotics are applied are American and European Foulbrood, caused by different bacteries and Nosema disease, caused by a microsporidian.

European Union do not allow any veterinary medicinal product containing antibiotics in beekeeping, and bee products. Anyway, at European level, a technical guide has been published by the Community Reference Laboratories [6].

The purpose of this technical guide is improving and harmonising the performance of analytical methods for substances for which MRLs have not been set. In this guide, recommended concentrations (RCs) in honey has been given for the tetracyclines (20 ppb), sulfonamides (50 ppb), streptomycin (40 ppb) and macrolides (tylosin and erythromycin, 20 ppb).

These RCs, however, have no real legal basis. Antibiotics are found in honey because are used in apiculture practice for treatment of bacterial diseases (in higher concentrations), or as "growth promotors" (in lower concentrations).

This is, however, an improper beekeeping practice, because antibiotics are forbidden to be used in the hive, due to their remanence in the bee-products [7].

The presence of antibiotics in bee products may have direct toxic effects for consumers [8], and their monitoring helps to assess the risk to human health and developing antibiotic resistance.

Contamination Level of Honey

Sulfonamine Contamination

It is known that the level of sulfonamides (sulfanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfamethazine, sulphametazine, sulfametoxazole) in honey decreases over time, if the sample is kept at room temperature [9].

But the reduction is only apparent, because glucose adducts are formed, and the antibiotic remains in the composition, but is bounded to sugars. For this reason, in the sample preparation one of the steps is hydrolysis (acidic), to ensure the complete release of bounded residues from the matrix.

Calibration curves of the standards, were performed for each sulfonamide in the range of 3-75 µg/kg. Correlation coefficient values were higher than 0.995. All compounds were in baseline separation, with a good resolution (Fig. 1).

Limits of detection were calculated using the HPLC soft. The chromatographic peaks in the samples were identified by comparing the retention data obtained for the standards and the spiked sample with the standards under same conditions and using the fluorescence detector to measure the spectrum while the mobile phase pass through the chromatographic column.

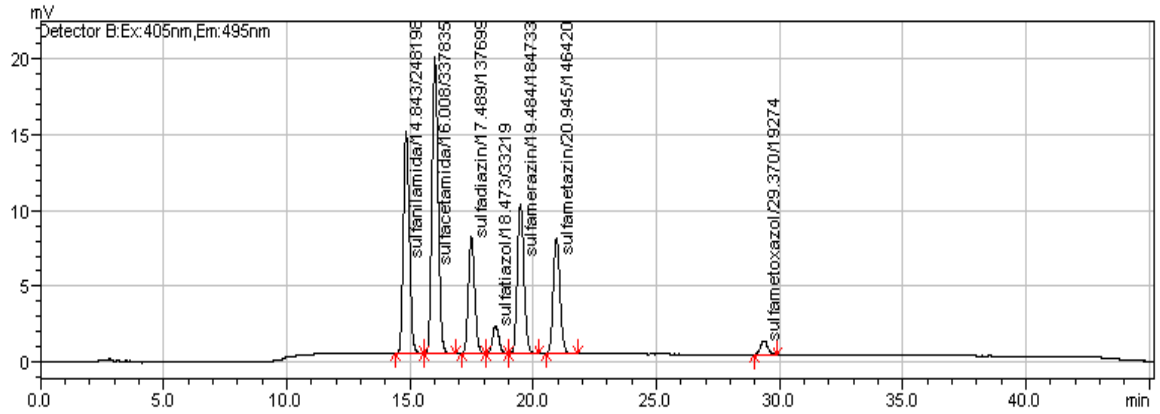


Fig. 1. HPLC-FL chromatogram of seven sulfonamide standards

Fifty-three samples of honey were screened for the presence of sulfonamides. No residues from this class of antibiotics were found present in the honey samples collected for this experiment. The method used in our laboratory, as well as the separation of the seven sulfonamides, was in accordance with the study of Posyniak *et al*. [10].

Tetracycline Contamination

In the last two years, many studies were made in our laboratory for determining the incidence of antibiotic contamination in bee products, namely honey and bee collected pollen. The same samples of honey were screened for the presence of tetracyclines (tetracycline and oxytetracycline) and 15.09% of them were found positive for oxytetracycline. No tetracycline residues were found in honey samples. Whenever is necessary, fortification of the samples which present suspicions or when checking the accuracy of the method, chromatograms are registered (Fig. 2).

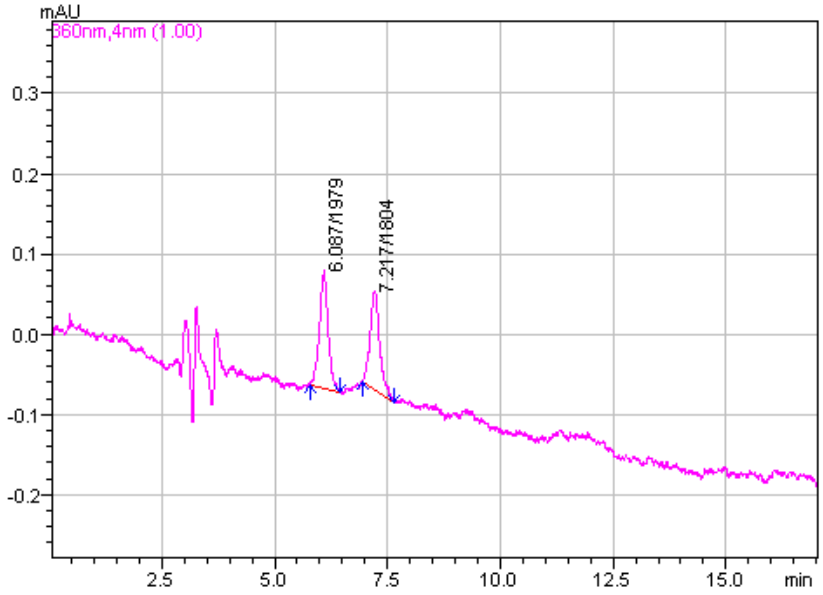


Fig. 2. HPLC-PDA chromatogram of tetracycline and oxytetracycline standards

From the 53 tested honey samples, 8 were found positive for oxytetracycline, and no tetracycline was detected in honey. The contamination level lies between 1.76 ppb and 13.1 ppb. The amount of oxytetracycline is shown in Table 2.

Table 2. Contamination level of honeys found positive for tetracyclines

Sample code	Tetracycline content (ppb)	Oxytetracycline content (ppb)
H5	-	12.99±0.01
H6	-	13.1±0.00
H8	-	1.76±0.02
H12	-	8.56±0.01
H23	-	5.82±0.01
H27	-	9.73±0.02
H28	-	8.82±0.01

Contamination Level of Beepollen

Thirty-eight samples of bee pollen (35 fresh pollen samples and 3 dried pollen samples), were collected from beekeepers and acquirers to be screened for the presence of tetracycline.

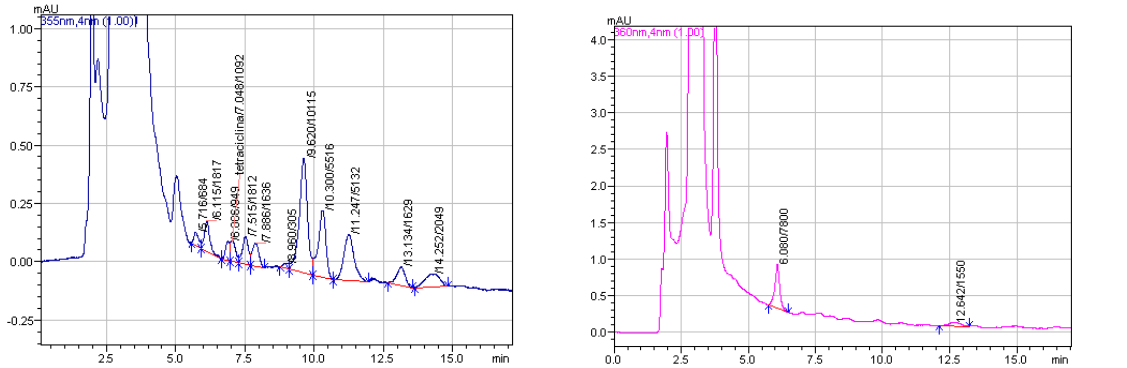
In 2018, one bee products acquirer in our area, notify our laboratory to verify a pollen sample (mixture from several beekeepers), which was found positive for tetracyclines by another laboratory. The sample presented a high concentration of oxytetracycline (66.74 ppb), and at our request, the acquirer provide us all the samples that consist the batch pollen mixture, separately. Analysing again the samples (4 distinct samples of bee pollen), three of them were found negative and one was positive, with an extremely high concentration of oxytetracycline (280.0 ppb) (Table 3).

At the end of the study, from the 38 analysed samples of bee pollen, 5 were found positive for oxytetracycline and one for tetracycline, representing 15.79% positive results from the analysed samples.

Table 3. Contamination level of bee-pollen found positive for tetracyclines

Sample code	Tetracycline content (ppb)	Oxytetracycline content (ppb)
P1	-	66.74±0.21
P5	-	280.0±0.31
P8	-	11.82±0.08
P16	-	290.59±0.84
P18	19.4±0.01	-
P32	-	53.49±0.23

Fig. 3 presents the chromatograms registered for two samples of bee pollen found positive for tetracycline (19.4 ppb) (A) and for oxytetracycline (66.74 ppb) (B).



A **B**

Fig. 3. Chromatograms registered for sample P18, found positive for tetracycline (A), and sample P1, found positive for oxytetracycline (B)

If the presence of antibiotics in honey is somehow logic (beekeeper treatment against different bee diseases, especially Fouldrood), finding tetracyclines in bee-pollen is at first sight questionable: where can be the source of this contamination?

Multiple answers were given by the beekeepers, but scientifically there is no study to confirm the theories. One study made in France [11] show the persistence of tetracycline residues in honey after treatments, indicating the persistence in the hive of the antibiotic, but also the diffusion into the apiary. This might be one of the answers for the presence on antibiotics in bee-pollen. If the bees take the antibiotic in their bodies, outside the hive, it may be mixed with flower pollen when combining with their own substances, in the formation of the pollen grain.

Other explanation may be the presence in the apiary vicinity of birds or animal farms with open air feeding with concentrated fodder and antibiotic substances. But the most credible way in which the antibiotic reached bee-pollen is that the bees collect pollen from plants that were treated with different insecticides that have in their composition these types of contaminants, if not in free form, then as metabolites belonging to different chemical classes. Such a study was made in Switzerland [12], confirming this hypothesis.

Conclusion

Although antibiotics are banned in apiculture, there still are beekeepers that use tetracycline and/or oxytetracycline in bee colony management.

Regardless the way of getting into bee products, these classes of contaminants are banned, and their presence makes the product un-usable for commercialization and human consumption.

Moreover, World Health Organization has recommended that antibiotics that are licensed in human medicine should be forbidden in treatment or as growth promoters in livestock [8].

Antibiotic residues cannot be eliminated completely from bee products, regardless of time, production and harvest techniques or storage. For this reason, proper monitoring is needed, by complying with quality assurance, certification and legislation.

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Green Synthesis of Silver Nanoparticles Using *Curcuma Longa* Plant Extract and their Possible Applications

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Abstract

Due to their wide spectrum antibacterial activity against distinct microorganisms and different mechanisms of action that may reduce the growth of resistant bacteria, silver nanoparticles may be a promising solution for the control and treatment of bacterial infections in livestock. Nanoparticle synthesis from plant extracts is tentatively offering a path for large-scale production of commercially appealing nanoparticles, such as silver nanoparticles (AgNP).

In this study we report our preliminary findings regarding the phytofabrication of silver nanoparticles (AgNPs) by *Curcuma longa*, emphasizing on their antioxidative characteristics as expressed by the hydrogen peroxide scavenging activity and the possible applications of silver green synthesized nanoparticles in the agri-food sector. The green synthesis of AgNPs was performed by mixing curcuma extract with $AgNO_3$ solution (1 mM) in a 1:9 proportion and adjusting the pH to 10, using NaOH 0.1N solution. The solution was kept under dark conditions at room temperature. The colour of the curcuma extract varied from bright yellow to pale yellow when the $AgNO_3$ was added and finally changed to dark red-brown after 24 hours, indicating complete reduction of silver ions to AgNPs. The ability of green synthesized AgNPs to scavenge hydrogen peroxide was evaluated using UV-VIS spectroscopy, by incubating the curcuma-AgNP with hydrogen peroxide. The biosynthesis of silver nanoparticles using *Curcuma longa* extract was confirmed by UV-Vis spectroscopy. The absorption spectra of AgNPs covered a wide range between 330-500 nm with a prominent peak at 428 nm, indicating the formation of AgNPs because this value is consistent with the range of the surface plasmon resonance (SPR) for AgNPs. Additionally, the spectroscopic analysis showed that the curcuma AgNP extract (500 μg/ml) has moderate hydrogen peroxide scavenging activity. Green synthesis of silver nanoparticles using *Curcuma longa* is a promising nanotechnological strategy that enables the simple, cost-effective and ecological production of silver nanoparticles that may be used afterwards for a multitude of applications, including the development of alternative antimicrobial agents by exploiting the antibacterial activity of *Curcuma longa* extracts and silver ions against pathogens, in the agri-food sector.

Keywords: green synthesis; plant extract; silver nanoparticles; antioxidant activity; animal husbandry

Introduction

Nanotechnology is the area of studies devoted to the designing, making, application and implementation on a large scale of these nanometric structures. Moreover, the use of nanoparticles, particles with at least one aspect in the range 1-100 nm and elevated surface to

volume ratios), is now widespread in areas such as medicine, industry, agriculture and pharmaceuticals [1], [2], [3], [4].

This promising and emerging technology has an enormous potential of revolutionizing the agri-food sector. Due to their physio-chemical characteristics such as size, distribution and morphology, as well as catalytic activity, optical properties, electronic, magnetic characteristics and of course antibacterial characteristics, the potential of noble metal nanoparticles, particularly silver nanoparticles (AgNPs), has been widely exploited [5], [6]. The wide spectrum antibacterial activity against distinct microorganisms and different mechanisms of action that may reduce the growth of resistant bacteria are making silver nanoparticles a promising solution for the control and treatment of bacterial infections in livestock [7], [8].

Curcumin, a plant-based product extracted from "turmeric", has been extensively used in medicine due to its non-toxicity and various therapeutic properties such as anti-oxidant, anti-inflammatory, analgesic, anti-inflammatory, anti-bacterial. Due to its high total polyphenol content, which may favorise the reduction of silver ions and synthesis of Ag NP, several protocols have been tested for the green synthesis (phytofabrication) of AgNPs from curcumin [9], [10].

In this study we report our preliminary findings regarding the phyto-fabrication of silver nanoparticles (AgNPs) by *Curcuma longa*, emphasizing on their anti-oxidative characteristics as expressed by the hydrogen peroxide scavenging activity and the possible applications of silver green synthesized nanoparticles in the agri-food sector.

Methodology

Chemical and plant extract

Pure organic turmeric powders were purchased from a local grocery store. $AgNO_3$ (99.98%) was used as a silver precursor, and was acquired from Merck (Darmstadt, Germany).

Preparations of the aqueous plant extract

The aqueous extract of turmeric powder was prepared by mixing 6.8 g of organic turmeric or curcumin powder with 100 mL distilled water. Subsequently, the mixture was boiled at 80°C for 15 minutes. After cooling at room temperature, the mixture was filtered using Whitman filter paper no.1 and centrifuged at 6000 G-force for 15 min, at 25ºC.

Phyto fabrication of silver nanoparticles

The phytofabrication of AgNPs through green synthesis was performed according to the methodology described by [10], with some modifications. Briefly, 1 ml of curcuma aqueous extract was mixed with 9 ml of AgNO3 aqueous solution (1 mM) (1:9 proportion), under moderate stirring, at room temperature. The pH of the solution was adjusted to 10, using NaOH 0.1N solution and the mixture was kept under dark conditions at room temperature until the complete synthesis of AgNPs. The colour of the curcuma extract varied from bright yellow to pale yellow when the AgNO3 was added and finally changed to dark red-brown after 24 hours, indicating complete reduction of silver ions to AgNPs.

Fig. 1. Green synthesis of silver nanoparticles (AgNps) using *Curcuma longa* extract

Hydrogen peroxide scavenging activity

The ability of green synthesized AgNPs to scavenge hydrogen peroxide was determined according to the method described by [11]. Briefly, 300 µl of synthesized AgNPs (500 µg/ml) was added to a hydrogen peroxide solution prepared in 0.1M phosphate buffer saline (pH 7.4, 40 mM and 0.6 ml). After 10 minutes, the absorbance of the control (hydrogen peroxide solution) was measured at 230nm against blank solution containing phosphate buffer without hydrogen peroxide. *Curcuma longa* aqueous extract was used for comparison. The percentage scavenging of hydrogen peroxide of AgNPs was calculated using the following formula:

$$\% \text{ scavenged } (H_2O_2) = [(Ao - A1) / Ao] \times 100$$

Ao – the absorbance of the control, A1 – Absorbance of the test sample

Results

Spectroscopic analysis

The UV-VIS spectroscopy may provide useful information regarding size, shape, and stability in aqueous suspensions of the green synthesized silver nanoparticles. Changes in colour, from pale yellow to dark red-brown are corresponding to the excitation of the surface vibration of plasmon in the metal nanoparticles generated during green synthesis, indicating the formation of Curcumin-AgNPs. The absorption spectra of AgNPs covered a wide range from 330 to 500 nm with a prominent peak at 428 nm, indicating the formation of AgNPs because this value is consistent with the range of the surface plasmon resonance (SPR) for AgNPs.

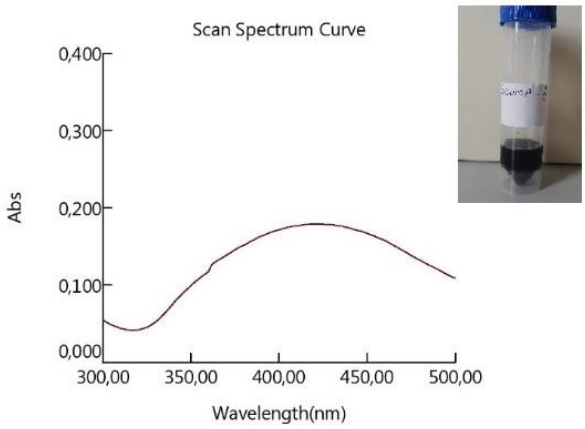

Fig. 2. Characterization of AgNPs by UV-Visible spectroscopy

Antioxidative activity

In our case, the spectroscopic analysis confirmed that green synthesized AgNPs by *Curcuma longa* presented 47.45% hydrogen peroxide scavenging activity, while the *Curcuma longa* extract presented 44.3% hydrogen peroxide scavenging activity. These results proved that the curcuma AgNP extract (500 μg/ml) has moderate hydrogen peroxide scavenging activity.

Discussions

For the synthesis of nanoparticles, chemical, physical and biological techniques have been employed, however, the chemical and physical techniques can be rather expensive because they require high cost equipment and specific conditions (high temperature and high pressure), moreover generating toxic by-products [12], [13], [14].

As the demand for nanoparticles has grown exponentially, research in the field of nanotechnology has also been reoriented in the direction of developing new methods for the synthesis of metal nanoparticles, through techniques that are environmentally friendly. Green synthesis, which is defined as the use of environmentally compatible materials such as bacteria, fungi and plants in the synthesis of nanoparticles, is a low-cost, safe and "green" approach that has been developed to synthesize stable metal nanoparticles with controlled size and shape [15].

According to some reports [16], [17], plant-based synthesis, that uses peel, leaf, root, inflorescence and fruit, is more efficient for the synthesis of stabilized nanoparticles as compared to microbe-based synthesis. Synthesis of nanoparticles from biologically derived extracts provides several benefits, such as fast synthesis, high yields and, most importantly, the absence of expensive downstream processing needed to generate the nanoparticles. As a result, nanoparticle synthesis from plant extracts is tentatively offering a path for large-scale production of commercially appealing nanoparticles.

Nanoparticles have been used for some time in the medical field as diagnostic and therapeutic tools, and more recently, their possible applications have begun to be explored in the field of veterinary medicine, animal husbandry and of course the food industry [18]. In the husbandry sector, nanoparticles have the potential to improve nutrition [19] and even replace the use of antibiotics [8]. AgNPs have been shown to have a potential wide spectrum of antibacterial activity against distinct microorganisms, their mechanism of action being effective even on antibiotic-resistant bacteria [20], [21] (Fig. 3). Silver nanoparticles presented high interest for the veterinary field as well, their biomedical applications including early clinical diagnosis, treatment, targeted anticancer drug delivery, medical device coating, wound dressings, smart bandages [22], [23], [24].

Furthermore, the increasing demand for enhanced fresh food shelf life and the need for improved foodborne illnesses protection has led to the emergence of active food packaging used in the food industry. Due to their specific proprieties (e.g., antimicrobial, anti-fungal, anti-yeast), silver nanoparticles can be combined with both edible and non-degradable polymers to create "smart" food packaging [25], [26], [27].

Nanotechnology has the potential to revolutionize the agriculture sector as well, its applications being beneficial for plant growth and disease control (Fig. 3). Metallic nanoparticles have been used for the development of environmentally friendly biocides that may be used in organic farming against phytopathogens. Silver nanoparticles are one of the most commonly used nanomaterials in the agricultural field, having multiple applications such as diagnosis, target drug delivery, pest control, virus disease, delivery system of pesticides (nano-pesticides) [28], [29].

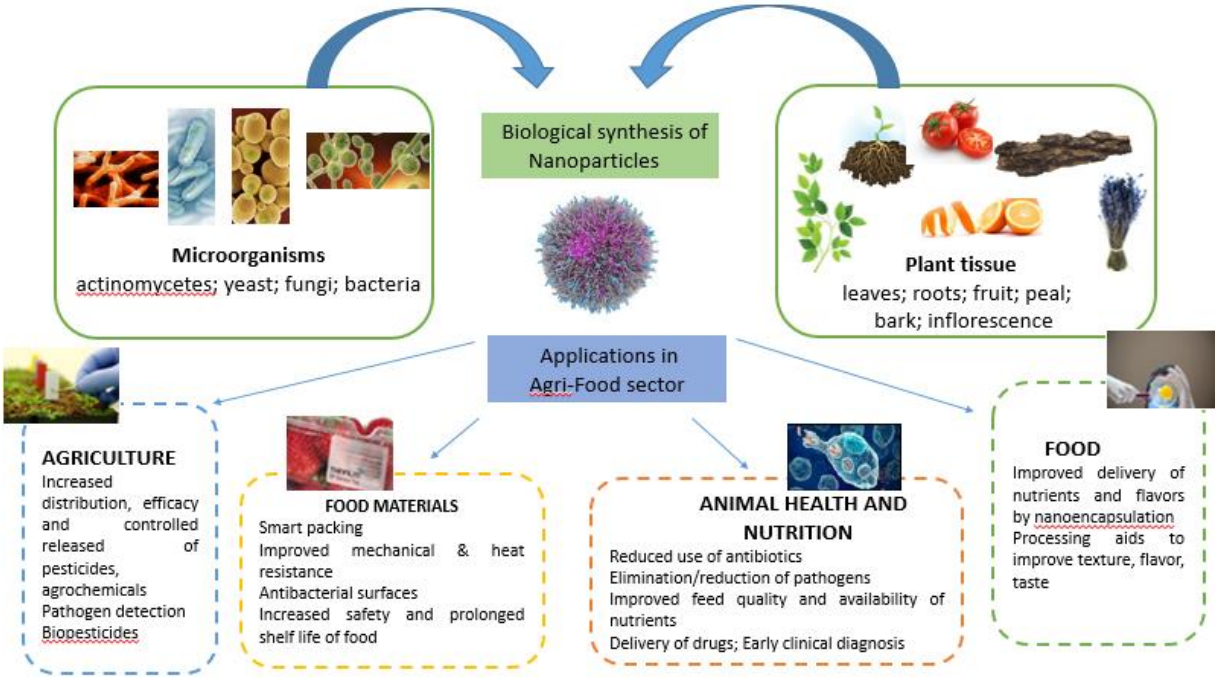

Fig. 3. Graphical presentation of biological synthesis of nanoparticles and their applications in agri-food sector

Conclusions

Green synthesis of silver nanoparticles using *Curcuma longa* is a promising nanotechnological strategy that enables the simple, cost-effective and ecological production of silver nanoparticles that may be used afterwards for a multitude of applications, including the development of alternative antimicrobial agents by exploiting the antibacterial activity of *Curcuma longa* extracts and silver ions against pathogens, in the agri-food sector.

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The Influence of the Disease State on the Maintenance Status for Rainbow Trout

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Abstract

The salmonids represent a valuable food source for humans, thanks to a high nutritional value and digestibility. In recent times, the authorities are preoccupied with ensuring the health and well-being of the consumers, by obtaining high quality, healthy and safe aquaculture products.

The aim of this paper is health status monitoring for rainbow trout (*Oncorhynchus mykiss*), during the disease caused by Yersinia rukeri, quantified through the biochemical composition of the meat. Yersiniosis is an illness that leads to significant economic losses in aquaculture industry.

The biological material – rainbow trout *Oncorhynchus mykiss* – was monitored monthly for 7 months. The biochemical analysis of trout meat was realized at the moment of identification of pathogenic bacterium Yersinia rukeri through microbiological methods, after 15 days following a 10-day treatment with antibiotics. The experiment demonstrated that the disease state compromises growth performance and nutritional value of the infected biological material. However, the results indicated that after a 15-day period following the treatment, the maintenance status of rainbow trout will get better, without recovering the weight loss determined by the disease.

Keywords: rainbow trout, yersiniosis

Introduction

In Romania, there are adequate conditions for salmonids growth and development, the second important branch of pisciculture.

Salmonids represent a very valuable food source for humans, thanks to its high content of proteins, high biological value and high digestibility.

In recent times, the authorities are preoccupied with ensuring the health and well-being of the consumers, by obtaining high quality, healthy and safe aquaculture products.

The monitored species was rainbow trout (*Oncorhynchus mykiss*), a freshwater predatory fish, belonging to the *Salmonidae* family, reared in the trout farm situated in NE Romania, in Bistrița drainage basin, supplied with water from this basin.

Fish brood necessary for basin population was purchased from trout farms equipped with incubation station. The fingerlings were reared in earthen basins with concrete margins. Feeding diets had a protein content between 45% and 65% and a lipid content between 12% and 15%, depending on the trout weight.

The purpose of this paper is the monitoring of rainbow trout (*Oncorhynchus mykiss*) condition status, quantified by meat biochemical composition, in the case of a disease caused

by *Yersinia rukeri* bacterium, which has a devastating effect economically and concerning the animal welfare [1].

Methodology

The biological material – rainbow trout *Oncorhyncus mykiss* – was monitored monthly for a 7 moths' period (April-October 2018). Water samples were collected every month. Water sample analysis was conducted according to work protocols indicated in standard analysis methods for surface waters [2].

pH determination was conducted according to SR ISO 10523:1997 standards, with a lab pH Meter – INO Lab pH 720 – with temperature probe.

Chemical Oxygen Demand was determined according to SR ISO 6060:1996.

Nitrogen and phosphorus compounds were determined according to standard methods for analysing water and residual waters/2005, using a DR 2800 spectrophotometer, equipped with the water quality kit HACH-LANGE.

To determine the rest of parameters that are used for establishing the water quality from a chemical point of view, the work protocols indicated in the current standard analysis methods for surface waters and the methods from specialized literature were respected.

Data interpretation was conducted according to Normative provisions regarding the classification of surface water quality in order to establish the ecological status of water bodies (Ord. MMGA no. 161/2006) [3] correlated with specialized literature data for waters used in fish farms.

Biochemical analysis of fish meat was conducted at the start of the disease, on healthy and sick specimens, after a 10 days treatment with antibiotic and after 14 and 21 days from the completion of the treatment.

Meat samples analysis was performed using the procedures indicated by standard analysis methods for fish meat.

The moisture was determined by Standard Official Methods of the AOAC (1990).

The total ash was determined by Furnace Incineration described by AOAC (1990).

The crude proteins content of the samples was determined using the Kjeldahl method of AOAC 17th edition, 2000, Official Method 928.08 Nitrogen in Meat (Alternative II), which involved protein digestion and distillation, where F (conversion factor), is equivalent to 6.25.

The total fats were determined using the Soxhlet method, equipped with Gerhardt Brand Multistate Controller, with modified ether extraction methods AOAC 960.39.

Statistical analysis

All analyses were carried out in triplicate. Statistical analysis was carried out using Microsoft Excel. The average values are compared with the standard deviations. The statistical interpretation of the considered data shows a variation within the allowable threshold of $P<0.05$.

Results and Discussions

Physicochemical analysis of water

One of the determining factors for salmonids growth is the presence of an adequate quality and quantity of water.

The physicochemical water proprieties have a significant influence on the fish growth parameters, fish health and fish meat quality, in the frame of food security.

The analysis of water physicochemical parameters, monitored monthly, reveal favourable values for comfortable trout growth during the entire experiment (Table 1).

Table 1. Values of the water physicochemical parameters in the water source and in the monitored trout rearing basin

Analysed parameters	***U.M.***	***Water source Average±SD****	***Rearing basin water Average±SD****	***Values of the physicochemical parameters of water for salmonids (Bud. I., 2007)***	
				Average	***Limits***
Temperature	^{0}C	9.36±2.25	10.2±3.15	10	4-20
Dissolved oxygen	mg/l	9.45±1.55	9.55±2.35	11	3-20
Ph	uPh	7.24±1.40	7.6±1.85	7.5	7-8
Total hardness	dGH	9.7±1.90	9.8±1.60	10	8-16
Nitrites, (N-NO_2)	mg/l	0.03±0.002	0.045±0,002	< 1	-
Nitrates, (N-NO_3)	mg/l	0.11±0.015	0,18±0.03	<0.5	-
Ammonia	mg/l	0.005±0.005	0.012±0.005	<0.5	-
Alkalinity	ml	1.95±0.5	2.2±0.4	-	-
Bicarbonates (HCO_3^-)	mg/l	122.2±15.0	144.3±18,1	-	-
Carbonates (CO_3^{2-})	mg/l	0	0	-	-
Calcium (Ca^{2+})	mg/l	24.6±2.62	20.50±2.25	-	-
Magnesium (Mg^{2+})	mg/l	0.50±1.4	0.73±2.21	0.8	0.6-1.0
Ca^{2+}/Mg^{2+}		9.84±2.01	4.33±2.23	-	-
Phosphate (PO_4^{3+})	mg/l	0.14±0.001	0.17±0.001	< 0.2	-
Chloride (Cl^-)	mg/l	0.53±0.02	0.82±0.035	<5.0	-

* *Standard deviation*

The water had a minimum concentration of 7.75 mg/l oxygen and a neutral pH during the entire experiment. The nitrogen compounds had values between the accepted limits, according to Ord. no. 121/2006 regarding surface water classification and according to Bud. I., 2007 [4].

The fodder was dosed correctly, the metabolism was almost complete, without expelling residue or toxins in water.

The supply of trout basins with water provided a complete replacement of water several times a day, water temperature reaching a minimum of 7.11^{0}C and a maximum of 13.35^{0}C.

Monitoring water quality is important because it allows trout farmers to take immediate decisions regarding corrective actions.

Analysis of the biologic material involved in the experiment

Yersiniosis, the disease known as „salmonid's enteric red mouth", is a septicemic bacteriosis, specific to salmonids. Rainbow trout (*Oncorhynchus mykiss Walbaum, 1792*) is a species susceptible to infection [5]. The disease first appeared in May. Fist symptoms were the separation of sick fish from the school and lethargy with the following clinical manifestation: swelling of the abdomen (stomach and intestines filled with gas and liquid), darkening of the tegument, modifications of the eye blood flow, gills, intestinal and peritoneal haemorrhage, oral cavity with haemorrhagic appearance. The fish with signs of disease were isolated in a quarantine basin. Following the identification of the pathogen bacterium *Y. ruckeri* through a bacteriological exam, the treatment was determined according to the antibiogram with the drug ENRODEM 50 (active component: enrofloxacin) for 10 days, administered in food.

The anatomoponderal and biochemical analysis of trout meat was performed on the healthy specimens and on those that were in the acute stage of disease, after 10 days of antibiotic treatment and after 14 and 21 days from the completion of the treatment.

Anatomoponderal analysis of biological material involved in the experiment

Rainbow trout (*Oncorhynchus mykiss*) from trout farms presents superior technological proprieties compared to other species [6]. The indices regarding meat percentage from healthy specimens, sick specimens, after 10 days of antibiotic treatment and at the completion of the treatment, are presented in table 2.

Table 2. Anatomoponderal parameters evolution for rainbow trout (*Oncorhynchus mykiss*) infected with *Yersinia* before and after treatment

Weight, g ***Average±SD****	***Head (g%) Average±SD****	***Torso (g%) Average±SD****	***Meat (g%) Average±SD****	***Scales (g%) Average±SD****	***Fins (g%) Average±SD****	***Skin (g%) Average±SD****	***Bones (g%) Average±SD****	***Organs (g%) Average±SD****
T_1HF – Healthy Fish (3 samples)								
122.4±3.57	14.13±0.25	62.45±2.10	56.63±0.16	1.44±0.16	1.23±0.03	9.74±0.15	4.88±0.07	11.95±0.12
T_1SF – Sick Fish (20 samples)								
138.81±4.15	19.97±0.56	61.12±1.75	46.29±1.12	0.76±0.15	1.51±0.02	7.11±0.35	7.72±0.075	16.64±0.15
T_2 SF – after 10 days of treatment (3 samples)								
120.70±3.40	21.33±1.02	61.71±1.65	49.11±2.01	1.08±0.09	1.39±0.20	8.42±0.45	4.18±0.24	14.49±1.22
T_3SF – 7 days after the completion of the treatment (3 samples)								
141.10±3.25	19.42±0.90	60.24±1.50	49.47±1.85	1.06±0.10	1.20±0.05	6.95±0.75	3.83±0.33	18.07±1.55
T_4SF – 14 days after the completion of the treatment (3 samples)								
158.00±3.22	16.44±1.15	65.15±1.45	51.93±1.55	1.15±0.075	1.34±0.08	7.79±0.54	5.05±0.25	16.30±2.15
T_5SF – 21 days after the completion of the treatment (3 samples)								
137.70±4.31	14.52±0.25	63.54±1.35	52.29±1.35	1.16±0.2	1.60±0.075	5.81±0.37	5.45±0.15	19.17±1.95
T_5HF – Healthy Fish (3 samples)								
174.10±5.80	14.2±0.45	70.71±0.96	60.35±0,55	1.55±0.3	1.6±0.22	8.1±1.66	4.18±0.30	10.02±1.64

** Standard deviation*

Meat percentage registered a minimum of 46.29±1.12 g% in the acute stage of disease, with 10,34 g% less than fish with no symptom of disease. Biomass accumulation stagnated for 17 days since the start of the disease, which also included the treatment period. After three weeks the completion of treatment, the biological material from quarantine presented a 2,5 g% higher meat percentage, without recuperating the 8,06 g% difference created between sick specimens and healthy ones. The values of meat indices for trout with no symptoms (56,63±0.16 g% – 60.35±0,55 g%) confirm that these fish have an adequate body development, are healthy and have a high meat percentage in accordance with the results obtained by Souza *et al*., 2015 [7] and Skałecki P. *et al*., 2013 [8].

The biochemical composition of biological material involved in the experiment

The biochemical analysis results for rainbow trout meat can be found in table 3.

The biochemical composition modifications were analysed in relation to the acute stage of disease, at the completion of treatment and post treatment.

Table 3. The biochemical composition and caloric value of rainbow trout meat

Weight, g ***Average±SD****	**Moisture, g %** ***Average±SD****	**Ash, g %** ***Average±SD****	**Protein, g %** ***Average±SD****	**Fats, g %** ***Average±SD****	***M/P***	**Energy value** kcal/100g**
T_1HF – Healthy Fish (3 samples)						
122.4±3.57	77.20±2.15	1.25±0.35	17.25±0.85	4.30±0.55	4.48	110.72
T_1SF – Sick Fish (20 samples)						
138.81±4.15	79.69±1.99	1.47±0.40	15.38±0.15	3.46±0.15	5.18	95.24
T_2SF - after 10 days of treatment (3 samples)						
120.70±3.40	77.96±1.85	1.50±0.20	14.78±0.65	3.76±0.32	5.25	95.57
T_3SF – 7 days after the completion of the treatment (3 samples)						
141.10±3.25	80.05±2.11	1.50±0.33	14.85±0.75	3.60±0.12	5.39	94.37
T_4SF – 14 days after the completion of the treatment (3 samples)						
158.00±3.22	80.18±2.54	1.61±0.40	15.14±1.15	3.07±0.60	5.30	90.63
T_5SF – 21 days after the completion of the treatment (3 samples)						
137.70±4.31	79.36±2.63	1.79±0.65	15.26±0.11	3.59±0.22	5.20	95.95
T_5HF – Healthy Fish (3 samples)						
174.10±5.80	76.65±1.95	1.25±0.55	17.85±1.11	4.30±0.20	4.29	113.18

** Standard deviation*

*** Calories conversion factors used; for proteins 4.1 kcal/g; for lipids 9.3 kcal/g*

The results for the samples harvested at the T1 moment from healthy specimens and those with first signs of disease, shows differences in protein content between healthy and sick specimens, because until the first clinical signs of disease the body was vulnerable, determining a decrease of meat nutritional value. The biochemical composition for healthy trout in similar to that obtained by R. Koshinsk *et al*.,2018 [9], but lower that the values for sick trout.

During the treatment and one week after treatment completion, the protein values (at T1-15.38±0.15 g% and at T2-14.78±0.65 g%) and lipid values (at T1-3.46±0.15 g% and at T2-3.76±0.32 g%) remain decreased. Protein concentration is 1.46 g% lower and lipid concentration is 3.17 g% lower compared to the values obtained by Ihuț A. *et al*., 2018 [10] for rainbow trout.

After 21 days from treatment completion, the energy value for trout meat (95,95 kcal/100g) was higher because of protein and lipid accumulation, but was lower than the energy value for the specimens with no signs of disease (113.18 kcal/100g) that did not receive antibiotic treatment.

Biochemical composition for trout meat after 21 days from treatment completion is similar to the biochemical composition obtained by Crețu M. *et al*., 2014 [11], in low density conditions.

Conclusions

- Rainbow trout *(Oncorhynchus mykiss)* infected with *Yersinia ruckeri* bacterium and treated with a drug premix, regained its technological value and can be commercialised after at least 3 weeks after the completion of the treatment.
- Biochemical analysis of trout meat highlighted the significant negative impact on protein and lipid content, mentioning that the nutritional value of infected and treated trout is 17,23 kcal/100g lower compared to the healthy trout.
- Awareness and compliance with retention time for administered drugs and are required for the restoration of farmed trout meat quality.
- Prophylactic measures and a strict management of the technological flow in salmonids culture are necessary in order to reduce the risks, diminish losses and ensuring the animals health and welfare, food safety and security.

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The Effect of Probiotic Supplementation on Meat Quality and Feed Efficiency

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Abstract

Probiotics is microorganism reported possessing the positive effect on gut morphology and subsequent performance of poultry birds. Therefore, the present study was carried out to evaluate the effect of feed efficiency and meat quality.

One hundred individuals of day-old commercial broiler chicks were allocated to 4 treatment groups in a complete randomized design (CRD) and each treatment was replicated 5 times and each replicate was filled 4 chickens.

The treatments consisted of fed: T-0 (basal ratio), T-1 (basal ratio with 100% fermented cow milk), T-2 (basal ratio with 50% fermented cow milk + 50 % fermented soy milk), and T-3 (basal feed with 50% fermented cow milk + 25% fermented soy milk + 25% fermented mung bean). There was no significant ($P>0.05$) effect on feed efficiency. However, it can improve feed efficiency.

Meanwhile, all treatments give significant ($P<0.05$) effect on meat quality. Probiotic supplementation can improve feed efficiency and meat quality on poultry.

Keywords: probiotic, feed efficiency, meat quality, broiler

Introduction

Probiotics contain lactic acid-producing bacteria, that serve to improve the digestive and nutrient absorption processes. Probiotics can increase the activity of enzymes such as sucrose, lactose, and tripeptidase in the small intestines.

Providing probiotics from the starter period has been assumed to adapt the broilers to probiotic microorganism and help them to improve the balance of intestinal microflora. Probiotics can increase the activity of digestive enzymes so that the absorption of nutrients being optimized in line with the increasing area of absorption as probiotics can influence the intestinal anatomy like increased density and size of small intestine villi, and intestine histology [1], [2].

In this study, Fermented milk uses probiotic such as *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidhophilus, and Bifidobacteria*. Soy is a group of oligosaccharides consisting of sucrose, stakiosa, and raffinose, which are hard to digest and to absorb in the intestine, and it helps within acting as a growth substrate for useful bacteria in the intestine. Lactic acid bacteria in fermented soy milk have a role in improving the isoflavone digestibility [3].

Isoflavones are secondary metabolite compounds that are widely synthesized by plants. In the soybean, isoflavone content ranges from 2 to 4 mg/g [4]. Various types of isoflavones are genistein, daidzin, and glisitin. Antioxidants have been reported to have an effect to improve intestinal villi, which in turn have an impact on increased absorption.

Probiotic can improve the structures of intestinal villi in the process of nutrient absorption.

Antibacterial herbs are able to suppress the growth of pathogenic bacteria in the intestine [5].

Fermented milk or soy contain flavonoid and vitamin, which can act as antioxidant, leading to the occurrence of a free radical attack on the cell membrane. Free radicals are an atom, a molecule, or a compound in which it contains one or more unpaired electrons, making it highly reactive [6].

Such radicals cause metabolic disturbances and cell disorders in the form of impaired DNA and protein function, causing mutations or cytotoxic and enzyme activity changes [7].

This can cause metabolic disorders in the body and suppress the growth of chickens.

Chickens consume most are to meet requirement of the protein and energy needs in ration.

Protein content in the ration is very influential on the achievement of chicken body weight, is required for tissue growth, tissue repair, and management of production and part of the enzyme structure, so that protein is known as one of the principal constituents of body cells and tissues [8].

Several studies have shown the benefit of probiotics on gut morphology and performance which suggest that by dietary means, it is possible to positively affect the development of the gut and provide the competitive advantage in favour of beneficial bacteria which can alter not only gut dynamics, but also many physiologic processes.

Methodology

Research Object

In this research used 100 head of broilers, divided into 4 groups and each group was repeated 5 times. Each cage contains 5 chickens which maintained until the age of 10 weeks. The coefficient value of the variation of initial body weight of chicken equal to 9.47%.

Trial and Ratio

One hundred individuals of day-old chicks were divided in to 4 treatment groups into a complete randomized design (CRD), and every treatment was replicated 5 times and filled 4 chickens.

1) T-0 = Basal Diet
2) T-1 = Basal ratio with 100% Fermented Cow Milk
3) T-2 = Basal ratio with 50% fermented cow milk + 50 % fermented soy milk
4) T-3 = Basal feed with 50% fermented cow milk + 25% fermented soy milk + 25% fermented mung bean

Experimental Design

Experiments were conducted experimentally design using Completely Randomized Design, consisting of 4 treatments and 5 replications.

Data were analysed using Varian Analysis and difference between treatments using Duncan Multiple Range Test.

Results

The Effect of Probiotic Supplementation on Meat Quality

The effect of probiotic supplementation on empty carcass, abdominal fat, and body weight gain can be seen in the Table 1.

Table 1. is shown the data regarding the effect of probiotic on carcass quality

Parameter	T-0	T-1	T-2	T-3
Empty Carcass	921.4[a]	915.1[a]	991.9[b]	905.8[a]
Abdominal Fat	22.5[bc]	21.7[c]	17.5[a]	18.35[ab]
Body Weight Gain	1241.6[a]	1242.3[a]	1294.3[b]	1238.3[a]

Note: T-0 (Basal Feed)
T-1 (Basal Feed with 100% Fermented Milk)
T-2 (Basal Feed with 50% Fermented Cow Milk + 50 % Fermented Soy Milk)
T-3 (Basal Feed with 50% Fermented Cow Milk + 25% Fermented Soy Milk + 25% Fermented Mung Bean)

Based on the ANOVA test, the use of probiotic gives a significant result ($P<0.05$) on all parameters. Then, T-2 the combination of Basal Feed with 50% Fermented Cow Milk + 50% Fermented Soy Milk has the best effect in increasing Empty Carcass and Body Weight Gain and decreasing Abdominal Fat.

The primary purpose of the supplementation of probiotics in poultry feed is to keep and improve the performance of the broilers [9], [10], and also to prevent and control pathogenic bacteria. The research on probiotic supplementation in poultry industry gives the effective result on the growth performance of broiler and their carcass [11], [12]. It could be related to the alteration of carcass quality. The supplementation of probiotics in the diet allows rapid development of beneficial bacteria in the digestive tract of the host and improving its performance.

T-2 is the treatment, which gives the best result in improving empty carcass and body weight gain if compared with the other treatments. These improvements might be as a result of the cumulative impact of probiotic's action in the combination of fermented cow milk and fermented soy milk such as, increased digestive enzyme ability, maintenance of beneficial non-pathogenic bacteria, and neutralized the effect of feed toxins in the gut environment to improve digestion and nutrient absorption [13], [14], [15]. Those findings are in line with the prior study of [16], [17] which have reported that *Lactobacillus* and *Saccharomyces* can increase the higher body weight and better carcass.

The combination of fermented cow milk and fermented soy milk also can decrease the fat level in carcass because the lactic acid produced by probiotics in fermented milk can make the low pH in the digestive tract and inhibit the growth of pathogenic bacteria. Then, it also makes the energy from the carbohydrate is converted into lactic acid so that the fat formation in carcass will decrease. Besides, Fermented soy milk can generate isoflavone, which is an active substance and has the function as a biological agent. Then, probiotics generate β-glucosidase enzyme, which can hydrolyse isoflavone to be free isoflavone compound called aglycone.

Aglycone has higher activity in lowering total cholesterol.

The fermentation process can also hydrolyse aglycone component to be glycoside, which shows the high antioxidant activity.

Another compound found in fermented soy milk is flavonoid. Flavonoid can inhibit the activity of the enzyme 3-hydroxy-3 methyglutaryl CoA, which plays a role in inhibiting cholesterol synthesis and acetyl CoA so that it can decrease the esterification of cholesterol in the intestine and live.

The Effect of Probiotic Supplementation on Feed Efficiency

The data regarding the effect of probiotic supplementation on feed efficiency is showed on the table 2 below.

Table 2. The effect of Probiotic Supplementation on Feed Efficiency

Parameter	*T-0*	*T-1*	*T-2*	*T-3*
Feed Consumption	*1858.52[a]*	*1799.69[a]*	*1893.01[a]*	*1822. 13[a]*
Feed-Conversion Ratio (FCR)	*1.42[a]*	*1.39[a]*	*1.38[a]*	*1.41[a]*

Note: T-0 (Basal Feed)
T-1 (Basal Feed with 100% Fermented Milk)
T-2 (Basal Feed with 50% Fermented Cow Milk + 50 % Fermented Soy Milk)
T-3 (Basal Feed with 50% Fermented Cow Milk + 25% Fermented Soy Milk + 25% Fermented Mung Bean)

ANOVA test shows that the probiotic supplementation is no significant difference (P>0.05) either on feed consumption or on feed conversion ratio. However, there is a tendency in T-2 to improve the feed consumption and FCR because T-2 has the best value in increasing feed consumption and has the lowest FCR.

The supplementation probiotic in T-2 can increase feed efficiency, feed conversion, and better quality of broiler meat [18]. Then, Wiseman [19] and Mudalgi [20] have reported that broilers feed supplemented with probiotic shows the improvement feed intake than control. The use of probiotics in feed has a beneficial effect on body weight gain of broiler from 4th to 6th week of age. Other studies have also reported that supplementation of probiotics in poultry feed generates a significant improvement in 42 days of body weight and feed conversion. Fermented milk contains Lactic Acid Bacteria such as Lactobacilli. The supplementation of Lactobacilli in feed can stimulate the favourable microbial balance in intestine and consequently improve feed efficiency and growth performance. It will increase weight gain and feed efficiency when compared to control [21].

Conclusion

The usage of 100% fermented cow milk (T-1) and combination 50% fermented cow milk + 25% fermented soy milk + 25% fermented mung bean (T-3), it does not give significantly different results on empty carcass and body weight gain compared to control. However, the giving of combination fermented cow milk (50%) + fermented soy milk (50%) (T-2) gives a significantly different result in decreasing abdominal fat.

All of the treatments do not give significantly different result on feed consumption ratio (FCR) and feed Consumption. But there is tendency improvement at the giving of combination fermented cow milk (50%) + fermented soy milk (50%) (T-2).

Acknowledgment

The authors would like to say thank to Norman Billi, who is very helpful in finishing this paper and to Academic Leadership Grant (ALG) project, Universitas Padjadjaran, Bandung for funding this research.

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Study on the Slaughter Results According to Sex and Age of Slaughter in Quails from Brown Jumbo Meat Population

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Abstract

The research was carried out on a number of 300 meat quails from the brown Jumbo population; sacrifices were made at the age of 42 days, 49 and 56 days separately by sex.

Following the research, the average live weight at 42 days was of 290.60±8.38 g/bird head in females and 245.00±7.90 g/head in males, at 49 days it was 326.00±11.41 g/bird in females and 256.80±6.14 g/bird in males, while at 56 days this was 332.80±8.51 g/bird in females and 269.60±4.18 g/head in males, the differences between sexes being very significant. The yield of the eviscerated carcass at 42 days was 70.45±1.46% in females and 70.98±1.89% in males, at 49 days it was 67.19±1.02% in females and 71.14±0.87% in males, and in 56 days this was 71.56±0.68% in females and 70.05±0.25% in males.

From the point of view of the evolution of the average daily weight gain during the period 1-56 days, as well as the weight of the carcass and its component parts, conclusions can be drawn regarding the optimal age of slaughter of Jumbo quails.

This is of 42 days in case of slaughtering both sexes, 42 or 49 days in case of females slaughtering and 42 days of males.

Keywords: quail, meat, slaughter, yield, age

Introduction

The rearing of quails in Romania has undergone a continuous development lately, starting from rearing as an activity related to other basic activities or as a hobby 30 years ago, to a specialized rearing with hundreds of thousands of eggs and thousands tons of meat annually and with investments of even millions of euros through European funds in equipment and slaughterhouses. In these conditions, however, the need arises for careful study of reproductive material and environmental factors before their farming in an organized and commercial way [9].

Thus, they have been brought to different breeding meat quails from other populations, without having been tested before and without designing a basic breeding technology for these birds, they being generally reared as mixed Japanese quails (used breeding density, mixed feed recipes, slaughter age etc. being the same as in mixed quails). The characteristics of the carcass and the chemical composition of the quail meat are influenced by many factors, including the genotype of birds [6], the feeding mode [2], [4] and the age of slaughter [3].

The purpose of this paper was to establish the results for slaughter separately for the two sexes, but also an optimal age for slaughtering quails from the brown Jumbo meat population.

Methodology

The research was carried out on a number of 300 quails from the Jumbo meat population (Fig. 1), sacrificing 50 males and 50 females at the age of 42 days, 49 days and 56 days. The researches took place within the quail farm Ioniță T. Lucian Individual Enterprise in Gherghița Commune, Jud. Prahova, Romania. The environmental conditions in which the researches were carried out were within the limits provided by the specialized literature [5, 11].

During the experiment, two compound feed recipes were fed namely quail starter (1-3 weeks) and quail grower (4-8 weeks).

The quail starter compound feed (c.f.) recipe had the following nutritional value: 3010 Kcal metabolizable energy/kg c.f., 24.80% crude protein, 5.10% crude fat, 0.59% methionine, 0.97% methionine + cystine, 1.58% lysine, 0.96 % calcium and 0.78% phosphorus, 0.03% choline, 0.40% salt.

The compound feed specific for the second phase of youth quail growth had the following calculated nutritional values: 3140 Kcal metabolizable energy/kg c.f., 22.50% crude protein, 6.10% crude fat, 0.64% methionine, 0.98% methionine + cystine, 1.33% lysine, 0.96% calcium and 0.75% phosphorus, 0.03% choline, 0.40% salt. [13].

Fig. 1. Female (left) and male (right) quail in brown Jumbo meat population

The live weight was determined for each bird separately, and then the carcasses were marked, determining the weight of the bleeding carcass, the plucked carcass and the eviscerated carcass.

Then the average data for both the male and female carcasses were calculated. The same has been done for the component parts of the carcass (breast, legs, wings, back). Finally, the proportions were established for the different components of the carcass. As for establishing the meanings of differences between the averages, it is a Student test [10], and data processing was done using Microsoft Excel 2007.

Results

The results are presented in the tables 1, 2, 3, 4 and Fig. 2.

Dynamics of live weight in Jumbo Quail meat population, throughout age span 1-56 days

If at the age of 1 day the weight of the chickens was 9.47±0.07 g/head (Table 1) (both genders), since the sexing was not performed at the age of 1 day, at the age of 42 days the weight of the chickens was 290.60±8.38 g in females, very significantly higher (t=3.960) with 15.70%, compared to the one of males (245.00±7.90 g/head) (Table 1).

Table 1. Average live weight at 1 day and 42, 49 and 56 days in female and male quails (g)

Age	Females	Males
1 day	9.47±0.07	
42 days	290.60±8.38***	245.00±7.90***
49 days	326.00±11.84***	256.80±6.14***
56 days	332.80±8.51***	269.20±4.18***
Statistical significance Student values	t calculated <1.982 – insignificant differences- ns t calculated = 1.982 - 2.871 – significant differences * (t0.05 = 1,982) t calculated = 2.871 – 3.390 – distinct significant differences ** (t0.010 = 2.871) t calculated >t 0.001 = 3.390 – very significant differences *** (t0.001 = 3.390)	

*Note. Between the values noted *** the differences are very significant at the same age*

At 49 days of age, the weight in females (326.00±11.84 g/head) was 21.23% very significantly higher (t=5.313) (compared to that of males (256.80±6.14 g/head). At 56 days the weight in females (332.80±8.51 g/head) it was 19.11% very significantly higher (t=6.701) compared to the weight of males (269.20±4.18 g/head).

Dynamics of average daily weight gain in Jumbo quail meat population, throughout age span 1-56 days

Depending on the average daily weight gain, which decreases after 6 weeks (Table 2), the optimal age of slaughter of Jumbo meat quail seems to be 42 days for both genders, 42 or 49 days for females (up to 7 weeks the weight gain decreases by only 3.44 % compared to 6 weeks) and 42 days for males (up to 7 weeks the weight gain decreases almost 3 times, with 9.98 %) (Table 2).

Table 2. Average daily weight gain from 1 day and up to 42, 49 and 56 days in female and male quails

Trait and age	Females		Males		Population mean	
	g	%	g	%	g	%
Average daily gain 1-42 days	6.69	100	5.61	100	6.15	100
Average daily gain 1-49 days	6.46	96.56	5.05	90.02	5.75	93.49
Average daily gain 1-56 days	5.77	86.25	4.64	82.71	5.20	84.55

Slaughter performances of Jumbo quail meat population at the age 42, 49 and 56 days

The weight of the eviscerated carcass (Tab. 3, Fig. 2) in the case of females (205.00±8.82 g/head) was at 42 days old, 15.22% higher compared to that of males (173.80±6.37 g/head), the differences being very significant (t=3.423). At 49 days of age, in females (219.20±9.31 g/head) it was 16.70% higher than in males (182.60±4.18 g/head) (t=3.585), while at 56 days the average weight of the eviscerated carcass it was 20.75% higher in females (238.00 4.79 g/head) compared to that of males (188.60±3.19 g/head), the differences between the sexes being very significant (t=8.591) at all ages of the experiment.

Table 3. Average slaughter results at 42, 49 and 56 days in Jumbo meat quails

Trait	Slaughter results 42 days		Slaughter results 49 days		Slaughter results 56 days	
	Female	Male	Female	Male	Female	Male
ALW (g)	290.60±8.38***	245.00± 7.90***	326.00±11.41***	256.80±6.14***	332.80±8.51***	269.60±4.18***
CBW (g)	279.80±8.14***	235.80±8.41***	309.00±11.00***	249.00±6.02***	318.60±7.86***	260.20±3.77***
PCW (g)	260.20±6.38***	220.00±7.34***	288.00±11.02***	227.00±4.97***	288.20±4.48***	237.40±4.50***
ECW (g)	205.00±8.82***	173.80±6.37***	219.20±9.31***	182.60±4.18***	238.00±4.79***	188.60±3.19***
SY (%)	70.45±1.46 ns	70.98±1.89 ns	67.19±1.02**	71.14±0.87**	71.56± 0.68 ns	70.05±0.25 ns
BW (g)	10.80±0.58***	9.20±0.80***	17.00±1.81***	7.80±0.20***	14.20±1.07***	9.00±0.45***
FW (g)	30.40±3.38***	25.00±5.76***	38.00±1.97***	29.80±1.39***	44.60±4.14***	31.80±1.66***
OIW (g)	44.40±1.94***	30.20±2.89***	45.00±1.52***	29.04±1.02***	46.00±0.71***	29.40±0.93***
BP (%)	3.72±0.18	3.79±0.41	5.21±0.55	3.04±0.07	4.26±0.25	3.34±0.12
FP (%)	10.40±0.98	10.11±2.35	11.69±0.65	11.58±0.31	13.31±0.93	11.82±0.63
OIP (%)	15.30±0.63	12.24±0.80	13.81±0.24	11.44±0.24	13.86±0.39	10.93±0.39
Statistical significance Student values	t calculated < 1.982 – insignificant differences – ns t calculated = 1.982-2.871 – significant differences * (t0.05=1,982) t calculated = 2.871-3.390 – distinct significant differences ** (t0.010=2.871) t calculated > t 0.001 = 3.390 – very significant differences *** (t0.001=3.390)					

Note: ALW – average live weight; CBW – carcass after bleeding weight; PCW-plucked carcass weight; ECW – eviscerated carcass weight; SY – slaughter yield; BW – blood weight; FW – flakes weight; OIW – organs and intestines weight; BP – blood proportion; FP – flakes proportion, OIP – organs and intestines proportion.

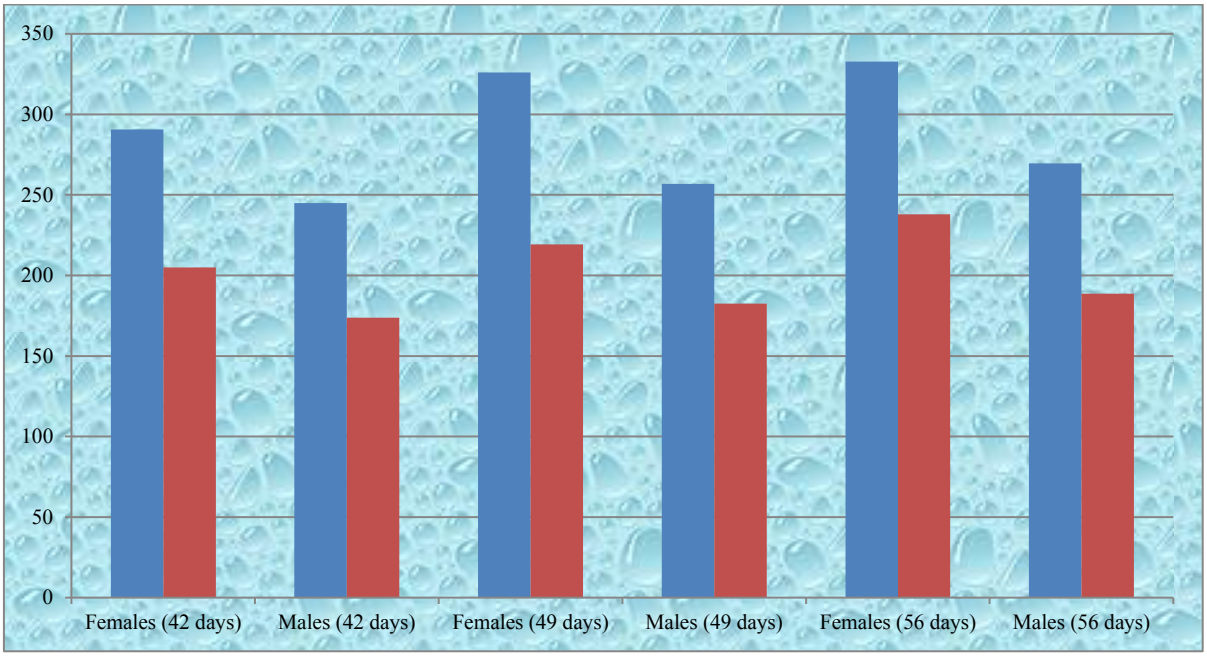

Fig. 2. The average live weight and the weight of the eviscerated carcass at the age of 42 days, 49 days and 56 days in the females and males from the Jumbo meat quail population

In a study conducted in Brazil [1] on a flock of meat quail *Coturnix* sp. from the Italian population, an average weight of the carcass was obtained in males similar to that in the present experiment in males (177.2 g/head at 42 days and 180.20 g at 49 days).

In a study carried out in Romania [8] on two batches of mixed and meat quail, in the group of meat quail, an average weight of 244.9 g/head (average weight for females and males) is mentioned at the age of 42 days.

In a study carried out in Turkey [12] on quails selected in the direction of meat production, a living weight of 42 days of 211.8 g/head is mentioned, a carcass weight of 146 g, a chest weight of 53 g and a weight of the wings of 33.7 g. At 49 days of age the average live weight was 220.5 g/head, the average weight of the carcass was 149.7 g/head, the average weight of the chest was 53.8 g, and the weight the average legs size was 34.3 g. At 56 days the average live weight was 225.6 g/head, the average carcass weight was 152.5 g/head, the average chest weight was 55.7 g, and the legs weight was 34.8 g.

A study in Egypt [7] showed that out of four different coloured quail populations, the white ones had the highest body weight, with the best carcass characteristics and meat quality.

The yield of the eviscerated carcass from the present experiment (Table 3) at the age of 42 days was 70.45±1.46% in females and 70.98±1.89% in males, the difference being not significant (t=0.220).

At the age of 49 days, it was 67.19±1.02% in females and 71.14±0.87% in males, the difference being distinctly significant (t=2.943), while at the age of 56 days it reached 71.56±0.68% in females and 70.05±0.25% in males, the difference being not significant (t=1.889).

Cuts parts in the carcass in Jumbo quail meat population slaughtered at 42, 49, and 56 days old

The average breast weight (Tab. 4) at 42 days was 116.80±6.11 g in females and 82.40±4.65 g in males, with 29.45% higher in females, the difference being very significant (t=4.478). The average breast proportion at the same age was 57.05±2.20% in females and 47.42%±2.00 in males.

The average breast weight at 49 days was 117.00±4.78 g in females and 93.00±1.70 g in males, 20.51% higher in females, the difference being very significant (4.724). The average breast proportion was 53.49±1.55% in females and 50.98±0.87% in males.

The average breast weight at 56 days was 117.80±2.21 g in females and 102.40±1.50 g in males, 13.07% higher in females, the difference being very significant (t=5.828). The average breast proportion was 53.82%±1.65 in females and 54.31%±0.44 in males.

The average legs weight (Tab. 4) at 42 days was 49.40±2.50 g in females and 41.00±1.22 g in males, 17 % higher in females, the difference being distinctly significant (t=3.015). The average proportion of the legs was 24.12±0.79% in females and 23.64±0.50% in males. The average weight of the legs at the age of 49 days was 42.60±1.29 g in females and 33.40±1.69 g in males, with 21.60% higher in females, the difference being distinctly significant (t=2.895).

The average proportion of the legs was 23.00±0.29% in females and 22.63±0.88% in males.

The average weight of the legs at 56 days was 50.00±1.00 g in females and 50.00±0.71 g in males, the difference being insignificant between the two sexes. The average proportion of the legs was 21.05±0.67 % in females and 26.52±0.21% in males.

The average back weight (Tab.4) at 42 days was 41.40±1.17 g in females and 29.20±1.74 g in males, with 29.47% higher in females, the difference being very significant (t=5.816).

The average proportion of back was 20.26±0.46% in females and 16.78±0.56% in males.

The average back weight at 49 days was 42.60±1.29 g in females and 33.40±1.69 g in males, 21.60% higher in females, the difference being very significant (t=4.327).

The average back proportion was 19.51±0.60% in females and 18.33±1.05% in males. The average back weight at 56 days was 37.40±0.87 g in females and 30.20±1.16 g in males, with 19.25% higher in females, the difference being very significant (t=4.968). The average proportion of back was 15.73±0.69% in females and 16.04±0.72% in males.

Table 4. The weight and the share of the carcass cuts at 42, 49 and 56 days in females and males of Jumbo meat quail

Specification	Slaughter results at 42 days		Slaughter results at 49 days		Slaughter results at 56 days	
	Females	Males	Females	Males	Females	Males
ABW (g)	116.80±6.11***	82.40±4.65***	117.00±4.78***	93.00±1.70***	117.80±2.21***	102.40±1.50***
ALW (g)	49.40±2.50**	41.00±1.22**	50.40±2.25**	41.40±2.20**	50.00±1.00ns	50.00±0.71ns
ABW (g)	41.40±1.17***	29.20±1.74***	42.60±1.29***	33.40±1.69***	37.40±0.87***	30.20±1.16***
ABW (g)	12.60±0.51 ns	11.60±0.55 ns	11.80±0.49 ns	11.4± 0.51 ns	12.40±0.51 ns	11.80±0.37 ns
ABP (%)	57.05±2.20	47.42±2.00	53.49±1.55	50.98±0.87	53.82±1.65	54.31±0.44
ALP (%)	24.12±0.79	23.64±0.50	23.00±0.29	22.63±0.88	21.05±0.67	26.52±0.21
ALP (%)	20.26±0.46	16.78±0.56	19.51±0.60	18.33±1.05	15.73±0.69	16.04±0.72
ABP (%)	6.19± 0.39	6.68±0.19	5.51±0.24	6.23±0.20	6.68±0.32	6.26±0.18
Statistical significance Student values	t calculated < 1.982 – insignificant differences - ns t calculated = 1.982-2.871 – significant differences * (t0.05 = 1,982) t calculated = 2.871 – 3.390 – distinct significant differences ** (t0.010 = 2.871) t calculated > t 0.001 = 3.390 – very significant differences *** (t0.001 = 3.390)					

Note: ABW – average breast weight; ALW – average legs weight; ABW – average back weight; AWW – average wings weight; ABP – average breast proportion; ALP-average legs proportion; ABP – average back proportion; AWP – average wings proportion.

The average weight of the wings (Tab. 4) at the age of 42 days was 12.60±0.51 g in females and 11.60±0.55 g in males, with 8.62% higher in females, the difference being insignificant (t=1.386). The average proportion of wings was 6.19±0.39% in females and 6.68±0.19% in males. The average weight of wings at the age of 49 days was 11.80±0.49 g in females and 11.40±0.51 g in males, 3.38% higher in females, the difference being insignificant (t=0.565).

The average proportion of wings was 5.51±0.24% in females and 6.23± 0.20% in males.

The average weight of the wings at 56 days was 12.40±0.51 g in females and 11.800.37 g in males, by 4.83% higher in females, the difference being uninsured statistically. The average proportion of wings was 6.68±0.32% in females and 6.26±0.18% in males.

Conclusions

From the point of view of the evolution of the average daily weight gain during the period 1-56 days, which decreases after 6 weeks, as well as the weight of the carcass and its component parts, conclusions can be drawn regarding the optimum age for slaughtering the Jumbo quails.

This seems to be 42 days for both sexes, especially in the case of breeding in common until slaughter, 42 or 49 days for females (up to 7 weeks the weight gain decreases by only 3.44% compared to 6 weeks) and 42 days for males (between 1 to 7 weeks the weight gain decreases almost 3 times, with 9.98%, compared to the one from 1 to 6 weeks), especially in the case of separate breeding by sex. It is necessary to continue the research in order to take into account other factors on which the age of slaughter depends in particular the nutritional value of the combined food formulas, or the type of feeding (two-phase, with two combined food recipes, or three-phase, with three combined food recipes, of which the latter is to be of finishing).

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Evaluation of Breeding Value of Youth Karakul Sheep after the Complex Selection Indices

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Abstract

The aim of the research was to reveal the methodological principles and to elaborate the formulas for evaluating the breed value of youth Karakul sheep according to the complex selection indices. The scientific researches were carried out on a batch of youth Moldavian Karakul sheep of the National Institute of Animal Husbandry and Veterinary Medicine from village Maximovca, Anenii Noi. The complex selection indices for youth Karakul sheep was built on the basis of three selected morpho-productive characters: furskin quality, expressed in points; own body mass (kg) and mothers milk production (kg). The complex selection indices of youth were built according to the formula $I_{cs}=(M_{fp}\cdot C_{fap})+(M_{fmc}\cdot C_{famc})+(M_{fpl}\cdot C_{fapl})$,
where:

- I_{cs} – complex selection indices of youth;
- M_{fp} – the phenotypic size of the furskin quality;
- C_{fap} – coefficient of aggregate phenotype of furskin quality;
- M_{fmc} – the phenotypic size of the body mass of the youth;
- C_{famc} – coefficient of aggregate phenotype of body mass;
- M_{fpl} – the phenotypic size of the mother's milk production;
- C_{fapl} – coefficient of aggregate phenotype of milk production of ewe-mothers.

Scientific research has shown that for the assessment of sheep youth's breeding value according to the complex selection indices it was necessary to establish the race standard after all three selected morpho-productive characters. Subsequently, the coefficients of the aggregate phenotype for each selected character were determined. As a result, it has been found that the determination of Karakul sheep youth's breeding value according to the complex selection indices is one of the most objective and effective methods, because it contains both phenotypic and genotypic elements of appreciation. The complex selection indices for youth sheep can be determined at any age, from birth to adult age. Determining the breeding value of Karakul lamb after complex selection indices is of scientific and practical importance because they are often marketed at early ages. The specificity of determining the aggregate phenotype coefficient for body mass is a particularity of the principles of construction of the complex selection indices of youth Karakul sheep. The size of the aggregate phenotype coefficient of body mass in youth sheep varies according to the standard of body mass not only at standard ages but also at intermediate ages.

Keywords: indices, complex, selection, youth, sheep, Karakul

Introduction

In zootechnics several methods of assessing the value of animals are known: according to the values of their own morpho-productive performances, expressed in absolute units of the International Metric System, in scores, percentages, or other relative units; according to the

morpho-productive characters of the collateral relatives, ascendants or descendants. In all cases of estimation of animal breeding value according to the simple parameters of the morpho-productive characters and the selection, either by the tandem method or by the independent boundary method of the selected character values, there is no guarantee of increasing the economic efficiency, because in these cases the economic values of the selection characters are not considered.

In the second half of the 20th century and the beginning of the 21st century the zootechnics increasingly began to apply the methods for estimating the value of breeding animals by complex selection indices, expressed in specific formulas, which reflects in themselves some ratios of values economic features of morpho-productive characters, combined with the heritability, repeatability, variability or other genetic parameters of populations [9, 10, 12].

A wider spread of selection methods according to complex indices of morpho-productive characters occurred in poultry [11, 14-17, 20], pigs [18, 25] and taurines husbandry [26]. For these animal species, the most diverse and complex selection indices have been built and applied.

Most specialists and researchers, who have developed and implemented animal selection methods according to complex indices, state that they provide greater efficiency in selection.

According to data [25, 26], the effect of animal selection on complex indices is about 10% higher than the selection based on the independent boundary method of selection characters.

Other authors [21, 23, 24] consider that the effect of selection by complex indices increases by 20-50%, and the third [19], states that the application of an integrated scheme to the genetic amelioration of sheep populations using indices selection based on computerized technologies allows the selection effect to be accelerated even 2-4 times.

Application of selection indices at Karakul race is not widespread. Only some publications on the use of selection indices for lambs' growth potential in the postnatal period of ontogenesis [27, 28] and on curl size, modelling and quality the fiber [23, 24] are known in karakulture. But these (indices) are not complex, therefore, they cannot be used to determine the overall breeding value of the animal.

In our previous research [8], we have shown that some researchers at Namibia's Karakul Sheep Research Center [23] have mentioned that "*general indices of Karakul lamb selection in ready form does not exist, nor does it need to exist. But, for the flock of the Neidam Experimental Station, such indices have been elaborate*". The author brings the selection index of Karakul lambs according to the qualities of furskin $I = 20 + 1Q + 4P + 2S$

where: I – the selection indice of Karakul lambs according to the qualities of furskin;

- 20 – the constant calculated by the author;
- Q – the quality of pilous fibers, appreciated in points;
- P – curls modeling, appreciated in points;
- S – the size of curls, appreciated in points.

Other Kazakhstan researchers [19, 21, 22, 27] have reported the application of selection indices of lambs and adult Karakul sheep after the harmony of body conformation, including some ratios of the measurements of external dimensions with the animal's body weight, as are: body mass, thorax perimeter and oblique length of the trunk.

The sheep type Moldavian Karakul possesses mixed (combined) productivity, which determines the need to select the animals according to a complex of characters and can be organized by different methods: in tandem, after the independent limits of the selected character value or by the complex indices of selection. The last method, as affirm many authors [19, 23-26], is considered one of the most effective in the selection. Determining the selection indices is a complex appreciation of the characters, both by productive value and by economic value.

At present, according to the Karakul Sheep Evaluation Instruction with amelioration principles in the Republic of Moldova in force [2], the sheep breeding value (class) is

determined only by the qualities of the furskin, without taking into account the most important morpho-productive selection, such as milk and meat production (body mass). The main drawback of these Instruction is that furskin production is considered to be the only basic character expressed by the lamb class, and the body mass and milk production are considered as secondary (secondary) characters and are never taken into account in the determination of the breeding value of the animal (class). Thus, between the values of the main morpho-productive characters and the breeding value of the animal, there is an obvious rupture, requiring bound access in an integral complex of the phenotypic, genotypic and economic values of the animal. Here is the urgent need to improve these Instructions, by developing and including in the selection methodology effective methods for assessing the value of the breed with the application of complex selection indices.

In our previous research [3, 7, 8] we have demonstrated the methods of assessing the breeding value of adult sheep (ewes and rams) according to complex selection indices.

At the same time, the method of assessing the breeding value of Karakul youth at different ages, from birth to adulthood (2-2.5 years), according to the complex selection criteria, presents a less elaborate problem, though quite important. Taking into account the fact that the method of assessing the breed value of Karakul youth after the complex selection criteria can be applied in estimating its marketing value, its elaboration becomes an innovative, practical and extremely current segment.

In this context, the purpose of the research was to reveal the methodological principles and to elaborate the formulas for evaluating the breeding value of the youth Karakul sheep according to the complex selection criteria.

Materials and Methods

The scientific researches were carried out on a batch of Moldavian Karakul youth sheep of the National Institute of Animal Husbandry and Veterinary Medicine from village Maximovca, Anenii Noi.

To proceed with the elaboration of complex selection indices, first, we examined the possibility of reducing the number of selected morpho-productive characters. Taking into account the recommendations of professor Iliev F.V. [13], referring to the decrease in the number of selected characters, and taking into account the fact that the efficiency of multiple character selection is inversely proportional to the square root of the number of selected characters ($1/\sqrt{n}$), we limited them to up to 3 main morpho-productive characters, such as: quality of lamb's furskin at birth, body mass of youth sheep, and ewes-mothers milk production.

The quality of lamb furskins Karakul was evaluated at 1-2 days after birth, according to the provisions of the official Instruction evaluation Karakul sheep with amelioration principles in the Republic of Moldova [2]. In order to appreciate the quality of the lamb furskin, the number of evaluated characters was reduced from 29 to only 7 synthetic characters, finally expressed by class and score [4].

Body weight of youth sheep was appreciated several times a year at different ages, with different technical scales, according to our own advanced methods [3, 6]. At birth, the lamb was weighed with the hand scale, with a capacity of 6-8 kg and a precision of 0.1 kg. At 20 days, the lambs were weighed individually with a medical weighing scale for children, with a capacity up to 15 kg and a precision of 0.1 kg. Starting at the age of 3 and 6 months, as well as in the autumn, at the end of October at the age of 1.5 and 2.5 years, youth sheep was annually weighed individually at technical scales with the capacity of 100-150 kg and an accuracy grade of 0.1-0.2 kg. To perform the weighing of the youth, a narrow cage (box) with two doors (entry and exit) was installed and fixed on the weighing platform. The dimensions of the cage were thus projected so that a sheep could be enclosed relatively tightly. In front of the scales was

arranged an enclosure (rodeo) (with a capacity of 50 sheep) with an entrance corridor. At the exit of the cage, another enclosure with a capacity of 50-200 sheep was built. At the simultaneous opening of the doors, the youth in the entrance vault, seeing through the cage the sheep from the opposite tide (from the exit), voluntarily entered the cage, after which the doors were immediately closed. After weighing, at the opening of the exit door, the sheep went out of the cage. This weighing technique allowed recording the animal's body weight without stress and trauma. Sheep weighing data were recorded in the Register of body weight of the sheep Karakul (F-10K).

Mother's milk production was determined by control milking, performed systematically on each sheep once every 15 days throughout lactation, according to method of T. Nică [15], with the improvements we developed [1]. The technical principle of this method is that sheep are subjected to control milking once a day, as a rule, in the morning milks. To determine the quantity of milk produced by sheep throughout the control day, the quantity of milk produced by it on the morning of the control day shall be multiplied by the control coefficient. This coefficient is determined by the formula:

$$K_c = \frac{Pt}{Pd} \cdot C_r \qquad (1)$$

where:

- K_c – control coefficient;
- P_t – the total quantity of milk from lactating ewes on the day of control;
- P_d – the amount of milk from lactating ewes on the morning of the control day;
- C_r – milk retention coefficient:
  - for ewes with infant lambs C_r=1,3;
  - for ewes in the first two weeks after weaning lambs C_r=1,2;
  - for the other lactating ewes C_r=1,0.

To control the quantity of milk, each ewe was individually milked in the cup, afterwards the milk was weighed to the electronic scales with capacity of 1000 g, after which the milk was poured into the storage can. The data on the matriculation number of each milking ewe and the quantity of milking milk under control were entered in the Milk Production Control Sheet (F-8K). Subsequently, the control sheet data on the amount of milking milk on the control day was transcribed into the Register of Milk Production of Karakul sheep (F-7K) where the individual milk production of each ewe on each control period was calculated. By summing the quantities of milk calculated in all control periods, the milk production of each ewe was deducted for the entire lactation.

The second step taken by us to build selection indices was to determine the *economic value of the three selection characters* and to determine its share in the total income obtained from one ewe per year [5]. By systemizing and generalizing research results, we inferred the following shares of the economic values of the selection characters:

- furskin quality – 12%;
- body mass – 28%;
- milk production – 60%.

Since these selection characters have different measurements and phenotypic values to construct summative complex selection indices, we have calculated the *coefficients of the aggregate phenotype* that allow the phenotypic size of the character to be transformed into the single weighted economic value of the complex animal selection indices.

As a benchmark for determining the coefficients of the aggregate phenotype, we used the standard phenotypic size (M_S) of the selection character, which represents the race standard (level class I), for each group of age and sex of the animals, developed by us for the sheep type Moldavian Karakul [3].

The coefficient of the aggregate phenotype of youth sheep was calculated for each selected character in part by the following formula:

$$C_{fa} = \frac{P_{ve}}{M_s} \quad (2)$$

where,

- C_{fa} – coefficient of the aggregate phenotype;
- P_{ve} – the weight of the economic value of the selection character;
- M_s – the standard phenotypic size of the selection character.

Given the coefficients of the aggregate phenotype for each selected character, we deduced the complex selection index for youth, according to the following formula:

$$I_{cs}=(M_{fp}\cdot C_{fap}) + (M_{fmc}\cdot C_{famc}) + (M_{fpl}\cdot C_{fapl}) \quad (3)$$

Where:

- I_{cs} – the complex selection index for youth;
- M_{fp} – the phenotypic size of the furskin quality;
- C_{fap} – coefficient of the aggregate phenotype of the furskin character;
- M_{fmc} – the phenotypic size of the body mass of the youth;
- C_{famc} – coefficient of aggregate phenotype of body mass character;
- M_{fpl} – the phenotypic size of the mother's milk production;
- C_{fapl} – coefficient of aggregate phenotype of milk production of ewes-mothers.

It should be noted that the coefficient of the aggregate phenotype of the quality of its own furskin of the youth sheep's remains constant from birth to life for life. At the same time, the coefficients of the aggregate phenotype for body mass and mother's milk production vary according to the age at which the young and the most productive lactation of the mother's lactations are evaluated.

Results and Discussions

Scientific research has shown that for the assessment of youth sheep breeding value, according to the complex selection criteria, it is necessary to establish the race standard after all three selected morpho-productive characters.

For the quality of the furskin, the Moldavian Karakul race standard is the average of the Classe I, expressed by the "appropriate" symbol and valued at 6 points.

Based on the results of the multiannual researches, parameters of Karakul Moldovenesc youth sheep race standard was developed and the two selected morpho-productive characters, such as body mass and milk production.

For body mass, the standard of the race varies according to youth age and is set in the following parameters (Tab. 1).

Table 1. Parameters of the minimum standard of body weight of Moldavian Karakul youth sheep at different standard ages, kg

Age of sheep	Class:		
	Elite	Class I	Class II
At birth (1-2 days)	4.7	4.5	4.0
At 2 months: male	17	16	15
female	16	15	14
At 6 months: male	34	32	30
female	31	29	27
At 18 months: male	65	60	55
female	47	45	42
Adult sheep (>2,5 years): male	80	75	-
female	50	48	45

Having the body mass parameters at standard ages, zootechnical specialist can deduct intermediate body mass parameters ($M_{st.inter}$) at any intermediate age by adding to the body mass of the lower standard age ($M_{st.min}$) of the calculated mass mass (M_{calc}) this being determined by multiplying the daily average additions in the period between the standard ages and the number of intermediate days ($N_{z.inter}$) that exceeds the standard at a lower age.

Standard body mass at intermediate age is calculated according to the following formula:

$$M_{st.inter}=M_{st.min}+\left(\frac{Mst.\max - Mst.\min}{Nz.st}\right)\bullet N_{z.inter} \quad (4)$$

where:

- $M_{st.inter}$ – standard body mass at intermediate age;
- $M_{st.min}$ – standard body mass at younger age;
- $M_{st.max}$ – standard body mass at older age;
- $N_{z.st}$ – the number of days of the intermediate period between the standard ages;
- $N_{z.inter}$ – the number of days of the interimediate period.

For an elucidation of this method, let's solve an example in practice. It is necessary to determine the standard value of the body weight of the ram no. 8448 at the intermediate age of 126 days.

From Table 1 we know the values of the body mass of the male lambs at the age of 2 months (60 days) equal to 16 kg and at the age of 6 months (180 days) equal to 32 kg. As we can see, the intermediate age of the ram in our example lies between the two standard ages of 2 and 6 months. The average daily body weight gain during this standard period is 133.3 g/day (16 kg: 120 days = 0.1333 kg). From the age of 60 days to 126 days, the ram gained 8.8 kg (0.1333 kg • 66 days). Therefore, the standard body weight of the ram at intermediate age of126 days is 24.8 kg (16+8.8 = 24.8 kg). Thus, after this example the standard body weight of youth sheep at any intermediate age can be calculated.

The race standard for the production of milk by mother-ewes has been developed and varies according to the successive lactation completed on the date of youth sheep breeding value using the complex selection index method. The data on milk production of mother-ewes are transcribed from the Register of milk production of ewes Karakul (F-7K), in which milk production of ewe is determined individually for the whole lactation, or from the Bulletin of general evaluation of Karakul sheep (F-8K). Parameters of the race standard after ewe's milk production are developed according to ewe's lactation (Tab. 2).

Table 2. Parameters of minimum standard of milk production of ewes Moldavian Karakul, kg

Depending on lactation	Class:		
	Elite	Class I	Class II
For ewes in III or higher lactation	70	60	50
For ewes in II lactation	63	54	45
For ewes in I lactation	53	45	40

If it is necessary to forecast (equalize) the production of milk by mature ewe, produced by young ewes in lactation I or II, then recalculate it by means of established correction coefficients. For the recalculation of milk production from lactation I to mature lactation, its value is multiplied by the coefficient 1.35. For the recalculation of milk production in lactation II, its value is multiplied by the coefficient 1.11. These coefficients are used to equate lactation of daughters for genotypic testing of ewes after milk production of the descendants.

By providing the standard values of the morpho-productive characters selected for sheep youth, we can proceed to construct the complex selection indices needed to assess its breeding value, especially at the marketing stage.

Complex selection index for lambs at birth

Breeders of Karakul sheep often practice lamb's commercialization (selling/buying) at 1-5 days after birth. If the buyer buys the lamb without a mother, it is further grown with the bottle.

When assessing the commercial value of the lamb, priority is given primarily to phenotypic characters such as furskin quality and body development. In some localities of the Republic of Moldova the sheep breeders organize fairs-exhibitions of animals where the lambs Karakul are exposed. Some of them are for sale, and some are exposed for public viewing and advertising.

Ram lambs exposed for public viewing, subsequently being raised for breeding, have a high demand (from Karakul ewes' owners) for use in autumn mating sheep and getting a better descendant.

Lately, some buyers of lamb Karakul, assisted by specialists in the field, are increasingly interested in mothers' milk production. In this case, a genotypic assessment of the breeding value of the lamb is already taking place, even if it is done unofficially. In elite farms, the breeding value of lambs is officially assessed after a complex of characters (furskin quality, body mass, mother's milk production). From breeding farms, Karakul lambs are exposed at fairs-exhibitions accompanied by a breed certificate, which includes evaluation dates with morpho-productive character selection indices. For young sheep of Moldavian Karakul breed of any age, the complex selection indices are built according to the methods developed by us.

For lambs Karakul at birth, the complex selection index is determined by the above-mentioned formula (3), based on the phenotypic character values of the character and the coefficients of the aggregate phenotype of the three selected morpho-productive characters.

First, we calculate the coefficients of the aggregate phenotype for each character, based on the weight of the economic value of the character and its standard phenotypic size.

Thus, we calculate the coefficients of the aggregate phenotype of the selection characters. The coefficient of aggregate phenotype for furskin quality will be,

$$C_{fap} = \frac{P_{vt}}{M_s} = \frac{12}{6} = 2.0$$

where: C_{fap} – the coefficient of aggregate phenotype for furskin quality of lamb;

P_{ve} – the weight of the economic value of the furskin character set by us = 12;

M_s – the standard phenotypic size of the furskin quality class I = 6 points.

As a result of the performed calculations, the coefficient of aggregate phenotype of the quality of the furskin is equal to 2.0.

The coefficient of aggregate phenotype for lamb body mass at birth, both for ewe lambs and for ram lambs, is the same, and constitutes:

$$C_{famc} = \frac{P_{ve}}{M_s} = \frac{28}{4.5} = 6.22$$

where: C_{famc} – the coefficient of aggregate phenotype for lamb body mass;

P_{ve} – the weight of the economic value of the body mass = 28;

M_s – the standard phenotypic size of body mass for lambs at birth is 4.5 kg.

The coefficient of the aggregate phenotype for mothers' milk production will be:

For ewes with I lactation,

$$C_{fapl} = \frac{P_{ve}}{M_s} = \frac{60}{45} = 1.33$$

where: C_{fapl} – coefficient of the aggregate phenotype for milk production;

P_{ve} – the weight of the economic value of milk production = 60;

M_s – the standard phenotypic size of milk production of ewe with I lactation = 45kg.

For ewes with II lactation,

$$C_{fapl} = \frac{P_{ve}}{M_s} = \frac{60}{54} = 1.11$$

where: C_{fapl} – coefficient of the aggregate phenotype for milk production;
P_{ve} – the weight of the economic value of milk production = 60;
M_s – the standard phenotypic size of milk production of ewe with II lactation = 54 kg.
For ewes with III lactation,

$$C_{fapl} = \frac{P_{ve}}{M_s} = \frac{60}{60} = 1.0$$

under: C_{fapl} – coefficient of the aggregate phenotype for milk production;
P_{ve} – the weight of the economic value of milk production = 60;
M_s – the standard phenotypic size of milk production of ewe with III lactation = 60kg.
Thus, the complex selection index of *lambs at birth* will have the following formula:

$$I_{mn}=(M_{fp}\cdot 2.0) + (M_{fmc}\cdot 6.22) + (M_{fpl} \cdot 1.33;\ or\ 1.11;\ or\ 1.0) \quad (5)$$

where: I_{mn} – the complex lamb selection index at birth;
M_{fp} – the phenotypic size of the quality of the own furskin, expressed in the score;
M_{fmc} – the phenotypic size of its own body mass, expressed in kg;
M_{fpl} – the phenotypic size of the mother's milk production, expressed in kg.

It should be mentioned that the numeric value of the index is expressed in figures without units of measurement, ranging from two to three full digits and one (tenths) or two (hundreds) digit rounded by comma. If the phenotypic size of the three selection characters coincide exactly with the race standard, the selection index value will be 100. Depending on the phenotypic size of the selection characters, the complex selection index may be less than or equal to 100. In principle, the complex selection index indicates the level of the breeding value of the animal compared to the breed standard, and at the same time shows the extent to which it yields or exceeds that standard. If the value of the selection index exceeds 100, we can conclude that the breeding value of the animal is higher than the breed standard and, conversely, if the animal's index is below 100, the breeding value of the animal does not match the breed standard.

According to the value of the complex selection index, the sheep youth of each age group can be divided into lines of ranks, showing the value of the animal in the sheep hierarchy.

Exaemples:

a) lamb Karakul no. 8145 has the furskin quality "exc.-8", the body weight at birth of 4.9 kg and the milk production of the mother in the second lactation equal to 68 kg. Based on these data, the complex selection index of this lamb will constitute,

$$I_{8145} = (8\bullet 2.0) + (4.9\bullet 6.22) + (68\bullet 1.11) = 16 + 30.48 + 75.48 = 121.96;$$

b) lamb Karakul no. 8216 has the furskin quality "red.-4", the body weight at birth of 4.2 kg and the milk production of the mother in the IV lactation equal to 52 kg. Based on these data, the complex selection index of this lamb will constitute,

$$I_{8216} = (4\bullet 2.0) + (4.2\bullet 6.22) + (52\bullet 1.0) = 8 + 26.12 + 52 = 86.12;$$

c) lamb Karakul no. 8206 has the furskin quality "potr.-7", the body weight at birth of 4.1 kg and the milk production of the mother in the first lactation equal to 46 kg. Based on these data, the complex selection index of this lamb will constitute,

$$I_{8206} = (7\bullet 2.0) + (4.1\bullet 6.22) + (46\bullet 1.33) = 14 + 25.50 + 61.18 = 100.68;$$

From the provided examples, we find that lamb no. 8145, having a complex selection index of 121.96 is the most valuable, far exceeding the standard of breed after the breeding value. On the contrary, lamb no. 8216 has a low breed value because the complex selection index of 86.12 is well below the breed standard. The third lamb, no. 8206, having the complex selection index equal to 100.68, after the breeding value is situated at the breed standard. In the decreasing series of the centralizing bulletin evaluation of the lamb's breeding value according to the complex selection index of the herd, the first lamb is among the highest ranks. The second lamb

is among the lower ranks of the herd, and the third lamb is found in the middle of the sheep flock.

Complex selection indexes for lambs at 2 months age

The age of 2 months is standard, as lambs of Moldavian Karakul reaching this age are weaned and separated from their mothers. For the 2-month lambs, complex selection indices are similar to those of lambs at birth, only the value of the aggregate phenotype coefficient of body mass is different, which is different in ram lambs and ewe lambs, constituting:

- ram lambs

$$C_{famc}\frac{P_{ve}}{M_s}=\frac{28}{16}=1.75$$

- ewe lambs

$$C_{famc}\frac{P_{ve}}{M_s}=\frac{28}{15}=1.87$$

Thus, the complex selection index for *2-month ram lambs* will have the following formula

$$I_{b2}=(M_{fp}\cdot 2.0)+(M_{fmc}\cdot 1.75)+(M_{fpl}\cdot 1.33;\ or\ 1.11;\ or\ 1.0) \quad (6)$$

where: I_{b2} – the complex selection index for 2-month ram lambs;

M_{fp} – the phenotypic size of the quality of the own furskin, expressed in the score;

M_{fmc} – the phenotypic size of its own body mass, expressed in kg;

M_{fpl} – the phenotypic size of the mother's milk production, expressed in kg;

The complex selection index for ewe lambs of 2 months will have the following formula

$$I_{m2}=(M_{fp}\cdot 2.0)+(M_{fmc}\cdot 1.87)+(M_{fpl}\cdot 1.33;\ or\ 1.11;\ or\ 1.0) \quad (7)$$

where: I_{m2} – the complex selection index for 2-month ewe lambs;

M_{fp} – the phenotypic size of the quality of the own furskin, expressed in the score;

M_{fmc} – the phenotypic size of its own body mass, expressed in kg;

M_{fpl} – the phenotypic size of the mother's milk production, expressed in kg.

Example: to calculate the complex selection index for ram lamb No. 7124 with the quality of furskin "exc.-9", which at the age of 3.5 months (105 days) had a body weight of 25 kg and the milk production of the mother in the second lactation is 63 kg.

The standard size of the coefficient of aggregate phenotype for furskin quality is 2.0.

To determine the aggregate phenotype coefficient of body mass, it is necessary to adjust the standard body mass at the 105-day intermediate age, which is between standard ages of 2 and 6 months. From Table 1, we see that standard daily additions to ram lambs during this period are 0.133 kg/day. From the standard age of 2 months to the intermediate age of 3.5 months, the lamb should add at least 5.98 kg (45 days • 0.133 kg = 5.98 kg). Therefore, the standard body weight of Karakul ram lamb at the intermediate age of 105 days is 21.98 kg (16+5.98).

Hence, the coefficient of aggregate phenotype of the ram lamb body mass at this age is:

$$C_{famc}=\frac{P_{ve}}{M_s}=\frac{28}{21,98}=1.27$$

The standard size of the aggregate phenotype of sheep in lactation II is 1.11.

Thus, with the phenotypic sizes of the selected ram lamb no. 7124 and aggregate phenotype coefficient sizes for these characters, we can proceed to calculate the complex selection index, which will constitute:

$$I_{7124}=(9\bullet 2)+(25\bullet 1.27)+(63\bullet 1.11)=18+31.75+69.93=119.68$$

Therefore, having the size of the complex selection index far above the breed level, the ram lamb no. 7124 has a high breed value and occupies a high rank in the descending row of the centralizing bulletin evaluating the value of the youth sheep of the flock.

Complex selection index for 6-month-old youths

The age of 6 months is considered to be standard, as it coincides with the assessment of the sheep's body development by weighing the entire flock in the autumn.

For the construction of 6-month-old youths complex selection indices for both ewe and ram lambs, the same coefficients of aggregate phenotype of furskin quality equal to 2.0 and maternal milk production according to lactation (*Lactation I =1.33; Lactation II =1.11; Lactation III =1.0*). The difference between ram and ewe lambs is only the coefficient of the aggregate phenotype of the body mass, which constitutes:

- at ram lambs $$C_{famc}\frac{P_{ve}}{M_s}=\frac{28}{32}=0.875$$
- at ewe lambs $$C_{famc}\frac{P_{ve}}{M_s}=\frac{28}{29}=0.965$$

Thus, the complex selection index for *6-month rams* will have the following formula:

$$I_{b6}=(M_{fp}\cdot 2.0)+(M_{fmc}\cdot 0.875)+(M_{fpl}\cdot 1.33;\ or\ 1.11;\ or\ 1.0) \quad (8)$$

where: I_{b6} – the complex selection index for 6-month rams;

M_{fp} – the phenotypic size of the quality of the own furskin, expressed in the score;

M_{fmc} – the phenotypic size of its own body mass, expressed in kg;

M_{fpl} – the phenotypic size of the mother's milk production, expressed in kg.

The complex selection index for *6-month ewes* will have the following formula:

$$I_{m6}=(M_{fp}\cdot 2.0)+(M_{fmc}\cdot 0.965)+(M_{fpl}\cdot 1.33;\ or\ 1.11;\ or\ 1.0) \quad (9)$$

where: I_{m6} – the complex selection index for 6-month rams;

M_{fp} – the phenotypic size of the quality of the own furskin, expressed in the score;

M_{fmc} – the phenotypic size of its own body mass, expressed in kg;

M_{fpl} – the phenotypic size of the mother's milk production, expressed in kg.

Examples of 6-month-old youths breeding value assessment after complex selection indices: a) the ram lamb no. 9057 had the quality of the furskin apreciated with the grade "exc.-8". At autumn weighing (at the age of 6 months) it had 35 kg. The milk production of the mother in the IV lactation was 61 kg. The coefficient of aggregate phenotype for furskin quality is the same as for lambs and is 2.0. The coefficient of aggregate phenotype for body mass at 6 months will be:

$$C_{famc}=\frac{P_{ve}}{M_s}=\frac{28}{32}=0.875$$

where: C_{famc} – coefficient of aggregate phenotype for body mass;

P_{ve} – the weight of the economic value of body mass = 28;

M_s – the standard phenotypic mass size for 6-month-old rams of 32 kg.

The coefficient of the aggregated phenotype for the ewe mother with lactation IV will be standard and equal to 1.0.

Thus, having the phenotypic size of the selected morpho-productive characters and the coefficients of the aggregate phenotype, the selection index of ram lamb no. 9057 will be:

$$I_{9057}=(8\bullet 2)+(35\bullet 0.875)+(61\bullet 1.0)=16+30.62+61=107.62$$

b) ewe lamb no.9101 had the quality of the furskin appreciated with the grade "potr.-5". At autumn weighing (at the age of 6 months) it had 28 kg. The milk production of the mother in the V lactation was 58 kg. The coefficient of aggregate phenotype for furskin quality is the same as for the above-mentioned ram lamb – 2.0. The coefficient of aggregate phenotype for body mass at 6 months will be:

$$C_{famc}=\frac{P_{ve}}{M_s}=\frac{28}{29}=0.965$$

where: C_{famc} – coefficient of aggregate phenotype for body mass;

P_{ve} – the weight of the economic value of body mass = 28;

M_s – the standard phenotip size of body weight for 6 months lambs equal to 29 kg.

The coefficient of the aggregate phenotype for the lactating V of ewe-mother will be a standard of 1.0.

Thus, having the phenotypic size of the selected morpho-productive characters and the coefficients of the aggregated phenotype, the complex selection index for lamb no. 9101 will be:

$$I_{9101} = (5•2) + (28•0.965) + (58•1.0) = 10+27.02+58 = 95.02$$

Therefore, from the given examples we see that the ram lamb no. 9057 has a higher breed value than Moldavian Karakul breed standard, because the complex selection index exceeds 100 points by 7.62 points. At the same time, the breeding value of ewe lamb no. 9101 is below the race standard because the complex selection index is less than 100 with about 5 points. In the raw ranks of 6-month-old ovine in the herd, the evaluated ram is situated among the advanced ranks, and the mentioned ewe, on the contrary, is among the lower ranks.

Complex selection index for 18-month-old rams

The age of 18 months is considered standard, since sheep youth is normally included in the reproductive process through directed mating. For this group of youth, the complex selection index will consist of the value of the quality of its own furskin, valued at its own level, of the body's own body mass and of the milk production of the mother. The coefficient of aggregate phenotype for own furskin quality will be the same as for other youth groups equal to 2.0. The coefficient of the aggregate phenotype for the body mass of the youth rams will be:

$$C_{famc} = \frac{P_{ve}}{M_s} = \frac{28}{60} = 0.467$$

where: C_{famc} – coefficient of aggregate phenotype for body mass;

P_{ve} – the weight of the economic value of body mass = 28;

M_s – the standard phenotypic mass of 18-month rams with 60 kg.

The coefficient of the aggregate phenotype for the milk production of the mother will depend on its lactation and will be the same as for the other groups of youth (*Lactation I =1.33; Lactation II =1.11; Lactation III =1.0*).

Thus, the complex selection index for 18-month-old rams will have the following formula:

$$I_{b18} = (M_{fp} \cdot 2.0) + (M_{fmc} \cdot 0.467) + (M_{fpl}\ 1.33;\ or\ 1.11;\ or\ 1.0) \qquad (10)$$

where: I_{b18} – the complex selection index for 18-month-old rams;

M_{fp} – the phenotypic size of the quality of the own furskin, expressed in the score;

M_{fmc} – the phenotypic size of its own body mass, expressed in kg;

M_{fpl} – the phenotypic size of the milk production of ram's mother, expressed in kg.

Example: the ram no. 5617, 18 months old, with furskin quality "exc.-8" with a body weight of 61 kg and milk production of mother in the second lactation of 85 kg, will have the following complex index of selection:

$$I_{b5617} = (M_{fp} \cdot 2.0) + (M_{fmc} \cdot 0.467) + (M_{fpl} \cdot 1.11) = (8 \cdot 2.0) + (61 \cdot 0.467) + (85 \cdot 1.11) =$$
$$16+28.49+94.35 = 138.84$$

Examining the value of the calculated index, we can conclude that the ram no. 5617 possesses a breeding value far superior to the standard of breed and is among the highest ranks in the decreasing order of the general bordering bulletin of 18-month-old rams.

Complex selection index for 18-month-old ewes

For ewes in this group of youth sheep the complex selection index will be made up of the same basic elements as the 18-month-old rams, namely: the quality of their own furskins appreciated at evolution, their own body mass and the milk production of the mother.

The coefficient of aggregate phenotype for the quality of own furskin will be the same as for other sheep, equal to 2.0.

The coefficient of aggregate phenotype for body mass will be:

$$C_{famc} = \frac{P_{ve}}{M_s} = \frac{28}{45} = 0.622$$

where: C_{famc} – the coefficient of aggregate phenotype for body mass;

P_{ve} – the weight of the economic value of body mass = 28;

M_s – the standard phenotypic standard of body mass for 18 months old ewes equals 45 kg;

The coefficient of the aggregate phenotype for the mother's milk production will be the same as for the 18-month-old rams and will, depending on the mother's lactation (*Lactation I =1.33; Lactation II =1.11; Lactation III =1.0*).

Thus, the complex selection index for 18-month-old ewes will have the following formula:

$$I_{m18} = (M_{fp} \cdot 2.0) + (M_{fmc} \cdot 0.622) + (M_{fpl} \cdot 1.33;\ or\ 1.11;\ or\ 1.0) \qquad (11)$$

where: I_{m18} – the complex selection index for 18-month-old ewes;

M_{fp} – the phenotypic size of the quality of the own skin, expressed in the score;

M_{fmc} – the phenotypic size of its own body mass, expressed in kg;

M_{fpl} – the phenotypic size of the mother's milk production, expressed in kg.

Example: Ewe no. 2809, 18 months old, the quality of the furskin "potr.-5" with a body mass 47 kg and the mother's milk production in the first lactation of 55 kg will have the following complex selection index:

$$I_{m2809} = (M_{fp} \cdot 2.0) + (M_{fmc} \cdot 0.622) + (M_{fpl} \cdot 1.33) = (5 \cdot 2.0) + (47 \cdot 0.622) + (55 \cdot 1.33) =$$
$$10 + 29.23 + 73.15 = 112.38$$

Based on this index, we can say that the breeding value of the ewe lamb no. 2809 is above the standard of the breed and is in the high ranks of the decreasing row of centralized bulletin evaluating the value of the youth sheep of the flock.

Analysing and generalizing the results of the researches regarding the breeding value of Moldavian Karakul youth sheep, we find that the principles of the construction of complex selection indices are the same as in the adult sheep. These principles are reflected in the previously published scientific papers [7, 8]. The resemblance refers, first of all, to the value of the aggregate phenotype for furskin quality, which is determined at evaluating (1-2 days after birth) for life. Secondly, the coefficients of the aggregate phenotype for mothers' milk production are also the same as for adult sheep.

At the same time, the aggregate phenotype coefficient for body mass of youth sheep varies greatly depending on its age because the body mass standard is different at different ages. It is important to note that one of the particularities of the principles of constructing the complex indicators of youth sheep selection is the specificity of determining the coefficient of aggregate phenotype for body mass and, in particular, the standard of this morpho-productive character at any age. Having developed standard body mass parameters at standard ages, the zootechnics specialist can determine the standard body mass of youth sheep at any marketing age, necessary to determine the aggregate phenotype and breed value after complex selection indices.

Conclusions

Determining the breed value of young Karakul sheep after the complex selection indices is one of the most objective and effective methods of assessing sheep quality.

The complex selection index for youth sheep can be determined at any age, from birth to adult age.

Determination of breeding value of Karakul lambs according to the complex selection indices is of scientific and practical importance, because it can be applied to their marketing at early ages.

The specificity of determining the aggregate phenotype coefficient for body mass is a particularity of the principles of construction of the complex selection indexes of youth Karakul sheep.

The size of the aggregate phenotype coefficient of body mass in youth sheep varies according to the standard of body mass not only at standard ages but also at intermediate ages.

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In Vitro Probiotic Properties of a Lactic Acid Bacteria Isolated from A Broiler Chicken

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Abstract

This study was conducted in order to evaluate in vitro the probiotic properties of lactic acid bacteria (LAB) isolated from ileum intestinal content tract of a healthy broiler (45-d-old Cobb 500). The isolate was assayed morphologically, culturally, biochemically. The tolerance to low pH an11d bile salt were tested as well. Phenotypically, the isolate strain was identified to be Lactobacillus acidophilus. The new strain was conserved as Lactobacillus acidophilus IBNA 64 in the Collection of INCDBNA.

It is Gram-positive Bacillus, thin, non-spore forming, appears isolated, rarely in the diploid form, in short chains or in small irregular piles in culture of 24h in Oxoid MRS broth and MRS agar medium.

The strain is an aerotolerant bacteria. The identification and analysis of the biochemical characteristics was performed by catalase assay, API 50 CHL Biomerieux strips, apiweb API 50 CHL V 5.1 soft (Lactobacillus acidophilus 1, 74.4% ID and L. crispatus 19.0% ID) and ABIS online (Lactobacillus acidophilus ~ 88.3%).

The strain showed good viability at pH 7±2 (8.32±0.330 log CFU/mL), respectively was able to resist at pH 3 (7.14±0.133 log CFU/mL) and 0.3% bile salts (7.56±0.19 log CFU/mL) during 3 h exposure. Results obtained provide some probiotic properties for L. acidophilus, but further studied will be done until to use for in vivo test in poultry feed.

Keywords: lactic acid bacteria, probiotic, chicken

Introduction

Lactobacillus spp. are part of normal poultry intestinal microbiota [1], being the largest reservoir of bacteria from animals [2]. The strains from this genus are characterized as Gram positive, catalase-negative able to produce lactic acid [3] as the main end product of carbohydrate fermentation [1, 4].

There is a balance between beneficial and non-beneficial bacteria in the gastrointestinal tract (GIT) of healthy and non-stressed broilers [2]. The lactobacilli are implied in normal microflora of animal status health [5] and could be considering probiotics with high benefits by improving intestinal microbial stability [3].

In general, probiotics based on lactic acid bacteria (LAB), used in poultry diets improve feed intake and digestion process [6], inhibit gastrointestinal pathogens and in the same time,

diminished susceptibility to diseases [7] by maintaining a healthy gut, maximize growth efficiency with beneficial effects on broiler performance [8].

Also, to obtain beneficial results, it is necessary that the probiotic candidate used as feed additive, to be removed some *in vitro* tests: resistance to low pH value from stomach, bile salts from intestine and their percentage of survival at these *in vitro* gastrointestinal conditions [9]; capacity to adhere to the host intestinal epithelium, to present antagonistic activity against pathogenic bacteria, to keep its viability during processing and storage of feed [9] etc.

According to the definition [10] "probiotics are live microorganisms when are administered in sufficient amounts, confer a health benefit on the host" [11].

Criteria for probiotic selection was to isolate, identify and phenotypically characterize a *Lactobacillus* spp. strain present, naturally, in the broiler GIT and to investigate their ability to colonize the chicken's gut (resistance to low pH, bile salts and percentage of survivability in these conditions).

Methodology

Bacterial strain media, growth conditions, isolation and identification

LABs present in GIT of a broiler chicken (45-d-old) was morphologically, culturally, and biochemically investigated. One g of ileum content from a healthy broiler was homogenized with 7 ml Oxoid BHI (Brain Heart Infusion) broth and 2 ml glycerol, and instantly frozen at –20^{o}C until testing (no more than three months) [12].

After defrost, the sample was supposed to decimal dilution in Oxoid PBS (Phosphate Buffered Saline) from 10-4 to 10-8 and from every dilution tube there were inoculated three Petri dishes with Oxoid MRS (de Man, Rogosa and Sharpe) agar. The culture was incubated overnight in microaerobic conditions (Jar with Anaerogen 2.5L from Oxoid). After 24 h incubation, the colonies were randomly selected from the plates and subcultured two times on a new MRS agar Petri dishes.

The cultural examination of the isolate was performed according to Bergey's Manual of Determinative Bacteriology by using the methods and criteria of Sharpe [12].

After morphological evaluation by Gram staining, the isolate strain from MRS agar was identified by biochemical tests (catalase assay, API 50 CHL Biomerieux strips), API 50 CHL V 5.1 and ABIS online soft [3, 13], according to manufacturer's instructions.

Preservation of bacterial strains

The pure culture was stored at room temperature and 4°C in MRS broth medium, respectively at -80°C with 20% (v/v) sterile glycerol, until the moment when the preservation viability will be tested. The strain can be found in the Collection of National Research Development Institute for Biology and Animal Nutrition Balotești (INCDBNA), Romania, under the code IBNA 64.

Determination of colony forming units (CFU/g intestinal content)

To determine the growth rate, the culture was cultivated on MRS medium (broth and agar), at 37°C, for 48h, in anaerobic conditions [3].

The catalase tests

The catalase test was performed according to the method described [3].

Acid tolerance

The overnight culture of *Lactobacillus* spp. [14] (7-8 log UFC/ml in PBS, pH 7.2), which was grown in anaerobiosis, was inoculated (1:10, v/v) in MRS broth adjusted to pH 3, with 1N HCl 37%.

The inoculated tube at pH 3, was incubated anaerobically at 37°C for 0 h, 1h:30 min. and 3h. After each incubation time, serial dilutions were performed (10-7) in sterile PBS. To determine the CFU/ml, 100 µl from 10-4-10-7 were dispersed on MRS agar plates (3 plates/dilution) and incubated at 37°C, 24 h, in anaerobiosis. Tolerance to low pH condition was estimated by comparing the CFU/ml after exposure to pH 3 with normal MRS broth (control, pH = 6.2±0.2), in the same growing conditions (37°C, 24 h, in anaerobiosis).

Bile salts tolerance

The overnight culture of *Lactobacillus* spp. was assayed according to the method [14], with minor modification. The isolate strain with a concentration of 7-8 log CFU/ml, was inoculated (1:10, v/v) in MRS broth with 0.3% (w/v) bile salts (oxgall, Sigma) at 37°C, for 0 h, 1:30 min. and 3h, in anaerobiosis.

The viability of *Lactobacillus* spp. strain was determined by estimating the number of colonies, by successive dilutions in sterile PBS (10-4 to 10-7), on MRS agar plates (3 plates/dilution), incubated at 37°C, 24 h, in anaerobic conditions.

The control sample was represented by the culture developed in MRS Oxoid broth (pH=6.2±0.2), without bile supplementation.

The survival percentage was calculated using the method presented [15]:

$$\text{Survival (\%)} = \frac{\text{Log}\, number\ of\ cells\ survived\ \left(\frac{CFU}{ml}\right) x\ 100}{\text{Log}\, number\ of\ initial\ cells\ inoculated\ \left(\frac{CFU}{ml}\right)}$$

Statistical Analysis

The analytical data were compared using variance analysis (ANOVA) with STATVIEW for Windows (SAS, version 6.0). The results were expressed as mean values and standard error of the mean (SEM), the differences between means considered statistically significant at $P<0.05$, using Tukey LSD test for untitled compact variable

Results

Bacterial strain media, growth conditions, isolation and identification

The taxonomic classification of bacterial strain in *Lactobacillus* spp. was performed by morphological (Gram positive bacilli, thin, non-spore forming rods, appears isolated, rarely in the diploid form, in short chains or in small irregular piles in culture of 24h in Oxoid MRS broth and MRS agar medium – Fig. 1), cultural (anaerobic growth) and biochemical characters (negative catalase test, [16]).

The isolate strain was identified by its ability to ferment different carbohydrates from API 50 CHL test kit (BioMerieux, S.A., France). The results are presented in Table 1. The strain was identified as *Lactobacillus acidophilus* as follow: *L. acidophilus* 1, -74.4% ID (% percentage of identification) by API 50 CHL V5.1, respectively *L. acidophilus*, -88.3% (% of similarity) by ABIS online. The fermentation capacity of our isolate was observed by a discoloration of the basal medium from purple to yellow, according to the manufacteur protocol.

The bacterial strain has been registered as *Lactobacillus acidophilus* IBNA 64 in IBNA Bacterial Collection.

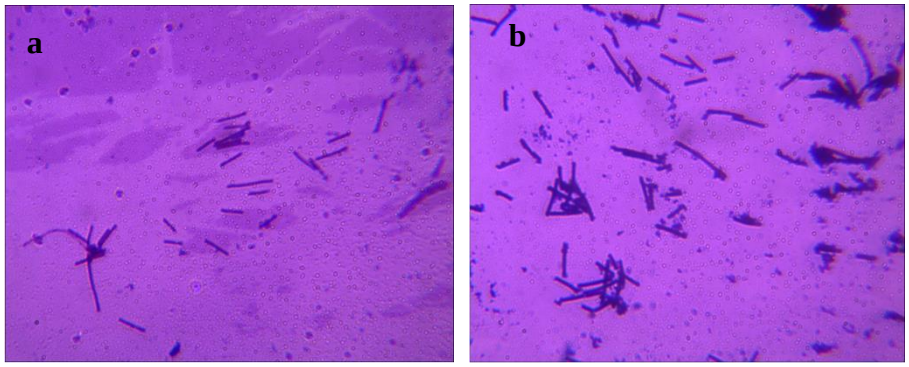

Fig. 1. *L. acidophilus* IBNA 64 anaerobe culture on MRS medium (Gram staining x 1000)
a) broth **b) agar**

Tab. 1. Biochemical characteristics of the *Lactobacillus* strain isolated from intestinal content of broiler chicken

Biochemical tests	Interpretation				
	24h	48h		24h	48h
Control	-	-	Esculin	+	+
Glycerol	-	-	Salicin	+	+
Erythritol	-	-	D-cellobiose	+	+
D-arabinose	-	-	D-maltose	+	+
L-arabinose	-	-	D-lactose	+	+
D-ribose	-	-	D-melibiose	-	+
D-xylose	-	-	D-saccharose (sucrose)	+	+
L-xylose	-	-	D-trehalose	+	+
D-adonitol	-	-	Inulin	-	-
Methyl-βD-xylopyranoside	-	-	D-melezitose	-	-
D-galactose	+	+	D-raffinose	+	+
D-glucose	+	+	Starch	?	+
D-fructose	+	+	Glycogen	-	-
D-mannose	-	+	Xylitol	-	-
L-sorbose	-	-	Gentibiose	?	+
L-rhamnose	-	-	D-turanose	-	-
Dulcitol	-	-	D-lyxose	-	-
Inositol	-	-	D-tagatose	-	-
D-mannitol	+	+	D-fucose	-	-
D-sorbitol	-	-	L-fucose	-	-
Methyl-αD-mannopyranoside	-	-	D-arabitol	-	-
Methyl-αD-glucopyranoside	-	-	L-arabitol	-	-
N-acetylglucosamine	-	-	Potassium gluconate	-	-
Amygdalin	+	+	Potassium 2-ketogluconate	-	-
Arbutin	+	+	Potassium 5-ketogluconate	-	-

"–" Negative test; "+" Positive test; "?" Weakly positive

In our study, the positive results were obtained for fermentation of amygdalin, D-melibiose, D-trehalose, starch and gentibiose, comparative with the literature [12] where these substrates were not fermented by another *L. acidophilus* strain.

Preservation of bacterial strains

The results of viability test for *Lactobacillus acidophilus* strain which are preserved at 4°C and room temperature are exposed in Table 2.

Tab. 2. The viability of *Lactobacillus acidophilus* preserved at 4°C and room temperature

4°C	Room temperature
-/66 day	-/45 days

L. acidophilus present only 66 days viability at 4°C *vs.* room temperature where the viability does not exceed 45 days. The strain isolated for intestinal content of broiler and its utilization as possible probiotic candidate must pass some in vitro tests for analyse its resistance under gastrointestinal conditions. A longer resistance of strains is an important probiotic trait for make it a good selection.

Determination of colony forming units (CFU/g intestinal content)

Lactobacillus acidophilus IBNA 64 present a good capacity for growth in MRS broth, $4.6x10^8$ CFU/g was registered by incubation at 37°C, 24 h, in anaerobic conditions.

Acid tolerance

To investigate the resistance of *Lactobacillus acidophilus* IBNA 64 in the presence of acid, the strain was exposed to low pH. The results obtained were presented in Table 3. The strain registered a good survival rate which differ significantly in comparison with the control ($P \leq 0.05$). However, with increase of incubation time at pH 3, the growth rate of *Lactobacillus acidophilus* IBNA 64 decreased.

Since, entering into the animal mouth [16], the lactobacilli must survive to difficult conditions as acidic environment from gastrointestinal tract.

The stomach has a low pH between 1.5-3.5, due to gastric juice secretion, and the intestine incline to alkaline pH values between 8-8.5 [16, 17]. Also, the pH of gastric juice depends on the animal feeding time, growing stage, between 2.0 to 3.5 [13]. In the present study, the results obtained confirm that the isolate strain presented a survival rate after 3 h (85.81%) at pH 3. One of the critical properties of a bacterial probiotic is the ability to tolerate the low pH from the stomach and in the same time, to survive to the high concentration of bile salts from GIT. These traits are in generally, evaluated as preliminary tests for selected a possible probiotic strain [19].

The lactobacilli have the properties to ferment the carbohydrates group to lactic acid [3]; by their development, lactobacilli determine acidification of raw materials from feed.

Tab. 3. The resistance of *Lactobacillus acidophilus* IBNA 64 to low pH

Strain	Initial $\log_{10}$ CFU/ml	pH 3				
		0 min.	1h:30 min.	3 h	*SEM*	*P value*
L. acidophilus IBNA 64	8.32^{a}	8.41^{b}	8.07^{c}	7.14^{d}	0.162	0.0007
	% of viability	101.08%	96.99%	85.81%	na	na

*Values are the means of three independent experiments (n=3). ^{abcd} Means in the same row differ significantly at $P<0.05$. na= not applied

Bile salts tolerance

The results from Table 4 showed that the isolate strain resist to 0.3% oxgall bile salts concentration. The value obtained after 3 h exposure to bile salts was 10.05% less than initial strain concentration.

Tab. 4. The resistance of *Lactobacillus acidophilus* IBNA 64 to bile salts

Strain	Initial log_{10} CFU/ml	0.3% bile salts				
		0 min.	1h:30 min.	3 h	*SEM*	*P value*
L. acidophilus IBNA 64	8.32^{d}	7.43^{a}	7.59^{b}	7.56^{c}	0.117	0.0029
	% of viability	89.30%	91.22%	90.86%	na	na

*Values are the means of three independent experiments (n=3). ^{abcd}Means in the same row differ significantly at P<0.05. na= not applied

The maximum survival rate was showed after 1h:30 min a good viability percentage 91.22%.

The ability to survive under high bile salts concentration and low pH, are the important characteristics for the successful passage through the gastrointestinal tract [20]. The strain was exposed to artificial simulated conditions and their viability was higher than 85% both to low pH and to bile salts. The assay on bile salts during 3 h of incubation at 37°C, in anaerobiosis conditions, showed that the isolate strain differ significantly ($P \leq 0.05$) between all times of incubation. The viability in our research was similar to literature data [21].

Conclusions

Gastrointestinal tract is a good source of lactic acid bacteria. From the result of our study, *Lactobacillus acidophilus* IBNA 64 presents high potential properties with a good viability at pH 7±2 (8.32 log CFU/ml), respectively was able to resist at pH 3 (7.14 log CFU/ml) and 0.3% bile salts (7.56 log CFU/ml) during 3 h exposure.

The obtained results, indicated that poultry intestine is a good resource to isolate lactic acid bacteria.

Our isolate strain provides some probiotic properties, but furthermore *in vitro* and *in vivo* studies must be performed until its usage as feed additive in poultry feed.

Acknowledgements

This study was funded by the Romanian Ministry of Research and Innovation through Program 1 – Development National Research-Development, Sub-program 1.2 – Institutional Performance – Projects funding excellence in R & D, Contract no. 17 PFE and Project 8PCCDI/2018 pc2.

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Preliminary Results of Artificial Insemination with Fresh Diluted Semen During Natural Estrous At Ewes

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Abstract

Based on the context of the development and extension of the ewe's exploitation system and on the application of the European legislation en force regarding the conditions for reproduction and certification from genealogical registry, the main concern of the sheep breeders became the assisted breeding with rams of known origin and genetic value. Thus, the interest for artificial insemination has increased among sheep breeders who are facing a shortage of rams with known genetic value, pure breed, in the application of selection and breeding programs. By intensive use of 5 rams in the natural breeding of 195 Palas Merinos, the male exhaustion and a rate of return to estrous cycle of 53.84% were obtained. After a 5 days of stimulating feeding diet and sexual rest of the rams, the semen was collected and qualitatively analysed. 105 ewes at the 2nd or 3rd estrous cycle were artificially inseminated. The insemination was performed during natural estrous, on the day of the detection of estrous, with diluted raw semen, with a motility of over 80%. The rate of non-returning to the estrous at 15-19 days from artificial insemination was of 84.76%. Diagnosis confirmation was made by transabdominal ultrasound at 45-50 days after artificial insemination and it indicated a gestation rate of 74.28%.

Keywords: ewes, artificial insemination, semen

Introduction

The present study was carried out as a result of the need to perform artificial insemination with semen from purebred rams with high genetic value. By the European Regulation 1012/2016 en force, for keeping animals in the racial genealogical register, females are allowed to be bred only with purebred males. By applying selection and improvement methods, the hierarchy and selection of the Merinos de Palas rams was done, so that a number of sheep breeders were unable to perform assisted natural breeding with the available rams from their own herd. Rams' physical and/or reproductive exhaustion have been reached by their excessive and irrational use for assisted natural breeding. In these conditions, it is necessary to verify the ram's semen quality in terms of mobility and viability [1], [2], [3] and to establish its suitability for artificial collection, dilution and insemination. Artificial insemination is an efficiently assisted breeding method that has expanded and become a common practice for sheep farmers in Europe. Thus, in France, in 2017, there were nine artificial insemination centers carrying out more than 800,000 insemination /year for ewes of dairy breeds [4], number which is maintaining almost constant for over 20 years. In France, where there is a network of insemination centers and semen collection and preservation centers, artificial insemination is

performed with frozen seminal material, thus allowing a better diffusion in time and space of valuable genetic material. In our country there is only the Institute of Research-Development for the Breeding of Ewes and Goats from Palas, Constanta and six Research and Development Stations for the Breeding of Ewes and Goats, which can carry out the actions of collecting and preserving semen. Because, breeding of small ruminants on Romanian territory has developed by increasing the number of herds raised traditionally, without moving to intensive agriculture with the application of breeding biotechnologies, the requirement for artificial insemination is quite low. A greater number of artificial inseminations was performed on goats, where the results were very good. So, after insemination with cooled semen achieved a rate of non-returning to estrous after two estrous cycles of 87% and a fecundity of 82% and after insemination with fresh diluted semen achieved a rate of non-returning to estrous after two estrous cycles of 92% and a fecundity of 88% [5].

Material and Methods

At the beginning of June, a sheep breeder from Constanta county, owner of a herd of 195 ewes and 5 rams Merinos de Palas, constituted 5 lots of females of 39 ewes, each lot being assigned a male. He carried out the assisted breeding by tracing the estrus every morning and evening, followed by the natural breeding of the ewes in estrus. The ewes found in estrus were not individually mounted, but on the day of the estrus they were kept together with the male, in a common box, for 5-6 hours, the ram carrying out the successive breeding of the same female.

Through the male effect, the synchronization of the females in the second cycle was achieved, so that a ram could mount 7-10 ewes/day, without giving the rest pause and no additional stimulating feeding was applied before and during the breeding period. In this activity, the breeder did not take into account the number of ewes/male/day and did not grant the necessary sexual rest in case of intense use, respectively performing 4-5 mounts/day for 3-5 consecutive days. Thus, during July, some of the ewes already mounted showed estrus, which demonstrates that the mount was not fertile. In these conditions, the sheep breeder called on the specialists from ICDCOC Palas, Constanta to complete the 2019 breeding campaign.

The first action carried out by the specialists of the Laboratory of Reproduction and Biotechnologies of ICDCOC Palas, Constanta was to verify the semen of the rams. Thus, after a 10-day sexual rest and stimulating feeding with a good quality fan, 500 g barley and 100 g carrot/ram/day, the semen was collected by artificial vagina and quantity (ejaculate volume) and quality (colour, density, motility, viability) were evaluated. The estimation of the seminal material was achieved both at the beginning of the collection from all 5 males as well as of each ejaculation collected during the entire period of semen collection and artificial insemination.

The quantitative assessment was made immediately after collection by reading the ejaculated volume directly on the gradations of the collecting glass, obtaining between 0.5 and 2.5 ml. The colour was appreciated through the transparency of the collecting glass, ranging from slightly white-yellow (normal colour) to white-brown (present in an ejaculate) [6], [7]. Once with appreciation of colour, the degree of viscosity was established too, being between the normal consistency of the cream and liquid, when the ejaculates contained seminal liquid. The motility and density were assessed firstly by microscopic examination between blade and lamella of a drop of raw semen, and secondly after dilution with TRIS extender. Ejaculates with high and very high spermatozoa concentration, over 1.2x109 spermatozoa/ml (thick and very thick semen) are diluted with TRIS extender in proportion of 1:4 and those with average concentration are diluted in ration of 1:3, so that they can reach a concentration of 800x106 spermatozoa/ml, respectively 400x106 spermatozoa/ml for an insemination dose. For the density assessment of over 3.5 billion of spermatozoa/ml, correspondent for a thick and very thick semen, the distance between spermatozoa must be smaller or equal to a spermatozoa head

length. In this situation the values of these parameters are similar to the results of the measurements made on the semen of 241 adult rams who recorded an average concentration of over 3x 109 spz/ml with a motility of over 80% [8]. The motility expressed by percentage, estimates the quantity of the mobile spermatozoa that performs forward movements. Viability was assessed by the vital coloration of eosin-nigrosine, by which the percentage of living spermatozoa was established. The qualitative parameters of the semen collected and diluted depends also on the type of extender used, respectively based on skimmed milk, citrate medium, TRIS medium) [9], [10] as well as on the period of time passed between collection-dilution and the time when artificial insemination is performed [11]. In the present study we used as dilution medium – TRIS solution and the insemination were performed at maximum 1.5 hours after collection, the diluted semen being maintained during this time on a water bath at 30-35^{0}C [12].

Following the first evaluation of ejaculates from the 5 rams, one ram was removed from reproduction, having yellow-brown, very rare semen. This male was examined during the entire artificial insemination, period at an interval of 3-4 days, observing an improvement of the sperm parameters, with an average dense semen and a motility of 40-60%. In these conditions the ram can be used on natural breeding with a moderate rate of use but it is not recommended the collection of semen and its dilution for artificial insemination. The artificial insemination of the ewes was done during natural estrus, following the detection of the ewes in heat by rams in heat trying with luck. Insemination was performed with 0.5 ml of diluted raw semen (in a ratio of 1: 4), with an average concentration of 400 x 10^6 spermatozoa/dose. For artificial insemination the vaginal speculum was used with its own light, visualizing the place of semen deposition, respectively at the level of the involved flora. The penetration of the insemination dropper into the cervix may vary according to its opening, respectively by the moment of the estrous cycle.

When the cervix was closed and the insemination dropper did not penetrate at least 0.5-1 cm, the semen was deposited at the vaginal level. These ewes showed estrus also the next day, so they were re-examined and artificially inseminated.

Results and Discussion

During July and the first third of August, the ewes in heat were detected, establishing a percentage of 53.85% rate of returns (105 ewes returned out of 195 ewes naturally mounted).

Of these ewes, 29 ewes (14.88% of the total number of mounted ewes and 27.62% of the returns respectively) were from the group assigned to the male who, when checking the semen, had the unfavourable results, having a very poor-quality semen. The gestation rate of the naturally mounted ewes with this male was 25.64%. At the other 4 batches, between 17 and 20 females returned in estrus, and only19-22 ewes/ram were considered pregnant, thus having a gestation rate of 48.72-51.28%. At the beginning of the action of estrus screening at ewes with trying rams, in all five groups of adult females from 2nd up to 4th parturition, a good synchronization of the estrous cycle is observed which demonstrates a good response to the male effect since the beginning of the breeding campaign. The manifestation of synchronized estrus in a large number of females and the lack of supervision of the breeding group explains the high rate of return to estrus [13]. At the beginning of the estrous screening followed by artificial insemination, the non-pregnant females were in the second or third estrous cycle.

Table 1 presents the structure of the groups of females that have been artificially inseminated, and formed didactically to evaluate the results. Thus, 79 ewes (40.51%) were in the second estrous cycle and 26 ewes were in the third estrous cycle (13.33% already had two unfertilized natural mounts).

After insemination of these 105 ewes, a non-return rate of 15.24% was recorded in the next estrous cycle, respectively 12 ewes inseminated in the second estrous cycle (11.43%) and 4 ewes in the third estrous cycle (3.81%). Because it was practiced the assisted breeding in cluster

(group) the rams often mounted the same ewes on the day when they were in heat having as consequence the physical and reproductive exhaustion of the males. The ewes that were in the second estrous cycle at the time of insemination had a delayed beginning of sexual activity and because the rams did not have the period of sexual rest for recovery they did not mount anymore or had improper ejaculation.

Table 1. Distribution of experimental batches of ewes

Total females	195	Return rate after NB* (%)	Return rate after AI*	
			Nr.	%
Total females artificially inseminated	105	53,84	16	15,24
Artificially inseminated ewes at the 2nd estrous cycle	79	40,51	12	11,43
Artificially inseminated ewes at the 3rd estrous cycle	26	13,33	4	3,81

NB – natural breeding, AI* – artificial insemination*

As a result of the artificial insemination with diluted raw semen, at the next detection of estrus, the first assessment of the gestation rate was performed between 16 and 20 days, which was 84.76% distributed in 63.81% of artificial inseminated ewes on the second estrous cycle (67 ewes) and 20.95% ewes inseminated on the third estrous cycle (22 ewes). (Table 2).

Table 2. Gestation rate in artificially inseminated ewes

Specification	Ewes No. AI	No. of pregnant ewes	GR* %	No of pregnant ewes ECO*	GR-ECO* %
Artificially inseminated ewes at the 2nd estrous cycle	79	67	63,81	64	60,95
Artificially inseminated ewes at the 3rd estrous cycle	26	22	20,95	14	13,33
Total females artificially inseminated	105	89	84,76	78	74,28

AI – artificial insemination, GR* – gestation rate, ECO* – ultrasound, GR-ECO* – GR after ECO*

During September, at 45-50 days after the artificial insemination, ultrasounds were performed, establishing the number of safe pregnant ewes by visualizing the uterus and the caruncular uterus. Thus, confirmation of pregnancy was achieved for 78 ewes (74.28%), respectively 64 ewes (60.95%) in the second estrous cycle and 14 ewes (13.33%) in the third estrous cycle. For the ewes artificially inseminated in the 2nd estrous cycle, 95.52% the diagnosis was confirmed by ultrasound (64 pregnant ewes out of 67 ewes non-returned in the estrus), while for the ewes inseminated in the third cycle the percentage of ultrasound confirmation of gestation is of 63.63% (14 pregnant ewes out of 22 ewes non-returned in the estrus). This higher percentage and the difference between the two female categories indicate the female's infertility. These results are similar to data reported by other authors. Thus, fertility at Turcana ewes, estimated basis on the non-return syndrome is 94.8% and the pregnancy rate established by ultrasound is 89.5% [14]. The rate of non-return to estrus for artificially inseminated goats, during non-breeding season, with hormonal induced estrous activity is of 94.3% and the gestation rate confirmed by ultrasound is of 84.3%.[15]. Other results regarding the variation of the reproduction indices according to the type of breeding and semen method of preservation indicate a gestation rate of 67% consecutive to natural breeding, 43% after artificial insemination with frozen semen and 78% after artificial insemination with freshly diluted semen when females had induced and synchronized estrus [16]. The gestation rate is influenced by maintaining the semen's viability during transportation, so that a longer time, of over 8 hours, from collection to artificial insemination causes smaller indicators. A gestation

rate of 86.70% was obtained after natural breeding, compared to a gestation rate of 64% after artificial insemination about 8 hours after semen's collection [17].

Conclusions

Based on the applicability of the new European legislative provisions, several farmers, sheep owners registered in the Genealogical Register, are facing a deficit of purebred rams, tested by performance. In order to ensure the assisted reproduction, it is necessary to synchronize the estrous cycle on groups of females according to the number of available males or to screen the estrus with male testers and to collect the semen of the males so as to ensure the artificial insemination of a large number of females in the estrous phase of the sexual cycle. An essential condition for the success of the breeding campaign is the analysis of the ram's semen that will be used for breeding. The rams selected on genealogy and productivity criteria may have poor reproductive performance, a semen with low density and motility, with an increased percentage of spermatozoa's abnormalities or even azoospermia. Therefore, performing the spermogram before using it in the natural breeding is mandatory. Particular attention should be paid to breeding preparation not only of the females but also of the males that should be provided with proper feeding quality and quantities. Very important is the intensity of use in the natural breeding or in the semen collecting programs so that it does not reach the physical and sexual exhaustion of rams. It is compulsory to correlate the rhythm of use at the mount or the semen collection with the rest periods that will ensure the recovery of the semen reserve and the maintenance of an adequate livelihood, without excesses.

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Endangered Romanian Cattle Breeds – Between Traditional Breeding and Genetic Conservation

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Abstract

In Romania, the Grey steppe and Pinzgauer cattle are included in a genetic program of conservation, due to their valuable genetic pool for increased resistance to diseases and high adaptability to severe environmental conditions. Also, these indigenous cattle breeds are part of the history of our country, as different varieties of them represent the same in their countries of formation. Methods applied worldwide in this aim of genetic conservation evolved considerably over the time, and the results reported are interesting on the view of their phylogeny, site of primary formation, and subsequently migration. The present work aims to review the most important aspects related to these two indigenous cattle breeds reared in Romania, the most important genetic markers and techniques which could be applied in further programs of their genetic characterization and preservation.

Keywords: indigenous cattle, resistance, conservation

Introduction

The Grey steppe cattle breed and, also the Pinzgauer, are considered threatened with extinction cattle breeds in Romania, in 2000 being maintained, by Food and Agriculture Organization of the United Nations (FAO), the "endangered-maintained" status for both of them. FAO reports normally draw the attention on the numerical decline of different species, various breeds being ranked, considering the number of individuals for each breed, the female/male ratio, and their including in active programs of preservation or maintenance by companies or research institutions, as one of the following categories (listed in their decreasing order of gravity): extinct, critical, endangered, critical-maintained, endangered-maintained, not at risk [1]. In the case of Grey steppe cattle, the presented FAO data showed a different status for individuals spread all over the Europe, ranging from a "critical" rank (Grey Steppe from Bulgaria, Greece, German cattle population of Hungarian Grey cattle) to and "endangered-maintained" one, such as for Romanian Grey Steppe population (or "Sura de stepă" as is named in our national language) (Table 1).

This classification was performed using data reported before 2000 year, but in many cases an appropriate number of animals is still reported in specific researches published up to today ([2], [3], [4], [5], [6], [7], [8]). But not all the Grey steppe varieties presented a continuous decline in their number. For example, the Hungarian variety even increase in its number after 1970 as a result of a good management of these genetic resources in order to serve touristic purposes and not only [9].

Table 1. Grey Steppe cattle populations size and their status according to FAO ranks

Grey Steppe breed	Country	Population data		Status
		Year	No. of animals	
***according to (1)**				
Iskar Grey *(Bulgarian Grey)*	*Bulgaria*	1994	245, 120♀, 5♂	*critical*
Istrian	*Croatia*	1995	110, 103♀, 7♂	*critical-maintained*
Slavonian Podolian *(Slavonian Syrmian)*	*Croatia*	1995	20, 12♀, 3♂	*critical-maintained*
Katerini	*Greece*	1995	80♀, 5♂	*critical*
Sykia	*Greece*	1995	90♀, 5♂	*critical*
Hungarian Grey Steppe	*Austria*	1994	10♀, 4♂	*critical-maintained*
	Germany	1997	36♀, 5♂	*critical*
Sura de stepă *(Moldavian variety)*	*Romania*	1993	350♀, 12♂	*endangered-maintained*
Ukrainian Grey	*Ukraine*	1990	1500, 684♀, 13♂	*endangered*

In the case of Pinzgauer, a polled variety named Jochberger Hummeln found in Bezirk Kitzbühel, Tirol, received a *"critical-maintained"* status, with 30 females and 4 males reported in 1994. In Germany, the Pinzgauer cattle received an *"endangered"* status, with 286 females registered in the herd book, and 6 males. A similar status was reported for Pinzgauer Fleischnutzung, with 906 females and 42 males registered in the herd book of Germany in 1997.

In Romania, the Pinzgauer cattle or *"Pinzgau de Transilvania"* as is reported in Romanian language, included 1092 females registered in the national herd book (Table 2), the semen of 23 males being stored at that times of 1993. However, the reported population trend was considered as decreasing [1].

Table 2. Pinzgauer cattle populations size and their status according to FAO ranks

Pinzgauer breed	Country	Population data registered in the herd books		Status
		Year	No. of animals	
***according to (1)**				
Jochberger Hummeln	*Austria*	1994	30♀, 4♂	*critical-maintained*
Pinzgauer	*Germany*	1997	286♀, 6♂	*endangered*
Pinzgauer Fleischnutzung	*Germany*	1997	906♀, 42♂	*endangered*
Pinzgau de Transilvania	*Romania*	1993	1092♀, 7♂	*endangered-maintained*

Both Grey steppe and Pinzgauer cattle are considered, in Romania, autochthonous, the first one – primitive, and the last one – improved. Their origin, aim of breeding, and main phenotypic characteristics were well [9], and presented throughout the time in many scientific papers. In

the same way it is worth mentioning the effort of characterization of these Romanian indigenous endangered cattle breeds in terms of various genetic markers ([10], [11], [12], [13], [14], [15], [16], [17], [18], [19]). In this context, the aim of this paper is to briefly present the most important aspects related to Grey steppe and Pinzgauer cattle breeding in Romania, the most important genetic markers for milk and meat production, and molecular techniques which could be applied in further programs of genetic characterization and preservation of these breeds in Romania.

Methodology

In order to achieve the assumed objectives of this study, there were consulted 53 references on the chosen topic. The most important aspects are presented in three different sections including: (i) Grey steppe and Pinzgauer cattle breeding, and their morphological and productive evaluation; (ii) genetic markers in the study of threatened with extinction cattle breeds; (iii) techniques used in cattle's DNA or protein analysis. In order to describe phenotypically and genetically the two breeds of cattle that are in genetic conservation in Romania, 4 books from the specialized literature were consulted, respectively 49 scientific articles from different national and international databases. Genetic markers associated with milk production have studied 26 scientific articles, while genetic markers associated with meat production have been highlighted in a number of 14 scientific articles. The techniques used in the analysis of DNA and polymorphism of major milk proteins respectively were studied from a number of 9 bibliographic sources.

Grey steppe and Pinzgauer cattle breeding, and their morphological and productive evaluation

The Grey Steppe breed represents one of the oldest indigenous breeds which belongs to Bos genus, Taurus subgenus, Primigenius species, the horned Taurine subspecies, being known in the popular language as "bour". Till 1850, the national herd book consisted of two indigenous breeds, the Grey steppe and Mocăniţa, the first one being more spread in the steppe and hill areas, and the other one, in the mountain areas. In the formation of these breeds were considered a higher influence of the environmental conditions, and less and even insignificant of man's intervention [14].

The Pinzgauer breed is found out in the Romania's mountain areas at over 1000 m altitude.

It originates from Austria, Salzburg, Tyrol, an alpine and subalpine area. The breed was firstly formed between 1690-1740 by the crossing of local red bulls with the Berna type of Switzerland; after 1740, the resulted animals were reproductive used in a true breed [20].

Nowadays, the interest of farmers all over the world is represented by specialized cattle breeds on milk or meat production, which makes financially profitable this activity of animal breeding. Although the Pinzgauer is considered improved indigenous cattle breed in Romania, its recorded productions are still lower than those of highly specialized cattle breeds reared in Romania and not only (2000-2500 kg milk/year, 3.8% fat, 400-500 kg the weight of cows, 650-700 kg the weight of bulls, 800-900 kg the weight of oxen).

Considering the Grey steppe breed, the recorded productions are even lower; the milk production of cows raised in households is of 800-900 kg/year, and of 1000-2500 kg for those raised in farms, with a content of 4-6% fat [9].

The main physical characteristics which emphasize some morphological differences between Grey Steppe males and females are shown in table 3, and of Pinzgauer cattle, in table 4.

Table 3. Some morphological differences of varied types of Grey Steppe cattle breed

Grey Steppe cattle breed	Average adult weight (kg)		Average wither height (cm)		Reference
	males	females	males	females	
Iskar Grey *(Bulgarian Grey)*	750	350	140	118	*
Istrian	900	625	148	138	*
Slavonian Podolian*(Slavonian Syrmian)*	600	460	135	128	*
	600-800 (1000 for oxen)	470	135-145	128	**
Katerini	400	285	123	113	*
Sykia	-	-	-	116	*
Hungarian Grey Steppe	900	600	150	140	*
Iugoslav Steppe	800	500	150	135	*
Romanian Steppe *(Moldavian variety)*	780	480	137	129	*
	744	488	-	-	***
Turkish Grey Steppe	470	375.07	126	117.98	****
Ukrainian Grey	780	480	137	129	*

* according to Scherf, 2000;
** according to Keros *et al*., 2015
*** according to Dascălu *et al*., 2012;
**** according to Soysal and Kök, 2008.

Table 4. Some morphological differences of varied types of Pinzgauer breed

Pinzgauer cattle breed	Average adult weight (kg)		Average wither height (cm)		Reference
	males	females	males	females	
JochbergerHummeln	1200	700	151	139	*
Pinzgauer	-	750	-	140	
PinzgauerFleischnutzung	-	-	-	-	
Pinzgau de Transilvania	900	500	134	127	

* according to Scherf, 2000; "-" unreported data in the studied reference

Excepting a fatty milk, which may be considered by some as an advantage for Grey steppe cattle, its meat is darker and not marbled, being less preferred by human consumers, although one of the original purposes of this breed raising was for meat production beside draught power.

The last decades mechanization of agriculture led even this final goal fall. Even so, researchers do not lose their interest in studying these animals, and also those of Pinzgauer breed, at their genetic level being considered a real reservoir of genes linked to various environmental factors resistance, including climatic conditions and various parasites or infectious pathogenic agents.

Genetic markers in the study of threatened with extinction cattle breeds

Techniques of genetic analysis evolved considerably over the time, allowing the genome to be sequenced in various species of interest. The phylogeny of many individuals was studied by researchers since 1980. Another types of genetic research were focused on the study of genetic markers associated to characters of productions, in the case of cattle breeds threatened with extinction this being useful for the appreciation of genetic resource conservation value related to animal origin, the degree of uniformity of breed and, corroborated with many other sensitive molecular markers, their place of formation and domestication.

In Romania, the study of genetic polymorphisms in the major proteins of milk (caseins, lactalbumin, and lactoglobuline) of various native farm species, and the possibility of using them as genetic markers for increasing milk quality or identifying the authenticity/origin of milk and other dairy products, were constantly worked by Ilie D.E. and Bâlteanu A.V. teams.

Isoelectric focusing (IEF) and PCR techniques were successfully tested in Romania for the characterization of milk protein polymorphisms. The allele α_{S1}-casein I^{SM} discovered in the Grey Steppe breed was not reported in any other European cattle breed, being an ancestral allele that originates directly from the wild ancestors of the breed, providing the first molecular proof of the phylogenetic position of the breed, which is extremely necessary in the context of its conservation ([10], [21], [22]). Although not in a Romanian population, it seems that Pinzgauer breed is also a carrier of a particular allele. IEF investigated milk samples of Pinzgauer individuals located in Austria and Bavaria, Germany by Erhardt (1996), revealed a new κ-casein variant (κ-casein G) with a frequency of 0.003, allele which was not found in milk samples of Limpurger, another endangered breed which was investigated. However, the family of milk proteins is large, with a significant influence on the milk composition and its physico-chemical properties, which were well documented in the past years for various alleles and genotypes at each locus in part. In an overview of all caseins, in most cases results of various associations are contradictory due to their location in linkage in the structure of the 6th bovine chromosomal pair. When considering caseins, for many scientists and cattle breeders is easier to take into account the genetic structure for κ-casein. Therefore, the κ-casein A allele is well known to be associated with increased milk yield ([23], [24]) and the B allele, with higher κ-casein concentration ([25], [26]), protein and fat yield ([27], [28]), a better reaction with chymosin, a significantly lower clotting time, and a higher rate of curd formation ([20], [30]). Considering Grey steppe cattle and Pinzgauer indigenous cattle breeds, their characterization on milk protein polymorphism [9] showed a higher frequency of B allele at κ-casein locus in Grey steppe cattle, and of its A allele, in case of Romanian Pinzgauer. This A allele was reported by [31] in the Original Pinzgauer cattle in a unique combination of alleles for milk protein loci located on the 6th chromosome (C-A^2-B-A, encoding the following proteins: α_{S1}-, β-, α_{S2}-, and κ-casein, respectively). In the case of whey proteins, β-lactoglobulin is mostly evaluated due to its polymorphism, the B type and BB genotype being reported in associations with desirable traits for milk industry, such as fat percentage [27], fat and cheese yield, shorter coagulation time and a higher thermal stability of proteins ([27], [32], [33], [34], [35]). In this case, the higher frequency of A allele in Grey steppe cattle was reported over the time, and of B allele, in Romanian Pinzgauer [9].

Beside these, there are many other genes associated with milk production, for example the Pituitary Transcription Factor and the Growth Hormone gene. The polymorphisms of Pituitary Factor 1 (POU1F1 or PIT1) and Growth Hormone Receptor (GHR) genes were investigated on Romanian Grey Steppe by ([36], [12]). The investigation of 60 blood samples showed two alleles at PIT1 locus, the B allele being prevalent to A variant also in Podolica breed [13], although the A allele was found to be desirable to milk production and body conformation, for example in Holstein Friesian, Polish Black and White, Romanian Simmental, and Maramureș Brown [12]. Even so, investigating 352 Holstein cattle for PIT1 gene polymorphism, Bayram *et al*., (2017) reported the largest frequency of B allele (0.68) as a part of these two identified alleles at this locus. Investigating the polymorphism in exon 6 of PIT1 gene in South Anatolian Red and East Anatolian Red cattle, a low linkage with dairy manufacturing traits was reported by [1]. Considering the GHR gene, the A allele was reported in several cattle breeds to be associated with higher fat and protein percentages; the genotyping of 60 blood samples collected from Romanian Grey Steppe revealed only the including T allele homozygous genotype [36].

In the case of meat production and its quality, it was established an influence of various alleles of some genes, for example Leptin (LEP) and Calpastatin (CAST), on fleshiness, succulence, degree of marinating, carcass quality, and body weight. Bayram *et al*., (2008) established a higher frequency of A allele of LEP gene in a Holstein Friesian population of 352 cows, considering only two alleles investigated at this locus (A and B). Investigating the

polymorphism of the bovine LEP gene at the level of Arg4Cys and Ala59Val amino acids, Komisarek *et al*., (2005) reported in 187 Black-and-White AI bulls with an average Holstein Friesian gene share of 96.4%, the following frequencies of the genes: for Arg4Cys, 0.55(C) and 0.45(T), and for Ala59Val, 0.73(C) and 0.27(T). The authors of the investigation concluded that the Arg4Cys TT genotype had a positive impact on milk yield growing and protein output, and no significant impact on butterfat yield, even of its greatly reducing. On the other hand, the Ala59Val polymorphism was not reported to affect milk and protein amounts, but was considered by the authors to be responsible for the quantity of obtained butterfat. No significant association was reported by [37] for the leptin receptor gene (LEPR) with reproductive parameters or daily weight gain in three cattle breeds (Brangus Ibagé, Charolais, and Aberdeen Angus). Considering the Calpastatin gene (CAST), in an investigated population of 71 Turkish Grey Steppe and 61 first-generation crossbreeds of Turkish Grey Steppe and Brown Swiss, two genetic variants (C and G) were reported by [38] for the CAST/Rsal polymorphism, with a higher frequency of C allele in both investigated groups. The aforementioned authors argued that the two single nucleotide polymorphisms found in the CAST gene in intron 5 between exons 5 and 6, which correspond to cross location change between cytosine and guanine, entitle C allele as the most favourable, the genotype CC yielded beef that was more tender than the beef obtained from GG genotype carriers, the CG genotype being correlate with intermediate beef tenderness.

Another such types of investigations on different types of Grey Steppe breed cover the study of haemoglobin (Hb), transferrin (Tf), and potassium (K) in erythrocytes polymorphisms ([39], [40], [41], [7]). Three Hb variants were reviewed in Grey Steppe cattle by [7], the highest average frequency being for A type (more than 90%), followed by B and C alleles. On the basis of vertical electrophoresys interpretation using polyacrylamide as a migration support, a single type corresponding to the A type was observed in Romanian Grey Steppe by [40]. Beside the common variants of Hb gene, an unusual hemogloblin polymorphism was recognized in Italian Podolic cattle, involving AY and A^{zebu} variants, which were not detected in other sampled breeds [39]. On the serum transferrin locus, seven alleles were reported in Grey Steppe cattle (A, B, D, D_1, D_2, E, and F), of which A and B variants were reported at a frequency ranged from 0.21 to 0.43, and 0.02 to 0.05 respectively, the average frequencies for other reported allele being in the following decreasing order: $D>D_1=D_2>E>F$. In Romanian Grey Steppe, three alleles were detected at serum transferrin locus, D variant being reported at more than a half of the investigated population; as in previous mentioned report, E allele was found at a lower incidence that A allele. The EE genotype was found to be associated with the highest milk yield, and any of E included allele in genotype seems to be associated with a good milk production, a higher fat percentage, and a higher performance for fat yield. The lowest performances were reported for cows with AD genotype, if there are considered the milk quantity and fat percentage features, and for DD genotype for fat yield. The potassium polymorphism in erythrocytes of Turkish Grey Steppe was investigated by [41], who identified two alleles, K^H and K^L, according to those different red cell potassium concentration types, LK and HK, with a lower and higher potassium concentration than 46 m-equiv l^{-1}, respectively. The reported frequencies in the investigated population of 39 individuals of Grey Steppe breed Turkish variety, showed a higher incidence of K^L allele at a just over ¾ of the individuals, which is in agreement with the highest frequency of K^L allele found by Gonzales and Vallego (1983) in Sayaguesa, Morucha, CordenaAndokza, and Blanca Cocerona breeds [41].

Techniques used in cattle's DNA or protein analysis

In the investigation of the genetic material usually are used several steps of working assuming: (i) the extraction of total genomic DNA from blood samples; (ii) the DNA quantification (purity and concentration determination) by spectrophotometry; (iii) the DNA

revealing by agarose gel migration technique; (iv) the amplification of genes of interest by PCR technique (Polymerase Chain Reaction).

(i) The extraction of total genomic DNA from bovine blood samples can be accomplished by several methods, the most well-known of them involving the extraction with Wizard ™ Genomic DNA Purification Kit; the rapid method of extraction of bovine blood DNA; and the automated method of extracting bovine DNA from blood.

(ii) The determination of the concentration and purity of DNA extracted samples can be performed by spectrophotometry by measuring the total absorbance of the extract at wavelengths of 260 nm and 280 nm. The purity of extracted DNA samples is estimated based on A260/A280 ratio (A=absorbance), and the sample concentration is automatically calculated using a specialized software. DNA integrity is appreciated by a technical migration in agarose gel [42].

(iii) Electrophoresis is a technique of separating molecules according to their molecular mass. Worldwide is a large number of electrophoretic methods applied to the analysis of various polymorphisms in cattle, the most commonly of them including: capillary electrophoresis; polyacrylamide gel electrophoresis under native conditions (NATIVE-PAGE); polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE); isoelectric focusing electrophoresis (IEF technique); agarose gel electrophoresis (AGE). Capillary electrophoresis combines separation mechanisms of electrophoresis and the automation of chromatography, with an extraordinary separation power, analytical speed, and extreme sensitivity (a single molecule by laser-induced fluorescence detection) [43]. NATIVE-PAGE uses the principle of protein separation based on their electrical charge, molecular mass, and spatial conformation. This technique was successfully applied to the research of major milk protein polymorphisms, to differentiate A, B, C and Dat the locus of β casein, and for the authentication of dairy products. Electrophoresis (SDS-PAGE) is based on the principle of protein separation based on molecular weight. A discontinuous polyacrylamide gel having a supporting medium role and a specific substance sodium dodecylsulfate (SDS) is used to denature proteins. This technique can be applied to both protein separation and separation of DNA and RNA molecules [44]. IEF electrophoresis separates proteins according to their isoelectric point (pI); these molecules are amphoteric ones, which means that they can have positive, negative or zero electrical charge, depending on the pH in the environment. The electrical charge of a protein is given by the sum of positive and negative electric charges, which characterize the amino acids in their constitution. Each amino acid has amino groups and terminal carboxyl groups, and depending on their prevalence in the composition of the protein, the total protein load may be positive (with more amino groups) or negative (with more carboxyl groups) [22]. Agarose gel electrophoresis (AGE) is a standard method of separating, purifying, and identifying DNA molecules, including mixtures that cannot be appropriately separated by other techniques. To verify the integrity of chromosomal DNA as well as to estimate the degree of RNA contamination, the extract is subjected to an agarose gel electrophoresis in a horizontal plate [42].

(iv) Amplification of genes of interest by PCR technique (Polymerase Chain Reaction). This technique was developed by K. Mullis in 1983 and considers the natural process of DNA replication, using the DNA polymerase enzyme in order to synthetize a new complementary strand of DNA using a native template one. As a result, will be formed many copies of DNA sequences (genes) of interest

[45]. There are known many variants of the basic PCR technique, the most used of them in the study of various polymorphisms in cattle being the Restriction Fragment Length Polymorphism (RFLP) technique and Amplified Fragment Length Polymorphism (AFLP) technique. RFLP technique is based on the hybridization properties that exist between two DNA fragments presenting a high degree of homology (RFLP-based hybridization) and the PCR technique, highlighting the polymorphisms existing at the restriction enzyme sites. AFLP technique is based on the selective PCR amplification of DNA fragments obtained by a particular restriction using two restriction enzymes and adapters, followed by the analysis of amplified fragments in polyacrylamide gel. It has a great advantage of its not requiring preliminary genome information, and its sensitivity in detecting polymorphism at the whole genome level [13].

Besides classical polymorphisms of genes or of their products represented by proteins or various enzymes, new codominant nuclear markers was found at high density and randomly dispersed across chromosomes, being increasingly used for genetic diversity studies, in particular for breeds that are on the brink of extinction, due to their highly degree of polymorphism and their neutral behaviour to selection proceedings ([46], [47]).When these microsatellite's analyses were considered, an increased number of alleles were observed, these loci having a good variability despite of the low populations size and the existed risk of inbreeding. The analysis of phylogenetic relationships established closer or further genetic distances within Grey Steppe breed, or between Grey Steppe and other breeds, being also confirmed some Grey Steppe varieties in Podolian cattle group [9].

Conclusions

Genetic markers are worldwide used in various programs of cattle's conservation and improvement. In Romania, the Grey steppe and Pinzgauer cattle breeds are included in a national genetic preservation program in order to maintain their genetic resources. The use and testing of new molecular markers research techniques will further help clarify aspects of their genetic diversity. At the same time, it is possible to establish associations between gene polymorphism and productive, reproductive or adaptation to environmental conditions of the two cattle breeds.

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Testing the Presence of SNP Polymorphisms in the 19th Intron of the Calpastatin (CAST) Gene on the Romanian Spotted Cattle, Simmental Type and Angus Breed

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Abstract

The tenderness of meat is under complex genetic determinism, being studied extensively in the last couple of years on various cattle breeds with respect to genetic variations associated with the trait. Only few of studied loci had major effect on tenderness, with calpains m and μ and calpastatin loci receiving special attention. Calpains m and μ intervene in the post-mortem proteolysis of myofibrils, being in direct relationship with their inhibitor – the calpastatin. The presence of potential Single Nucleotide Polymorphisms on intron 19 of the calpastatin gene (CAST) in mixed cattle breed (meat & milk/Romanian Spotted breed, Simmental type) and specialized meat breed (Angus) were investigated in this study. DNA samples from two cattle breeds were used to determine the SNP polymorphisms in the 552 bp fragment of the 19th intron of the CAST gene. The presence of mutations was investigated by testing the modifications occurring in *Bsh*F1 (GGCC) and *Rsa*I (GTAC) restriction enzyme's sites. All individuals analysed have had the same electrophoretic profile suggesting that no genuine mutations occurred in both restriction enzyme's sites of CAST gene (BTA7) from the Bos taurus populations.

Keywords: CAST, BshF1, RsaI, tenderness, SNP Polymorphism

Introduction

Improvement of complex traits such as the ones tied to organoleptic properties of the beef, which have low heritability and are unrepeatable, is difficult and their standardisation is even more so. In the case of cattle breeds the process is more complex, because of the long generative cycle as opposed to other species. Traditional methods of selection and breeding were based on the estimated breeding value, taking into consideration the phenotypical values of the animals or its' collateral relatives [1]. Palatability attributes of beef, valued by consumers (juiciness, tenderness, flavour, etc.) have received special attention in the last couple of years for the identification of favourable genetic variation that can be introduced in selection and breeding [2]. Studies have been focused firstly on identifying loci of candidate genes with major effect, which can be used in Marker Assisted Selection. In the last 15 years numerous polymorphisms have been tested for association, some of which are available commercially today as genetic tests (IGenity/GENE-STAR). Out of all the quantitative trait loci studied just a few have been proven to have a major effect in genetic variation. Among them, calpains loci and the calpastatin locus can be found. A major factor which contributes to meat tenderization is post mortem proteolysis of myofibrils [3; 4] and evidences of the existing direct relationship with tenderness in livestock include association with calpains and calpastatin genes variation. For these reasons many research projects proposed to introduce molecular information in breeding programmes in last ten years, to improve this parameter. They are involved in a wide range of physiological

processes including muscle growth and differentiation, pathological conditions and post-mortem meat aging [5]. In the calpain proteolytic family, μ-calpain (CAPN1) is responsible for the breakdown of myofibril proteins, while calpastatin (CAST) inhibits μ-calpain and m calpain (CAPN2) activity and therefore regulates post-mortem proteolysis [6; 7; 8]. Therefore, increased tenderness of the beef that reaches consumers tables is the result of the two endogenous proteases, whose activity is dependent of their inhibitor. The calpastatin gene (CAST), through genetic variants, modulates the post mortem meat tenderization, being identified several genetic variants and many polymorphisms associated with a tender meat or a tougher one. These evidences are addressed specifically to SNPs polymorphisms of calpastatin and also to calpains genes. Various studies identify specific polymorphisms in CAST gene and establish association with beef tenderness in many breeds and crossbreed animals [7; 9; 10; 11; 12; 13; 14; 15]. In a recent study (2014) the CAST and CALP gene polymorphisms have been tested for association with beef tenderness in two Spanish breeds (Parda de Montana and Pirenaica). Out of 31 polymorphisms identified in CAST gene, none were found in the coding region. Five polymorphisms from CAST gene have been associated with tenderness, 7 day after slaughtering [6]. The polymorphisms are situated in the 5th and 12 introns, 7th exon and 3' UTR region. The polymorphism (g98579663A>G) of CAST gene, described for the first time by Barendse in 2002, modifies the target putative site for miRNA (bta –miR 542-5P), being associated with beef tenderness in many breeds and crossbreed animals [14; 6; 10; 17]. The A>G mutation is in the 3' UTR region (CAST_5). Also, a SNP polymorphism in the 5th intron of CAST gene (g282CNG) was associated with post mortem beef tenderness in crossed breeds *B. taurus* populations [9]. Alongside two polymorphisms, Calvo *et al*., in 2014, described three other polymorphisms in CAST gene, namely in CAST 2&3, in the 7th exon and in CAST _4, 12th intron, which were tested for association with tenderness in two Spanish breeds. Two polymorphisms, in the exon 7 (g98535683A>G) and on 3'UTR region (g.98579663A>G) was significant associated with a tougher meat [6]. This mutation consists in an amino acid modification Thr182Ala. However, to utilize these polymorphisms in breeding programs it is mandatory that each of them is tested in different populations. In the present work we tested for possible presence of SNP polymorphisms in 19th intron of the CAST gene on Romanian Spotted cattle, Simmental type, and comparative with the Angus breed.

Methodology

DNA samples

35 animals from different populations were used to test the presence of putative genetic polymorphisms. (25 animals of Romanian Spotted cattle, Simmental type – a mixed breed for milk and meat and 10 animals of Angus meat breed). The blood samples from which DNA was extracted come from animals included in different experiments conducted between years 2016-2018 at Laboratory of Molecular genetics and Biotechnologies of our University. *Genomic DNA extraction from blood* -200 μl of blood samples collected on K_2EDTA, from each animal, were subjected to extraction with Quick DNA Microprep Plus Kit following the manufacturer's instructions (BioZyme). The DNA samples were then analysed on Spectrophotometer NanoDrop ND1000 to determine the quantity and quality of the DNA. All samples analysed had optimum purity, ranging between 1.8 and 2 and the quantity, ranging between 50-78 ng DNA/μl.

Primer design and PCR amplification

Nucleotide structure of the intronic region of CAST gene, taken into account in this study was identified in the NCBI database. The polymorphism study was conducted initially using the genetic database to identify the DNA fragment to be amplified. The region selected to be

amplified by PCR was identified based on Accession Number-AH014256.2 of CAST gene sequence that corresponds to the 19th intron and a small previous region (of 19 nucleotides).

For this study, the previous regions with polymorphisms associated with tenderness were excluded [18; 9; 19; 11; 6]. The fragment to be amplified by PCR had 552 bp and the primers design to amplify this fragment of the CAST gene was realised with Primer3 Premier Software. Their sequence is presented in Table 1.

Table 1. The 5'-3' primer's structure for amplification of 552 bp fragment of the 19th intron of CAST gene (BTA7)

	Primer structure	**Ta**
Forward	5' ATCCAGAAGACGGAAAGCCT 3'	58ºC
Reverse	5' CTCACGATCCTCTTC TTTGG 3'	

PCR Amplification

All the DNA samples were amplified by PCR in a 25 µl final volume with following composition: 5x FirePol Master Mix (BioZyme) – 5µl; 0.6 µl of each (forward and reverse) primer (from 10pmol/µl solution), DNA template – 2µl and H_2O -16.8 µl. The PCR thermal cycling conditions were: 95ºC (5 min) – 1 cycle; 35 x 94ºC – (30 s); 58ºC (45 s) and 72ºC (45 s); and final extension at 72ºC for 8 min, maintaining 4ºC thereafter. The PCR reactions were performed in an Eppendorf MasterCycler thermocycler (Eppendorf, Germany). The amplimers of 552 bp were than subjected to digestion with *Bsh*FI and *Rsa*I restriction enzymes. The possible SNP mutations in the GGCC site of *Bsh*F1 restriction enzyme and also in the GTAC site of *Rsa*I restriction enzyme site were analysed.

RFLP analysis

The PCR products were submitted to digestion with two restriction enzymes to identify the possible causative mutations abolishing the restriction sites. The possible SNP mutations were searched in the positions 149 and 235 bp, with *Bsh*F1 restriction enzyme (GGCC) and also in the positions 242, 314 and 491 bp, which correspond to *Rsa*I restriction enzyme site (GTAC) in the analysed fragment. The following protocol for enzymatic digestion was applied. The reaction mixture was set for a final volume of 20 µl for each restriction experiment, with 10 µl PCR product, 2 µl restriction enzyme's buffer, 0.6 µl restriction enzyme (for each digestion experiment) and 7.4 µl H_2O. The samples were incubated at 37ºC for 3 h and the digestion products were submitted to electrophoresis in a 2.5% agarose gel. After restriction analysis performed on the two breeds all electrophoretic profiles reveal no SNP mutations, being all monomorphic. The presence of restriction sites in the 552 bp fragment, corresponding to the two enzymes, was confirmed by expected fragments after digestion. The *Bsh*F1 enzyme has two restriction sites in the fragment (position 149 and 235 bp) and *Rsa*I has three restriction sites in the same fragment (positions 242, 314 and 491 bp).

Results

After digestion of 552 bp fragment with *Bsh*F1 restriction enzyme, 3 fragments of 316, 149 and 86 bp were obtained for all samples (Fig. 1). The monomorphic profile of each sample denotes that no mutation was occurred in GGCC restriction site of *Bsh*F1 enzyme. For *Rsa*I restriction enzyme, 3 fragments of 314 bp, 177 bp and 60 bp respectively were obtained for each sample (Fig. 2). These profiles show also that no mutations have occurred in the *Rsa*I restriction site, and all individuals have the same profile.

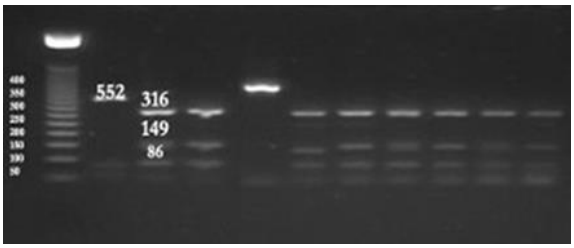

Fig. 1. The *Bsh*FI restriction profile of 552 bp

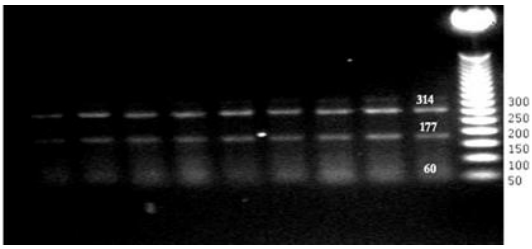

Fig 2. The *Rsa*I restriction profile of 552 bp sequence

sequence from 19th intron of CAST gene from 19th intron of CAST gene (line 10-50 bp DNA) (line 1-50 bp DNA ladder; line 2 PCR product, ladder; line 1-9 restricted PCR products of 19th intron and CAST/*Bsh*FI fragments of 316, 149 and of CAST gene from Romanian spotted cattle, 86 bp from Romanian spotted cattle, Simmental Simmental type and Angus individuals (314, 177 and type and Angus individuals 60 bp).

In the literature, three polymorphisms on the CAST gene are well documented. All of these were associated with increased tenderness of the beef in various breeds and crossed breeds.

These are BTA7 g.98533962C>G in the 5th intron [9], BTA7 g.98535683 A>G in the 7th exon and mutation g.98579663A>G in the exon 30/3'-UTR region [18; 10]. Among the studied breeds are: Angus, Limousin, Charolais, Simmental [9]; Brahman Belmond Red, Angus, Hereford [18], Jersey x Limousin, Angus x Hereford cross-cattle [20]; Chinese commercial cattle [11], Brahman, Nellore, Angus and crossed breeds: Angus x Nellore, Rubia Galega x Nellore, Canchim, Brown Swiss x Nellore [10], Hereford, Charolais, Gelbviech and Simmental [14], Parda de Montana and Pirenaica [6], Hanwoo breed [21]. Another study analysed 28 SNPs, grouped in 3 LD blocks on the CAST gene (BTA7). The three blocks lie between the 3rd intron and 9th exon (LD block 1); intron 12 and exons 20 and 22 (LD block 2) and finally, in the third block between intron 25 to exon 31 (including a segment of 3' UTR region). Out of the 15 initially identified polymorphisms in the 3rd block, 4 of them were associated with tenderness on the Angus-Brahman hybrids [22].

Conclusions

In conclusion, the extensive study of CAST gene polymorphisms suggests the still unexplored potential of genetic determinism for establishing associations with meat tenderness in different breeds or crossed breeds. A recent study in three French meat breeds has also indicated that distinct effects of the calpastatin markers are attributed to the breed and cannot be extended to all Bos taurus breeds [17]. Another conclusion about meat tenderness is drawn by Pintos and Corva (2011) in marker association studies for meat tenderness and growth traits on Angus breed. They suggest that any recommendations to improve beef tenderness, using molecular markers in the CAPN1 and CAST genes, should take into account the fact that there could be a correlated response in growth traits. Consequently, the further exploration of the genetic polymorphisms in the calpastatin and calpain genes, in the Romanian spotted cattle – Simmental type, remains open, with the potential to identify new genetic polymorphisms.

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Biotechnological Potential of Apilarnil and Royal Jelly Used in Obtaining Some Functional Foods

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Abstract

Bees, through their activity, provide us with bee products containing a variety of biologically active compounds, which, in different proportions, have a positive impact on general human health, on the physical performance or mental status but also from a nutritional point of view.

Through this research paper, it was sought the obtaining of two functional foods based on apilarnil, royal jelly and multifloral pollen with biotechnological potential.

The products prototypes produced were characterized from a physico-chemical and organoleptic point of view. As a result to the analyses, numerous polyphenolic compounds have been identified in this jellies, namely: rutin, chlorogenic acid, isoquercetin, naringenin, and which give antidepressant, immunomodulatory, antitumoral, anti-inflammatory, hypolipidemic, antioxidant, DNA protection, proliferation of peroxisomes activities, compared to commercial jelly, where the amount and concentration of polyphenolic compounds is greatly diminished.

Keywords: innovation, apilarnil, biotechnological potential, functional food, royal jelly

Introduction

In recent decades, the concept of functional foods has offered a new and practical approach to achieving optimal health by promoting the use of natural products with physiological benefits thus reducing the risk of various chronic diseases [1-3].

According to the definition, functional food is a part of human diet and is demonstrated to provide health benefits and to decrease the risk of chronic diseases beyond those provided by adequate nutrition [4-6]. Functional foods include: usual foods with naturally occurring bioactive substances (e.g. dietary fibre), foods supplemented with bioactive substances (e.g., probiotics, antioxidants), and derived food ingredients introduced to conventional foods (e.g., prebiotics) [5].

The health benefits such as decrease of cancer risk, improvement of heart health, enhancement of immune system [5, 7], reducing of menopause symptoms, enhancement of gastrointestinal health, preservation of urinary tract health, anti-inflammatory influences, diminution of blood pressure, protection of vision, antibacterial and antiviral activities, decline of osteoporosis and anti-obesity influences [6-7].

Among foods that possess the characteristic of functionality, we may include all those originating in the beehive: bee pollen, royal jelly and apilarnil.

Bee pollen is a good source of healthy compounds due to the potential biotechnological such as phenolics, terpenes and flavonoids [8, 9], that are relevant for clinical applications against inflammatory diseases [10-12] source of proteins, essential amino acids, essential fatty acids, vitamin complexes, lipids, trace elements [10].

Royal Jelly is one of the most challenging bee products for biotechnologies [13], it is an important functional food item that possess several health promoting properties [9]. Biological activities of RJ are mainly attributed to the bioactive fatty acids, proteins and phenolic compounds as quercetine, kaempherol, galangine, pinocembrine, narynin, apigenin, hysperdine, acacetin, crisina, luteoline [9]. RJ has been demonstrated to possess numerous functional properties such as antibacterial activity, anti-inflammatory activity, vasodilative and hypotensive activities, disinfectant action, antioxidant activity, antihypercholesterolemic activity and antitumor activity [13, 14].

On the other hand, apilarnil is a natural bee product obtained by triturating larvae of drones harvested under special conditions, in whole, on the seventh day of hatching [9, 15].

Apilarnil has a higher nutitional value and biological active compounds as essential amino acids (treonin, leucine, isoleucine, methionine), vitamins (vitamin A, betacaroten, B1, B6, PP and choline), minerals (calcium, phosphorus, sodium, zinc, manganese, iron, copper and potassium) and it is also rich in sex hormones like testosterone, prolactin, progesterone and estradiol [16, 17].

In this research study, it was desirable to optimise some recipes of jellybeans based on bee products (royal jelly, apilarnil, multifloral pollen) that would bring added benefits due to the content in biologically active compounds with antioxidant, antiinfectious, vitalizant, biostimulatory and energizing properties, balancing of the endocrine and nervous system and falling within the category of functional foods.

Material and Methods

Chemicals and Bee Products Samples

All chemicals and reagent were analytical grade or chromategraphic grade purity and ultrapure water, purchased from Sigma, Cromatec and Merck. Fresh royal jelly and apilarnil were obtained from beekeepers; multifloral pollen from UASVM apiary. Other materials used were: *Sambucus L.* syrup, *Salvia officinalis* extract and gelling agent.

Bee Products Sample Preparation

Royal jelly and apilarnil were lyophilized using the method described by Nascimento *et al*., [18], for obtaining a powder and bee pollen was subjected to heat treatment at 40 Celsius degrees.

Jelly Technology

This process assumed the following steps: raw materials preparation, ingredients incorporation, raw materials mixing, boil mixture, cooling, apilarnil or RJ (0.15 gr/jelly) adding, bee pollen adding, mold transfer and refrigeration. The jelly weight varies between 10-15g.

Chemical Characterization of Experimental and Commercial Jelly

The two varieties of jellybeans based on royal jelly and apilarnil were analysed by high-performance liquid chromatography with refractive index detection for sugar spectrum [19].

The instrument used was a Shimadzu apparatus equipped with a column: modified Alltima Amino 100Å, 5 μm, 250 x 4.6 mm; mobile phase flow: 1.3 ml/min; mobile phase: acetonitrile/water (75/25; v/v); column temperature: 30^{o}C; injection volume: 20 μl; separation

time: 60 min. Sugar standard solutions are prepared similar to the analysed sample. Results are expressed in g/100 g sample.

Biological active compounds

Identification of polyphenolic compounds from jellybeans based on royal jelly and apilarnil and the commercial jelly was made after Campos and Markham method [20], modified in our laboratory, using a liquid chromatograph Shimadzu 2010 EV (Kyoto, Japonia) coupled with Photodiode Array Detector (PDA). Compounds separation was achieved on a MEDITERRANEA SEA C18 reverse phase column. A binary gradient of two mobile phases was used: water at pH 2.5 (phase A) and acetonitrile (phase B). Elution was carried out with a flow rate of 1ml/min at 24°C, with an injection volume of 10 µl. Spectral data for all peaks were registered in the range of 220-600 nm.

Determination of mineral content

To determine the levels of micro and macroelements: Na, Mg, K, Ca from obtained jellybeans, the atomic absorption spectrometry method was used. The mineralization of the samples was performed in a microwave furnace, Berghof digestion system MWS-2.

Approximately 0.3 grams of the homogenized samples were placed in special Teflon tubes, 2 ml of 65% HNO_3 was added and let to react for 15 minutes, after which 3 ml of H_2O_2 was added before the container was sealed [21, 22].

At the end of the initiated program, the solution is transferred into plastic containers and the sample is diluted with ultrapure water to a volume of 50 ml. An Analyst 800 Atomic Absorption Spectrometer from Perkin-Elmer was used, equipped with a cross-linked graphite furnace. In the graphite furnace the sample is inserted into a small tube of electrically heated graphite. By increasing the tube temperature, the sample passes through the drying, pyrolysis and atomization phases [23].

Lipid and Protein Content

The total lipids and proteins for obtained jellybeans were determined by Soxhelt method and Kjeldahl method described by Bobiș *et al*., in 2018 [23]. The moisture content was also determined by gravimetric method for calculating nutritional value of obtained jellybeans.

Sensory analysis

A number of 30 people, aged 18-40 years, by different sexes and occupations were questioned following the consumption of jellybeans with bee products.

Results and Discussions

Two prototypes of products were achieved. One prototype contains: 160ml/200ml *Sambucus L.* syrup, 3 ml/200ml *Salvia officinalis* extract, 10g/200ml gelling agent, 25g/200ml multifloral pollen and 2g/200ml royal jelly and the other prototype contain: 160ml/200ml *Sambucus L.* syrup, 3ml/200ml *Salvia officinalis* extract, 10g/200ml gelling agent, 25g/200ml multifloral pollen and 2g/200ml apilarnil.

Those two functional foods (jellybeans with apilarnil and royal jelly) have a smaller nutritional value 179.6 kcal/100g for royal jelly and multifloral pollen jelly, and 141.9 kcal/100g for apilarnil and multifloral pollen jelly, compared to a commercial jelly product (according to the label), whose nutritional value is 340 kcal/100g. Chemical composition of experimental jellybeans with bee products are presented in Figure 1.

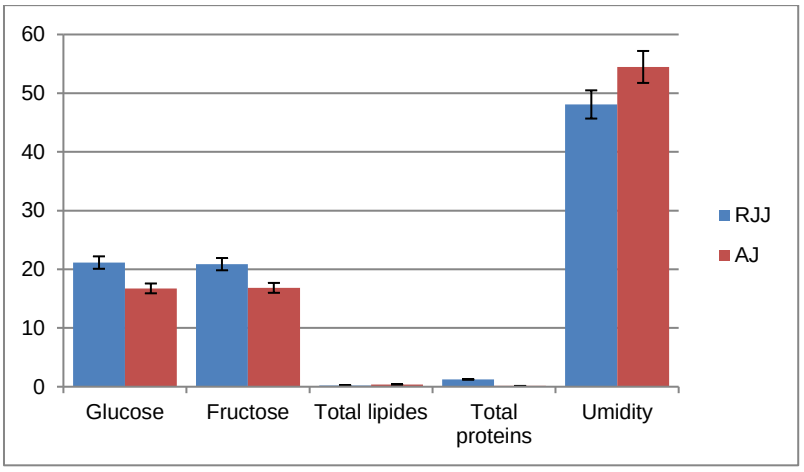

Fig. 1. Sugar content, total lipids, total proteins and humidity (%) of jellybeans with bee products.
*RJJ = royal jelly and multifloral pollen jelly; AJ = apilarnil and multifloral pollen jelly

The royal jelly and multifloral pollen jelly contain humidity in small amounts, but a higher content in total lipids (0.234±0.02%), total proteins (1.25±0.01%) and total sugars (42.03±0.06%) compared to apilarnil and multifloral pollen jelly.

The content of some minerals from jellybeans with bee products are represented in Figure 2.

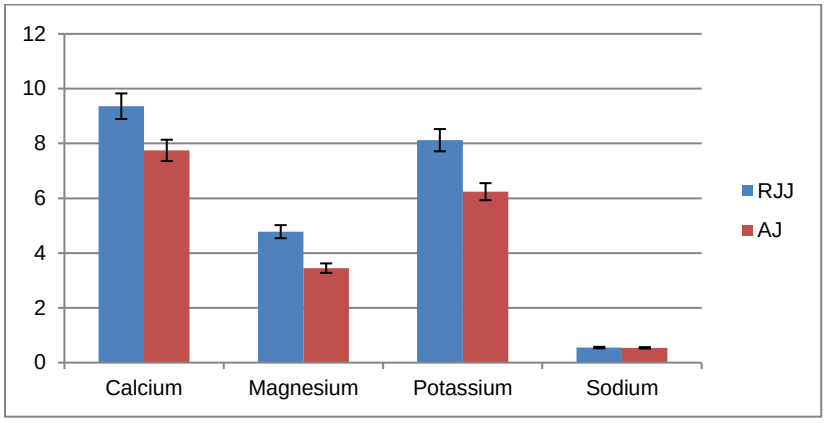

Fig. 2. Mineral content (µg/g) of jellybeans with bee products
*RJJ = royal jelly and multifloral pollen jelly; AJ = apilarnil and multifloral pollen jelly

The distribution of the elements among the two functional food were in favour of royal jelly and multifloral pollen jelly, much higher amounts being determinate in royal jelly and multifloral pollen jelly compared to apilarnil and multifloral pollen jelly.

The biologically active potential of the two jelly samples obtained has been highlighted by identifying polyphenolic compounds (Table 1) and comparing the results achieved with those of an already existing on the market (Table 1). So, the royal jelly and multifloral pollen jelly have higher concentration in rutin, chlorogenic acid and isoquercetine while the cafeic acid and myricetin were found in small amounts. Rutin is a flavonoid predominantly found in plants, bee pollen [25] and presents antioxidant, cytoprotective, vasoprotective, anticarcinogenous, neuroprotective and cardioprotective activity [26]. On the other hand, chlorogenic acid present antibacterial, antioxidant and anticarcinogenous activity, specially hypoglycemic and hypolypidemics [27]. Also, isoquercetin have neuroprotective, cardioprotective, antioxidant, anti-inflamtory, chemopreventive and antiallergic effects [27].

Analyses performed to apilarnil and multifloral pollen jelly confirmed higher amounts in rutin, chlorogenic acid and isoquercetine as well as naringenine, possessing both

antidepressants, immunomodulatory, antitumor, anti-inflammatory, hypolypidemic and antioxidant activity, DNA protector and improves memory [28].

Table 1. Polyphenolic compounds of experimental and commercial jelly, containing plant extracts and bee products

Retention time (min)	Specification	Experimental jelly (apilarnil and multifloral pollen) [µg/g]	Experimental jelly (royal jelly and multifloral pollen) [µg/g]	Comercial jelly [µg/g]
11.11	protocatecuic acid	0.52	0.33	0.14
17.5	p-OH Benzoic acid	1.17	0.59	0.04
21.78	chlorogenic acid	6.79	7.01	0.14
23.48	caffeic acid	0.03	0.10	0.01
26.79	vanilin	-	0.40	1.04
29.37	p-coumaric acid	-	0.50	0.25
30.74	rutin	22.59	21.16	0.64
30.86	ferulic acid	-	-	0.17
31.2	isoquercetine	2.69	2.86	0.10
33.57	myricetin	0.17	0.25	-
41.26	naringenine	5.56	1.50	-
42.1	kaempferol	2.12	0.27	-

Comparing the bioactive compounds results for both functional foods, much higher concentrations were found in apilarnil and multifloral pollen jelly except for protocatecuic acid and isoquercetine.

In order to highlight the biologically active potential of jellybeans studied, comparisons were made with the results of polyphenolic compounds analysis for a commercial jelly (Table 1). It can be observed that, with regard to all the compounds recovered, concentrations are much lower range between 0.1-1.1 (µg/g) for commercial jelly, while the concentrations for RJJ and AJ are found between 0.03-23 (µg/g).

The main quality characteristics of jellybeans from consumer's perspective consist of being equilibrate in terms of aroma (21/30 responses), containing natural ingredients, without preservatives (20/30 replies) and not contain sugar (19/30 responses). Regarding organoleptic analysis of the two products, the replies received were mostly in the category "Excellent taste" (66.7% for RJJ and 60% for AJ), 30-40% expressed "Pleasant taste". The attention was equally given to both jellybeans of 73.3%; With regard to the changes they would like on these products, 56.7% were in favour of their shape, 23.3% on consistency and only 10% of respondents would bring improvements to taste.

Conclusions

Bee pollen, royal jelly and apilarnil are food products obtained from bees. All of them are important not only for their nutritional properties but also for their functional and biological properties. For their beneficial effects, the presented bee products can be used as potential ingredients for some functional foods, in our case jellybeans. Biotechnological potential of the functional foods obtained following this study are mainly attributed to the phenolic compounds such as flavonoids and phenolic acids and this potential of food can promote health, improve general well-being and reduce the risk of developing certain illnesses.

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Impact of Climate Change of Atmospheric Precipitations on the Vital Activity of Bees Families

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Abstract

The present study aims to determine the correlation between the parameters of monthly atmospheric precipitation at different times of the year and the evolution of morpho-productive characters of bee families, thus elucidating the impact of the climate change on atmospheric precipitations on the vital activity of bee colonies Apis mellifera. The scientific researches were performed at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova, located in the Center area of Moldavian Codri. The obtained results have demonstrated that the precipitation in February of this year had a weak and directly negative influence on the wintering resistance of the bee families (r_{xy}= -0.468±0.276; t_r=1.70; P<0.1) and the precipitation from July last year – indirect influence (r_{xy}= -0.482±0.271; t_r=1.78; P<0.1).

The queen bee prolificacy is significantly negatively and directly influenced by the atmospheric precipitation in May and June of this year (r_{xy} = -0.811±0.121; t_r=6.70; P<0.001) and indirectly positive by the annual volume of atmospheric precipitation from last year (r_{xy}= 0.525±0.256; t_r=2.05; P<0.05). The colony strength is negatively and directly influenced by the precipitations in April of this year (r_{xy} = -0.564±0.241; t_r=2.34; P<0.05) and indirectly positive by the atmospheric precipitation in December of the previous year (r_{xy}= 0.629±0.214; t_r=2.94; P<0.01). The disease resistance of bee families is influenced negatively and directly by the atmospheric precipitation in January of this year (r_{xy} = -0.712±0.174; t_r=4.09; P<0.001), negative and indirectly by July precipitation (r_{xy}= -0.642±0.208; t_r=3.09; P<0.01) and, positively and indirectly, by the precipitation from August of last year (r_{xy} = 0.660±0.199; t_r = 3.32; P<0.001), as well as by the annual quantity of atmospheric precipitation from the previous year (r_{xy}= 0.621±0.215; t_r=2.89; P<0.01). The brood viability is influenced negatively and directly by the atmospheric precipitation in the months of January (r_{xy} = -0.557±0.244; t_r=2.28; P<0.05), February (r_{xy} = -0.573±0.237; t_r = 2.42; P<0.05) and Mai (r_{xy} = -0.491±0.268; t_r=1.83; P<0.1) of current year, and positive and indirect by atmospheric precipitation in October (r_{xy} = 0.499±0.265; t_r=1.88; P<0.1) and November (r_{xy} = 0.648±0.205; t_r=3.16; P<0.01) of previous year. The production of honey, accumulated in the nest by the bee families, is negatively and directly influenced by the atmospheric precipitation from months of February (r_{xy} = -0.797±0.128; t_r=6.23; P<0.001), Mai (r_{xy} = -0.507±0.262; t_r=1.94; P<0.1) and June (r_{xy} = -0.507±0.263; t_r=1.93; P<0.1) of current year, and, positively and indirectly by atmospheric precipitation in September (r_{xy} = 0.732 ± 0.164; t_r=4.46; P<0.001) and November (r_{xy} = 0.627±0.214; t_r=2.93; P<0.01) of the previous year.

Keywords: bees, climatic changes, atmospheric precipitation, correlation, characters

Introduction

The notion of climate change means complex modification of air temperature, of atmospheric precipitation regime and of extreme weather phenomena, or irregular events such

as drought, storms, tornadoes, hail, floods, etc [10, 14, 15, 17]. Numerous scientific researches [2, 11-13, 19] demonstrates that climate change is caused by global warming, which is a direct or indirect result of human activities (burning fossil fuels, changing land use, fermenting organic substances, etc.) that emit enormous quantities of greenhouse gases into the atmosphere. These determines the change in the composition of the global atmosphere, to which the natural variability of the climate, observed over a comparable period of time, is added. The greenhouse effect occurs because of the selective absorption by the greenhouse gas molecules of the thermal radiation emitted by Earth, and its isotropic remittance in both the extra-atmospheric space and to the Earth. Increasing the concentration of these gases into the atmosphere, intensifies the greenhouse effect, which perturb the transport of energy and humidity in the system, which determines imbalances in the climate system.

The impact of climate change are reflected in: increasing global average temperature with significant regional variations, reducing freshwater resources for the population, reducing the volume of glacier calving and increasing ocean levels, modifying the hydrological cycle, increasing arid areas, anomalies in the deployment of seasons, increasing the frequency and the intensity of extreme climatic phenomena, with a particularly negative impact on flora and fauna, expressed by reducing biodiversity, decreasing agricultural productivity etc. [3, 16, 20].

It is worrying that, despite the Paris Agreement – the United Nations Framework Convention on Climate Change [1] – greenhouse gas emissions and global warming continue to increase.

Thus, in an official publication of the European Environment Agency (EEA), total greenhouse gas emissions in the European Union (EU) increased by 0.7% in 2017 compared with 2016. Greenhouse gases emissions in the EU are mainly due to higher industrial and transport emissions, the increasing of which have been recorded for the fourth consecutive year [13].

Along with global warming, there is concern over the climate change of atmospheric precipitation. Clouds, which represent the more condensed form of tiny water molecules suspended in the air, are the main source of atmospheric precipitation. Minuscule water particles play an important role in the way and duration of clouds formation, in the amount of solar radiation the clouds can reflect, and determine the type of generated precipitations.

Concentrations and particle composition may even climate change the time and place where precipitation occurs [6].

Climate changes in the frequency and volume of precipitation has attracted the attention of multiple specialists and researchers in the field [5, 6, 21-23] as they cause really economic and social costs, affecting food production and their global price.

The report of the European Environment Agency "Climate change, impact and vulnerability in Europe 2012" reveals there are quite pessimistic forecasts of climate change that all regions of Europe are affected by climate change, and higher temperatures across Europe have been set, in combination with decreasing of precipitation in southern regions and increasing precipitation in Northern Europe. In addition, icecaps and glaciers are melting and sea level is rising. All these trends are expected to continue [11]. According to the same Report, climate change of atmospheric precipitation has a direct impact on the physiology, phenology, and biodiversity distribution of fauna and flora as well as on the overall human society.

The pessimistic predictions of climate change are complemented by Romanian researchers [3-6, 17, 19-23], who, for the most part, affirm that during the hot season there is a tendency for decrease the precipitation, which, generally, will be accentuated towards the end of the XXI century. Under these conditions, the trend of predictions is associated with the climate change signal, determinate by the increase of global greenhouse gas concentrations, with the regional signal of reducing of precipitations in the zone, as well as the negative impact on agriculture, natural ecosystems and society as a whole.

In Technical Report of Greenpeace Research Laboratories [10] it was mentioned that: "climate change, such as increasing temperatures, changes in rainfall patterns and more erratic or extreme weather events, will have impacts on pollinator populations. Some of these changes could affect pollinators individually and ultimately their communities, becoming reflected in higher extinction rates of pollinator species".

In our previous research, it has been demonstrated that the excessively high summer-summer temperatures of a droughty year caused a drastic decrease in the values of the main morpho-productive bee family indices by 20-46% [7].

We also found that changes in air temperature in different months of the year have different impacts on the vital activity of bee families, depending on the period of the year and the air temperature [8].

Appreciating the research results of the above-mentioned multiple authors, we can report that they have provided useful information on the impact of climate change on ecosystems in general and on pollinators in particular. At the same time, in the accessible for us bibliographic sources, information on the concrete influence of changes in the atmospheric precipitation regime on the vital activity of bee families is missing.

In this context, the aim of our research was to determine the correlation between the parameters of monthly atmospheric precipitation at different times of the year and the evolution of morpho-productive characters of bee families, thus elucidating the impact of the precipitation regime on the vital activity of bee colonies *Apis mellifera.*

Materials and Methods

The scientific researches were carried out on bee families *Apis mellifera carpatica,* at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova, located in the central part of Moldavian Codri, Forest District Ghidighici, Canton no. 8, Forest Sector no. 21. At the apiary there were a total of 50 bee families. During the years 2010-2018, each year, at the end of June, each bee family were individually evaluated the main morpho-productive reproduction and developmental characters (queen prolificity, family strength), wintering and disease resistance, brood viability, as well as productivity of honey accumulated in nest, according to our methods [9] described in the Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011 [18]. Then the average value of each evaluated morpho-productive character per apiary was calculated.

To study the impact of climate change on the vital activity of bee families, monthly and annual average of atmospheric precipitations data in the last 8 years (2010-2017), from the nearest hydrometeorological station in Bravicea, Dist. Călărași, at a distance of 27 km from the apiary, were used. During this period, for each month, Pirson's linear correlation coefficients were calculated between the monthly average of atmospheric precipitations and the average values per apiary of each of the 6 main morpho productive characters of bee families, such as: queen prolificity, colony strength, wintering resistance, disease resistance, brood viability and honey production of bee families. For the months of the first half of the year, correlation coefficients were calculated between atmospheric precipitations and values of morpho-productive characters, evaluated in the same year at the end of June, with the exception of wintering resistance, which was assessed at the end of March. Given that in the second half of the year the climatic factors don't influence the morpho-productive characters, already evaluated in this year, the atmospheric precipitations variable in July-December was calculated in correlation with the value of the morpho-productive characters of the bee families from next year. The same correlation coefficients were also calculated for the average annual

precipitations and the average values per apiary of the above-mentioned morpho-productive characters.

Pirson's linear correlation coefficient (r_{xy}) was calculated using the "STATISTICA 12" computer software. For each correlation coefficient the criterion of certainty of the correlation (t_r) and the certainty threshold (P) after Student was calculated in part.

The obtained in experience data were statistically processed and evaluated their certainty, according to variation biometric statistics, by methods of Plokhinsky H.A. [24].

Results and Discussions

The analysis of the research results showed that during the years 2010-2017, in the area monitored by us, climate change caused a quite various annual and monthly atmospheric precipitations (Tab. 1).

It was found that the annual quantity of atmospheric precipitation in the area ranged from 437.0 mm minimum in 2011 to 734.9 mm maximum in 2010. The difference between the volume of atmospheric precipitation (variability) was 40.5%.

Table 1. Annual and monthly atmospheric precipitation registered at the Hydrometeorological Station "Bravicea", Dist. Călăraşi, during the years 2010-2017, mm

Month of the year	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
January	87.3	19.6	18.1	31.4	51.1	26.5	32.8	29.5
February	40.8	45.7	78.1	33.8	5.9	21.3	27.4	22.5
March	18.7	24.8	15.0	65.1	12.9	57.7	25.7	30.8
April	41.4	27.6	84.2	29.1	55.1	41.8	39.8	112.5
May	97.0	22.9	44.1	62.1	88.3	12.6	64.5	42.9
June	147.2	119.8	64.3	142.8	27.4	34.8	188.0	89.7
July	64.0	64.6	44.1	9.5	91.7	83.2	19.6	85.5
August	43.4	50.5	39.7	78.2	18.9	38.0	120.7	33.9
September	49.7	12.2	6.3	114.6	10.3	20.2	9.0	29.7
October	51.7	24.2	36.5	4.9	44.3	42.8	134.5	62.3
November	37.1	4.9	23.3	60.7	91.8	56.6	36.5	31.7
December	56.6	20.2	99.2	6.6	41.3	2.7	11.2	81.2
Total annual	**734.9**	**437.0**	**552.9**	**638.8**	**539.0**	**438.2**	**709.7**	**651.6**

Data analysis demonstrates that over the years, the monthly volume of atmospheric precipitations varies considerably. In the analysed period (2010-2017), the highest variability of the monthly precipitation volume was registered in December, ranging from 2.7 mm in 2015 to 99.2 mm in 2012, the variation constituting 97.3%. The lowest variability in atmospheric precipitation was registered in April, ranging from 27.6 mm in 2011 to 112.5 mm in 2017, with a variation of 75.5%.

Also, atmospheric precipitations during this period had significant variability in the months: October, from 4.9 mm in 2013 to 134.5 mm in 2016, with a difference of 96.4%; November, from 4.9 mm in 2011 to 91.8 mm in 2014, with a variation of 94.7%; September, from 6.3 mm in 2012 to 114.6 mm in 2013, with a variation of 94.5% and in February, from 5.9 mm in 2014 to 78.1 mm in 2012, with the variation of 92.4%. In the other months of the year, the variability in the quantity of atmospheric precipitation in this period (2010-2017) fluctuated from 79.3% in January to 89.6% in July.

During the monitored period, two terrible droughts were registered, in the years 2012 and 2015, the annual precipitations volume was relatively low, compared to the other years, but not the smallest. Terrible droughts were registered in the years when the least quantity of atmospheric precipitations fell during the warm period (May-August). Despite the fact that in 2011 the lowest quantity of annual precipitations (437.0 mm) was registered, the drought did

not occur, because during the warm period of this year, fell sufficient quantity of precipitations (257.8 mm). However, in 2012 and 2015, when the lowest precipitation quantity during the warm period of all the monitored period, 192.2 and 168.6 mm respectively, was registered, the severe drought triggered.

The climate change in air temperature, atmospheric precipitation, as well as the extreme phenomena triggered in the area of location of experimental apiary have caused a significant variation in the vital activity of the bee families, expressed by different levels of morpho-productive characters development (Tab. 2).

From the presented data, it can be observed that the average per apiary of queen's prolificacy varied during this period, from 1371 eggs/24ore in 2016, to 1806 eggs/24ore in 2011.

The variability of this character at bee families was 24.1%.

The colony strength, expressed by the number of bees present in the nest, fluctuated from 2.20 kg in 2016 to 3.14 kg in 2018, with the variability of 30%. Wintering resistance varied, albeit to a smaller extent, from 80.1 percentage points in 2010 to 93.3 percentage points in 2014, with the variability being 14.2%.

Of the researched morpho-productive characters, the brood viability had the slightest variability in this period, fluctuating from 85.1 percentage points in 2010 to 95.8 percentage points in 2015, with a variability of 11.2%.

Table 2. Average indices of morpho-productive characters in bee families at the experimental apiary of the Institute of Zoology, during the years 2010-2018

Year	Prolificacy, eggs/24 h	Colony strength, kg	Wintering resistance, %	Brood viability, %	Disease resistance, %	Honey production, kg
2010	1583	2.83	80.1	85.1	76.8	38.8
2011	1806	2.97	82.5	91.0	89.4	32.8
2012	1740	2.37	86.2	88.6	87.4	23.9
2013	1661	3.03	91.1	91.0	90.5	35.5
2014	1781	3.13	93.3	92.3	91.6	57.4
2015	1711	3.04	88.6	95.8	86.3	44.2
2016	1371	2.20	84.1	95.7	89.2	31.0
2017	1678	2.36	86.8	95.5	92.6	34.2
2018	1781	3.14	88.4	95.4	91.7	39.7

Resistance to diseases (hygienic instinct) of bee families oscillated, during the nominated period, from 76.8 percentage points in 2010 to 92.6 percentage points in 2017, the variability being 17.1%. On the whole, we notice that climate change during this period fas caused a quite obvious variability of honey production accumulated in nest, from at least 23.9 kg in 2012 to a maximum of 57.4 kg in 2014, the variability being 58.4%.

In order to elucidate the concrete relationships impact of the climate change atmospheric precipitation on the vital activity of the bee families, the linear correlation coefficients (r_{xy}) between the monthly quantity of atmospheric precipitation and the medium value per apiary of the morpho-productive characters of the bee families was calculated (Tab. 3).

Table 3. The correlation coefficient (r_{xy}) between the monthly quantity of atmospheric precipitation in the first half of the year and the medium value of morpho-productive characters bee families

Morpho-productive character	**$r_{xy} \pm m_r$**	**t_r**	**P**
Precipitations in January			
Wintering resistance	-0.274±0.327	0.84	>0.1
Queen prolificity	-0.288±0.324	0.89	>0.1
Colony strength	0.227±0.335	0.68	>0.1
Disease resistance	-0.712±0.174	4.09	<0.001
Brood viability	-0.557±0.244	2.28	<0.05
Honey production	0.425±0.289	1.47	>0.1
Precipitations in February			
Wintering resistance	-0.468±0.276	1.70	<0.1
Queen prolificity	0.046±0.353	0.13	>0.1
Colony strength	-0.356±0.308	1.15	>0.1
Disease resistance	-0.303±0.311	0.97	>0.1
Brood viability	-0.573±0.237	2.42	<0.05
Honey production	-0.797±0.128	6.23	<0.001
Precipitations in March			
Wintering resistance	0.351±0.310	1.13	>0.1
Queen prolificity	0.105±0.349	0.30	>0.1
Colony strength	0.338±0.313	1.08	>0.1
Disease resistance	0.156±0.345	0.45	>0.1
Brood viability	0.359±0.308	1.16	>0.1
Honey production	0.012±0.353	0.03	>0.1
Precipitations in April			
Queen prolificity	0.097±0.350	0.28	>0.1
Colony strength	-0.564±0.241	2.34	<0.05
Disease resistance	0.292±0.323	0.90	>0.1
Brood viability	0.179±0.342	0.52	>0.1
Honey production	-0.198±0.339	0.58	>0.1
Precipitations in May			
Queen prolificity	-0.811±0.121	6.70	<0.001
Colony strength	-0.383±0.301	1.27	>0.1
Disease resistance	-0.335±0.313	1.07	>0.1
Brood viability	-0.491±0.268	1.83	<0.1
Honey production	-0.507±0.262	1.94	<0.1
Precipitations in June			
Queen prolificity	-0.811±0.121	6.70	<0.001
Colony strength	-0.383±0.302	1.27	>0.1
Disease resistance	-0.233±0.334	0.70	>0.1
Brood viability	-0.160±0.344	0.46	>0.1
Honey production	-0.507±0.263	1.93	<0.1

Research results have shown that January's atmospheric precipitation had a significant negative impact on disease resistance and brood viability. The coefficient of linear correlation of the quantity of atmospheric precipitation with disease resistance is negative at high level, having a significance of the highest threshold of certainty after Student (r_{xy}= -0.712±0.174; t_r=4.09; P<0.001). The correlation between the quantity of atmospheric precipitation in January

and the viability of the brood is also negative at the medium level with the significance of threshold one after Student (r_{xy}= -0.557±0.244; t_r=2.288; P<0.05).

February's atmospheric precipitation provokes a significant negative impact on the brood viability and honey productivity of bee families. Thus, the linear correlation coefficient between the quantity of atmospheric precipitations in this month and the brood viability is negative on medium level and quite significant (r_{xy}= -0.573±0.237; t_r=2.42; P<0.05). At the same time, between the quantity of atmospheric precipitation in February and the honey production of bee families, a negative high-level correlation was registered, with the highest threshold of certainty after Student (r_{xy}= -0.797±0.128; t_r=6.23; P<0.001). In addition, it was determinate a tendency of atmospheric precipitation of this month to negatively influence on the wintering resistance of bee families (r_{xy}= -0.468±0.276; t_r=1.70; P<0.1).

Research has shown that atmospheric precipitations in March does not exert any negative or positive influences on the vital activity of bee families, because the coefficients of linear correlations between the quantity of precipitation and the morpho-productive characteristics of bee families did not have significant values (P>0.1).

It was found that the atmospheric precipitations in April had a negative impact on the bee colonies strength. The linear correlation coefficient of the quantity of atmospheric precipitation this month with this morpho-productive character is negative on medium level and quite significant (r_{xy}= -0.564±0.241; t_r=2.34; P<0.05).

It is important to note that the atmospheric precipitations in May had a particularly negative influence on the queens prolificity. The coefficient of linear correlation of the atmospheric precipitation quantity this month with the prolificity of the queens is negative at a relatively high level with a significance of the highest confidence threshold after Student (r_{xy}= -0.811±0.121; t_r=6.70; P<0.001). This negative impact is explained, by us, by the excessive increase in the humidity in the nest, caused by the intense atmospheric precipitation. In addition, the May atmospheric precipitations had a tendency to influence negative on the viability of the brood (r_{xy}= -0.491±0.268; t_r=1.83; P<0.1) and the quantity of honey accumulated in the nest.

The correlation coefficient of the atmospheric precipitations with the last morpho-productive character is close to the significance of the first threshold of certainty after Student (r_{xy}= -0.507±0.262; t_r=1.94; P<0.1).

The atmospheric precipitations in June had a similar impact to May. Thus, the linear correlation coefficient between the quantity of atmospheric precipitations in June and the prolificity of the queens is negative, of a rather high threshold and with a significance of the highest degree of certainty after Student (r_{xy}= -0.811±0.121; t_r=6.70; P<0.001). This means that with the increase in the quantity of atmospheric precipitation in June, there will be a significant decrease in the prolificity of queens. At the same time, the quantity of atmospheric precipitations in June has a negative impact on the quantity of honey accumulated in the nest.

The correlation coefficient of these two variables is negative, approaching the significance of the first threshold (r_{xy}= -0.507±0.263; t_r=1.93; P<0.1).

Generalizing the impact of climate change on atmospheric precipitation in the first half of the year, we can conclude that high quantity of precipitations during this period, especially in January, February and May, has a negative impact on the vital activity of bee families. The negative impact primarily influences the disease resistance, brood viability, prolificity of queens, colony strength, and honey production. Under the influence of atmospheric precipitation, the humidity in the nest increases, inhibiting the development of most morpho-productive characters of bee families.

Beginning with the second half of the year, the July-December atmospheric precipitation can no longer impact the morpho-productive characteristics previously assessed (at the end of June), but may have a direct impact on the vital activity of bee families related to the consolidation of

colonies strength and their preparation for wintering, as well as indirectly on the evolution of morpho-productive characters in the next year (Tab. 4).

Table 4. Coefficient of correlation between the quantity of monthly atmospheric precipitation in the second half of the current year and the morpho-productive value of bee families in the following year

Morpho- productive character	$r_{xy} \pm m_r$	t_r	**P**
Precipitations in July			
Wintering resistance	-0.482±0.271	1.78	<0.1
Queen prolificity	-0.126±0.348	0.36	>0.1
Colony strength	-0.021±0.353	0.06	>0.1
Disease resistance	-0.642±0.208	3.09	<0.01
Brood viability	0.255±0.331	0.77	>0.1
Honey production	-0.372±0.305	1.22	>0.1
Precipitations in August			
Wintering resistance	0.123±0.349	0.35	>0.1
Queen prolificity	-0.015±0.354	0.04	>0.1
Colony strength	0.346±0.311	1.11	>0.1
Disease resistance	0.660±0.199	3.32	<0.001
Brood viability	0.388±0.300	1.29	>0.1
Honey production	0.040±0.353	0.11	>0.1
Precipitations in September			
Wintering resistance	0.393±0.298	1.32	>0.1
Queen prolificity	0.284±0.325	0.87	>0.1
Colony strength	0.396±0.298	1.33	>0.1
Disease resistance	0.331±0.317	1.04	>0.1
Brood viability	-0.153±0.345	0.44	>0.1
Honey production	0.732±0.164	4.46	<0.001
Precipitations in October			
Wintering resistance	-0.333±0.317	1.05	>0.1
Queen prolificity	-0.080±0.351	0.23	>0.1
Colony strength	-0.345±0.311	1.11	>0.1
Disease resistance	0.433±0.287	1.51	>0.1
Brood viability	0.499±0.265	1.88	<0.1
Honey production	-0.286±0.325	0.88	>0.1
Precipitations in November			
Wintering resistance	0.172±0.343	0.50	>0.1
Queen prolificity	0.008±0.354	0.02	>0.1
Colony strength	0.261±0.330	0.79	>0.1
Disease resistance	-0.262±0.330	0.79	>0.1
Brood viability	0.648±0.205	3.16	<0.01
Honey production	0.627±0.214	2.93	<0.01
Precipitations in December			
Wintering resistance	0.165±0.344	0.48	>0.1
Queen prolificity	0.334±0.317	1.05	>0.1
Colony strength	0.629±0.214	2.94	<0.01
Disease resistance	0.068±0.352	0.18	>0.1
Brood viability	-0.156±0.345	0.45	>0.1
Honey production	-0.061±0.352	0.17	>0.1
Total annual precipitations			
Wintering resistance	0.020±0.353	0.06	>0.1
Queen prolificity	0.525±0.256	2.05	<0.05
Colony strength	0.455±0.280	1.62	>0.1
Disease resistance	0.621±0.215	2.89	<0.01
Brood viability	0.143±0.346	0.41	>0.1
Honey production	0.350±0.310	1.13	>0.1

Analysing the variability of monthly quantity of atmospheric precipitations in the second half of this year in correlation with the evolution of morpho-productive characters of bee families in the first half of the next year, we found that these (atmospheric precipitations) also had a rather evident impact on the vital activity of bee families.

Thus, the quantity of atmospheric precipitations in July had a significant negative impact on the disease's resistance of bee families in the next year. The coefficient of linear correlation between the quantity of atmospheric precipitations in July and the diseases resistance of bee families is negative at the supramedia level and quite significant (r_{xy}= -0.642±0.208; t_r=3.09; P<0.01). At the same time, the precipitations of this summer month also had a tendency to influence negative on the wintering resistance of the bee families in the following year (r_{xy}= -0.482±0.271; t_r=1.78; P<0.1).

August's atmospheric precipitation, on the contrary, had a positive impact on the resistance of bee families to diseases next year. The linear correlation coefficient between the quantity of atmospheric precipitations in August and the resistance of the bee families to the disease is positive above average level and quite significant, with the highest confidence threshold after the Student (r_{xy}= 0.660±0.199; t_r=3.32; P<0.001). This means that with the increase in the quantity of atmospheric precipitation in August this year, it will increase the diseases resistance of bee families in the following year.

Research has shown that the atmospheric precipitation in September of this year had a positive impact on the quantity of honey accumulated in the nest by bee families the following year. The linear correlation coefficient between the quantity of atmospheric precipitations in September and the quantity of honey accumulated in the nest by the bee families is positive at a high level and quite significant, with the highest certainly threshold after Student (r_{xy}= 0.732±0.164; t_r=4.46; P<0.001).

It was found that the atmospheric precipitation in October in general had no significant impact on the vital activity of the bee families, since, the linear correlation coefficients of this month's precipitations and the morpho-productive character of the family's bees are not significant. Only a positive tendency to influence of the quantity of atmospheric precipitations this month on the viability of the brood was observed (r_{xy}= 0.499±0.265; t_r=1.88; P<0.1).

Research data analysis shows that November's atmospheric precipitations has a positive influence on the viability of brood and honey productivity in the coming year. Thus, the linear correlation coefficient between the quantity of atmospheric precipitations in November and the viability of brood in the bee families is positive at the supramedia level and quite significant, with the second threshold of certainty after Student (r_{xy}= 0.648±0.205; t_r=3.16; P<0.01). Also, the linear correlation coefficient between the quantity of atmospheric precipitations in November and honey production of bee families in the following year is positive and significant, with the second certainly threshold after Student (r_{xy}= 0.627±0.214; t_r=2.93; P<0.01). This means that as the quantity of atmospheric precipitations increases in November this year, there will be an increase in the viability of the brood and the productivity of honey accumulated in the nest by bee families the following year.

The atmospheric precipitations from the month of December positively influences the vital activity of the bee families, which is related to the reproduction and developing characters of the brood, ultimately expressed through colony strength. The coefficient of linear correlation between the quantity of atmospheric precipitation in December and the strength of the bee families in the following year is positive and very significant, with the second threshold of certainty after Student (r_{xy}= 0.629±0.214; t_r=2.94; P<0.01).

Regarding climate change the annual atmospheric precipitations, we have established that these have a concrete impact on some morpho-productive characters of bee families of the following year. It has been found that there is a positive correlation between the quantity of annual atmospheric precipitations, on the one hand, and the value of the queen's prolificity as

well as the resistance to disease on the other. Thus, the linear correlation coefficient between the annual quantity of atmospheric precipitation and the prolificity of the queens is positive of medium level with the significance of the first threshold after Student (r_{xy}= 0.525±0.256; t_r=2.05; P<0.05). Between the annual quantity of atmospheric precipitation and disease resistance of bee families was revealed a positive linear correlation coefficient of the supramedia level with the significance of the second threshold after Student (r_{xy}= 0.621±0.215; t_r=2.89; P<0.01). In addition, it was observed that the annual quantity of atmospheric precipitation tends to have a positive influence on bee colony strength in the following year (r_{xy}= 0.455±0.280; t_r=1.62; P>0.1). This means that with the increase of the annual quantity of atmospheric precipitations in the next year there will be an increase in the prolificity of queens, colonies' strength and disease resistance.

Therefore, generalizing the data on the correlation between climate change atmospheric precipitation and the values of the main morpho-productive characters of the bee families, we can conclude that it (climate change) have a significant influence on functions of the vital activity of bee colonies. The variability in the quantity of atmospheric precipitation in different months of the year had a different influence on the development of morpho-productive characters of bee families. Moreover, climate change the atmospheric precipitation in the first half of the year directly influences the variability of the morpho-productive characters values assessed by the end of June, and the atmospheric precipitation in July-December indirectly influences the variability of these values in the next year.

Knowing the impact of climate change on atmospheric precipitation and its influence on the variability of values of morpho-productive characters of bee families during different concrete times of the year, will enable beekeepers to undertake certain mitigation measures by applying of special procedures, and directed feeding of bee families according to the specific periods of the year.

Conclusions

1. The wintering resistance of bee families is low and directly influenced by the atmospheric precipitations in February of current year (r_{xy}= -0.468±0.276; t_r=1.70; P<0.1) and indirectly by atmospheric precipitation in July of last year (r_{xy}= -0.482±0.271; t_r=1.78; P<0.1).
2. The queen prolificity of bee families is negatively affected directly and significantly by the atmospheric precipitation in May and June of current year (r_{xy}= -0.811±0.121; t_r=6.70; P<0.001) and positive indirectly by the annual precipitations amount of the previous year (r_{xy}= 0.525±0.256; t_r=2.05; P<0.05).
3. The strength of bee colonies is negatively and directly influenced by the atmospheric precipitations in April of current year (r_{xy}= -0.564±0.241; t_r=2.34; P<0.05) and indirectly positive by the atmospheric precipitation in December last year (r_{xy}= 0.629±0.214; t_r=2.94; P<0.01).
4. The disease resistance of bee families is influenced negatively and directly by the atmospheric precipitations in January of current year (r_{xy}= -0.712±0.174; t_r=4.09; P<0.001), negative and indirect by the atmospheric precipitation in the month of July previous year (r_{xy}= -0.642±0.208; t_r=3.09; P<0.01) and, positively and indirectly, by the atmospheric precipitation from August last year (r_{xy}= 0.660±0.199; t_r=3.32; P<0.001), as well as by the annual amount of atmospheric precipitations from the previous year (r_{xy}= 0.621±0.215; t_r=2.89; P<0.01).
5. The brood viability is negatively and directly influenced by the January (r_{xy}= -0.557±0.244; t_r=2.28; P<0.05), February (r_{xy}= -0.573±0.237; t_r=2.42; P<0.05) and May atmospheric precipitations precipitations (r_{xy}= -0.491±0.268; t_d=1.83; P<0.1) of current

year and, positively and indirectly by the atmospheric precipitations in months October (r_{xy}= 0.499±0.265; t_r=1.88; P<0.1) and November (r_{xy}= 0.648±0.205; t_r=3.16; P<0.01) of last year.

6. The production of honey accumulated in the nest by the bee families is negatively and directly influenced by the atmospheric precipitation in February (r_{xy}= -0.797±0.128; t_r=6.23; P<0.001), May (r_{xy}= -0.507±0.262; t_r=1.94; P<0.1) and June (r_{xy}= -0.507±0.263; t_r=1.93; P<0.1) of current year, and, positively and indirectly, by the atmospheric precipitations in September (r_{xy}= 0.732±0.164; t_r=4.46; P<0.001) and November (r_{xy}= 0.627±0.214; t_d=2.93; P<0.01) of last year.

Acknowledgements

Scientific researches have been carried out within the fundamental institutional project 15.817.02.12F "Diversity, structure and functioning of complex natural and anthropogenic fauna in the context of strengthening of the national security strategy of the Republic of Moldova" funded from the state budget.

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First Embryos Produced in Romania by Ovum Pick-Up and *In Vitro* Fertilization in Holstein Friesian Cattle

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Abstract

Reproductive technologies, such as ovum pick-up (OPU) and in vitro fertilization (IVF) can rapidly enhance genetics of cattle through both the female and male linage. In this study, we describe the results of first bovine embryo production in Romania by IVF after retrieval of oocytes by OPU.

Also, we evaluated the embryo production by OPU followed by IVF in cows stimulated with an FSH-based treatment (experimental group, n=5) compared to non-stimulated cows (control group, n = 4). Our results suggest that the follicular growth stimulation with FSH improves the number of the punctured follicle (9.8±0.8 vs. 2.5±0.8, $P<0.05$) and the hatched blastocyst rate (41.9% vs. 16.7%). No effect was registered in the oocytes recovery rate in experimental vs. control group (63.3% vs. 60%). In conclusion, the use of follicular growth stimulating program is recommended in order to improve the number of recovered oocytes and the blastocyst rate at Holstein Friesian cattle. This is the first report of embryo production in Romania, using OPU in association with IVF in cattle.

Keywords: dairy cows, ovum pick-up, in vitro fertilization, bovine embryos, hatched blastocyst

Introduction

Both ovum pick-up (OPU) and *in vitro* fertilization (IVF) are seen as mature technologies currently applied in cattle which can be used like an important instrument to drive genetic progress. *In vitro* embryo production has remarkably expanded in the last decade compared to *in vivo* embryo production. This is supported by the total number of transferable OPU and IVF bovine worldwide embryos, 326.623 fresh embryos and 121.490 frozen embryos in 2016 [1].

According to Qi *et al*., [2], Brazil dominated the *in vitro* embryo production by performing 53.019 OPU sessions averaging 15 oocytes and 6 embryos per session.

Although there is a large variation between donors, some IVF labs have achieved the performance to produce over 50 calves per donor cow per year by combining the two technologies, OPU and IVF.

Most of the studies identify donors with highly potential for oocyte production during oocytes retrieval protocols [3], [4]. However, for stimulating follicular growth in the cows from all hormone used in the research studies, FSH has usually given the best results in terms of number of follicles aspirated and oocytes retrieved. Due to the individual variation to FSH stimulation, the number of recovery oocytes varied from 0 to 26 [5]. Most regimens for FSH involved multiple treatments, either 12 or 24h apart, over 2-4 days [6], [7], [8].

In this study we highlight the results of first embryos production in Romania by combining OPU and IVF at Holstein (*Bos taurus*) breed. Also, we test the involvement of FSH follicular growth stimulating program for improving the number of recovered oocytes and the rate of hatched blastocyst.

Methodology

This study was performed at the Research and Development Station for Cattle Breeding Dancu-Iasi (SCDCB Dancu-Iasi), Romania, which owns a population of 600 Holstein Friesian cows (recognized as a Bălțată cu Negru Românească breed).

In this experiment we used a standard protocol (IVF Bioscience, UK) for obtaining bovine embryos through IVF which is according to the current literature.

Ovarian stimulation program and oocytes recovery

The cows from this experiment were part from the SCDCB Dancu-Iasi culling group with no milk production during our study. Each animal from experimental group (E-group, n=5) was treated during the luteal phase to prevent spontaneous ovulation. Cows were stimulated for 3 days with FSH (Pluset, Laboratorios Calier, Spain), 2 administration per day at 12 hours interval following the next doses: day 1 – 3ml/3ml; day 2 – 2.5ml/2.5ml and day 3 – 2ml/2ml. Also, in day 3 a dose of PgF2α (PGF Veyx forte, GmbH) was administered for each cow from experimental group. OPU was performed at 24 h after PgF2α administration. The cows from control group (C-group, n=4) did not receive any treatment.

The oocytes were harvested from the living donors by OPU. A real-time B-mode ultrasound scanner (Aloka Prosound 2) equipped with a 5 MHz convex transducer was used during the transvaginal ultrasound guided follicular aspiration. The transducer was mounted in a metal handle with stainless needle guide. A Cova OPU Needle of 17G x 600 mm was attached by a silicone hose to a 50 ml plastic tube (Falcon). Follicular fluid was aspirated using continuous negative pressure, 50-90 mm Hg, applied with a suction pump (Craft Suction Pump-Rocket Medical) and collected in a 50 ml tube containing OPU medium (IVF Bioscience, UK)

In vitro embryo production

After recovery, all the collected cumulus oocytes complexes were cleaned of debris by washing them three time in Bo-Wash medium (IVF Bioscience, UK) and transferred for maturation step in 4 well NUNC dishes, which contained 500 µl/well BO-IVM medium (IVF Bioscience) and placed in incubator at 38.8ºC, 5% CO_2 and 90% relative humidity for 24 h.

Spermatozoa were selected by using BO-SemenPrep medium (IVF Bioscience, UK) as following: for each 250µl frozen semen straw, two tubes with 4 ml and respectively 2 ml Bo-SemenPrep, were prepared; the semen for one straw was centrifuged at 328g for 5 minutes in 4 ml Bo-SemenPrep tubes; after centrifugation the supernatant was removed until 350-700µl sperm suspension remained in 4 ml tubes; after this procedure we added the additional preheated BO-SemenPrep (from 2 ml tubes), resuspended the pellet and centrifuged; following the second centrifugation, we removed again the supernatant until the same volume of 350-700µl sperm suspension remained; we resuspended the pellet in this volume and used for IVF.

The fertilization of matured oocytes was conducted in 90 µl drops of BO-IVF medium (IVF Bioscience, UK) under mineral oil. Before fertilization, the matured oocytes were washed three times in 100 µl BO-IVF medium and then transferred to the fertilization microdrops. After this procedure, a concentration of 2×10^6 sperm/ml was used for the *in vitro* fertilization and then the gametes were co-incubated for 20 h at 38.8ºC, 5% CO_2 and 90% relative humidity.

For culture of presumptive zygotes in BO-IVC medium (IVF Bioscience, UK), cumulus cells were removed by vortexing the cumulus oocytes complexes for 2 minutes in the same solution.

The culture of presumptive zygotes was carried out in NUNC dishes, which contained 500 µl/well BO-IVC medium under mineral oil and placed into the incubator at 38.8ºC, 5% CO_2 and 90% relative humidity for 7-9 days. The results of the IVF procedure were evaluated in days 7, 8 and 9 (the day of fertilization was considered day 0).

The statistical significance of the differences in means of two groups was evaluated by one-way analysis of variance (ANOVA) and by Tukey-Kramer Multiple Comparisons Test.

Results

Nine sessions of OPU associated with IVF procedures were performed, in which a total number of 59 follicles were punctured (49 in E-group and 10 in C-group). In experimental group the follicular growth stimulation protocol with FSH generated an average of 9.8±0.8 punctured follicles (Fig. 1) per session ($P<0.05$) compared with C-group in which only 2.5±0.8 follicles per session were punctured (Table 1). In this experiment we observed no differences in the recovery rate in E-group (63.3%) vs. C-group (60%), but in the number of recovered oocytes, which was superior ($P<0.05$) for E-group (6.2±0.8) compared with C-group (1.5±0.2).

In our opinion this is a promising result considering that it is a premiere in Romania. In domestic animals, four methods for collection of oocytes have been described: aspiration of the oocyte from the follicles of living cows by transvaginal ultrasound guided follicular aspiration (a procedure also called ovum pick-up, OPU) [9], [10], slicing the ovaries [11], [12], [13], puncture of visible surface follicles [14], [15] and laparoscopic ovum pick-up [16]. However, the OPU seems to be preferred for commercial purpose [1] as it allows a prolonged use of a certain donor cows for IVF compared to other methods. Several studies reported a high variation in oocyte retrieval per OPU across breeds [17], [18], [19]. For example, Watanabe *et al*., [20] presented a high number of recovery oocytes (n = 19.3±0.6) per OPU session at Holstein Friesian cattle, which is superior compared to us. Also, this research highlights the fact that the result was influenced by the decision to use for OPU session only the donor cows with greater potential for oocyte recovery per OPU.

This may determine IVF success in some cattle breeds yielding fewer oocytes per OPU [20].

Thus, further research is needed to explore the relationships between the number of oocytes recovered per OPU session and with IVF efficiency, as well as with field fertility (pregnancy results following embryo transfer).

It will be an important step in decisions making regarding the donor's selection for improving the results of *in vitro* production of bovine embryos.

Although we did not select the oocytes according to A, B, C quality grade [21], [22] we obtained an acceptable embryo development rate (Table 1) objectified by 41.9% hatched blastocyst (n = 13, $P<0.05$) in E-group compared with only 16.7% hatched blastocyst in C-group (n=1).

In our opinion, this result is influenced by the higher number of fertilized ova in E-group compared to C-group. Similar results were obtained by Watanabe *et al*., [20], which concluded that the number of blastocysts per OPU is greater for dairy donors with higher number of oocytes recovered per OPU. Our best performance was to obtain 6 hatched blastocysts (Fig. 3) from 9 recovered cumulus oocytes complexes (Fig. 2), generating a hatched blastocyst rate of 66.6%.

Although the number of samples was suboptimal to obtain high impact results, our study generates a hatched blastocyst rate at E-group similar with those from the study of Watanabe *et al*., [20], which have been achieved in a specialized laboratory.

Further efforts will be focused at SCDCB Dancu-Iasi for improving the results of OPU in association with IVF and to transfer the obtained embryos to the recipient cows.

Table 1. The results of first OPU and IVF procedures at SCDCB Dancu Iasi in Holstein Friesian cattle

OPU group	Crt. no. of OPU session	Punctured follicles	Recovered oocytes	Oocytes recovery rate	Hached blastocysts	Hatched blastocyst rate
E-group	1	9	5	55.5%	1	20%
	2	13	9	69.2%	6	66,6%
	3	9	4	44.4%	1	25%
	4	10	6	60%	3	50%
	5	8	7	87,5%	2	28.5%
	Average± SEM	9.8±0.8*	6.2±0.8*	63.3%	2.6±0.9*	41.9%
C-group	6	2	2	100%	0	0
	7	2	1	50%	0	0
	8	1	1	100%	0	0
	9	5	2	40%	1	50%
	Average± SEM	2.5±0.8	1.5±0.2	60%	0.25±0.2	16.7%

* P<0.05 statistically significant; E-group is experimental group; C-group is control group

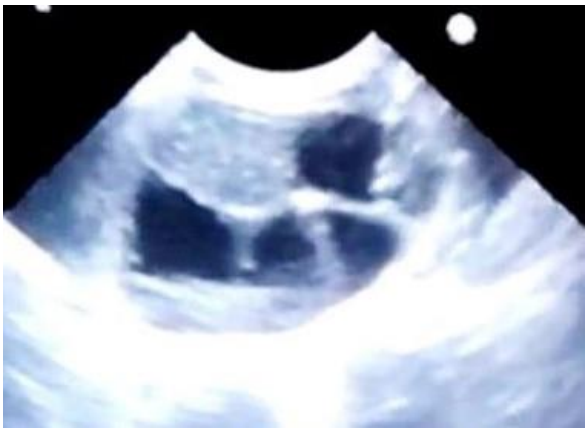

Fig. 1. Representative image from OPU session on an FSH-stimulated ovary (E-Group)

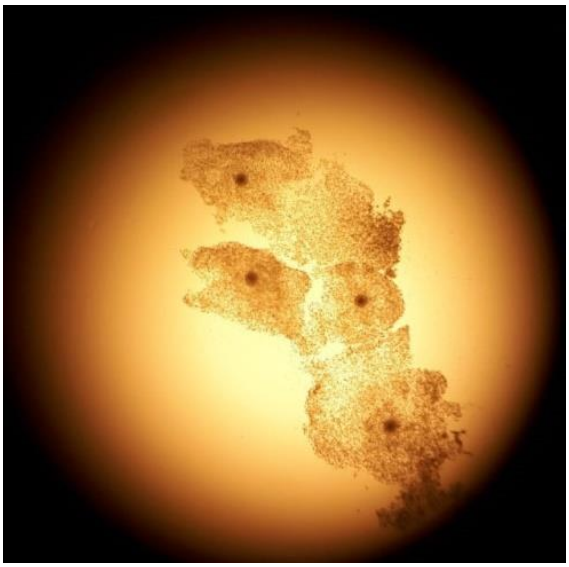

Fig. 2. Representative image of matured cumulus oocyte complexes, recovered by OPU, during *in vitro* embryo production procedures

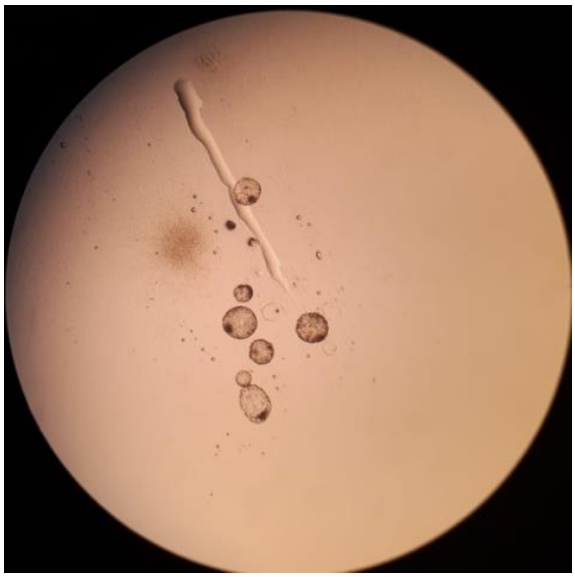

Fig. 3. Representative image of hatched blastocysts during *in vitro* embryo production procedures

Conclusion

This is the first report of bovine embryos production (hatched blastocysts) in Romania by the association of OPU and IVF. This could be a corner stone for implementation of embryo production by OPU and IVF in Holstein Friesian cattle in north-eastern Romania to extend elite genetics provided by both proven donors and rare or expensive sires. Although a small sample size was used, this paper is a proof of the fact that at SCDCB Dancu-Iasi the *in vitro* embryo production in *Bos taurus* species is in a continuous development for further attempts to produce genetically highly valuable animals.

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Monitoring the Qualitative Parameters of the Refrigerated Ram Semen During Non-Breading Season

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Abstract

The association of the biotechnology of artificial insemination with the conservation of the semen allows the use of a small number of males with which a large number of females can be inseminated. Artificial insemination gives numerous genetic and economic benefits for animal production, being the safest method to bring into the herd the superior genes, of valuable individuals. The aim of this study was to evaluate the effect of Tris-based extender containing 2% glycerol and 20% egg yolk on the motility and viability of rams' semen collected in non-breeding season and refrigerated at 4^0C. During 30 days, a total of 16 ejaculates from 6 Texel rams were collected. The semen was collected by artificial vagina during May-June 2019. The main qualitative parameters: motility and viability were evaluated immediately after collection and also at 24, 48, 72, 96 and 120 hours. The results were statistically processed and the extender's influence and storage time were analysed. In conclusion, the Tris-based extender containing glycerol and egg yolk showed a satisfactory protective effect on rams' semen collected in non-breeding season and refrigerated at 4^0C. Storage time ($P<0.05$) has significantly affected the qualitative parameters of the semen recording a 10-15% decrease of the qualitative parameters every 24 hours.

Keywords: Semen quality; ram; non-breeding season

Introduction

Artificial insemination with frozen sperm in cattle has been successfully and widely used.

Unlike cows, artificial insemination in sheep using frozen sperm is not common due to the difficulty of the method and low fertility rates [12]. Ram semen cryopreservation is of high interest, particularly in European countries, aiming to increase productive parameters by animal genetic improvement in selected flocks. Additionally, the need for widespread performance of sheep artificial insemination (AI) over extended periods or at different times of the year, stimulated more research on semen preservation.

Therefore, in ewes breeding, instead of frozen semen, native or liquid preserved semen has been used and a 60% or higher fertility rate can be achieved [10]. The greatest difficulty with liquid storage is the 10% to 35% loss of sperm fertility if the storage time is over 24 h. Even though semen can remain motile for up to a week, its fertility capacity can decrease [11].

Although successful fertility rates have been reported after a storage period for more than 24 h [10] in some studies, contradictory or low fertility results have also been reported [10]. It is necessary to extend the liquid storage time to benefit from artificial insemination techniques on

a wider platform. More research on the subject is required to achieve optimum fertility rates in storage periods over 48 h.

In ewes breeding, due to the large scale of the herd to be inseminated and breeding performed over long distances, sperm must be transported without any problems and loss in its fertility capacity. Moreover, to take advantage of the rams for longer periods and in various times of the year, sperm storage technologies should be more advanced. The basic principle of sperm storage is to reduce the spermatozoa metabolism, thus extending its life. For this purpose, sperm is stored at low temperatures (4-22 C, liquid storage) or frozen (-196°C, long-term storage) [12]. The success of short- and long-term storage methods is, on the whole, dependent on the storage temperature, cooling rate, chemical composition of the extender, reactive oxygen species (ROS), and seminal plasma composition. The protein and chemical composition of seminal plasma and its effect on sperm function are dependent on the secretory activity of the accessory sex glands, epididymis and testis. This varies with breed, animal and ejaculate, due to numerous sources of intra- and inter-animal variation including climate (high temperatures and humidity negatively affect sperm quality), plane of nutrition, sexual maturity, health status, frequency of collection and ejaculate number [5].

The aim of this study was to evaluate the effect of Tris-based extender containing 2% glycerol and 20% egg yolk on the motility and viability of rams' semen collected in non-breeding season and refrigerated at 4^0C.

Methodology

Six Texel rams were used in the present study. The rams were housed at ICDCOC Palas Constanta. From May to June in the non-breeding season, the semen was collected twice a week using an artificial vagina and was kept in a water bath at 37°C until use. The volume of the ejaculate is appreciated right after collection, by reading the divisions on the graduated test tube of the collecting glass. Raw semen was analysed and used if the following criteria were met: volume bigger than 0.5 ml, motility and membrane integrity ≥70%, and concentration ≥2.5 × 109 spermatozoa/mL.

In order to assess the semen quality, the physical and morphocytological parameters were evaluated at collection, and after 24, 48, 72, 96 and 120 hours of refrigeration at 4^0C. The analysis of the morphological parameters was performed by optical microscopy techniques.

Tris-citric-glucose-egg yolk (20%) – glycerol 2% was used as dilution medium. Motility was assessed by manual evaluation technique [13] in wet environment, under the optical microscope (Novex, Holland) (x100 magnification) equipped with heating plate maintained at 37°C and camera. The evaluation of motility must take into account the speed, linearity and lateral movements of spermatozoa.

Structural integrity of plasma membranes (viability) was assessed by the eosin-nigrosine staining method [4].

The results were statistically processed using IBM SPSS Statistics 20 software.

Results and Discussion

Several studies have demonstrated that season has an influence on ram's reproductive characteristics [7], [14]. They reported that the standard method of evaluating the fertility of male breeding is the examination of sperm production [3].

The semen collected for dilution came from 6 Texel rams between 2.5 and 3 years old. For each male, the ejaculate was collected using the artificial vagina. The collections were performed during non-breeding season, twice a week. From each ram, an average of 15

ejaculate were collected. The morpho-cytological parameters of the collected semen are shown in Table 1.

Table 1. Morpho-cytological parameters of semen in Texel rams collected during non-breeding season

	Spermatozoa indices	Male 1	Male 2	Male 3	Male 4	Male 5	Male 6
1.	Ejaculates (n)	16	14	15	16	15	16
2.	Volume (ml)	1.21±0.04	1.89±0.31	1.34±0.25	1.31±0.16	1.56±0.22	1.17±0.09
3.	*pH*	7.0±0.04	7.1±0.10	6.9±0.21	6.9±0.17	7.1±0.08	7.0±0.25
4.	Concentration (mild/ml)	2.99±0.11	4.12±0.22	3.01±0.34	3.23±0.19	4.62±0.08	2.86±0.29

The results are presented as mean ± standard deviation.

Semen extenders have been designed to protect and maintain spermatozoa during the processing and storage of semen [9]. Extender as a medium creates optimal conditions for the extension of the life of sperm and, most importantly, preserves the reproductive ability of semen. Extender replaces the seminal plasma and assumes its role. In other words, extender maintains motility and fertilizing capacity, and preserves sperm membrane integrity. It is also necessary to dilute semen to obtain a larger number of doses from the ejaculate.

The extender's composition is critical for the success of ram's liquid semen conservation at low temperature. Tris-based and skimmed-milk extenders are currently the most used for preserving goat semen. In addition to Tris, the extender also contains fructose or glucose, egg yolk, antibiotic and citric acid. Antioxidants can be used to remove reactive oxygen species generated from the intracellular compartments of spermatozoa. In order to be protected against cold shock, in addition to lipids from egg yolk, the dilution environment must contain also other cryoprotectant substances. The medium contains glycerol as cryoprotectant substance. The glycerol concentration in the dilution medium is 2% (v/v) which leads to a final concentration in diluted semen of about 1%. For preparation, the volume of glycerine required is calculated and added to the egg yolk medium (20%), heated to 300 ºC, to facilitate homogenization. The glycerol used is of purity p.a. (Sigma, Germany) to prevent the environment's contamination.

The volume of ejaculates collected during non-breeding season from Texel rams varied between 1.17 and 1.89, and the pH of the semen ranged between 6.9 and 7.1. The semen concentration was established by assessing the consistency of the semen. It resulted an average concentration of 4.15 billion spermatozoa / ml. Concentration (density) is an important feature on which the level of dilution and the number of fractions subsequently depend.

Motility was assessed by manual evaluation technique [13] in wet environment, under the optical microscope (Novex, Holland) (x100 magnification) equipped with heating plate maintained at 37°C and camera. An average motility of 91.7% resulted. The minimum permissible motility for the semen to be diluted for refrigeration is of 80%.

Determination of viability is one of the basic elements of semen quality assessment, being of great importance, in order to distinguish between dead and living immotile speramatozoa, especially for the samples in which many immotile spermatozoa are found. In order to assess viability, we used the eosin-nigrosine staining method.

The semen from the 6 rams was diluted with Tris-based dilution medium and then refrigerated at +4ºC. At 24-hour intervals, the motility and viability of the diluted semen were evaluated. The obtained results are presented in Table 2.

Table 2. Variation of motility in relation to storage time

Refrigeration Period (h)	Male 1	Male 2	Male 3	Male 4	Male 5	Male 6
0	92±2.54	93.9±1.49	90.7±2.01	88.1±1.74	90.1±1.54	93.1±1.94
24	80.3±3.17	82.7±2.23	79.2±2.29	75.9±2.26	79.4±1.98	81.9±2.81
48	65.2±2.96	67.8±2.44	65.7±2.94	62±2.01	65.5±1.86	67.5±2.65
72	51.4±2.24	52.8±2.10	51.6±2.84	48.3±1.54	51.7±1.81	52.9±2.30
96	37.1±1.73	38.5±2.20	37.2±1.71	35.1±1.56	37.3±1.49	38.3±1.72
120	22.9±2.20	27.6±1.59	25.3±2.13	20.3±2.37	23.5±1.65	26.8±1.95

The results are presented as mean ± standard deviation.

According to these data it can be observed that within the 5 days in which the semen was refrigerated and tested, there was a decrease of motility between 65-70%, from the moment of collection until the day 5, but with significant differences for each male in part. There were no significant differences regarding the rate of decreased motility from 0 to 120 hours, between male 2 and male 5, between male 2 and male 6 and between male 5 and male 6 ($p>0.05$).

Determination of viability is one of the basic elements of semen quality assessment, being of great importance, in order to distinguish between dead and living immotile speramatozoa, especially for the samples in which many immotile spermatozoa are found. In order to assess viability, we used the eosin-nigrosine staining method. The viability of the diluted semen was evaluated at 24-hrs intervals. The obtained results are presented in Table 3.

Table 3. Variation of viability in relation to storage time

Refrigeration Period (h)	Male 1	Male 2	Male 3	Male 4	Male 5	Male 6
0	93.4±1.34	94.7±1.32	91.8±1.40	89.2±1.68	91.5±1.28	94.4±1.65
24	81.7±2.43	82.8±1.96	80±2.35	78.4±1.78	80.3±1.39	83.3±2.13
48	67.2±2.32	68.5±2.138	66.2±2.01	64.6±2.23	66.1±1.70	68.5±1.87
72	52.5±1.82	53.7±1.93	52.3±1.90	50.6±2.13	51.7±2.08	53.7±1.52
96	38.2±1.42	38.5±1.22	37.8±1.70	36.9±2.01	37.5±1.39	38.7±1.68
120	24.5±1.55	28.3±1.64	26.5±1.65	21.7±2.36	25.2±1.25	28.1±2.26

The results are presented as mean ± standard deviation.

According to these data it can be observed that within the 5 days in which the semen was refrigerated and tested, there was a decrease of motility between 65-70%, from the moment of collection until the day 5, but with significant differences for each male in part. There were no significant differences regarding the rate of decreased viability from 0 to 120 hours, between male 2 and male 5, between male 2 and male 6 and between male 5 and male 6 ($p>0.05$).

The most commonly used non-penetrating cryoprotective for freezing ram semen is egg yolk due to its protective effect on plasma and acrosomal membranes [12]. Due to the interactions between this cryoprotective and semen plasma enzymes, such as egg yolk coagulation enzyme (EYCE), it was concluded that a very important factor is the percentage of egg yolk in the extender A variety of results were observed regarding concentrations between 2% and 20% of egg yolk in extender [1]. Glycerol is the most widely used permeable cryoprotectant for the preservation of farm animals' semen because it prevents phase changes of the extender when added to the medium in concentrations below 3% [6].

Concentrations greater than 3% lead to decreased survival during preservation and deterioration of acrosomes, resulting in decreased fertility [6]. Glycerol has an osmotic effect and has a direct effect on the plasma membrane by binding to membrane phospholipids.

Although the main cryoprotective effect of glycerol is expressed at the extracellular level, it can enter the cell and remain bound to the plasma membrane or cytoplasm [2].

Liquid storage of sperm up to 2-4 days is the main goal of artificial insemination in sheep breeding programs. However, decrease in sperm fertility in durations longer than 24 h of liquid

storage is the most important problem. For this purpose, improvements in both the artificial insemination techniques and the storage techniques are required. Mitochondria of spermatozoa are different from those of the somatic cells in terms of morphology and biochemistry.

Mitochondrial energy metabolism plays a vital role in the continuation of sperm functions.

In liquid storage, spermatozoa need to be able to maintain their energy reserves and their mitochondria function fully to survive for an extended period of time without losing their motility. In a study conducted by Maxwell and Salamon [8], it was reported that more than 24 h of storage rapidly decreased fertility. The rate of decline in fertility was between 10% and 15% per day. In our study there was recorded a decrease rate between 10% and 15%. Researches will continue with the quality assessment of semen during breeding season (August-September).

Conclusions

The Tris-based extender containing glycerol and egg yolk showed a satisfactory protective effect on rams' semen collected in non-breeding season and refrigerated at 4^0C. The volume of ejaculates collected during non-breeding season from Texel rams varied between 1.17 and 1.89, and the pH of the semen ranged between 6.9 and 7.1. The average semen concentration was 3.47 billion spermatozoa/ml, and the average motility was 91.3%, with variations between 88.1 and 93.9. After 5 days of preservation by refrigeration at 4^0C, there was a decrease of viability between 60-70%, and a decrease of motility between 60-70% with a daily rate of decrease between 10-15%. The decrease in motility and viability depends on the individual, in our study there were significant differences ($p<0.01$) between the studied rams.

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Semen Quality of Mature Carpathian Bucks During Non-Breading Season

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Abstract

The recognition of the differences specific to each breed and those caused by the estrous season in terms of the breeding performance of the bucks, leads to the improvement of the management of individual semen samples used in the artificial insemination programs. The aim of the study was to evaluate the quality of the semen collected during non-breeding season, from 3 Carpathian bucks, 3 years of age. The semen was collected by artificial vagina between June and July 2019. Two methods of semen dilution, with and without seminal plasma were compared, in order to highlight the effect of seminal plasma in the refrigeration process. Tris-based extender was used, with and without egg yolk. The quality of the semen was evaluated immediately after collection by assessing the volume ejaculated, concentration, pH and motility and viability immediately after collection and also at 24 and 48 hours, after refrigeration at a temperature of 4^0C. The results showed a decrease of the cytological parameters (motility, viability) by 15-20% every 24 hours of refrigeration in the semen samples from both methods. In conclusion, for insemination during non-breeding season with refrigerated semen, within 48 hours after collection, there is no need for centrifugation in order to remove the seminal plasma during semen's preparation process.

Keywords: Semen quality; buck; non-breeding season

Introduction

The photoperiod controls the sexual activity and is the main environmental factor which established the seasonal feature of goat's reproduction. Although the seasonality is less important at buck than goat, the bucks present a seasonal reduction of sexual behaviour and spermatogenesis during the same period of the year when the females are in out of seasons [1].

The used extenders for buck semen will contain egg yolk or skimmed milk powder.

However, the dilution of buck semen with extenders that contain egg yolk can be harmful for spermatozoa. This thing happens because the buck seminal plasma presents characteristics that differentiate it from other species, the most important one being the presence of photolipase a secreted by bulbourethral glands. This phospholipase is called also Eyce (Egg yolk coagulating enzyme) or BUSgp60 (bulb urethral gland secretion) and is responsible for spermatic cells viability decrease that have been diluted with extenders containing egg yolk or milk powder [2]. Because the seminal plasma is removed or diluted during processing and because this contains elements that prevent premature capacitation [3], it is considered to be a physiological fluid that could protect the spermatozoa from handling induced stress.

The objectives of recent research on animal reproduction wanted to establish the physiological functions of seminal plasma and to examine the possibility that this complex fluid can be used as a biotechnological tool in order to improve the function of semen during processing for the use in artificial reproductive technologies (ART). *In vitro* processing of spermatozoa for preservation by refrigeration, cryopreservation or sexing leads to important changes in the semen sample extracellular fluid medium. The most obvious result is the dilution of semen plasma proteins in the sperm environment due to the addition of optimized extender for reproductive technologies. *In vitro* manipulation attempts to copy the signals and protective aspects of the *in vivo* environment to maintain the shape and function of the semen. The processing of the semen also leads to fluctuations in temperature, pressure, osmolality and pH which can damage seminal plasma membranes and limit the fertilizing life of the processed spermatozoa. These *in vitro* modifications have many similarities with *in vivo* capacitation, including impaired lipid mobility, cholesterol efflux and tyrosine phosphorylation. Because these changes occur *in vitro* prior to deposition in the female tract, rather than at the site of *in vivo* fertilization (the oviduct), the fertilizing capacity of processed spermatozoa is considerably decreased [4].

The aim of this study was to evaluate the quality of the semen collected during non-breeding season, after 48 hours of refrigeration at 4°C, with and without seminal plasma.

Methodology

The semen was collected from 3 Carpathian bucks of 3 years old, during June and July 2019.

The semen was collected using female goats with estrus induced by hormonal treatments.

From each male, two ejaculates were collected, at an interval of 15-20 minutes, which, after the first dilution, were processed together. If the motility differences between the ejaculates of the same buck were greater than 15%, the ejaculates were processed separately. The ejaculates were collected by artificial vagina.

The processing for refrigeration was done by two methods: the classical method, by centrifugation to remove the seminal plasma and dilution with Tris extender with 20% egg yolk and 1% glycerol and the method without centrifugation and dilution with Tris extender without egg yolk, with 1% glycerol.

In order to assess the quality of the semen, the physical and morphocytological parameters were evaluated at collection, and after 24 and 48 hours of cooling at 4°C. The analysis of the morphological parameters was performed by optical microscopy techniques.

The volume of the ejaculate is established immediately after collection, by reading the divisions on the graduated test tube of the collecting glass. Only the volumes larger than 0.5 ml were used for processing. The concentration of spermatozoa in semen sample was estimated by haemocytometer method and is expressed in billions of spermatozoa/ ml semen.

In order to measure the motility, the semen is classified as non-motile, with progressive motility or with non-progressive motility. Also, the total percentage of spermatozoa with progressive motility is estimated. Motility was assessed by manual evaluation technique [5] in wet environment, under optical microscope (Novex, Holland) (x100 magnification) equipped with heating plate maintained at 37°C and camera.

Structural integrity of plasmatic membranes (viability) was assessed by the eosin-nigrosine staining method [6].

The morphological examination of the spermatozoa consists in establishing the number of spermatozoa with abnormal appearance.

The results were statistically processed using IBM SPSS Statistics 20 software.

Results and Discussion

The morpho-cytological parameters of the semen collected during non-breeding season from the 3 Carpathian bucks are shown in table 1.

Table 1 showed that mean of semen volume per ejaculate ranged from 0.95±0.26 to 1.35±0.34 ml. Semen volume per ejaculate differed significantly ($p<0.05$) among the bucks.

Highest semen volume was obtained in buck 3 followed by 2, and 1. The three bucks belonged to the same breed and of similar age, their management and nutritional status and general health condition were also similar. So, the difference in volume of semen might reflect their different genetic potentiality and genetically superior bucks could produce higher volume of semen. The result of the present study agrees with the studies of previous workers [7, 8]. In terms of sperm concentration, there were also significant differences ($p<0.05$) between all 3 males.

Table 1. Morpho-cytological parameters of Carpathian bucks' semen collected during non-breeding season

No.	Spermatozoa indices	Male 1	Male 2	Male 3
1.	Ejaculates (n)	32	30	35
2.	Volume (ml)	0.95^{a}±0.26	1.1^{b}±0.23	1.35^{ab}±0.34
3.	*pH*	7.1±0.06	7.1±0.11	7.0±0.21
4.	Concentration (billions/ ml)	4.99^{a}±0.16	5.12^{a}±0.19	3.23^{a}±0.19
5.	Abnormalities (%)	7.66±0.92	7.36±0.12	7.21±0.58

The results are presented as mean ± standard deviation. Means with different superscripts within the same row differed significantly ($p<0.05$)

Usually, the percentage of morphological abnormalities in the semen of a buck with normal fertility should be less than 5% during the breeding season. If collections are made during the summer, the percentage of abnormalities can be expected to be higher. The percentage of morphological abnormalities in the semen of below average and poorly fertile bucks may be 10-15%. Our study recorded values between 7.21 and 7.66%, which are normal for the season.

There were no significant differences between the semen from the 3 males neither for anomalies nor for pH.

The results regarding the effect of refrigeration, in the presence and absence of seminal plasma, on the male 1 semen's quality are shown in table no. 2. According to these data, it can be observed that within the 2 days during which the semen was tested, for the batch with refrigerated semen in the presence of seminal plasma, without the addition of egg yolk, a decrease of viability by approximately 37% is observed, from 90.61% to 53.63%, while for the second alternative with the refrigerated semen without seminal plasma, there is a decrease of viability of 35%. There are no significant differences between the experimental options.

Motility is a very important sperm indicator on which the fertilizing capacity depends. The assessment of motility must take into account the speed, linearity and lateral movement of spermatozoa. During the 2 days of monitoring, there was a decrease of motility by 34% for the refrigerated batch without seminal plasma and by 35% in the refrigerated batch with seminal plasma (table 2).

Table 2. Motility (%) and viability (%) of the male no. 1 semen according to dilution method

Refrigeration period (h)	Motility (%)		Viability (%)	
	Without seminal plasma	With seminal plasma	Without seminal plasma	With seminal plasma
0	88.21±2.04	88.82±1.75	90.21±2.14	90.61±2.01
24	72.30±2.11	72.42±3.02	74.40±2.27	73.92±1.96
48	54.31±2.66	53.33±2.41	55.40±3.62	53.63±3.06

The results are presented as mean ± standard deviation (n=32).

The results regarding the male 2 semen's quality are shown in table no. 3.

According to these data it can be observed that during the 2 days in which the sperm was tested, for the batch with refrigerated semen in the presence of seminal plasma, without the addition of egg yolk, a decrease of viability by approximately 37% was observed, while for the second alternative with refrigeration without seminal plasma, there is a 35% decrease. There are no significant differences between the experimental options. During the 2 days of monitoring, there was a decrease in motility by 35%, from 89.71% to 54.50%, in the refrigerated lot without seminal plasma and by 37% in the refrigerated lot with seminal plasma (table 3).

Table 3. Motility (%) and viability (%) of male no. 2 semen depending on dilution method

Refrigeration period (h)	Motility %		Viability %	
	Without seminal plasma	With seminal plasma	Without seminal plasma	With seminal plasma
0	89.71±1.88	90.50±1.35	90.71±2.00	91.30±1.25
24	72.73±2.16	73.52±2.75	74.00±2.66	74.02±2.35
48	54.50±2.79	53.13±2.02	55.90±2.33	54.11±1.53

Results are presented as mean ± standard deviation (n = 30).

The results regarding the male 3 semen's quality are shown in table no. 4.

According to these data it can be observed that during the 2 days in which the sperm was tested, in the batch of semen refrigerated in the presence of seminal plasma, without the addition of egg yolk, a decrease of viability was observed by approximately 33,6% while for the refrigeration variant without seminal plasma, there is a decrease of 34%. There are no significant differences between the experimental options. During the 2 days of monitoring, there was a decrease of motility by 34,2%, from 84.61% to 50.45%, in the refrigerated lot without seminal plasma and by 35,5% in the refrigerated lot with seminal plasma (table 3).

Table 4. Motility (%) and viability (%) of the male 3 semen according to dilution method

Refrigeration period (h)	Motility (%)		Viability (%)	
	Without seminal plasma	With seminal plasma	Without seminal plasma	With seminal plasma
0	84.61±1.34	84.91±2.23	86.51±1.26	84.71±1.15
24	70.53±1.43	69.62±2.22	71.31±1.05	72.42±1.77
48	50.45±1.34	49.41±0.69	52.90±1.96	50.51±1.90

Results are presented as mean ± standard deviation (n = 35).

Refrigeration at low temperatures provides some suppression of semen metabolism compared to physiological temperatures, but the maintenance of energy metabolism following storage is still the most important criteria for successful preservation. Because glucose is an energy source for spermatozoa, higher efficiency energy metabolism would support sperm mobility during preservation. [9].

Researchers have long sought to identify the specific factors in seminal plasma that influence semen's function and fertility. As the information on seminal plasma function were accumulated, it has become clear that dilution of seminal plasma during semen's processing for artificial reproduction (e.g., sexing and cryopreservation), to some extent, may explain the altered function and fertile status of the processed spermatozoa.

As a result, a considerable investigation of the effect of supplementation with seminal plasma on the survival and function of spermatozoa processed for controlled reproduction has been observed in recent decades Our study has shown that the decrease of semen's quality during refrigeration is the same in the presence and absence of seminal plasma. Other studies obtained higher percentages of motility and viability after 56 hours of refrigeration in the presence of seminal plasma [10]. Moreover, by avoiding centrifugation, a stress-generating

stage is eliminated and the processing time is optimized. Our results recorded a 34-37% decrease in motility and viability without significant differences between the three males. Other studies have shown that semen's motility during refrigeration (24 h, milk at 15°C) is male dependent and may be correlated with the differences existing in the seminal plasma proteome.

The same studies have shown that several seminal cell membrane proteins that interact with the cytoskeleton, glycolysis enzymes, and spermatozoa associated proteins involved in capacitation are positively correlated with good results after refrigeration and can be considered as seminal biomarkers in semen conservation [11].

The proteomic evaluation of spermatozoa and the environment has made considerable progress toward these goals and allowed a better understanding of their physiological function [13]. Many plasma proteins have been identified as diagnostic predictors of semen's function and have been isolated and applied *in vitro* in order to prevent damage of semen due to the application of artificial reproduction technologies. A higher concentration of glycolytic enzymes in the seminal plasma of seminal samples that have high in vitro motility may be related to an increased abundance of glycolytic pathways in the spermatozoa or may represent an increased spermatozoa production [12]. Proteomic characterization of ram's seminal plasma has identified over 700 proteins [11], the most abundant seminal proteins being secreted by accessory sex glands. Comparative proteomic analysis has shown that spermatozoa-associated protein complexes are predominantly associated with higher semen's preservation capacity [14, 15]. Our research must continue to identify these proteins in the buck seminal plasma.

Conclusions

The proteomic evaluation of the spermatozoa and the environment has made considerable progress toward these goals and has allowed a better understanding of the physiological function of the spermatozoa. Our study has shown that the variation of semen's quality during 48 hours of refrigeration, in the presence and absence of seminal plasma, is the same, which demonstrates the importance of seminal plasma during preservation. Furthermore, by avoiding centrifugation, a stress-generating stage is eliminated and the processing time of the semen to be used in artificial insemination is optimized.

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Section 4
VETERINARY MEDICINE

Microbiological Risk Assessment of Some Fast Food Products for the Public Health

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Abstract

Fast-food products are ready to eat products, appreciated by a large part of consumers (especially children and young people), according to the sensorial characteristics, fast serving and a relatively low price, when compared to the dishes offered by restaurants. Considering the chemical composition, rich in carbohydrates, proteins and fats, extremely favorable for the growth of pathogens, in case of not following the good hygiene practices (GHP), consumption of these products can increase the risk of foodborne illnesses, with *Staphylococcus aureus, E. coli, Listeria monocytogenes, Salmonella* spp. etc.

The research aimed to perform a microbiological risk assessment of the most consumed fast-food products

The research material, collected from fast-food units located in Transylvania, consisted of 63 samples of the following dishes: hamburger, chicken shawarma, and roasted chicken. The samples were collected from February to May 2019, from 7 different batches (with a frequency of 2 batches/month), 3 samples were collected from each lot.

The objectives of this work were: evaluation of the safety criteria: *Salmonella* Enteritidis/Typhimurium and *Listeria monocytogenes*, as well as evaluation of the hygienic criteria of the technological process: aerobic plate count, *E. coli*, Enterobacteriaceae and *Staphylococcus aureus*.

Aerobic plate count in hamburger samples ranged between 2.85±1.36 log cfu/g and 4.07±0.17 log cfu/g, Enterobacteriaceae ranged between 1.66±1.45 log cfu/g and 3.02±0.3 cfu/g, E. coli ranged between 1.68±0.35 cfu/g and 2.01±0.28 cfu/g and for *Staphylococcus aureus*, ranged from 0.33±0.57 log cfu/g and 0.93±0.83 log cfu/g. In the case of chicken shawarma samples, aerobic plate count ranged between 4.01±0.74 log cfu/g and 2.86±0.74 log cfu/g, Enterobacteriaceae between 1.03±1.79 log cfu/g and 3.90±1.75 cfu/g, *E. coli* between 0.85±0.35 cfu/g and 1.5±0.38 cfu/g and *Staphylococcus aureus* between 0.85±1.47 log ufc/g and 2.41±0.57 log cfu/g. *Salmonella* Enteritidis and *Listeria monocytogenes* were isolated from a one chicken shawarma sample (4.76%).

Non-compliance values of Enterobacteriaceae (>5 cfu/g) were exceeded in 2.3% of the total of the analysed samples.

Based on our results, the microbiological risk of fast-foods in producing foodborne illnesses for fast food consumers is considered to be relatively low.

Keywords: fast food, microbiological risk assessment, pathogenic bacteria, public health

Introduction

Consumers prefer fast-food because of the speed with which the dishes are served but also for the variety and the possibility of purchasing a complete menu in well-positioned locations.

The reasons why young people choose to eat at fast food are because of the good taste, the cheap price (their budget is not enough to eat at the restaurant daily), the speed of serving, the environmental conditions, the cleanliness, etc. Fast-food products are tasty, at affordable prices, we find them everywhere, but they are poor in nutritional values, especially in fiber, vitamins, minerals, and high in fat, salt, and food additives. Among the types of fast food, the sandwich is among the most popular choices of consumption due to its easy-to-eat form and the ingredients it contains. Diseases spread through this type of food can affect groups of people, which can lead to disability or even death. Foodborne illnesses represent a global problem, especially in developed countries, where gastrointestinal diseases are the leading cause of mortality and morbidity. These hazards can be reduced by monitoring the microbiological quality of food and by increasing awareness among people about the fundamental principles of health and hygienic quality of foods [1]. The ingredients used in the manufacture of fast-foods, for example, meat, eggs, raw vegetables, etc., are sometimes contaminated and not kept at proper temperature. Recent studies have shown that ready-to-eat foods and food preparation surfaces can be an important source for microbial cross-contamination [2]. Fast food from some Asian countries has been tested for various microorganisms important for public health, including fecal coliforms, *Escherichia coli, Salmonella, Staphylococcus aureus* and *Bacillus cereus* [2]. Every year, a large number of cases of illness in each country is due to germs transmitted through food. According to the World Health Organization estimation, in 2010, the number of foodborne illnesses exceeded 600 million and resulted in 420,000 deaths [3]. These pathogens can induce foodborne illnesses, whose origin is difficult to identify, without changing the organoleptic characteristics of the product. It is estimated that in industrialized countries one-third of the population has at least one annual episode of disease caused by biological risk factors, transmitted through food consumption [4]. Recently, the problem of food safety has become more important, due to the dependence on fast food consumption, and to the fact that consumers have no control regarding the manufacture of these products. With the lifestyle of these days, very busy, always in a hurry, many people eat more in cities than at home. If fast-food is not handled hygienically or stored at the proper temperature, foodborne illnesses are likely to occur [2]. This research aimed to carry out an assessment of the microbiological hazards in fast food products, at the level of a region of Transylvania. The main objectives were represented by the evaluation of safety criteria and process hygiene criteria.

Material and Methods

During February-May 2019, 63 samples were collected from fast-food units located in the center of Transylvania, from the following fast-food products: hamburger, chicken shawarma and roasted chicken, as follows: each product was sampled from 7 different batches (with a frequency of 2 batches/month), and from each batch 3 samples were taken. The selection of the fast-food products selected for laboratory examinations was made based on consumer preferences. The samples were transported in isothermal bags to the Food Inspection laboratory at FMV Cluj-Napoca, where they were processed and subjected to standardized laboratory techniques. In order to carry out an analysis regarding the microbiological risk of these foods, the following microbiological analyses were performed: total viable count, Enterobacteriaceae, *E. coli*, *Staphylococcus aureus, Salmonella* Enteritidis/Typhimurium and *Listeria monocytogenes* [5; 6; 7; 8; 9; 10]. Statistical analysis of the results was realized using the Origin 8.5 software program by comparison of means by analysis of variance through ANOVA test.

The interpretation of the results was realized according to the probability indicator: $p \leq 0.05$ (confidence level 95%).

Results and Discussions

Results regarding the microbiological risk from the hamburger

In the hamburger samples, total viable count ranged between 2.85±1.36 and 4.07±0.17 log cfu/g, (Fig. 1).

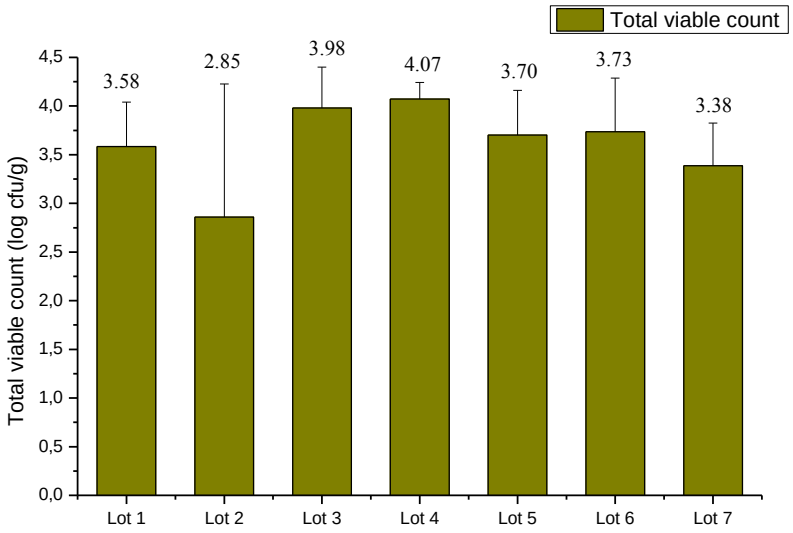

Fig. 1. Mean values of total viable count (±SE) of hamburger samples (n=21)

Higher contamination, ranged between 10^5-10^7 cfu/g was reported by Tamminga *et al.*, (1982), on 750 samples that were subjected to a thermal preparation process for 2-5.5 minutes [11]. In the case of Enterobacteriaceae, 66.60% of the hamburger analyzed samples were positive (14 samples). The mean values ranged between 1.66±1.45 and 3.02±0.3 cfu/g (Fig. 2).

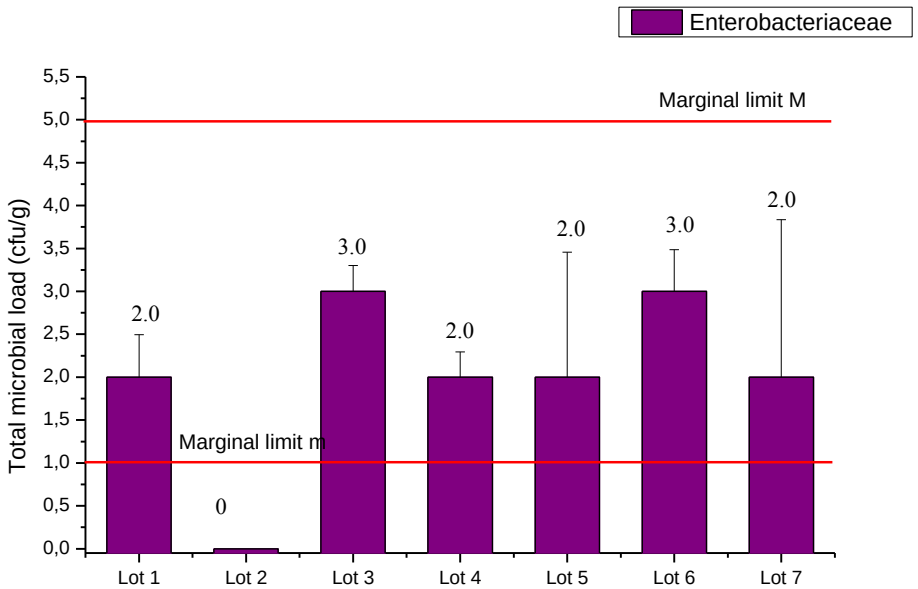

Fig. 2. Mean values of Enterobacteriaceae count (±SE) of hamburger samples (n=21)

Considering that the marginal limit m is 1 cfu/g, and the marginal limit M is 5 cfu/g, we found that out of the positive samples, 10 exceeded the marginal limit m [12]. Thus, in the case of hamburger samples, 28.57% were with non-compliances (6 samples).

Regarding the results for *E. coli*, it was found that 28.57% of the examined samples were positive (6 samples), with values between 1.68±0.35 and 2.01±0.28 cfu/g (Fig. 3).

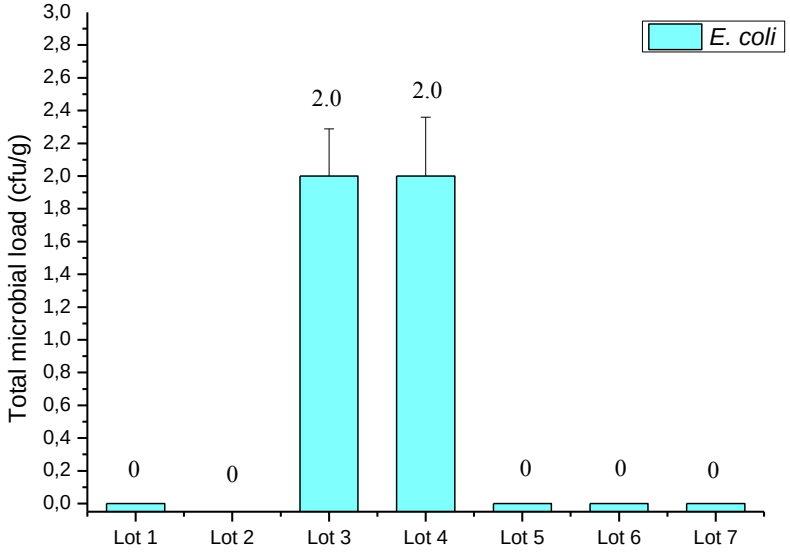

Fig. 3. Mean values of *E. coli* count (±SE) of hamburger samples (n=21)

Different results were obtained by Tamminga *et al.*, (1982), in the case of hamburger samples heat-treated at 80°C, all the samples were confirmed to be negative [11]. This fact confirms that the high temperatures inactivate pathogenic microorganisms that can cause foodborne illnesses in humans. It should be noticed that *Salmonella* Enteritidis/Thyphimurium and *Listeria monocytogenes* were not isolated from all analysed hamburger samples. Similar results were presented by Min *et al.*, (2013), in a study on the prevalence of *E. coli* and *Listeria monocytogenes* in hamburger [13]. The results indicate that hamburgers sold in different stores in the Caterbury region had a satisfactory microbiological quality, all samples being negative for *E. coli* and *Listeria monocytogenes*. Different results were obtained by Ozbey *et al*., (2013), out of a total of 35 hamburger samples, from which *Listeria monocytogenes* was isolated in 5.7% of the samples taken in the study [14]. Also, Tamminga *et al*., (1982), from samples prepared on the grid, isolated *Salmonella* spp. in 9.4% of the analysed samples [11].

In the case of *Staphylococcus aureus*, out of the total samples, 38.09% were positive (8 samples). Microbial load ranged between 0.33±0.57 and 0.93±0.83 log cfu/g (Fig. 4).

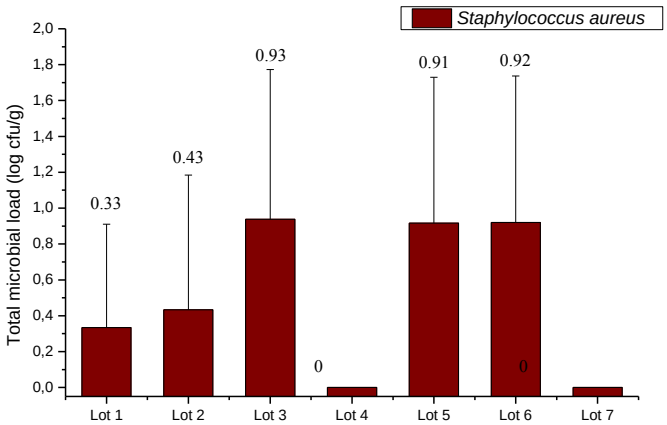

Fig. 4. Mean values of *Staphylococcus aureus* count (±SE) of hamburger samples (n=21)

Results regarding the microbiological risk from the shawarma

According to the standards of the American Public Health Association, regarding the acceptable limits for *Staphylococcus aureus* from hamburger, (maximum 2.0 log cfu/g), we found that 23.80% exceeded this value, which can lead to a possible foodborne outbreak [15].

In a study performed by Min *et al.*, (2013), out of 4 samples of hamburger, two were contaminated with *Staphylococcus aureus*, with a microbial load ranged between $1.05x10^2$ and $2.30x10^2$ cfu/g [13].

The mean values of total viable count in the chicken shawarma ranged between 4.01±0.74 log and 2.86±0.74 log cfu/g (Fig. 5).

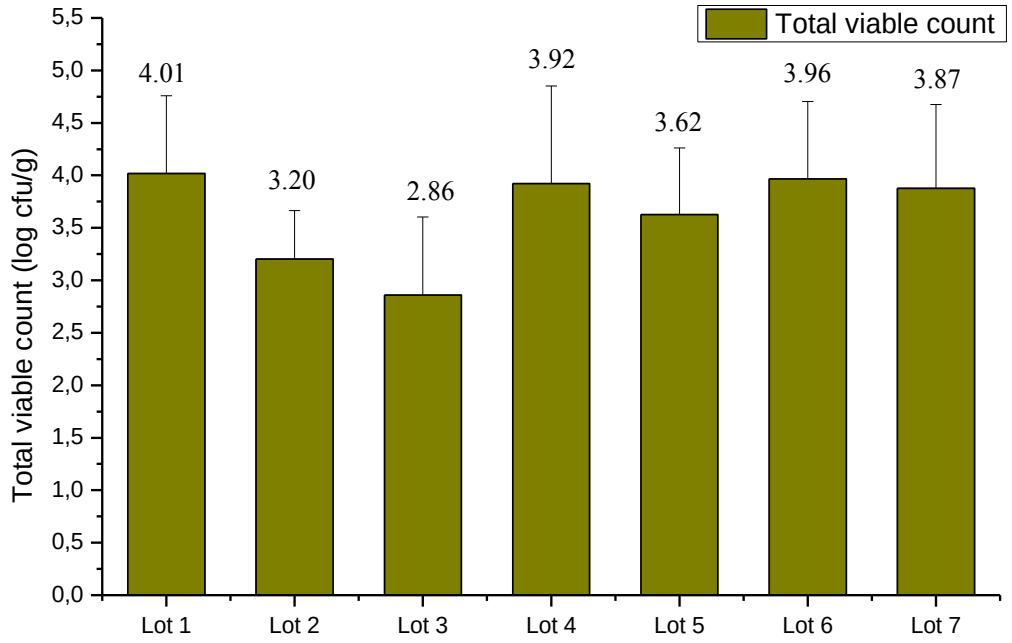

Fig. 5. Mean values of total viable count (±SEM) of shawarma samples (n=21)

The results were in accordance with the standards of the American Public Health Association, regarding the acceptable limits for viable count in the shawarma (up to 5.0 log) [15]. Different results, with a higher microbial load, were reported by Odu *et al.*, (2012), on 12 shawarma samples, collected randomly from 3 local restaurants, in which total viable count ranged between $2.0x10^3$ and $1.8x10^6$ cfu/g [16]. In general, vegetables have the highest value of bacteria, up to 1.8x106 cfu/g. Rashmi *et al.*, (2013), mentioned in a study that the total viable count does not increase significantly unless employees do not comply with GHP and GMP [17].

Similar results were mentioned by Sharaf and Sabra (2012), where the mean of the total viable count was $1.2x10^5$ cfu/g, for chicken shawarma samples [18].

In the case of Enterobacteriaceae, out of the total samples, 38.09% were positive (8 samples), with a microbial load ranged between 1.03±1.79 and 3.90±1.75 log cfu/g (Fig. 6). Out of the total positive samples, three were below the marginal limit m, four (19.04%) were between the marginal limit m and M, and one sample exceeded the limit of 5 cfu/g (4.76%). Thus, we established that 9.5% of the total samples were not compliance. Similar results were published by Emam *et al.*, (2013), with values of $5.0x10^1$ cfu/g of Enterobacteriaceae [19]. Different results, with a higher microbial load ($2x10^4$ cfu/g), were reported by Sharaf and Sabra *et al.*, (2012), on a sample of 20 chicken shawarma samples [18].

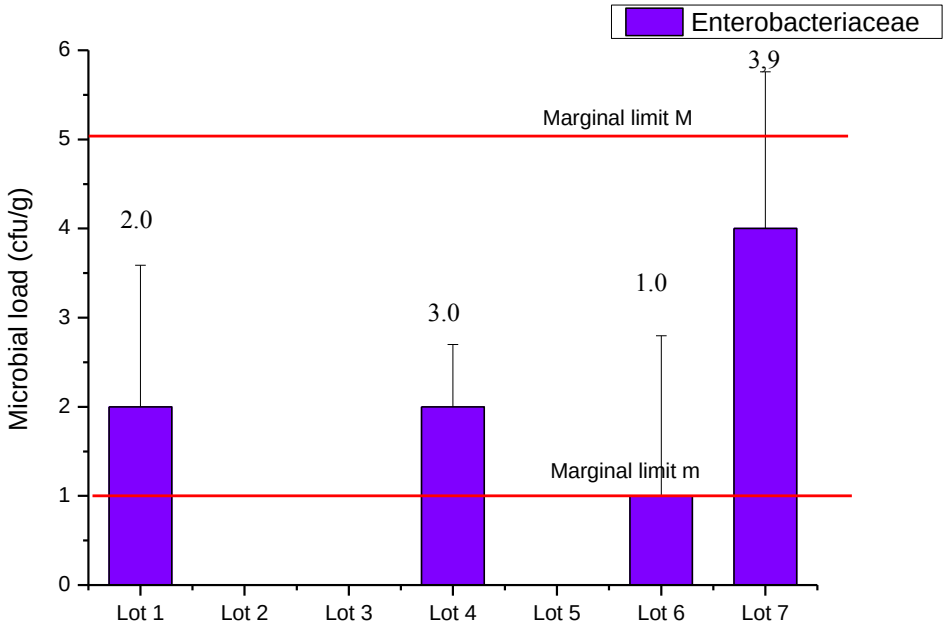

Fig. 6. Mean values of Enterobacteriaceae count (±SE) of shawarma samples (n=21)

E. coli was detected in 19.04% of the samples (4 samples), with a microbial load ranged between 0.85±0.35 and 1.5±0.38 cfu/g (Fig. 7).

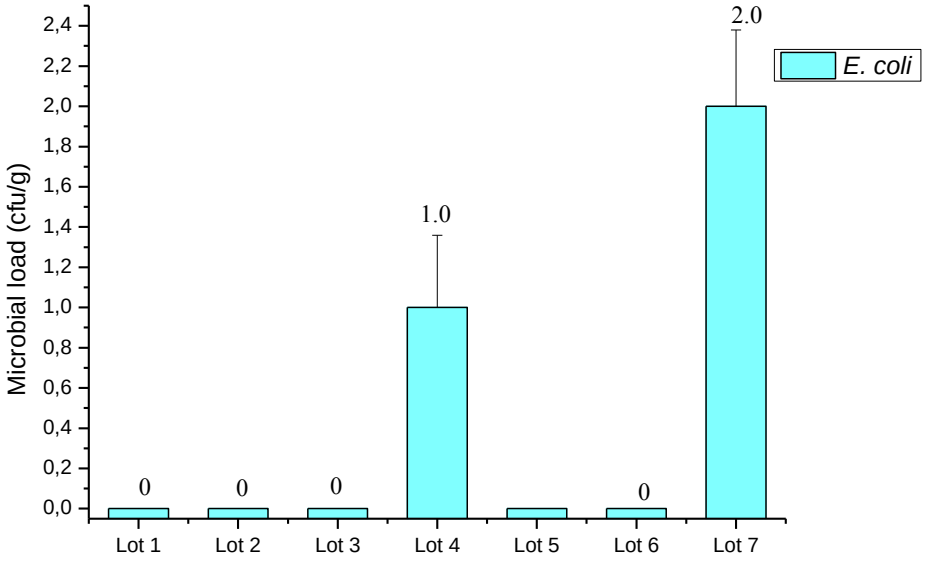

Fig. 7. Mean values of *E. coli* count (±SE) of shawarma samples (n=21)

Different results were reported by Odu *et al*., (2012), in which *E. coli* was isolated in 13.6% of the samples [16]. Also, Zakaria *et al*., (2018), isolated *E. coli* from 17.1% in chicken shawarma [20]. Similarly, Sharaf and Sabra (2012), in a study on the prevalence of *E. coli* in chicken shawarma samples, mentioned values of 20% for *E. coli*, with an average microbial load of 3.9x10² cfu/g [18].

Explanation for these different results can be represented by different initial microbial load of raw materials, different storage temperatures, compliance with good hygiene and manufacturing practices in the fast-food units.

Staphylococcus aureus was isolated from 38.09% of the samples (8 samples), with a contamination level ranged between 0.85±1.47 and 2.41±0.57 log cfu/g (Fig. 8).

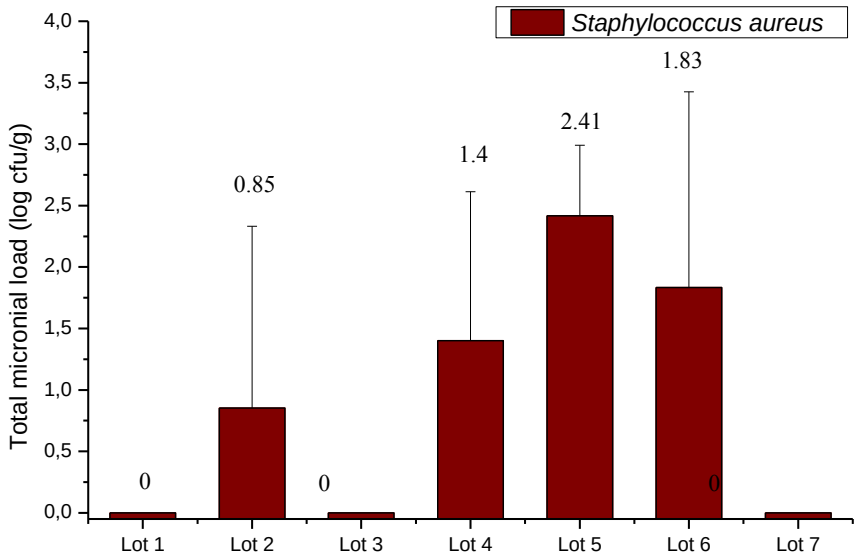

Fig. 8. Mean values of *Staphylococcus aureus* count (±SE) of shawarma samples (n=21)

These coagulase positive staphylococci values may be considered to have a low risk of producing foodborne illnesses in consumers, given that the toxin that is responsible for triggering the infection is produced only when values exceed 5 log cfu/g. Our results are in accordance with studies reported by Odu *et al*., (2012), in which *Staphylococcus aureus* ranged between $1.9x10^3$ cfu/g to $5.3 x 10^3$ cfu/g [16]. Different results, with a higher microbial load, were reported by Sharaf and Sabra *et al*., (2012), where 70% of the samples were positive, with a microbial load of $6.2x10^4$ cfu/g [18]. *Salmonella* Enteritidis and *Listeria monocytogenes* were isolated from one chicken shawarma samples (4.76%).

Results regarding the microbiological risk from the roasted chicken

In case of roasted chicken samples, the mean value of total viable count ranged between 2.4±0.6 and 3.70±0.56 log cfu/g (figure 9). Different results were obtained by Muna *et al*., (2018), for grilled chicken, with values ranged between $8.9x10^3$ and $2.7x10^4$ cfu/g [21].

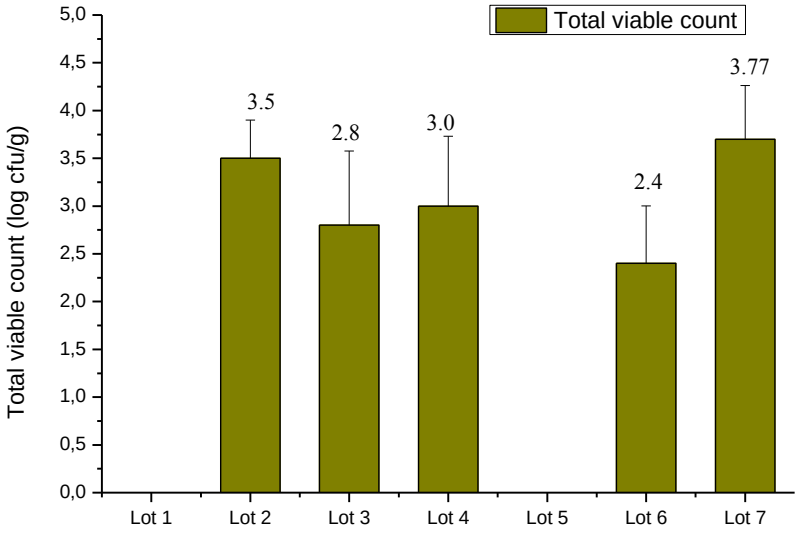

Fig. 9. Mean values of total viable count (±SE) of roasted chicken samples (n=21)

Enterobacteriaceae were isolated from 19.04% samples (4 samples), with a microbial load ranged between 0.3±0.12 and 0.84±0.12 cfu/g. All samples were below the marginal limit m.

Different results, (with a higher microbial load, were obtained for Enterobacteriaceae by Muna *et al*., (2018), with values of $1.9x10^2$ and $5.5x10^2$ cfu/g [21]. *Staphylococcus aureus* was isolated from three samples (14.28%), and the average microbial load was 1.25±0.25 log cfu/g (Figure 11). Different results were obtained in the study by Muna *et al.*, (2018), where the load of *Staphylococcus aureus* was between 9.8x101 and $2.1x10^2$ cfu/g [21]. Also, the samples were analysed for *Salmonella* spp., but all were negative [21]. *E. coli*, *Salmonella* Enteritidis and *Listeria monocytogenes* were not isolated from roasted chicken samples. Based on these results, we can state that the temperatures used in the case of roasted chicken manufacture was effective to inactivate the pathogens.

Conclusions

The prevalence of Enterobacteriaceae was: roasted chicken (9.52%), shawarma (38.09%), and hamburger (66.60%). Non-compliances regarding Enterobacteriaceae were recorded in the case of 2.3% of the total samples. The prevalence of *E. coli* was: roasted chicken (9.52%), shawarma (19.04%), and hamburger respectively (28.57%). The prevalence of *Staphylococcus aureus* was: roasted chicken (14.28%), respectively shawarma and hamburger (38.09%). Based on these results we can state that the microbiological risk of producing foodborne illnesses for consumers of fast food products is relatively low. Further extended studies are necessary to evaluate the real risk of pathogens in fast-food products for the consumers.

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Case Report: *Demodex Cornei* of Dog Can Also Affect Humans

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Abstract

Mites from the genus *Demodex* are ectoparasites of many mammals, including humans.

There are over 100 *Demodex* species, which demonstrate strong specificity in host selection.

Demodex in the dog is a common infestation of the dog's skin with tiny, cigar-shaped, eight-legged mites. Dog, 8 months diagnosed with generalized dermatitis. After direct microscopic examination was identified *Demodex cornei*. Because *Demodex* sp. *"cornei"* reside within the stratum corneum, superficial skin scraping or tape impression offers a better method for detecting these mites. *D. cornei* was identified by based on the morphological characters including short opisthosoma with blind and round terminal end. The boy, 10 years old, with erythema of the face with follicular plugging and discreet fine, whitish scale; after direct microscopic examination was identified *Demox cornei* (The Laboratory Praxis- human medical analysis laboratory from Iași). *Demodex "cornei"* of dogs may be contagious.

Keywords: Demodex cornei, contagious, mites

Introduction

Canine demodicosis is a parasitic skin disease caused by the characteristically cigar-shaped mite *Demodex canis* (family Demodicidae) in dogs. The disease it manifests when the parasites develop excessive in hair follicules and sebaceous glands (3,10). Other species have been suggested (e.g., Demodex injai, and Demodex cornei), but some authors consider that they are only morphological variants of D. canis (4).

Immunodeficiency is a predisposing factor which is probably hereditary in young dogs (under 2 years old) and acquired in adult dogs, following development of an underlying cause (e.g., excessive glucocorticoid therapy, Cushing's syndrome, diabetes mellitus and neoplasia).

It is common but also under-diagnosed and can be very serious medically (9, 10).

Differentiation of the both the mites (*D. cornei* and *D. canis* mainly based on their size, inhabitant or location of the mite and morphological difference. *Demodex canis* has short legs, arranged in the shape sometimes described as resembling the Brandenburg cross. Demodex mites mainly feed on scale and sebum, the production of which they help increase. They never feed on blood and are incapable of living off their host (2, 6). *Demodex cornei* has elongated body with short stumpy legs on podosoma and shorter opisthosoma. The D. cornei inhabits in stratum corneum of epidermis (1, 8).

The mite collection technique can also give a useful diagnostic data, because D. cornei inhabits in stratum corneum of epidermis, the suitable collection technique for D. cornei is superficial skin scraping or using tape preparation techniques, while the habitat of D. canis is hair follicles and sebaceous glands which move deeper into layer of dermis, so it may concluded

that the suitable collection techniques for D. canis are deep skin scraping or hair-plucking examination (5, 7).

Materials and Methods

The case was presented at the Faculty of Veterinary Medicine, Parasitology Clinic, in June 2018.

Patient: Dog, male, mops breed, 8-month female.

Symptoms: generalised dermatitis, with hyperkeratosis and pustular folliculitis, erythema, alopecia, hyperpigmentation, erosions, lichenification and cellulitis (fig. 1, fig. 2). Distribution of lesions observed on face, ears, chin region, fore limbs, neck and lateral abdomen. Treated 2 months ago with dectomax (doramectine), being suspect of infestation with demodex, a shot per week for 1 month. A slight amelioration could be noticed in the first month, but with relapse after stopping the treatment.

From the same family, 11-year-old boy, with lesions of erythema with squamous aspect, slightly itchy (fig. 3). The dermatologist recommended treatment with antimycotic ointment.

Under treatment, the lesions extended from the face to neck and upper arms.

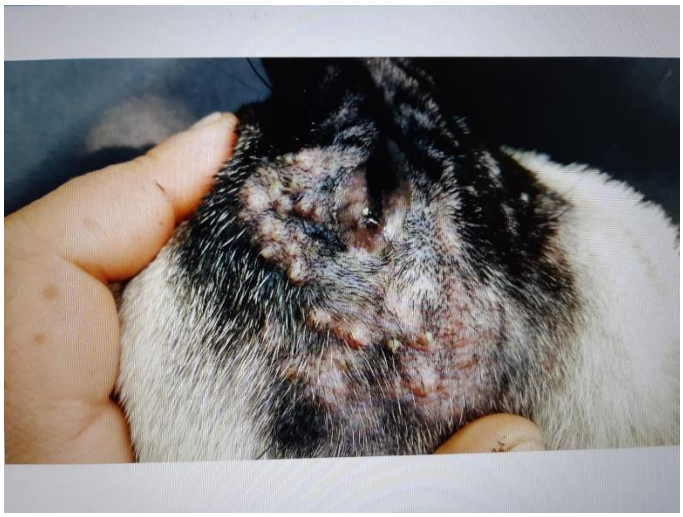

Fig. 1. Generalized dermatitis, with hyperkeratosis and pustular folliculitis

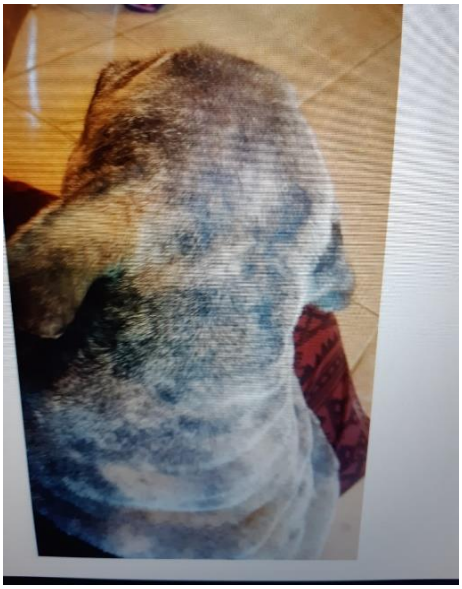

Fig. 2. Erythema, alopecia, hyperpigmentation

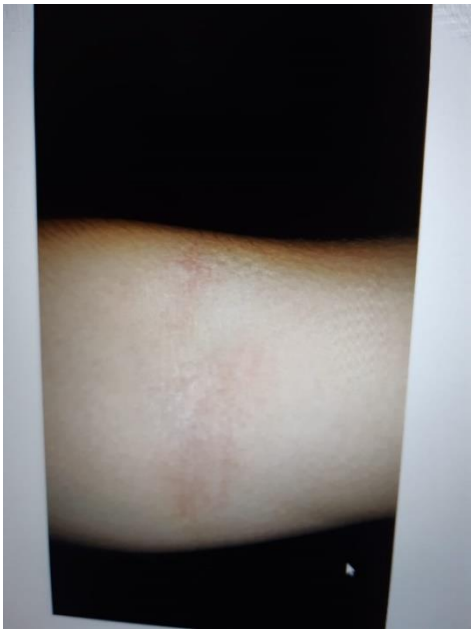

Fig. 3. Lesions of erythema with squamous aspect, slightly itchy

Results and Discussions

Dog-Skin scrapings, tape impression smears and hair plucks were collected from the affected dog for laboratory examination. Scrapings were collected with scalpel blade dipped in liquid paraffin and collection of scrapings was continued until there was slight ooze of blood from dermal capillaries. Material was suspended in a few drops of liquid paraffin on a microscopic slide, a coverslip was applied and the preparation was examined under low and high power (10X, 40X) of microscope. The sticky surface of the tape was pressed on the suspected lesions, and tape was then mounted directly on a glass slide. The glass slides were examined under compound microscopes with 10X and 40X of magnification. Few tape impression smears were stained with methylene blue for 1 min and examined under 40X. D. canis (Fig. 6) were found in hair pluck examination technique. The tape impression technique of the dogs revealed a greater number of short-tail Demodex mites (D. cornei). Mites with short tail were identified as D. cornei (Fig. 7) based on other morphological characteristics. Mites present in the tape impression smears had elongated body with short stumpy legs on podosoma and shorter opisthosoma.

Treatment:

- Ivermectin tablets 0.4 mg/kg every 7 days for 28 days.
- NexGard is given orally once a month, for three treatments every 28 days.
- HEPATIALE FORTE Advanced 1tablet/days for 90 days (Fig. 4).

Fig. 4. Dog-after treatment

Boy-Skin scraping and Scotch test was use-*Demodex cornei* (Fig. 5) was identified with Scotch tape test. A special examination was recommended at Praxis laboratory of human tests, where the diagnostic was of demodicosis, other species than *Demodex folliculorum*. It was recommended a treatment with 2% ivermectin ointment, one application per day, and the lesions disappeared after 3 weeks of treatment.

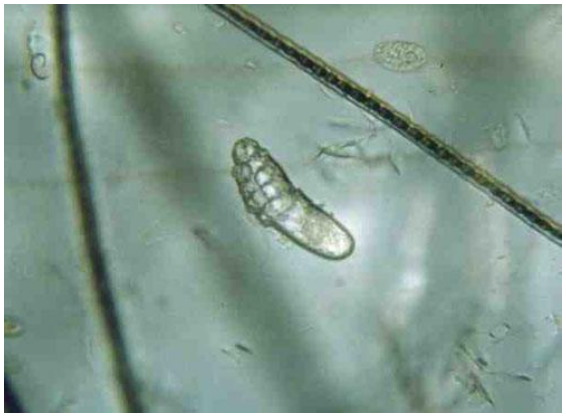

Fig. 5. *Demodex cornei* in human

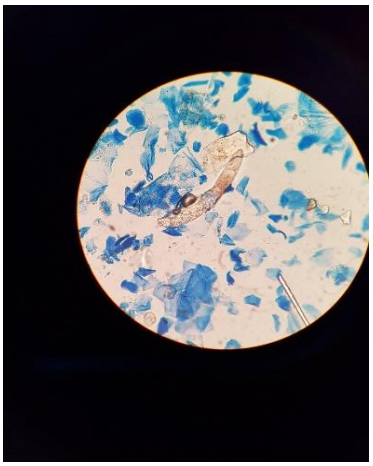

Fig. 6. D.canis has short legs, arranged in the shape sometimes described as resembling the Brandenburg cross. The mean total body length was (214.32±13.81 μm) of D. canis.

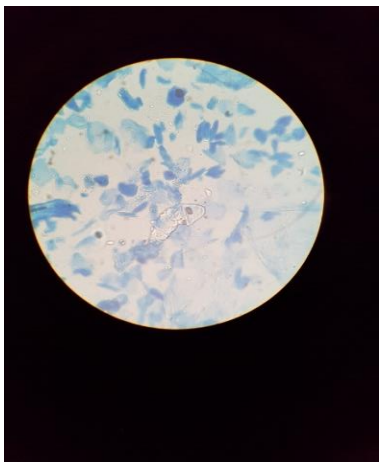

Fig. 7. Demodex cornei has elongated body with short stumpy legs on podosoma and shorter opisthosoma, with blind and round terminal end.The mean total body length was (132.21±14.6 μm) of D. cornei.

Conclusions

In the sample collected from human only *Demodex cornei* was identified, though in the dermal scraping from the dog both species, *Demodex canis* and *Demodex cornei*, were identified. In the literature, demodicosis in dogs is not considered zoonosis, signalling species specificity.

In human, the presence of *Demodex cornei* was signalled on the face, neck and upper arms with tendency of expansion.

The conclusions highlight the zoonotic potential of *Demodex cornei* in human, with higher tendency of generalisation than in the case of infestation with the species common to human *Demodex folliculorum*, which is grafted especially on the face.

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The Role of Thermography in the Evaluation of Inflammatory Processes of the Knee in Dogs

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Abstract

The thermography or thermal imaging appeared only a few decades ago and, as they were perfected, they occupy an ever-wider area of applicability, being used in veterinary medicine with very good results. Thermography has become a tool in the medical environment after a process of continuous refinement, both in the application of principles and in the methods of acquisition and processing of digital images. This accumulation process, in the sense of improving the technique, is on the rise worldwide. The aim of the study is to evaluate and interpret the temperature values at the knee level of the posterior limb, with the help of the thermograph camera, in order to identify changes that could indicate an ongoing pathology.

Depending on the condition or pathology, local or regional temperature may be negatively or positively influenced compared with the normal values found in healthy tissue. The study clinically assesses symptoms, the local temperature at the level of the knee, using the infrared camera, and corelate the findings with the changes found on the radiographic examination of the region. For the purpose of the work in this study, 23 patients were examined, all with posterior leg pathologies. The result show increased local temperature in cases diagnosticated with acute pathology of the knee.

Keywords: thermography, thermal imaging, dog, knee

Introduction

Medical thermography is a method of measuring and recording heat at the skin level, produced by different parts of the body, by using a device sensitive to infrared radiation. The image obtained is called the thermogram, which, in practice, is a temperature distribution map at the skin level.

According to a study, thermography, like any other method of diagnosing, requires a minimum of criteria to be followed, before being used. These factors need to be recognized and controlled to avoid false negative or false positive values. The main factor is the room temperature, where the thermographic examination is carried out. It should have a value of about 21°C (±5°C), preferably without windows or free airflow. Other factors that need to be considered for good interpretation are: humidity, solar radiation and air movement [1].

Infrared thermography reflects, in real time, the superficial microcirculation of the skin. The skin is a dynamic organ, in which the temperature is constantly adjusted and modified due to metabolic processes, to allow a harmonious balance between the internal and external temperature of the body, through the process of vasoconstriction and vasodilation [2, 3].

High temperature zones are associated with increased local metabolism and circulation, which may be clinically correlated with an inflammatory process. On the other hand, a regional decrease in temperature may indicate a decrease in tissue perfusion (heart failure or changes in the autonomic nervous system) [3].

Material and Methods

The biological material was represented by 23 dogs of various breed, age and sex, that where brought for consultation accusing different degree of limping. The clinical examination concludes the location of the limping.

For thermographic evaluation we have used the FLIR E6-XT thermographic camera, with a resolution of 240x180 pixels (fig. 1).

Fig. 1. Thermographic camera FLIR E6-XT

In order to interpret the thermographs obtained in the study, we used the FLIR QuickReport 2.1 program (FLIR 2012). We have chosen this program because it can be downloaded easily and free of charge from the official FLIR website (https://www.flir.com/). At the same time, the respective program is included with the thermographic camera, upon its purchase, being the default application for FLIR thermographic evaluation.

The thermography will be done after 15-20 minutes at the place of examination, in order for the body to adjust to the room temperature. This work takes a few minutes. The images are taken and stored on the thermographic camera and after that are transferred on the computer and analysed using the camera software.

After the thermography was performed, the patients were examined using radiography, for each patient a latero-lateral and a dorso-ventral imaging of the knee region was obtained. The temperatures changes registered on thermographic image were corelated with the changes found on the radiographic images.

Results and Discussion

The thermography principle is based on increasing of the metabolic activity and vascular circulation at the level of the affected region. The need for nutrients in the affected area is much higher than the healthy tissue, which will cause an increase of the local temperature. It is known that an inflammatory process (which occurs secondary to many conditions) is accompanied by local hyperthermia, and with the help of the medical thermograph you can obtain information about the vascularization of certain segments or about a possible inflammatory process (muscular or joint), useful for establishing a diagnosis and monitoring in time the evolution of the disease [4].

Modern thermographic devices record the temperature ranging from -40°C to +1500-2000 °C, with very high accuracy (0.1°C). the infrared can find its practical use in any area in which

the heat appears or changes its distribution as a result of a chemical, physical, biological or other process. Modern thermography is an absolutely harmless and easy to perform examination, which includes both functional and imaging diagnostics. The infrared camera is used for this purpose, which converts the heat emitted by the patient's body into electrical impulses, which in turn are displayed on the monitor computer in the form of colour images.

The momentary and precise visualization allows the qualitative and quantitative evaluation of the temperature changes of the skin coating [5]. The colour spectrum is the index of the quantity of the infrared flux, emitted by the given area, and the thermogram as a whole reflects the specificity of the structure of the subcutaneous areas, so each individual has its individual thermal image. Noticing the earliest and smallest disturbances, thermography is the only method that anticipates the clinical manifestation of the disease [5-7].

The temperature was recorded on the cranio-lateral side of the knee, the normal temperature was recorded in 5 dogs without knee pathology (fig. 2) being close to the values reposted by literature [3, 7]. The mean recorded value was 31.2±0.98°C.

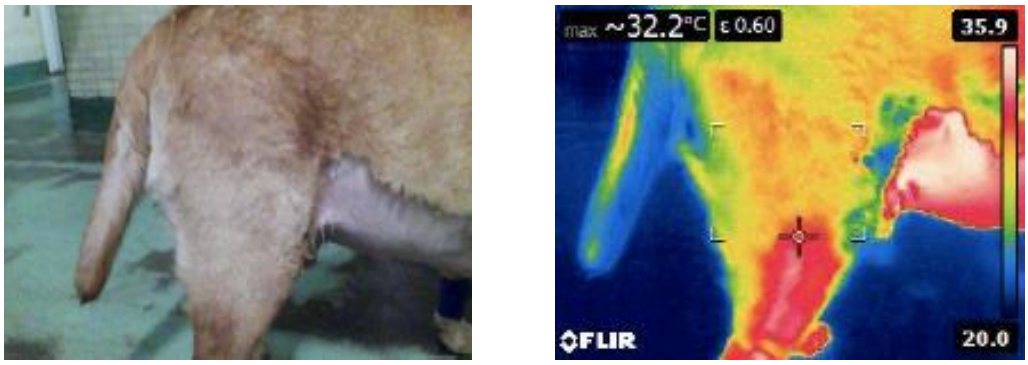

Fig. 2. Recording normal knee temperature in dog knee

From the examined dogs the main pathology found in the knee region was rupture of the cruciate ligament (N-13, 56,52%) followed by degenerative processes (N=7, 30.43%) and the lest common in our study group was avulsion of the tibial crest (N=3, 13.04%).

The mean registered temperature in case of cruciate ligament rupture was higher compared with the degenerative processes, especially in case of an acute process (fig. 3), the mean value being 38,9±2.26 °C.

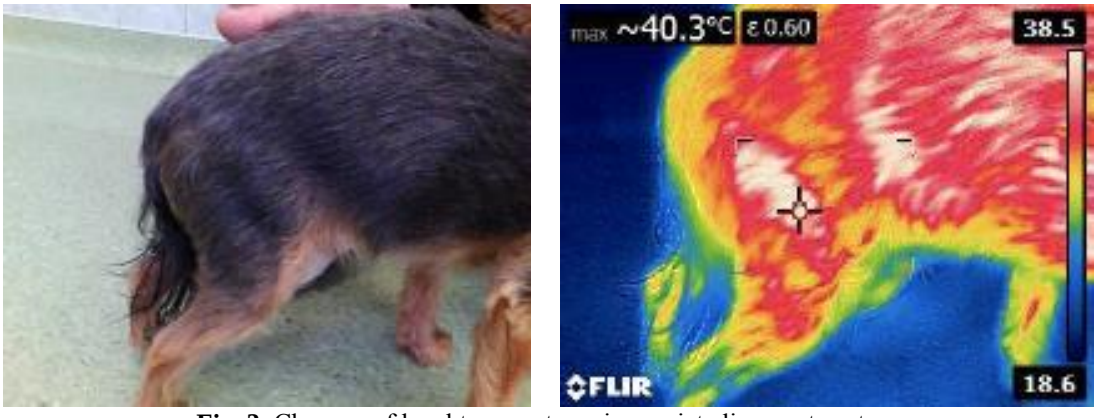

Fig. 3. Changes of local temperatures in cruciate ligament rupture

The radiographic examination show destruction of the cruciate ligament and intraarticular reaction (fig. 4).

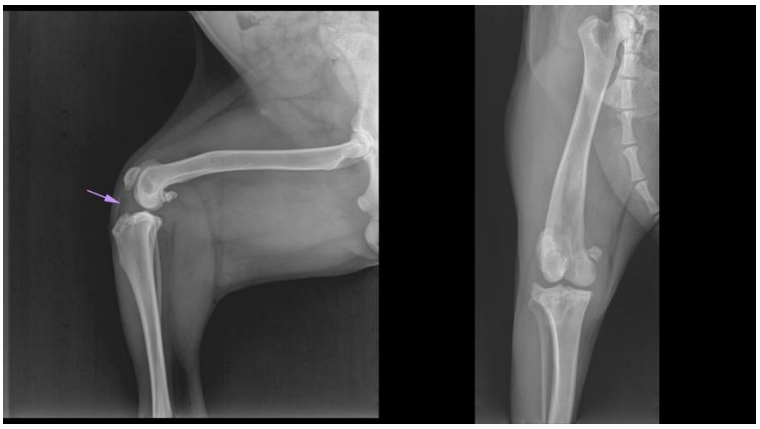

Fig. 4. Rapture of the cranial cruciate ligament

In case of degrative processes the mean temperature, value was 34.2±1.26 °C (fig. 5, fig. 6) closer to the normal temperature range, the temperature was lower due to the chronic process.

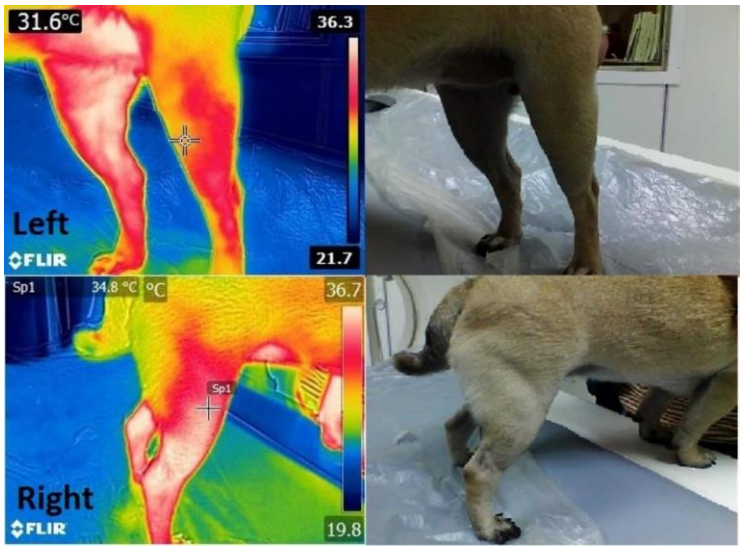

Fig. 5. Changes of recorded temperature in case of degenerative process (right limb) compared with the normal values (left limb)

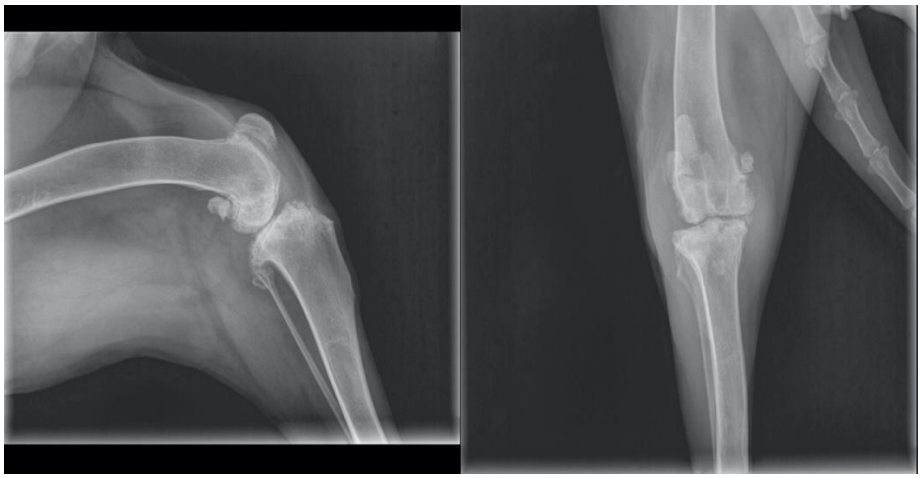

Fig. 6. Degenerative process of the knee (right limb)

In case of trauma and the avulsion of tibial crest, the mean registered temperature was 36.7±1.78 °C, the temperature is higher compared with that registered in the chronic process (fig. 7, fig. 8).

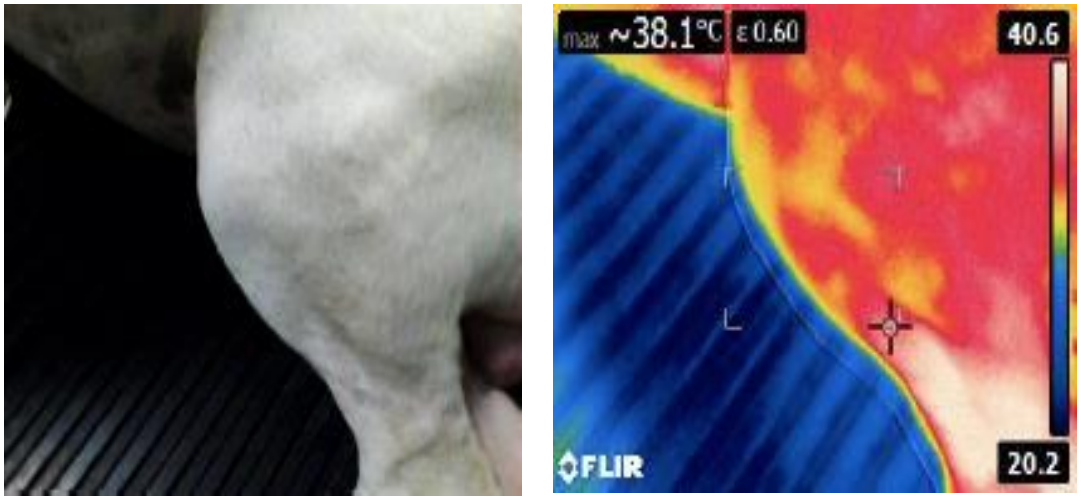

Fig. 7. Changes of temperature in case of traumatic processes

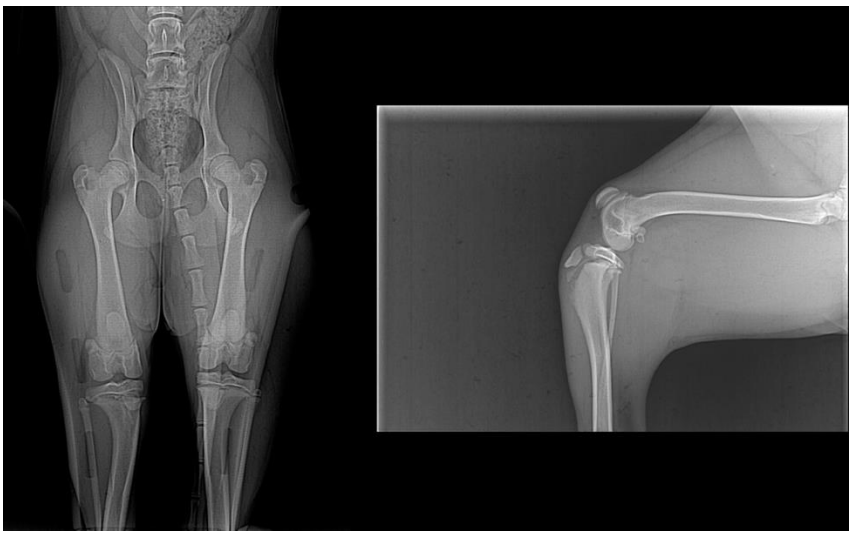

Fig. 8. Avulsion of the tibial crest and tibial valgus

Conclusions

The thermography is a useful tool in determine the changes of knee temperature that could indicate a certain pathology. The higher values of temperature were registered in case of acute processes like cruciate ligament injury or avulsion of the tibial crest. In case of chronic process, the temperature was slightly increased above normal range. Further study, on a larger number of cases, are required in order to establish an exact interval of temperature that can be corelated with a certain type of pathology. Also creating a temperature map that can be associated with a certain type of pathology is important for a non-invasive diagnostic of the knee pathology in dogs.

Acknowledgement

The studies were conducted in the laboratory of Medical Imaging – Radiology and are part of the internal grand research conducted by the Radiology laboratory.

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Comparative Antinematodal Effect Assessment of Some Plant Extracts and a Modern Medicinal Product in Pigs

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Abstract

The present study investigated the therapeutic efficacy of some plant products and an anti-parasitic substance on the intestinal nematodes of pigs. 6 groups were used, of 10 individuals each: group 1 treated with *Cucurbita pepo* oil, group 2 treated with *Calendula Officinalis* oil, group 3 treated with extract of *Artemisia absinthium* flowers, group 4 treated with *Tagetes patula* hydro-alcoholic extract, group 5 treated with a benzimidazolic derivate and group 6 untreated lot. Parasitic contaminations were determined via copro-parasitological examinations. Methods like Willis and Mc Master were used both ante and post therapeutic in day 7, 14 and 28. Eggs of *Oesophagostomum sp.*, *Trichocephalus suis*, *Ascaris suum* were observed. The greatest efficacy as anti- parasitic has had benzimidazolic derivate for all identified parasites in samples, followed by hydro-alcoholic extract of the *Tagetes patula* against *Ascaris suum* and *Oesophagostomum sp.* and the hydro-alcoholic extract of the Wormwood (*Artemisia absinthium*) against *Ascaris suum*. *Cucurbita pepo* oil, *Calendula offcinalis*, and Tagetes *patula* extracts presented a low antinematodal efficacy.

Keywords: plant extracts, antiparasitic substances, digestive nematodes, swine

Introduction

The importance of parasitic diseases in animals is given by the great losses that they cause, although there are programmed measures and to prevent parasitic infestations, they are still insufficient.

Parasites like the ones from Ascaris Genus affect more than 1 billion people worldwide causing high morbidity. The current treatment with mass drug administration of synthetic anthelmintic drugs is problematic due to re-infection and the threat of drug resistance [1], [2].

The medicinal plants were used in the treatment of parasitic diseases with some degree of success since ancient times, making the idea of using these products, alone or in combination, for therapeutic purposes not new [3]. Although medicinal plants have been used to control the parasitic infestations both in animals and humans there is a limited number of scientific studies to confirm their efficacy [4], [5].

There is large source of natural substances present in nature, mostly of plant origin that can be easily obtained by local communities in order reduce the accentuation of drug resistance [1].

Although technology of drug artificial synthesis is evolving rapidly still two thirds of the new chemicals identified yearly were extracted from higher plants [6].

In livestock there are subjects infested with a small number of parasites, with unaffected health that disseminate invasive elements in the external environment and contaminate the rest

of the herd [7] thus residing the necessity to investigate alternative ways for control parasites in pigs.

The aim of the investigation is to do a comparative study on evolution of the antinematodal effect of some plant extracts and modern medicinal products in pigs raised in the small farms and households from Romania.

Material and Methods

The study was conducted between March 2008 and June 2008 at the University of Veterinary Medicine in Cluj-Napoca (Romania) – *Department of Parasitology* and Parasitic Diseases, and in small pig farms from 4 villages from Bistrița-Năsăud and Cluj Counties.

Study population

The study was conducted on a total number of 60 pigs up to 5 months old males and females.

In the pig breeding units studied, the classical conditions of extensive exploitation are maintained, with some differences from one area to another. The shelters provide microclimate conditions only based on the biological heat of the animals, without mechanical ventilation; there are no special fodder or watering facilities, the works in the shelters are done manually.

Experimental protocol

In order to evaluate pig parasite infection, biological samples were collected from a number of small pig farms. Groups were formed and starting day 0 plant extracts were administered as well as a chemical medicinal product. In day 7, 14 and 28 biological samples (faeces) were again collected to evaluate parasite infestation dynamic. The animals were divided in 6 groups of 10 individuals each: group 1 treated with *Cucurbita pepo* oil (50ml / animal), group 2 treated with *Calendula officinalis* oil (10 ml/animal), group 3 treated with hydro-alcoholic extract of *Artemisia absinthium* flowers (10 ml/animal), group 4 treated with *Tagetes patula* hydro-alcoholic extract (20 ml/animal), group 5 treated with a benzimidazolic derivate (Oxibendazol 15%) and group 6 untreated group.

Biological samples

The samples were taken in plastic packaging, hermetically sealed, labelled and identified.

The coproparasitological examination was performed in the Parasitology Department of the Faculty of Veterinary Medicine in Cluj-Napoca. To highlight the eggs of digestive nematodes, the Willis ovohelmintoscopic concentration method [8] was used. To calculate the intensity of the parasite infestation, McMaster method was used, which consists of counting parasitic elements in a specific amount of faecal matter [9]. For each batch, parasite and therapeutic moment, the extensivity and intensivity of parasitism were established.

Results and Discussion

Initial coproparasitological examination performed in the pigs under study, eggs of *Oesophagostomum sp.*, *Ascaris suum*, *Trichocephalus suis* were identified. The antiparasitic treatment carried out with 4 types of plant extracts: Pumpkin seed oil (*Cucurbita pepo L.*), Marigold oil (*Calendula officinalis L.*), hydro-alcoholic extract of Wormwood (*Artemisia absinthium*), hydroalcoholic extract of French marigold (*Tagetes patula*) and a medicinal product based on benzimidazole derivatives (15%) have shown in variable results regarding the intensity and extensivity of infestations (Tab. 1).

Tab. 1. Evolution of extensivity and intensivity of digestive nematodes before and after treatment

Day of Sampling	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
	E (%)	I (EPG)	E (%)	I (EPG)	E (%)	I (EPG)	E (%)	I (EPG)	E (%)	I (EPG)	E (%)	I (EPG)
Oesophagostomum sp.												
0	70	455	40	40	50	75	70	55	80	350	50	65
7	30	400	50	35	60	55	30	35	20	30	50	60
14	30	435	20	10	60	85	30	35	10	15	60	65
28	40	270	10	5	20	15	10	5	0	0	40	50
Trichocephalus suis												
0	30	45	40	30	-	-	40	40	-	-	60	45
7	30	30	30	20	-	-	30	35	-	-	20	30
14	0	0	0	0	-	-	30	35	-	-	30	40
28	10	5	10	5	-	-	30	20	-	-	40	45
Ascaris suum												
0	40	230	90	715	40	70	60	50	60	295	70	150
7	30	75	50	360	30	25	30	20	30	25	70	880
14	30	80	50	645	10	5	10	5	10	5	90	500
28	30	20	70	410	10	5	0	0	0	0	90	535

Legend: E – Extensivity (%); I – intensivity; EPG – eggs per gram of faeces

All the results of antiparasitic treatment performed with herbal extracts and a medicinal product based on benzimidazole derivatives (15%) were compared with an untreated control group. In all the groups taken in the study, the poliparasitism was present similar to the results of Morariu in Timisoara [10].

The administration of pumpkin oil (*Cucurbita pepo L.*) in group 1 had average efficacy on the evolution of trichocephalosis and esophagostomosis, and quite low on ascariasis. Used in diet wad described to contribute to the prevention and treatment of conditions such as: intestinal parasites, liver disorders, oedematous heart failure. Also, the increased content of vitamin E and selenium confers antioxidant effect on the protection of hormones, enzymes and vitamins in the body. The pumpkin oil used by us was obtained by cold pressing, this method of preparation is recommended, because it fully preserves the active principles of pumpkin seeds [11]. In an experimental research on canine tapeworms, Diaz [12] observed an antiparasitic effect at a minimum inhibitory concentration of 23 grams of pumpkin seed oil (*Cucurbita maxima L.*) in 100 ml. of distilled water. Other studies confirmed that *C. pepo* was found to exhibit nematodicidal properties [13].

In the second group, in which the Marigold oil (*Calendula officinalis*) was administered, we observed that the extract was not effective in the case of esophagostomosis. Trichocephalosis regressed at 3rd sampling but reappeared 28 days after therapy. In ascariasis the oil of the Marigold has had moderate to low efficacy. Marigold is worldwide known for its medicinal importance containing various phyto-chemicals including carbohydrates, carotenoids,

terpenoids, flavonoids, quinones and many others. It presents an important biological activity in wound healing, immuno-stimulant, spasmogenic and spasmolytic, hepatoprotective, anti-inflammatory, anti-oedematous, anti-bacterial and anti-fungal, antioxidant, antidiabetic, anti-HIV and anti-cancerous, nephron-protective, prevention of oropharyngeal mucositis, hypoglycaemic and gastroprotective activities with no toxic effect [14]. Calendula was described to have efficiency against some nematode worms (*Heligmosomoides polygyrus*) [15] but a positive antinematodal action in pigs is still to be found.

The hydroalcoholic extract of Wormwood (*Artemisia absinthium*) administered to group 3 had an average efficacy on *Ascaris suum* but not on *Oesophagostomum sp.* Wormwood is considered to be effective as: vermifuge, anti-inflammatory of the gastrointestinal mucosa, antiasthenic, choleretic, diuretic, detoxifying, laxative, stimulates the body's resistance capacity, very good antirheumatic, gut antiseptic and urinary [11].

The anthelmintic activity of Atemisia is considered to be caused by lactones related to santonin, which is found in wormwood. In addition, thujone can stun roundworms, which can then be expelled by normal intestinal peristalsis [16].

The 2015 Nobel Prize in Physiology or Medicine was awarded for the discovery of artemisinin and avermectin, two substances of natural origin. This discovery changed the way people thought of antiparasitic treatments showing that plants are a great source of medicaments.

The *Tagetes patula* extract administered to group 4, had a better antiparasitic effect than the other extracts both against ascariasis and against esophagostomosis. On the infestation with *Trichocephalus suis* it had a diminished effect. The aerial parts of the plant, harvested during flowering, contains an essential oil rich in tagetone, ocimene, myrcene, linalol, limonene, feather, carvone, citral, camphor, valerianic acid, salicylic aldehyde, flavonoids, camferitrine, quvercetagetin, patuletin, helene (dipalmitic acid ester of lutein). Substances in the terpenoid group (linalol, limonene) are considered to have antiparasitic effects [3]. Williams has described a number of plants like *Clausena anisata*, *Zanthoxylum zanthoxyloides* and *Punica granatum* which traditionally are used in medicinal form in *Ascaris*-endemic regions – Ghana (Africa) which have direct anthelmintic activity against *A. suum* [1].

In the case of group 5 (treated with Oxibendazole 15%), the results were superior to those observed after the administration of the plant extracts, the intensity and extensivity of the parasitic infestation reaching 0 at the last sampling. Similar studies testing the efficacy of Oxibendazole in swine revealed that the intensity and the extent of these parasitic diseases dropped to null in 28 days, following the treatment, thus proving a 100% efficacy of the used substance [17].

In the control group, normal variations of the copro-eliminations were observed in all the samplings.

The difficulty of introducing into practice the alternative methods (naturalistic or biological) has determined hyper-dependence of antiparasitic substances and the increase of chemo-resistance phenomenon [18].

Plants are investigated for their medicinal properties against parasites around the world with promising results. Plant extracts are obtained through laborious technics and the chosen method may affect the success in isolating the efficient compound against parasites [19]. Our investigation shows that using plant extracts separately as the only way of treating parasites is not efficient in simultaneous infestations. Recommendable would be to combine or alternate the two methods of treatment, with chemical compounds and with plant extracts leading to the reduction of used chemical substances.

Conclusions

The extracts from *Cucurbita pepo*, *Calendula Officinalis, Artemisia absinthium* and *Tagetes patula* had a reduced efficacy on the evolution of the digestive nematodes on the pigs taken in study compared to the modern medicinal product tested.

Extracts with average efficacy on one parasite have a weak antiparasitic effect on other nematode infestation in simultaneous contaminations; therefore, their overall efficacy is debatable.

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Mycological Investigation and Determination of Total Aflatoxins and Fumonizins in Dried Food Coming from Supermarkets and Small Shops

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Abstract

Mycotoxins are toxic biological compounds, produced by certain species of fungi, which may accumulate in their spores or on the developed substrate. Of these, a major impact on the food chain and human health presents aflatoxins produced by fungi of the genus *Aspergillus* and fumonizins produced by *Fusarium* genus.

The present study was conducted on 10 samples of preserved foods from supermarkets and small shops. For mycotoxicological examination test RIDASCREEN®FAST Aflatoxin and RIDASCREEN®FAST Fumonisin were used, competitive enzyme immunoassay tests for the quantitative determination of total aflatoxin and fumonizins in cereals and food. Following mycological examination performed on preserved foods, it was found the presence of mycetes from *Penicillium*, *Aspergillus* and *Fusarium* genera in 6 samples: dried peppers, bulk roasted corn, dried tomatoes, bulk corn flour (Alba county), bulk corn flour (Cluj county), extra degermed corn, the preserved foods being considered unfit for human consumption because of high and very high fungal loads, colony-forming unit (CFU)/g product exceeding tens, hundreds and thousands of times the maximum admitted value of 100 (CFU)/g of product.

Total Aflatoxins were found in all analysed samples, with a maximum value of 2.6 ppb and an average of 1.32 ppb, none of the samples exceeding the maximum values imposed by European legislation. Fumonizins were identified in 7 analysed samples with very low values, even in different maize samples investigated.

Keywords: mycetes, mycotoxins, total aflatoxin, fumonizins, preserved food

Introduction

Drying vegetables is one of the oldest and most widely used methods of preserving them. In time, the stability of dehydrated vegetables offers the possibility of being marketed at any time of the year, thus, by dehydration, diversifying the consumption of vegetables. During dehydration, the vegetables subjected to this process lose a certain amount of water and a physico-chemical state is created that is beneficial for maintaining nutritional values and sensory qualities, such as taste, smell, aroma [1].

Water activity is closely correlated with microbial development and food preservation, being influenced by osmotic pressure, pH and temperature. The development of microorganisms can be found in a range of water activity of 0.62-1, depending on the species. Thus, the bacteria require a water activity of not less than 0.85 because at values lower than this, their development can be inhibited, compared to yeasts that can withstand a value of 0.78 and moulds 0.65 [2].

Of the natural pollutants that raise a major problem on human health, the mycotic and mycotoxic are separate categories.

Many fungi have the ability to produce mycotoxins, but this depends on the type of substrate that provides fungi with the nutrients needed to grow or to elaborate the toxin [3]. The most common fungal contaminants among spices and herbs are species of the genera *Aspergillus*, *Penicillium* and even *Fusarium*, but only some of them are capable of biosynthesizing toxic metabolites [4].

Aflatoxins comprise compounds produced by the fungi species of the genera *Aspergillus* and *Penicillium*, which are naturally found in the substrate (forage) [3] and are referred to as "storage fungi", which are capable of growing and to produce toxins in low humidity conditions [5].

Reports of microbial contamination of dry vegetables are rare. Fumonizins are a family of carcinogenic mycotoxins, first isolated in 1988 from cultures of *Fusarium verticillioides*, strain MRC 826, following the Mycotoxin and Carcinogenic Experiment Program in Tygerberg, South Africa. These mycotoxins were isolated from maize foods. Nowadays, it is known that fumonizins can also be produced by several other *Fusarium* species: *F. proliferatum, F. napiforme, F. oxysporum, F. dlamini, F. nigamai* [6].

Aflatoxins have been detected in a large number of agricultural products, from vegetables and cereals to nuts and oil products. Aflatoxin B1 is the major metabolite of moulds of the genus *Aspergillius* and has a mutagenic, teratogenic and carcinogenic potential [3]. In Italy, assortments of buttermilk have been confirmed as being contaminated at a rather high level with fumonizins. Taking into account the incidence of oesophageal cancer in this country, a direct correlation was made with the presence of fumonizins in maize foods [7].

Materials and Methods

Given that human nutrition includes many products of plant origin, we considered it appropriate to study the incidence of mycotoxins in these. The purpose of this study was to identify the level of total aflatoxins and total fumonizins, known as factors favouring hepatocarcinoma and oesophageal cancer in humans. We also considered a mycological examination that aimed at determining the number of fungi as well as identifying the dominant fungi.

The analyses were performed on a number of 10 dried food samples, of vegetal origin, collected from supermarkets and small shops, as follows: one sample of dried pepper, two samples of fried corn, two samples of corn flour, one sample of corn flour extra degermed, one dry red tomato sample, a dry sliced carrot sample, a dry celery sample and a dry onion sample.

For the collection of the samples, the hygiene norms in force were strictly observed, only sterile containers were used so that the results are as conclusive as possible.

For the organoleptic examination, all 10 preserved food samples were subjected, following changes in the appearance, colour, odor, taste, consistency.

To perform the mycological examination, the working technique consisted of a sequence of steps, as follows: the weighing of the sample required for each determination was 10 grams, over which 90 ml of peptone water were placed. After 30 minutes, serial dilutions were made.

Subsequently, samples were placed on Sabouraud culture medium in Petri plates, aerobically incubated at 25°C for 5 days, after which the former colonies were counted and genera and species were identified based on cultural characteristics.

For the quantitative analysis of total aflatoxins and total fumonizins, all 10 preserved food samples were used. For the laboratory analyses we used the RIDASCREEN®FAST Aflatoxin test, respectively RIDASCREEN®FAST Fumonisin, competitive inmmunoenzymatic ELISA kits for quantitative determination of aflatoxins and fumonizins in cereals and foods. At the

base of the test stands the antigen-antibody reaction, the wells of the plate being labelled with anti-aflatoxin antibodies.

The results were read on a spectrophotometer equipped with Rida Soft, following the spectrum at an absorbance of 450 nm. The average value of the absorbance obtained for the standards and samples is divided by the absorbance value of the first standard and multiplied by 100. The zero standard is equal to 100% and the values of the absorbance are expressed in percent. The absorbance is inversely proportional to the concentration of fumonizine in the sample.

Results and Discussion

At the organoleptic examination, there were no changes in the appearance, consistency and odor of most of the analysed samples, considering their conditioning for long storage. Corn flour (bulk) was the only sample that showed particle agglomerations which denotes improper storage. Following the mycological examination performed on the 10 samples of dried foods, we found the presence of the *Penicillium*, *Aspergillus* and *Fusarium* fungi genera in 6 of them: dried peppers, roasted corn (bulk), dried tomatoes, corn flour (Alba); corn flour (Cluj), extra degermed corn flour.

Other studies also confirmed the isolation of these fungi species from dried and/or fresh vegetables [9, 10]. In the dry pepper sample the Colony Forming Units (CFU)/g mycotic load was over 5000 times higher (568,181 CFU/g) than the maximum allowed value [8], at the sample of roasted corn was more than 1,100 times higher (118,181 CFU/g), in the dried tomatoes more than 10 times (7,818 CFU/g), the maize flour (Alba) of more than 400 times (46,363 CFU/g), in the maize flour sample (Cluj county) more than 150 times higher (157,500 CFU/g) and in the extra degermed corn sample, the mycotic load was more than 70 times higher (7,500 CFU/g).

Following the mycotoxicological examination, the presence of total aflatoxins was found in all 10 dried food samples, the values being: 0.297 ppb – corn flour (Cluj); 0.892 ppb – extra dehydrated corn; 0.909 ppb - bulk roasted corn; 1,961 ppb – dried carrots; 1.11 ppb – corn flour (Alba); 1.23 ppb – dried onion; 1.53 ppb – dried peppers; 1.72 ppb – roasted corn with chili powder; 2.6 ppb – dry celery (Fig. 1). None of the evidence exceeded the maximum limit allowed by European legislation of 10 ppb. Many foods have been reported positive for contamination with aflatoxins.

Through most contaminated foods with aflatoxins, most commonly have been reported herbs and spices, cereals and nuts [11]. One study confirmed the presence of total aflatoxins in dried green bell pepper, with values ranging from 0.81 to 2.42 ppb [12]. Total aflatoxins content was determined in some typical African dried vegetable, with values ranging from 18.17 to 185.5 [13], way higher than the ones found in our samples.

Fumonizins reached a maximum value of 0.508 ppm in the extra-degraded corn sample and a minimum value of 0.013 ppm in the dried tomatoes. The average value is 0.1238 ppm and the standard deviation was 0.1627.

In three of the samples, namely the corn flour from Cluj and Alba counties, respectively the roasted corn with chilli flavour, the value 0 was registered, being below the detection limit (Fig. 2), the values obtained did not exceed the maximum limit imposed by Legislation in force.

Our results concluded once more the presence of fumonizins in foods such as corn, dried vegetables and fruits, confirmed by other studies, where values range from 10 to 5990 ppb [14, 15].

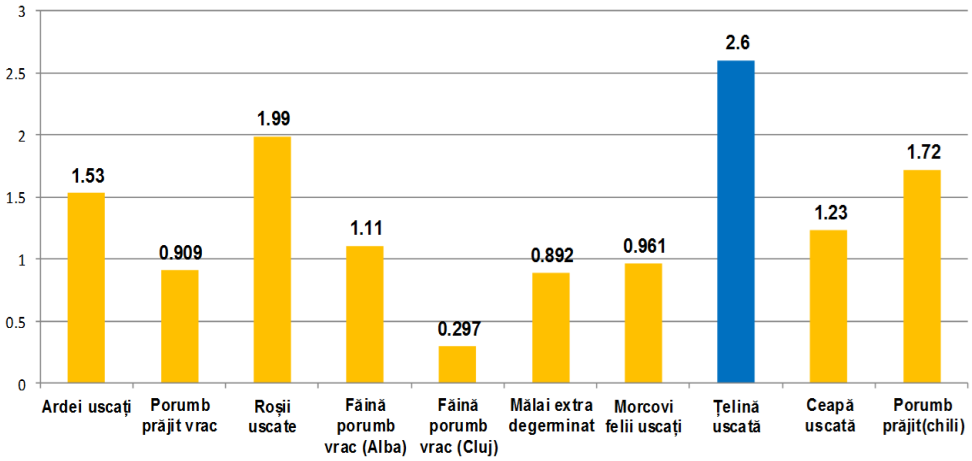

Fig. 1. Level of total aflatoxins found in tested samples

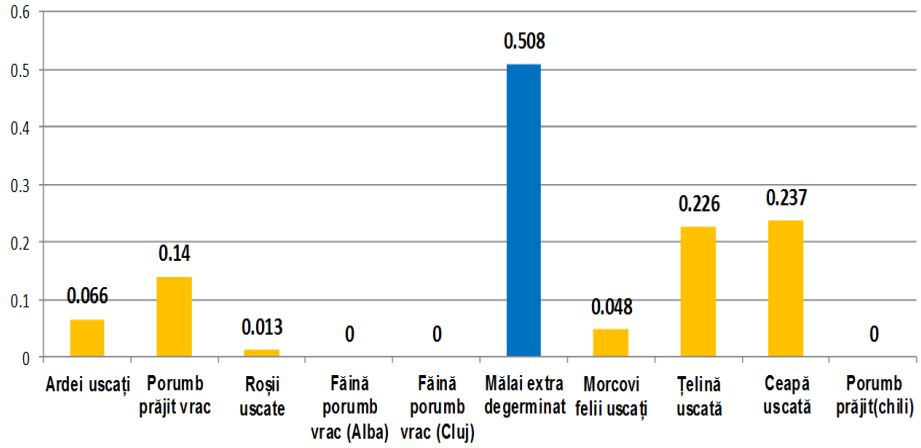

Fig. 2. Level of total fumonizins found in tested samples

Conclusion

Following the organoleptic examination, only one sample showed particle agglomerations, which indicate an improper storage. Following the mycological examination of the 10 samples analysed, the presence of the *Penicillium*, *Aspergillus* and *Fusarium* fungi genera were found in 6 of them: dried peppers, loose roasted corn, dried tomatoes, corn flour (Alba); corn flour (Cluj), extra degermed corn flour. Following the examinations, the level of total aflatoxins and fumonizins did not exceed the maximum allowed by the European Legislation of 10 ppb in the case of aflatoxins and 3000 ppb in the case of fumonizins, in any of the samples, which indicates that in none of the stages of the food chain did not exist contamination or thermal processes to which they were subjected would have eliminated some of the mycotoxins. Of the ten samples analysed, six should be eliminated from consumption due to the high mycological load, well above the maximum admissible value of 100 CFU/g. The fact that in these samples the level of total aflatoxins and fumonizins was below the maximum level allowed by the European legislation, but correlated with the presence in these samples in very large amounts of the genera *Aspergillus*, *Penicillium* and *Fusarium* may indicate that these samples could contain other mycotoxins, such as ochratoxins, tricotecene mycotoxins or zearalenone, which could make the subject of another possible study.

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Polyclonal Antibody Production in Several Rabbit Models

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Abstract

The rabbit is the most commonly used animal for polyclonal antibody production (pAbs).

The study did not find any difference between the antibodies' optical densities ($p>0.05$) of several allergens in both male and female rabbits. In the case of the pre-immune serum, the difference of average optical densities of pAbs, at 10^5 dilution, was statistically significant; older rabbits had higher optical densities (0.7716±0.0105 vs.0.0457±0.0019, at $p=0.010$). For the 38- and 59-days test, the trend of optical densities was statistically accepted only for 10^3 dilution; the 6-8 months old rabbit had a higher optical density at 38 (2.8859±0.1876 vs. 2.1726±0.0787, at $p=0.019$) and 59 days (3.1705±0.3058 vs. 2.1198±0.0787, at $p=0.019$). For the equal of more than 10^4 optical densities of pAbs the study did not find any statistical accepted differences. In the studied sample the gender and age variables did not manifest the expected effect on the pAbs production against allergens extracted from pollen of Ambrosia artemisiifolia. Nevertheless, the younger than 6 months old have to be preferred for pAbs production.

Keywords: rabbit model, polyclonal antibody, Ambrosia artemisiifolia

General

Experimental unit of *Horia Cernescu* Research Unit is a research infrastructure running projects under Authorization no. 535/19th of May 2016. Obtaining the polyclonal antibody in rabbits animal models is considered to superficial levels of pain, suffering or distress; by Directive 2010/63/EU of the European Parliament and of the Council regarding the *Protection of animals used for scientific purposes* and national legislation (Law 43/2014 and Order 97/2015 of National Authority for Veterinary and Food Safety) the classification of severity of procedures, over the entire polyclonal antibody production, was *mild* [1]. The main objective of antibody production in rabbit model is obtaining antiserum (pAbs; antisera) with a high titre and a high degree of affinity for experimental use or in diagnostic tests such as ELISA-type assays; Western blots; immunohistochemical and immune-precipitation procedures; and in immunofluorescence and immunoelectron microscopy). Specific target of the study was to compare the technological parameters of animals and pABs production by gender and age of animals.

Materials and Methods

The rabbit is the most commonly used animal for the production of pAbs, as it is easy to handle, to bleed and produce an adequate volume of high-titre, high affinity, antiserum. In a typical bleed, yield should be approximately 250mg of pAb. In a terminal bleed time using saline displacement, yield should be approximately 1g of pAb [5].

Polyclonal antibody production schedule

Polyclonal antibody production schedule is described in Fig. 1; the standard protocol follows the time schedule showed in such figure. The blood collection was performed at 7-10 days, following a booster injection for maximum results, depending upon the titter response curve generated by animal and antigen. The ELISA-type assay was performed for obtaining optical densities of the produced antibodies.

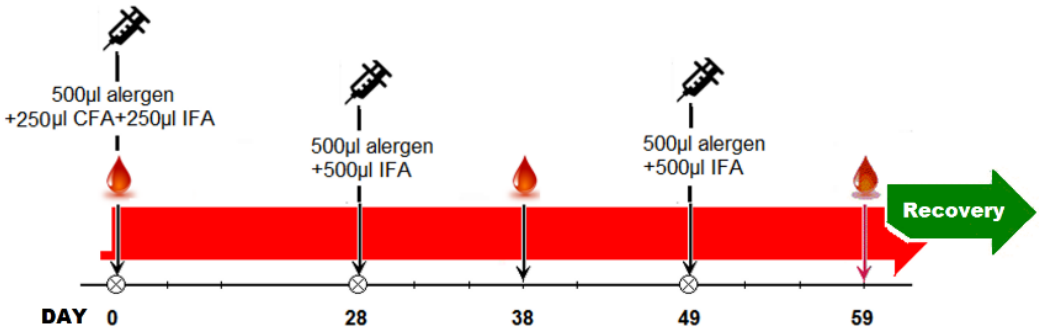

Fig. 1. Standard polyclonal antibody production schedule

- **Legend:**
- Polyclonal antibody production in rabbits started at their 4 and 6 months of age, scheduled for 59 days:
- Day 0 – pre-immunization bleed (preimmune serum) and mix of antigen injection with Freund's complete (CFA) and incomplete adjuvant (IFA) in a mixture of 500 µl antigen + 250 µl CFA + 250 µl IFA; 5 allergens extracted from pollen of the plant *Ambrosia artemisiifolia* were inoculated as antigens;
- Day 28 – second antigen injection with Freund's incomplete adjuvant (500 µl antigen + 500µl IFA);
- Day 38 – bleed test;
- Day 49 – the third antigen booster (allergen and Freund's incomplete adjuvant – (500 µl antigen + 500µl IFA);
- Day 59 – blood collection.

Alternation of antigen inoculation and blood collection can be prolonged if an animal fails to produce a satisfactory titter of antibody. Thus, the third antigen inoculation and final bleeding are depending on the titter response curve. Any animal which is not able to produce a satisfactory titter after the fort's booster should be considered unsuitable for the antigen and taken out of the polyclonal antibody production group.
Source: Experimental Unit Specific Procedure [1].

Sampled animals and data collection

Two groups of five males and five females[1] (sample 10 out of 26 White New Zeeland rabbits) were used, for measuring the technological indexes (body weight, average daily gain and feed conversion) and characteristics associated to pAbs production. Initially 6 rabbits were treated and 4 animals were the control group. The immunological performances of the control group came 4 months later; the control group started the antibody production one week after the first group had the final blood collection. In the pre-immunisation day, all sampled animas were monitored and clinically healthy [1]; they were micro chipped and had normal growth

[1] The sample included 6 rabbits used in polyclonal production (1:1 sex ratio) and 4 (two males and two females) as a control group for monitoring the technological variables and health. – Chondrotissue and C – Control); each group had 7 animals.

parameters (average daily gain, feed consumption, and body mass) during the accommodation period.

Housing and feeding

The rabbits were kept individually in a three tiers Tecniplast® X-type cage, L x l x h = 784 x 820 x 1.830 mm and 4.264 cm2 space, walls and floors made of transparent (side panels) or opaque polycarbonate (rear panels, discontinuous floor and trash and purine trays). In the rabbits' compartment, the environmental temperature and humidity were continuously monitored (every half an hour) by multi-functional wireless digital device *Weather Station PCE-FWS 20.* The environmental temperature was 24.21±1.31ºC (X±SD for all study period, with each ½ hour measurements) and the study did not sustain significant differences between days of measurements. Air currents speed in the room of rabbit was 0.01 m/s (one measurement per day). The rabbit consumed daily 160-180 g of pelleted feed with digestibility up to 65%.

The metabolic energy density of feed was 1.980±50 kcal/kg. The calories came from protein -23%, fat -10% and carbohydrates -67% [3]. During the production period, each cage had an elevated platform and two times per week acacia branches were brought for environmental enrichment.

Statistical Analysis

Mann-Whitney U analysis was used to assess differences in mean values for gender, treatment and age variables. The study used *IBM® SPSS® Statistics* software for statistical analysis. Significance of differences was considered at a value of $\alpha = 0.05$.

Results and Discussion

In practice female rabbits are more often used due to their docility; there are also suggestions that female is much more sensitive to lower doses to antigen and many have significantly higher and more prolonged response to immunization than males [1, 2]. All the animals were clinically healthy during the experiment. The polyclonal antibody production started in the rabbits' 4^{th} and 6^{th} month of age, after one month of accommodation. At the 4^{th} months the body mass of the sampled animals was 2,661.50±55.66 g and in the 6^{th} months 3,288.50±88.21 g.

The pAbs production effect on technological parameters had the estimated impact, without any statistical significate differences. The difference was found insignificant, between treated (immunised group) vs. untreated animals (control group), in terms of initial body weight (2606.66±78.14 vs. 273.75±64.92, p=0.352), final body weight (3,205.83±134.91 g vs. 3,412.50±65.62 g, p=0.352), total gain (599.16 ±94.35 g vs. 668.75±18.75 g, p=0.762), average daily gain (13.61±2.14 g vs. 15.20±0.43 g, p=0.762), feed consumption (4.512±242 g vs 5498±481 g, p=0.114) and feed conversion (8.94±1.89 vs. 8.20±0.59, p=0.762). Thus, in the study, even if the value of body weight, gain and feed conversion are a little bit favourable to untreated animals (control group), the immunization and antibody production did not influence drastically the technological parameters.

The gender variables had not the estimated impact. Working with the male rabbits can be a little bit difficult for the inexperienced technicians, while well trained personnel will easily manage the restriction and manipulation of the males during bleeding, boosting or weighting.

The samples of the study did not sustain the difference between body mass of males and females in the beginning (p=0.151 in Mann-Whitney U test) but it became obvious in the end of the experiment (p=0.016). The result can be better explained if associated to normal difference in growing curve of males and females [4]. In the sampled animals of the study, average daily gain, total gain, feed conversion and level of pAbs production quantified by

ELISA assay in terms of optical densities (OD) for 10^3, 10^4 and 10^5 dilutions, were not associated with the gender variable (Mann-Whitney U test with value of $p = 0.421$ to 1.000).

Retroactive comparisons between 4-6 month and 6-8-month rabbits were run after the control group ended its polyclonal antibody production, two month later. The study did not find differences between antibodies' optical densities (*p* more than 0.05 for all five antibodies in all three considered optical densities). In the case of pre-immune serum, the difference of average optical densities of pAbs at 10^5 dilution was statistically significant; older rabbits had higher optical densities (0.7716±0.0105 vs.0.0457±0.0019, at p=0.010). For the 38- and 59-days test, the trend of optical densities was statistically accepted only for 10^3 dilution; the 6-8 months older rabbit had higher optical densities at 38 (2.8859±0.1876 vs. 2.1726±0.0787, at p=0.019) and 59 days (3.1705±0.3058 vs. 2.1198±0.0787, at p=0.019). For the equivalent of more than 10^4 optical densities of pAbs the study did not find any statistical accepted differences.

Although the study was not able to sustain the hypothesis of association between age and level of pAbs production, the younger animals have to be preferred.

Conclusions

- Polyclonal antibody production can be obtained equally from male and female rabbits.
- Older rabbits can be used for the convenient level of antibody production against allergens extracted from pollen of the plant *Ambrosia artemisiifolia*.

Ethic Statement and Acknowledgments

Study protocol was designed and followed in strict accordance with the Specific Procedures of Experimental Units under Veterinarian Authority Authorization no. 001/29.09.2017 and was supported by the project *INSPIRED – Strategii inovative pentru prevenția, diagnosticul și terapia afecțiunilor respiratorii induse de polenul de ambrozia,* ID: P_37_747, cod MySMIS: 103663, contract no. POC 92/09.09.2016. The research was run within Laboratory Animal Unit, part of *Horia Cernescu* Research Unit, in Banat University of Agricultural Science and Veterinary Medicine *King Michael I of Romania*, Timisoara.

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Efficacy Assessment of Afoxolaner (Nexgard®) in Dogs Naturally Infected with *Sarcoptes Scabiei*

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Abstract

Sarcoptic mange in dogs continues to represent a serious skin problem that if left untreated, could end up with the death of the animal. In Romania, it is a common dermatitis of stray dogs, where it evolves usually without control, being easily passed to dogs with owners. The treatment recommended for curing sarcoptic mange includes quite a big number of different acaricid molecules. However, the novel introduced acaricids from the isoxazoline family seem to have very good results, with a minimum compliance of the owner. In our study, we evaluated the efficacy of afoxolaner, a molecule from the isoxazoline family, which has been recently licensed (2018) in the European Union for use in treating sarcoptic mange in dogs. We conducted the study at the end of 2017, and included 16 dogs in an open pre-treatment versus post-treatment study, dogs naturally infected with *Sarcopes scabiei* as confirmed by the microscopic examination, which received afoxolaner (Nexgard®) adapted to their body weight (between 2.7 and 6.9 mg/kg), at day 0 (D0) and at day 28 (D28). The skin scraping was made from three body sites (head, trunk, legs) at D0 and D28 and the presence of sarcoptes mites was confirmed or infirmed. Their general health and dermatological status (evaluated through a specific clinical score) was evaluated at D0 and D28 and photos of each dog were taken. The efficacy of the afoxolaner treatment was evaluated at D28 through the evolution of the clinical score, which diminished with about 63.6% from its initial value from D0. Furthermore, even though we did not perform a mite count, we could observe that at D28, we found no more mites in the samples collected.

Keywords: Sarcoptes scabiei, afoxolaner, mange, dog, treatment

Introduction

Sarcoptic mange in dog is a highly contagious, parasitic skin disease, characterized by intense pruritus, hair loss, scales and crusts [1].

It is caused by *Sarcoptes scabiei var. canis*, a burrowing mite that lives during all stages on the host, having a life cycle of approximately 14 days [2].

Mange is a disease that evolves worldwide, usually in crowded shelters and big communities of animals, where dogs are in close proximity and disinfection is a problem. Privately owned dogs can also get easily infected by direct contact with a dog or fox suffering from mange, or through fomites [3].

Clinical signs of mange typically occur on the head (especially the ear pinnae), on the elbows, the hooks, on the ventral part of the abdomen and on the legs, but can easily spread all

over the body. The signs in mange are almost constantly represented by intense pruritus, alopecia, papules (that transform into crusts after the dog scratches them) and scales [4].

The authorized treatment available for curing sarcoptic mange in dog, at the moment, in the European Union includes the following molecules: selamectin (spot-on), imidacloprid/moxidectin (spot-on), sarolaner (tablet) and the newly approved afoxolaner and afoxolaner/milbemycin oxime (tablet). The latter 3 molecules belong to the isoxazoline family, a new chemical class of acaricides and insecticides, introduced in the 2000s, that have proven efficacy against some ectoparasites such as ticks, fleas, mites [5], [6].

The use of afoxolaner in treating sarcoptic mange in dogs has been previously investigated.

In 2016, Beugnet *et al*., studied 20 dogs naturally infected with sarcoptic mange (10- control group, 10- treated group). The treatment with afoxolaner (Nexgard®) was given at a monthly interval, at the labeled dose, for 2 months, and it was observed that even after the first month, there was a complete cure of mites (from an average of 166.9 to 0 in the treated group at both D28 and D56) and in 2 months, the clinical signs resolved in the treated dogs [7].

The second study, from Hampel *et al*., 2018, considered both afoxolaner (Nexgard®) or afoxolaner/milbemycin oxim (Nexgard Spectra®) for the treatment of 65 dogs with sarcoptic mange, monthly, for 2 months. The efficacy of the treatment, illustrated in reduction of geometric mean live Sarcoptes mite counts was 98.9% and 99.7% for NexGard® treated (n=38) and 99.6% and 100% for NexGard Spectra®-treated dogs (n=27) at one month and two months after treatment initiation. Both treatments marked a rapid significant improvement in papules, crusts and pruritus at one month and two months after treatment. In conclusion, afoxolaner or afoxolaner/milbemycin oxim have remarkable effects on mange mites [8].

The aim of our study was to evaluate the efficacy of afoxolaner, at the commercial recommended dose, for two months' administration, in dogs that are naturally infected with *S. scabiei*. The study included dogs with mild to very severe lesions, and the efficacy was evaluated based on the resolution of some characteristic clinical signs at D28, compared to D0. We also noted the presence or absence of mites at the two time points.

Materials and Methods

There were 16 dogs included in the study, both dogs that were presented in consultation at the Parasitology Clinic from the Faculty of Veterinary Medicine of Iași, and dogs that were examined during field work. The criteria of inclusion consisted in the presence of typical signs of mange (alopecia, pruritus, crusts), confirmed by microscopical identification of *S. scabiei* from skin scrapings.

A questionnaire was filled regarding the history of the dog including general presentation, breed, sex, age, number of in contact animals, possible source of contamination with mange, the moment when the first lesions appeared, if the owner noticed skin problems in himself or in the members of the household.

For confirmation of the diagnosis, identification of *S. scabiei* mites is required, through the direct examination at the microscope. Several samples were collected from three body sites (head, trunk, legs), from the areas that presented characteristic signs of sarcoptic mange. Skin scrapings were made with a sterile scalpel until capillary bleeding resulted, on areas of approximately 4 cm^2. The samples were then put on a slide and macerated and spread in lactophenol or mineral oil, then covered with a coverslip and examined at the microscope, with the 10X objective. The sampling was done at D0 and at D28.

The identification of *S. scabiei* mites was done by morphological identification of adults (male or females), nymphs, larvae or eggs.

Concerning the clinical examination, a general health exam was performed in all dogs, at D0.

As for the dermatological exam, an original clinical score adapted for dogs, but initially designed for pigs infected with *S. scabiei* was used [9]. This score includes grades from 0 to 4, with 4 being the most severe, given to some characteristic signs that appear in mange in dogs such as: the skin area affected by sarcoptic mange, alopecia, skin erythema, and crusts or scales.

These signs were evaluated on different parts of the body (head, trunk, legs and tail), resulting in a total clinical score that could be between 0 and a maximum of 60.

The scores were assessed at D0 and at D28 and photos of each dog were also taken.

The treatment protocol consisted in the use of the commercial product NexGard® (afoxolaner), administrated orally according to commercial registration labeling, taking into account the weight of the animal, resulting in a dose between 2.7 and 6.9 mg/kg. The treatment was given at D0 and at D28. All other in contact dogs were equally treated. The owners of the dogs consented in the off label use of the product, as at the time of the study, 2017, the molecule was not accepted officially for the treatment of *S.scabiei* in dogs.

Results

The individual dog was the experimental unit. From the 16 dogs, all remained in the same environment as before mange has been diagnosed, with the exception of one dog, which was hospitalized in our clinic (A10).

Among these 16 dogs, 9 were from the same countryside household from Vaslui County (Fig. 1, Fig. 2). At the moment when the owner noticed the first clinical signs, there were a total of 19 dogs with ages between 2 months and 10 years present in the yard of the house. The source of the disease remained unknown. In the end, we caught and sampled 9 dogs (Table 1).

The other dogs were also given the treatment, the tablet being hidden in a piece of meat.

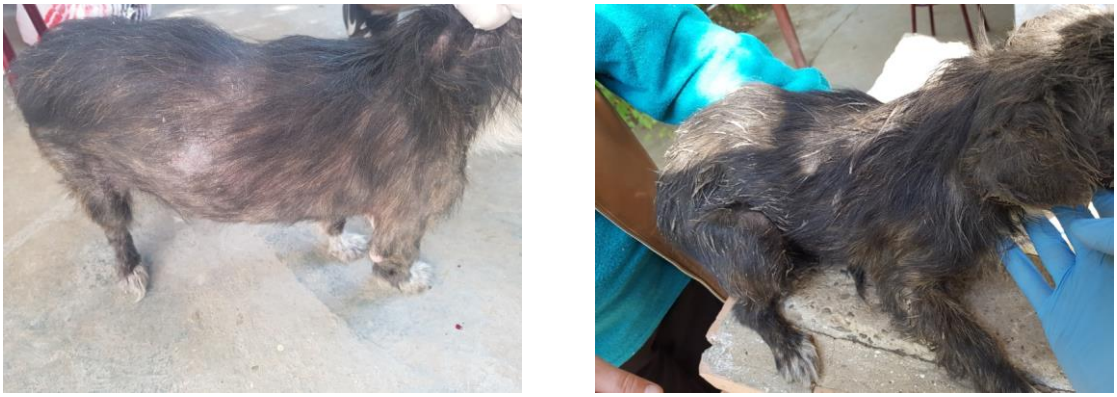


Fig. 1. Dog A6, at D0 (left) and at D28 (right)

Fig. 2. Dog A9, at D0 (left) and D28 (right)

Another dog included in the study was a stray dog from Vrancea County (A10, Table 1), which was brought to our clinic with severe skin lesions due to the infection with *S. scabiei*.

The history of the dog was unknown. He was hospitalized for a long period of time, approximately 4 months.

Furthermore, we had included 3 dogs from Public shelters, an adult male (from Tomești shelter, A11) and from another shelter, one puppy and his mother (A14, A15) (Table 1).

Also, we had 2 dogs from different households from Iași, a puppy (A12) and an adult female (A16) infected with *S.scabiei* (Table 1).

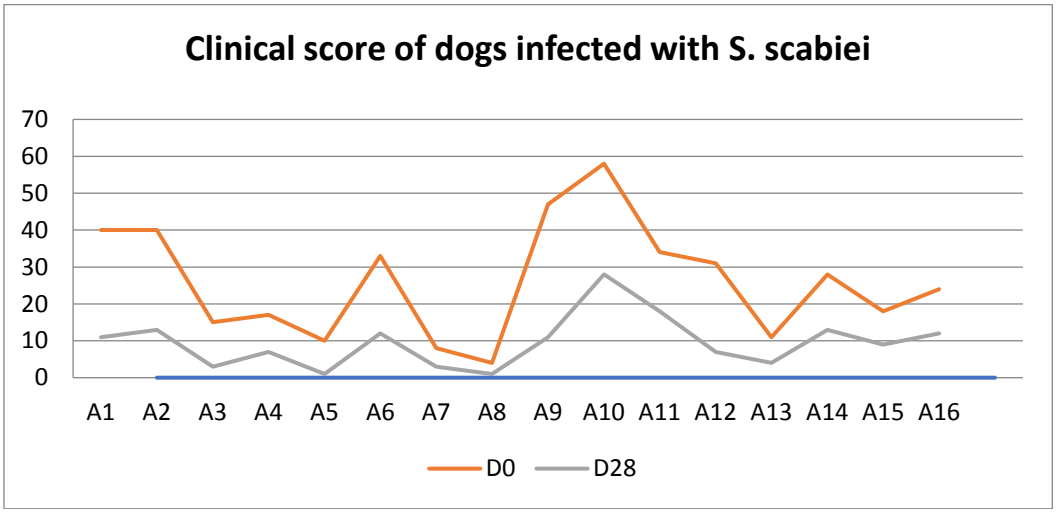
Table 1. Characterization of the dogs included in the study

ID	Breed	Age	Sex	Location	Clinical score D0	Clinical score D28
A1	Common	4 Y	M	Vaslui (owner)	40	11
A2	Common	3 Y	F	Vaslui (owner)	40	13
A3	Common	6 Y	M	Vaslui (owner)	15	3
A4	Common	5 Y	F	Vaslui (owner)	17	7
A5	Common	6-7 Y	F	Vaslui (owner)	10	1
A6	Common	4 Y	F	Vaslui (owner)	33	12
A7	Common	3-4 Y	F	Vaslui (owner)	8	3
A8	Common	6-7 Y	F	Vaslui (owner)	4	1
A9	Common	4 m	M	Vaslui (owner)	47	11
A10	Cross breed	2 Y	M	Vrancea (stray dog)	58	28
A11	Common	4 Y	M	Iasi, Public Shelter	34	18
A12	Common	2m	M	Iasi (owner)	31	7
A13	Cross breed	4 m	M	Suceava (owner)	11	4
A14	Common	2m	M	Iasi Public Shelter	28	13
ID	**Breed**	**Age**	**Sex**	**Location**	**Clinical score D0**	**Clinical score D28**
A15	Common	4 Y	F	Iasi Public Shelter	18	9
A16	Cross breed	5 Y	F	Iasi (owner)	24	12
					Average D0=26.12	Average D28=9.56

At D28, the clinical score was reassessed for each dog and after that, compared with the clinical score from D0. Overall, we noticed a significant resolution of the intensity of the clinical signs, proved by the fact that the average clinical score at D28 was only 36.6% of the value of the average of the clinical score at D28.

The maximum value of the clinical score is 60, and the dog A10 almost reaches it, having a resolution of the clinical signs of 51.8% from the initial score by D28. The scores are highly

variable across individuals, because we included dogs that were highly affected by sarcoptic mange like A10, and dogs that showed very little signs of the disease like A8 (Graphic 1).



Graphic 1. Clinical score of the studied dogs at D0 and D28

Furthermore, the clinical efficacy of the treatment was evaluated based on the resolution of the clinical score from baseline, calculated as [(clinical score at D0 – clinical score at D28)/clinical score at D0] * 100. Efficacy varied from 47% to 90%.

Even though our study didn't include a mite count, we did however resample skin scrapings from the 3 body sites at D28 from all dogs and found 0 mites present.

Discussion

There were 16 dogs included in our research, which is a number comparable to that of the first study of afoxolaner in dogs infected with *S. scabiei*, which included 20 dogs from South Africa divided into a treatment group (treated with the product Nexgard® at D0 and D28 according to body weight) and a control group [7]. The most recent study from Hampel *et al*., from 2018, included 65 dogs from Portugal and Germany, among which, 38 were treated with Nexgard® at D0 and D28 and 27 with Nexgard Spectra® (afoxolaner and milbemycine oxime) at D0 and D28 according to body weight [8].

Concerning the diagnosis method used in the two studies on the efficacy of afoxolaner on *S. scabiei* mites in dogs, it consisted on identifying positive skin scrapings from 5 different body sites/dog. Comparing to our study, the diagnosis method used was the same, but the number of body sites included was less in our work (3 compared to 5).

The clinical score used by us included an original aspect by evaluating the extent of the skin affected by sarcoptic mange. Also, we evaluated the degree of erythema present on the skin.

What our study has in common with the other two studies mentioned, is the evaluation of the alopecia (hair loss) and crusts and scales. However, a minus is that we did not include a pruritus score, this clinical sign being an important evaluating factor for the efficacy of the studied molecule, as shown by the two studies. Furthermore, the evaluation of the dogs was made at D0 and D28 in our experiment, comparing to the evaluation made by the other authors at D0, D28 and after the second treatment at D56.

Concerning the treatment, afoxolaner, a member of the isoxazoline family, is a relatively newly introduced molecule, a safe drug, effective against fleas, ticks and most recently approved for the treatment of other external parasites, such as mites (*Demodex spp. and S. scabiei,* 2018) [10], [11], [12], [13]. At the moment of our study, the product was not officially approved for the treatment of sarcoptic mange in dogs.

In conclusion, these studies support our findings, showing a very high efficacy of afoxolaner.

In both studies, mite counts were used as primary criteria in evaluating the success of the treatment. Even though we didn't include this step in our study, we did observe a complete disappearance of the mites at D28. Furthermore, in these studies the efficacy was assessed also by comparing the score of the characteristic lesions in sarcoptic mange at the beginning of the trial D0, at the time of the second administration of afoxolaner D28, and at the end of the treatment at D56. The clinical signs were already significantly improved at D28, which is consistent with our findings.

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Large Prostatic Cyst with Sarcomatous Transformation in a Golden Retriever

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Abstract

A 12-year-old intact male Golden retriever dog was presented for evaluation of persistent tenesmus and a caudal abdominal wall contour deformation. Presurgical evaluations included complete physical examinations, serum biochemistry and abdominal ultrasonography. A large paraprostatic cyst and multiple intraglandular small fluid-filled cavities were diagnosed. The results of histopathology of tissue samples obtained during exploratory laparotomy showed a sarcomatous transformation of the paraprostatic cyst. To the authors' knowledge, the case presented is the first documented case of prostatic cyst with sarcomatous transformation. Short-term outcome was very good after resection of the cystic wall and omentalization but the prognosis must be guarded because of sarcomatous transformation.

Keywords: dog, prostate, paraprostatic cyst, sarcoma

Introduction

Although multiple cystic changes are commonly found both within and on the surface of the prostate in association with benign prostatic hyperplasia, the development of large solitary cysts is less common (White, 2018). Their cause still remains unclear, but the accumulation of glandular secretions through progression of microscopic cystic changes and disordered drainage caused by obstruction of the ducts in the presence of benign prostatic hyperplasia seems the most plausible explanation currently [1]. Distinctions have been drawn between "paraprostatic" cysts, which appear to develop separately from the prostate and do not communicate with the parenchyma but usually have some attachment to the capsule, and "prostatic" cysts, which develop within the capsule of the gland itself. This distinction on the sole basis of anatomic location may in fact be artificial; it seems more plausible that secretory accumulations on the glandular surface may develop as separate "paraprostatic" entities within the caudal abdomen or pelvic region, but intraparenchymal cystic accumulations, which often show some form of communication with the prostatic urethra, remain surrounded by capsular tissue. The more general term *prostatic* cyst is therefore probably more appropriate for all types of larger discrete cysts found in association with the prostate gland. Irrespective of the cyst's location in relation to the gland, its capsule is often thick and fibrous and, in some cases, may even become mineralized. The interior structure often contains trabecular strands and is filled with quantities of brown to chocolate-coloured fluid. Some cysts may become secondarily

infected. Isolated cases of prostatic cysts resulting from *Echinococcus multilocularis* have been reported [2]. The speculation that paraprostatic cysts represent an anomaly of the uterus masculinus that develops in the urethral midline in the region of the seminal hillock from embryonic remnants of the Müllerian duct [3, 4] has never been substantiated in dogs. In one report [5] the term uterus masculinus was used to describe a single or double ("horn-like") cylindrical or tubular structure originating from the craniodorsal aspect of the prostate gland and extending cranially. In that study the structure was not felt to be contributing to any clinical signs seen in those dogs.

This report describes the presence of a large prostatic cyst with sarcomatous transformation in an old Golden retriever. According to our knowledge, a simultaneous sarcomatous transformation of a large prostatic cyst in a dog was not previously reported.

Methodology

Case presentation

A 12-year-old intact male Golden retriever dog was presented to the veterinary clinic Echovet Cluj-Napoca with a four-week history of tenesmus, and a firm, mobile, large mass on the prepubic region. The non-painful prostate gland was palpable rectally and located intra-abdominally. The results of routine serum biochemistry and haematology were unremarkable, and no abnormalities detected on thoracic radiographs were present. Abdominal ultrasonography confirmed the presence of a well encapsulated, fluid-filled large soft tissue mass in in the caudal abdomen in the region of the prostate area. Asymmetrical prostatomegaly and multiple other small fluid-filled cavities within the prostatic parenchyma were obvious at ultrasonography (Fig. 1) A presumptive diagnosis of paraprostatic cyst was established ratory

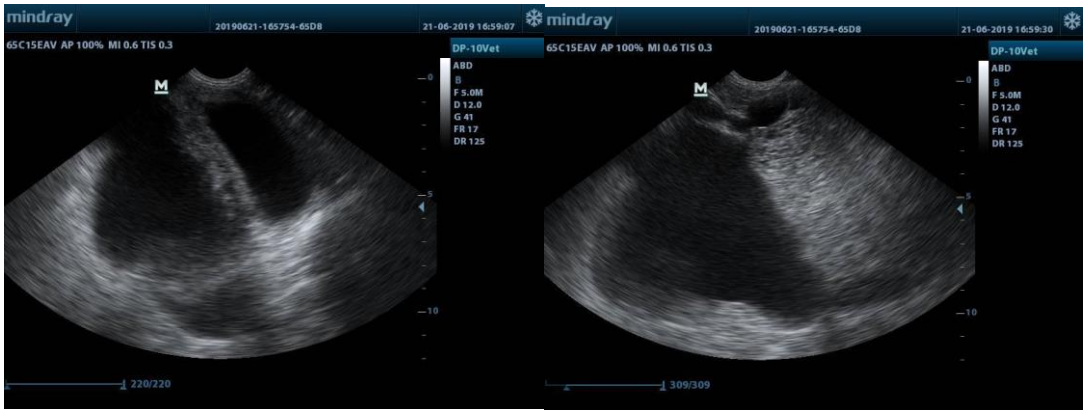

Fig. 1. Ultrasonograph taken via the caudal abdomen. The prostate is enlarged and the large cyst is lying against the abdominal wall. The cyst wall can be seen as a separate echogenic linear interface, and the cyst contents are anechoic. Multiple other small fluid-filled cavities within the prostatic parenchyma are also present

Asymmetrical prostatomegaly and multiple small fluid-filled cavities within the prostatic parenchyma were obvious at ultrasonography.

Following general anaesthesia, the entire abdomen, including the scrotum, was clipped and prepared for aseptic surgery. The dog was positioned in dorsal recumbency and preoperative intravenous antibiotics were administered in cases 1 and 2 (cephazolin 20 mg/kg [UCF Borsceagov SAI, CSP]). Analgesia was provided using a combination of systemic opioids and non-steroidal anti-inflammatory drugs. A urinary catheter was placed to help identification of the urethra. A caudal celiotomy extending from the umbilicus to the pubic brim was performed.

Large left dorsolateral cyst was present and attached to the prostate. Multiple cystic cavities located within the prostate were also observed. The largest cyst was easily identified and partially exteriorized. After it has been packed off from the remainder of the abdominal cavity

with moist sponges, suction was used to remove the contents (Fig. 2 and 3). The prostate gland was carefully examined and palpated during the surgical procedure for any evidence of neoplastic disease that may underlie the development of the cyst. No gross evidence of neoplastic infiltration was seen but incisional biopsy was performed. The large cyst was partially resected, leaving the attachment of its base to the prostate gland intact (Fig. 4). Digital disruption of small cystic cavities within the prostatic parenchyma was also performed. A leaf of momentum was packed into the cyst remnant (Fig. 5) and secured into its prostatic base with absorbable suture material in a mattress pattern. Before abdominal closure, the peritoneal cavity was lavage with warm saline solution. The celiotomy wound was closed routinely and prescrotal castration was performed.

The cystic wall and prostatic biopsy were submitted for histological exams.

Histopathology

Formalin-fixed sections of the cystic proliferation and prostate were processed into paraffin blocks using routine histology techniques, sectioned to 4-µm thickness, mounted on histological slides, and finally standardly stained with hematoxylin-eosin (H&E).

Immunohistochemistry

For the immunohistochemistry, a Leica Bond-Max automated immunostainer (Leica Microsystems) was used. The immunohistochemistry staining was performed for vimentin (Clone SRL33) (Vim) and multicytokeratin (Clone AE1/AE3) (CK). 3,3- diaminobenzidine (DAB) was used as chromogen, and slides were counterstained with hematoxylin. Bright-field histological images were obtained using a UC30 Olympus Digital Camera mounted on an Olympus BX4-light microscope further processed by Stream Basic software.

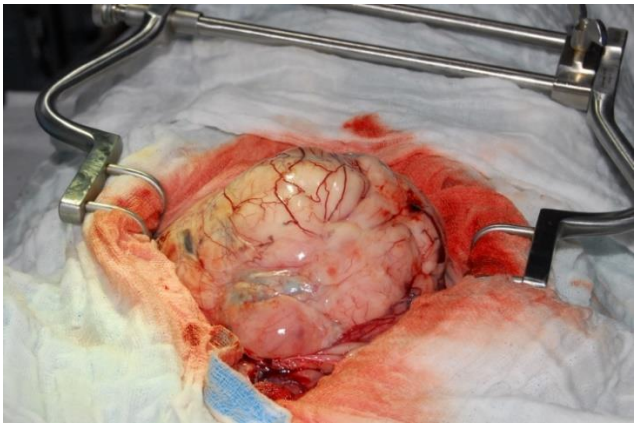

Fig. 2. Caudal laparotomy exposure of prostatic cyst surrounded by adipose tissue

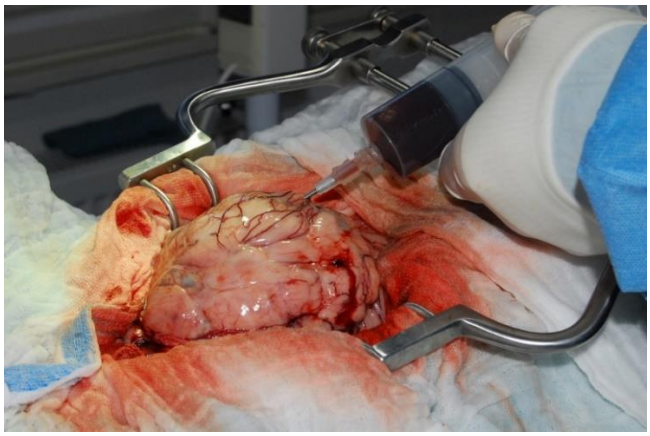

Fig. 3. Drainage of brown to chocolate-coloured fluid inside the cyst

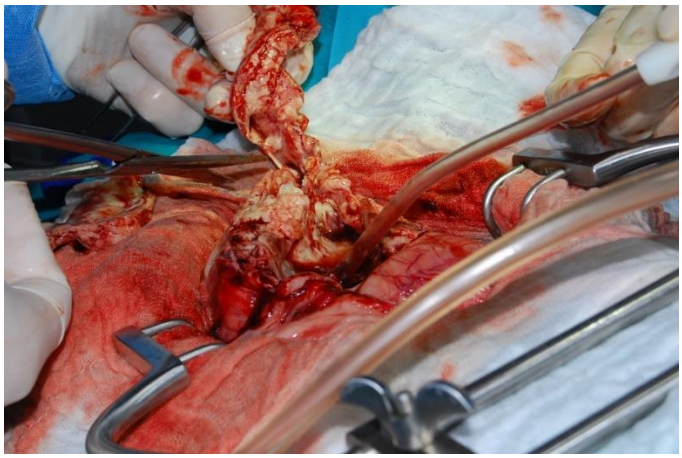

Fig. 4. Resection of capsular tissue from cyst wall

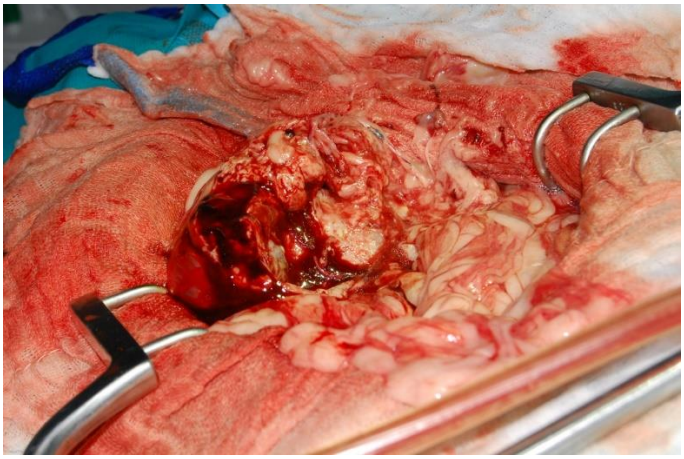

Fig. 5. A leaf of omentum was packed into the cyst remnant

Results

The dog was discharged in the day of surgery. Microscopy and culture failed to demonstrate bacteria in the cystic fluid and urine, while histopathology of the cyst and prostatic biopsy yielded a diagnosis of paraprostatic cyst with sarcomatous transformation (Fig. 6) and benign prostatic hyperplasia, respectively.

The dog clinical signs significantly improved postoperatively. Repeated rectal examination and abdominal ultrasonography demonstrated the presence of fluid-filled spaces within the gland, but without clinical signs. Follow-up 2 months after surgery, revealed that the dog was in good health, urinary continent and no tenesmus. Ultrasonographic examination of the prostatic region two months after surgery revealed several cystic foci in the prostate.

The wall of the cystic tumour consists of poorly defined sheets of polymorphic cells separated by a small amount of myxoid extracellular matrix. Tumour cells are polygonal to fusiform and stellate (marked cellular polymorphism), have well-defined margins and a large amount of acidophilic cytoplasm (fibrillary or homogeneous). The nuclei are oval to kidney-shaped, contain vesicular chromatin and a large amphophilic nucleolus. Rarely tumour cells are elongated, with centrally arranged multiple nuclei ("nuclear rowing"). Multifocally, tumour cells infiltrate the vascular wall (occasionally with vascular tumour embolism). Necrotic foci (≤5%) are present inside the tumour. Mitoses are rare (1/10HPF). All tumour cells were intensely positive to vimentin and negative to cytokeratin (Fig. 6).

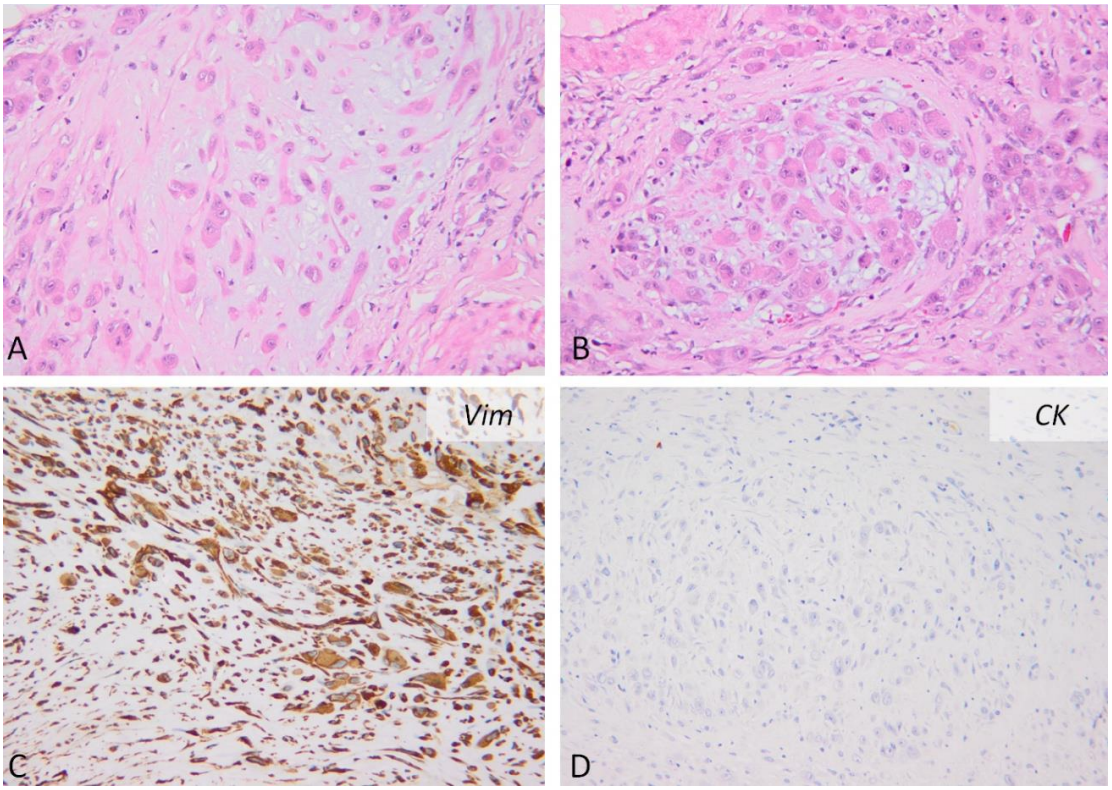

Fig. 6. Paraprostatic tumour: histopathological images of H&E histology (image A and B) and immunohistochemistry for vimentin (Vim) and multicytokeratin (CK) (image C and D). Obx 40 for images A and B and obx20 for images C and D.

Discussion

This unique case describes the sarcomatous transformation of a large paraprostatic cyst in a dog. Prostatic disease is a common problem in older entire male dogs. However, canine paraprostatic cysts are relatively uncommon compared with other prostatic diseases, such as benign prostatic hyperplasia and prostatitis [4, 6, 7]. Paraprostatic cysts are commonly located intra-abdominally [7]. They have also been reported as extending through the pelvic canal to the perineum, although this is rare [6]. In the present case, thorough clinical examination, including rectal palpation and ultrasonography, the mass was easely identified as a fluid-filled in the caudal abdominal area. Castration was considered an essential component of therapy [8, 9].

The sonographic appearance of the paraprostatic cyst was similar to that in previous studies; an anechoic structure with a thin echogenic wall [7, 10].

Mineralization of paraprostatic cysts has been reported to be uncommon [4, 6, 12, 13]. Of nine abdominal prostatic cysts in a previous study, two were mineralized, while three of four intrapelvic/perineal prostatic cysts were mineralized [6]. In this study, three of six abdominal cysts were mineralized, as were three of five intrapelvic/perineal cysts. Calcified or osteocollagenous cysts have been described as typically having regularly distributed mineralization that may resemble an eggshell [8]. In our case, we did not observed patterns of mineralization. It was not the target of our study to assess relationship between the type of prostate gland disease and mineralization. According to other studies [10] paraprostatic cysts are frequently mineralized. Utrasonography is useful in the assessment of prostatic disease, but does not replace survey and contrast radiography in the assessment of a suspected prostatic cyst.

As well as ultrasound being inferior at demonstrating spatial relationships, the detection of acoustic shadowing due to prostatic cyst mineralization seems unreliable.

The prostate was omentalised. This technique has been described for the treatment of prostatic retention cysts and prostatic abscesses in dogs [11] and was performed in the present case as a prophylactic measure.

Although urinary incontinence may be seen in dogs after surgery for large prostatic cysts (White, 2018) because of the original anatomic changes in the urethra caused by the development of the cyst, we did not notice this complication in our patient.

Histopathological exams showed a sarcomatous transformation of the prostatic cyst. To the authors' knowledge, this is the first report of a sarcomatous transformation of a paraprostatic cyst in a dog. Based on this case, we strongly recommend the histopathological examination of every paraprostatic cyst sample in the dog. Surgical removal of the cyst and prostatic omentalisation may ensure a temporary relief of clinical signs and good quality of life. Anyway, prognosis should be considered guarded.

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Histological Structure of the Central Nervous System in Adult Zebrafish (Danio Rerio)

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Abstract

The increasing popularity of zebrafish in the field of scientific research makes a good knowledge of its anatomy and histology essential. The detailed knowledge of the zebrafish neuroanatomy is a valuable tool in its studies on the nervous system, allowing the changes made at various levels to be easily detected. 20 adult zebrafish were taken in our study from which histological sections of the brain were obtained. The most rostral telencephalic divisions are the pair of olfactory bulbs.

The rest of the telencephalon is composed of two subdivisions: the dorsalis area and the ventralis telencephali area. The diencephalon comprises a total of 5 major regions that are organized dorso-ventrally in the adult brain.

These are: epitalamus, dorsal thalamus, ventral thalamus, posterior tubercle and hypothalamus. The mesencephalon includes dorsal and ventral optic tectum (TeO), torus semicircularis and tegmentum.

TeO is the most complex stratified structure in the brain of zebrafish. The rhombencephalon is often divided into a rostral metencephalon and a caudal myelencephalon. With the exception of the cerebellum, the rest of the ventral part of the metencephalon can only be arbitrarily separated from the more caudal myelencephalic portion of the medulla oblongata. Medulla oblongata and tegmentum are considered to make the brain stem.

The first spinal roots, dorsal and ventral, are located caudally from Haller's very small commissure. The second dorsal root is located caudally at 500-800 mm. Currently, the use of this animal model is augmenting considerably in the research of neurological and neurodegenerative diseases in humans, due to its notable easier husbandry and breeding, but more important, genetic similarity with human.

Keywords: Zebrafish (Danio rerio), central nervous system, histology

Introduction

Zebrafish (Danio rerio) is currently one of the most popular animal models in the world of science because of the many advantages and qualities it has shown over the last 40 years of research. This small fish is native to South Asia and is found in freshwater sources.

The first to perform innovative studies using zebrafish as an animal model were George Streisinger and Charles Kimmel in the early 1970s and so far zebrafish have significantly contributed to understanding the development of the nervous system and the regulation at genetic level in at least three areas: differentiation of the first neurons, pathways and associated synapses in the zebrafish embryonic brain; description of neuromeres and expression of regulatory genes in the embryo; generation of mutations through saturation mutagenesis [1]-[9].

The zebrafish are vertebrates with a hard skeleton (Teleostei) and, therefore, have a high degree of sequential and functional homology with mammals, including humans. Due to the conservation of cellular biological processes and developmental processes in all vertebrates, studies on these fish can provide important insights into human disease processes. For example, so far, all the proteins studied have a similar function in fish and mammals [10]-[13].

The zebrafish shows the basic organization of the nervous system like other vertebrates.

Many neural circuits and cell types relevant to the study of human diseases are conserved in the central nervous system (CNS) of the zebrafish. In addition, many genes involved in human neurological disorders have highly conserved orthologues in the zebrafish genome, suggesting that the biochemical events underlying the pathogenesis could be reiterated in the zebrafish nervous system [9], [11], [12].

Materials and Methods

20 adult zebrafish taken in study were euthanized by immersion in cold water (4°C). The fixation was performed with 10% neutral formalin and Bouin solutions. Cross or longitudinal (sagittal or coronal plane) sections through the fish were performed. Samples dehydration was performed by usual method with alcohol series and cleared with xylene. Paraffin cubes were prepared and cut in slices of 5 μm by microtome.

For histological examination, sections were stained with hematoxylin-eosin and examined in light microscopy. The main areas of the brain were evaluated and most significant parts were illustrated (fig. 1).

Results and Discussions

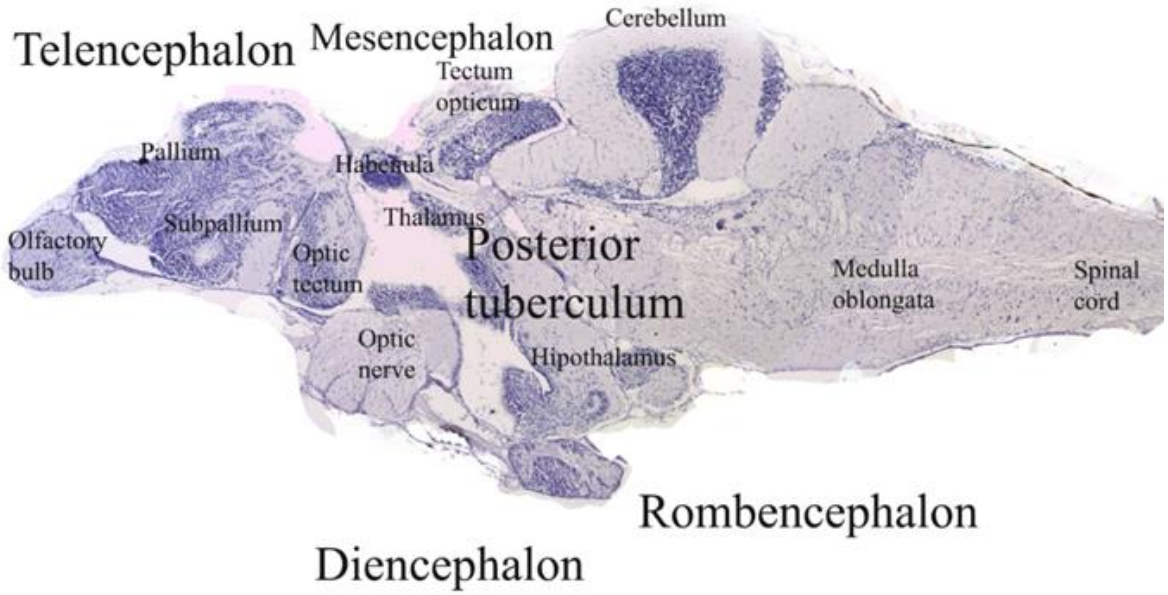

Fig. 1. Sagittal section of the brain in adult zebrafish with the main regions and areas, H&E, X40

I. Telencephalon

In teleost fish, the topography of the telencephalon (Tel) is strongly distorted. In the rest of the vertebrate groups, the telencephalic hemispheres develop by bilateral evagination and by thickening of the most rostral embryonic neural tube, each hemisphere containing a lateral diverticulum of the central ventricle. In teleosts (including zebrafish), the dorsal plate of the embryonic telencephalon extends laterally and, as a result, the pair of wing plates forming the hemispheres' walls extends latero-ventrally in a process called inversion. Thus, it is very

difficult to make correlations between the topography of adult telencephalon and the homologous structures of other vertebrates, although considerable progress has recently been made [14].

The most rostral telencephalic divisions are actually the pair of olfactory bulbs. Primary olfactory fibers that enter the olfactory bulbs are axons of olfactory receptors and, by definition, do not form part of the CNS. The rest of the telencephalon is composed of two subdivisions: the dorsalis area and the ventralis telencephali area.

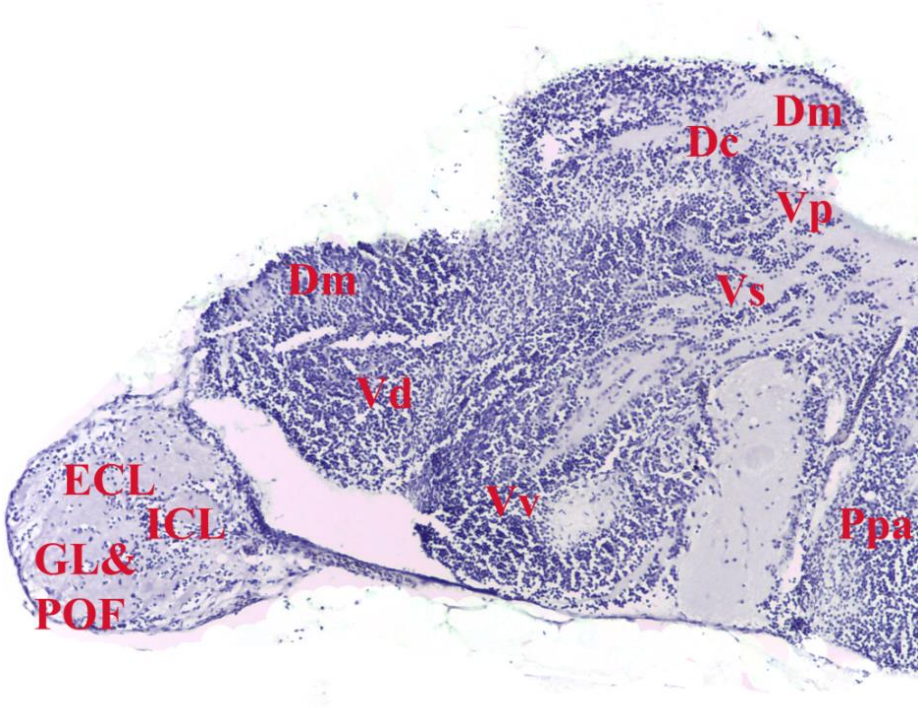

Fig. 2. Sagittal section of telencephalon in adult zebrafish. Dc – central zone of dorsal telencephalic area; Dm-medial zone of dorsal telencephalic area; ECL – external cellular layer of olfactory bulb including mitral cells; ICl – internal cellular layer of olfactory bulb; GL – glomerular layer of olfactory bulb; POF – primary olfactory fiber layer; PPa – parvocellular preoptic nucleus, anterior part; Vd – dorsal nucleus of ventral telencephalic area; Vp – postcommissural nucleus of ventral telencephalic area; Vs – supracommissural nucleus of ventral telencephalic area; Vv – ventral nucleus of ventral telencephalic area

II. Diencephalon

The diencephalon comprises a total of 5 major regions that are organized dorso-ventrally in the adult brain. These are: epitalamus, dorsal thalamus, ventral thalamus, posterior tubercle and hypothalamus.

The praeoptic area, although usually considered to be part of the hypothalamus, is in this case an intermediate area between telencephalon and diencephalon. It surrounds the most rostral diencephalic ventricle and can be divided into a ventral series of parvocellular preoptic nuclei and, respectively, a dorsal series of magnocellular preoptic nuclei [15].

The epithalamus is composed of a dorsal nucleus and a ventral nucleus of the habenula and two dorsal projections, epiphysis and saccus dorsalis. The epiphysis is a light-sensitive endocrine organ.

The dorsal thalamus extends below the caudal portion of the ventral habenular nucleus and is composed of the anterior nucleus and two more caudal nuclei: the dorsal posterior thalamic nucleus and the central posterior thalamic nucleus.

The ventral thalamus is located ventral to the dorsal thalamus, but only at its caudal level and consists of: an intermediate nucleus, a ventromedial nucleus and a ventrolateral one.

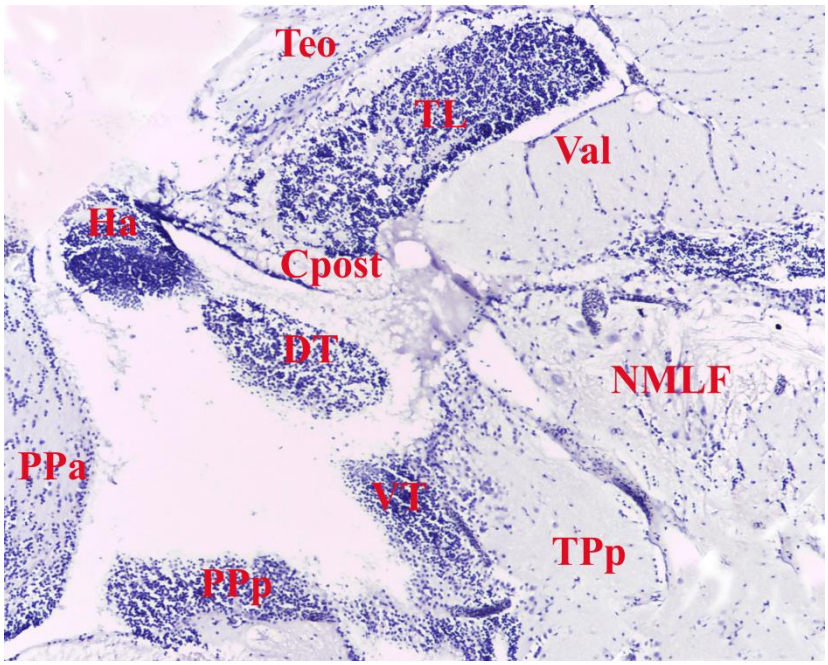

Fig. 3. Sagittal section of diencephalon and mesencephalon in adult zebrafish. Cpost – commissura posterior; DT – dorsal thalamus; Ha- habenula; NMLF – nucleus of medial longitudinal fascicle; PPa – parvocellular preoptic nucleus, anterior part; PPp – parvocellular preoptic nucleus, posterior part; TeO – tectum opticum; TL – torus longitudinalis; TPp – periventricular nucleus of posterior tuberculum; Val – lateral division of valvula cerebelli; VT – ventral thalamus

The zebrafish have a posterior tubercle much larger than the dorsal and ventral thalamus. Its periventricular part is composed of two nuclei, both located between the ventral thalamus and the hypothalamus. The periventricular part also contains a paraventricular organ [14].

The hypothalamus of the zebrafish is the largest diencephalic area and includes ventral, dorsal and caudal areas. While the ventral and caudal areas represent most of the medial tubular portion of the hypothalamus, the dorsal area extends laterally and includes the pair of lower lobes of the hypothalamus.

The pituitary gland is attached ventrally to the ventral and caudal hypothalamic areas.

Sinencephalon is defined as a series of intermediate structures between the dorsal and mesencephal diencephalon. These structures are located near the posterior commissure and include the medial longitudinal bundle nucleus, the periventricular protectum, the paracomisural nucleus, and the sub-commissure organ. A periventricular pretectum, central and superficial pretectum are observed [14].

III. Mesencephalon

The mesencephalon includes dorsal and ventral optic tectum (TeO), torus semicircularis and tegmentum.

TeO is the most complex stratified structure in the brain of zebrafish. It consists of 4 zones: periventricular gray zone, deep white zone, central zone and superficial gray and white zone, which can be further subdivided into another 15 layers. Unlike the other vertebrates, the most superficial tectal layer in the teleosts does not contain retinal fibers. This marginal layer is composed of axons whose pericarions are located in torus longitudinalis (TL). The latter is composed of a longitudinal eminence of granular cells attached to the tectum [14].

The ascending octavolateralis system terminate in the mesencephalon in the sensory torus semicircularis (TS), located above the lateral tegmentum, where it extends into the tectal ventricle. In the Cyprinidae the central nucleus is associated with hearing, whereas the ventrolateral nucleus corresponds to mechanoreception.

The ventral mesencephalon is separated from this upper area by the ventricle and forms the tegmentum. It plays a very important role in motor functions.

The tegmentum includes numerous motor structures such as oculomotor and trochlear nerve nuclei, the parasympathetic Edinger Westphal nucleus, the rubus nucleus, and the most rostral portion of the superior reticular formation. While the axons of the oculomotor nerve exit the ventral brain between the tegmentum and the lower lobe, the axons of the trochlear motor nucleus extend dorsally where they intersect in the cerebellum valve, move caudo-laterally, and leave the CNS in the form of the trochlear nerve between torus semicircularis and rombencephalon [14].

The tegmentum is rostrally adjacent to the sinencephalon, the dorsal thalamus and the posterior tubercle; ventral hypothalamus; dorsolateral of torus semicircularis. Caudal tegmentum is continued with the medulla oblongata without observable morphological differentiation. The interpeduncular nucleus and the trochlear nucleus are often considered to be the most caudal tegmental nuclei [15].

IV. Rombencephalon

The rhombencephalon is often divided into a rostral metencephalon and a caudal myelencephalon. With the exception of the cerebellum, the rest of the ventral part of the metencephalon can only be arbitrarily separated from the more caudal myelencephalic portion of the medulla oblongata. Medulla oblongata and tegmentum are considered to make the brain stem [15].

The cerebellum of the zebrafish is composed of 3 parts: the vestibulolateral lobe (which includes: the medial caudal lobe (LCA) and the pair of granular emineas (EG)), the corpus cerebelli and the cerebelli valve that has the medial (Vam) and lateral subdivisions (Val). The cerebellar commissure is located within the ventral border between the valve and the corpus cerebelli.

Although the valve extends into the tectal ventricle, its histology (presence of granular and molecular layer and aggregations of large Purkinje cells and Euridendroid cells) and caudal attachment to the rostral oblongata medulla prove the valve's belonging to the cerebellum.

While both the vestibulolateral lobe and the corpus cerebelli are homologous to other vertebrates, the cerebelli valve is present only in actinopterigens [14].

Medulla oblongata contains sensory and motor nuclei of the trigeminal, abducens, facial, VIII, IX and X. The posterior and anterior lateral nerves (ALLN/PLLN) are separated by other cranial nerves.

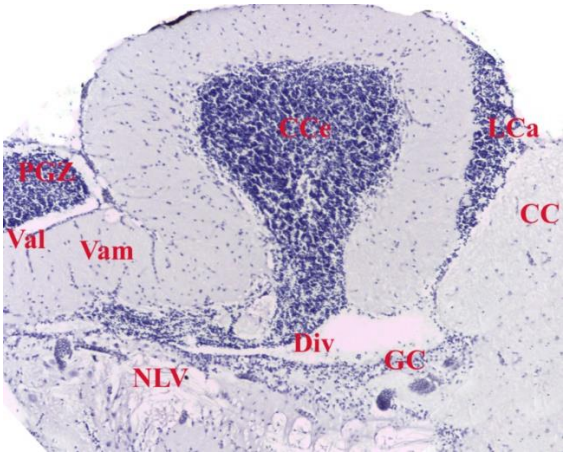

Fig. 4. Sagittal section of rombencephalon in adult zebrafish. CC – crista cerebellaris; CCe – corpus cerebelli; Div – diencephalic ventricle; GC – griseum centrale; LCa – lobus caudalis cerebelli; NLV – nucleus lateral is valvulae; PGZ – periventricular gray zone of optic tectum; Val – lateral division of valvula cerebelli; Vam – medial division of valvula cerebelli

There are two distinct trigeminal motor nuclei, one located dorsal to the lateral longitudinal bundle and the other located at the ventrolateral edge of the same bundle, and four trigeminal sensory nuclei. Pyriform neurons of the mesencephalic nucleus of the trigeminal nerve contain sensory fibers that extend peripherally to the trigeminal nerve.

The abducens nerve is composed of two separate populations of motor neurons. The rostral motor nucleus and its root are at the level of the upper lattice formation, and the caudal motor nucleus and its root respectively are located at the level of the intermediate lattice formation.

At the exit of the brain root, these neural roots join and continue rostrally.

The sensory root of the facial nerve enters the brain root along with the nerves of the anterior lateral line. The facial sensory root initially extends to the median of the cerebral root, from where it extends caudally to the facial lobe [14].

Ventral to the facial sensory root and dorsal to the ventral rhombencephalic commissure and the intermediate reticular formation, is found the facial motor nucleus. The nerve VIII enters the brain of the zebrafish through an extended rostrocaudal region. From that start connections to 5 primary sensory nuclei: the anterior, magnocellular, descending and posterior nuclei, plus the tangential nucleus. Large neurons of the tangential nucleus are located at the periphery of the cerebral root, immediately ventral to the entrance of the anterior portion of the nerve VIII.

The smaller neurons are located more caudally and are part of the descending octave nucleus, which is by far the largest octave system. It extends not only ventrally and dorsally around the octave root, but also very medially [15].

V. Medulla spinalis

The first spinal roots, dorsal and ventral, are located caudally from Haller's very small commissure. The second dorsal root is located caudally at 500-800 mm [14].

At the level of the second secondary dorsal root, the gray dorsal and ventral horns are clearly visible around the central canal. The longitudinal tracts were rearranged substantially compared to their position in the brain root. They are located in the white peripheral substance. The white matter of the spinal cord can be divided into funiculi: dorsal, lateral (this in turn being composed of one ventral and one lateral), and ventral, as in mammalian neuroanatomy.

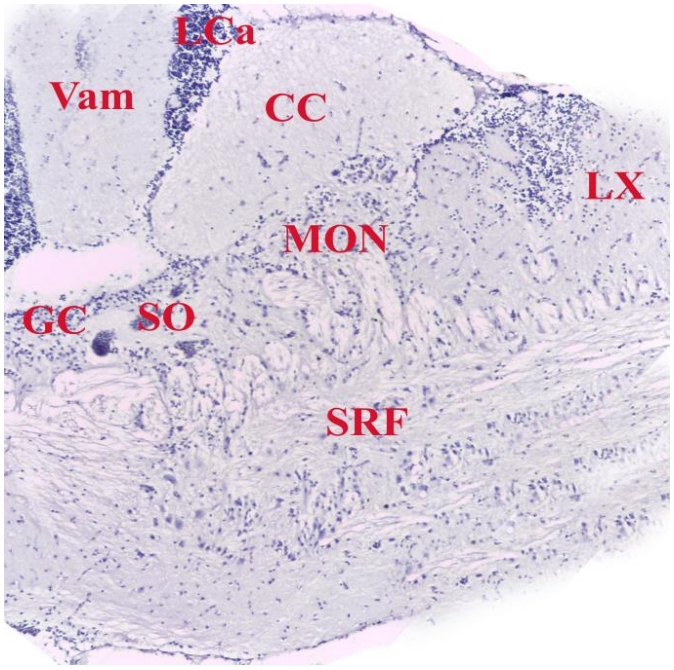

Fig. 5. Sagittal section of medulla oblongata in adult zebrafish. CC – crista cerebellaris; GC – griseum centrale; LCa – lobus caudalis cerebelli; LX – vagal lobe; SO – secondary octaval population; SRF – superior reticular formation; MON – medial octavolateralis nucleus; Vam – medial division of valvula cerebelli.

Conclusions

The study of zebrafish neuroanatomy is of interest due to its use in numerous researches, particularly on neurodegenerative diseases. Rodent models have been used extensively to investigate the cause and mechanisms underlying Alzheimers disease. Despite many years of intensive research in which these models have been used, a complete understanding of the events leading to neurodegeneration is still lacking. Although zebrafish does not have the complexity of advanced cognitive behaviours evident in rodent models, it has proven to be a very useful model for studying human diseases because zebrafish possess orthologous genes for those who have undergone mutations in familial Alzheimer's disease, and research using zebrafish has revealed the unique characteristics of these genes that have been difficult to detect in rodent models. Danio rerio is becoming an increasingly popular model for research in neurological diseases and will complete studies that use other models to help understand those diseases.

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Morpho Functional Features of the Kidney in Zebrafish (Danio Rerio)

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Abstract

The study focused on the histological structure of the kidney in zebrafish. It consists of 3 main regions: the cranial, the central and the caudal region. The morphofunctional unit is the nephron formed by the Malpighi corpuscle, a proximal and a distal convoluted tubule and two collecting ducts. Unlike mammals, in fish the nephron has the ability to regenerate after contact with nephrotoxic substances. In fish the kidney is also a major hematopoietic organ being the functional counterpart of the hematogenous bone marrow in mammals. The proliferation of hematopoietic cells was evidenced with anti-PCNA antibody, showing the highest positivity in the cranial region.

Keywords: Zebrafish (Danio rerio), kidney, immunohistochemistry, hematopoiesis

Introduction

Zebrafish (*Danio rerio*) is a teleostean freshwater fish widespread in tropical and subtropical areas of South Asia, including India, Nepal, Bangladesh and North Burma [1]. Zebrafish are known worldwide as model animal for the study of cell biology, physiology, genetics, neuroscience, toxicology etc. In vivo studies using zebrafish have some advantages over those using mice. Thus, zebrafish produce large numbers of small embryos, allowing drug screening and functional analysis of specific genes. They have a short lifespan (42 to 66 months) [2] and develop rapidly, requiring 90 days to develop in adults [2], shortening the periods required for experiments. Their embryos are transparent and grow outside the uterus, allowing researchers to visualize their development and genetically manipulate them. Finally, many functions of zebrafish genes are conserved in mice and humans, allowing researchers to transpose the results obtained in zebrafish studies into mammalian contexts. At present, through large-scale mutagenesis, have been established several models of human diseases in zebrafish, which allows the development of new therapies.

Kidney diseases continue to be an important medical problem. Because zebrafish are known for their capacity for renal regeneration, understanding the mechanisms behind these processes can be an important step for regenerative medicine in humans.

Another major role of the kidney is that of a definitive hematopoietic organ, homologous to the hematogenous bone marrow in mammals. Like the generation of other types of blood cells, zebrafish erythropoiesis occurs in the mesoderm and is classified into two sequential stages: early and definitive. The early, embryonic stage generates erythrocytes and macrophages, and ultimately the hematopoietic stem cells are produced, which can be differentiated in each blood cell type (erythrocytes, granulocytes, lymphocytes and platelets) that maintain homeostasis throughout the life.

During development, the vertebrate species possess a series of up to 3 renal structures that appear sequentially: the pronefros, the mesonephros and the metanephros [2]. Nephron serves as a basic structural and functional unit after successive degeneration of the intermediate stages developed during the fetal period [3]. The inferior vertebrates, amphibians and fish, develop the functional embryonic pronephros, followed by mesonephros which serves as an adult organ [4]-[7].

The use of adult zebrafish in experimental studies is valuable because it allows the examination of hundreds of nephrons (about 300-500 depending on the age of the fish), compared to the 2 nephrons found in embryos.

Materials and Methods

10 fish were euthanised by imersion in cold water (4°C). Every fish had the abdominal wall sectioned from the anus to the heart and has been subjected to the usual fixation, dehydration and embedding techniques. Sections were cut at 5 μm each with the microtome. 5 microscope slides from each paraffin block were stained using the standard hematoxilin-eosin protocol and examined under light microscope Olympus CX41. We performed also an immunohistochemical staining using anti-PCNA antibody (Gene Tex, Inc.). Sections were dewaxed and microwaved for 10 minutes at 95^0C in 10 mmol citrate acid buffer pH6, left 20 minutes to cool, washed twice in PBS for 5 minutes and incubated with primary antibodies, diluted 1:100, overnight at 4^0C in humid chamber. Next day slides were washed 3 times in PBS for 5 min and incubated with the secondary antibodies. The antigen-antibody complexes were visualised as brown using an avidin-biotin peroxidase complex solution from the Vectastain ABC Kit, Vector. Sections were developed with 3,3'-diaminobenzidine (DAB) and finally counterstained with hematoxylin.

Results and Discussions

The kidney in zebrafish presents the cranial, central and caudal area. The cranial area of the kidney (fig. 1) is characterized by a hematopoietic tissue that predominates among the urinary tubules. Malpighi corpuscles are very rare. In the central area of the kidney, Malpighi corpuscles, urinary tubules and hematopoietic tissue are all present. In the caudal area frequently appear urinary tubules, rare Malpighi corpuscles and peritubular hematopoietic tissue.

The morphostructural unit of the kidney is the nephron. It consists of the Malpighi corpuscle and urinary tubule. The first segment of the nephron, the Malpighi corpuscle, is a true blood filter. Centrally, the glomerulus consists of capillaries containing circulating blood, a space in which the filtrate is collected, known as the capsular space and the Bowman capsule, which continues with the proximal convoluted tubule. One of the types of cells that make up the glomerulus are the podocytes, which form a complex network with a sieve role and specialized extensions known as pedicles that wrap around the capillaries. The proximal convoluted tubule is lined by a prismatic epithelium with a prominent brush border, often with dilated lumen. The cells of the distal convoluted tubule are cubic, lighter, without brush border. Two major collecting ducts cross the length of the kidneys and drain hundreds of nephrons.

Expression of proliferative cellular nuclear antigen (PCNA) in zebrafish circumscribes the proliferative hematopoietic component. The most intense expression was found in the cranial region (fig. 2), and decreases progressively in the other areas, medial (fig. 3) and caudal (fig. 4).

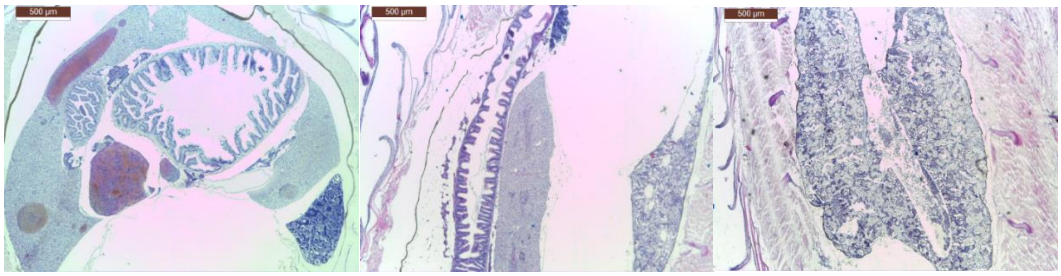

Fig. 1. The three areas of the kidney in zebrafish: cranial, central and caudal. IHC staining. Negative control x40 (a, b) < x100 (c)

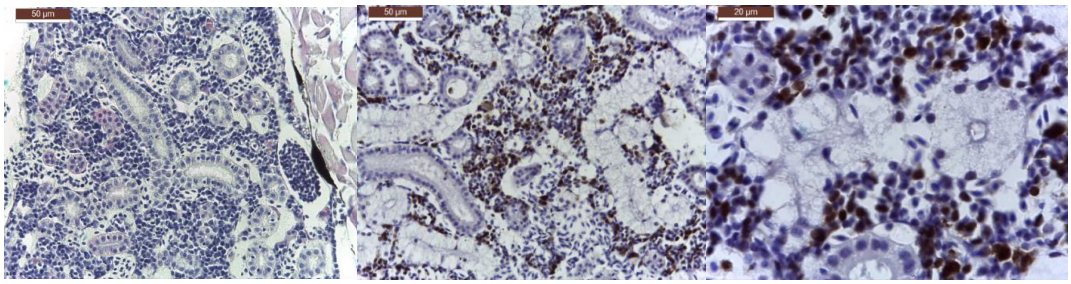

Fig. 2. Cranial area of the kidney. Hematopoietic tissue predominates among urinary tubules. A. negative control x400; b. IHC staining with PCNA x400; c) x900

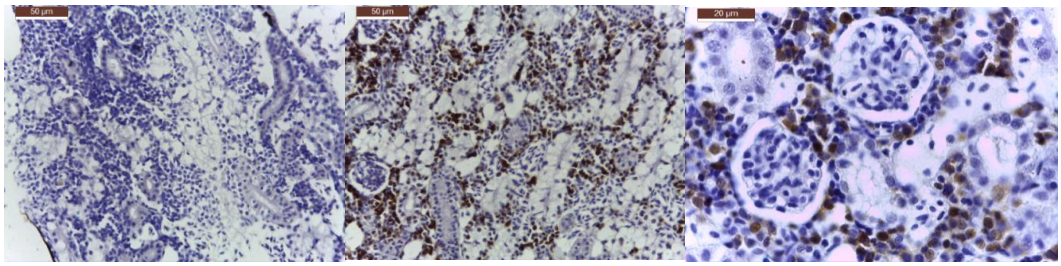

Fig. 3. Central area of the kidney. Hematopoietic tissue between the urinary tubules and Malpighi corpuscles. A. negative control x400; b. IHC with PCNA x400; c) x900

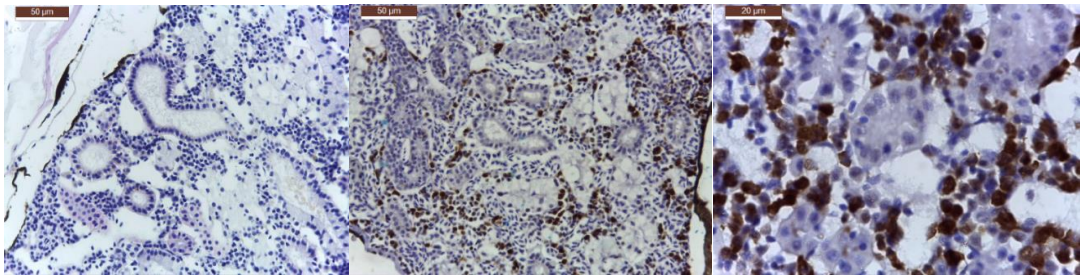

Fig. 4. Caudal area of the kidney. Hematopoietic tissue between the urinary tubules and rare Malpighi corpuscles. A. negative control x400; b. IHC with PCNA x400; c) x900

Discussions

The mesonephric kidney of adult zebrafish is a unique, flattened structure, which is attached to the body wall by connective tissue. Anatomically, the kidney is made up of 3 main parts: cranial, body or the so-called "*saddle*" and caudal region. Mesonephros-type nephrons are similar to those found in the embryonic kidney; however, adults are strongly bifurcated and drained by 2 collecting ducts [8]-[10]. As the zebrafish become older, new nephrons, which appear from the renal progenitors, are added. It is believed to be interspersed in the interstitial stroma between the existing nephrons [9], [10]. This neo-nephrogenesis process can be induced by renal aggression. An initial phase of the lesion is described, of cell death and in which the

denudation of the basal membrane occurs in the proximal convoluted tubule. Subsequently, the brush border flattens and loses, followed by a repopulation of the basement membrane.

Complete restoration of the proximal convoluted tubule is done in 2 weeks. Groups of cells (which have been named nephrogenic aggregates) appear and grow and elongate in a process that recapitulates mesonephric nephrogenesis [9], [10].

After exposure to nephrotoxins, goldfish exhibit tubular necrosis with luminal debris.

Subsequently, new nephrons have been identified as coming from groups of basophilic cells that grow, form lumen, and finally, eosinophilic tubules appear, very similar with fully mature nephrons [11].

In zebrafish, new nephrons firstly appear as groups of renal progenitors near existing mesonephron tubules [9], [10]. These groups express early markers of renal development, such as lhx1a, pax2a, wt1b and pax8, suggesting that their presence reflects the regeneration process [12]. These groups then expand into S-shaped cell bodies, which mature into nephrons that fuse with pre-existing nephrons [9], [10], [12].

Based on parallel studies in mammals, intratubular cells are likely to renew damaged regions, however, the cellular mechanism remains controversial [12]. In mammalian nephron lesions, they have limited potential for self-repair [13].

After the death of a nephron, fibrosis appears which can cause a chain reaction that causes partial degeneration and eventually decreases organ function [1], [14]. The expression of proliferative cellular nuclear antigen (PCNA) in zebrafish was investigated to delineate the proliferative hematopoietic component in adults.

The renal tubules in adult fish are surrounded by endothelial cells and are thus separated into hematopoietic and excretory compartments. PCNA was expressed in hematopoietic progenitor cells, but not in neutrophils, eosinophils or erythroid cells. Some PCNA positive cells are spread in the hematopoietic compartment of the kidney, while others are closely associated with renal tubular cells. Therefore, with its role in cell proliferation and DNA synthesis, PCNA can be used to mark cell proliferation in zebrafish hematopoietic tissues and to identify a population of progenitors whose significance should be further investigated [15].

Despite the clear similarities between zebrafish and mammalian blood cell development, definitive hematopoiesis occurs in a stromal compartment of the kidneys in adult zebrafish [15], meanwhile in the mouse and human take place in the hematogenous bone marrow. However, it is likely that the hematopoietic strain and progenitor cells have retained the function and underlying molecular pathways responsible for the continuous production of blood cells in adult fish.

Hematopoietic cell lines can usually be identified in mice and humans, using antibodies against cell surface epitopes. However, this method has not been widely applied to zebrafish due to the lack of specific antibodies. Rather, researchers have developed a wide range of fluorescent, transgenic, zebrafish lines that label certain types of blood cells [18]-[22]. Despite the great utility of fluorescent lines for dynamic cell imaging, in vivo, these approaches often fail to identify the heterogeneity of blood cell types.

Conclusions

The kidneys in zebrafish are made up of three areas: cranial, medial and caudal region.

Malpighi corpuscles predominate in the central area, the urinary tubules have been highlighted throughout the organ. The urinary tubules are surrounded by a simple prismatic epithelium that delimits the urinary segments from the hematopoietic one.

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Biocompatibility of Some Titanium Based Alloys in Bone Tissue: An Experimental Study on Pigs

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Abstract

The aim of this study was to investigate and assess by classical histological staining methods and examination, bone cell response and bone healing process to some titanium alloys inserted into the tibia of 18 pigs. The evaluation was performed at 4 weeks post-implantation, on 18 pigs divided into 4 groups: LM-control group (3 pigs) and 3 experimental groups (5 pigs/group): LE1 (Zr45Ti), LE2 (Zr5Ti), LE3 (Zr25Ti).

Special attention was drawn to describe and define by the use of general stain methods like Vankossa, hematoxylin and eosin and Movat's pentachrome, the process of cell proliferation, new bone formation on peri-implant areas and osseointegration in all 4 groups. Without relevant reactivity differences between the groups, histological results showed a good biocompatibility in all experimental groups. LE1 (Zr45Ti) group showed an enhanced peri-implant bone formation process and a higher number of forming osteons compared to the same peri-implant areas of LE2 and LE3 experimental groups.

Keywords: ossification, histology, biocompatibility

Introduction

Bone healing is a complex biological process that takes place according to a specific regeneration pattern.

There can be a direct healing pattern – *primary bone healing* or *intramembranous osteogenesis* and an indirect healing pattern – *secondary bone osteogenesis*, which involves both intramembranous and enchondral bone formation, characterized by periosteum and surrounding soft tissue response.

Indirect bone formation is the most common form of bone healing, encountered in large bone defects and especially after an implantation procedure.

Clinical studies indicate that when an implant is placed into the bone tissue, the periosteum reacts by cell proliferation, which sustains and increases bone formation in the first post-implantation days, thus ensuring initial implant stabilization [1].

Materials and Methods

The animals were divided into 4 lots - 1 control group (3 pigs/group) and 3 experimental groups (5 pigs/group): LM, LE1 (Zr45Ti), LE2 (Zr5Ti), LE3 (Zr25Ti).

All animals were anesthetized, and each one received a titanium-based alloy implant on each tibia. The implant placement on each animal was the tibial crest. At 4 weeks post-implantation, all animals were euthanized and the implants and peri-implant bone tissue were harvested and processed for histological analyses.

The tibia-implant blocks were fixed in buffered formalin 10%, and subsequently decalcified with EDTA sol 15% for 3 weeks. The tibia-implant blocks were subsequently included in paraffin, sectioned and stained with H&E, PAS, Movat's pentachrome and VanKossa methods.

Histological analyses were carried out using a Leica microscope.

Results

Slides from control group, illustrated a thickened periosteum with mesenchymal stem cells that will undergo differentiation into osteoblasts involved in both synthesis of collagen fibers and osteoid (bone matrix proteins), and matrix mineralization.

The mineralized matrix contains a number of gaps called lacunae, which house an osteocyte involved in bone metabolism.

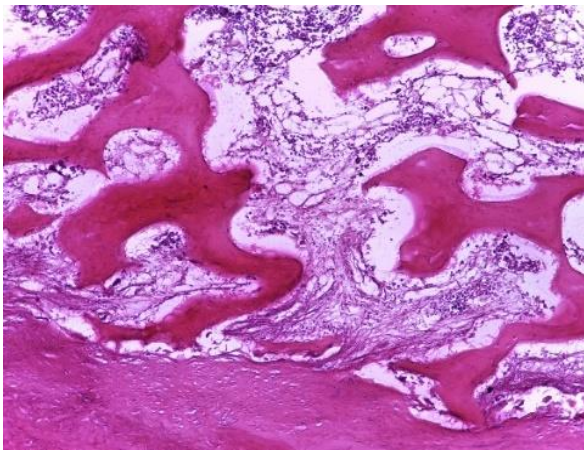

Fig. 1a. Control group (LM). Periosteum and different stages of bone formation. H&E x40.

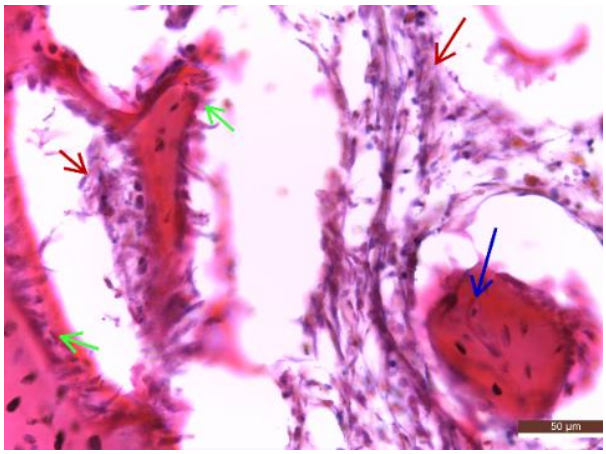

Fig. 1b. Mesenchymal stem cells (red arrow) and active osteoblast cells (green arrow) lined up along the surface of small, irregular shaped forming bone island/trabeculae (blue arrow). H&E x400

Osteoblasts are active secretory cells, with prismatic or cuboidal shape that can be identified as 1-2 rows of cell aggregation on the surface of the forming bone.

About 2mm from newly formed bone, can be identified irregular shaped large bone cavities with numerous bone marrow cells and adipocyte. Newly formed bone trabeculae are characterized by mineralization gradient consisted by osteoid and mineralized bone matrix. (Fig. 1c)

13 798 2034. LE1 (Zr45Ti) experimental group

Histological images illustrated a thickened periosteum with increased mesenchymal stem cells densities, which are migrating into bone cavities. Some of these mesenchymal cells synthesize the periosteal extracellular matrix.

These mesenchymal stem cells will undergo differentiation into osteoblasts lineage, which can be identified on newly formed bone trabeculae surface. In periosteum proximity, a thin layer of spongy bone is forming followed by small osteons consisting of 2-6 osseous lamellae arranged concentrically around a Haversian canal.

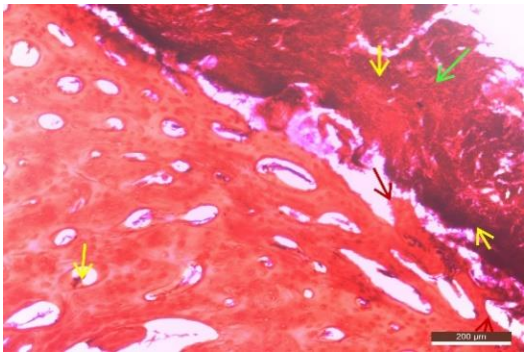

Fig. 2a. Experimental LE1 group. Thickened periosteum, spongy bone in periosteum proximity. About 500-1000µm from spongy bone area, there can be observed small osteons with 2-6 concentric lamellae. The Haversian canals contain blood vessels, nerve, and connective tissue. Movat x40

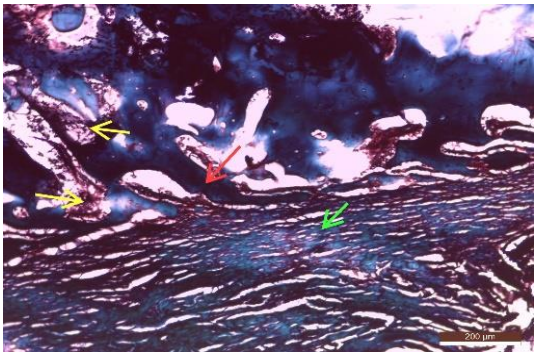

Fig 2b. Experimental LE1 group. Thickened periosteum, extracellular matrix (green arrow), spongy bone tissue in periosteum proximity (red arrow). Mesenchymal stem cells in bone cavities and osteoblasts cells attached to bone spicule surface (yellow arrow). Pentachrome Movat stain modified with Alsacian blue x40.

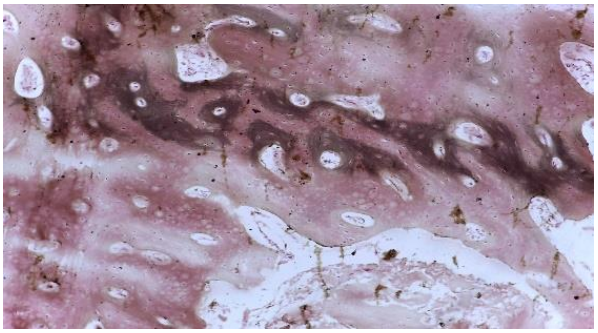

Fig. 2c. Cancelous and compact bone tissue from periosteum proximity. Mineralized bone lamellae are coloured in dark brown. Van Kossa x40

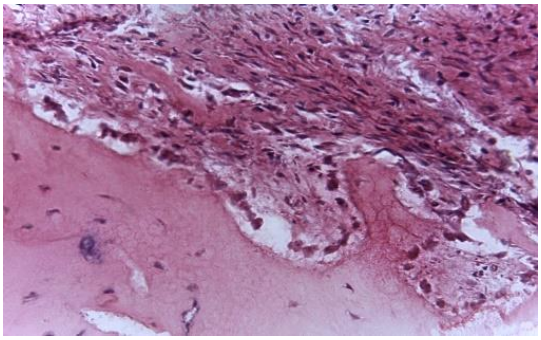

Fig. 2d. Periosteal mesenchymal stem cells proliferation and mesenchymal cells differentiated into osteoblast attached to osseous lamellae. H&E x100

In LE1 implant proximity, there have been observed thin forming bone spicules and a large number of mesenchymal stem cells. The peri-implant spicules are initially characterized by a partially mineralized matrix and a smaller diameter who is progressively incresing size as getting distance.

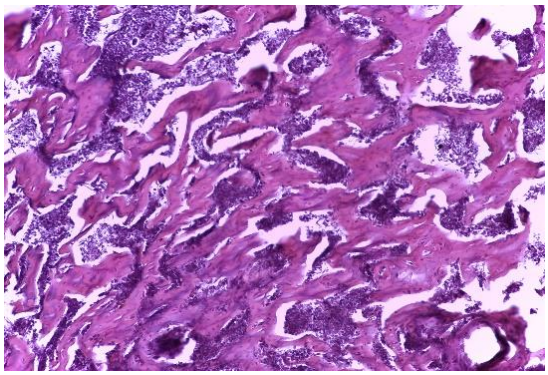

Fig. 2e. Peri-implant thin bone trabeculae that progressively increase in diameter as getting distance and a large number of mesenchymal stem cells. H&E x100.

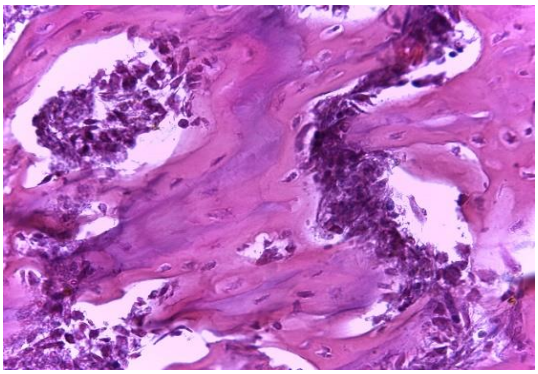

Fig. 2f. Implant proximity: thin bone newly forming trabeculae and large number of mesenchymal stem cells. The thicker bone spicules are made of spongy bone tissue. Unineralized bone – light pink colour, mineralized bone-dark pink colour. H&E x400

13797 2046. LE2 (Zr5Ti) experimental group

Histological illustrations show a thickened periosteum with numerous mesenchymal stem cells that are also found in the neighbouring spongy bone tissues cavities, some of these differentiated into extracellular matrix secretory osteoblasts (Fig. 3a). The peri-implant area has a particular histological feature: hypertrophic hyaline cartilaginous tissue, followed by spongy and lamelar bone tissue (Fig. 3b and Fig. 3c). The compact bone tissue is structured in small osteons formed by 2-3 osseous lamellae arranged concentrically around a Haversain/osteonic canal (Fig. 3d)

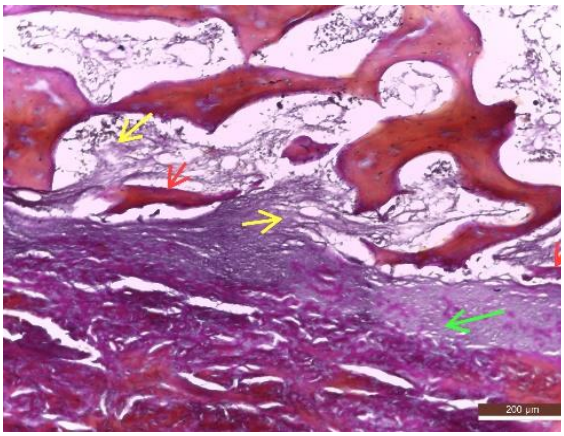

Fig. 3a. Modified periosteal area. Bone cavities with mesenchymal stem cells differentiated into secretory osteoblast. H&E x100.

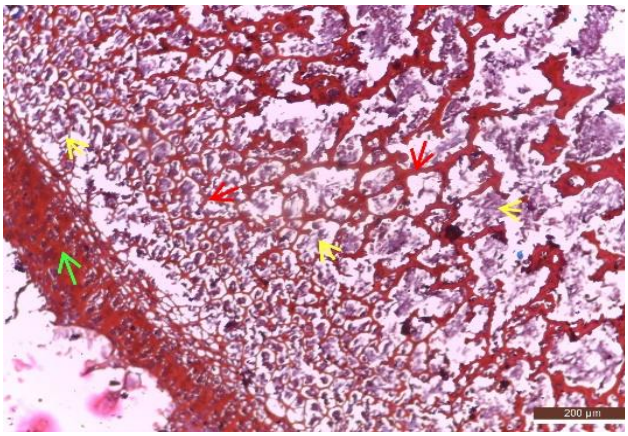

Fig. 3b. Peri-implant area. Cartilaginous tissue (green arrow), spongy bone tissue (red arrow), compact bone tissue, mesenchymal stem cells (yellow arrow) in bone cavities. Movat x40

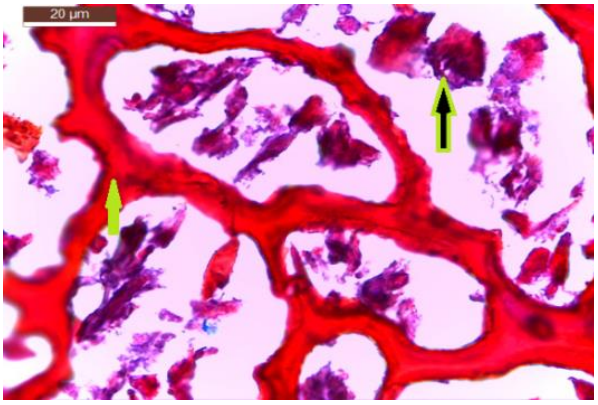

Fig. 3c. Cancelous bone tissue in implant proximity. Thin bone spicule (green arrow) and bone cavities with differentiated mesenchymal stem cells (black arrow). Movat x1000

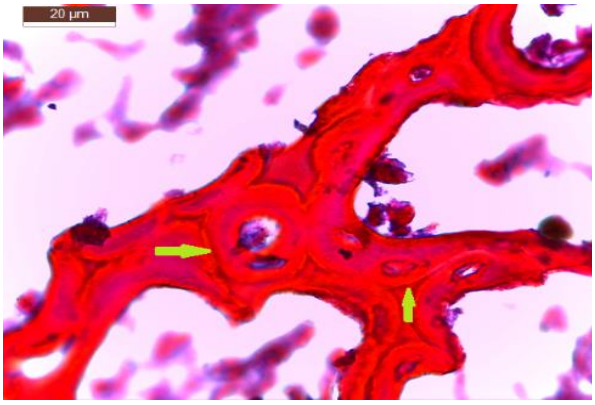

Fig. 3d. Compact bone tissue in implant proximity. Forming osteons with 2-3 concentric lamellae (green arrow). Movat x1000

2039. 13814. LE3 (Zr25Ti) experimental group

LE3 experimental group shows a much-thickened periosteum with numerous proliferated mesenchymal stem cells that are migrating in the adjacent spongy bone tissue cavities (Fig. 4a, Fig. 4b).

The peri-implant area is characterized by a small band of hyaline cartilaginous tissue followed by thin bones trabeculae. Mesenchymal stem cells are noticed in spongy bone cavities (Fig. 4c).

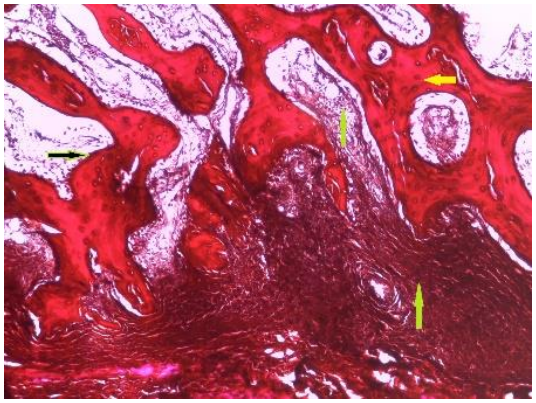

Fig. 4a. Thickened periosteum and incresed mesenchymal stem cell proliferation. Mesenchymal cells in bone tissue cavities (green arrow). Pentachrome Movat stain modified with Alsacian blue x40.

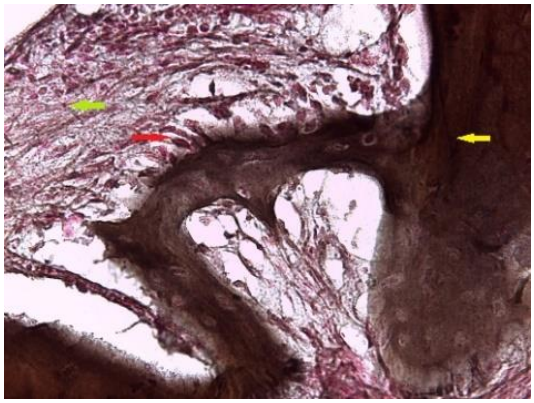

Fig. 4b. Mesenchymal stem cells in bone areolas (green arrow), active secretory osteoblasts (red arrow), bone trabeculae (yellow arrow).

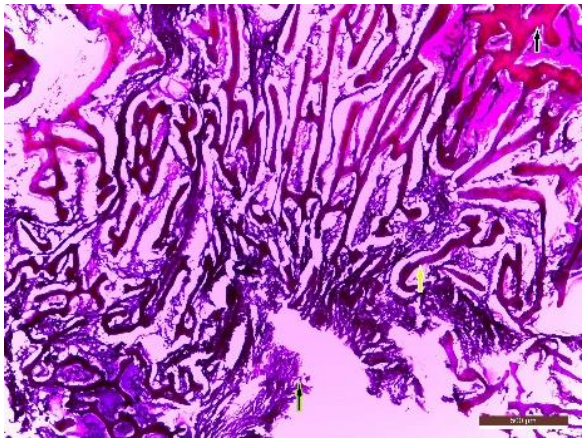

Fig. 4c. Thin cartilaginous tissue (green arrow) followed by thin trabeculae of spongy bone (yellow arrow) and lamellar bone tissue with 1-2 osseous lamellae/osteon (black arrow). Pentachrome Movat stain modified with Alsacian blue x40.

Discussions

Periosteal cellular reactivity persisted after 4 weeks post-implantation, suggested by the proliferation of mesenchymal stem cells, observed in all experimental groups from this study. Most of the periosteum proliferating cells differentiate into chondrocytes, which will subsequently be removed and replaced by bone tissue through the process of enchondral ossification. Some authors assume that the hypoxia caused by the interruption of the vascularization by any periosteum lesion (in this case implantation surgical act) favours the differentiation of mesenchymal osteochondroprogenitor cells into the chondrogenic lineage [2], [3]. Others place the periosteal chondrogenic response on account of the modification of the mechanical tension through the implantation surgical act [4]-[6].

Another explanation regarding the presence of cartilaginous cells in periosteum is that the dead osteocytes in the implant surrounding area block the nutrients and oxygen supply for osteoblasts, and as a consequence the periosteum cells differentiate into chondrocytes.

At 4 weeks post-implantation, no cartilaginous tissue was detected in the periosteum, only extracellular matrix in LE1 and LE2 experimental groups.

Secondary bone healing can be described in 4 overlapping stages: Stage I – Initial inflammatory response: includes local hematoma formation, inflammation and recruitment of mesenchymal stem cells (MSCs). This stage involves a release of a wide range of pro-

inflammatory cytokines, growth factors, differentiation factors, and chemotactic mediators [7], [8].

Some of the most important inflammatory mediators with an important contribution to bone healing process are IL-1 and IL-6. IL-1 is secreted by macrophages and induces IL-6 production in osteoblasts, promotes primary cartilaginous callus formation and angiogenesis at the site of bone injury, by activating either of its two receptors: IL-1RI. or IL-1RII [9], [10], [11]. IL-6 stimulates angiogenesis, vascular endothelial growth factor (VEGF) production, and differentiation of osteoblasts and osteoclasts [12].

Mesenchymal stem cells recruitment, proliferation and differentiation into osteogenic lineage are three essential steps for bone regeneration as most data indicates and as it was also highlighted by the histological analysis of this study. Even though it is not fully understood exactly where these cells come from, most data indicate that these cells originate from bone marrow and surrounding soft tissues.

Stage II – soft callus formation: involves both intramembranous ossification and enchondral ossification processes, chondroclast, chondrocytes, osteoblast, osteocytes and osteoclast activity. Once soft callus formation is achieved, the cartilaginous callus is subject to a controlled hypertrophy and mineralization process. Cartilaginous degradation by chondroclast activity and chondrocyte apoptosis allows blood vessels in-growth and osteoblast differentiated mesenchymal cells migration at the repair site in order for woven bone synthesis to start.

Stage III – hard callus formation it is defined by woven bone transformation into mature lamellar bone.

The main mediating molecules of this stage are MCSF (macrophage colony stimulating factor), OPG (osteoprotegerin) and TNF-alfa, with a role in mineralized soft callus resorption and mesenchymal cell with osteogenic potential recruitment needed for woven bone synthesis and mineralization. Woven bone mineralization involves mitochondria activity that accumulates and release calcium-containing vesicles into the bony matrix in order to precipitate with phosphate and forming the initial mineral deposit.

Stage IV – bone remodelling: characterized by osteoblastic and osteoclastic activity, immature bone tissue resorption with mature cortical and trabecular bone tissue formation in order to assure and restore biomechanical properties of the normal bone [13].

Bone remodelling is a well-balanced process between hard callus resorption by osteoclast and a progressive replacement by lamellar bone synthesized by osteoblasts.

In LE2 experimental group, in the implant surrounding area it was identified a hypertrophic cartilaginous tissue area continued with cancellous and compact bone tissue. In LE3 experimental group, the cartilaginous tissue area was very thin and was followed by cancellous bone tissue with thin bone trabeculae and compact bone with small forming osteons. In LE1 experimental group, it could not be identified any cartilaginous tissue. Cancelous bone tissue was characterized by small osseous trabeculae continued with thickened trabeculae. No forming osteons were identified.

The presence of compact lamellar bone and mature osteocytes near the implant surface indicates a good biocompatibility. No signs of inflammation have been identified, thus concluding a good compatibility.

Conclusions

All 3 titanium-based alloys implanted in the tibial crest in this experimental study can be considerate biocompatible. In all experimental groups it was noticed an intense mesenchymal stem cells activation, proliferation, migration and differentiation.

LE2 implant demonstrated a good biocompatibility but a slower rate of cancellous and compact bone formation in the periosteum proximity and peri-implant area.

In LE3 experimental group we identified small hyaline cartilaginous tissue islands continued with thin cancellous bone tissue trabeculae and compact bone with small forming osteons.

In LE1 group, histological results indicate a superior bone formation inducing potential of the Zr45Ti alloy. Peri-implant cancellous bone tissue presented a more advanced bone consolidation process compared to other two groups as well as the periosteum proximity area which presented a higher number of forming osteons compared to the same area of the LE2 and LE3 implants.

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Systemic Mycobacteriosis Combined with Intestinal Tumours Induced by Heterakis Allinarum in a Golden Pheasant (Chrysolophus Pictus)

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Abstract

In pheasant's intestinal infections with *Heterakis spp.* are associated with a peculiar form of proliferative, nodular typhlitis, consisting of atypical nodular mesenchymal proliferation and rarely potentially metastasizing sarcomatous tumours. In this manuscript, we describe the pathological features of a rare case of systemic mycobacteria's in a golden pheasant associated with multiple cecal tumours induced by a *Heterakis gallinarum* infection. The parasitic-induced tumours consist of spindle-shaped cells which by immunohistochemistry show positive immunolabeling for vimentin and alpha-smooth muscle actin (α-SMA), supporting the diagnosis of intestinal multifocal leiomyoma.

Although the oncogenetic mechanism is poorly understood, chronic inflammation and a stromal atypically proliferative response of the intestinal mesenchymal cells are considered to be the key events in the development of the cecal tumours associated with *Heterakis spp.* infection in pheasants.

Keywords: Mycobacteriosis, nodular typhlitis, Heterakis spp, intestinal mesenchymal tumours

Introduction

Infection with *Heterakis gallinarum* and *Heterakis isolonche* in pheasants and quails is occasionally associated with cecal proliferative lesions that are manifested as exuberant granulomas or occasionally as intestinal tumours [1, 2].

There has been a long controversy regarding the nature of the nodular intestinal lesions caused by *Heterakis spp*, historically being diagnosed as nodular typhlitis or verrucose typhlitis, atypical nodular mesenchymal proliferation, parasitic granuloma or leiomyoma [1, 3].

Recently, additionally to the expected granulomatous enteritis and atypical nodular mesenchymal proliferation, *Heterakis spp* infection was associated in a ring-necked pheasant (*Phasianus colchicus*) with malignant mesenchymal neoplasia with pulmonary and hepatic metastasis [4].

The oncogenic mechanism of such parasite-induced intestinal tumours is largely unknown, nevertheless, chronic inflammation induced by long term intramural parasite persistence is generally accepted to be an important factor in transformation [2, 4].

Mycobacteriosis (avian tuberculosis) is a major infectious avian disease, affecting most of the companion, and domestic birds. Is usually produced by *Mycobacterium avium-intracellulare* and *Mycobacterium genavense* [5] although more than 10 other mycobacterial species haves been reported to produce infections in birds [6].

In avian mycobacteria's, lesions are typically located within the gastrointestinal tract, liver, spleen and bone marrow [7], although systemic forms are not rare.

Recently, there are reports that in humans indicating the fact that pulmonary tuberculosis is a major risk-factor for lung cancer [7, 8].

The mechanism of this process is considered to be most-likely associated with both immune-system modulations induced by chronic-tuberculosis, and disturbance of local antiproliferative mechanisms. In veterinary medicine, such a correlation between tuberculosis and increased risk for cancer is not yet reported.

In this article, we describe the gross and histological features of a rare case of disseminated mycobacteriosis in a golden pheasant combined with multifocal cecal tumours induced by a *Heterakis gallinarum* infection. We also discuss the hypothetical pathological connection between the mycobacterial/*Hetarakis* coinfection, highlighting the role of macrophage polarization in tuberculosis, parasitism and their involvement in oncogenesis.

Materials and Methods

Case presentation

A two-year-old male golden pheasant (Chrysolophus pictus) from a local zoo with a history of chronic weight loss and recurrent diarrhoea was submitted for complete necropsy to the Pathology Department of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.

Histopathology and Cytology

Following necropsy, all tissues were briefly fixed in 10% Neutral buffered formalin, followed by routine embedding into paraffin-wax using a routine histology technique. The paraffin-wax blocks were sectioned to 4-μm thickness by a rotary microtome, and finally stained by hematoxylin-eosin (H&E). Ziehl-Neelsen-stained smears were prepared during necropsy from the mycobacterial-suspected granulomas.

Morphologic assessment of the intestinal parasites

Adult Heterakis sp. worms were collected and clarified in lactophenol and identified based on morphometry and anterior and posterior characters according to Yazwinski and Tucker [9].

Immunohistochemistry

For immunohistochemical evaluation of the intestinal masses, a fully automated Leica Bond-Max immunostainer was used. Following a 20 minutes heat induced antigen retrieval, the immunohistochemical labelling was provided for vimentin using a rabbit polyclonal antibody (Abcam, ab45939) in a 1:50 dilution.

For the assessment of α-SMA a mouse monoclonal antibody (clone 1A4) (Abcam, ab76549) was used. Internal controls were used and both antibodies were validated by the immunolabeling of normal tissues from the same bird.

Microscopical images were taken with Olympus SP 350 digital camera mounted on an Olympus BX51 microscope.

Results

During necropsy, marked cachexia and anaemia were observed.

The lesions in the caeca consist of multiple, grey to yellow, rubbery, coalescing nodules, expending from the ceca wall into serosa and lumen of the cecum. Occasionally, adult viable

Heterakis spp. parasites were embedded into the tumour or coiled under the covering serosa of the tumour (Fig. 1).

Based on previously-described morphological keys by Yazwinski and Tucker [9], the cecal-harvested nematodes were identified as *Heterakis gallinarum* (Fig. 1). Within the intestine, *Capillaria spp* was also present, but with unknown importance in the pathogenesis of this lesion.

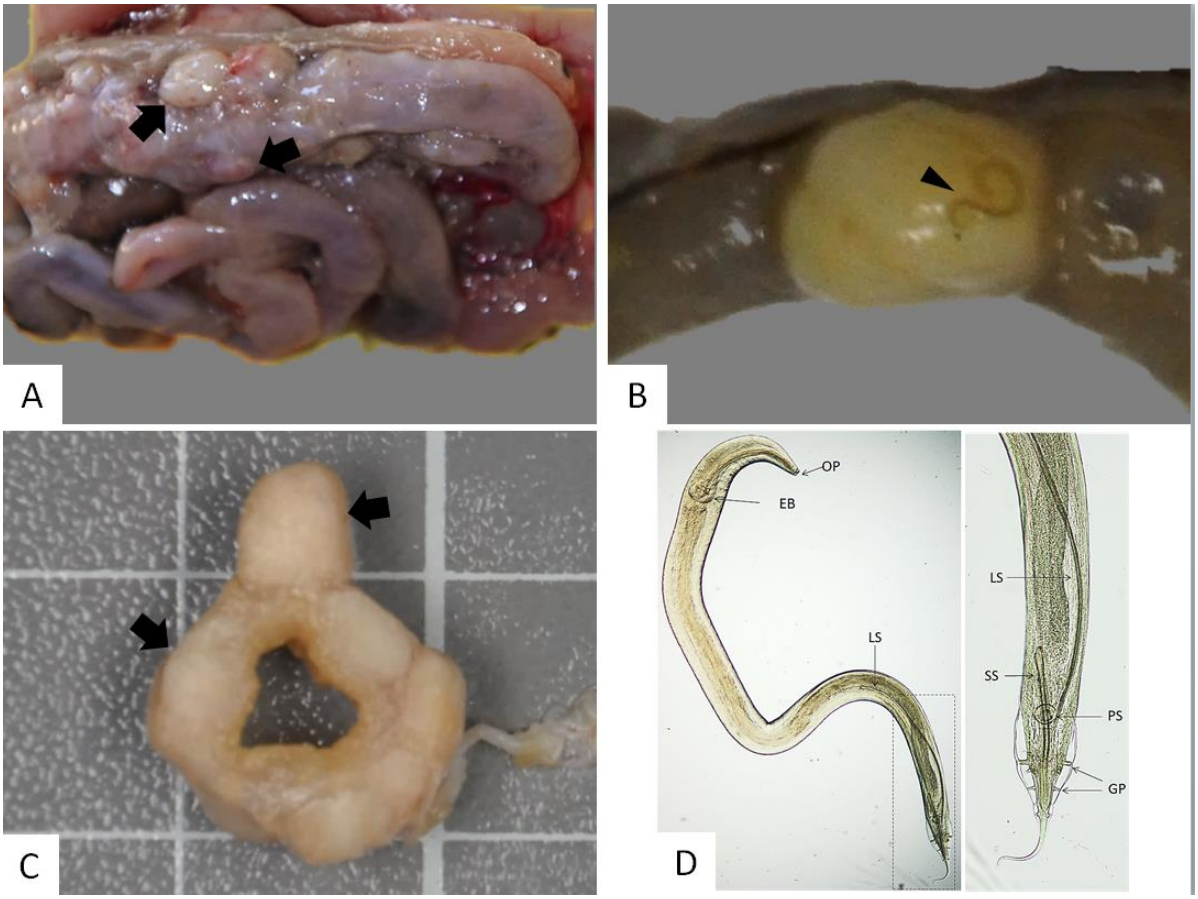

Fig. 1. Gross images of the nodular typhlitis (A-C) and microscopical examination of a Heterakis gallinarum male harvested from the cecal content.
Image A-C: within the wall of the cecum and bulging through the serosa, there are multifocal to coalescing, often transmural, white-grey nodules (arrows), occasionally containing a distinctive, partially coiled, nematode (arrowhead, image B).
Image D: morphological assessment of the parasite; Abbreviations: large esophagus bulb (EB), LS-Long spicule, SS-Short spicule, OP-Papillary pours, PS- pre-cloacal sucker, GP-Great caudal papillae and caudal alae.

Associated with the cecal masses, multifocal to coalescing, poorly defined yellowish granulomas with occasional central areas of caseating necrosis were present within the spleen, liver, abdominal serosa and bone marrow. On Ziehl-Neelsen-stained smears harvested from the above-mentioned granulomas, multiple, intra and extracellular, acid-fast rod-shaped bacteria consistent with Mycobacterium spp. are present (Fig. 2).

On histology, the multifocal-coalescing tumours are markedly expanding the intestinal wall and consist of poorly defined bundles and streams of spindle-shaped cells separated by a small amount of collagenous stroma and multifocally by a variable number of lymphocytes and macrophages.

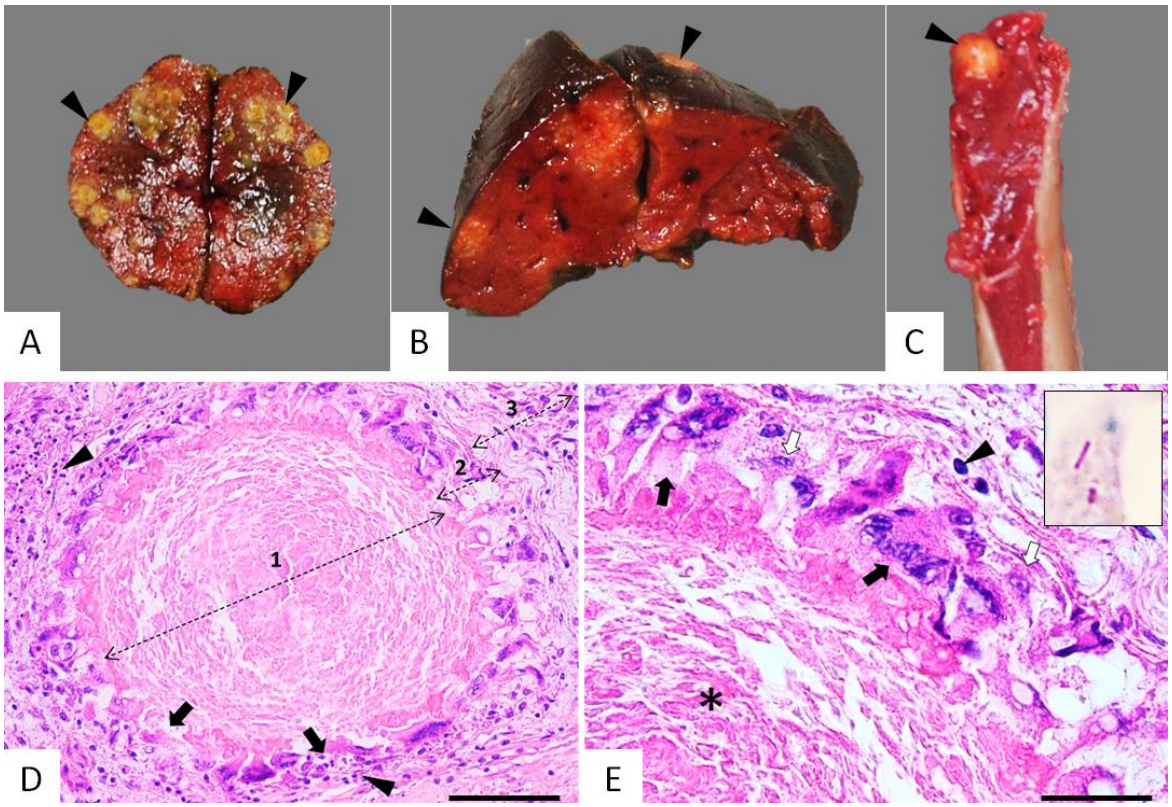

Fig. 2. Visceral mycobacteriosis: gross (A to C) and histopathological (D and E) images presenting multifocal-coalescing, yellowish, well demarcated granulomas (arrow head) randomly disseminated within the splenic (A) and liver parenchyma (B) and tibial bone marrow (C). Image D and E: Histological features of the tuberculoid granuloma, consisting of a central area of caseating necrosis (zone 1) surrounded by a reactive rim (zone 2) of multinucleated giant cells (Langhans type)(black arrows) admixed with fewer histiocytes, macrophages and lymphocytes (arrowhead) and bordered by a partially formed fibrous capsule (zone 3) focally infiltrated by the above-mentioned cells (detailed in image E); the inset from image E present an acid fast rod-shaped bacterial within the macrophages. Hematoxylin and eosin, obx 10 for image A (scale bar=200 µm) and obx40 for image B (scale bar=50 µm); inset: Ziehl-Neelsen stain, ob x100.

Histologically the tumoral cells are poorly demarcated, have a moderate amount of pale-eosinophilic cytoplasm and an oval nucleus with inconspicuous nucleoli and vesicular chromatin. The neoplastic cells generally demonstrated few cytological features of malignancy.

Few mitoses are generally observed (usually less the 1 mitosis/HPF) within the tumour. In some of the intestinal proliferations which harbour the parasites, the histiocytic inflammation which separates the atypical nodular mesenchymal proliferation is particularly prominent. The large tumours (up to 1 cm diameter) eliciting minimal inflammation. Multifocal areas of granulomatous and lympho-histiocytic typhlitis are also present in the small intestine (ileum), cecum and colonic wall. By immunohistochemistry, the intestinal tumours are, at least focally, positive for vimentin and alpha-smooth muscle actin (α-SMA), supporting the diagnosis of intestinal leiomyoma (multifocal). The immunolabeling in both cases was cytoplasmic, assessed as intense for both markers used (Fig. 3).

The poorly-defined coalescing granulomas containing the acid-fast bacteria consist histologically in generally irregularly arranged histiocytes admixed with lymphocytes, necrotic cell debris and occasionally with multinucleated giant cells (Langhans type) and heterophils.

Within the bone marrow the granulomas have a particularly-distinct central area of caseating necrosis surrounded by a well-demarcated rim of multinucleated giant cells (Langhans type) admixed with fewer histiocytes, macrophages and lymphocytes. The above describe inflammation is overall bordered by a partially formed fibrous capsule (Fig. 2).

On histology, the intratumoral nematode have prominent, irregular intensely-eosinophilic cuticle with lateral alae (equal) and cylinder-shaped coelomyarian muscles. The intestine is lined by a single layer of columnar cells with a single-nucleus.

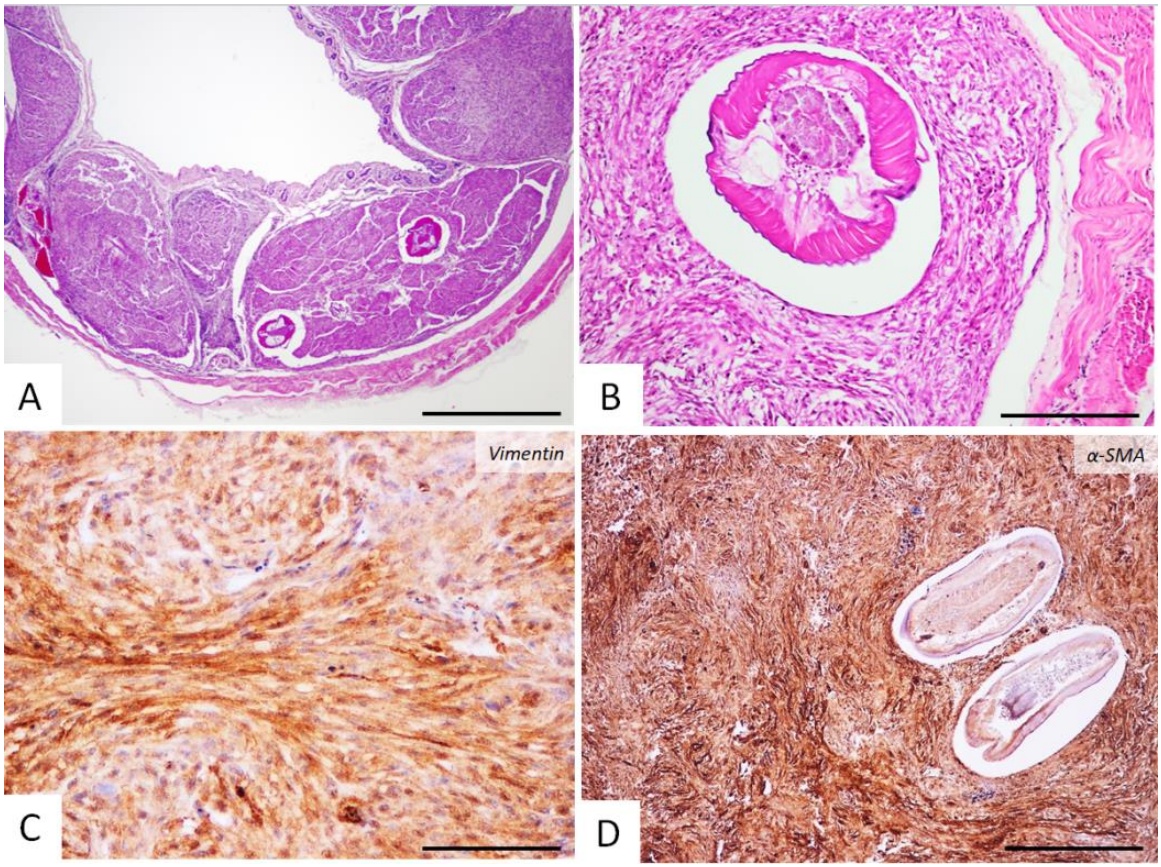

Fig. 3. Histological (A-C) and immunohistochemical (D) images of the cecal masses induced by Heterakis gallinarum.
Image A-B: the submucosa and tunica muscularis are expanded by multifocal tumours contain multiple nematode parasites; the tumoral tissue surrounding the parasites consists of bundles of spindle-shape cells focally admixed with lymphocytes and macrophages.
Image C and D: positive immunoreactivity of the tumoral cells for vimentin (C) and α-SMA (D). Hematoxylin and eosin (A-B), Vimentin (C) and α-SMA (D) immunohistochemistry; obx 2 for image A (scale bar=1000 µm), obx20 for image B (scale bar=100 µm), obx40, images C (scale bar=50 µm), and obx 10 for image D (scale bar=200 µm).

Discussions

Despite the fact that recent reports in humans indicates the pulmonary tuberculosis as an important risk factor for lung cancer, there are no similar data in veterinary literature. In this manuscript we describe the gross and histological features of a rare case of nodular typhlitis induced by a *Heterakis gallinarum* infection associated with a disseminated mycobacteriosis in a golden pheasant.

Heterakiasis is a major intestinal parasitic disease of glinaceous birds, being associated with nodular enteritis, granulomatous hepatitis [10], and occasionally with malignant mesenchymal tumours [4].

Regarding the parasitic-induced cecal tumours, the type of cells responsible for the formation of the nodular lesion and finally of the tumours has long being disputed, historically being considered to be fibroblasts (immature mesenchymal cells) [1], smooth muscle-cells [2, 3, 11] or neurofibroblastic cells [4]. In our case due to the positivity of the proliferative cell to both vimentin and α-SMA, smooth muscle cells or reactive myofibroblasts are considered to be the

most likely origin of the cecal tumours. Although the morphological and immunohistochemical observations are consistent with the diagnostic of leiomyoma, other tumours as inflammatory myofibroblastic tumour ("inflammatory pseudotumor") [12, 13] can exhibit myogenic markers.

Interestingly, the cecal tumours induced by *Heterakis* in birds show several morphological and behaviour features with the inflammatory myofibroblastic tumours [12, 14]. Further immunohistochemical (actin and desmin) and ultrastructural assessment of the cells can bring additional data regarding the origin of this peculiar tumour.

The primary intestinal spindle-shape tumours show considerable morphologic overlaps. This is the case of fibrosarcoma, leiomyoma/leiomyosarcomas, nerve sheath tumours and sarcomas of neurogenic origin, which often require immunophenotyping for confirmation of the tissue origin [15,16]. The pathological diagnostic becomes especially challenging in the intestinal parasitic pathology induced by *Heterakis spp*, since both pseudoneoplastic nodules (reactive fibroplasia) and tumors coexist in the same case [17].

In our case, as in most of the previous reports, the inflammatory lesions coexist with hyperplastic, atypical mesenchymal nodules and finally with the intestinal mesenchymal tumours. This shows a most-likely dynamic of lesions, highlighting a possible inflammatory pathogenesis of such neoplastic cases.

Although the morphologically-identified parasites were harvested from the cecal content to avoid the distortions following removal from tumours, the histological features of the nematode are consistent with the previously-described morphology of *Heterakis spp* [18, 19]. As in the previously described cases [4, 18], packed around the *Heterakis larvae*, there were large, multilobular, masses of spindle shaped cell separated by a fine fibrovascular stroma, making the parasitic origin the most likely cause of the tumoral transformation and progression.

The mechanism which hypothetically links the chronic tuberculosis with increased risk for cancer is considered to be linked with the increased levels of IL-17 and TNFα, which will determine an inhibition of cellular expression of caspase-3 by reducing the mitochondrial oxidative activity [20]. It is well known that M2 macrophage functional-polarization (Th2 inflammatory response) plays a critical role in both parasitic survival (and induction chronic proliferative lesions) and chronic tuberculosis, this type of functional polarization being possible linked with such a cytokine dysregulation.

The main drawback of the current manuscript and its conclusions is the limited immunohistochemical study of the intestinal tumours, the lack of microbiological characterization of the Mycobacterium spp. responsible for the disseminated infection and, of course, the limitations of a case-only pathologic scenario. For immunohistochemistry, the assessment of expression for several other immunomarkers such as C-Kit, CD34, SMA, S100 protein or desmin could bring additional data regarding the tissular origin of cecal tumours.

Also, further cytogenetical analysis could confirm the clonal nature of cells and fibaly-prove the true-neoplastic nature of cecal masses.

In the context of routine diagnosis, in addition to lymphosarcoma caused by avian leukosis virus, one of the main differentials for nodular typhlitis in pheasants is digestive tuberculosis [4].

This case brings the attention of a possible association of the two infections, highlighting the diagnostic pitfalls in such peculiar cases.

In conclusion, to the authors' knowledge, this is the first report of a systemic and gastrointestinal mycobacteriosis in a golden pheasant associated with multiple intestinal tumours induced by a *Heterakis gallinarum* infection. Anyway, although a synergic oncogenic mechanism is hypothetically possible in such cases, such an intriguing assumption should be further studied.

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Determining the Degree of Hip Dysplasia in Dogs

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Abstract

Hip dysplasia in dogs is a polygenically inherited disease characterized by discongruence of the articular surfaces of the femoral head and acetabulum. Large and giant dog breeds are more susceptible to this pathology. The problem of dysplasia is very relevant in pedigree dog breeding in connection with genetic inheritance. Of particular importance is the timely diagnosis of this pathology, the identification of the severity and consideration of these data when breeding in order to control the transmission of dysplasia genetically. By the methods of proper preparation and placement of the patient for the study and the methods of X-ray diagnosis of hip dysplasia in dogs, the possibility of X-ray examination and the diagnosis of hip dysplasia in dogs is shown.

Keywords: dogs, computer radiography, dysplasia, true position, Norberg angle

Introduction

In recent years, in Moldova there has become a demand for determining the presence of hip dysplasia in dogs. Every dog breeder knows about this problem. For admission to breeding, he needs to take an X-ray for hip dysplasia. Fused to the lateral surface of the pelvis for each animal.

Dysplasia can affect all dogs, but purebred large and giant dogs are more susceptible. For breeding dogs involved in breeding, the final conclusion indicating the degree of dysplasia is issued by a licensed classification specialist approved by one of 3 organizations: FCI (Federation Cynologique Internationale), OFA (Orthopedic Foundation for Animals), BVA/KC (British Veterinary Association/The Kennel Club). [5, 9]

When symptoms of dysplasia occur in animals not participating in breeding, without documents of origin, mestizos, veterinarians are faced with the need to diagnose and determine the severity of the pathology in such patients.

The purpose of the work was to visually familiarize with the technique of measuring on a radiograph for diagnosis and assessment of the degree of hip dysplasia in dogs.

Materials and Methods

During the work, 2 dogs were examined. X-ray diagnostics was carried out on the basis of images of the pelvis, thigh bones with capture of the knee joints in a direct ventro-dorsal projection. An X-ray was obtained using a Philips PCR Eleva F digital installation at the "Esculap Vet-Vasile Buza" Veterinary Center, Chisinau. There were used digital X-ray cassettes with a size of 53.4x 53.4 cm. The examined animals were sedated with Meditin in accordance with the instructions in the doses indicated in Tab. 1.

Table 1. Number of animals sedated

Breed	Age	Weight	Meditin, ml
Pembroke Welsh Corgi	1 year	12 kg	0,5
Shiba-Inu	1 year 1 month	9 kg	0,4

The animals were laid using a special fixing table (Fig. 1, 2, 3).

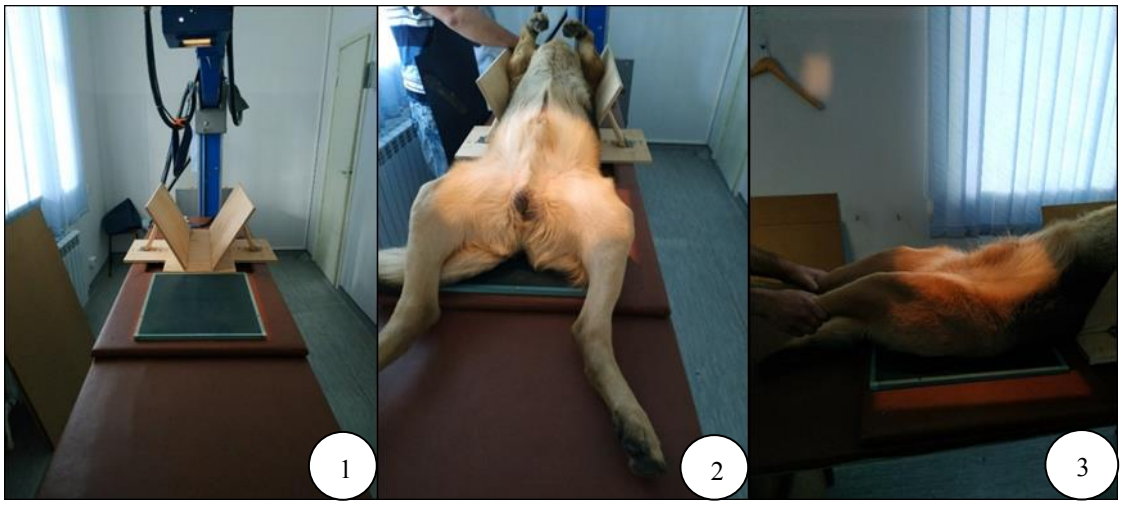

Fig. 1, 2, 3. The use of a special table for the correct fixation of animals during X-ray examination

The pelvic limbs of animals were located parallel to the plane of the cassette and parallel to each other. At the same time, the knee joints rotated inwards by about 15 degrees so that the kneecaps were located symmetrically along the axes of the femurs.

Results

The measurements were carried out according to 6 parameters and quantified each radiological sign in points, according to the evaluation system Flukiger L., 1999 [4], modified by V. Mitin (Tab. 2). [5]

Table 2. Quantification of each radiological sign in points

Parameter 1	Parameter 2	Parameter 3	Parameter 4	Parameter 5	Parameter 6	Points
Norberg angle	Hip head insertion index	Tangential angle anterior outer edge of the cavity	Vault Arch Lock Plate Status	Head shape and hip architectonics	Exostoses on the neck	
		The edge is sharp, covers the head	Normal, uniformly thickened	The head is round, trabecula systems are represented by three systems	Indistinguishable, the transition of the head to the neck is clearly expressed	0
Joint gap uniform or uneven			Evenly thickened	The head is round, angular, the outline is fuzzy, architectonics is not represented perfectly	Indistinguishable, neck cylindrical transition unchanged	1

		Tangential angle horizontal	Laterally slightly thickened, medially slightly reduced	The head is slightly flattened, architectonics reinforced by I and II systems of trabeculae	Thin pointed border up to 1mm. transition slightly modified	2
		Tangential angle horizontal edge slightly rounded or changed	Laterally strongly thickened, medially-medium reduced	The head is flattened, architectonics is presented in the lower 1/3 of the head (1 system)	Border width up to 3mm, transition slightly changed	3
		Tangential angle is positive, the edge is very rounded, the contours are clearly bifurcated	Laterally strongly thickened, medially complete reduction	The head is flattened, Architectonics is presented only along the edge of the head	Border more than 3 mm wide. transition is moderately modified	4
	I.I. 0,5 thigh laterally, dorsal edge of the acetabulum (10mm) ¼ thigh head covered	Tangential angle is not detected, the edge is missing, all contours are bifurcated	Fused to the lateral surface of the pelvis, sometimes absent	The head is very flat, architectonics is not defined	The border is layered on the edge of the neck due to large exostoses	5

Norberg angle – the angle between the straight line connecting the geometric centers of the femoral heads and the line drawn from the center of the head along the front-outer edge of the articular cavity (Fig. 4), with a norm> 105. [6, 8].

The index of insertion of the femoral head into the cavity is determined by the ratio of the size of the covered part of the femoral head to the outer edge of the acetabulum to the radius of the femoral head. Normally, the ratio is equal to unity, that is, the size of the covered part of the femoral head with the upper edge of the acetabulum is equal to the radius of the femoral head or half of the femoral head is covered with the acetabulum (Fig. 5), with a norm of IV> 1.0 [3, 10].

The tangential angle is located between the horizontal drawn through the front-outer edge of the articular cavity, and the tangent, which is a continuation of the cranial contour of the joint space. Normally, the tangent passes below the horizontal, forming a negative angle, or coincides with it, forming an angle equal to zero. A tangent directed above the horizontal forms a positive angle characteristic of the pathological process (Fig. 6) [6], normal: the tangential angle is negative, the edge is sharp, covers the head [1].

Changes in the state of the end plate of the acetabulum arch – *sclerosis* – are determined on the radiograph in the form of an intense light strip along the end plate of the acetabulum. It characterizes the uneven distribution of pressure on the cavity during loading and is an indirect symptom of a hidden subluxation of the hip, normal tangential angle is negative, the edge is sharp, covers the head. [6, 8]

The shape of the head and changes **in the architectonics of the proximal femur** (Fig. 7) are characterized by the state of the trabecular apparatus (I, II and III of the trabecular system).

They reflect the pattern of change in the shape of the femoral head depending on its different position in the joint (unstable position, subluxation, dislocation), normally the head is round, the trabeculae system is represented by three systems [1, 6].

Exostosis (Greek *exostosis*, from *exo* – outside and *osteon* – bone) – bone or bone-cartilaginous growth of a non-tumour nature on the surface of the bone (in the form of linear, spherical and other formations) [1], normally they are indistinguishable, the transition of the head to the neck is clearly expressed [6].

Thus, a radiologist with a digital image and software needs a minimum of time to make a preliminary diagnosis of DTBS, which allows the attending physician to prescribe treatment and give recommendations on the first day of seeking help (Fig. 8, 9, 10, 11).

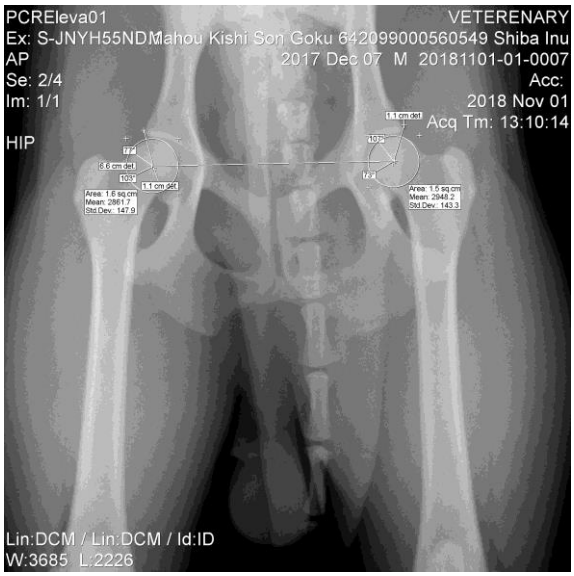

Fig. 4. Measurement of the Norberg angle of the hip joints by X-ray of a dog breed Shiba-Inu

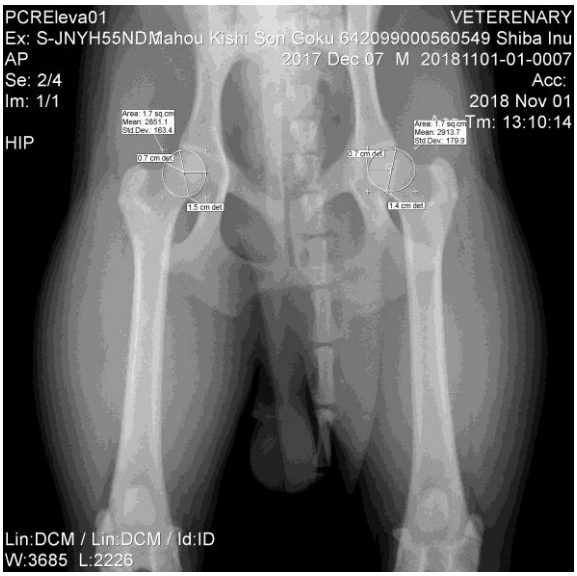

Fig. 5. Measurement of the index of insertion of the femoral head into the cavity on the X-ray of a dog of the Shiba-Inu breed

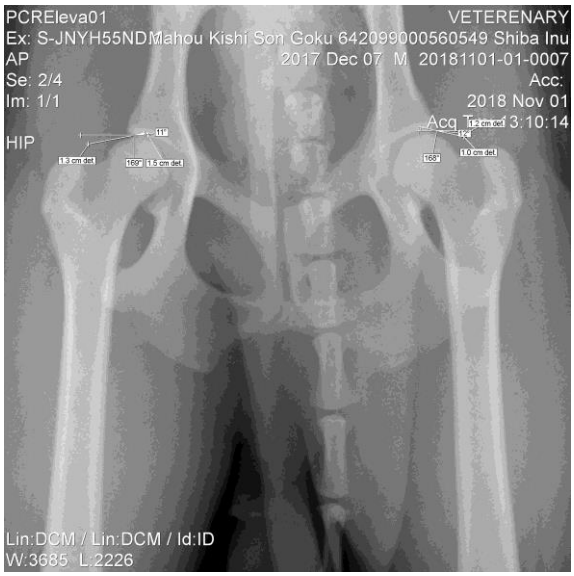

Fig. 6. Measurement of the tangential angle on the X-ray of a dog of the Shiba-Inu breed

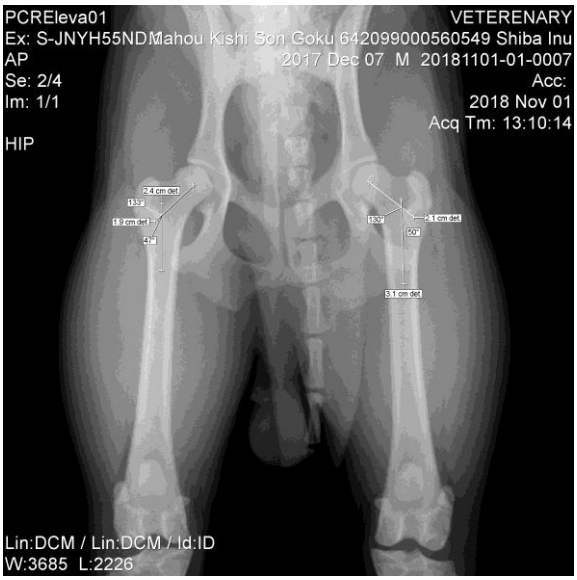

Fig. 7. Measurement of the cervical-diaphyseal angle on the X-ray of a dog of the Shiba-Inu breed

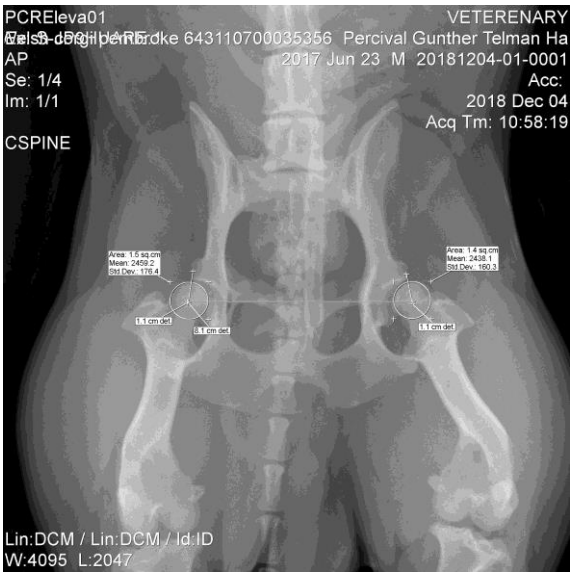

Fig. 8. Determination of the Norberg angle on the roentgenogram of a dog of the breed Welsh Corgi Pembroke

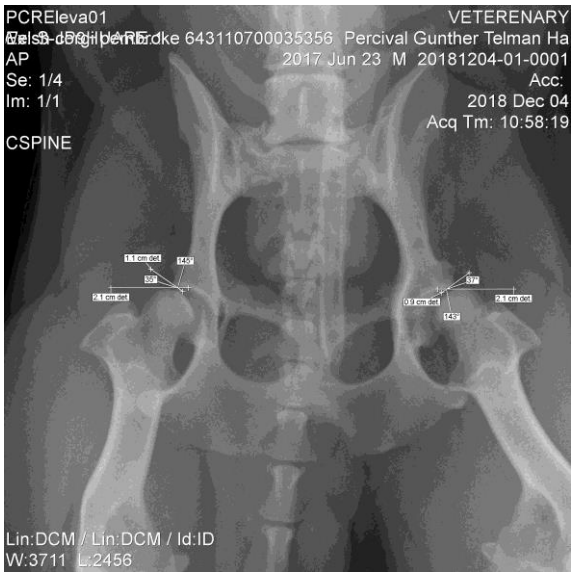

Fig. 9. Determination of the tangential angle on the roentgenogram of a dog of the breed Welsh Corgi Pembroke

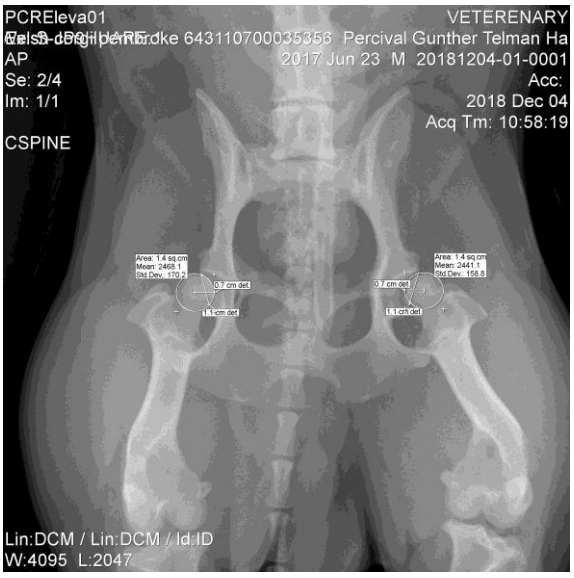

Fig. 10. Determination of the index of insertion of the femoral head into the cavity on the radiograph of a dog of the breed Welsh Corgi Pembroke

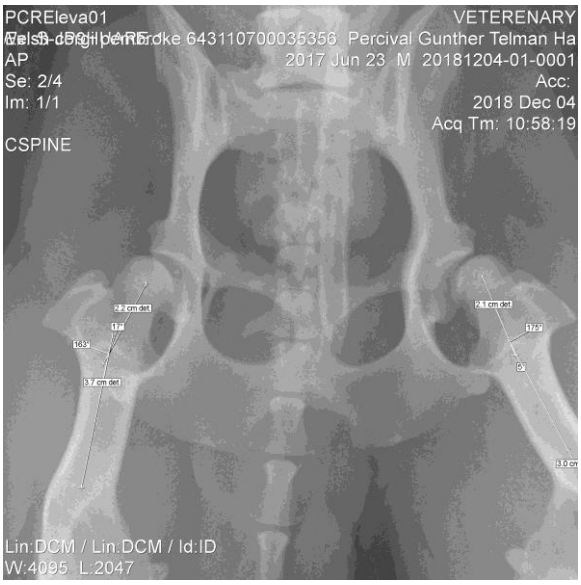

Fig. 11. Determination of the cervical-diaphyseal angle on the radiograph of a dog of the breed Welsh Corgi Pembroke

Table 3. The final assessment of hip joint dysplasia in dogs aged 12-18 months

Total points	Degree of dysplasia	Interpretation
0-2	A	Lack of dysplasia
3-6	B	Borderline case, suspicion
7-9	C1	Mild of hip joint dysplasia
10-12	C2	Mild of hip joint dysplasia (not for reproduction)
13-18	D	Medium Cull
More 18	E	Severe rejection

Assessment of the degree of hip joint dysplasia of a Shiba-Inu breed dog is presented in Tab. 4:

Table 4. Assessment of the degree of dysplasia of hip joint in the Shiba Inu breed

	Left hip joint	**Total points**	**Right hip joint**	**Total points**
Norberg angle	107	0	103	0
The index of insertion	=1	1	=1	1
The tangential angle	negative	0	negative	0
Vault Arch Lock Plate Status	Laterally slightly thickened, medially slightly reduced	2	Evenly thickened	1
Head shape and hip architectonics	The head is round, angular, the outline is fuzzy, architectonics is not represented perfectly	1	The head is round, angular, the outline is fuzzy, architectonics is not represented perfectly	1
Exostoses on the neck	Indistinguishable	0	Indistinguishable	0
Total points		4		3
Degree of dysplasia		B		B

Table 5. Assessment of the degree of dysplasia of hip joint dogs of the breed Welsh Corgi Pembroke

	Left hip joint	**Total points**	**Right hip joint**	**Total points**
Norberg angle	79	5	81	4
The index of insertion	0,71	3	0.7	3
The tangential angle	Positive, the edge is very rounded, the contours are clearly bifurcated	4	Positive, the edge is very rounded, the contours are clearly bifurcated	4
Vault Arch Lock Plate Status	Laterally strongly thickened. Medially complete reduction	4	Fused to the lateral surface of the pelvis	5
Head shape and hip architectonics	The head is round, angular, the outline is fuzzy. Architectonics is not presented perfectly	1	The head is round, angular, the outline is fuzzy. Architectonics is not presented perfectly	1
Exostoses on the neck	Indistinguishable, the neck is cylindrical, transition unchanged	1	Indistinguishable, the neck is cylindrical, transition unchanged	1
Total points		18		18
Degree of dysplasia		D		D

Conclusions

After evaluating the X-ray of a Shiba-Inu breed dog, a quantitative assessment of each radiological trait was performed in points, each joint was evaluated separately according to 6 parameters. The number of points was summarized. Best score 0 points, worst 30 points. Taking into account the accumulated points, one of the degrees of dysplasia is assigned. The conclusion on the degree of dysplasia in a Welsh Corgi Pembroke dog is determined by the worst joint.

Starting from the sum of 10 points, the animal cannot be used in breeding.

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Antibiotic Resistance Patterns of ESBL and Ampc-Producing *Escherichia Coli* Isolated from Slaughtered Pigs

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Abstract

The presence of extended-spectrum beta-lactamase (ESBL) enzyme-producing Escherichia coli strains in production animals represents a public health problem. Moreover, these strains are associated with multiple antibiotic resistance. The aim of our study was to phenotypically characterise non-beta-lactam antibiotic resistance of ESBL/AmpC-producing E. coli strains isolated from slaughtered pigs. 128 samples of caecum content collected from slaughtered pigs have been analysed for ESBL/AmpC screening using the MacConkey medium (Oxoid, Basingstoke, UK) with addition of cefotaxime. The bacterial strains obtained after screening were confirmed as being E. coli using the MIU and TSI tests, and the phenotypical confirmation as being presumptive ESBL/AmpC-producing strains was carried out using Sensititre plates (Trek Diagnostic System) and EUVSEC2 panels, respectively. In order to characterise non-beta-lactam antibiotic resistance of ESBL/AmpC-producing E. coli strains, we used the broth microdilution method. Following investigations, we obtained 51/128 (39.84%) presumptive ESBL/AmpC-producing Escherichia coli strains, which were resistant to antibiotics from the sulphonamide (resistance to sulfamethoxazole: 39/51 (76.47%), and 31/51 (60.79%) to trimethoprim) and fluoroquinolone classes (29/51 (56.86%) resistant to ciprofloxacin, and 27/51 (52.94%) to nalidixic acid). Although at a low percentage of 5.88% (3/51), there were also strains resistant to Colistin (cyclic polypetides). All isolated strains were sensitive to the action of tigecycline (glycylcyclines). In conclusion, the ESBL/AmpC-producing E. coli isolated strains were also associated at a high percentage with non-beta-lactam antibiotic resistance.

Keywords: E. coli, ESBL, AmpC, non-beta-lactam antibiotic resistance

Introduction

The isolation of extended-spectrum beta-lactamase enzyme-producing *Escherichia coli* (ESBL *E. coli*) strains from production animals represents a public health problem. Moreover, the World Health Organization (WHO) acknowledges and classifies these strains as being pathogens with critical priority [20]. Additionally, the presence of ESBL enzymes is accompanied by the presence of AmpC or carbapenemase enzymes, which also have an increasing prevalence [3]. The occurrence and high prevalence of ESBL enzymes is associated with the excessive antimicrobial use (AMU), as well as the inappropriate use of cephalosporins in production animals [11]. The transmission of genes that encode ESBL enzymes from animals to humans can be carried out either through contaminated food, or through direct contact [7; 10]. A few studies have signalled the presence of ESBL *E. coli* on the one hand in production

animals, especially in pigs, free-range poultry and bovines, and on the other hand in food products worldwide. Their transmission from animals to humans has also been highlighted [8; 9]. Furthermore, ESBL-producing *E. coli* strains are commonly associated with resistance to other classes of non-beta-lactam antibiotics, such as aminoglycosides, tetracyclines and fluoroquinolones, the latter being used mostly in the veterinary field [6]. In Romania, information regarding the prevalence and characterisation of ESBL-producing *E. coli* isolates has been analysed for the strains obtained from chicken and pets [12; 13; 4; 5]; however, information regarding the ESBL *E. coli* strains isolated from pigs has not been concluded yet.

Therefore, in order to adopt the optimal measures in the management of antibiotic use in production animals (pigs, poultry, bovines), it is important that the epidemiology and importance of ESBL *E. coli* isolates for public health are understood.

The aim of the study was to analyse the non-beta-lactam antibiotic resistance pattern for some positive ESBL *E. coli* strains isolated from slaughtered pigs.

Methodology

In order to analyse the non-beta-lactam antibiotic resistance pattern for the ESBL/AmpC *E. coli* isolates, we first aimed at identifying the extended-spectrum beta-lactamase enzyme-producing strains, and the AmpC enzymes, respectively. On this line, caecum samples have been collected in a sterile manner from slaughtered pigs, their contents being subsequently inoculated in peptone water for pre-enrichment. After the pre-enrichment stage, we carried out ESBL/AmpC screening using the selective medium MacConkey (Oxoid, Basingstoke, UK), with addition of cefotaxime. The colonies that had typical morphology for *Enterobacteriaceae* on the MacConkey medium have been confirmed as being *E. coli* isolates based on the biochemical properties, using the triple-sugar-iron (TSI) agar test, motility-indole-urea (MIU) medium, and for atypical strains Api 20E tests have been used. Phenotypical confirmation of the obtained isolates as being ESBL/AmpC was carried out based on the synergy between a cephalosporin and the cephalosporin potentiated with clavulanic acid. Thus, Sensititre plates (Trek Diagnostic System) and EUVSEC 2 panels have been used containing cefotaxime, ceftazidime and clavulanic acid in combination with cefotazime (CTX) and ceftazidime (CAZ) to detect the production of ESBL enzymes, and cefoxitin (FOX) and cefepime (FEP) to detect AmpC. Moreover, EUVSEC 2 also contains imipenem (IMI), meropenem (MERO) and ertapenem (ETP) necessary to verify the phenotyping of presumptive carbapenemase-producing strains. The results of the minimum inhibitory concentrations have been read with the Biomic device.

The isolates that have been phenotypically confirmed as being ESBL/AmpC have been tested for resistance to non-beta-lactam antibiotics through the microdilution in broth method, using Sensititre plates (Trek Diagnostic System) and EUVSEC panels containing the following antibiotics: gentamicin (GEN), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP), nalidixic acid (NAL), chloramphenicol (CHL), azithromycin (AZI), tigecycline (TGC), and colistin (COL). The levels of the obtained minimum inhibitory concentration (MIC) have been interpreted according to the epidemiological limit levels of the European Committee on Antimicrobial Susceptibility Testing [17].

Results

128 samples of caecum content collected from slaughtered pigs aged between 3 and 8 months have been examined. Following ESBL screening, 51/128 (39.84%) presumptive ESBL/AmpC-producing *Escherichia coli* strains have been isolated. Establishing the non-beta-lactam

antibiotic resistance pattern was carried out for all 51 *E. coli* isolates, which were phenotypically confirmed as being ESBL/AmpC.

Following analysis of the minimum inhibitory concentration results, it has been noticed that the level of resistance for animal isolates is high for the antibiotics from the classes: sulphonamides [resistance to sulfamethoxazole: 39/51 (76.47%), and 31/51 (60.79%) to trimethoprim], fluoroquinolones [29/51 (56.86%) strains were resistant to ciprofloxacin, and 27/51 (52.94%) strains to nalidixic acid (NAL)] and phenicols [23/51 (45.1%) strains were resistant to chloramphenicol] (table 1).

A very low resistance of *E. coli* isolates was noticed for gentamicin (aminoglycosides) (8/51; 15.68%) and azithromycin (macrolides) (15/51; 29.41%) (table 1). For the glycylcycline group, which contains tigecycline, no resistance was registered, all tested strains being sensitive. In addition, all strains were sensitive to the antibiotics from the carbapenem class, the results being noticed with the help of the EUVSEC 2 panel used to phenotypically confirm ESBL/AmpC isolates.

A particular situation represents the resistance of isolates to Colistin (polymyxins), an antimicrobial considered a last resort in antibiotic treatment for multi-drug resistant (MDR) Gram-negative bacteria [15]. Colistin is not administered only in human medicine, its use has also been described in veterinary medicine. Moreover, it has been suggested that uncontrolled use of colistin in animals has played an important role in the global occurrence of plasmid-mediated resistance to colistin [2]. In addition, the World Health Organization has recently added polymyxins on the list as being critically important antibiotics, which are used in production animals worldwide [2]. Following the conducted investigations, it has been noticed that 3/51 (5.88%) (table 1) ESBL-AmpC *E. coli* strains isolated from slaughtered pigs are resistant to colistin. Although the number of strains resistant to colistin is low, the results obtained phenotypically practically suggest the administration of this antibiotic in swine farms.

Moreover, the strains analysed in this study are ESBL/AmpC enzyme-producing strains, which means that they are resistant to beta-lactam antibiotics, including third generation cephalosporins. In fact, similar studies have revealed that the coexistence of ESBL and mcr-1 genes on the same plasmid facilitates the dissemination of colistin-resistant strains through the co-selective pressure applied with the administration of colistin, as well as through the use of non-beta-lactam antibiotics [6].

Table 1. Resistance of ESBL/AmpC -producing bacterial strains to non-Beta-Lactam Antimicrobials

Type of antibiotic	**Resistant strains n=51**	**Sensitive strains n=51**
SMX	***39 (76.47%***	***12 (23.53%)***
TMP	***31 (60.79%)***	***20 (39.21%)***
CIP	***29 (56.86%)***	***22 (43.14%)***
NAL	***27 (52.94%)***	***24 (47.06%)***
GEN	8 (15.68%)	43 (84.32%)
CHL	*23 (45.1%)*	*28 (54.9%)*
AZI	15 (29.41%)	36 (70.59%)
TGC	0 (0%)	51 (100%)
COL	***3 (5.88%)***	***48 (94.12%)***
IMI	0 (0%)	51 (100%)
MERO	0 (0%)	51 (100%)
ETP	0 (0%)	51 (100%)

Legend: SMX – sulfamethoxazole; TMP – trimethoprim, CIP – ciprofloxacin, NAL – nalidixic acid, GEN – gentamicin, CHL – chloramphenicol, AZI – azithromycin, TGC – tigecycline, COL – colistin, IMI-imipenem, MERO – meropenem, ETP – ertapenem

Similar studies carried out in Europe have also revealed the presence of ESBL or AmpC *E. coli* strains, and the association between beta-lactam antibiotic resistance and non-beta-lactam antibiotic resistance, respectively [14; 15].

The use of antibiotics without a medical prescription represents a problem, and it is increasingly clear that careless application of antimicrobials during the production cycle can increase the occurrence of resistant bacteria in farms, especially in the "growing" period, immediately after weaning. Selective pressure caused by the consumption of antibiotics adds to the occurrence and spread of bacterial resistance; in this regard, pigs are suggested as a potential source of resistant bacteria [1].

Antimicrobial resistance is a real problem in veterinary medicine as well. Moreover, in addition to the research in the antibiotic resistance field, official warnings call attention on the consumption of antibiotics in animals. Thus, the World Health Organization recommends decreasing and restrictively administering important antibiotics for the prophylaxis of human infectious diseases as growth promoters in farm animals [19]. According to the list of the World Health Organization, critically important antimicrobials for human medicine (OMS CIA list) mainly include extended-spectrum cephalosporins, macrolides, ketolides, glycopeptides and polymyxins [18]. By controlling the consumption of antibiotics in the veterinary sector, the WHO aims at reducing the occurrence of antibiotic resistance on the one hand, but also at preserving the efficacy of important antibiotic classes for infectious treatment in human medicine, on the other hand.

Conclusion

This study revealed a high prevalence of ESBL/AmpC-producing *E. coli* strains associated with non-beta-lactam antibiotic resistance, including colistin, for slaughtered pigs.

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Epidemiological Study of Canine Leishmaniosis in the South of Romania

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Abstract

Like for many other vector-borne diseases, the distribution and prevalence of canine leishmaniosis in Europe is changing. Romania is considered a non-endemic country but there are historic reports, between 1912-1968 of autochthonous Leishmania infantum infections in dogs and humans from the south of Romania. Between 2013 and 2017 there have been new reports of autochthonous L. infantum infections, in dogs but also in one human and one jackal from the south of Romania. These recent reports of L. infantum indicate that there is a risk that canine leishmaniosis could become endemic in the south of Romania. We present here a comprehensive epidemiological study of canine leishmaniosis in stray dogs from the south of Romania.

Venous blood samples and conjunctival swabs were collected from 300 stray dogs in municipal shelters, in four counties from the south of Romania. Sampling locations were chosen to be representative for all regions in the south of Romania and to take into account the location of previous leishmaniosis reports. Serum samples were screened by serology for anti-L. infantum antibodies and blood and conjuncival swabs were screened by qPCR for L. infantum.

All 300 sampled dogs were negative for anti-L. infantum antibodies. Sampled dogs were also negative for L. infantum by qPCR from the blood and conjunctival samples.

In spite of the negative results in this study, recent reports of L. infantum infections in human and animals as well as new reports of vector-competent sand-flies indicate that the risk factors associated with the re-emergence of leishmaniosis in the south of Romania are still present.

In order to monitor the state of L. infantum infection in the south of Romania it is important to continue monitoring new human and animal leshmaniosis cases and to monitor the distribution of vector-competent phlebotomine sand-flies. There have been reported changes in several factors that can influence the distribution of L. infantum infections and future epidemiological studies focused on canine leishmaniosis would also be beneficial.

Keywords: canine, leishmaniosis, Romania, serology, qPCR

Introduction

Leishmania infantum is a trypanosomatid protozoan that is transmitted by phlebotomine sandflies and is the causative agent of canine leishmaniosis (CanL) in Europe. *L. infantum* is endemic in countries of the Mediterranean basin and is considered to be one of the most important canine vector-borne disease of zoonotic concern [1, 2]. The distribution and prevalence of CanL in Europe is changing as it is for other vector-borne diseases [3, 4]. These

changes can be influenced by modifications of habitat, in particular modifications that can induce changes in sand-fly distribution and increased movement of dogs between endemic and non-endemic regions [3, 5].

Changes in the distribution and prevalence of CanL have been reported in western European countries, like Spain or Italy and eastern European countries like Bulgaria, Hungary and Romania [4, 6-9]. These changes have been reported both in countries that are already endemic, from the south of Europe but there are also increasingly frequent reports of CanL cases in countries considerede to be non-endemic [4, 6, 7, 9, 10].

Romania is now considered a non-endemic country but there are historic reports of human and canine leishmaniosis. The first report of human leishmaniosis is from 1912 and the first report of CanL is from 1935 [10].

Other human cases have been reported in the south of Romania between 1944 and 1955, including one outbreak in the southern county of Dolj [10, 11]. Epidemiological studies of CanL in 1967 and 1968, in the south and south-west of Romania reported prevalence's of 1.2% and 2.2% [10].

After 1968 no more autochthonous human or canine leishmaniosis cases were reported. This can be attributed to the mass use of insecticides employed during the Malaria eradication campaign of 1949-1954 [8, 12]. Reports of *L. infantum* infections in both humans and dogs are not infrequent now but most of them report people or dogs that have previously travelled to endemic areas in the south of Europe [13-15].

The first recent case of autochthonous human leishmaniosis was reported in 2013 in a patient that was from the northeast of Romania but had previously worked in the southern county of Dolj [14].

In 2014 the first recent case of autochtonous CanL was presented in a stray dog from the town of Râmnicu Vâlcea, in the southern county of Vâlcea, neighbouring the previously mentioned county of Dolj [10]. The first recent epidemiological study of CanL was published in 2016 and investigated the prevalence of *L. infantum* in clinically healthy dogs from Râmnicu Vâlcea [8]. This study reported a 3.7% seropositive, a 8.7% prevalence by qPCR performed on conjunctival swabs and a 1.2% prevalence by qPCR performed on blood [8].

Besides human and canine leishmaniosis cases, *L. infantum* infection has also been reported in a golden jackal (*Canis aureus*), again in the county of Dolj [16].

New reports of autochthonous human and canine leishmaniosis cases indicate that the re-emergence of leishmaniosis in the south of Romania is a definite possibility.

This study aims to investigate the presence of *L. infantum* infections in stray dogs, taking into account historical data and other previous studies, in order to determine if CanL is indeed re-emerging in the south of Romania.

Material and Methods

Study area and sample collection

Venous blood samples and conjunctival swabs were collected from 300 stray dogs in municipal shelters in four counties from the south of Romania between the 10th and the 20th of July 2017.

The samples were collected from dogs in municipal shelters of the city of Drobeta-Turnu Severin, Craiova, Roșiorii de Vede, Alexandria and Slobozia. The locations of the collection sites can be seen in Fig. 1 and the number of samples collected from each location are described in Tab. 1.

Tab. 1. number of samples collected from each location

County	City and geographic coordinates	Samples
Mehedinți	Drobeta-Turnu Severin (44°37'53.9"N22°37'55.2"E)	64
Dolj	Craiova (44°20'32.4"N 23°43'02.6"E)	100
Teleorman	Rosiorii de Vede (44°07'04.8"N 24°59'58.5"E)	50
	Alexandria (43°57'14.5"N 25°20'43.6"E)	41
Ialomița	Slobozia (44°34'18.1"N 27°20'51.4"E)	45
Total		**300**

Blood was taken by cephalic venipuncture in EDTA tubes for molecular biology testing and clot tubes for serology. Conjunctival samples were obtained from all 300 dogs using sterile swabs as previously described [17]. Blood samples in EDTA tubes and swabs were kept at -20°C until processed for DNA extraction. Blood samples in clot tubes were centrifuged and serum was also kept at -20 C° until used for serology.

The sampled dogs did not show clinical signs compatible with CanL including skin ulcerations, onychogryphosis or exfoliative dermatitis. All sampled dogs were captured from the corresponding city, outskirts and nearby villages up to two weeks before the sample collection and received no antiparasitic or prophylactic treatments.

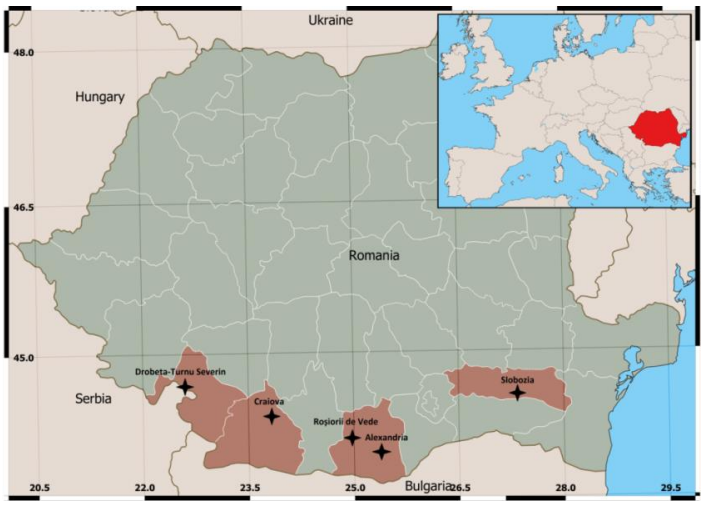

Fig. 1. Location of sampling sites

Serology

Serum anti-leishmanial antibodies were determined by ELISA using crude leishmanial antigen as previously described [18]. Dog sera were diluted to 1:100 and incubated in *L. infantum* antigen coated plates and incubated one hour at 37°C. The plates were washed with 0.1% Tween 20 in 50 mM phosphate-buffered saline (PBS), pH 7.2, and incubated with Protein A conjugated to horseradish peroxidase. The plates were washed again with Tween and PBS and the plates were developed by adding the substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS). The plates were read at a wavelength of 405 nm when the absorbance of the positive control reached a value between 0.95 and 1.0. A positive and negative control was

added in each plate in order to monitor inter-assay variation. The optical density of the samples was calibrated against the positive control. Samples with calibrated optical density ranging between 0.2 and 0.6 were considered borderline and samples over 0.6 were considered positive.

DNA extraction and PCR

DNA was extracted from blood in EDTA tubes using a commercial kit (Ilustra Blood Genomic Prep Mini Spin Kit, GE Healthcare, Buckinghamshire UK) according to the manufacturer's instructions. DNA from conjunctival swabs was extracted using the phenol-chloroform-isoamyl alcohol method as previously described [17].

DNA extracted from both blood and conjunctival swabs was assessed by real-time quantitative PCR, targeting the kinetoplast minicircle DNA of *L. infantum*, using LEISH-1 and LEISH-2 primers and TaqMan-MGB probe, as previously described [19].

Results and Discussion

All of the 300 sampled dogs were negative for *L. infantum* DNA in both blood and conjunctival swabs and all serum samples were seronegative.

Romania is considered a non-endemic country but historical studies report that human and canine *L. infantum* infections were present in the south of Romania. This indicates that autochthonous *L. infantum* infections can happen in this region and that there is a risk that the south of Romania can become endemic.

Historical studies report human leishmaniosis cases in the southern counties of Dolj, Prahova and Giurgiu as well as two epidemiological studies in Dolj and Mehedinți reported prevalence of 1.2% and 2.2%, in clinically healthy dogs, respectively. Historical studies also report sandfly vectors of *L. infantum*, such as *Phlebotomus neglectus* and *Phlebotomus perfiliewi* in southern counties such as Mehedinți, Tulcea, Constanța and Teleorman [11].

More recent studies report one human autochthonous leishmaniosis case in 2013 in Dolj county [14] and one *L. infantum* infection in a golden jackal in 2017 [16].

The first recent case of autochthonous canine leishmaniosis was reported in 2014 in the town of Râmnicu Vâlcea (Vâlcea county) [10]. This study was followed in 2016 by an epidemiological study on dogs from the same town of Râmnicu Vâlcea and reported 3.7% seroprevalence, 8.7% prevalence by PCR from conjunctival swabs and 1.2% prevalence by PCR from blood [8]. In 2019 the presence of *P. perfiliewi* was confirmed again in the southern counties of Mehedinți, Giurgiu and also in the eastern county of Vaslui [20]. *P. neglectus* was also reported again but only in Mehedinți [20].

These studies between 2013 and 2017, that report autochthonous *L. infantum* infections in the south of Romania raise a question regarding the possibility that the south of Romania could become endemic again.

The dog is considered to be the main reservoir for *L. infantum* because it is highly susceptible to infection and a large proportion of infected individuals are asymptomatic but can still be a source of infection [21]. Because of this, in territories that are endemic to leishmaniosis the prevalence of *L. infantum* infections in dogs is often high [21]. Studying the prevalence of *L. infantum* infections in stray dog populations can explain if the recent reports of *L. infantum* infections are due to a re-emergence of this infection in Romania. Alternatively, the recent reports could represent isolated cases of local transmission between an imported case and a local animal or human.

In order to understand if these recent isolated cases of *L. infantum* represent a situation of disease re-emergence in Romania, we undertook a comprehensive epidemiological study on *L. infantum* infections in stray dogs from the south of Romania.

Our epidemiological study was designed to take into account the location of previous reports of *L. infantum* infections in humans, canine and wildlife, as well as the location of previous sand-fly reports. Sampling locations were in counties where previously *L. infantum* infections or sand-flies have been reported or neighbouring counties where such reports have previously been made. The sampling locations were also chosen so that all the regions in the south of Romania would be represented in the study.

Despite the negative results of this study, the risk factors that could determine the re-emergence of leishmaniosisin the south of Romania are still present: There are reports of autochthonous *L. infantum* infections in non-endemic areas from eastern Europe [6, 7, 10].

There is frequent travel of animals and humans between Romania and regions where leishmaniosis is endemic. There are no national prevention or monitoring programs and many clinicians are unaware of the risk of this infection.

Because the risk factors are still present, further epidemiological studies should be carried in the future. It would also be useful to continue to monitor both human and canine autochthonous leishmaniosis cases and to test dogs that have travelled to endemic areas. Studies on the ecology, prevalence and distribution of vector-competent sand-flies should also be carried out.

Conclusion

In spite of recent reports of autochthonous *L. infantum* infections in dogs as well as one human and one jackal, no infections were found in stray dogs from the south of Romania after a comprehensive epidemiological survey. This study demonstrates that the south of Romania is probably non-endemic to *L. infantum* but the risk factors for this disease are still present. It is important to continue to monitor all CanL cases and future epidemiological studies are also recommended.

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Case Report – Intracranial Meningioma in a Cat

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Abstract

The meningioma is the benign tumour of the meninges and may develop intracranially or inside the spinal canal, causing compression on the nervous tissue and subsequent severe clinical signs which may sometimes cause the death of the animal.

Following the necropsic exam performed on a cat euthanized due to the increasing severity and frequency of epileptic seizures, the presence of a single intracranial tumour was assessed, exerting compression on the right cerebral hemisphere. The formation was non-adherent to the nervous tissue, mobile, well delimited, eliptic in shape, with irregular surface, low vascularization and about 2cm/1 cm in size.

The histopathological exam showed that the tissue structure and cellular aspects of the tumoral formation were those of a psammomatous type meningioma. The cells were either spindle shaped or elliptic, mostly uniform in size and shape, displayed in nests and swirls connected in between them through conjunctive fibers. No mitotic figures that would indicate a high cellular proliferation rate were observed.

Keywords: intracranial meningioma, epileptic seizure, cat

Introduction

The meningioma is a tumour that originates from the cells of the arachnoid. Its development is extranevraxial [1] and its character benign [2]. It may be localized intracranially or adjacent to the spinal cord [3] and may cause clinical neurological signs like: ataxia, epileptic seizures, sight disfunctions, depression, coma, aggressivity, circling [1], [4]. Even so, there are many cases (21%) in which the meningioma may be found incidentally during the necropsic exam [4].

The tumour may be single or multicentric and sometimes associated with other types of intracranial neoplasias (gliomas). It's much more frequent in older cats, with ages over 9 years [5] and a slight predisposition in males has been observed [1].

The frequency of intracranial tumours in cats is estimated by some studies as being close to 2.5% and out of these, that of the meningiomas is of 56-58%. These neoplasms are generally limited to the surface of the meninges, non-infiltrative, with well delimited margins, with a pediculated or large base ("en plaque"), ferm or rubber-like consistency [4], lobulated, encapsuled, sometimes with cysts inside [1].

Histopathologically, the meningioma is characterized by the presence of spindle shaped or epithelioid cells, organized as islets, nests or swirls (which may have a mineralized, hyaline or necrotic center) separated by collagen fiber bundles, sometimes mixed with fibroblasts [5].

Materials and Method

In the Internal medicine clinic of the Faculty of Veterinary Medicine of Iași a male cat, aged 13 years, was presented showing severe neurological symptoms that had been evolving for about 2-3 days. These included epileptic seizures that lasted about 30 minutes each, opisthotonus and forced extension of all four limbs. In between seizures partial recovery of consciousness, circling and refusal of water were observed.

Blood samples were collected (simple and on EDTA) for serum biochemistry and CBC (complete blood count) analysis.

A radiological exam was performed in order to observe changes in the thoracic area and a symptomatic treatment based on prednisone (0.5 mg/kg/day) and diazepam (1 mg/kg when needed) was recommended.

The cat was euthanized a day after the consultation due to the increasing severity and frequency of the epileptic seizures and a necropsic exam was performed in order to confirm the presumptive neurological diagnostic of meningioma. From the tissue and organ areas that showed modifications, samples were harvested for the histopathological exam. The fragments were fixated in a 10% formaldehyde solution for 48 hours, included in paraffin, sectioned at 5 microns and stained using the Masson's Trichrome method.

Results and Discussions

During the clinical neurological examination several signs were observed among which a visual proprioception deficit, a tactile sensitivity deficit in the left for limb, inconsistent response to threat reflex, low response following the stimulation of the right nare, circling on the right side, without the tendency to fall, low response to nociception stimulation on the left hemithorax.

The seric concentrations of proteins, albumins, ureea, creatinin, bilirubin, ALT, AST and lactate dehydrogenase were investigated through blood biochemistry. Also, a CBC was done, both analyses showing no abnormal values for the age category of the patient.

In the necropsic exam, after opening the cranial cavity we observed the presence of a lobulated formation, of a pinkish-grey colour, with a large base, of aproximatelly 2cm/1cm in size, located on the right cerebral hemisphere, without protruding in its structure (Fig. 1).

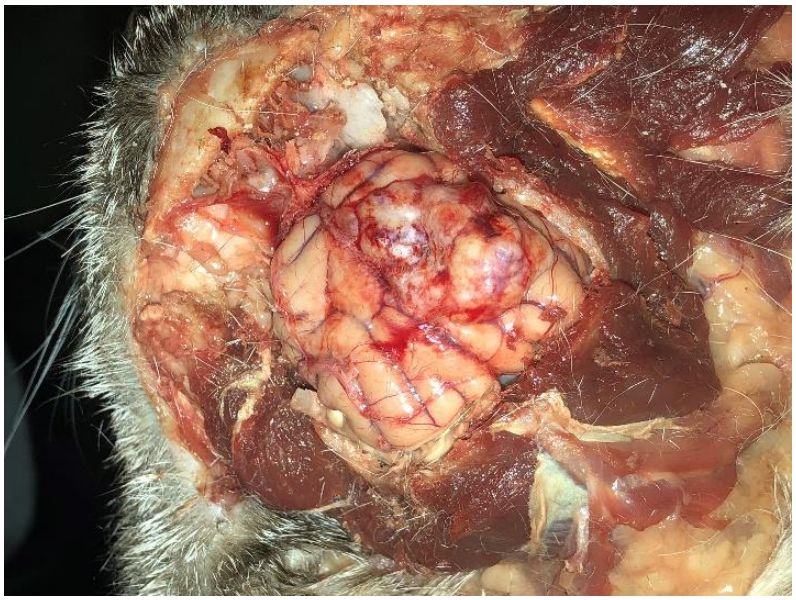

Fig. 1. Meningioma located on the right cerebral hemisphere

The formation was attached to the meninges and could be removed along with them, without showing any type of adherence to the nervous substance. On the right cerebral hemisphere, a concave print could be observed, formed due to the compression exerted by the neoplasia (Fig. 2, 3).

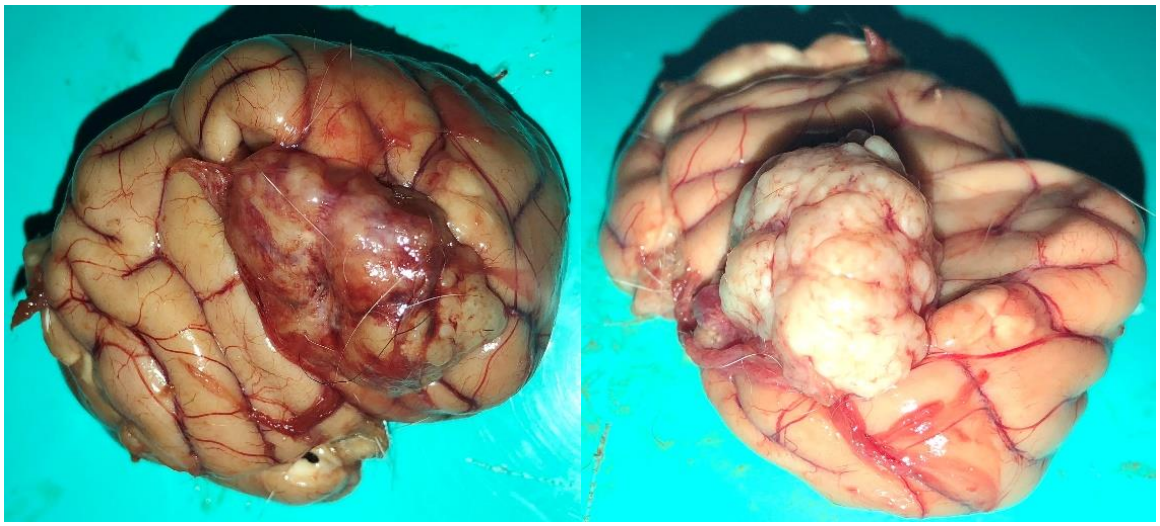

Fig. 2, 3. Meningioma partialy compressing the right cerebral hemisphere, leaving a concave print, suggesting nervous compression

On the histopathological exam we could see that the cells of this formation were spindle shaped and organized in nests or swirls, structures that were tied to each other and delimited by a fine, discrete conjunctive stroma (Fig. 4-13).

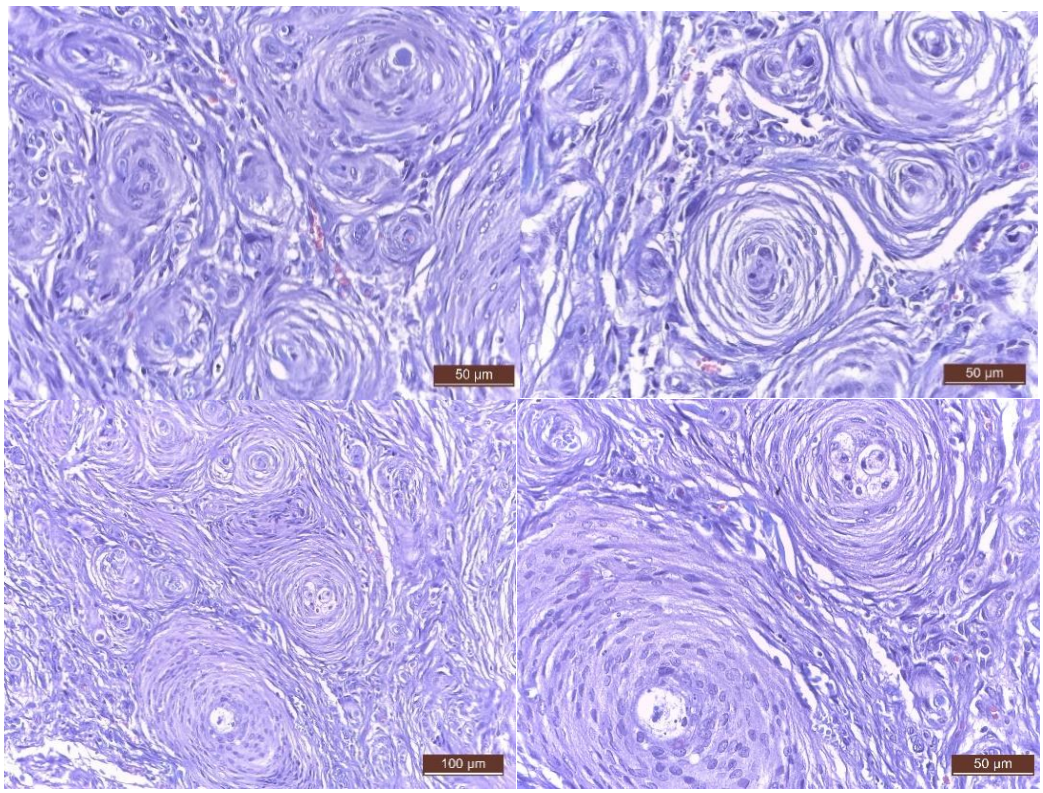

Fig. 4-7. Histopathological aspects of a meningioma indicating a psammomatous type, Masson's trichrome stain

The general microscopical aspect corresponds to the psammomatous type meningioma (Fig. 4-7), because of the classical cellular arrangement in nests and spirals. Still, some areas were more similar to a transitional meningioma [6], characterized by alternative territories of thick conjunctive fibers with a linear orientation (Fig. 9) or abundant eliptic and spindle shaped cells, that formed no particular structure (Fig. 12-13), sometimes manifesting anisocytosis and anisokaryosis and cytoplasmatic extensions (Fig. 9, 12, 13). Also, hyalin (Fig. 8) or necrotic areas were observed (Fig. 10-11).

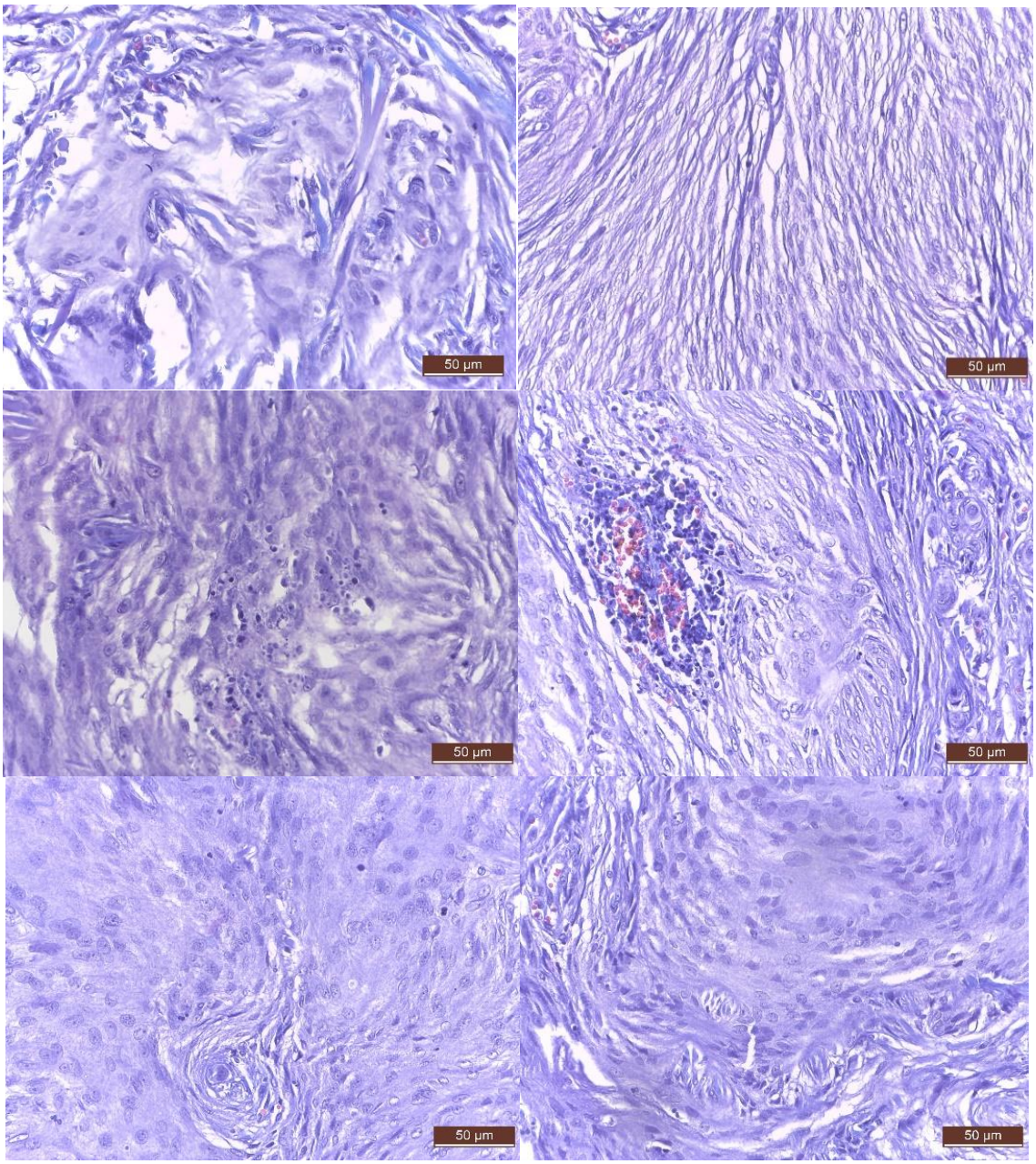

Fig. 8-13. Histopathological aspects of areas showing characteristics of mixt meningioma, with necrosis, hyalinisation, eliptic cells, lower concentration of collagen fibers and lack of organization into nests or swirls, Masson's trichrome stain

The margins of the tumour (Fig. 14-16) were characterized by an abundance of collagen fibers, the presence of neo-formation blood vessels and even in these areas we could see tumoral cells organized in swirls. On the surface of the formation a pseudo-capsule of compressed conjunctive fibers could be observed.

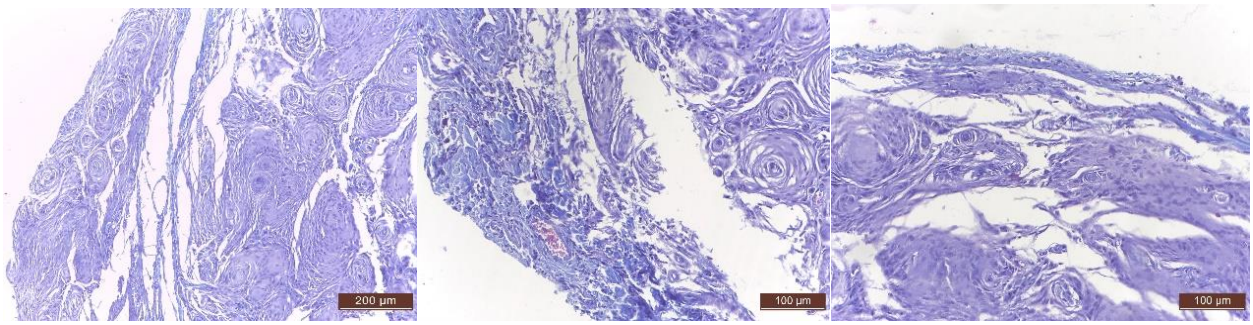

Fig. 14-16. The margins of the meningioma characterized by the presence of a pseudo-capsule of collagen fibers and compressed tissue, a few microcapillaries and tumoral cells displayed as swirls, Masson's trichrome stain

The benign character of the meningioma results from the fact that the tumour was well delimited, non-adherent to the surrounding tissues (except the meninges from which it originates), non-infiltrative, single, with a low vascularization and from a histological point of view, no mitotic figures, that would suggest an active cellular proliferation rate, could be observed.

Conclusions

The meningioma is a tumour that may, despite its benign character, cause the death of the animal that it affects through its localization, adjacent to the nervous tissue, causing compression and subsequent severe neurological signs that may prove lethal.

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LATE ARRIVALS

SECTION 1
AGRICULTURE AND FOOD ENGINEERING

The Role of Genetic Resources for the Development of Organic Farming and Decentralized Food Production

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Abstract

Nowadays, modern varieties of crops have been bred over a short period they have not respected local environmental conditions. The varieties are usually adapted to farming technologies and they are not able to respond to unfavourable environmental conditions.

Therefore, the most valuable varieties of genetics resources should be conserving by the on-farm method which assures a dynamic process. Our results are composed from more studies made in organic farming system from 2006 till now. We have been working with many varieties of cereals. Evaluation of landraces was oriented to analysis of its technological and nutritional quality and possibility of processing their products by small scale processing methods. Our results show potential of some landraces of cereals to be grown in organic farming system – high resistance to common diseases and competition ability against weed plant. Landraces had lower yield potential than modern varieties of cereals. Different situation was on less quality soils or low level of plant nutrition. The main advantage of landraces was quality of grain and they have potential to be grown in organic farming and used for the preparation of local high-quality products. In this case there will be combination of two important aspects – unique value of genetics resources and added value of organic growing methods.

Keywords: organic farming, genetics resources, cereals, quality, processing

Introduction

In the history of agriculture, the cropping pattern changed considerably. In a majority of agricultural crops, intensive and one-sided breeding results in narrowing the genetic base of the current range of varieties. This process is called genetic erosion and was confirmed by a number of studies [1]. Losses or damage of the genetic base of those resources are connected with reduction of possibilities of further genetic improvement of agricultural crops and their adaptation to the changing conditions and needs [2]. The modern varieties which are resistant to diseases and other stresses [3], give high yields that were mostly obtained by improving the current properties by the genes of wild species [4], and have the high antioxidant activity [5].

The protection of the gene pool takes place on two levels – in situ (on site preservation) and ex situ (off-side preservation) [6].

Cereals are good example of management and use of genetics resources in organic farming.

There is a group of hulled wheat species – *Triticum monococcum* L., *Triticum dicoccum* Schrank (Schuebl) and *Triticum spelta* L. [7]. Hulled wheats rank among often overlooked crops which, however, have a potential for future utilization in food industry. Due to their low demands on the environmental conditions, they are suitable for cultivation in areas less favourable for agriculture. Generally, hulled wheats give lower but stable yields of high-quality production [8]. The high quality of the production is interesting particularly with regard to healthy nutrition. In general, not only have hulled wheat a thicker aleurone layer [9] but also more the insoluble rest fractions than modern common wheat [10].

Over the last decades, the humankind has been increasingly addressing the matter of sustainable development in connection with the protection of the environment and its components. The most common sustainable farming method is organic farming. Organic farming often grows hulled wheat species, due to their low yield potentials in conventional farming, replaced by hybrids with higher yields [6]. Despite the lower yields, these species as example are interesting for farmers for several reasons. Given the complicated legislation regulating organic farming, the farmers find it more convenient to grow species from the group of genetics resources showing greater ecological plasticity. Their cultivation has a lower negative impact on the environment [11], and they give lower but stable yields [12].

The aim of the study is to evaluate the combination of yield and quality parameters of genetics resources of wheat and assessment of their value for use as source for the preparation of local food products. The second objective was also to evaluate its value for sustainable development of rural regions if local product was replaced by global product.

Methodology

Grain of genetics resources of hulled wheat species were produced in organic farming. Used varieties: mean values of four varieties of einkorn, eight varieties of spelt and seven varieties on spelt. As control we used two varieties of bread wheat. We use small plot trials in randomized plot design. Experiments were carrying out in organic farming system. We use four varieties of einkorn. The quality analysis of harvested grain was tested by The International Association for Cereal Chemistry (ICC) methods. Protein fractions were measured according methodology developed by Osborn.

Results and Discussion

According to CSN 46 1100-2 (Czech standard of quality), an amount of N-substances in wheat for food use should reach 10.8-13.7 %, which corresponds with the sample of bread wheat flour. The fact that hulled wheat is higher in protein has been confirmed within this study “Fig. 1”. The tested wheat varieties contained 16% of N-substances. The most important than protein content is also composition of protein fractions in the grain (Table 1). It is one from the main advantages of hulled wheat species from the group of genetics resources.

The results of determination of baking quality are summarized in Figure 1. The lowest protein content was measured in bread wheat flour. This fact is also confirmed by the classification in a statistically different group ($p<0.05$). Similarly, the lowest protein content was detected in white spelt flour. The observed result is consistent with the data published in the literature, where the protein content is normally referred above the limit of 15%.

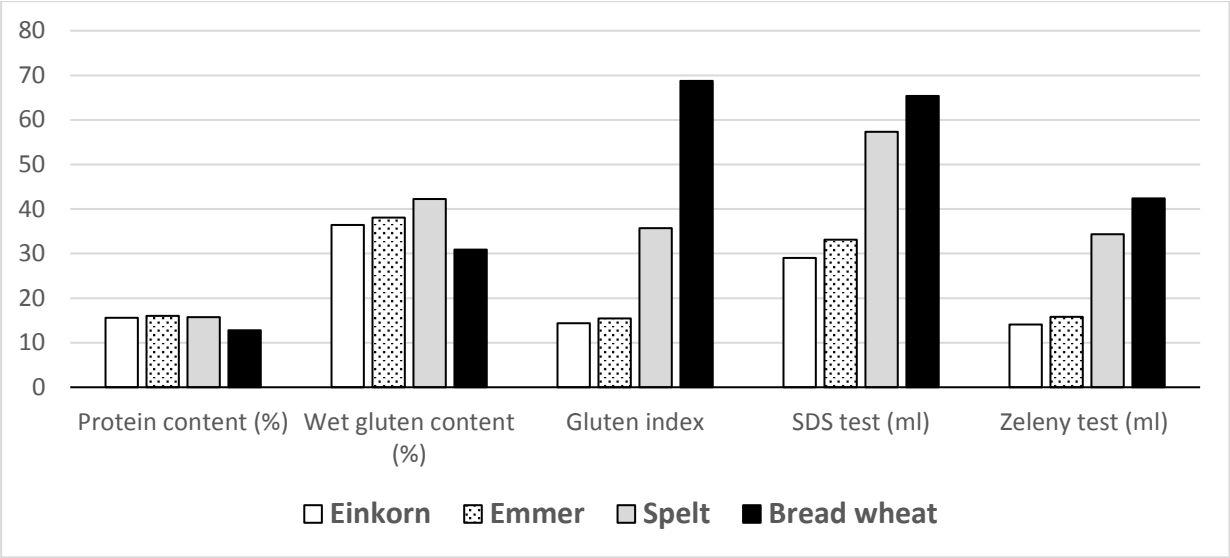

Fig. 1. Baking quality characteristics of different wheat species (mean and SD, three localities; four years

Table 1. Baking quality characteristics of different wheat species (mean and SD, three localities; four years)

Species	Gluten content (mg.100g^{-1})	Albumins + Globulins (%)	Gliadins (%)	Glutenins (%)	Insoluble rest (%)
Einkorn	153,45a	26,67b	27,93a	27,64a	17,76d
Emmer	194,86b	28,67c	30,06b	27,70a	13,67c
Spelt	248,17c	27,04c	31,03bc	29,95b	12,12b
Bread wheat	248,52c	21,38a	34,67c	36,96c	6,99a

Remark: Within column values followed by the same letter are not significantly different at P < 0.05 (Tukey HSD test).

The amount of wet gluten in the samples was in optimum quantity excluding white bread wheat flour that showed a statistically significant difference from white spelt flour. The values resulting from the Zeleny's test were generally low. Only bread wheat reached higher values.

Low values of sedimentation are general problems of hulled wheat, which are due to the genetic background to some extent. The gluten index was determined to assess the gluten quality. The highest amount of gluten was found in bread wheat flour. However, the value of Gluten index in whole-wheat bread flour was surprisingly low. A partial explanation was found based on the correlation analysis, because the flour had low Zeleny values. The correlation between these values and the values of Gluten index was statistically significant (r=0.89). Flour of both wheat varieties showed high values of the falling number – an indicator of damage to the starch grains due to the pre-harvest sprouting. The values are very high (exceed the standard). Such a high falling number may have negative effects on loaf volume, as well as the sensory evaluation of bread crumb. The most objective parameter of the baking quality is determination of the loaf volume. Whole-wheat bread showed the highest values. Conversely, the lowest values were found in whole-wheat spelt flour. The results did not fully correspond with the values regarding the Gluten index and Zeleny's test "Fig. 1". Correlation analysis results indicate negative correlation (r=-0.69) between the bread volume and protein content. A possible explanation is that spelt is higher protein content, but it is of lower baking quality than bread wheat "Fig. 2".

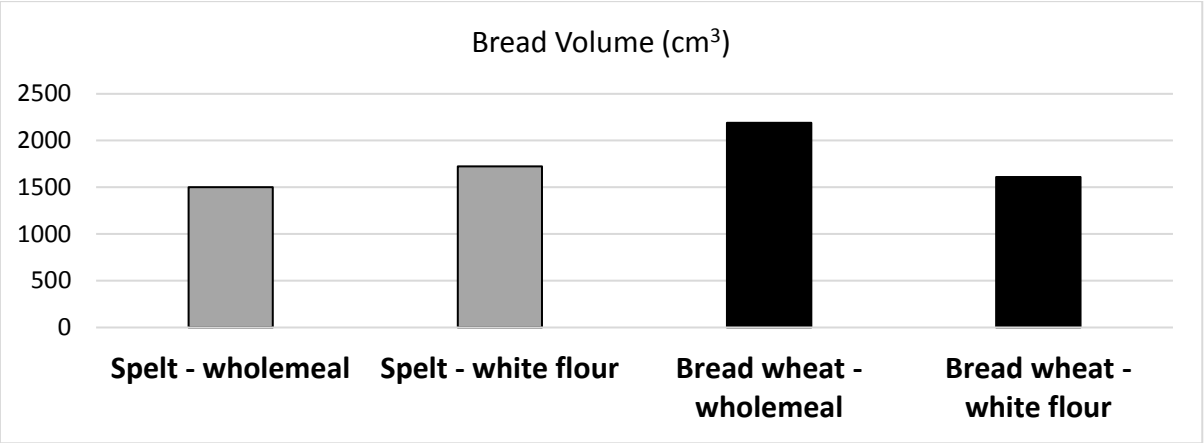

Fig. 2. Comparison of volume of different kinds of bread

Generally, the sedimentation index was low in hulled wheat samples. It may be therefore stated that these varieties are not suitable for baking purpose. Other values did not affect the quality of pasta to a great extent. However, Gluten index and the amount of sediment have an impact on the quality of pasta. Negative correlation indicates that increased Gluten index decreases an amount of sediment with 99.9% probability when cooking pasta. Gluten index of einkorn wheat is very low, which consequently resulted in the relatively large amount of sediment. The interesting is the dependence of wet gluten on the amount of water bound by pasta during boiling. The amount of water absorbed by pasta thus increases due to the higher wet gluten content together with the weight of pasta. Spelt showed the highest values of wet gluten and thereby the highest binding, conversely, the lowest amounts were found in bread wheat.

Discussions

It has been proven that hulled wheats have higher protein content (Figure. 1) than common wheat when grown in the same agronomic conditions [13]. Spelt wheat grains have high protein content (13-20%). The results from Italy [7] show that spelt wheats with a high protein content of grains can also be used for the production of pasta. The protein content of emmer wheat ranges widely from 9 to 18% [14]. However, according to Cubadda and Marconi [15], emmer's protein even reaches up to 20.6-21.9%. The protein content of emmer wheat is very variable and depends on the given site conditions. Einkorn wheat contains 13.2-22.8% proteins [16].

Because of the lower quality of gluten, which is rather running, the einkorn wheat flour is not suitable for the production of yeast doughs and yeast products [17]. Low values of SDS and Zeleny test and worse rheological properties are also typical of emmer wheat (this is also true for einkorn wheat) [18]. For example, einkorn wheat is suitable for the production of children's and special food due to the good transmission of properties connected with high protein and carotenoid content [17]. Einkorn wheat can also be used for the production of non-yeast products, biscuits and flakes. In macrobiotic diet, its germinated grains are used [19]. The only exception is spelt wheat, which could be used for the production of yeast products in a mixture with common wheat. In the study of Cubadda and Marconi [15] described a high baking quality of spelt wheat despite that fact that the spelt gluten is less firm and the dough has worse rheological properties.

The important aspect of hulled wheat growing and processing in organic farming is also its dynamic management as source of genes for the future. In case of perspective and most valuable genetics resources is supported conservation.

Conclusions

Hulled wheat from the genetics resources constitutes an interesting alternative for the preparation of organic food. Due to the lower requirements for growing conditions, they may also be grown in less favourable areas for the cultivation of cereals. In addition to the production aspect itself, the cultivation of hulled wheats in organic farming shows a large number of other positives which are further studied. The use of hulled wheats in nature-like farming systems not only contributes to sustainable development but can also help the regional economy due to the processing of products right at the place of origin. So, a part of the profit can remain with the local farmers. At the same time, it is an interesting alternative e.g. in the current development of agritourism at farms (a tourist is also a purchaser of products). In this respect, organic farming, including the cultivation of hulled wheats, is an irreplaceable element of this large chain.

Ackowledgement

Our work was supported by the research project No. NAZV QK1910046 of the Ministry of Agriculture of the Czech Republic and the University of South Bohemia in České Budějovice (project No. GAJU 059/2019/Z).

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Wheat Rusts: The Effect of Climatic Conditions Variability on Wheat Rust Pathogens

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Abstract

The climatic conditions specific to wheat cropping area of north-east of Romania favor the occurrence and development of many pathogens which lead to quantitative and qualitative losses. The aim of this paper is the characterization of eleven Romanian winter wheat varieties: Bezostaia 1 (control variant), Glosa, Izvor, Miranda FDL, Otilia, Pajura, Pitar, Semnal, Ursita, Andrada and Codru. Nowadays some of these cultivars are widely distributed around the country (e.g., Glosa, Miranda FDL, Izvor, etc.), while others are newly created and look like they have a good production potential (Pitar, Pajura, Ursita, etc.), and last but not least this study relied on Bezostaia 1 as its case control variant, an old winter wheat cultivar extensively used in experimental trials, due to its high yield potential and superior quality traits. The research spanned over four agricultural years, 2015-2019. The climatic conditions specific for the interval of time made it to be present all three pathogens which produce diseases know as wheat rusts, although in this part of Romania is usually present just Puccinia triticina Ericks.

Keywords: winter wheat, rusts, Puccinia triticina, Puccinia striiformis, Puccinia graminis

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple grains and represent the leading source of calories plant-derived protein in human food [1], [2]. Wheat crop is affected by a many diseases that cause significant quantitative and qualitative losses. Also diseases damaging the food, feed and seed production. From the many diseases present in wheat crops, the rusts, are responsible for yields decrease and also contribute to quality parameters decrease.

Wheat rusts, brown/leaf rust, yellow/stripe rust and black/stem rust caused by *Puccinia triticina* Ericks., *Puccinia striiformis* Westend. f.sp. *tritici* and *Puccinia graminis* f.sp. tritici Ericks. and Henn. are important diseases and severe infestation has been reported in the last years in different areas worldwide [3], [4], [5], [6]. This biotrophs reduce the total photosynthetic area, consume plants assimilates and interrupt with normal growth of host, leading to reduction in yield grains. The reduction in yield due to the rusts may lead to severe economic losses as reported in areas where these diseases were present. Wheat yield losses associated to rusts pathogens reach up to 70% in susceptible cultivars, when are not applied the correct measures to control the diseases [7], [8].

The relationships between climatic conditions and wheat rust diseases have been extensively studied [9], [10] and the most important factors for the infection process and progress of wheat rusts are air temperature, precipitation and relative humidity [11]. Generally, optimum air temperatures for the germination of wheat rusts uredospores ranged from 12^0C to 15^0C, with the germination process stopping at temperatures over 35^0C [12]. Another important aspect is

represented by leaf wetness induced by air moisture which is essential for uredospores germination and leaf penetration [10].

Methodology

Study site and disease monitoring

Between 2015 and 2019 the occurrence and frequency of wheat rust pathogens was studied.

A total number of eleven winter wheat cultivars, represented by Romanian varieties (from N.A.R.D.I. Fundulea: Glosa, Izvor, Miranda FDL, Otilia, Pajura, Pitar, Semnal, Ursita, respectively from A.R.D.S. Turda: Andrada, Codru), as well by the old Russian variety Bezostaia 1, used as a long-term control variant in comparative crops, were the biological materials used in the study (Table 1).

The experience was placed in the experimental field of the Iasi Didactic Station, the "Ezăreni" farm, being organized according to the randomized block diagram, in three replicates, each wheat cultivar representing an experimental variant. In the experience, the specific technology of wheat cultivation was applied; no treatments against pathogens were performed.

Table 1. Wheat varieties assessed for behaviour in relation with rusts, during 2015-2019

No.	Variety	Parentage-Pedigree	Rusts Gene
1.	BEZOSTAIA 1	LUTESCENS-17/SKOROSPELKA-2	**Sr5**, **Sr9**; **Sr5+**; **Sr8b**; **Sr42**; **SrTmp**; **Lr1**; **Lr3a**; **Lr34**; **Lr10**; **Lr22**; **Yr9**; **Yr18**;
2.	GLOSA	DELABRAD "S"/F508U1-1 //DELABRAD "S"	**Lr34;**
3.	IZVOR	Karl/201R2-111//508U1-1	**Lr34;**
4.	MIRANDA FDL	ERYT26221/96869G1-//GLOSA	**Lr34;**
5.	OTILIA	F96052G16-2/FAUR	**Lr34;**
6.	PAJURA	IZVOR/F-96012-G-2-2//GLOSA	**Lr34;**
7.	PITAR	02555-GP-2/00099-GP-2	**Lr34;**
8.	SEMNAL	05511GP4/LITERA	-no data found
9.	URSITA	00628G34-2/2*GLOSA	-no data found
10.	ANDRADA	DROPIA/T-57-90	-no recorded gene
11.	CODRU	FUNDULEA-4/T-56-95	-no recorded gene

The observations to identify the presence of pathogens were conducted between March-June each year. In order to determine Frequency (F%), Intensity (I%) and to calculate the Degree of Attack (DA%), were made observation with metric frame (50x50 cm) in each variant of the three replications. The genotype reaction was estimated by F.A.O. scale rate, with 9 attack classes in which, 1 = very resistant; 9 = very sensitive [15], [16].

Regional climatic conditions

The effects of climate change represent a significant threat to global food security, not only by increasing global average temperatures, estimated at 1.5-2℃ during the 20th and 21st centuries, but also through increasing the frequency and severity of extreme weather events.

The increase of heat waves, drought, floods and the pressure exerted by pests create direct demands on crops, which leads to reduced yields [13].

The first decade of the 21st century has positioned the north-eastern area of Romania in an unprecedented position. The region of Moldova is forced, lately, to face very hot and drought and very humid and rainy periods. These climatic events in some cases exceed the relevant meteorological observations, some of them varying strongly from the multiannual average values recorded so far [14].

The characteristics of the climate of a region are very important for the geographical environment of the respective region, for the life of the people in that area, as well as for the economic activities, or of another's activities developed in that area. As a whole, the climate of the north-eastern part of Romania can be characterized by the presence of favourable features, but on this background are frequently observed climatic events which limited the high potential of this geographical area. Sometimes, the manifestations of climatic elements and phenomena are quite unusual, creating real difficulties for people and damaging the economy.

Having in view that the experiments were performed under field conditions, the results were influenced by the climatic factors specific to the analyzed period. Table 2 presents the air temperatures, rainfall and relative humidity registered at Ezareni Farm – Iasi Didactic Station during March-July, 2015-2019. The climatic data on the air temperature, rainfall and relative humidity recorded during the studied period were carefully analyzed for comparisons between the four studied agricultural years, as well as for observing the trends of evolution of air temperature over time.

Table 2. Climatic conditions at Ezareni Farm – Iasi Didactic Station, during 2016-2019

		March	**April**	**May**	**June**	**July**
Air Temperature (^{0}C)						
Multiannual average (^{0}C) (last century)		**3.1**	**10.2**	**16.0**	**19.5**	**21.2**
2016	Month average (^{0}C)	6.5	13.3	15.3	20.9	22.6
	Deviation (^{0}C)	+3.4	+3.1	-0.7	+1.4	+1.4
2017	Month average (^{0}C)	8.0	10.0	16.1	21.1	21.6
	Deviation (^{0}C)	+4.9	-0.2	+0.01	+1.6	+0.04
2018	Month average (^{0}C)	1.2	15.4	18.7	20.8	21.3
	Deviation (^{0}C)	-1.9	+5.2	+2.7	+1.3	+0.1
2019	Month average (^{0}C)	7.3	10.6	16.1	21.9	21.2
	Deviation (^{0}C)	+4.2	+0.4	+0.1	+2.4	0.0
Rainfall (mm)						
Multiannual sum (mm) (last century)		**28.4**	**43.9**	**55.9**	**82.6**	**69.3**
2016	Month sum (mm)	33.8	76.2	70.4	142.4	24.0
	Deviation (mm)	+5.4	+32.3	+14.5	+59.8	-45.3
2017	Month sum (mm)	107.0	140.4	72.8	71.6	84.4
	Deviation (mm)	+78.6	+96.5	+16.9	-11.0	+15.1
2018	Month sum (mm)	56.8	18.0	16.8	216.0	136.6
	Deviation (mm)	+28.4	-25.9	-39.1	+133.4	+67.3
2019	Month sum (mm)	40.4	62.6	125.2	113.8	24.2
	Deviation (mm)	+12.0	+18.7	+69.3	+31.2	-45.1

Table 2. continuation

HC Relative Humidity (%)						
Multiannual average (%) (last century)		**80.0**	**72.0**	**70.0**	**72.0**	**72.0**
2016	Month average (%)	72.9	69.0	70.7	76.0	60.0
	Deviation (%)	-7.1	-3.0	+0.7	+4.0	-12.0
2017	Month average (%)	80.1	65.7	65.9	65.6	69.0
	Deviation (%)	+0.1	-6.3	-4.1	-6.4	-3.0
2018	Month average (%)	85.3	60.0	59.5	73.1	80.1
	Deviation (%)	+5.3	-12.0	-10.5	+1.1	-8.1
2019	Month average (%)	61.5	65.4	80.9	78.2	70.8
	Deviation (%)	-18.5	-6.6	+10.9	+6.2	-1.2

Results and Discussion

The experiments were performed under natural field conditions, and the infection with rust pathogens was made under natural infection conditions, the inoculum has been provided by the nearby winter wheat fields.

Under the climatic conditions of the four agricultural years studied (2015-2019), following the observations made on the eleven winter wheat cultivars, the presence of wheat rust pathogens was observed with different values of frequency and severity of attack.

Puccinia triticina is the rust pathogen which is wide-spread in this area. The disease is noticed every year in wheat fields with different frequency and intensity of the attack. Of the three rusts of wheat, *Puccinia triticina* has been observed in all years of research (Table 3).

Of the four years of research, the highest values of the DA% were recorded under the climatic condition of spring 2016. In this year with the exception of wheat cultivar Pajura, where the pathogen was not present, in all cultivars, the pathogen was recorded with different values of DA%.

Table 3. Leaf rust caused by *Puccinia triticina* – (DA%), during 2015-2019

No.	Variety	***Puccinia triticina*** **Degree of Attack (%)**							
		2016		**2017**		**2018**		**2019**	
1.	**BEZOSTAIA 1**	**3.57±0.57**	**Cv**	**1.40+0.28**	**Cv**	**0.29+0.02**	**Cv**	**1.80±0.51**	**Cv**
2.	GLOSA	3.64±0.29	Ns	1.16+0.21	Ns	0.67+0.24	*	1.34±0.40	Ns
3.	IZVOR	2.28+0.52	Ns	0.00+0.00	**	0.00+0.00	Ns	0.00±0.00	***
4.	MIRANDA FDL	2.14+0.79	Ns	**2.39+0.53**	Ns	0.44+0.17	Ns	1.69±0.31	Ns
5.	OTILIA	1.58+1.41	*	0.00+0.00	**	0.00+0.00	Ns	0.00±0.00	***
6.	PAJURA	0.00+0.00	***	0.00+0.00	**	0.00+0.00	Ns	0.00±0.00	***
7.	PITAR	0.06+0.06	***	0.00+0.00	**	0.14+0.01	Ns	0.00±0.00	***
8.	SEMNAL	0.71+0.19	**	0.00+0.00	**	0.00+0.00	Ns	0.32±0.32	***
9.	URSITA	0.45+0.26	**	0.00+0.00	**	0.00+0.00	Ns	0.00±0.00	***
10.	ANDRADA	4.27+0.66	Ns	1.92+0.94	Ns	0.68+0.19	*	0.84±0.18	*
11.	CODRU	4.20+0.34	Ns	1.11+0.28	Ns	**1.70+0.20**	***	1.22±0.15	Ns

Ns - Not Significant (P>0.05) Cv – control variant
* - Significant (P>0.01)
** - Distinguish significant (P>0.001)
*** - Very significant (P<0.001)

In the last three years of research *Puccinia triticina* was recorded with smaller values of DA% and not in all cultivars studied.

The highest values of DA% recorded in 2017 was 2.39+0.53% by Miranda FDL wheat cultivar.

The highest values of DA% recorded in 2018 was 1.70+0.20% by Codru wheat cultivar.

The highest values of DA% recorded in 2019 was 1.80+0.51% by Bezostaia 1 wheat cultivar.

In the last two years of research another wheat rust was recorded in the field (Table 4).

Yellow rust of wheat caused by *Puccinia striiformis* f.sp. *tritici* are usually recorded in the south part of Romania, but under the conditions of climate change we can observe that this disease is present and another areas where in the past didn't meet the conditions necessary for the production of infections.

Table 4. Yellow rust caused by *Puccinia striiformis* f.sp. *tritici* – (DA%), during 2015-2019

No.	Variety	***Puccinia striiformis* f.sp. *tritici* Degree of Attack (%)**							
		2016		**2017**		**2018**		**2019**	
1.	**BEZOSTAIA 1**	**0.00±0.00**	**Cv**	**0.00±0.00**	**Cv**	**1.55+1.02**	**Cv**	**1.37±0.27**	**Cv**
2.	GLOSA	0.00±0.00	■	0.00±0.00	■	0.00±0.00	*	0.00±0.00	***
3.	IZVOR	0.00±0.00	■	0.00±0.00	■	0.78+0.19	Ns	0.47±0.14	***
4.	MIRANDA FDL	0.00±0.00	■	0.00±0.00	■	0.58+0.30	Ns	0.32±0.10	***
5.	OTILIA	0.00±0.00	■	0.00±0.00	■	0.00±0.00	*	0.00±0.00	***
6.	PAJURA	0.00±0.00	■	0.00±0.00	■	1.20+0.76	Ns	0.78±0.08	**
7.	PITAR	0.00±0.00	■	0.00±0.00	■	0.95+0.59	Ns	0.00±0.00	***
8.	SEMNAL	0.00±0.00	■	0.00±0.00	■	0.00±0.00	*	0.00±0.00	***
9.	URSITA	0.00±0.00	■	0.00±0.00	■	0.53+0.26	Ns	0.00±0.00	***
10.	ANDRADA	0.00±0.00	■	0.00±0.00	■	0.00±0.00	*	0.00±0.00	***
11.	CODRU	0.00±0.00	■	0.00±0.00	■	0.00±0.00	*	0.00±0.00	***

Ns - Not Significant (P>0.05)
* - Significant (P>0.01)
** - Distinguish significant (P>0.001)
*** - Very significant (P<0.001)

Cv – control variant
■ the pathogen was not recorded in that year

The third rust of wheat, black or stem rust, caused by *Puccinia graminis* f.sp. *tritici*. This pathogen is also not usually found in the north-east area of Moldova, but in the 2016-2017 agricultural year, he was recorded just in a case, represented by Glosa wheat variety. For this pathogen was calculated the frequency of attack, which was 48.24%.

Conclusions

Leaf rust (*Puccinia triticina* Ericks): Based on observations regarding the wheat cultivars reaction to this disease during 2015-2019, under natural infection, we can see that the strongest attack took place in 2016. In the next years, the leaf rust attack was recorded with lower values of attack, due to unfavourable for diseases climatic conditions, but in these years the climatic conditions were favourable for the other two wheat rusts yellow rust (*Puccinia striiformis* Westend. f.sp. *tritici*) and black rust (*Puccinia graminis* f.sp. tritici Ericks. and Henn).

The winter wheat varieties exhibited a wide variability over the studied pathogens, during the study period, variability determined by the characteristics of the agricultural year and by each winter wheat variety.

In conclusion, researchers and farmers need to monitor the presence of rusts in wheat crops, because data presented in recent years show that these pathogens expand their range and are capable to produce important yield losses in the areas where they were out of control.

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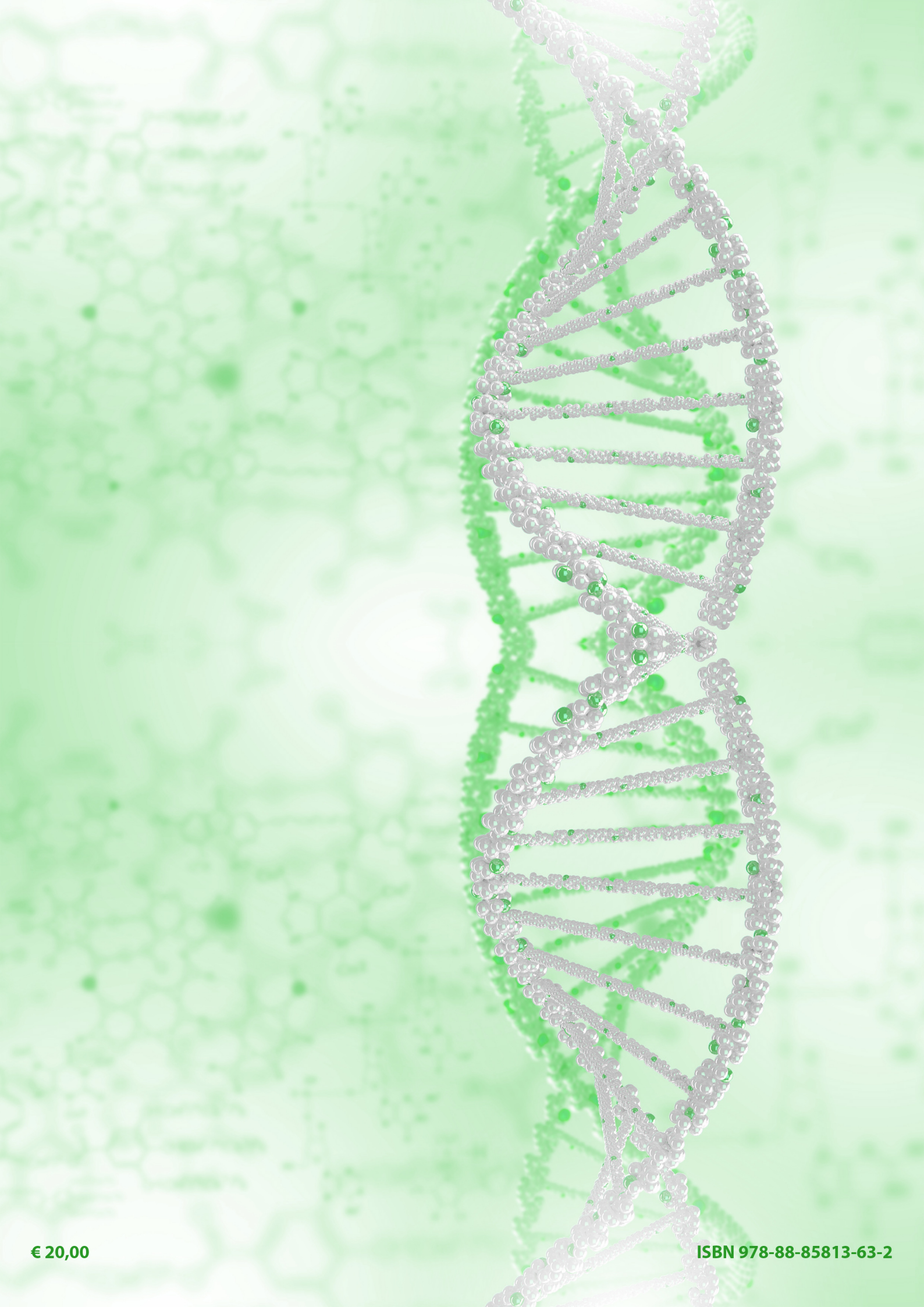

€ 20,00

ISBN 978-88-85813-63-2