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„RESEARCHES REGARDING COW, SHEEP AND GOAT MILK CYTOMORPHOLOGY AND ITS HYGIENICAL QUALITY"

Thesis for acquiring the title of „ Doctor in Sciences"
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ABSTRACT

The milk represents a mammary gland product and constitutes from a physical – chemical point of view, a heterogeneous dispersed system in which lactose and mineral salts form true solutions, with proteic substances in coloidal phase and fat under the form of emulsion. In raw milk, right after milking all those compounds consisits in a homogeneous mixture.

For a long time, various statistics accepted milk consumption on a resident head as a index of welfare and prosperity.

By the contained elements milk represents a complete food, being the ideal aliment of the new-born in the first months of life and recomended to childs and adults irrespective age, work conditions and with few exceptions, health state.

From this cause the milk was called „white blood" or „the elixir of life and child health". This is because the fact it contains almost all the substances necessary to human and animal organism in a easy intake form.

It is observed, more and more, consumers preocupation for a milk that is satisfactory not only for quantitativ desideratum, but specially for hygienic quality (health state).

It is accepted to be consumed only that milk which respects the limits of determined germs number, a established cells number, a milk without additional water, with well precised acidity values, etc.

All these conditions weren't easily imposed, being need a restrictive legislation, scientifical fundamented, to convince the producers and milk processors, that quality indicators of hygienical quality must pass compulsory before quantity index, when is about milk consumption and health.

To establish milk quality in function of somatic cell number and total germs number from milk, it's necesary to be identified the factors that have influence over their content in milk and, what are the economical losses produced by cells and germs high level.

The thesis is composed of two different parts. In the first one, **The Stage Of Knowledge** developed during 3 chapters, comprising 58 pages on the base of bibliographical datas, it's made a synthesis of speciality literature regarding knowledge actual level in the researched theme. Between those there are refferences regarding mammary gland morphophysiology (**chapter I**), total germs number (**chapter II**), somatic cells number (**chapter III**) and factors that influence this parametres.

Mammary gland and it's function is an extraordinary biological type, because makes possible the study of organogenesis, functional relations between different cell types,

secretion mechanisms, hormonals messages transmission (specially prolactine), hormonal receptors, genetic expression control, metabolism and mother behaviour, immunological mechanisms of mammae and new-born protection.

The importance of total germs number and somatic cells number for the healh of mammary gland and implicit of milk hygienical quality was observed relative late and it is now one of the hygienical quality index of milk for human consumption.

The second part "***Own researches***" is developed during 5 chapters, comprising 157 pages and presents ***aim and research objectives; dinamic of milk NTG individual level, in taurines, ovines, goats populations; dinamic of milk SCN individual level, in taurines, ovines, goats populations*****; *citomorphological expression of NCS at the monitored taurine, ovine and caprine populations***

In the 4th chapter intituled "***Aim and research objectives***" it is presented the importance of studying parameters, somatic cell number and total germs number, from the point of view of hygienical quality of milk and proposed objectives to accomplish the goal.

In the 5th chapter " ***Dinamic of milk NTG individual level, in taurines, ovines, goats populations***" it was described the researched biological material, represented by milk samples individually obtained from taurines, ovines and goats. Researches developed in Suceava and Iaşi counties. For each specie were taken for study 2 populations of animals breeded and exploited in similar conditions.

There were presented work methods, classical and modern.

To determine total germs number using quick method, on the milk samples obtained from taurines Bactocount 50 devise was used.

For the milk samples obtained from goats and ovines BactoCount IBCm devise was used.

Datas obtained after laboratory analysis were performed statistically. For statistic performing S.A.V.C. programe (Statistic, Variance analysis and Covariance) elaborated during 2000 – 2003 by Conf. Dr. Vasile Maciuc from Zootehny Faculty USAMV Iaşi was used.

To establish total germs number were harvested 2 samples monthly, 1200 samples from taurines, 652 from ovines and 664 from goats.

Obtained results analysis shows a number of 1080 (90%) from the 1200 samples from **taurines** acomplish European Union hygiene satandards (100000 germs/ml), the rest number of 120 (10%)samples being not suitable for human consumption. The most decreased values were observed during winter period, and the most increased during summer period.

For the samples came from **ovines**, after analyzing average monthly values and variability estimation of the total germs number, was revealed the fact that the milk from studied animals was generally a „healthy milk", because the average germs number was under 1500 x 10^3 germs/ml.

From the 652 samples, a number of 49 (7,51%) samples overreached the limits of total germs number admitted by European Union, the rest number of 603 (92,49 %) samples being allowed for human consumption.

Although the average values are between the limits of a milk considered healthy, there are evidence whose analyses showed a high increasing of the germs number. Those values showed the existence of some sick animals. After treatment the sick animals, in the next period decreased the germs number from milk.

Regarding the milk samples from **goats**, after analysing the obtained results performing datas, it was observed that the total germs number computed monthly registered inferiour values toward admited limit (1500 x 10^3 germs / ml).

A number of 44 (6,62 %) samples from the 664 samples overreach the normal limit (1500 x 10^3germs/ml), the rest number of 620 (93,37%) samples being in the recomanded norms (1500 x 10^3 germs /ml).

In the 6th chapter was followed to establish somatic cell number from the taurines, ovines, goats milk.

To determine somatic cells number from milk classical and modern methods were used.

Somatic cells number in cow milk was establish using **Somacount 150** device.

For the sheep and goat milk **BactoCount IBCm** device was used.

The results allowed us, after systematization and performing, an individual and dynamic presentation of the somatic cells number for each plot, during each lactation.

To determine somatic cells number in **taurines** milk 600 samples were taken, 326 from ovines and 331 from goat.

The average monthly values and variability estimation show the fact that the milk from **taurines** in 2007, from the point of view of the somatic cells number is not in the Order 682 /2006 admitted limits (400000 cells / ml milk).

From the 300 milk samples collected during 2007 from **taurines** a number of 91 (30,3%) samples doesn't fulfilled the hygienical conditions imposed by E.U. (400000 cells /ml milk), being not suitable for human consumption. The most frequent cause for not hygienical milk is the health state of the mammary gland. From the factors identified in our

study which could influence the values of the milk somatic cells number and that appear in the speciality literature

are the animal age, mammary repause, knowing that the values of somatic cells number increases before anf after mammary repose, incomplete milking.

From the 300 samples harvested during 2008, a number of 59 (34,35) samples are not corresponding from the hygienical point of view. This procent is much bigger then the one obtained during 2007 (34,35 %)

After analysing the 163 **ovine milk samples**, regarding somatic cells number, it was observed that a number of 56 (34,35 %) samples are not suitable for human consumption because of the increased number of somatic cells because of the mammary gland health state, eventually defective milking and advanced age of animals.

From the 168 samples harvested during 2007, 31,54% weren't suitable for human consumption because of the somatic cells number.

From the 163 samples, just 69,33% may be used for human consumption, the rest being not suitable from hygienical point of view.

Somatic cells number from milk presented variations in function of the mamary gland health state and from a harvesting to another. In animals at fisrt lactation cells number is smaller, increasing progresivly with lactation number.

Milk somatic cell number increasing over admitted values is abnormal and indicates the evolution of un inflammatory process inflamator at the mamary gland level. This increasing of the somatic cells number reaches maximum values at the begining of the inflammatory process, and the decreasing is gradual made, cells number being increased after healing too, during more days and even weeks.

Because of the neutrophils increased number, 50-70% of NCS toward just 5-20% in normal cow milk (**Dulin and col., 1983; Poutrel and Lerondelle, 1983**), goats are less predisposed in mamary infections.

Cytomorphological expression of the milk obtained from the three species taken in study (7th chapter) was realized using lactocytograma on the blood smears.

The staining method used was May – Grünwald – Giemsa, which wasn'n able, till now, to be substituted with any other method to study cells morphology.

In the milk collected from the healthy mamary gland, after cytomorphologic exam were observed neutrophile with multilobate nucleus, well individualized lobular segments, amphophile cytoplasma, hypercromatic.

Likewise were identified macrophages in cours of activation, with hypercromatic nucleus and vacuolized cytoplasma. Lymphocites were rarely encountred on lactosediment

and perform compact, hypercromatic, round shape nucleus, with basophile cytoplasma, ring shape.

Neutrophile polymorphonuclear cells were encountred beside epithelial cells. These cells appear in milk because of the physiological peeling off mamary canalicular mamar – alveolar, canalicular, cysternal epithelium.

In forms of incipient mamitis at **taurines** increased total germs and somatic cells number, reaching values of 856979 germs/ml, respectivly 989576 cells/ml. In microbiologic exam Escherichia coli was identified.

From the cytomorphological point of view, se observă preponderența celulelor PMN neutrofils and macrophages cells preponderence was observed, activated for germ microphagocitosis.

Cytomorphologic, in cases of incipient mamitis, the preponderence of PMN neutrophile cells and macrophages, activated for germs microphagocytosis was observed.

Cellular population is numerous with intense phagocytosis activity, activated and hyperactivated macrophages in various phases of necrobiosis and necrosis.

In cronical streptococcal mamitis, somatic cells number reaches values of 867567 cells/ml, and total germs number reaches till 598567 germs/ml. Morphologically it was observed the presence of an increased number of PMN neutrophile and macrophages with intens fagocytic activity.

In **ovines**, milk with normal aspect processed in hygienical conditions presented the lactosediment in reduced amount, sometimes barely visible, who was easily flaunted giving a homogenous smear, without crowds and precipitate.

In cytological exam, the smear contains a rare and dispersed cellular population, with cells in groups of 2 – 3 each, composed by neutrophils, with intens sequenced nucleus, lymphocites, even eosinophils, with intens basophil stained nucleus.

In milk came from clinical healthy **goats**, examining the smear un increased number of neutrophils with multilobed nucleus, neactivated and in course of activation macrophages, rare lymphocites were observed.

In subacut phase of streptococcal mammitis, it's interesting that besides polymorfonuclear neutrophile cells appear also 10 – 15 % eosinophile cells from PMN population. Probabilly, these appear because of the massive epithelial distruction in mammary gland.

In the context of the cellular population in mammitic milk appear hyperactivated monocytes – macrophages in groups of 2-3 cells with well consolidated cytoplasmatic bounds.

On the respective milk samples determinations regarding NCS and NTG were made.

In this situation these two parametres values overreached admitted values in our country, respectivly, NCS - 1132000 cells/ml over $1000x10^3$ cells/ml and NTG – 1132000 germs/ml over 1500 x 10^3 germs /ml.

Using cytomorphologic exam it was observed the association streptococcal infection process with staphylococcic germs, the process gaining character of acute streptococcal and staphylococcic mammitis. Cytomorphologic exam of the sediment confirmed too the direct relationship with the increased cells number. Morphologically it was observed the presence of an increased number of PMN neutrophile with great phagocytic activity and numerous epitheliale cells in various phases of necrosis and necrobiosis and various microorganisms.

Lactosediment cytomorphologic exam realised from retension milk, at the end of the lactation, showed that cells morphological aspects is not very different from the ones in normal milk.

The cytosediment was significant increased similar to mamitis cases, producing a reach smear with homogeneous aspect.

In 8th chapter "***Final conclusions***", are syntetised the results of the research made on milk samples collected from taurines, ovines and goats, performed using classical and modern methods, conform to effectual normatives.