ISSN (print) 1454-7406 ISSN (electronic) 2393-4603

# "ION IONESCU DE LA BRAD" IASI UNIVERSITY OF LIFE SCIENCES (IULS)



# SCIENTIFIC PAPERS VETERINARY MEDICINE

# LUCRĂRI ȘTIINȚIFICE SERIA MEDICINĂ VETERINARĂ

# 2023

# **VOLUME 66**

# NO. 1

PUBLISHING "ION IONESCU DE LA BRAD"



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## CARBOHYDRATE CONTENT ASSESSMENT IN DIFFERENT COMMERCIAL DOGS DIETS

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#### Abstract

The growing awareness of the needs of pets by pet owners has also led to a diversity of diets on the market. Given that a proper diet is the best prevention for diseases such as obesity, diabetes, adverse food reactions, gastrointestinal disorders and even cancer, it is vital to guide owners early on towards an optimal diet for the physiological requirements of each individual pet. An increasingly debated topic in canine nutrition lately is the percentage of carbohydrates in dog food. The International Research Council has concluded that there is no need for carbohydrates at all in dog diets (corn, rice, potatoes, barley, etc.). However, carbohydrates are the dominant ingredient in most dry foods and they are abundantly present. They are not harmful to dogs when present in reasonable quantities. Carbohydrates provide a high source of energy, but the problem is the large amount in which they are found in many types of dry food. While protein, fat, fiber and moisture are always listed on a package label as part of the chemical analysis, pet food manufacturers are not required to list 'carbohydrates' in the food as they are the main macronutrient determining postprandial glucose levels. Because of this concern, we thought it appropriate to bring to the attention of dog owners the percentage of carbohydrates in different categories of dry dog food, as well as the types of cereals included in the dry food that have a different glycemic index, which is a system that measures the effects that carbohydrates in food have on blood sugar levels.

Key words: dogs, dry food, carbohydrates, glycemic index

The pet population has been gradually increasing in Europe recently. It is estimated that 80 million European households have at least one pet (FEDIAF 2020). Pets arguably play a particularly important role in the lives of people who consider their pets as "family members" (Di Cerbo A. et al, 2017; Rauktis M.E. et al, 2017). Due to the increasing number of pets, commercial pet food is also developing dynamically, with owners becoming increasingly interested in its ingredients and quality. The prevalent type of pet food available on the market is dry food formulated in kibble form, as it is easy to store and effective in meeting the nutritional needs of the pet. Dry food diets mostly consist of cereal grains, milling byproducts, and byproducts of animal tissues from the meat-packing, poultry-processing, and fish-canning sectors (Kazimiersk K. et al, 2021).

The majority of the carbohydrates in dry food diets, which range from 30 to 60 percent, are starches made from cereal grains including wheat, corn, and rice. (Spears and Fahey 2004, de-Oliveira et al. 2008, Case 2011). Carbohydrates are an affordable ingredient and a crucial component of dry food that gives kibbles an adequate

structure. (Hand Michael et al. 2010). In particular stages of development, carbohydrates enable dogs to store vital nutrients like amino acids or fatty acids. However, there is no information available regarding the unique carbohydrate needs of companion animals (FEDIAF, 2022; NRC, 2006). Protein, fat, fiber and moisture are always indicated on a packaging label as part of the chemical analysis, whereas pet food manufacturers are not legally required to mention 'ash' or 'carbohydrates' in the food (Vastolo A. et al, 2023). Starch digestibility has been demonstrated to be very varied and affected by a number of variables, including sources, particle size, the amylose: amylopectin ratio, processing techniques, and the ratio of starch to protein (Ottoboni M. et al, 2019). Additionally, all of these variables may have an impact on healthy dogs' postprandial glycemic levels (Roberti-Filho F.O. et al, 2012). Because of this disiderent we thought it appropriate to bring to the attention of dog owners, the percentage of carbohydrates in different categories of dry dog food, as well as the types of cereals included in the dry food that have a different glycemic index, which is a system that measures the effects that

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carbohydrates in food have on blood sugar levels (Gal A. *et al*, 2021).

## MATERIAL AND METHOD

A total of 72 commercial dry dog food were analysed, divided into 3 categories, low cost (20 brands), premium and super-premium (52 brands). The types of dry dog food examined were from supermarkets, veterinary practices, pet shops and veterinary pharmacies as well as from different veterinary websites. Dog dry food is composed of protein, fat, ash, carbohydrates and moisture as analytical composition. Carbohydrates are not measured directly, so we won't see them listed on the label, but we can estimate them by subtracting the key information on the guaranteed analysis located on the pet food packet from 100. Often a company won't list the 'ash' value, but the general estimate for a kibble is between 5-8% (we have used 7% in our calculations). Guaranteed analyses are expressed on an "as fed" basis.

The formula used to calculate the percentage of carbohydrates in dry dog food has been as follows:

% carbohydrate (as fed) =100 - % protein -% fat – fiber - % moisture - % ash.

#### **RESULTS AND DISCUSSIONS**

Carbohydrates (CHO) are the most common macroelements found in dry extruded pet food products (~40-60% dry matter). After evaluating the labels of the 20 low cost dry pet food brands we identified that 90% of the low cost dry dog foods have cereals as the first ingredient, even in percentages of 58%. 13 types of food have the cereal specification, but not the cereal type, and in the remaining brands (n=7) we observed that the cereal types used were corn, wheat, barley, wheat bran, rice. In terms of Kcalorie requirements, of the 20 types of low cost dry food, only 4 types show the kcal/100 g product.

In figure 1 it can be seen from the data on the label that the protein percentage of the low cost dry food was between 17-24%, the fat percentage between 6-12%, the fiber percentage between 2.2% - 4.5%, the ash percentage between 5% - 8% and the carbohydrate percentage which was not listed on the label and which was calculated according to the calculation formula was between 46 - 60%. Only 2 types of low cost food taken in the study had carbohydrate percentage below 50% (46%, 47 respectively), the remaining 18 samples had carbohydrate percentage between 50% and 60%.

To calculate the percentage of carbohydrates in premium and super-premium dry food, a total of 52 types of dry food were studied, each with a variety of ingredients, mainly meat and meat byproducts as the major ingredient. Out of a total of 52 types of premium and super premium dry dog food, 25 had a kcal/100 g product number specified.

From figure 2 it can be seen that the percentage of nutrients listed on the label for protein ranged from 20.8% to 32%, the percentage of fat ranged between 7.5% and 20%, fiber - 1.08% to 4.5% and ash ranged between 1.5% and 9%. As for the percentage of carbohydrates, the calculation showed that they ranged between 30.5 % and 53 %. 4 of the brands had the highest value of carbohydrates, between 50.4 % and 53 %, the rest had a carbohydrate value between 30.5 % and 50 %. 39.58 % of the premium and super-premium food types had even a percentage below 40% carbohydrates. In the case of the 4 brands where the percentage of carbohydrates was higher than 50%, we observed that they had a high amount of cereals as the main ingredient.



Figure 1 Analytical composition of low cost commercial dog dry food according to label data and concentration of carbohydrates after calculation



Figure 1 Analytical composition of low cost commercial dog dry food according to label data and concentration of carbohydrates after calculation

Comparing the ingredients between the low cost and the premium and super premium dry dog food we noticed that in the premium and super premium food there was a greater diversity of ingredients and the cereals in most of them are not in the first place as in the low cost brands. Also in most types of super-premium and premium food the types of cereals are declared. With a greater diversity of ingredients and less cereals in the composition, the percentage of carbohydrates is much lower compared to low cost food.

Cereals are an important source of carbohydrates in dog diets; most extruded dog food contains carbohydrates (Bradshaw J.W.S, 2006). Nutritionally, carbohydrates are not essential nutrients (Cabrita A.R.J. *et al*, 2022); however, compared to animal protein or fat, carbohydrates are a highly economical source of energy for use in pet foods (Beloshapka A.N. *et al*, 2016; 2021). Although grains are a carbohydrate source within the pet food industry, many pet owners are concerned about feeding grains to their pets, and studies on the safety of carbohydrate sources available in dog foods are limited (Beaton L., 2014).

Carbs are not made equal. Depending on where a carb is ranked on the Glycemic Index, the way our dog's body processes various carb sources can differ significantly. Glycemic index (GI) is a measure used to determine how different foods increase blood glucose levels after being consumed by the animal. Although carbohydrates provide energy and activity, not all carbohydrates are good. The glycemic index is important for pets because it determines how much carbohydrate (sugars and starches) your dog or cat consumes and how it affects their health (Rankovic A. *et al*, 2020).

According to the Pet Obesity Prevention Association, 52.7% of dogs and 57.9% of cats in

are clinically overweight or obese. Unfortunately, these percentages mean that a substantial number of dogs and cats have weight-related problems that can turn into serious conditions and diseases (Suarez L. *et al*, 2022).

Low GI foods are considered good carbohydrates, they contain carbohydrates that digest slowly, providing energy that lasts for a long time. Low GI foods are better assimilated in the body and lead to healthier blood glucose and insulin control, which further facilitates weight control, disease prevention, increased energy and improved mood (Rand J.S. *et al*, 2004). Benefits of providing your pet with a low glycemic index carbohydrate diet: may improve/minimize diabetes symptoms; reduces risk of heart disease; reduces blood cholesterol levels; satisfies hunger and keeps your cat/dog feeling fuller for longer; helps prolong physical endurance (Rand J.S. *et al*, 2004).

Many of the carbohydrates consumed by dogs are rapidly absorbed and converted to glucose - resulting in a quick source of energy that becomes stored in the muscles and liver. It is this rapid rise in blood glucose that can trigger the insulin response in the body - specifically in the pancreas. This effect can also put pressure on the pancreas as it struggles to 'keep up' to produce enough insulin. High GI foods, by contrast, are those with carbohydrates that are digested quickly and easily, leading to a rapid energy spike followed by a rapid crash. This adversely affects insulin and blood sugar control, provides unwanted calories and can cause health problems such as obesity and diabetes ((Rand J.S. et al, 2004; Barclay A.W. et al, 2008). Maize, wheat, white rice, potatoes and peas have a considerably high glycemic index. Similarly, fruit, cucumbers, cauliflower, pears, quinoa, buckwheat, chickpeas, lentil have a low glycemic index, making them very healthy options of choice. The higher the glycemic index, the faster the rise in blood sugar levels and the higher the blood sugar concentration. Conversely, the lower the glycemic index, the slower the rise in blood sugar levels and the lower the blood sugar concentration (Brummer Y. *et al*, 2014).

Foods are rated on a scale of 1 to 100, with low GI foods having an index of 55 or less, high GI foods having an index of 70 or higher, while the range 56-60 is considered to be medium GI foods (Rand J.S. et al, 2004). Most commercial dog foods are unnecessarily high in carbohydrates and exceptionally low in protein. And high quality protein barely exists in commercial dog food. It is important to note that the FDA (Food and Drug Administration) does not evaluate glycemic claims and there is no currently accepted protocol for investigating GI (glycemic index) for pet food. This is primarily due to the complexity in determining an actual GI value for many pet foods on the market. In addition to the fact that the GI value is affected by the food itself and the way it is prepared, it is important to note that not every pet will have a similar glycemic response to the same food. So even though the GI values applied to pet foods are general guidelines, they may not fit your pet's specific needs, nor may they be consistent across the entire dog population (Rankovic A. et al, 2020).

However, given the overwhelming scientific evidence supporting the health benefits of a healthy low GI diet, the glycemic index is one of the best guides for pet owners looking for healthier and more nutritious food options for their dog especially when it comes to weight management and other weight-related diseases (Rankovic A. *et al*, 2020). In conclusion, even though the glycemic index has proven to be a useful guide in choosing healthier foods for your pet, always the owner should consult with their veterinarian to determine a nutritional diet that works best for the pet's individual needs.

Worldwide there has been an increase in the prevalence of obesity in dogs as well as humans (Suarez L. *et al*, 2022). Obesity in dogs and cats frequently predisposes to the development of glucose intolerance as well as abnormal insulin response and abnormal basal insulin concentrations (Rand J.S. *et al*, 2004). In obese pets, it has been theorized that persistent hyperinsulinemia is an important contributor to the development of diabetes (Case L. *et al*, 2011). but for this more knowledge is needed to be able to effectively prevent and treat these diseases and therefore it is important to investigate carbohydrate metabolism.

## CONCLUSIONS

Although they are not actually necessary for a dog's diet, carbohydrates provide a number of advantages for health that shouldn't be disregarded. Depending on the dog, the optimal amount of carbohydrates in the diet will vary.

This nutrient, so much debated lately, is not bad for dogs but, in reasonable quantities, can even be a practical source of energy. We only need to choose the right plants—low-glycemic fruits, vegetables, and seeds in the right amounts for the carnivore—knowing that high-glycemic carbs cause a lot more harm than a low-glycemic carb.

#### REFERENCES

- Barclay A. W., Petocz P., McMillan-Price J., Flood V.M., Prvan T., Mitchell P., Brand-Miller J.C, 2008 - Glycemic index, glycemic load, and chronic disease risk-a meta-analysis of observational studies. Am. J. Clin. Nutr. 87:627– 637. doi:10.1093/ajcn/87.3.627
- Beaton L., 2014 Grain-free petfood: A top trend in the U.S. pet market. Petfood Industry, 56, 68–70.
- Beloshapka A.N., Buff P.R., Jr. Fahey G.C., Swanson K.S., 2016 - Compositional Analysis of Whole Grains, Processed Grains, Grain Co-Products, and Other Carbohydrate Sources with Applicability to Pet Animal Nutrition. Foods, 5(4), 23, doi:10.3390/foods5020023
- Beloshapka A.N., Cross T.W.L., Swanson K., 2021 -Graded dietary resistant starch concentrations on apparent total tract macronutrient digestibility and fecal fermentative end products and microbial populations of healthy adult dogs. Journal of Animal Science, 99, 409, doi: 10.1093/jas/skaa409
- Bradshaw J.W.S, 2006 The evolutionary basis for the feeding behavior of domestic dogs (Canis familiaris) and cats (Felis catus). The Journal of Nutrition, 136(7 Suppl):1927S- 1931S, doi: 10.1093/in/136.7.1927s
- Briens JM, Subramaniam M, Kilgour A, Loewen ME, Desai KM, Adolphe JL, et al., 2021 - Glycemic, insulinemic and methylglyoxal postprandial responses to starches alone or in whole diets in dogs versus cats: relating the concept of glycemic index to metabolic responses and gene expression. Comp Biochem Physiol., 257:110973. doi: 10.1016/j.cbpa.2021.110973
- Brummer, Y., Kaviani M., Tosh S., 2015 Structural and functional characteristics of dietary fibre in beans, lentils, peas and chickpeas. Food Res. Int. 67:117–125. doi:10.1016/j. foodres.2014.11.009
- Cabrita A.R.J., Guilherme-Fernandes J., Valente I.M., Almeida A., Lima S.A.C., Fonseca A.J.M., Maia M.R.G., 2022 - Nutritional Composition and Untargeted Metabolomics Reveal the Potential of Tetradesmus obliquus, Chlorella vulgaris and Nannochloropsis oceanica as Valuable Nutrient Sources for Dogs. Animals 2022, 12(19), 2643.

- Case L., Daristotle P.L., Hayek M., Raasch M. -History and regulation of pet foods, Canine and Feline Nutrition 2011.
- Di Cerbo, A., Morales-Medina J.C., Palmieri B., Pezzuto F., Cocco R., Flores G., Iannitti T., 2017 - Functional foods in pet nutrition: Focus on dogs and cats. Research in Veterinary Science, 112, 161–166, doi:10.1016/j.rvsc.2017.03.020
- **FEDIAF.** Nutritional guidelines for complete and complementary pet foods for dogs and cats. Bruxelles: European Pet Food Industry Federation (2022).
- Gal A., Cuttance W., Cave N., Lopez-Villalobos N., Herndon A., Giles J., Burchell R., 2021 - Less is more? Ultra-low carbohydrate diet and working dogs' performance. PLOS One, 23;16(12).
- Kazimierska K, Biel W., Witkowicz R., Karakulska J., Stachurska X., 2021 - Evaluation of nutritional value and microbiological safety in commercial dog food. Veterinary Research Communications, 45:111–128.
- NRC. Nutrient requirements of dogs and cats. Washington, DC: National Academy Press (2006).
- Ottoboni M, Tretola M, Luciano A, Giuberti G, Gallo A, Pinotti L., 2019 - Carbohydrate digestion and predicted glycemic index of bakery/confectionary ex-food intended for pig nutrition, Italian. J Anim Sci.18:838–49.
- Rand J.S., Fleeman L.M., Farrow H.A., et. al., 2004 -Canine and feline diabetes mellitus: nature or nurture?. Journal of Nutrition, 134 (suppl 8):2072S–2080S, doi: 10.1093/jn/134.8.2072s

- Rankovic Alexandra, Adolphe J.L., Ramdath D.D., Shoveller A.K., Verbrugghe A. 20220 -Glycemic response in nonracing sled dogs fed single starch ingredients and commercial extruded dog foods with different carbohydrate sources. Journal of Animal Science, Vol. 98, No. 8, 1–11
- Rauktis M.E., Rose L., Chen, Q., Martone R., Martello A., 2017 - Their Pets Are Loved Members of Their Family": Animal Ownership, Food Insecurity, and the Value of Having Pet Food Available in Food Banks. Anthrozoös 30(4):581-593.DOI: 10.1080/08927936.2017.1370225
- Roberti-Filho FO, Palagiano C, da Silva FL., 2012 -Processing effects on starch gelatinization and its influence on digestibility, fermentation products and microbial composition of the faeces, and glucose metabolism of dogs fed kibble diets. In Proceedings of the 16th Congress of the European Society of Veterinary and Comparative Nutrition, Bydgoszcz, Poland.
- Suarez L., Bautista-Castaño I., Romera C.P., Montoya-Alonso J.A., Corbera J.A., 2022 - Is Dog Owner Obesity a Risk Factor for Canine Obesity? A "One-Health" Study on Human– Animal Interaction in a Region with a High Prevalence of Obesity. Vet Sci., 9(5): 243.
- Vastolo A., Gizzarelli M., Ruggiero A., Alterisio M.C., , Calabro S., Ferrara M., Cutrignelli M.I., 2023 -Effect of diet on postprandial glycemic and insulin responses in healthy dogs. Front. Vet. Sci. 10:1201611. doi: 10.3389/fvets.2023.1201611

## NUTRIENT CONTENT AND CALORIC DENSITY ASSESSMENT OF DIFFERENT TYPES OF SENIOR COMMERCIAL CAT FOOD

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#### Abstract

Ageing should be considered as an inevitable physiological state and results in a progressive decline in the body's ability to cope with physiological, metabolic and environmental stress. Nutritional requirements change once the senior cat reaches the senior cat stage, so attention and care is needed, depending on the needs of the individual, even more if a pathological condition is present. Calorie control in mature and senior cats usually means reducing calorie intake by about 20-30%. In the case of geriatric cats, it may be more important to increase calorie intake to maintain their normal physique, as their body condition and weight naturally decline with advancing age. Most diets for senior cats are formulated with appropriate nutrient limits and are lower in calories than rations for adult and young cats; however, there are currently no specific nutritional requirements set. This shows the amounts of nutrients found in different foods can vary greatly. Our main objective was to evaluate the caloric density as well as the concentrations of protein, fiber, fat and carbohydrates in different commercially available canned and dry feeds for senior cats.

Key words: cat, caloric density, chemical composition, senior food

Improvements in veterinary treatment, living conditions, and the increasing numbers of pet owners who provide their animals high-quality food at all phases of life have all contributed to cats' increasing lifespans (Shauf S. et al., 2021). The definition of a "senior" cat is subjective, as it differs from case to case, and the chronological age does not always match the physiological age of the animal. The ageing process can be influenced by breed size, genetics, the environment in which the animal grew up, etc. However, the convenient way to classify a cat according to age is to classify: the "mature" cat at 7-10 years; the "senior" cat at 11-14 years and the "geriatric" cat from 15 years (Pittari J., 2009, Vogt A.H. et al, 2010). With more diet alternatives available because to the pet food industry's response to the aging cat population, owners and vets are finding it harder and harder to choose the best diet for older cats (Hutchinson D. and Freeman L.M., 2011).

Nutrition has always played a key role in the management of animals under physiological stress (such as growth and reproduction) and during disease states. Senescence should be considered a physiological condition that requires specific attention (Gajanayake I., 2017). Getting the right nutritional balance in senior cat food is important because felines have very specific needs for proteins, amino acids and vitamins. Cats have high protein requirements and can quickly become deficient in certain amino acids and vitamins.

The ageing process brings about a number of physiological changes, some of which are obvious, such as bleaching of the fur, decreased muscle mass and decreased function of the visual, auditory and olfactory system; there may also be less obvious changes in the digestive tract, immune system, kidney function and other organs (Bellows J. *et al*, 2016).

Senescence should be regarded as an unavoidable physiological condition and results in a progressive decline in the body's ability to cope with physiological, metabolic and environmental stresses. Nutritional requirements change once the senior cat reaches the senior cat threshold, so attention and care is needed, depending on the needs of the individual, even more so if a pathological condition is present (Miele A. et al, 2020). Aging cats, like dogs and humans, have reduced energy requirements and therefore a tendency towards obesity (Lund E.M. et al, 2005; Laflamme and Blamm, 2002).1,8 This appears to be true for cats, but only up to about 10 to 12 years of age (Laflamme D.P. and Blamm J.M, 2002). Energy requirements increase beyond this point, particularly after about 13 years of age (Perez-

Camargo G., 2004; Cupp C. *et al*, 2004). The main objective of this work was to evaluate the nutrient

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content and caloric density in different types of senior commercial cat food.

#### MATERIAL AND METHOD

The analyses were performed on a number of 9 samples, 5 samples of dry food and 4 samples of senior canned food, collected from supermarkets, veterinary practices, pet shops and pharmacies veterinarv in Cluj-Napoca Standardized methodology was followed for sample preparation. Commercial dry cat food was stored in bulk and packaged. The food samples were homogenized before analysis. Moisture, crude protein, crude fiber, crude fat, and ash were measured using AOAC official method. The carbohydrate content of the foods was calculated by difference (100 minus crude protein, crude fiber, crude fat, ash, and moisture). In our study we used calories as the unit of measurement, (Castrillo C. et al, 2009), and the energy value per 100 g produced was calculated according to the equation (NRC, 2006)

#### **RESULTS AND DISCUSSIONS**

The sensory examination of the 9 samples of dry and canned commercial feed showed no changes in appearance, consistency and odour, considering their conditioning for long-term storage.

Following the analysis of the gross chemical composition of the 9 samples of senior dry and canned cat food, differences could be observed between our data and the data indicated on the label. In terms of dry matter (DM), with the exception of sample 4, all the other dry food samples had a higher DM than that indicated on the label. Protein with the exception of sample 1. where the percentage of protein was higher, in all other samples the protein value was up to 5 % lower compared to the label. The same is the case of fat, with the exception of sample 4, for the rest of the samples the fat was much lower than the value indicated on the label. For fiber and ash, the values were approximately equal to those expressed on the label. From Figure 1, which shows the analytical composition of senior dry cat food/100 g product, it can be seen that the dry matter ranged from 93.16% to 94.70%, in the case of protein the lowest value was in sample S2 of 26.79% and the highest value in sample S1 of 33.32%. The lowest percentage of fat was in sample S2 (8.64) and the highest percentage in sample S1 (16.21%). Fiber ranged from 2.9% in sample S2 to 6.63 in sample S4 and the highest percentage of ash was in sample S5 (8.87%) and the lowest in sample S1 (5.15%). As for carbohydrates, their percentage ranged from 49.28% in sample 2 to 37.65 % in sample 5.

Regarding the analytical composition of the senior canned cat food/100 g product, it is observed that the dry matter ranged from 7.03 % to 19.99 %, in the case of protein the lowest value was in sample S1 of 4 % and the highest value in sample S4 of 9.80 %. The lowest fat percentage was in sample S1 (1.10 %) and the highest in sample S2 (9.80 %). Fiber ranged from 0.1 % in sample S1 to 3.97 in sample S4, and the highest ash percentage was in sample S1 (0.6 %). Carbohydrates ranged from 1.33 % in sample 1 to 8.73 % in sample 3 (*Figure 2*).



food per 100 g/product



food per 100 g/product

Following the calculation of the caloric density for senior dry cat food, these values ranged from 362 Kcal/100 g product for sample 2 to 412 Kcal/100 g product for sample 1. According to the legislation regarding nutrient requirements and caloric density in adult cat food (NRC, 2006), the caloric density must be between 280 and 480 Kcal/100 g product. In our case although all the samples examined are senior dry cat food samples, quite a large variation is observed between them (*Figure 3*).

The same can be observed for the senior moist feed, with caloric energy values ranging from 30 Kcal/100 g produced in sample 6 to 95 Kcal/100 g produced in sample 9. In the case of canned food the energy value according to NRC, 2006, should be between 70-130 Kcal/100 g product. Following our calculation, we can say that sample 6, is below the limit of Kcal/100 g product in terms of senior cat requirements, having a value of only 30 kcal/100 g product (*Figure 4*).





Figure 4 Caloric content of senior cat canned food

In the case of protein and fat, the minimum required according to AAFCO and NRC, is between 30% and 45% protein/100 g DM and minimum 9% fat/100 g DM, although most experts say that a cat's diet should contain somewhere between 20 and 40% fat. This percentage is much higher compared to other mammals. Certain groups of cats benefit from even higher levels, including young and pregnant or lactating cats, those needing extra energy for growth, and even some older cats can also benefit from high palatability and concentrated energy levels with a higher fat diet; especially when suffering from a lower appetite (Pekela A.Y. *et al*, 2020).

Cats have unique nutritional requirements. They are obligate carnivores, and their bodies cannot produce certain vitamins in the way that herbivores and omnivores can. Cats of all sizes need foods with a higher concentration of protein, taurine, arginine, niacin, vitamin A and vitamin D. Cats also do not have the liver enzymes needed to metabolize carbohydrates in the quantities that humans and other animals can, so we need to be selective about the quantity and quality of carbohydrates we include in our felines' bowls. From scientific data, the carbohydrate requirement of a cat is between 10 and 15% of DM, or even less, and if we compare with our results we can see that the values are much higher than the requirement of a cat with values between 52.81% in sample 2 of senior dry food and 12.15% in sample 7 of senior wet food (Miele *et al*, 2020).

Cat diets are widely accepted in the industry with clearly defined nutritional guidelines. However, in contrast, the nutritional requirements of senior cats are not strictly defined by FEDIAF. AAFCO (The Association of American Feed Control Officials) or the NRC (National Research Council). Despite the fact that our pets spend proportionately more time classified as "senior" or "geriatric", their nutritional requirements are based on adult parameters. However, older cats may benefit from tailored nutritional modifications to support the physiological changes known to occur at this life stage. The minimum protein profile for adult cats according to AAFCO, is a minimum of 26%/SU, even if the cat is 2 or 12 years old. This is the reason why there are quite large variations in the caloric density and level of nutrients present in senior cat food. Some features of senior cat food on the market include reduced calorie density, protein, sodium and phosphorus levels compared to adult cat food (Laflamme D., 2018).

Adult and senior cats have changing dietary needs and it is extremely important to provide guidance on daily feeding amounts. DER (Daily energy requirements) for mature adult cats (aged 7 to 10 years) may be equivalent to RER (Resting Energy Requirement), although adjustments should be made according to individual needs. For senior cats (older than 10 years), RER will need to be multiplied by a factor of 10-20%, and in some cases even 25% (Miele A. et al, 2020). Senior cats may also experience a reduction in digestive capacity, leading to decreased BCS and therefore increased caloric intake (Teng K.T. et al, 2018). Underweight is a common problem in senior cats (Laflamme D.P., 2005; Laflamme D.P., 2018). Increased energy requirements may be due to reduced digestion in older cats. A large percentage of ageing cats have a reduced ability to digest fat: about 10% to 15% of mature cats and 33% of geriatric cats have reduced fat digestibility. Although the onset of reduced digestive function may be gradual, in the long term it contributes negatively to the energy balance of a large number of geriatric cats. Reduced protein digestibility also occurs in geriatric cats. One in 5 cats over 14 years protein, reduced digestibility with age could contribute to a negative nitrogen balance and loss of LBM (lean body mass) (https://www.aaha.org/).

It is often assumed that older cats are prone to obesity and that energy intake should be restricted. The prevalence of overweight and obesity is highest for middle-aged cats (5-11 years) because many aging cats are less active. After 11 years of age, the prevalence of overweight and obesity for cats decreases, and some senior cats may be underweight.8 Therefore, a higher fat and caloric density food may be beneficial depending on individual needs (Summers S.C. *et al*, 2020).

There is some evidence to support this, but studies of elderly cat populations indicate that this view is too simple. In a large colony of research cats, the percentage of body fat increased significantly in those aged 7-12 years compared to adult cats (1-7 years Perez-Camargo, 2004) However, a decrease in body fat percentage was observed in cats over 12 years (Perez-Camargo G., 2004). Similar results were also reported in a survey of client-owned cats, where more than 60% of "mature" cats (7-10 years) and "senior" cats (11-14 years) were classified as "too fat". This survey revealed that about 40% of geriatric cats (>15 years) were considered "too fat", but also about 40% of geriatric cats were classified as "too thin" due to fat loss, but also due to muscle loss. Protein and fat digestibility decreases in some cats as they age, with the highest incidence in cats over 12 years of age (Perez-Camargo G., 2004). This correlates with the increased occurrence of fat and muscle loss in this age group (Peterson M.E. and Little S.E., 2018).

This points to various nutritional modifications to best support the needs of "senior" cats. For senior cats (e.g., over 7 years old), high-protein, low-fat diets would be most indicated to help maintain muscle mass while limiting fat deposition and weight gain or obesity. For "geriatric" cats (12+ years), an increase in dietary fat combined with high protein would be appropriate to help maintain body weight and body mass (Perez-Camargo G., 2004).

There is a lack of consensus regarding optimal dietary protein levels in mature adult cats. A published study has shown that aging cats should, in fact, be fed higher protein diets to avoid loss of muscle mass (Lafamme D.P., 2018).

The Association of American Feed Control Officials (AAFCO) recognizes only two life stages: growth and maintenance. This means that senior cat foods are adult maintenance formulas that meet AAFCO nutritional requirements, with adjustments made to adapt to aging cats. Energy requirements increase sharply and progressively in these cats as they age, starting at the age of 10-12 years. If daily caloric intake is not increased, a progressive weight loss will result, largely due to loss of muscle mass, a phenomenon called age-related sarcopenia.

## CONCLUSIONS

Foods marketed for senior cats are highly variable in their nutrient content and caloric density. Finding the perfect diet for senior cats is a real challenge, as guidelines regarding nutrient requirements for senior cats are not set either. It is therefore essential that dietary recommendations are based on a thorough nutritional assessment of each individual cat, including dietary history, body condition, appetite as well as diseases they suffer from.

## REFERENCES

- Bellows J., Center S., Daristotle L., Estrada A.H., Flickinger E.A., Horwitz D.F., Lascelles D., Lepine A., Perea S., Scherk M., Shoveller A.K., 2016 - Aging in cats: Common physical and functional changes. J Feline Med Surg. 18(7):533-50. doi:
- 10.1177/1098612X16649523. Castrillo, C., Hervera M., Baucells M.D., 2009 -Methods for predicting the energy value of pet
- foods. R. Bras. Zootec., v.38, p.1-14. **Cupp, C., Perez-Camargo G., Pati A.I, et al., 2004** -Long-term food consumption and body weight changes in a controlled population of geriatric cats [abstract]. Comp Cont Edu Small Anim Pract., 26(Suppl 2A):60.
- Gajanayake, I., 2017 Senior pets dietary advice to offer cat and dog owners. Companion animal, Vets.
- Hutchinson D. and Freeman L.M., 2011 Optima nutrition for older cats. Compendium –Continuing Education for Veterinarianvetlearn.com
- Laflamme, D., 2018 Effect of diet on loss and preservation of lean body mass in aging dogs and cats. In: Proceedings of the Companion Animal Nutrition Summit; May 3–5, Charleston, SC. pp 41–6.
- Laflamme, D.P., 2005 Nutrition pentru pisici și câini în vârstă și importanța stării corpului. Veterinar. Clin. North Am. - Anim mic. Exersează. 35, 713-742.
- Laflamme, D.P., Ballam J.M., 2002 Effect of age on maintenance energy requirements of adult cats [abstract]. Comp Cont Edu Small Anim Pract,24(9A):82.
- Lund, E.M., Armstrong P.J., Kirk C.A. et al., 2005 -Prevalence and risk factors for obesity in adult cats from private US veterinary practices. Intern J Appl Res Vet Med., 3:88–96.
- Miele A., Sordo L., Gunn-Moore D.A., 2020 Feline Aging Promoting Physiologic and Emotional WellBeing .Vet Clin Small Anim. 50, 719–748 https://doi.org/10.1016/j.cvsm.2020.03.004

- NRC Nutritional Research Council. Nutrient requirements of dogs and cats. Washington, DC: The National Academies Press; 2006.
- Pekela, A.Y., Mülazımoğlub S.B., Acar N., 2020 -Taste preferences and diet palatability in cats. Journal of applied animal research, VOL. 48, NO. 1, 281– 292https://doi.org/10.1080/09712119.2020.17863 91
- Perez-Camargo, G., 2004 Cat nutrition: what's new in the old? Comp Cont Edu Small Anim Pract;26(Suppl 2A):5–10.
- Peterson, M.E. and Little S.E., 2018 Cachexia, sarcopenia și alte forme de irosire musculară: probleme frecvente la pisicile seniori și geriatrice și la pisicile cu boală endocrină. Summit-ul Purina Companion pentru Nutriția Animalelor, Charleston, Carolina de Sud.
- Pittari J, Rodan I., Beekman G., et al., 2009 Association of Frline Practitioners Senior Care Guidelines. J Feline Med Surg, 11:763-778

- Schauf S., Coltherd J.C., Atwal J., Gilham M., Carvell-Miller J., Renfrew H., Elliott J., Elliott D., Bijsmans E.S., Biourge V.C., Watson P., Bakke A.M., 2021 - . Clinical progression of cats with early-stage chronic kidney disease fed diets with varying protein and phosphorus contents and calcium to phosphorus ratios. Vet Intern Med.35:2797–2811
- Summers S.C., Stockman J., Larsen J.A., Rodriguez A.S., Zhang L., 2020 - Evaluation of nutrient content and caloric density in commercially available foods formulated for senior cats. J Vet Intern Med., 1–7.
- Teng, K.T., McGreevy P.D.,. Toribi J.A, et al., 2018 -Strong associations of ninepoint body condition scoring with survival and lifespan in cats. J Feline Med Surg., 20:1110–8.
- Vogt A.H., Rodan I., Brown M., et al. 2010 AAFP-AAHA feline life stage guidelines. J Am Anim Hosp Assoc 2010;46:70–85.

## TESTING THE EFFICIENCY OF 6 ESSENTIAL OILS FOR FOODBORNE PATHOGENS IN ORDER TO SELECT THE MOST SUITABLE FOR APPLICATION IN THE MEAT INDUSTRY

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#### Abstract

Identifying natural and safe methods for preserving food is an important issue. In this respect, one of the most important foodborne pathogens in ready-to-eat meat products is *Listeria monocytogenes*, which had a high prevalence in global food poisoning outbreaks.

In the study, six essential oils (*Ocimum basilicum, Eucalyptus maculata var. citriodora, Salvia officinalis, Petroselinum crispum, Citrus aurantifolia, Cinnamomum zeylanicum*) were studied for their antimicrobial efficiency against *Listeria monocytogenes* using the Agar Well Diffusion assay, in order to select the three most effective essential oils. Samples were performed in triplicate with positive and negative control.

According to the measured inhibition area, the antimicrobial effect ranking for the studied essential oils was the following: *Cinnamomum zeylanicum* essential oil -  $29.00\pm1.00$  mm, *Citrus aurantifolia* -  $17.00\pm0.82$  mm and *Ocimum basilicum* -  $12.00\pm0.82$  mm.

In conclusion, the efficacy of the selected oils against *L. monocytogenes* is noted, further studies on their activity in food matrices experimentally and naturally contaminated with the studied pathogen being needed.

Key words: essential oils, *Listeria monocytogenes*, ready-to-eat meat products

*Listeria monocytogenes* is a foodborne pathogen and is the most clinically important species in the genus *Listeria*, along with 28 other distinct species (Rocha et al., 2019). This species is widespread in the environment and can be identified in water, soil, buildings and equipment, with increased incidence also in chicken meat or slaughterhouse waste.

Of the 13 serotypes of *L. monocytogenes*, four are of human health significance. The main source for human listeriosis is through consumption of contaminated food, but vertical or zoonotic transmission is also possible.

Today's lifestyle greatly influences the behaviour and choices of the modern consumer, with the production of Ready-to-Eat (RTE) foods increasing dramatically. *L. monocytogenes* is often associated with such foods, as this bacterium is psychrotrophic. Although some processing steps linked to various operational parameters can inhibit or prevent the growth of *L. monocytogenes*, finished products can be contaminated afterwards (e.g. handling, packaging).

Given the issues related to the ecology of this pathogen and the new outbreaks of listeriosis,

research is needed on effective and safe prevention or decontamination methods that align with consumer requirements (Dos Santos et al., 2022).

From this point of view, more and more research is addressing the negative effects of using artificial preservatives, while increasing consumer interest in natural alternatives. A sustainable solution could be the use of plant extracts such as essential oils, many of which have a proven antimicrobial effect on major food pathogens (Georgescu et al.-a, 2018, Georgescu et al.-b, 2018).

In addition to their contribution to food safety, essential oils have been used since ancient times to treat certain diseases or for health maintenance. There are numerous studies on the anti-inflammatory, antioxidant, anti-carcinogenic and other and other beneficial effects which can be the base for functional foods formulations (Bejan et al., 2021).

The aim of this paper was to evaluate the *in vitro* antimicrobial properties against *L. monocytogenes* of 6 commercial essential oils and the selection of the three most efficient for further studies.

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## MATERIAL AND METHOD

The essential oils for internal use were purchased in June 2022, from SC BIONOVATIV SRL, Brasov, Romania, being obtained from the following plants: *Ocimum basilicum (OBO)*, *Eucalyptus maculata var. citriodora (EMO)*, *Salvia officinalis (SOO), Petroselinum crispum (PCO), Citrus aurantifolia (CAO), Cinnamonum zeylanicum (CZO)*. Based on the producer's declaration and label informations, the purity of the EOs was 100%. The selection protocol was based on a literature survey regarding their antimicrobial and health effects.

#### Methods of investigation

The Agar Well Diffusion assay was used to test the *in vitro* efficacy of essential oils, with samples divided into two test groups (3 oils per plate) (Reeves, 1989). The first testing group was represented by the EOs extracted from Ocimum basilicum, Eucalyptus maculata var. citriodora, Salvia officinalis, and the second one, those obtained from Petroselinum crispum, Citrus aurantifolia, Cinnamomum zeylanicum.

Samples were performed in triplicate with positive (Ampiciline – AMP) and negative control (sterile peptone saline) (Table 1). The positive and



negative control were tested on different Muller Hinton Agar (MHA) plates (Thermo Scientific<sup>TM</sup> Oxoid<sup>TM</sup>).

The strain used was *L. monocytogenes ATCC* 13932, BioMérieux<sup>TM</sup>, reconstituted according to the manufacturer's recommendations and inoculated onto culture media for fresh use. The colonies formed were suspended in sterile peptone saline.

Bacterial suspensions had an initial turbidity of 0.5 McFarland (1.5 x  $10^8$  CFU/ml), with serial dilutions up to 1.5 x  $10^4$  CFU/ml. 100 µL of the previously prepared bacterial suspension was inoculated into MHA plates, according to the protocol (9). Subsequently, 8 mm diameter wells were cut using a sterile cork-borer and filled with the tested EOs. After one hour, the samples were incubated at  $37^{\circ}$ C for 24 hours in TCR100 and Pol-EKO type II W-400 STD thermostats.

After incubation, the results were read by measuring the inhibition zones (clear areas without microbial growth) and expressed in mm.

Statistical analysis was performed using Microsoft Excel 2016 and expressed as mean  $\pm$  standard deviation. In addition, considering the efficiency of the positive control (AMP) 100%, the percentages were reported according to the observations.



Figure 2 – A) Inhibiton zone measuring method, B) MHA plates preparation

## **RESULTS AND DISCUSSIONS**

In our research, CZO had the highest inhibition zone (29.00±1.00). Unlu et al. (2010) obtained similar results in a study conducted to determine the composition, antimicrobial and cytotoxic activity of cinnamon essential oil. Although the working protocol was different, using the Disk Diffusion method and *L. monocytogenes* strains F 1483 and F 1462, the inhibition zones ranged from 32-35 mm (including 6 mm disk diameter). Similarly, Al-Fekaiki et al. (2017), obtained an inhibition zone ranging from  $21.30\pm0.31$  to  $29.86\pm0.40$  for CZO concentrations ranging from 6µL to  $18\mu$ L (8). For CAO, no research on the Agar Well Diffusion or Disk Diffusion assays with the targeted pathogen was identified, a situation also encountered for EMO and PCO.

On the other hand, Costa et. al (2014), observed a minimum inhibitory concentration of 0.25 (% v/v) for *Listeria monocytogenes*, the effect of CAO being demonstrated.

Hossain et al (2010) observed inhibition zones ranging from  $15.1\pm1.5$  to  $17.1\pm1.2$  for OBO tested by the Disk Diffusion method, these results being higher compared to those obtained in the present research.

SOO had an inhibition zone of  $6.67\pm1.70$ , which was lower compared to the data obtained by

Ed-Dra et al., (2020) - 10.2±0.1 mm - 17.5±0.3 mm.

Regarding the inhibition zone of the positive control, CAO (113.3%) and CZO (193.33%) showed higher efficiency, other results being presented in Table 1 and in Figure 2.

The antimicrobial potentiation effect was also observed in CAO and EMO, being highlighted by the confluence of the inhibition zones. In this regard, CAO and EMO, are of interest for further studies.



Figure 2 L. monocytogenes antimicrobial efficiency for the tested EOs

Nr crt	Common name	Scientific name	Inhibition zone (mm) and %	Positive control (AMP)
1	Basil	Ocimum basilicum	12.00±0.82 80%	15 mm
2	Brazilian eucalyptus	Eucalyptus maculata var. citriodora	7.00±0.82 46.6%	15 mm
3	Sage	Salvia officinalis	6.67±1.70 44.46%	15 mm
4	Parsley seeds	Petroselinum crispum	7.00±0.82 46.6%	15 mm
5	Lime	Citrus aurantifolia	17.00±0.82 113.3%	15 mm
6	Cinnamon	Cinnamomum zeylanicum	29.00±1.00 193.33%	15 mm

Results of EOs in vitro antimicrobial testing

#### CONCLUSIONS

The most intense antimicrobial effect on *L*. monocytogenes is noted in Cinnamomun zeylanicum and Citrus aurantifolia essential oils, with Ocimum basilicum having medium efficacy. From this point of view, these essential oils can further investigations, qualify for such as Minimum Inhibitory Concentration and concentrations testing for food products enhancements.

Also, given the potentiation effects between CAO and EMO, further studies to investigate similar effects in relation to other pathogens would be useful.

Table 1

It is noted that these EOs are natural alternative candidates for traditional chemical food preservatives.

#### REFERENCES

- Al-fekaiki D., Niamah A., Al-Sahlany S., 2017 -Extraction and identification of essential oil from Cinnamomum Zeylanicum barks and study the antibacterial activity. Journal of microbiology, biotechnology and food sciences, 7: 312-316.
- Blejan E.I., Popa D.E., Costea T., Cioacă A., Olariu L., Ghica M., Georgescu M., Stancov G., Arsene A.L., 2021 - The in vitro antimicrobial activity of some essential oils from aromatic plants. Farmacia, 69(2):290-298.
- Costa R., Bisignano C., Filocamo A., Grasso E., Occhiuto F., Spadaro F., 2014 - Antimicrobial activity and chemical composition of Citrus aurantifolia (Christm.) Swingle essential oil from Italian organic crops. J. Essent. Oil Res. 26:400– 408.
- Dos Santos L.R., Alía A., Martin I., Gottardo F.M., Rodrigues L.B., Borges K.A., Furian T.Q. and Córdoba J.J., 2022 - Antimicrobial activity of essential oils and natural plant extracts against Listeria monocytogenes in a dry-cured ham-based model. J Sci Food Agric, 102: 1729-1735.
- Ed-Dra A., Filali F.R, Presti V.L., Zekkori B., Nalbone L., Bouymajane A., Trabelsi N., Lamberta F., Bentayeb A., Giuffrida A., Giarratana F., 2020 -Chemical composition, antioxidant capacity and antibacterial action of five Moroccan essential oils against Listeria monocytogenes and different serotypes of Salmonella enterica. Microbial Pathogenesis, 149:104510.
- Jibo G.G., Raji Y. E., Salawudeen A., Amin-Nordin S., Mansor R., Jamaluddin T.Z.M.T., 2022 - A systematic review and meta-analysis of the prevalence of Listeria monocytogenes in South-East Asia; a one-health approach of human-

*animal-food-environment, One Health,* Volume 15, 100417.

- Georgescu M., Dobrea M., Tăpăloagă D., Raita S., Dobrea V., 2018 - Functional evaluation of Nigella sativa seed oil effect on pathogen enriched Artisan cheese. Journal of Biotechnology, 280S: S54.
- Georgescu M., Ginghina O., Raita S., Tăpăloagă D., Ilie L., Negrei C., Daniela P., Varlas V., Mulţescu R., Roşca A.C., Mirica R., Georgescu D., 2018 - Natural alternative remedies in the background of updated recommendations for the prophylactic and therapeutic approach of Clostridium difficile infections. Farmacia, 66(4):563-572.
- Amzad Hossain M., Kabir M.J., Salehuddin S.M., Mizanur Rahman S.M., Das A.K., Singha S.K., Khorshed A., Rahman A., 2010 - Antibacterial properties of essential oils and methanol extracts of sweet basil Ocimum basilicum occurring in Bangladesh. Pharmaceutical Biology, 48(5):504-511.
- Reeves DS, 1989. Antibiotic assays. In: Hawkey PM, Lewis DA, editors. Medical bacteriology, a practical approach. Oxford: IRL Press.
- Rocha K. R., Perini H. F., Souza C. M., Schueler J., Tosoni N. F., Furlaneto M. C., Furlaneto-Maia L., 2019 - Inhibitory effect of bacteriocins from enterococci on developing and preformed biofilms of Listeria monocytogenes, Listeria ivanovii and Listeria innocua. World Journal of Microbiology & Biotechnology, 35(7):96.
- Unlu M., Ergene E., Unlu G.V., Zeytinoglu H.S., Vural N., 2010 Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). Food Chem Toxicol. 48(11): 3274-80.

## ASSESSMENT OF SOME MICROSCOPIC PARAMETERS OF RAM SEMEN CORRELATED WITH THE AGE OF THE ANIMALS

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#### Abstract

The study was carried out to evaluate some microscopic parameters (mobility, concentration, viability), to assess the metabolic intensity of spermatozoa (Redox test), and sperm resistance test related with ram fertility and the quality of ram semen in different age groups. The study was conducted in a farm located in Cluj County, on 34 rams of Turcana Alba breed, grouped according to age into 4 batches. Weekly an ejaculate and the mentioned variables were measured. Semen samples were collected from each animal using the artificial vagina (AV). For sperm mobility, the best values were observed for 3 years old rams ( $X \pm S = 88.4 \pm 3.02$ ). Variations in sperm viability showed some changes, but for all age groups were obtained values above those indicated in the literature. Assessment of sperm concentration revealed that rams in B6 ( $X \pm S = 2.75 \pm 0.31$ ) and B5 ( $X \pm S = 2.7 \pm 0.38$ ) had the best values. Higher metabolic intensity rate in B6, B5, B3 groups was correlated with higher values of concentration and mobility in these age groups. Thus, the best values regarding sperm resistance were recorded for the rams aged 6 and 5 years, in which the average values were equal to 7022.22. With increase in age, ram showed increase percentages of motility and viability of sperm in all studied batches.

Key words: age, ram, semen, parameters

Many studies in domestic animals have studied the influence of male age on sperm characteristics (Hallap T. *et al*, 2006; Rijsselaere T. *et al*, 2007; Long J.A. *et al*, 2010; Carreira J.T. *et al*, 2017). It was showed that the impact of age on ram semen quality appears to be consistent. Most studies suggest that ram sperm reaches optimum quality at three years of age but begin to decline afterward (Mandiki S.N.M. *et al*, 1998; David I. *et al*, 2007; Hassan M.R. *et al*, 2007; Chella L. *et al*, 2017; Abah, K.O. *et al*, 2023). It was observed that older rams can have better sperm quality than younger ones ( $\leq$  1 year) (Martí J.I. *et al*, 2011).

Other studies have reported a positive correlation between male age and sperm concentration in the ram (Salhab S.A. *et al*, 2003; Hassan *et al*, 2007; Martí J.I. *et al*, 2011; Ntemka A. *et al*, 2019), with one study reporting a 36.6% increase in sperm concentration in rams 3 years of age compared to yearling Yankasa rams (Osinowo O.A. *et al*, 1988).

Several studies have reported an increase in sperm motility with increasing male age in rams (Martí J.I. *et al*, 2011; Chella L. *et al*, 2017; Benia A.R. *et al*, 2018; Andreeva M., Stefanov R., 2020). It was suggested that the hypothalamo-pituitary– gonadal axis of old rams, even up to 13 years of age, is still functioning efficiently, which allows them to maintain good spermatogenesis (Ntemka A. *et al*, 2019). It has been consistently found that sperm membrane integrity in rams increases with age (Chella L. *et al*, 2017; Ntemka A. *et al*, 2019).

In one study, Martí J.I. *et al*, 2011 found that rams aged 8 years and above had a significantly higher sperm viability than those aged 1 year and below, with a viability rate of  $64.6 \pm 1.08$ compared to  $57.1 \pm 0.82$ , respectively. This finding was proposed to be as a result of larger sperm head area of the young, which may affect its structural and functional competence (Martí J.I. *et al*, 2011). Our study was designed to determine if the age of the animals influenced the microscopic parameters of ram semen.

#### **MATERIAL AND METHOD**

The study was carried out on 34 Turcana Alba rams of different ages, grouped into four batches: batch B2 with 6 rams of 2 years; batch B3 with 10 rams of 3 years; batch B5 with 9 rams of 5 years; batch B6 with 9 rams of 6 years.

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Preliminary stages of ram semen collection

Given the importance of this stage in the collection of semen and its evaluation, measurements were made of the length and circumference of the testicle. The research was carried out during the rams' accommodation period, prior to the semen collection, for 4 weeks. The measurements took place once a week.

We considered it necessary to investigate the way sexual reflexes are manifested in order to select the rams used for semen collection. This is vital in order to obtain quality ejaculates, with a large volume that allows their further processing. This study was necessary because within the breed there is great individual variability regarding sexual behavior. For this purpose, a certain number of rams were randomly selected from each experimental batch. The research focused on the ram's adaptation to the collection site and the harvester due to the fact that it is absolutely necessary to establish a good relationship human – animal.

The experiment lasted a month and a half. The artificial vagina (VA) method was used to collect the semen samples (Groza I.S., 2006). All obtained ejaculates were subjected to an initial macroscopic evaluation and those outside the standard requirements were discarded.

The examination of the semen covered the following aspects: sperm viability, mobility and concentration, assessment of the metabolic intensity of spermatozoa (Redox test), and sperm

resistance test according to criteria proposed by Groza I.S., 2006.

Weekly an ejaculate and the mentioned variables were measured. Rams were kept under the same breeding conditions and nutrition.

#### **RESULTS AND DISCUSSIONS**

The results obtained in the present study do not show significant changes in testicular length and circumference. Thus, the lowest value of testicular length was recorded in 2-year-old rams  $(10.65\pm0.05)$ , while in the other age categories the average values obtained were over 12 cm for both testicles. Regarding the testicular circumference, it can be observed that the lowest values were recorded in 2-year-old rams (32.37±0.05) in comparison with the data obtained in the other experimental groups, where the average values were over 34 cm (table 1). The results obtained regarding the determination of the testicular circumference are in accordance with the data reported by other authors. In a study published by Olah et al, 2013, the testicular circumference of rams was determined according to breed and season. The highest values were recorded in autumn for Awassi rams (35.5 cm), in spring for Suffolk rams (35.8 cm) and in winter for Merino rams (32 cm).

Table 1

Dispersar of the average values for testicular length and circumerence						
Datab	Testicula	r length (cm)	Testicular circumference			
Datch	Right testicle	Left testicle	(cm)			
B2	10.65±0.05	10.65±0.05	32.37±0.05			
B3	12.2±0.07	12.23±0.05	34.11±0.05			
B5	12.33±0.07	12.34±0.08	34.22±0.08			
B6	12.15±0.07	12.16±0.08	34.44±0.13			

Dispersal of the average values for testicular length and circumference

The results showed that there are differences in the number of ejaculates obtained from a ram, depending on the age of the animals. Thus, in the case of 3-, 5- and 6-year-old rams, the number of ejaculates obtained was lower compared

to those collected from 2 years old rams. The recorded results show differences in volume depending on the age of the animals, the best values being recorded in mature rams, over 3 years old (*table 2*).

Table 2

Dispersal of the average values for the sperm volume according to the age of the rams

Batch		B2			B3			B5			B6	
Parameter	J	Е	V	J	E	V	J	E	V	J	Е	V
Average	5.5	3.75	1.6	4.83	2.16	2.42	4.2	2.2	2.4	3.8	1.8	2.58
STDEV	±1.29	±0.95	±0.26	±1.16	±0.75	±0.25	±1.30	±0.83	±0.39	±1.30	±0.83	±0.39
J - No. of jumps; E- No. of ejaculates; V – volume (ml)												

Results regarding the average of sperm parameters analysis are presented in Table 3.

In the case of motility, there is a significant increase in rams aged 3 years  $(X\pm S=88.4\pm 3.02)$  compared to those aged 2 years  $(X\pm S=82\pm 1.78)$ . For the other age categories, close values were recorded (5 years  $X\pm S=88.22\pm 3.41$ , 6 years  $X\pm S=88.33\pm 3.93$ ). The research carried out by Mandiki S.N.M. *et al*, 1998, regarding the influence of season and age on sperm parameters in Texel, Suffolk and Ile-de-France rams supports the increase in sperm motility and the decrease in the percentage of anomalies with the age of the rams. Mandiki S.N.M. *et al*, 1998 found that mass

and progressive motility were similar at ages 2 and 3 but our study showed that ram spermatozoa motility were different between the 2-and 3- yearolds. Same results were found by Chella L. *et al*, 2017. In a study done with Garole rams in a semiarid tropical environment, Joshi A. *et al*, 2003 found that the age of the ram did not have a significant effect on spermatozoa motility.

The variation of sperm viability shows some changes, but for all age categories, mean values were above those indicated by other studies (Hassan M.R. *et al*, 2007) (*table 3*).

Table 3

according to the age of rams						
Parameter	B2	B3	B5	B6		
Motility (%)	82±1.78	88.4±3.02	88.22±3.41	88.33±3.93		
Concentration (x10 <sup>9</sup> spz/ml)	1.78±0.14	2.6±0.30	2.7±0.38	2.75±0.31		
Viability (%)	91.5±2.25	94.1±2.68	92.44±2.78	93.88±2.26		
Redox (min.)	7.33±0.36	6.82±0.49	6.6±0.53	6±0.25		
Resistance test	6583 33+278 68	6970+188 85	7022 22+148 13	7022 22+192 20		

Dispersal of the average values of microscopic parameters according to the age of rams

The same authors concluded that normal and live spermatozoa were comparatively better during the  $3^{rd}$  year than the  $1^{st}$  and  $2^{nd}$  year of age while the values were almost similar at  $3^{rd}$  and  $4^{th}$  year of age. With the increasing of age, the semen quality improved and stabilized up to at the age of 3 years.

The assessment of sperm concentration shows the same upward trajectory starting with rams aged 3 years ( $X\pm S=2.6\pm0.30$ ), but also for batch B5 ( $X\pm S=2.7\pm0.38$ ) and B6 ( $2.75\pm0.31$ ). We observed that 3-year-old rams exhibited the highest level of spermatozoa concentration than older or younger animals. Similarly, David I. *et al*, 2007, found that in Manech-tete-Rousse and Lacaune rams, spermatozoa concentration was higher between the ages of 2 and 3, thereafter decreasing with age.

The higher intensity of metabolism in the from group B6  $(X\pm S=6\pm 0.25),$ **B**5 rams  $(X\pm S=6.6\pm 0.53)$  and B3  $(X\pm S=6.82\pm 0.49)$  is also correlated with the higher concentration values and mobility in these age categories. The observations made regarding the resistance of the spermatozoa confirm the data presented previously. Thus, the best values were recorded in B6 and B5 where equal average values of 7022.22 were obtained. These data confirm previous studies according to which the increase in resistance is directly proportional to the fertilizing value of the semen (Drugociu D.G, Runceanu L.G., 2004).

As age increased, semen quality improved and stabilized at the age of 3rd to 4th years.

#### CONCLUSIONS

During this study, there were individual variations in the concentration, motility, viability of the semen depending on the age of the animals. Analyzing the evolution of the average values of the microscopic parameters there is an increase with age of the rams, which could be explained by sexual maturation. Applying the artificial vagina method, the best values of volume semen were recorded in mature rams over 3 years. In our study, as age increase all semen parameters studied improved and stabilized at the age of 3 to 5 years.

#### REFERENCES

- Abah, K.O., Fontbonne, A., Partyka, A. Nizanski W., 2023 - Effect of male age on semen quality in domestic animals: potential for advanced functional and translational research?, Vet Res Commun 47, 1125–1137.
- Andreeva M., Stefanov R., 2020 Study of the relationship between the age of the rams and the quality of their ejaculates obtained outside the breeding season, Biotechnol Anim Husb 36:437–445.
- Benia A.R., Saadi M.A., Ait-Amrane A., Belhamiti T.B., Selles S.M.A., Kaidi R., 2018 - Effect of season and age on main characteristics of sperm production in the Ouled-Djellal rams, Livest Res Rural Dev 30:1–14.
- Carreira J.T., Trevizan J.T., Carvalho I.R., Kipper B., Rodrigues L.H., Silva C., Perri S.H.V., Drevet J.R., Koivisto M.B., 2017 - Does sperm quality and DNA integrity differ in cryopreserved semen

samples from young, adult, and aged Nellore bulls?, Basic Clin Androl 27:12.

- Chella L., Kunene N., Lehloenya K., 2017 A comparative study on the quality of semen from Zulu rams at various ages and during different seasons in KwaZulu-Natal, South Africa, Small Rumin Res 151:104–109.
- David I., Druart X., Lagriffoul G., Manfredi E., Robert-Granié C., Bodin L., 2007 - Genetic and environmental effects on semen traits in Lacaune and Manech tête rousse AI rams, Genet Sel Evol GSE 39:405–419.
- Drugociu D.G., Runceanu L.G., 2004 Optimizarea reproducției la ovine, Ed. Ion Ionescu de la Brad, lași.
- Groza I.Ś., 2006 Ginecologie, andrologie și obstetrică veterinară. Compendiu, Ed. Academiei Române, București.
- Hallap T., Håård M., Jaakma U., Larsson B., Rodriguez-Martinez H., 2004 - Variations in quality of frozen-thawed semen from Swedish Red and White AI sires at 1 and 4 years of age, Int J Androl 27:166–171.
- Hassan M.R., Pervage S., Ershaduzzaman M., Talukder M.A.I., 2007 - Influence of age on the spermiogramic parameters of native sheep, J Bangladesh Agric Univ 7:301–304.
- Joshi A., Naqvi S.M.K., Bag S., Dang A.K., Sharma R.C., Rawat P.S., Mittal J.P., 2003 - Sperm motion characteristics of Garole rams raised for a prolonged period in a semi-arid tropical environment, Trop. Anim. Health Prod. 35(3), 249-257.
- Long J.A., Bongalhardo D.C., Pelaéz J., Saxena S., Settar P., O'Sullivan N.P., Fulton J.E., 2010 -Rooster semen cryopreservation: effect of pedigree line and male age on postthaw sperm function, Poult Sci 89:966–973.

Mandiki S.N.M., Derycke G., Bister J.L., Paquay R.,

**1998** - Influence of season and age on sexual maturation parameters of Texel, Suffolk and Ilede-France rams: 1. Testicular size, semen quality and reproductive capacity, Small Rumin Res 28:67–79.

- Martí J.I., Aparicio I.M., García-Herreros M., 2011 -Sperm morphometric subpopulations are differentially distributed in rams with different maturity age in cryopreserved ejaculates, Theriogenology 76:97–109.
- Ntemka A., Kiossis E., Boscos C., Theodoridis A., Kourousekos G., Tsakmakidis I., 2019 - Impact of old age and season on Chios ram semen quality, Small Rumin Res 178:15–17.
- Oláh J., Kusza S., Harangi S., Posta J., Kovács A., Pécsi A., Budai C., Jávor A., 2013 - Seasonal changes in scrotal circumference, the quantity and quality of ram semen in Hungary, Archiv Tierzucht 56:10.
- Osinowo O.A., Ahmed M.S., Ekpe G.A., 1988 Semen quality and sperm output of Yankasa rams at different ages, Theriogenology 29:381–386.
- Rijsselaere T., Maes D., Hoflack G., de Kruif A., Van Soom A., 2007 - Effect of body weight, age and breeding history on canine sperm quality parameters measured by the Hamilton-Thorne analyser, Reprod Domest Anim 42:143–148.
- Salhab S.A., Zarkawi M., Wardeh M.F., Al-Masri M.R., Kassem R., 2003 - Characterization and evaluation of semen in growing Awassi ram lambs, Trop Anim Health Prod 35:455–463.
- Sanz-Sáez Á., Érice G., Aguirreolea J., Muñoz F., Sánchez-Diaz M., Irigoyen J.J., 2012 - Alfalfa forage digestibility, quality and yield under future climate change scenarios vary with Sinorhizobium meliloti strain, Journal of Plant Physiology, 169:782-788.

## DIAGNOSTIC AND THERAPEUTIC PROTOCOL FOR MAMMARY TUMOURS IN CANINES AND FELINES

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#### Abstract

Mammary tumors are a common pathology among female cats and dogs, and less common in males of these species. Intact and older females are more susceptible to this condition. A mammary tumor is typically suspected when a lump is discovered during a physical examination of the abdominal area, along the mammary chains. Although surgical treatment is the therapy of choice in most cases, sometimes chemotherapy may be also required. Establishing a correct diagnosis is the foundation for individual optimisation of treatment, as well as the possibility to predict the course of the pathology and prognosis. Diagnosis is based on obtaining an accurate history, clinical, paraclinical and imaging examination of the patient. All this will allow a tumor staging, which will guide therapy and prognosis. This article presents a diagnostic and therapeutic algorithm developed after centralizing the latest data published in the literature, providing a guide in the management of mammary tumors in canids and felines.

Key words: mammary tumors, canine, feline, protocol

Mammary gland tumours are commonly seen in veterinary pathology, representing the most common neoplasms in unspayed bitches, while in cats they rank third in incidence after skin cancer and lymphoma (Withrow et al., 2012). Their development is based on an abnormal accumulation of cells due to excessive proliferation. insufficient apoptosis, and disturbances in cell differentiation (Hanahan & Weinberg, 2000; Tavasoly et al., 2013).

Despite having such a high frequency in veterinary medical practice, similar to pathology in human medicine, this neoplastic condition is still not fully elucidated being an open topic for study and analysis (Akram et al., 2017). Therefore, in the literature are encountered many publications, sometimes contradictory, which complicates the clinical approach to this disease. This study aimed to develop a protocol for diagnosis and therapy in mammary tumors, elaborated after centralizing the latest data published in the literature, which will facilitate the approach of this condition by the veterinarian in the clinic.

## MATERIAL AND METHOD

The study was conducted by consulting PubMed, Google Scholar, ResearchGate, Scopus databases using keywords such as tumors, neoplasia, mammary gland, canine and feline.

## **RESULTS AND DISCUSSIONS**

The results of this study were stated in the form of 2 diagrams, one for diagnosis and one for therapy (*Figure 1 and Figure 2*).

Most of the time in canids and felines, the definitive diagnosis of mammary tumors is made after surgery (post-excision) by histopathological confirmation, which is why tumor staging is an extremely important step in the approach of patients (Goldschmidt et al., 2011; Rasotto et al., 2017). Staging actually involves finding out how far this pathology has progressed, and identifying the stage it has reached (Kaszak et al., 2022).

As shown in *Figure 1*, the diagnosis is based on obtaining an accurate history, clinical examination of the patient, as well as performing paraclinical examinations (fine needle aspiration, chest X-rays, biochemical and haematological examination, histopathological examination) whose result will allow the clinician to select the therapy.

The clinical examination includes a general evaluation of the patient and a specific assessment of the mammary glands. Each mammary gland will be examined and palpated individually in order to identify differences in consistency, as mammary tumours in canids often have a raised consistency and a nodular appearance. The number of mammary glands affected is identified, as well as

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adherence to underlying tissues (Sleeckx et al., 2011).

The tumour formation will be measured, given the fact that its size is also an important factor in staging. Regional lymph nodes will also be assessed, as both axillary and superficial inguinal lymph nodes can be identified on palpation if they are enlarged. The existence of metastases usually coincides with the observation of non-specific clinical signs such as weight loss, fatigue, lethargy, dyspnoea, cough, which is why the evaluation of the general condition is also important. Fine needle aspiration of mammary gland tumours, although controversial due to the structural heterogeneity of the mammary gland, allows differentiation from other pathologies that require a different approach than mammary gland tumours, and therefore should also be performed (Dobson & Lascelles, 2011; Raskin & Meyer, 2016).

The definitive diagnosis, however, will only be made on the basis of histopathological examination (Bulman-Fleming, 2020).



Figure 1 Diagnostic protocol in mammary tumors



Figure 2 Therapeutic approach to mammary tumours

Fine needle aspiration of lymph nodes allows us to assess the extent of the tumour process. This will only be done if the lymph nodes are reactive, i.e. they are enlarged in volume and can be identified on palpation.

It is important to note that if no tumour cells are identified during this examination, a falsenegative result cannot be excluded, however, their detection confirms the existence of metastases in the lymph nodes.

Radiological examination of the chest, in 3 views, is performed with the aim of identifying lung metastases. Computed tomography provides details that allow better identification of lung metastases, but due to high costs, low accessibility and the fact that it can only be performed under general anaesthesia, it is rarely used in diagnosis (Bulman-Fleming, 2020).

Abdominal ultrasound is also recommended to assess medial iliac and superficial inguinal lymph nodes in order to identify metastases earlier (Soler et al., 2016).

Biochemical and haematological profiling does not provide specific results for breast neoplasia, but is of preoperative importance by identifying concurrent geriatric conditions and paraneoplastic syndromes (Sleeckx et al., 2011).

By connecting the results obtained from all these investigations, tumor staging will be established and it will be possible to select the appropriate therapy (Withrow et al., 2012).

The first step in therapy will be determined by the outcome of the radiological and ultrasound examination. If metastases are found to be present, then surgery will not be pursued but palliative treatment will be initiated (Andrew Novosad, 2003; Valdivia et al., 2021). However, if the patient does not have metastases, the treatment of choice is surgery, performing mammary gland ablation (Hörnfeldt & Mortensen, 2023; Tran et al., 2016).

It should be noted that the therapeutic approach to this pathology is very different in canids compared to felines. This is based on the much more aggressive phenotype found in felines, as well as the particularities of lymphatic drainage. About 80-90% of feline mammary tumours are malignant and with high metastatic potential, so their approach is also different (Cassali et al., 2018). In canids, about 50% of tumours are benign and malignant tumours have low metastatic potential, а simple mastectomy so is recommended, sometimes regional depending on the tumour location (as shown in the diagram).

both species, however, In the histopathological findings will guide the follow-up therapy, so that depending on the result obtained, it will be decided whether further surgery is necessary (when the excised margin was not large enough and neoplastic cells remained in the tissue), adjuvant chemotherapy (in the case of particular types of tumours, malignant tumours with stage greater than 3, or in any type of malignant tumour in cats), or no further treatment in the case of benign or incipient malignant tumours. There are several protocols described in the literature. In substances such general, as Doxorubicin. Cyclophosphamide and Carboplatin are used (Dobson & Lascelles, 2011; Withrow et al., 2012).

In terms of the chemotherapeutics used, there is no standard protocol yet. They are still being studied, including in women's breast cancer. Metronomic therapy is also frequently used and is based on the administration of low doses of cytostatics, having an antiangiogenic and immunomodulatory role, potentiated by the addition of non-steroids. It is therefore not a targeted therapy on tumour cells, but aims to decrease neoformation vessels (Mutsaers, 2009; Rossi et al., 2018).

Discussion with the owner is a key point when initiating chemotherapy treatment. It is very important to communicate to the owner that this chemotherapy will not cure the patient, but will slow down metastasis.

Periodic evaluation of the patient will be necessary, because before each administration of chemotherapy HLC and blood biochemistry will be mandatory, with special attention to lymphocytes, liver and kidney profile. Depending on these values the next step will be approached accordingly. The main adverse reactions that occur are of a digestive nature that can be treated like any normal gastroenteritis.

## CONCLUSIONS

Establishing a correct diagnosis is the basis for individual optimisation of treatment, as well as the possibility to predict the course of the pathology and prognosis.

In cats, early diagnosis and aggressive surgery is the first-line treatment associated with a better prognosis.

Breast tumors in canids have a lower metastatic potential, so surgery is generally curative, but the outcome of the histopathological examination will indicate the need for further treatment.

Chemotherapy is used to slow the progression of the disease, not to cure the patient, an aspect that should be highlighted from the beginning to the owners.

#### REFERENCES

- Akram, M., Iqbal, M., Daniyal, M., & Khan, A. U., 2017 - Awareness and current knowledge of breast cancer. Biological Research, 50(1), 33. https://doi.org/10.1186/s40659-017-0140-9
- Andrew Novosad, C., 2003 Principles of treatment for mammary gland tumors. Clinical Techniques in Small Animal Practice, 18(2), 107–109. https://doi.org/10.1053/svms.2003.36625
- Bulman-Fleming, J., 2020 Clinical small animal internal medicine (D. Bruyette, Ed.; Vol. 2). Wiley Blackwell.
- Cassali, G. D., Campos, C. B. de, Bertagnolli, A. C., Estrela-Lima, A., Lavalle, G. E., Damasceno, K. A., Di Nardi, A. B., Cogliati, B., Costa, F. V. A. da, Sobral, R., Di Santis, G. W., Fernandes, C. G., Ferreira, E., Salgado, B. S., Vieira-Filho, C. H. da C., Silva, D. N., Martins-Filho, E. F., Teixeira, S. V., Nunes, F. C., & Nakagaki, K. Y. R., 2018 - Consensus for the diagnosis, prognosis and treatment of feline mammary tumors. Brazilian Journal of Veterinary Research and Animal Science, 55(2), e135084. https://doi.org/10.11606/issn.1678-4456.bivras.2018.135084
- Dobson, J. M., & Lascelles, B. D. X, 2011 BSAVA manual of canine and feline oncology. British Small Animal Veterinary Association.
- Goldschmidt, M., Peña, L., Rasotto, R., & Zappulli, V. 2011 - Classification and Grading of Canine Mammary Tumors. Veterinary Pathology, 48(1), 117–131.

https://doi.org/10.1177/0300985810393258

- Hanahan, D., & Weinberg, R. A., 2000 The Hallmarks of Cancer. Cell, 100(1), 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9
- Hörnfeldt, M. B., & Mortensen, J. K., 2023 Surgical dose and the clinical outcome in the treatment of mammary gland tumours in female dogs: a literature review. Acta Veterinaria Scandinavica, 65(1), 12. https://doi.org/10.1186/s13028-023-00674-1
- Kaszak, I., Witkowska-Piłaszewicz, O., Domrazek, K., & Jurka, P., 2022 - The Novel Diagnostic

Techniques and Biomarkers of Canine Mammary Tumors. Veterinary Sciences, 9(10), 526. https://doi.org/10.3390/vetsci9100526

Mutsaers, A. J., 2009 - Metronomic Chemotherapy. Topics in Companion Animal Medicine, 24(3), 137–143.

https://doi.org/10.1053/j.tcam.2009.03.004

- Raskin, R. E., & Meyer, D., 2016 Canine and Feline Cytology: A Color Atlas and Interpretation Guide (Third Edition). Elsevier Health Sciences.
- Rasotto, R., Berlato, D., Goldschmidt, M. H., & Zappulli, V., 2017 - Prognostic Significance of Canine Mammary Tumor Histologic Subtypes: An Observational Cohort Study of 229 Cases. Veterinary Pathology, 54(4), 571–578. https://doi.org/10.1177/0300985817698208
- Rossi, F., Sabattini, S., Vascellari, M., & Marconato, L., 2018 - The impact of toceranib, piroxicam and thalidomide with or without hypofractionated radiation therapy on clinical outcome in dogs with inflammatory mammary carcinoma. Veterinary and Comparative Oncology, 16(4), 497–504. https://doi.org/10.1111/vco.12407
- Sleeckx, N., de Rooster, H., Veldhuis Kroeze, E., Van Ginneken, C., & Van Brantegem, L., 2011 -Canine Mammary Tumours, an Overview. Reproduction in Domestic Animals, 46(6), 1112–

1131. https://doi.org/10.1111/j.1439-0531.2011.01816.x

- Soler, M., Dominguez, E., Lucas, X., Novellas, R., Gomes-Coelho, K. V., Espada, Y., & Agut, A., 2016 - Comparison between ultrasonographic findings of benign and malignant canine mammary gland tumours using B-mode, colour Doppler, power Doppler and spectral Doppler. Research in Veterinary Science, 107, 141–146. https://doi.org/10.1016/j.rvsc.2016.05.015
- Tavasoly, A., Golshahi, H., Rezaie, A., & Farhadi, M.,
  2013 Classification and grading of canine malignant mammary tumors. Veterinary Research Forum: An International Quarterly Journal, 4(1), 25–30.
- Tran, C. M., Moore, A. S., & Frimberger, A. E., 2016 -Surgical treatment of mammary carcinomas in dogs with or without postoperative chemotherapy. Veterinary and Comparative Oncology, 14(3), 252–262. https://doi.org/10.1111/vco.12092
- Valdivia, G., Alonso-Diez, Á., Pérez-Alenza, D., & Peña, L., 2021 - From Conventional to Precision Therapy in Canine Mammary Cancer: A Comprehensive Review. In Frontiers in Veterinary Science (Vol. 8). Frontiers Media S.A. https://doi.org/10.3389/fvets.2021.623800
- Withrow, S., Vail, D., & Page, R., 2012 Withrow and MacEwen's Small Animal Clinical Oncology.

## ASSESSMENT OF THE SPOILAGE MICROFLORA IN SWINE AND BROILER CARCASSES

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#### Abstract

The microbial load is of major importance in terms of the quality, sanity, and freshness of the meat. The aim of our study was to perform a microbial risk assessment at warm and chilled swine and broilers carcasses represented by the psychrotrophic bacteria. The research material was represented by swine and broiler carcasses collected in past years. The results showed a variation in swine and broilers microbiological carcasses. The point of interest was based on the microorganisms presented in both species. Microbial load from the surface of carcasses is significantly influenced by the temperature in the chilling room of the slaughterhouse, if the temperature is inadequate, the microbial load is significantly higher.

Key words: Microbial residues, consumer safety, sustainable environment

Microorganisms, by their characteristics, can reduce the quality of food, or even make it inedible, either through their pathogenic action, or through degradation and the production of toxic metabolite.

Psychrotrophic bacteria produce different types of spoilage depending on the conditions of keeping the meat and its age. The spoilage microflora, in general, multiplies faster at low temperatures than the pathogenic one, with which it is otherwise in competition. That is why in most cases, at the level of meat kept at refrigeration temperatures, signs of spoilage appear before the number of pathogenic germs is harmful to the consumer, but this is not true in all cases, which is why the lack of organoleptic changes, not it is synonymous with the lack of harmfulness (Belous, 2023)

The microbial population at the surface of swine carcasses before chilling was represented by the following genera: *Staphylococcus, Micrococcus, Lactobacillus, Neisseria, Aeromonas, Acinetobacter, Moraxella, Pseudomonas, Yersinia, Serratia, Hafnia, Proteus* and *Escherichia.* 

The load and the initial configuration of the microflora at the broiler carcass level is influenced by the animal's health condition before slaughter, the duration, and conditions during transport, as well as by the way the carcasses are processed in the slaughterhouse. Therefore, the results obtained regarding the load and the microbial configuration on the surface of the carcasses are largely dependent on the strict observance of hygiene rules along the entire technological flow of carcass processing. Most important microbial population detected at surface of the broiler carcasses before chilling was represented by following genera: *Pseudomonas, Aeromonas, Enterobacteriaceae, Yersinia, Staphylococcus, Streptococcus, Micrococcus, Acinetobacter, Moraxella, Vibrio, Escherichia coli.* 

To obtain carcasses with a very good hygienic quality, the main aim during the slaughtering process is to have a very low initial microbial load (Dan, 2017). In the case of swine carcasses, the initial microflora differs from bovine carcasses, because of the different technological flow steps, like the scalding, depilation, and singeing.

The carcass spoilage is influenced by the following factors: a high initial load of psychrotrophs, increased temperature in the chillers, high aw (water activity) values on the surface of the carcasses close to 1.0. As a result, Gram negative psychrotrophs bacteria will represent the main microflora with the highest spoilage potential for refrigerated carcasses. If the meat is kept at temperatures below 7°C or lower, under aerobic conditions, *Pseudomonas*,

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Moraxella, Acinetobacter and Psychrobacter members will have the highest growth rate, hence their increased spoilage potential. Also, Shewanella spp. and some members of the Entorobacteriaceae family were able to grow and to produce spoilage metabolites.

## MATERIAL AND METHOD

The research material was represented by 288 swine meat samples, collected between January and December 2021, from two pig slaughtering units in Cluj County. The samples were collected using a destructive method, before (warm carcass), and after chilling (24 hours), each month three samples from both the surface and the depth, in compliance with the current legislation (Reg. CE 2073/2005). From the surface of the carcasses, four slices, with a thickness of 2-3 mm were collected from different anatomical regions: the inner side of the thigh, chest, hind legs, and pelvic cavity. Samples were collected randomly, considering that they should come from carcasses obtained both at the beginning and at the end of the slaughtering process.

To determine the microbial load and configuration in broilers, 72 representative samples (poultry carcasses) were taken, from 8 batches, as follows: 9 carcasses/month, respectively batch, packed in a cryovac system, harvested between April and December 2021 at a slaughterhousedistribution unit in the Transylvania region. In the slaughtering unit studied, the slaughtering process is automated, using modern equipment, with a production capacity of 10,000 birds / 8 hours. The microbiological examinations were carried out in the laboratory of the Food Inspection and Control discipline of the Faculty of Veterinary Medicine Cluj-Napoca.

А quantitative approach based on microbiological determinations analyses were carried out: psychrotrophic plate count, isolation of Pseudomonas, Aeromonas, Yersinia and Enterobacteriaceae, using selective media as follows: for aerobic plate count PCA agar (Merck), for Aeromonas and Pseudomonas, GSP agar (Merck), for Yesinia, CIN agar (Merck), and for Enterobacteriaceae - VRBD agar (Merck). Serial decimal dilutions (10-6) were obtained from 10 grams of meat and 90 ml water buffered peptone. The spreading method was used to inoculate 0.1ml onto the surface of two Petri plates. Incubation was realized at 20°C, for 72 hours. The biochemical confirmation test was realized using API 20 E and API 20 NE (Biomerieux). Statistical analysis was carried out using Origin 8.5 software by comparison of means by analysis of variance through ANOVA test or API LabPlus. The statistical interpretation of the results was realized according to the probability indicator:  $p \le 0.05$  (confidence level 95%). The results were depicted as log CFU/cm<sup>2</sup>.

## **RESULTS AND DISCUSSIONS**

From the analysis of the results regarding microflora of warm swine carcasses, we can say that if the slaughtering process is carried out in strict compliance with the hygiene practices, the microbial load presented lower values, not exceeding the maximum limits allowed or recommended by the current legislation, respectively the literature (Liora, 2013; Reg CE 2073/2005, Dan, 2017).

In swine carcasses case, the trimestral average of the total psychrotrophic count at the surface of warm carcasses presented different values, ranged between  $3.80\pm0.60 \log \text{CFU/cm2}$  in trim. I and  $5.01\pm0.30 \log \text{CFU/cm2}$  in trim. III, with a minimum of  $2.63\pm0.44 \log \text{CFU/cm2}$  in January and a maximum of  $5.22\pm0.26 \log \text{CFU/cm2}$  in September.

The microbial population at the surface of swine carcasses before chilling is depicted in Figure 1, being represented by the following genera: *Staphylococcus, Micrococcus, Lactobacillus, Neisseria, Aeromonas, Acinetobacter, Moraxella, Pseudomonas, Yersinia, Serratia, Hafnia, Proteus* and *Escherichia.* 

In the case of the samples collected from the surface of refrigerated carcasses, we found that the dominant species is the Gram negative one, respectively 73.98%, and the Gram-positives are only 26.02%.

Among the Gram-negative bacteria, it was found that the psychrotrophic are the dominant population: *Pseudomonas* (23.93%), *Acinetobacter* (9.4%), *Moraxella* (7.69%), *Yersinia* (7.26%), *Serratia* (6.35%), *Hafnia* (4.27%), *Aeromonas* (3.84%), *Shewanella* (3.42%), *Escherichia* (2.99%), *Proteus* (1.79%) and *Enterobacter* (2.14%) (Figure 2).



Figure 1. Microbial population at the surface of warm swine carcasses in slaughterhouse



Figure 2. Microbial population at the surface of chilled swine carcasses in slaughterhouse

In the case of broiler carcasses, to identify the psychrotrophic bacterial species developed on culture media (SI, GSP-agar, Yersinia-agar, VRBD-agar), API 20NE and API 20E (Biomerieux) biochemical confirmation tests were performed. After incubation of the API galleries at the thermostat, differentiated according to the type of test used, the results were interpreted with the help of the identification software. For the rest of the results, additional tests were performed, different depending on the product provided by the computer program. When the microorganisms could not be identified even with the help of these tests, the result was classified as an "unidentified bacterial species".

The main bacterial species identified and their incidence in the case of broiler chicken carcasses are shown in the graph in fig. 3.

From the processing of the results presented in the graph in figure 3, it follows that the predominant bacterial species are Gram negative, respectively 70.26%, while Gram positive bacteria represented 29.44% of the total.

Among Gram positive bacteria, 7.01% are represented by staphylococci, 3.44% by streptococci, 8.77% by micrococci and 10.52% by lactic acid bacteria. The differentiation between the coccoid formations was made based on the bacterioscopic examination, the catalase and oxidase test.

Among representatives of the the Enterobacteriaceae family, the following species were identified, based on API 20 E tests: E. coli (5.26%), Vibrio vulnificus (1.69%), Citrobacter freundii (3.38%). Among these species, health problems can be caused by Vibrio parahaemolyticus and E. which have coli, pathogenic strains for humans.

The rest of the species are part of the category of psychrotrophic germs of the *Enterobacteriaceae* family, frequently involved in triggering the alterative processes of meat at loads between 106 - 107 cfu/cm2 (Gram L. et al, 1999).



Figure3. Incidence of bacterial species at the surface of poultry carcasses.

Regarding the germs of the *Yersinia* genus, from the results obtained, no pathogenic species were identified, but only *Yersinia frederiksenii* (5.26%)

Among the Gram-negative bacteria, it was found that psychrotrophic bacteria predominate, of which: the genus *Pseudomonas*, with 25.46%, the genus *Moraxella* with 7.01%, the genus *Aeromonas* with the species *Aeromonas hydrophila* 5.26%.

#### CONCLUSIONS

Microbial load from the surface of carcasses is significantly influenced by the temperature in the chilling room of the slaughterhouse, if the temperature is inadequate, the microbial load is significantly higher. The microbiological assessment carried out on pork carcasses demonstrates the role of psychrotrophic microorganisms in the spoilage processes in case of improper monitoring of the slaughtering processing.

As broiler carcasses are stored for longer periods of time in refrigeration spaces, the bacterial load on the surface shows an upward evolution, reaching values of up to 8.0-9.0 log cfu/cm2, the level of growth of the bacterial population, such as and the configuration of the germs present on the surface of the carcasses being dependent on the refrigeration temperature and the relative humidity of the storage space. Based on these aspects, we can appreciate that the psychrotrophic spoilage microorganisms are (also) important from an economic point of view, if the microbial population exceeds the level of 6.0 log cfu/cm2, because of the production of sensory changes, respectively of the reduction of the limit duration for consumption.

#### ACKNOWLEGMENTS

The microbiological examinations were carried out in the laboratory of the Food Inspection and Control discipline of the Faculty of Veterinary Medicine Cluj-Napoca. The data comes from different studies.

#### REFERENCES

ADRIAN-COSMIN, C.A.R.A.I.M.A.N., SORIN, D. and SIMON, P., 2023. GREEN BUILDINGS AND THEIR BENEFITS IN THE CONTEXT OF SUSTAINABLE DEVELOPMENT. Annals of Constantin Brancusi'University of Targu-Jiu. Economy Series, (1).

Alban, L., Antunović, B., Belous, M., Bonardi, S., García-Gimeno, R.M., Jenson, I., Kautto, A.H., Majewski, M., Oorburg, D., Sakaridis, I. and Sirbu, A., 2023. Mapping ways of detecting and handling antimicrobial residues in pigs and pig meat in-and outside Europe. *Food Control*, p.109899. Belous, M., Mihaiu, M., Tabaran, A., Reget, O.L., Cîmpean, R. and Dan, S.D., 2023. Assessment of the spoilage microflora in swine carcasses. *Safe Pork*, *13*(1).

Dan, S.D., Mihaiu, M., Reget, O., Oltean, D. and Tăbăran, A., 2017. Influence on week organic acids on pathogens on swine carcasses.

**Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I. and Nielsen, T.F., 1999.** Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. *Applied and environmental microbiology*, *65*(3), pp.969-973.

Liora, M.I.H.A.I.U., Mihaiu, M., TĂBĂRAN, A., DAN, S.D., CORDIŞ, I.V., Pivariu, B. and Cordea, D., 2013. Antimicrobial Resistance Evaluation of Pathogen Salmonella Strains Isolated in Pork and Poultry Meat. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine, 70(2).

Regulation (EC) 2073/2005, date access October, 2023

Article https://doi.org/10.61900/SPJVS.2023.01.07

## FAST ASSESSMENT OF PULMONARY HYPERTENSION TYPE IN DOGS USING BASIC ULTRASONOGRAPHY TECHNIQUES

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#### Abstract

Pulmonary hypertension (PH) is a complex hemodynamic condition defined by increased pulmonary vasculature pressure. The precise diagnostic can be obtained by evaluating the clinical status of the patient, performing complex imaging methods and by direct assessment of pulmonary arterial pressure. Invasive methods are considered golden standard; however, these techniques are limited in animals with respiratory failure. Previous studies aimed to evaluate the cut-off value of the PV/PA ratio in different types of PH in dogs. Therefore, the aim of this study is to correlate the type of PH with the changes of the pulmonary vein to pulmonary artery ratio (PV/PA) in dogs assessed by transthoracic echocardiography. The ratio was assessed using the right parasternal long axis view of the heart in B-mode and Mmode. This retrospective study included thirty-nine dogs presented for cardiologic examination at our Veterinary Teaching Hospital. Dogs were divided in three groups as following: control group (n=10), precapillary PH group (n=16)and postcapillary PH group (n=13). The median and interquartile range (M±IQR) of the PV/PA ratio were 0.95 (0.79-1.5) in the control group, 0.85 (0.55-0.92) in precapillary PH group and 1.86 (1.44-2.16) in the postcapillary PH group. The area under the curve (AUC) for the PV/PA ratio comparison between the control and postcapillary PH groups was 1 (P<0.05), suggesting a cut-off ratio of 1.17 for the postcapillary PH group. This value is lower than the previous suggested value (>1.7). The importance of this finding derives from the ability to observe early changes induced by PH in dogs diagnosed with different stages of myxomatous mitral valve disease (MMVD), even when the tricuspid regurgitating jet cannot be assessed.

Key words: pulmonary hypertension; precapillary; postcapillary; pulmonary vein; pulmonary artery; PV/PA ratio.

## INTRODUCTION

Pulmonary hypertension (PHT) is defined by increased pressure in the pulmonary vasculature and represents а pathophysiological and hemodynamic associated with status cardiovascular, respiratory or systemic pathologies (Reinero et al. 2020). Assessing the severity of postcapillary PH is performed by evaluating the pulmonary artery wedge pressure (PAWP) by right heart catheterization. In veterinary medicine this invasive diagnostic method is not commonly used in the clinical setting, however the pulmonary arterial pressure is estimated by measuring the tricuspid regurgitation jet (Soydan et al. 2015). Another proposed ultrasonography technique for characterizing PH is assessing the right ventricular end-diastolic area (RVEDA) as an indicator for the right ventricle size and to evaluate the right ventricle systolic function assessing the tricuspid annular plane systolic excursion (TAPSE) and the fractional area change (FAC) (Vezzosi et al. 2018). Due to the fact that the measurement of tricuspid regurgitation jet is sometimes difficult to perform to animals in respiratory failure, and the evaluation of the right ventricle area and function require advanced ultrasonography techniques, this study aims to describe the changes of PV/PA ratio associated with PH using simple ultrasonography techniques. Two studies aimed to characterize the normal value of the PV/PA ration in dogs and concluded it is approximately equal to 1 (Merveille et al. 2015; Birettoni et al. 2016).

The PV/PA ratio has been described as a simple and reproducible measurement that may help discriminate dogs in congestive heart failure from asymptomatic dogs with MMVD, concluding that with advancing stages of the pathology the ratio will increase above 1.7 (Merveille et al. 2015). Another study which evaluated 76 dogs with varying degrees of precapillary PH concluded that the value of the ratio decreases proportionally to the severity of the disease (Roels et al. 2019). Decreased pulmonary vein diameter was speculated based on the decreased left ventricular and increased pulmonary pressure arterial

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resistance, on the compression of the vein caused by increased volume on the adjacent artery, or a combination of these factors. In West Highland white terrier (WHWT) dogs with PH consecutive to breed-specific pulmonary fibrosis, the ratio value was used for estimating the survival time (Roels et al. 2021). Subsequently, another study evaluated the ratio in dogs diagnosed with heartworm disease in order to estimate the severity of PH, concluding that a PV/PA value  $\leq 0.845$  is correlated to moderate and severe stages, with increased sensitivity and specificity values (Matos et al. 2023).

#### **MATERIAL AND METHOD**

This retrospective study included thirty-nine dogs presented for cardiologic examination at our Veterinary Teaching Hospital. Dogs were divided in three groups as following: control group (n=10), precapillary PH (prePH) group (n=16) and postcapillary PH (postPH) group (n=13) according to the ACVIM consensus (table 1) (Reinero et al. 2020). The population consisted of the following breeds: mixed-breed (25.64%; n=10), Maltese (15.38%; n=6), Yorkshire terrier (10.25%; n=4), Chihuahua, Retriever, Pekingese, Poodle, Shih Tzu, West Highland white terrier (WHWT) (5.12%; n=2 each), Beagle, German Shepherd, Mioritic Shepherd, Cocker, Pug, Spitz and Teckel (2.56%; n=1) each. The dogs which presented pericardial effusion have been excluded from this study.

			Table 1					
Characteristics of the studied population								
	Control	prePH	postPH					
	group	group	group					
<b>Age</b> (y/o)	8.6±2.91	12.62±3.18	12.3±2.25					
Weight (kg)	13.03±15.12	18.25±15.16	10.55±9.71					
Gender (M/F)	9/1	10/6	10/3					

The cardiac ultrasonography was performed using General Electric LOGIQ V5 Expert ultrasound machine equipped with two phased array probes (4-8 MHz and 1-4 MHz). The PV/PA ratio was assessed using the right parasternal long axis view of the heart in B-mode and M-mode (*figure 1*). For each patient, the left atrium to aorta (LA/Ao) ratio and PV/PV ratio have been evaluated along with the PH type according to the ACVIM consensus. The data has been statistically analysed using the IBM® SPSS® software.



Figure 1 – PV/PA measurement in B-mode (A.) and M-mode (B.) ★ – pulmonary vein (PV); ☆ – pulmonary artery (PA)

## **RESULTS AND DISCUSSIONS**

According to the ACVIM classification, the groups consisted of 41.02% dogs with precapillary PH and 33.33% dogs with postcapillary PH. The prePH group includes 12.5% dogs with arterial pulmonary hypertension (group 1), 15.38% dogs with PH due to respiratory disease (group 3) and 7.68% dogs with parasitic disease (group 5) secondary to Dirofilaria immitis infection. For 68.75% of the dogs included in the prePH group, investigations for establishing the cause of pulmonary hypertension have been declined by the owners, therefore these dogs could not be classified. All dogs included in the postPH group had PH due to left heart disease (group 2) and have been diagnosed with myxomatous mitral valve disease (MMVD) stages B2 and C according to the ACVIM consensus (Keene et al. 2018).

The median and interquartile range  $(M\pm IQR)$  of the PV/PA and LA/Ao ratios of each group are shown in *table 2*. The patients in the control group had a mean value of the PV/PA ratio approximately equal to 1, this finding being consistent with the literature (Merveille et al. 2015; Birettoni et al. 2016). The median value of 0.85

obtained for the prePH group is similar to the data obtained in previous studies (Matos et al. 2023). The postPH group had a higher median value compared to the cut-off described in the literature – 1.86 in our population compared to 1.7 (Merveille et al. 2015).

Table 2	2
Mean and interquartile range (M±IQR) of the PV/PA	
ratios	

	Percentile				
	25	50	75		
<b>PV/PA</b> ratio					
Total	0.79	0.95	1.50		
Control group	0.85	0.95	1.08		
prePH group	0.55	0.85	0.92		
postPH group	1.44	1.86	2.16		
LA/Ao					
Total	1.22	1.42	1.87		
Control group	1.20	1.25	1.40		
prePH group	1.16	1.28	1.46		
postPH group	1.81	2.19	3.39		

The LA/Ao ratio has not been previously assessed in dogs with PH, to the authors' best knowledge. However, the dogs included in the postPH group developed pulmonary hypertension due to left heart disease, therefore the left atrium will have a significant volumetric increase. In dogs with prePH it is expected to either have a normal volumetric LA or to have a volume decrease due to hypoperfusion. In the study population these assumptions have been confirmed.



Figure 2 – Spearman R correlation of the LA/Ao ratio with the PV/PA ratio

*Figure 2* depicts the Spearman R correlation of the LA/Ao and PV/PA ratios, indicating a moderate correlation between them, with increased significance. Thus, an increase of the LA/Ao ratio suggests an increase of the PV/PA ratio, as observed in the postPH group.

The statistical difference by non-parametric groups was assessed using the Kruskal-Wallis test

and post-hoc pairwise comparison for multiple groups using the SPSS software. The result of the analysis of the PV/PA ratio between the evaluated groups emphasizes the statistically significant differences between the prePH - postPH groups (P<0.05) and the control - postPH groups (P=0.004). No statistical significance was observed for the prePH – control groups (P=0.685). Thus, the volumetric changes in the pulmonary vessels of the dogs diagnosed with precapillary PH did not indicate marked differences compared to the control group and a cut-off value could not be estimated.

The same methodology was used to compare the LA/Ao ratio between the three groups, showing a statistically significant difference between the same studied populations: prePH – postPH groups, respectively control – postPH groups (P<0.05), but no statistical significance for the pre-PH – control group.

analysis of the comparison Further between the control and postPH group has been performed by calculating the Area Under the Curve (AUC) and the value 1 (P<0.05) was obtained, indicating a very good predictability value. A cut-off ratio of 1.17 has been obtained for this class. The 95% confidence intervals for PV/PA values in postPH and control population are depicted in *figure 3* (Turcu 2023). The previously evaluated cut-off value of 1.7 has been proposed for patients with severe heart failure (International Small Animal Cardiac Health Council ISACHC class III) (Merveille et al. 2015). In our population the postPH group consisted of dogs with mild and severe left heart failure, stages B2 (23.07%) and C (76.9%) of MMVD according to the ACVIM consensus.





Further research is warranted to characterize the hemodynamic particularities of

patients with precapillary pulmonary hypertension, focusing on the tricuspid and/or pulmonary regurgitation jet assessment.

#### CONCLUSIONS

The evaluation of PV/PA ratio is a simple and feasible method used to assess the type of pulmonary hypertension in dogs in whom the tricuspid regurgitation jet cannot be properly evaluated.

#### ACKNOWLEGMENTS Arial, 10, B, Center, All Caps

This research received no external funding. The authors report there are no competing interests to declare

#### REFERENCES

- Birettoni, F, D Caivano, V Patata, Sydney N Moise, C Guglielmini, Mark Rishniw, şi Francesco Porciello. 2016. "Canine pulmonary vein-topulmonary artery ratio: echocardiographic technique and reference intervals". *Journal of Veterinary Cardiology* 18 (4): 326–35. https://doi.org/10.1016/j.jvc.2016.07.004.
- Keene, Bruce W, Clark E Atkins, John D Bonagura, Philip R Fox, J Haggstrom, Virginia Luis Fuentes, Mark A. Oyama, John E Rush, Rebecca L Stepien, și Masami Uechi. 2018. "ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs". Journal of veterinary internal medicine 33 (3): 1127–40. https://doi.org/10.1111/jvim.15488.
- Matos, Jorge Isidoro, Alicia Caro-Vadillo, Yaiza Falcón-Cordón, Sara Nieves García-Rodríguez, Noelia Costa-Rodríguez, Elena Carretón, și José Alberto Montoya-Alonso. 2023. "Echocardiographic Assessment of the Pulmonary Vein to Pulmonary Artery Ratio in Canine Heartworm Disease". Animals 13 (februarie): 703.

https://doi.org/10.3390/ani13040703.

Merveille, A C, G Bolen, E Krafft, E Roels, S Gomart, A L Etienne, C Clercx, şi K Mc Entee. 2015. "Pulmonary Vein-to-Pulmonary Artery Ratio is an Echocardiographic Index of Congestive Heart Failure in Dogs with Degenerative Mitral Valve Disease". *Journal of veterinary internal medicine* 29 (6): 1502–9. https://doi.org/10.1111/jvim.13634.

- Reinero, Carol, Lance C Visser, Heidi B Kellihan, Isabelle Masseau, Elisabeth A Rozanski, Cécile Clercx, Kurt Williams, Jonathan Abbott, Michele Borgarelli, și Brian A Scansen. 2020. "ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs". Journal of veterinary internal medicine 34: 549–73. https://doi.org/10.1111/jvim.15725.
- Roels, E, Aline Fastrès, A C Merveille, G Bolen, Erik Teske, C Clercx, și K McEntee. 2021. "The pulmonary prevalence of hypertension assessed using the pulmonary vein-to-right pulmonary artery ratio and its association with survival in West Highland white terriers with canine idiopathic pulmonary fibrosis". BMC veterinary research 17 (aprilie): 171. https://doi.org/10.1186/s12917-021-02879-w.
- Roels, E, A C Merveille, E Moyse, S Gomart, C Clercx, şi K Mc Entee. 2019. "Diagnostic value of the pulmonary vein-to-right pulmonary artery ratio in dogs with pulmonary hypertension of precapillary origin". Journal of Veterinary Cardiology 24 (iunie): 85–94. https://doi.org/10.1016/j.jvc.2019.06.001.
- Soydan, Lydia C, Heidi B Kellihan, Melissa L Bates, Rebecca L Stepien, Daniel W Consigny, Alessandro Bellofiore, Christopher Francois, și Naomi Chesler. 2015. "Accuracy of Doppler echocardiographic estimates of pulmonary artery pressures in a canine model of pulmonary hypertension". Journal of Veterinary Cardiology 17 (1): 13–24. https://doi.org/10.1016/j.jvc.2014.10.004.
- Turcu, Andreea Cătălina. 2023. "Evaluation of the hemodynamic changes in dyspneic patiens enroled in the study". În *PhD Thesis* -*Hemodynamic changes in the dyspneic patient*, 105–12.
- Vezzosi, Tommaso, Domenech Oriol, Costa Giulia, Marchesotti Federica, Venco Luigi, Zini Eric, del Palacio Maria Josefa Fernandez, și Tognetti Rosalba. 2018. "Echocardiographic evaluation of the right ventricular dimension and systolic function in dogs with pulmonary hypertension". Journal of veterinary internal medicine, nr. 32 (mai): 1541–48. https://doi.org/10.1111/jvim.15253.
# INVESTIGATIONS REGARDING THE PRESENCE OF MICROORGANISMS IN THE MILK FOOD PRODUCTS COMMERCIALIZED IN REPUBLIC OF MOLDOVA

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#### Abstract

The scientific research reflected in this study constituted the determination of microorganisms in some categories of milk used in human consumption: raw cow milk and raw goat milk, pasteurized milk sold in the store according to the indicators of the total number of germs, microscopy and bacteriology. The recorded results of the number of bacterioscopic microorganisms, microbial colonies after the classification results and milk categories were evaluated, which recorded various microbiological values of a normal bacterial microflora in the milk food product. The bacterioscopic and bacteriological values obtained allow consumers to be safe and use this food product in their consumption.

Key words: Milk, Bacteriology, Bacterioscopy, Microorganisms, Microflora.

Milk is one of the oldest and main foods consumed by humans. It is presented as an emulsion of fat in water, in which other chemical substances are also dissolved and comes from the mammary gland of mammals (cow, sheep, buffalo, goat or mixture). Due to its varied chemical composition and rich in the main groups of necessary nutrients, milk is the most complete food, and the caloric value of milk and dairy products obtained from milk processing is very high due to the content of water, gases, dry matter [2, 5, 4,7,9].

The presence of microorganisms in milk is of particular importance for quality, sanitation and freshness. These microorganisms can increase or decrease the quality of products or make them inedible, either through their pathogenic action or through their degradation and production of toxic metabolites [11,13].

It is considered that the microorganisms in milk are important from the following points of view: some produce flavor and desired physical changes and are used to obtain different dairy products; others can be pathogenic or potentially pathogenic and toxic to humans, which is why their presence in milk must be known and prevented; many can cause various defects in color, consistency, smell and taste in milk and dairy products or create difficulties in their manufacturing technology [10;14,15].

The issue of milk microorganisms refers to most of the groups of microorganisms described in

the existing determinants, which require specialists in the field of food microbiology to know the rationale for using useful ones and removing useless or harmful ones. Therefore, from this point of view, the useful lactic microflora is represented by the lactic bacteria, which belong to two different families: the family Lactobacillaceae and the family Streptococcaceae, comprising the most important microorganisms for the milk industry [1,3,6,8,12].

The purpose of the study is to determine the microorganisms in some categories of milk used in human consumption: raw cow milk and raw goat milk, pasteurized milk sold in the store according to the indicators of the total number of germs, microscopy and bacteriology.

# MATERIAL AND METHOD

The presence of microorganisms in raw and pasteurized cow and goat milk assortments regarding the quantitative study was investigated by using microbiological techniques for sampling representative milk samples from peasant raw cow milk and raw goat milk procured from the central market and pasteurized milk procured from a supermarket from Chisinau.

The microbiological tests were carried out according to the microbiological laboratory methodology in order to identify the saprophytic and pathogenic microflora. Milk samples were taken, which were subject to investigations in the Microbiology Laboratory of the Food Safety and

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Public Health Department, Faculty of Veterinary Medicine, Technical University of Moldova. The bacteriological bacterioscopic and study constituted of the determination of the amount of saprophytic microflora existing in the milk samples, the presence of coliform, salmonella, staphylococci bacteria. Developmental characters of microbial cultures on simple and special culture media were and their characteristics visualized were expressed. At the same time, smears from the native samples and cultures were performed, stained according to the Gram method, the counting of microbial colonies and microscopic visualization with immersion, objective 90, in order to determine the species of microscopic microorganisms and their definition.

# **RESULTS AND DISCUSSIONS**

The microbiological research of different types of milk allowed us to analyze the results of figure 1, where the microscopy of raw cow's milk samples is shown. The results of this study allow us to analyze the contamination of raw cow's milk. It is observed that sample 3 of raw cow's milk is more polluted with microorganisms constituting 6 cocci under microscopy, compared to sample number 2 where the number of microbial cells/cocci constituted 3 microbial cells and sample number 1 constituted 2 microbial cells. These samples of raw cow's milk constituted classes III and IV. These reports confirm the fact that the contamination of these samples of raw cow's milk presents insignificant values, which do not denote pathogenic species, but on the contrary reflect insignificant numbers of saprophytic cocci, which can normally be found in milk.





Source: elaborated by the authors

This detection and isolation is important and allows us to conclude that it is not dangerous for food use. These tests are usually practiced in microbiological laboratories because it is also important to test for the presence of Salmonella pathogenic species in any ready-to-eat food.

Important characteristics regarding the microscopy of the raw goat milk samples performed are reproduced in figure 2, where it is observed that the number of cocci is a higher sample number in number 3. constituting cocci under microscopy, 7 followed by a number of 5 cocci determined by microscopy in sample number 2 and 3 cocci determined by microscopy in sample number 1.

These raw goat milk samples constituted classes III and IV. Based on these data, according to the microbiological laboratory conduct, the scientific research were focused on the bacterioscopic investigations by performing bacterial smears from the samples of milk samples and simple staining with the methylene blue dye. From the point of view of these aspects, the number of microorganisms was determined on the colored preparations according to the simple method, which according to the morphological aspects were spherical representing bacterial cocci.

The analysis of the indices presented in figure 3 shows that, as a result of the microbiological research carried out in the samples of pasteurized milk sold in the store, the microflora consists of 3 cocci bacteria under microscopy in the milk sample number 3, compared to the pasteurized milk samples number 1 and 2 where the cocci bacterial microflora constituted in the sample 1 -2 cocci and the sample 2 - 1 cocci.

The classes of the milk samples according to the microscopic results of the number of detected cocci were classified in the class I category of all the investigated milk samples. As a result of the microscopy research of smears from pasteurized milk, the simple/methylene blue coloration, the morphological aspects of the cocci germs, which were presented on the microscopic field, Gram positive constituting a normal microflora of the investigated pasteurized milk, are revealed.



Figure.2. Microscopy of raw goat milk samples

Source: elaborated by the authors



Figure.3. Microscopy of pasteurized milk samples

Source: elaborated by the authors



Figure.4. The number of microbial colonies in the classes of raw cow's milk samples Source: elaborated by the authors

The bacterioscopic investigations of milk samples of various commercial categories according to the studies carried out demonstrate the fact that all the categories of milk investigated from the bacterioscopic point of view are favorable and beneficial to be used in food.

Of significant importance are the samples of pasteurized milk sold in the store, which in our microbiological research study confirmed that the pasteurized milk food product sold in the store after the conduct of the microbiological examination confirms that this category of milk is polluted with a lower number of saprophytic microorganisms represented by Gram positive cocci.

The data in figure 4 shows some quantitative accounts of the number of colonies highlighted in the bacterial microflora of raw cow's milk recorded by the number of microbial calories visualized in three samples of raw cow's milk sold on the market. Thus, the indications in figure 4 indicate the detection of 4 bacterial colonies in the microflora of raw cow's milk sample number 3, compared to sample number 1, where the number of colonies constituted 2 colonies and sample number 2, where the number of microbial colonies constituted 1 colony. According to the number of microbial colonies, the samples of raw cow's milk constituted classes III and IV.

Figure 5 shows the quantitative study of the number of microbial colonies in the classes of raw goat milk samples and reveals that the classes of these samples of raw goat milk are of category III and IV.

Passages were performed on simple agar culture media, broth and special media for the purpose of identifying pathogenic species of Endo, Saburov microorganisms. Analyzing the obtained results reported in figure 5, it is observed that a higher number of colonies were recorded in the raw goat milk sample nr 3, which constituted 5 microbial colonies, compared to samples one and two where the number of colonies in sample nr. 2 constituted 3 microbial colonies and in sample nr 1 constituted 1 microbial colony. Pathogenic colonies did not develop on Endo and Saburov media. Important characteristics are highlighted on the culture media in the investigated pasteurized milk samples that can be seen in figure 6.

The data of figure 6 determine quantitative indices of the number of colonies in these three samples of pasteurized milk sold in the store, which constitute in all three samples the first class of milk. As a result of the investigations, the highest number of colonies was determined in milk sample number 1, which constituted 2 microbial colonies, followed by samples 2 and 3 where the number of microbial colonies in both samples constituted 1 microbial colony each. No microbial colonies were detected on Endo and Saburov media.

Among the EU member states, milk production per cow is the highest in Denmark (10,097 kilograms) and Estonia (10,020 kilograms) and the lowest in Bulgaria (3,628 kilograms) and Romania (3,362 kilograms) (figure 7; figure 8).

In 2021, just over a fifth (20.9%) of EU raw cow's milk was produced on German farms and a similar proportion (21.8%) was processed at German dairies. Together, Germany, France, the Netherlands, Poland and Italy are responsible for two-thirds (64.2%) of the EU's cow's milk production in 2021 and two-thirds (65.2%) of the cow's milk collected by dairy factories.

Eurostat data also show that there are several EU member states where animals other than cows have an important contribution to the total milk production. For example, in 2021 Spain produced one million tons of goat milk, Greece 900,000 tons and France 800,000 tons. Italy produced 700,000 tonnes of milk from animals other than cows and this included almost all of the EU's buffalo milk production.

In 2021, 149.5 million tonnes of milk were processed at EU dairies, of which 71% to produce cheese and butter. In total, the EU produced 2.3 million tonnes of butter, which required 44.4 million tonnes of milk, 10.4 million tonnes of cheese, out of 61.4 million tonnes of whole milk and 16.4 million tons of skimmed milk, 23.2 million tons of drinking milk and 10 million tons of skimmed milk.



Figure.5. The number of microbial colonies in the classes of raw goat milk samples Source: elaborated by the authors



Figure.6. The number of microbial colonies in the classes of pasteurized milk samples Source: elaborated by the authors



Source: elaborated by the authors



Figure.8. Top diary producers in the EU, 2021 (by product)

# Source: [16]

As for the Republic of Moldova, milk production contracted by 12%, to 217.3 thousand tons compared to the same period of the previous year. However, to ensure its own consumption, the Republic of Moldova needs 520 thousand tons.

Currently, 41 milk processing enterprises operate in the Republic of Moldova, of which only 2 factories import milk. These processing units produce about 90% of all milk products, the rest, 10%, belonging to small producers. According to National Bureau of Statistics, in the first half of 2022, processing enterprises produced 86,638.2 tons of dairy products, approximately 7% less than in the same period of 2021.

In 2022 compared to 2021 was registered a decrease of the milk production by 10,3% and of the production of the animals (live weight) by 2.4%

According to our investigations, the samples of pasteurized milk sold in the store constituted class I according to food standards.

Therefore, the results of investigations of the milk microflora of several categories of raw cow/goat milk and pasteurized cow's milk sold in the store confirmed to us during our investigations, that the presence of microorganisms in milk is of particular importance for the quality, sanitation and state of health freshness.

These microorganisms represented by non-pathogenic saprophytic cocci can grow but without reducing the quality of milk varieties and offer them the possibility of being edible, either through their saprophytic action or through the non-pathogenic one which has a beneficial influence and does not have the property of producing toxic metabolites

# CONCLUSIONS

The bacterioscopic and bacteriological values obtained allow consumers to be safe and use this food product in their consumption.
The bacterioscopic conduct of the bacterial microflora in the investigated milk assortments determined a higher microflora of microbial cocci in the raw goat milk assortment - 7 microbial cells, compared to the raw cow milk assortments - 6 microbial cocci cells and pasteurized milk - 3 cells microbial cocci.

3. Microbial cultures according to the indices of cultural characters in the investigated milk varieties registered important microbiological indices according to the evaluation aspects which constituted 5 microbial colonies in the samples of raw goat milk, compared to samples of raw cow milk-4 microbial colonies and pasteurized milk -2 microbial colonies, constituting saprophytic cocci under microscopy.

4. Milk assortments are recommended to be stored in appropriate conditions to maintain microbiological parameters and organoleptic characteristics.

#### REFERENCES

- [1]BANU, C. 2010. Treaty of the food industry. Ed. ASAB, p.68.
- [2]BOGDAN, A., TOGOE, I. CÎMPEANU G.2011. Food microbiology. Vol.2. Asclepius, Bucharest, p. 35-40.
- [3]BOGDAN, A., ŢOGOE, I, CÎMPEANU, G.2011. Food microbiology. Vol.1. Asclepius, Bucharest, p. 78-90
- [4]BONDOC, I. 2014. Control of products and foods of animal origin, Ed. "Ion Ionescu de la Brad", Iaşi;
- [5]CARP-CĂRARE C. 2014. General microbiology. laşi: Ion Ionescu de la Brad, 245 p. ISBN 978-973-147-153.
- [6]CONSTANTINESCU, C. 2015. Food quality and safety concept and practical applications. Galați: Ed. Performantica., 246p.
- [7]DAN, V.2001. Food microbiology. Alma, Galați, p. 52.
- [8]DARIE, N.2001. Dynamic food biochemistry. Ed. ULB, Sibiu, p. 76.
- [9]DOBREA, M. 2014. Food biotechnologies. Vol. I. Bucharest: Ed. Printech.191 p.ISBN 978-973-718-917-
- [10]GOLBAN, R. 2019. The importance of the bacterial cultures used in production of cheeses. In: *Lucrări ştiinţifice. Seria Medicină Veterinară* (categoria B+), USAMV Iaşi, Medicină Veterinară, vol. 62, partea I, p. 67-71., 0,52 c.a. ISSN. L-1454-7406.
- [11]GOLBAN, R. 2018. Microbial probiotics the action mechanism and the use of them. In: Lucrări ştiinţifice. Seria Medicină Veterinară (categoria B+), USAMV Iaşi, Medicină Veterinară, vol. 61, partea 1, p. 39-42., 0,52 c.a. ISSN 1454-7406.
- [13]GOLBAN, R. 2015. Food microbiology. Course of lectures, UASM, Chişinău: uasm.moodle.md, 142p., 4,7 c.a.
- [14]IMRE, C. 2019. Inspection and control of food products of animal origin 2. Didactic manual.Timişoara: Editura Eurobit. 153 p., 2019, ISBN 978-973-132-497-5.
- [15]TAŞBAC, B., TOGOE, I.2018. Food microbiology. Bucharest: Ed. Larisa Câmpulung Muscel, 101 p.
- [16]https://ec.europa.eu/eurostat/web/products-eurostatnews/-/ddn-20221114-2#:~:text=EU%20farms%20produced%20an%20

estimated,increase%20of%200.7%20million%20t onnes.

# THE IMPORTANCE OF PHAGOCYTOSIS IN ASSESSING CELLULAR IMMUNITY AT LAMBS

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# Abstract

The scientific research reflected in these investigations constituted the study of some immunological aspects of the immune defence mechanisms in various periods at lambs. As a result of the immunological investigation, various indices characteristic of phagocytic activity and intensity were revealed. It was found that the phagocytic activity in these periods of the lambs' life is primarily attributed to neutrophils, the rest being carried out by macrophages. At the same time, the investigations have demonstrated that the defence mechanisms of the investigated animals are not triggered enough to protect the aggression of microorganisms, viruses and other pathogenic agents. The results of the study determined that the regulation of the humoral or cellular immune response is a complex modulation process involving a series of means through which the body's specific defence is maintained at a certain level and with a certain duration, in order to achieve homeostasis and preserve health status.

Key words: Phagocytosis, Phagocytic activity, Phagocytic intensity, Macrophages.

The immune response is considered a cellular and humoral defense mechanism, through which the body recognizes what is foreign to itself. In maintaining the phenotypic homeostasis, the animal organism is endowed with various defense mechanisms and is constantly requested by factors of the external environment, which, coming into contact with its defense mechanisms, favor mechanisms against what is non-proper for the cellular constituents, having as resulting in their neutralization and elimination [5,7,9].

The activity of the mechanisms favoring some infections has an important role in the regulation of the immune response. Immunological and autoimmune pathology is of current interest and interests all fields of both human and veterinary medicine. Therefore, the recognition of the self from the non-self is specific to each organism and is the basis of the innate immunological tolerance towards the own immunocompetent cells, favoring immune defense substances reactions against with foreign intervention [1,3,4,6].

Non-specific immunity presents an immediate means of defense in the immune response, and the most important mechanisms are considered to be external and internal. The external mechanisms are represented by the skin, mucous membranes and body fluids. These constitute natural barriers that prevent the penetration of pathogenic agents into the tissues. Once these mechanisms are defeated, an attempt is made to remove the pathogens through the internal mechanisms of non-specific immunity: phagocytosis, inflammation, physiological factors, constituting the immune defense mechanisms that act complexly on the host organism's defense mechanisms [2,8].

Scientific research on the mechanisms of phagocytosis and its intervention in protecting the human and animal body subjected to the harmful influence of external and internal environmental factors is currently an important topic [10].

For these reasons, the main objectives of these researches are to study the aspects of phagocytosis in the assessment of cellular immunity at lambs.

# MATERIAL AND METHOD

The scientific investigations were carried out in the Faculty of Veterinary Medicine in the microbiology and immunology laboratory of the Technical University of Moldova. To carry out the investigations, blood samples from lambs in various age periods were used. Blood samples were collected from the jugular vein with heparin based on the calculation of 0.3 ml of heparin per 10.0 ml of blood for the purpose of anticoagulation. The blood samples were used to perform the opsono-phagocytic test constituted by the cellular mechanism of phagocytosis and the cells involved in this process

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Accordina to specialist studies. the neutrophil cells, monocytes and macrophages involved in this process constitute the first line of defense against pathogenic organisms. The elimination of bacterial infections through the process of phagocytosis involves the recruitment of neutrophils from the bloodstream and from the hematogenous marrow through chemotaxis to the site of infection. Thus, the phagocytic activity of neutrophils is potentiated by the complement system and by antibodies.

To perform the opsono-phagocytic test, 1.0 ml of blood stabilized with heparin and 0.1 ml of E.coli microbial culture suspension from the calculation of 1.0 ml of physiological solution at 500 million microbial cells were used. The tubes were shaken, then incubated in a thermostat for 30 minutes at T-37 C and centrifuged at 1500 rpm. The supernatant was removed by means of a Pasteur pipette. Stained smears were performed according to the Romanovschii-Giemsa method, fixed with methyl alcohol and stained for 30 minutes. Preparations from blood samples were visualized under a microscope, immersion 90. The test determined the number of phagocytosed microorganisms per 100 neutrophils. The index of phagocytic activity and intensity was determined by determining the percentage of neutrophil cells, which participate in the phagocytosis process. At the same time, the phagocytic intensity was determined by the number of microorganisms engulfed by a single neutrophil. The calculation was made by expressing the ratio of the amount of phagocytosed microorganisms to the number of neutrophils participating in the reaction.

# **RESULTS AND DISCUSSIONS**

Immunological investigations, regarding the study of immune phagocytic indices in different age periods at lambs, revealed characteristic indices of phagocytic activity and intensity.

The indices of phagocytic activity at lambs determined significant values in investigated periods of age and time shown in figure 1. These data denote that the animals possess resistance to infectious germs. The important factor of the cellular system of protecting the body is represented by the opsono-phagocytic reaction of leukocytes.

The dynamics of these indices demonstrate the fact that in the neonatal period at calves aged 10 days, 6/12 hours the phagocytic activity was  $50.33 \pm 0.60/47.23 \pm 0.54$ , compared to the age of 20 days, 6/12 hours, which constituted  $40.67 \pm 0.65/37.13 \pm 0.31$ , which denotes a reduction expressed by

various aspects of the external factors, which act on the newborn animal in the first days of life. Following the dynamics of the phagocytic activity indices at the age of 20 and 30 days, it was found that the values were 40.67  $\pm$ 0.65/37.13 $\pm$  0.31, and 38.44  $\pm$  0.47/34.11 $\pm$  0, 21, which confirms the reduction of phagocytic processes at these animals.

In immunological aspect, it can be found that the phagocytic activity in this period of life of the lambs is primarily attributed to neutrophils, the rest being carried out by macrophages. Therefore, the phagocytic mechanisms induce phenomena that can be achieved through two ways, depending on the resistance of the bacteria: the first way, without opsonization through the direct interaction between the phagocytic cell and the and the second antigen; way. with opsonization, is the interaction that requires an additional molecule, opsonin, which plays the role of adapter between the bacterium and the leukocyte. In this context, phagocytosis continues with adhesion, then with the phase in which the pseudopods surround the bacterium. The final destruction phase provides complete digestion of the bacterium.

Relevant data were recorded, regarding the phagocytic intensity at the investigated lambs in various periods of age and time reported in figure 2.

From the obtained results, it can be found that the phagocytic intensity in neonatal animals at the age of 10 days, 6/12 hours determined significant values  $2.13 \pm 0.01/1.95$  $\pm 0.01$ , compared to animals aged 20 and 30 days, 6/12 hours, where these values were 1.83  $\pm 0.02/1.82 \pm 0.01$  and  $1.56 \pm 0.01/1.45 \pm$ 0.01. Therefore, in neonatal animals, the defense mechanisms are not triggered enough to protect the aggression of microorganisms, viruses and other pathogenic agents.

The development of immunity or tolerance is subject to fine-tuning mechanisms because the immune response to self-antigens or the tolerance of a potential pathogen can have unfavorable consequences for life. The regulation of the humoral or cellular immune response is a complex process of modulation involving a series of means through which the body's specific defense is maintained at a certain level.



Figure. 1. Indices of phagocytic activity at lambs at different ages and time periods Source: elaborated by the author



Figure. 2. Indices of phagocytic intensity at lambs at various age periods and timp Source: elaborated by the author

# CONCLUSIONS

1. The first defense weapon in the immune response is non-specific immunity through external and internal mechanisms, which, preventing the migration of pathogenic germs in the animal's body. 2. The results regarding the phagocytic activity in different age periods of the lambs are characteristic of the activity of the phagocytic cells and their mechanisms.

3. The phagocytic mechanisms depend on the resistance of the bacteria through direct contact between the phagocytic cell/antigen and the bacteria/leukocyte process.

4. The activity of the phagocytic intensity at lambs in different age periods determined significant values, which confirms that the defense mechanisms are not sufficient to protect the aggression favored by various pathogenic agents.

#### REFERENCES

- [1]ANDRIEŞ, L., 2014. Clinical immynology. Chişinău: Central Typography, 556p. ISBN 978-9975-53-383-6.
- [2] ANDRIEŞ, L., OLINESCU, A. 1992. Compendium of Fundamental Immunology. Chişinău: Ştiinţa, 476 p.
- [3]BROCAW, A., 2013. Immunology: Use Howard Hughes Medical Institute Resurces to Teach. Ohio. 37 p.Disponibil: // www.hhmi.org/biointeractive/teacher-guideimmunologzy.
- [4]CRISTEA, V., CRIŞAN, M. 2011. Immunology course for the students of the Faculty of Medicine. Cluj – Napoca, 2011, 255p.
- [5]GĂJĂILĂ, G. 2003. The immune system at pigs. Bucharest: Cartea Universitară, 131p. ISBN 973-86231-7-0.
- [6]GĂJĂILĂ, G. 2002. Analytical immunology. Fundamental and methodological aspects. Bucharest: Printech, 224p. ISBN 973-652-583-X.
- [7] ROSEN, R., 2008.Transplantation Immunology: Whatthe Clinician Needs to Know for Immunotherapy Gastroenterology. 134:1789 – 180.
- [8]SILOŞI, I. 2014. Immunology. Craiova: SITECH, 266p. ISBN 978-606-11-3717-6.
- [9]SILOŞI, I.2013. Laboratory investigations in clinical immunology. Craiova: Ed. a II-a, 241p.
- [10]TAŞBAC, A. 2014. Guide for the Veterinary Immunology Laboratory. Bucharest: Larisa, Câmpulung Muscel, 170p. ISBN 978-606-715-271-5.

Review https://doi.org/10.61900/SPJVS.2023.01.10

# CURRENT TRENDS IN THE APPLICATION OF VACCINIUM MYRTILLUS AND ARONIA MELANOCARPA AS NATURAL PRESERVATIVES IN MEAT AND MEAT PRODUCTS: A REVIEW

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#### Abstract

In the contemporary food industry, preservation of meat and meat products remains a paramount concern for ensuring safety, quality, and extended shelf life. Against the backdrop of consumer demand for natural and clean-label food additives, this comprehensive review investigates the current trends in utilizing *Vaccinium myrtillus* (bilberry) and *Aronia melanocarpa* (chokeberry) extracts as natural preservatives in meat and meat products. The review begins by elucidating the intrinsic antioxidant and antimicrobial properties of these berry extracts, emphasizing their relevance in the preservation of meat products. Recent research highlights their efficacy in reducing lipid oxidation, extending the shelf life of meat products, and inhibiting microbial proliferation. Furthermore, it explores various application methods, including their incorporation into meat matrices and the development of edible coatings, shedding light on innovative techniques and emerging technologies. The practicality of deploying these natural preservatives in meat processing is substantiated through industrial applications and illustrative case studies. Recognizing the promise of these natural preservatives, the review acknowledges the hurdles faced, including formulation optimization, cost-effectiveness, and consumer perceptions. Overcoming these challenges is crucial for their widespread adoption. The review concludes by summarizing key findings and underscores the pivotal role of *Vaccinium myrtillus* and *Aronia melanocarpa* in shaping the landscape of meat preservation while ensuring consumer safety and satisfaction.

Key words: meat preservation, Vaccinium myrtillus, Aronia melanocarpa, natural preservatives

## **INTRODUCTION**

*Vaccinium myrtillus*, commonly known as bilberry, and *Aronia melanocarpa*, also referred to as black chokeberry, have garnered attention as potential natural preservatives within the meat industry. These berries are renowned for their remarkable abundance of bioactive compounds, particularly phenolic antioxidants, recognized for their multifaceted antimicrobial and antioxidant attributes (Bujor et al., 2019; Gumus & Kızıl, 2022).

Extensive research has demonstrated the efficacy of *Vaccinium myrtillus* extracts in impeding the growth of various pathogens, encompassing bacteria and fungi (Gumus & Kızıl, 2022). The phenolic constituents present in bilberries, such as anthocyanins and flavonoids, have been discerned as potent inhibitors of spoilage bacteria, thereby substantially extending the shelf life of meat products (Gumus & Kızıl, 2022). Moreover, *Vaccinium myrtillus* extracts have exhibited the capacity to curtail the formation of heterocyclic aromatic amines,

potential carcinogens formed during meat cooking processes (Gumus & Kızıl, 2022).

Conversely, investigations into Aronia melanocarpa extracts have also unveiled their potential as natural preservatives in meat products. The phenolic compounds inherent to Aronia melanocarpa, including anthocyanins, chlorogenic acids, and proanthocyanidins, manifest robust antioxidant and antimicrobial properties (Efenberger-Szmechtyk et al., 2021). These compounds have been identified as effective growth inhibitors of spoilage bacteria and as agents capable of retarding lipid oxidation in meat products, thus substantially augmenting their shelf life (Efenberger-Szmechtyk et al., 2021).

The utilization of *Vaccinium myrtillus* and *Aronia melanocarpa* extracts as natural preservatives in the meat industry holds considerable promise. These natural preservatives afford robust antioxidant protection, deter microbial proliferation, and mitigate the formation of deleterious compounds during meat processing and storage. Additionally, their application aligns

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harmoniously with the escalating consumer demand for clean label products featuring natural ingredients.

Nevertheless, it is imperative to underscore the need for further research to fine-tune the application of *Vaccinium myrtillus* and *Aronia melanocarpa* extracts in meat products, including the determination of optimal concentrations and incorporation techniques. Furthermore, the incorporation of these natural preservatives into the meat industry must be approached with due consideration of regulatory aspects and consumer acceptance.

The primary aim of this paper is to comprehensively review and elucidate the burgeoning trend in the utilization of Vaccinium myrtillus and Aronia melanocarpa extracts as natural preservatives in the meat and meat products industry. By synthesizing the latest research findings and insights, this review seeks to provide a holistic understanding of the multifaceted advantages offered by these extracts. Additionally, it aims to shed light on their mechanisms of action, including their potent antimicrobial and antioxidant properties, which play pivotal roles in prolonging the shelf life of meat products. Furthermore, this paper endeavors to emphasize the alignment of these natural preservatives with the growing consumer preference for clean label and natural ingredients in food products. Ultimately, this review aims to serve as a valuable resource for researchers, food technologists, and industry stakeholders seeking to explore innovative and sustainable approaches preservation while addressing to meat contemporary consumer demands for healthier and more natural food choices.

# ANTIOXIDANT PROPERTIES

Vaccinium myrtillus, commonly known as bilberry, is recognized for its robust profile of phenolic compounds, comprising anthocyanins, flavonoids, and phenolic acids (Bujor et al., 2019). These constituents serve as formidable antioxidants, employing multiple mechanisms to counteract oxidative stress (Scalzo et al., 2013; Bunea et al., 2013; Jaakola et al., 2010; Tarusico et al, 2004). They effectively scavenge reactive oxygen species (ROS), chelate transition metal ions, and inhibit enzymes associated with oxidative stress (Bujor et al., 2019; Zheng et al., 2002; Kähkönen et al., 2003). Remarkably, showcased the remarkable studies have antioxidant potential of blueberry extracts. particularly those rich in anthocyanin-pyruvic acid adducts and vinylpyranoanthocyanin-catechins

(Faria et al., 2005). These extracts have demonstrated profound antioxidant properties with applications in the food industry, where they mitigate free radical activity and thwart lipid peroxidation (Faria et al., 2005).

In a parallel vein, *Aronia melanocarpa*, abundant in polyphenols including phenolic acids and flavonoids, especially anthocyanins (Bushmeleva et al., 2021), exhibits potent antioxidant attributes, potentially alleviating immune system disorders (Bushmeleva et al., 2021). Investigations have revealed the radical-scavenging abilities and immunomodulatory potential of *Aronia melanocarpa* extracts, firmly establishing their credentials as antioxidants (Bushmeleva et al., 2021).

Extending the purview to the domain of meat products, studies have delved into the antioxidant efficacy of Vaccinium myrtillus. For instance, wild blueberry extracts, including those derived from Vaccinium myrtillus, have been appraised for their antioxidant prowess within meat products (Dróżdż et al., 2017). These extracts have demonstrated significant DPPH radical scavenging ability and showcased a robust reducing capacity, indicative of their potential to deter oxidative processes in meat (Dróżdż et al., 2017). Additionally, bilberry extracts housing anthocyanins from Vaccinium myrtillus have displayed potent antioxidant attributes, even inhibiting platelet aggregation (Matsunaga et al., 2009). These findings underscore the promise of Vaccinium myrtillus extracts in safeguarding meat integrity by thwarting oxidative harm.

Similarly, the antioxidant potential of Aronia melanocarpa extracts in meat products has garnered attention (Skalski et al., 2019). In comparative analyses, Aronia melanocarpa twig and leaf extracts demonstrated notable antioxidative capabilities, marking them as promising candidates for diverse food applications, including meat preservation (Skalski et al., 2019).

# ANTIMICROBIAL PROPERTIES

The potential antimicrobial properties of *Vaccinium myrtillus*, a member of the *Vaccinium* berry family, have garnered attention within the scientific community. Rimando et al. (2004) conducted an analysis that unveiled the presence of notable antimicrobial compounds such as resveratrol, pterostilbene, and piceatannol in various *Vaccinium* berries, including *Vaccinium myrtillus*. While their study didn't explicitly explore the effects of *Vaccinium myrtillus* on meat spoilage microorganisms and pathogens, it did

illuminate the berry's latent antimicrobial efficiency (Rimando et al., 2004).

Further support for the potential antimicrobial attributes of *Vaccinium myrtillus* can be found in the comprehensive review by Bujor et al. (2019), which delved into the pharmaceutical and biological properties of wild *Vaccinium species*, including *Vaccinium myrtillus*. While this reference doesn't delve into the specifics of how *Vaccinium myrtillus* interacts with meat spoilage microorganisms and pathogens, it lends credence to the notion of its antimicrobial potential (Bujor et al., 2019).

Continuing in the realm of natural antimicrobial candidates, Aronia melanocarpa, emerges as another promising contender in the battle against meat spoilage microorganisms and pathogens (Kulling & Rawel, 2008). The antimicrobial potential of this fruit, though not expounded upon with respect to its effects on meat spoilage microorganisms and pathogens in the reference by Kulling & Rawel (2008), beckons attention due to the presence of a plethora of phenolic compounds. These include anthocyanins, flavonoids, and phenolic acids, all of which are intricately linked to the fruit's innate antimicrobial properties (Kulling & Rawel, 2008). Intriguingly, these compounds, so conspicuously found in Aronia melanocarpa, are recognized within scientific literature for their role in combating microorganisms and pathogens. The notion that constituents. which have such showcased antimicrobial capabilities in various contexts, could potentially be harnessed in the preservation of meat and meat products is tantalizing. However, it is imperative to underscore that the reference by Kulling & Rawel (2008) primarily hints at the general antimicrobial prowess of chokeberry.

A more specific inquiry into how *Aronia melanocarpa* and *Vaccinium myrtillus* interact with meat spoilage microorganisms and pathogens in the context of meat preservation would be pivotal. Consequently, dedicated research efforts aimed at elucidating the precise mechanisms and efficacy of the discussed berries within the scope of meat preservation are warranted.

# **APPLICATION METHODS**

The application of *Vaccinium myrtillus* and *Aronia melanocarpa* extracts into meat and meat products entails a multifaceted approach that leverages their inherent antioxidant and antimicrobial attributes to elevate the quality and safety of these food items. Several techniques have emerged as promising avenues for this purpose, each offering distinct advantages in terms of stability, controlled release, and enhanced bioavailability of the bioactive compounds found in these extracts.

One avenue involves the direct addition of *Vaccinium myrtillus* and *Aronia melanocarpa* extracts to meat formulations. This can be executed by seamlessly integrating the extracts into meat batters or marinades, ensuring their uniform dispersion throughout the product matrix. The extracts may be introduced in liquid form or as lyophilized powders, obtained through the dehydration and grinding of the berries. This approach facilitates the direct transfer of bioactive compounds to the meat matrix, thereby conferring their invaluable antioxidant and antimicrobial properties.

Encapsulation emerges as another promising technique, serving to safeguard the stability and controlled release of Vaccinium myrtillus and Aronia melanocarpa extracts. Microencapsulation methods, including spray drying or freeze drying, are instrumental in encapsulating the extracts within protective matrices like maltodextrin or alginate. This encapsulated form can be seamlessly integrated into meat products, where it facilitates the gradual and controlled release of bioactive compounds over time. thereby sustaining desired antioxidant the and antimicrobial effects throughout storage and consumption.

Intriguingly, emerging technologies, notably in the realm of nanotechnology, offer novel prospects for the incorporation of these extracts products. Nanoemulsions into meat or nanoparticles, adeptly loaded with Vaccinium myrtillus and Aronia melanocarpa extracts, serve their solubility, stability, to amplify and bioavailability. These advanced nanocarriers can be seamlessly integrated into meat formulations, ensuring improved dispersion and interaction with meat constituents. This, in turn, translates to heightened antioxidant and antimicrobial efficacy, further enhancing the preservation and safety of meat products.

The versatility of these techniques underscores the potential for *Vaccinium myrtillus* and *Aronia melanocarpa* extracts to revolutionize meat preservation strategies, catering not only to the quest for enhanced quality and safety but also aligning with the burgeoning consumer preference for natural and clean-label ingredients in meat and meat products. However, it is paramount to embark on rigorous research endeavors aimed at optimizing these methods, determining the most efficacious concentration levels, and ensuring compliance with regulatory standards, thereby

facilitating the seamless integration of these berry extracts into the meat industry.

## CHALLENGES AND FUTURE DIRECTIONS

The integration of Vaccinium myrtillus and Aronia melanocarpa extracts as natural preservatives in meat products is an area of scientific exploration that presents both challenges and promising avenues for future research. Within this realm, several challenges must be navigated. One significant hurdle is determining the optimal dosage and formulation of these extracts. Striking the right balance between effective preservation, sensory attributes of meat products and possible individual reactions is critical for consumer Consequently, researchers must acceptance. meticulously investigate the concentration of blueberry and chokeberry extracts required to maintain both safety and sensory appeal. Moreover, complex matrix interactions come into play. The interaction between the bioactive compounds in these extracts and the meat matrix can be influenced by variables such as pH, temperature, and the presence of other ingredients. This complexity necessitates a deep understanding of these interactions to ensure the stability and efficacy of the extracts in preserving meat products. Furthermore, the sensory effects of these extracts pose a challenge. While they offer preservative benefits, blueberry and chokeberry extracts may induce alterations in the flavour, colour, or texture of meat products. These sensory changes should be carefully considered, as consumers often have strong preferences for these attributes in meat products. Another challenge relates to regulatory compliance. Ensuring that meat products incorporating these extracts adhere to regulatory standards and labeling requirements is essential. Achieving this requires a delicate balance between complying with regulatory norms and maintaining high standards of efficacy and quality.

Looking ahead, promising future directions in this field include the refinement of extraction methods. Researchers are continually exploring more efficient methods, such as ultrasoundassisted extraction supercritical fluid or extraction, to obtain high-quality blueberry and chokeberry extracts. Advancements in encapsulation technologies also hold potential. Techniques like microencapsulation and nanotechnology-driven solutions can enhance extract stability and enable controlled release within meat products. Additionally, synergistic approaches involving blueberry and chokeberry extracts alongside other natural preservatives, such as rosemary extract or essential oils, merit exploration to bolster preservation efficacy. Consumer education plays a vital role in fostering acceptance. Educating consumers about the advantages of natural preservatives and their contributions to food safety and quality can help gain their trust and appreciation. Furthermore, emphasizing clean labeling can resonate with health-conscious consumers seeking minimally processed food items. Highlighting the presence of blueberry and chokeberry extracts as natural preservatives aligns with this trend.

Expanding research into processed meat products, such as sausages and cured meats, broadens the scope of these extracts' applications, particularly in products with extended shelf lives.

Sustainability is an increasingly important aspect to consider. Investigating the environmental implications of large-scale extract production and exploring sustainable sourcing alternatives aligns with the growing demand for eco-friendly food products.

# CONCLUSIONS

In conclusion, the integration of *Vaccinium myrtillus* and *Aronia melanocarpa* extracts as natural preservatives in meat products represents a dynamic and evolving field of research with significant potential. While it offers promising avenues for enhancing the safety and quality of meat products, several challenges and considerations must be addressed.

The challenges encompass optimizing dosage and formulation, managing complex matrix interactions, mitigating sensory alterations, ensuring regulatory compliance, and educating consumers. Overcoming these challenges requires a multidisciplinary approach, combining food science, sensory analysis, regulatory expertise, and consumer behavior studies. Looking ahead, future directions in this area involve refining extraction methods, harnessing encapsulation technologies, exploring synergistic approaches with other natural preservatives, educating the benefits of natural consumers about preservatives, and expanding research into processed meat products. Additionally, sustainability considerations will play an increasingly vital role in shaping the future of these extracts' applications in the meat industry.

Despite these challenges, the pursuit of innovative solutions using *Vaccinium myrtillus* and *Aronia melanocarpa* extracts holds great promise. These extracts, rich in bioactive compounds, can contribute to extending the shelf life, enhancing the safety, and aligning with consumer preferences for clean label and natural ingredients in meat products. As the field continues to evolve, collaboration among researchers, food manufacturers, regulatory agencies, and consumers will be essential in realizing the full potential of these natural preservatives.

#### REFERENCES

- Bujor, O., Tanase, C., & Popa, M. 2019. Phenolic antioxidants in aerial parts of wild Vaccinium species: towards pharmaceutical and biological properties. Antioxidants, 8(12), 649.
- Bunea, A., Rugină, D., Sconţa, Z., Pop, R. M., Pintea, A., Socaciu, C., & VanCamp, J. 2013.
  Anthocyanin determination in blueberry extracts from various cultivars and their antiproliferative and apoptotic properties in B16-F10 metastatic murine melanoma cells. Phytochemistry, 95, 436-444.
- Bushmeleva, K., Vyshtakalyuk, A. B., Terenzhev, D., Belov, T., Parfenov, A. A., Sharonova, N., & Zobov, V. V. 2021. Radical scavenging actions and immunomodulatory activity of Aronia melanocarpa propylene glycol extracts. Plants, 10(11), 2458.
- Dróżdż, P., Šėžienė, V., & Pyrzynska, K. 2017. Phytochemical properties and antioxidant activities of extracts from wild blueberries and lingonberries. Plant Foods for Human Nutrition, 72(4), 360-364.
- Efenberger-Szmechtyk, M., Gałązka-Czarnecka, I., Otlewska, A., Czyżowska, A., & Nowak, A. 2021. Aronia melanocarpa (Michx.) Elliot, Chaenomeles superba Lindl. and Cornus mas L. leaf extracts as natural preservatives for pork meat products. Molecules, 26(10), 3009.
- Faria, A., Mateus, N., Neves, P. Ä., Gameiro, P., Ferreira, I. C., Freitas, V. d., & Mateus, N. 2005. Antioxidant properties of prepared blueberry (Vaccinium myrtillus) extracts. Journal of Agricultural and Food Chemistry, 53(17), 6896-6902.
- Gheorghe-Irimia, R. A., Tăpăloagă, D., Tăpăloagă, P. R., Ilie, L. I., Şonea, C., & Serban, A. I. 2022. Mycotoxins and Essential Oils—From a Meat Industry Hazard to a Possible Solution: A Brief Review.
- **Gumus, D., & Kızıl, M. 2022.** Reduction of heterocyclic aromatic amines formation in chicken thigh meat by Vaccinium myrtillus L. extract. Journal of Food Processing and Preservation, 46(11).
- Jaakola, L., & Julkunen-Tiitto, R. 2010. Phenolic composition and antioxidant capacity of bilberry (Vaccinium myrtillus) leaves in northern Europe following foliar development and along environmental gradients. Journal of Chemical Ecology, 36(9), 1017-1028.
- Kähkönen, M. P., Heinämäki, J., Ollilainen, V., & Heinonen, M. 2003. Berry anthocyanins: isolation, identification and antioxidant activities. Journal of the Science of Food and Agriculture, 83(14), 1403-1411.
- Kulling, S. E., & Rawel, H. M. 2008. Chokeberry (Aronia melanocarpa) – a review on the characteristic components and potential health effects. Planta Medica, 74(13), 1625-1634.
- Matsunaga, N., Tsuruma, K., Shimazawa, M., Yokota, S., & Hara, H. 2009. Inhibitory actions of bilberry anthocyanidins on angiogenesis. Phytotherapy Research, 24(S1), S42-S47.

- Rimando, A. M., Kalt, W., Magee, J. J., Dewey, J., & Ballington, J. R. 2004. Resveratrol, pterostilbene, and piceatannol in Vaccinium berries. Journal of Agricultural and Food Chemistry, 52(15), 4713-4719.
- Scalzo, J., Stevenson, D. K., & Hedderley, D. 2013. Blueberry estimated harvest from seven new cultivars: fruit and anthocyanins. Food Chemistry, 139(1-4), 44-50.
- Taruscio, T. G., Barney, D., & Exon, J. H. 2004. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest Vaccinium berries. Journal of Agricultural and Food Chemistry, 52(10), 3169-3176.
- Zheng, W., & Wang, S. Y. 2002. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. Journal of Agricultural and Food Chemistry, 51(2), 502-509.

Article https://doi.org/10.61900/SPJVS.2023.01.11

# MORPHOLOGICAL EVALUATION OF RAM SEMEN RELATED TO THE **COLLECTION METHOD**

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#### Abstract

The study was carried out to perform a morphological evaluation of ram semen related to the collection method. A total of 20 Turcana Alba rams aged 3 to 6 years old were used in the study. The study was conducted in a farm located in Cluj County. Semen samples were collected from each animal using two collection methods: the artificial vagina (AV) and the electrostimulation (E). The average variation of normal spermatozoa (%) for the artificial vagina method was  $X\pm$ S=94.31 $\pm$ 2.07, and by electrostimulation  $X\pm$ S=93.62 $\pm$ 3.19. Regarding the percentage of primary anomalies recorded, no significant changes were found:  $X\pm S=2.27\pm1.05$  in the case of AV and  $X\pm S=2.83\pm1.61$  for E. The dynamic of secondary anomalies (%) present in the analyzed semen was as follows: for AV it was  $X\pm S=2.94\pm1.14$ , while when collected by electrostimulation an average of  $X\pm S=3.04\pm 1.36$  was found. The percentages of immature spermatozoa were approximately equal:  $X\pm S=0.57\pm0.25$  for AV and  $X\pm S=0.59\pm0.40$  for the electrostimulation method. The results showed that both sperm collection methods can be used in field conditions, with the mention that the values obtained were superior for the artificial vagina method.

Key words: artificial vagina, electrostimulation, ram, semen

The first step for having good quality sperm is the use of an effective method for the collection of the ejaculates. Artificial vaginas (AV) are widely used for semen collection from ruminants (Leboeuf B. et al, 2000), but this technique requires a previous training period (Wulster-Radcliffe M.C. et al, 2001). This is a practical method and use of this technique does not lead to alterations in semen quality compared to that when there is natural mating. Another method of collection such as the electroejaculation (EE) involves an alternative when males are not trained to AV or for wild species, and may be a viable method of repeatedly collecting ejaculates from individual specimens without causing death (Santiago-Moreno et al, 2009; Abril-Sánchez S. et al, 2019).

Motility and morphology of spermatozoa are accepted as markers of fertility for a long time. It has been shown that these parameters of semen are strongly associated with successful conception in vivo (Davis R.O., Siemers R.J., 1995; Abadieva D. et al, 2014). Additionally, the authors indicated a

coefficient between the high correlation morphology and fertilization capacity of the spermatozoa (Zhang B.R. et al, 1998; Bohlooli S.H. et al, 2012). Also poor morphology has been associated with deviant kinematic and inefficient penetration of both cervical mucus and the zona pellucida (Morales P.K., Overstreet D.R., 1998).

Evaluation of sperm morphology is part of the assessment of fertility in animal reproduction. Sperm morphological evaluation determines the percentage of normal and abnormal sperm (Koziol J.H., Armstrong C.L., 2018; Barth A.D., Oko R.J., 1989). Microscopic examination of ejaculates indicated that sperm morphological assessment has discrepancies, even within the same ejaculate, and these discrepancies create difficulties in determining bull fertility potential (Barth A.D., Oko R.J., 1989; Auger J., 2010). Various stains and methods were used for sperm morphological analysis, resulting in ambiguous outcomes (Gatimel N. et al, 2017a; Gatimel N. et al, 2017b). In this regard, eosinnigrosin (ENS) staining has remained the most

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commonly used technique for detecting sperm morphological abnormalities (Koziol J.H.. Armstrong C.L., 2018; Barth A.D., Oko R.J., 1989). However, it should be noted that sperm morphological evaluation methods can critically affect sperm morphology outcomes (Freneau G.E. et al, 2010; Brito L.F. et al, 2011; Brito L.F., 2016). The Spermac staining method, on the other hand, was first used for seminal evaluations in domestic animals and has since been applied to a range of species including goats, horses, bulls, dogs, boars, and humans (Chan P.J. et al, 1999; Schaäfer S., Holzmann A., 2000). A study on cat spermatozoa demonstrated that this method provides a clear view of cell morphology, particularly the acrosome (Schaäfer S., Holzmann A., 2000; Agarwal A. et al, 2022). The study was carried out to perform a morphological evaluation of ram semen using the Spermac stain.

## MATERIAL AND METHOD

Animals: The study was conducted in a farm located in Cluj County, on 20 rams of the breed Turcana Alba divided equally into two batches according to the collection method: BAV (n=10 rams) and BE (n=10 rams).

The including criteria of the rams in the study were: males, sexual mature, clinically healthy.

The excluding criteria from the study were: sexual imature rams or at andropause.

Semen collection and processing: Semen samples were collected from each animal using two collection methods: the artificial vagina (AV) and the electrostimulation (E) according to methodology proposed by Bogdan et al, 2020 and Garde J.J. et al, 2003 with several adaptations. For EE, we used a manual electroejaculator standardized for small ruminants. The electrical stimulation is made by the user and not by a program, like in electronic electroejaculators. A local toiled was performed by shaving and washing the prepuce with sodium bicarbonate 3% followed by the drying of the area. An enema was performed to eliminate the feces and for a better conductivity. The electrode of the electroejaculator (30 cm long and 2 cm in diameter) was inserted into rectum after the lubrication, for about 15 cm. The EE regime consisted of consecutive series of 5 seconds pulses of similar voltage, each separated by 10 seconds break. The semen was collected after 3-5 stimuli, and the process occurs without erection.

All obtained ejaculates were subjected to an initial macroscopic and microscopic evaluation and those outside the standard requirements were discarded. From each ram 4 ejaculates were collected and a total of 68 ejaculates were included in the study.

Morphological parameters: In our study, the morphology of ram sperm was evaluated using the this 200 Spermac stain. In way spermatozoa/smear were evaluated, and the staining allowed the observation of spermatozoa primary and secondary morphological with changes represented by: lack of head, double head, lack of tail, bifid tail, twisted tail, proximally or distally flexed tail. The presence of the protoplasmic drop, which denotes the immaturity of the spermatozoa, was also determined.

Spermac®, which is a metachromatic stain, is a rapid, easy and reliable staining technique and is used to observe different levels of acrosome defects (Oettle E.E., 1986). For the Spermac method, slides were air-dried at room temperature, then fixed by immersing in a formaldehyde solution for 5 min. The slides were air-dried, then stained by immersing for 1 min in solutions A, B, and C. Solution A was composed of ultrapure water, ethyl alcohol, rose Bengal, and neutral red. Solution B was composed of ultrapure water, ethyl alcohol, pyronin Y, orange G, and Phosphomolybdic acid. Solution C was composed of ultrapure water, janus green, and fast green FCF. The slides were washed in distilled water between each staining process (7 times). Finally, the slides were washed again and air-dried at room temperature. The results from the semen samples collected by the two different methods were compared using one-way ANOVA procedures.

#### **RESULTS AND DISCUSSIONS**

The results revealed that there are differences in the number of ejaculates obtained from a ram, depending on the sperm collection method. In all the rams included in the study, the average volume of ejaculates obtained was higher for the artificial vagina method than with the electrostimulation method. Analyzing the average obtained during the study, it was found that in the case of the BAV group,  $X\pm S= 1.8\pm 0.83$  ejaculates were obtained, which resulted in X±S=2.58±0.29 ml of sperm, while for the BE group there were collected  $X\pm S= 2.8\pm 0.44$  ejaculates from which  $X\pm S= 1.2\pm 0.18$  ml of semen were obtained. The evolution of the averages of microscopic parameters (mobility, concentration, viability, redox test, sperm resistance test), reveals that the results obtained are influenced by the collection method (*table 1*). The data distribution indicates that for all the studied variables the recorded results are in favor of the artificial vagina collection method.

Parameter	Collection method	Min.	Max.	AVERAGE STDEV
Mobility $(9/)$	VA	83	94	89.2±3.90 <sup>a</sup>
MODIIILY (76)	E	83	92	87.7±3.85 <sup>a</sup>
Concentration	VA	2.5	3.5	2.79±0.33 <sup>a</sup>
(x10 <sup>9</sup> spz/ml)	E	2.1	2.8	2.46±0.25 <sup>b</sup>
	VA	94	98	95.5±1.27ª
Viability (%)	E	89	94	91.8±1.87 <sup>b</sup>
Deday (min )	VA	5.7	7	6.54±0.48 <sup>a</sup>
Redox (min.)	E	6.2	7.8	6.87±0.44 <sup>a</sup>
Resistance test	VA	6800	7300	7020±172.04 <sup>a</sup>
	E	6700	7200	6960±142.82 <sup>a</sup>

# Characteristics of ram semen collected by electrostimulation and artificial vagina

 $^{a, b}$ Row means with different superscripts, differ significantly (P < 0.05)

In a study conducted by Matthews N. et al, 2003, regarding the comparison of the two methods of semen collection in rams, it was found that both collection methods were effective. However, the semen collected through the artificial vagina has a significantly (P < 0.05) better concentration and viability than through the electrostimulation method. Analyzing the results obtained in this study, it can be assumed that the electroejaculation method can be used in satisfactorily in order to increase viability in the breeding season, but with some reservations related to sperm concentration and viability. That is why the artificial vagina method is preferred because it leads to higher concentrations (more insemination doses) and a higher percentage of live spermatozoa (better sperm quality). The research carried out by Gordon I., 1983, supports the same recommendations offered by these researchers. In other studies, a larger volume of semen was obtained by the electroejaculation method, but with a lower concentration than that collected with the artificial vagina (Mattner P.E., Voglmayr J.K., 1962; Salamon S., Morrant A.J., 1963; Memon M.A., Ott R.S., 1981). In his research, Bertschinger H.J., 1995 claims that the application of the electrostimulation method leads to acceptable semen, but it can rarely be compared to that collected through the artificial vagina. He also supports the same ideas promoted by his predecessors, regarding obtaining a larger volume of sperm, but of a questionable concentration.

Table 2

Parameter	Collection method	Min	Max	AVERAGE STDEV					
Normal anarmatazoa (%)	VA	90	98	96.4±1.62ª					
Normal Spermatozoa (%)	Е	88	97	93.9±3.04 <sup>b</sup>					
Drimory observalities (9()	VA	0.7	1.9	1.47±0.4 <sup>a</sup>					
Filmary abnormalities (%)	E	0.9	2.4	1.69±0.49 <sup>a</sup>					
Secondary obnormalities (9()	VA	1.3	2.7	1.99±0.33 <sup>a</sup>					
Secondary abnormanilies (%)	Е	1.5	2.3	2.14±0.37 <sup>a</sup>					
Immeture enermetezee (9()	VA	0	0.9	0.36±0.33 <sup>a</sup>					
minature spermatozoa (%)	E	0	0.9	0.48±0.30 <sup>a</sup>					

Dispersal of the average values of morphological parameters according to sperm collection method

<sup>a, b</sup>Row means with different superscripts, differ significantly (P < 0.05)

Table 1

The results of the morphological exam are presented in table 2. In the morphological examination of the semen, the results showed that there are no major differences in the studied parameters depending on the collection method. The morphologically appropriate spermatozoa had: a well-defined head, with the acrosome colored in dark green, the nuclear portion of the sperm head in red, and the equatorial zone in pale green. The intermediate part and the tail correctly attached, in a straight position, colored in dark green (*figure 1*, a - b).



Figure 1 (a, b). Morphologically appropriate spermatozoa

The primary morphological changes identified by this staining were represented by the appearance of the double head, detached head, while the secondary anomalies identified were detached tail and tail flexion (*figure 2, a-f*).



Figure 2 (a-f). Spermatozoa with abnormalities appearance

#### CONCLUSIONS

The results showed that both sperm collection methods can be used in field conditions, with the mention that the values obtained in our study were superior for the artificial vagina method.

#### REFERENCES

- Abadjieva D., Chervenkov M., Stefanov R., Metodiev N., Kistanova E., Kacheva D., Raycheva E., 2014 - Effect of breeding season on the kinematic parameters and morphology of ram' sperm from Synthetic Population Bulgarian Milk sheep breed. Bulgarian Journal of Agricultural Science, 20 (No 4), 967-972.
- Abril-Sánchez S., Freitas-de-Melo A., Giriboni J., Santiago-Moreno J., Ungerfeld R., 2019 -Sperm collection by electroejaculation in small ruminants: A review on welfare problems and alternative techniques, Animal Reproduction Science, Volume 205, 1-9.
- Agarwal A., Sharma R., Gupta S., Finelli R., Parekh N., Selvam M.K.P., 2022 - Sperm morphology assessment in the era of intracytoplasmic sperm injection: Reliable results require focus on standardization, quality control, and training. World J Men's Health. 40(3):347–60.
- Auger J., 2010 Assessing human sperm morphology: top models, underdogs or biometrics? Asian J Androl;12:36–46.
- Barth A.D., Oko R.J., 1989 Abnormal morphology of bovine spermatozoa. 1<sup>st</sup> edition, Ames, Iowa, USA; Iowa State University Press. 285.

Bertschinger H.J., 1995 - Breeding soundness and

*andrology of the bull.* Department of Theriogenology. Faculty of Veterinary Science. University of Pretoria. 69 pp.

- Bogdan L., Ciupe S., Blaga Petrean A., Bogdan S., Berean D.I., 2020 - Biotehnicl în reproducția animalelor domestice. Ed. Colorama, Cluj-Napoca, ISBN 978-606-9056-54-7.
- Bohlooli S.H., Cedden F., Bozoğlu S., Razzaghzadeh S., Pishjang J., 2012 - Correlation between conventional sperm assay parameters in cryopreserved Ram Semen. Annals of Biological Research, 3 (2): 884-889.
- Brito L.F., Greene L.M., Kelleman A., Knobbe M., Turner R., 2011 - Effect of method and clinician on stallion sperm morphology evaluation. Theriogenology;76:745–750.
- Brito L.F.C., 2016 A multilaboratory study on the variability of bovine semen analysis. Theriogenology ;85:254–266.
- Chan P.J., Corselli J.U., Jacobson J.D., Patton W.C., King A., 1999 - Spermac stain analysis of human sperm acrosomes. Fertil Steril. 72(1):124–8.
- Davis R.O., Siemers R.J., 1995 Derivation and reliability of kinematic measures of sperm motion. Reprod Fertil Dev., 7: 857–869.
- Freneau G.E., Chenoweth P.J., Ellis R., Rupp G., 2010 - Sperm morphology of beef bulls evaluated by two different methods. Anim Reprod Sci;118:176–181.
- Garde J.J., Soler A.J., Cassinello J., Crespo C., Malo A.F., Espeso G., Gomendio M., Roldan E.R.S., 2003 - Sperm Cryopreservation in Three Species of Endangered Gazelles (Gazella cuvieri, G. dama mhorr, and G. dorcas neglecta). Biology of reproduction 69, 602–611.
- Gatimel N., Mansoux L., Moreau J., Parinaud J., Leandri R.D., 2017a - Continued existence of significant disparities in the technical practices of sperm morphology assessment and the clinical implications: results of a French questionnaire. Fertil Steril;107:365.
- Gatimel N., Moreau J., Parinaud J., Leandri R.D., 2017b - Sperm morphology: assessment, pathophysiology, clinical relevance, and state of the art in 2017. Andrology;5:845–862.
- Gordon I., 1983 Controlled breeding in Farm Animals (1st Ed.). Pergamon International Library. Oxford, N.Y. pp. 199.

- Koziol J.H., Armstrong C.L., 2018 Manual for breeding soundness examination of bulls. 2<sup>nd</sup> edition, Montgomery, AL, USA; Society for Theriogenology.
- Leboeuf B., Restall B., Salamon S., 2000 Production and storage of goat semen for artificial insemination. Animal Reproduction Science, Volume 62, Issues 1–3, 113-141.
- Morales P.K., Overstreet D.R, 1998 The relationship between the motility and morphology of spermatozoa in semen. J. Androl., 9: 241-247.
- Matter P.E., Voglamyr J.K., 1962 A comparison of ram semen collected by the artificial vagina and by electro-ejaculation. Aust. J. Exp. Agri. Anim. Husb. 2, 78-81.
- Matthews N., Bester N., Schwalbach L.M.J., 2003 A Comparison Of Ram Semen Collected By Artificial Vagina and Electro-Ejaculation. SA-ANIM SCI, vol 4.
- Memon M.A., Ott R.S., 1981 Methods of semen preservation and artificial insemination in sheep and goats. Wld. Rev. Anim. Prod. 17, 19-24.
- Oettle E.E., 1986 Using a new acrosome stain to evaluate sperm morphology. Vet. Med. 81: 263-266.
- Santiago-Moreno J., Coloma M.A., Dorado J., Pulido-Pastor A., Gómez-Guillamon F., Salas-Vega R., Gómez-Brunet A., López-Sebastián A., 2009 Cryopreservation of Spanish ibex (Capra pyrenaica) sperm obtained by electroejaculation outside the rutting season. Theriogenology, Volume 71, Issue 8, 1253-1260.
- Salamon S., Morrant, A.J., 1963 A comparison of two methods of artificial breeding in sheep. Aust. J. Agr. Res. 13, 72-77.
- Schaäfer S, Holzmann A., 2000 The use of transmigration and Spermac stain to evaluate epididymal cat spermatozoa. Anim Reprod Sci. 59(3–4):201–11.
- Wulster-Radcliffe M.C., Wiliams M.A., Stellflug J.N., Lewis G.S., 2001 - Technical note: artificial vagina vs. a vaginal collection vial for collecting semen from rams. J. Anim. Sci. 79, 2964–2967.
- Zhang B.R., Larsson B., Lundeheim N., Rodriguez Martinez H., 1998 - Sperm characteristics and zona, pellucida binding in relation to field fertility of frozen, thawed semen from dairy AI bulls. Int. J. Androl., 21: 207-216.

# MAMMARY GLAND DEVELOPMENT IN DOGS AND CATS IN RELATION TO THEIR SEXUAL CYCLE

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#### Abstract

The mammary gland is a modified sweat gland specialized in milk secretion, whose development is synchronized with the sexual function, being directly influenced by the stages of the sexual cycle in both canids and felines. Knowledge of morphology and physiology is essential for a correct approach of the mammary diseases, an important pathology affecting two categories: the adult female and the newborns.

There are numerous controversies in the literature regarding the terminology and duration of the dog and cat sexual cycle, leading to the existent variability of the information. However, for the bitch it is unanimously accepted that most canine breeds have two estrous periods per year, usually in spring and autumn. This period can be systematically divided into proestrus, estrus, luteal phase and anestrus. In this species, the proestrus and estrus periods are long and necessarily accompanied by a long luteal phase. These are then followed by anestrus, which is not influenced by the season, unlike the cat. The queen is a seasonally polyestrous animal, which in the absence of mating goes into estrus every 2-3 weeks throughout the breeding season. She will have repeated cycles in the breeding season, which will only be interrupted by gestation, pseudo-gestation or various ailments.

The aim of this review is to describe the estrus cycle in dogs and cats, and its connection with mammary gland development, a gland with unique growth due to the fact that the final stage of development is reached in the adult female only during gestation.

Key words: mammary gland, dogs, cats, estrus cycle

The mammary gland is an accessory gland of the genital tract, an extensively modified sweat gland specialized for milk secretion, whose morpho-physiology is directly influenced by the stages of the sexual cycle in both cats and dogs (Drugociu & Drugociu, 2015; Raskin & Meyer, 2016).

Some of the international literature classifies bitches as monoestric animals, a term that defines the existence of only one estrus period per sexual cycle. Compared to mammals like horses, cattle and pigs, that have estrus cycles every three weeks during the breeding season, unless they remain pregnant, canines are different (Pretzer, S. D., 2008).

The cat is a polyestrous animal that usually has 2 estrous periods per year. However, under modern conditions of maintenance, cycles can occur all year round. Under normal circumstances, the anestrus extends over a period of 3-4 months in winter, when the daylight is short (Rosca, 2005).

A thorough knowledge of the reproductive physiology of the female cat and dog is necessary

to enhance success of diagnostic or therapeutic purposes, and also for assisted reproductive technologies. The aim of this article is to provide an overview of dogs and cats sexual cyclicity in relation to the mammary gland, in order to improve the veterinarians approach of the reproductive pathologies.

#### **MATERIAL AND METHOD**

An electronic search for English and Romanian publications was conducted in Web of Science, Scopus, PubMed and Google Scholar databases, using terms such as estrus, oestrus, cycle, mammary gland, reproductive, dog, cat, bitch and queen, and the articles and book chapters were analyzed.

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## **RESULTS AND DISCUSSIONS**

# Mammary gland development in canids and felines

Canids and felines have several pairs of mammary glands, arranged in two symmetrical, bilateral rows extending from the ventral region of the thorax to the inguinal area (Raskin & Meyer, 2016).

The mammary gland in the bitch is usually represented by 5 pairs of mammae, arranged in 2 thoracic or pectoral, 2 abdominal and 1 inguinal pair. Sometimes 6 or 4 more pairs may be found. (Drugociu & Drugociu, 2015; Silver, 1966) Unlike the bitch, the cat has 4 pairs of mammary glands: 2 pectoral and 2 abdominal, with the nipples traversed by 4-6 papillary ducts. (Drugociu & Drugociu, 2015)

The ratio between the secretory and stromal components of the mammary gland shows major variations depending on the stage of the sexual cycle (Rehm et al., 2007). In each gestation, duct proliferation, differentiation of secretory acini, secretion of milk by them, and involution of the secretory component of the gland at the end of lactation, with preservation of the ducts (Sorenmo et al., 2011). By puberty, the mammary gland grows in accordance with the body's development and the ducts develop in a circular pattern at a short distance from the nipple. Vascularisation at this level is similar to that of the skin (Silver, 1966).

At birth, the animal's mammary gland has only the large ducts formed. These extend only a short distance from the nipple into the subcutaneous mesenchymal tissue. With puberty and secretion of oestrogens, cell proliferation of the ductal end is activated. Ductal development and the formation of lobulo-alveolar units will influence of occur under the increased progesterone levels during diestrus and gestation. A third stage is undergone under the influence of gestational prolactin when it will cause differentiation of secretory alveolar cells so that at parturition the mammary gland is characterized by a secretory lobulo-alveolar ductal structure (Donald J. Meuten, 2017).

## Sexual cycle in canids

The bitch is a diestric animal (when referring to the number of estrous cycles per year), with an average length of sexual cycle of about 7 months, with many particularities (Runceanu et al., 2007). Because of this, the values found in the literature show major differences. However, the interval between two successive cycles should be constant in the same individual. (Roşca, 2005) The

intervals resulting from the overlapping different literature sources are: Proestrus (4-7-10 days), Estrus (5-9-14 days), Diestrus (1-2 months) and Anestrus (2-4-5 months) (England et al., 2010; Noakes et al., 2019; (Rehm et al., 2007).

In bitches, the proestrus and oestrus periods are long and always accompanied by a long luteal phase - diestrus (whether the bitch is pregnant or not) (England et al., 2010; P. Concannon, 2009).

Proestrus is the phase of intense follicular activity, with estrogenic dominance, but the female does not accept mating. Clinically, the beginning of this period is marked by the appearance of the first vaginal bleeding, and ends when the female will accept the male (Feldman and Nelson, 2004). Now, under the action of FSH and LH, follicular growth and development takes place, followed by secretion of estrogens from the follicular granulosa. These reach their maximum serum concentration at this stage, then they decrease.

Estrogens then induce a proliferation of epithelial cells, which is more obvious in the vaginal mucosa, changing its epithelium from cuboidal to a layered squamous epithelium that will become hyperplastic (most likely in order to prevent trauma to the vaginal mucosa during mounting) (Concannon, 2011). This is important because it allows the clinician to identify the stage of the cycle. Similar studies are also in queens (Zambelli, D., & Cunto, M., 2005). The earliest evidence of entry into the proestrus is the presence of erythrocytes among the superficial and basal epithelial cells sampled (most commonly using a moistened cotton swab) (England et al., 2010).

High LH and FSH concentration is crucial for follicular growth and development. However, as follicles mature, inhibin is secreted, a specific inhibitor of FSH, so towards the end of proestrus, FSH secretion becomes stationary. (England et al., 2010; Runceanu et al., 2007)

Estrus is characterised by an increased concentration of oestrogens, which decline before ovulation. Clinically, the beginning of this period is marked by the female's acceptance of reproduction, and ends when she no longer accepts it (Feldman and Nelson, 2004). At this stage, there is congestion and swelling of the external genitalia, secretion of a characteristic fluid (smelly, bloody mucus at the beginning of estrus that becomes clear towards the end of estrus). The female becomes restless, constantly searching for the male, and in its proximity takes the characteristic position for mounting (Noakes et al., 2019).

Ovulation in the bitch is spontaneous, occurring at the end of the heat cycle, and is stimulated by a spike in LH secretion - which occurs after a maximum concentration of estrogen is reached. Ovulation occurs 2 days after the LH peak. Of interest is the fact that in bitch, the LH peak is of prolonged duration - 24-48 h, leading to a pre-ovulatory luteinization of the follicles and an increase in serum progesterone concentration before ovulation, so that at the time of ovulation, the concentration of progesterone is close to 5.4 ng/ml, changes that are essential for the bitch's sexual behaviour (England et al., 2010) Thus, in the bitch both oestrus and diestrus are of progesterone dominance. (Feldman and Nelson, 2004)

In bitches, unlike other species, the ovules are not fertilisable immediately after ovulation, but need to mature within 1-5 days, depending on the individual (Ciupe et al., 2020) This is due to the fact that unlike other species in which ovulation of a secondary oocyte (of order II) occurs, in the bitch ovulation of a primary oocyte (of order I) takes place, which will have to mature, becoming a secondary oocyte capable of being fertilised after 96 to 108 hours. This secondary oocyte usually remains viable for 24-48 hours (day 5-6 after LH peak) Figure 1. Most of the time, an equal number of luteal bodies can be seen on the two ovaries. This is important because it has been observed that often the number of fetuses in a uterine horn is not always identical to the number of corpora lutea on the corresponding ovary. Thus, embryo migration into the contralateral uterine horn is a common occurrence. (Noakes et al., 2019; Pretzer, 2008; Reynaud et al., 2020)



Figure 1 - Representative diagram in bitch, with key events on segments ovary-oviduct-uterine horn.

This delay between ovulation and fertilisation in bitches leads to relatively frequent over-fertilisation, which results in the fertilisation of eggs with semen from several males, producing offspring of different breeds. Therefore, in artificial insemination with refrigerated semen, the female is inseminated on the second day after ovulation, the lifespan of the spermatozoa covering days 2 and 3. In artificial insemination with frozen semen, as the thawed spermatozoa have a very short lifespan, insemination is carried out on day 3. (Ciupe et al., 2020; Pretzer, 2008)

In bitches, estrous behaviour is prolonged for about 7 days after ovulation. After ovulation, the luteal phase of the sexual cycle (diestrus) begins, when the concentration of progesterone continues to increase. In bitches, the term diestrus is preferred, precisely because of the particularities of the sexual cycle, namely the fact that progesterone secretion starts as early as the oestrus phase (luteal follicles). Metestrus on the other hand, describes a period of luteal body activity as a distinct entity, not applicable in this situation. (Feldman and Nelson, 2004)

Progesterone modifies the characteristics of the cervical mucus (viscous), decreases muscle excitability, preparing the uterus for embryo implantation. The diestrus phase is considered to begin when oestrus signs have ceased, and ends when progesterone concentration returns to basal levels (<1.0 ng/ml). (Feldman and Nelson, 2004) During the early part of the luteal phase, progesterone concentration is the same in pregnant and non-pregnant females, unlike in other species such as cats. (England et al., 2010)

The luteal stage in the pregnant bitch lasts for about 66 days from ovulation to parturition, while in the non-pregnant bitch it lasts for about Date of

63 days. In the second part of the luteal phase in pregnant bitches. the concentration of progesterone starts to decrease, while at the same time there is an increase in the concentration of prolactin. Inhibition of prolactin secretion may result in the termination of the luteal phase, or of gestation if it has been established. After parturition, newborn breastfeeding induces an increase in prolactin secretion, which lasts for about 6 weeks and gradually decreases as milk production diminishes until weaning (Chastant, 2023; England et al., 2010).

Anestrus is the period between the end of the luteal phase and the onset of the return to proestrus. The onset of this period is difficult to identify clinically in the un-estrus female because there are no obvious changes between diestrus and anestrus. When the previous cycle was accompanied by gestation, the first part of anestrus includes lactation (Concannon, 2011; England et al., 2010; Feldman and Nelson, 2004).

## Sexual cycle in cats

The cat is a seasonal polyestrous animal, which in the absence of mating enters oestrus every 2-3 weeks throughout the breeding season. In the northern hemisphere this season usually starts in January (as daylight increases) and continues until September. In breeding cats there are usually only 2 estrous periods (and therefore 2 possible gestations) per season. The age of first cycle onset, or puberty, depends not only on physical maturity but also on the season (Table 1).

Table 1

Age of puberty in cats The first estrus If they and will have their first estrus at 6 MONTHS OLD. specific





Figure 2 - The estrous season of the cats from the Northern Hemisphere. E - estrus, M - mating, P - parturition

The stages of the cat's sexual cycle are proestrus, estrus, (metestrus = interestrus), diestrus (a short period of sexual inactivity). However, since the cat has a induced ovulation, in the absence of mating luteal phase will not follow, so estrus will be followed by a period of sexual inactivity (interestru). which differs endocrinologically from the luteal phase - after interestru the cat enters estrus again. The luteal phase, however, occurs when ovulation occurs and the female remains, or does not become, pregnant. The cycle repeats itself before the end of the reproductive period, after which the last metestrus of the reproductive period is followed

by a period of longer sexual inactivity, i.e. anestrus. So, cats have three varieties of sexual cycle Figure 3 (England et al., 2010; Reynaud et al., 2020; Johnson, 2022).

Therefore, if after ovulation, fertilisation of oocytes does not occur, or for other reasons pregnancy does not occur, the female will enter pseudopregnancy for about 45 days. Ovulation is therefore followed by the formation of the corpus luteum both in and out of gestation. If gestation does not set in, CL reaches peak progesterone activity in 10-15 days, then begins to decline, reaching baseline values in davs 30-35. Behavioural changes and mammary gland

enlargement in pseudopregnancy are not observed in the cat as they are in the bitch, but only slight swelling of the mammary gland can be observed (Arikan et al., 2009).

Follicular growth and development occur in the proestrus due to FSH and LH secretion. During this period the cat intensifies its rubbing of the head by objects or owners, often interpreted as affectionate behaviour. On the other hand, the proestrus may continue without the cat showing any change in behaviour. It is, however, the period when the male is attracted to unreceptive females (Rosca, 2005).

During oestrus the oestrogen concentration is increased and clinically, specific meowing is noticed and the cat accepts the male. Up to 5-7 matings can occur within 1-2 hours if the couple is not disturbed (England et al., 2010).



Figure 3 - Stages of the cat reproductive cycle

Ovulation occurs as a result of mechanical stimulation of the vaginal walls (Vansandt, 2022). It is reported that about 50% of cats ovulate after a single copulation, while for the rest the release of sufficient LH for ovulation is achieved when about 4 copulations occur in 2-4 hours. Ovulation will take place about 27 hours after mating. After ovulation, oocytes can be fertilised immediately, as they are mature (England et al., 2010).

At the end of the estrus there are therefore 3 possible outcomes (Roşca, 2005):

1. Lack of mating: proestrus (0-1 days), oestrus (2-6 days), interestrus (8-15 days).

2. Infecund mating: proestrus, estrus, diestrus, interestru.

3. Fecund mating: proestrus, oestrus, gestation.

Anestrus is the seasonal period of ovarian inactivity that usually occurs in winter (Noakes et al., 2019). Interestrus is the period between one oestrus and the next oestrus (8-15 days) in females that have not ovulated or had fertile breeding (England et al., 2010).

The data collected in this review will be beneficial for research on the reproductive pathologies, artificial insemination, in dogs and cats, but also for the therapeutical approach of the diseases.

#### CONCLUSIONS

In order to truly recognize what is abnormal in reproductive diseases, medical practitioners must have an understanding of what is considered normal for the canine and feline estrous cycle.

The particularity of the cat's sexual cycle comes from the fact that the cat has induced ovulation. During copulation, receptors located in the vulva will stimulate the release of LH from the anterior pituitary, triggering ovulation.

There are at least 3 particularities in the sexual cycle of canids: each cycle lasts a minimum of five months, the diestrus period is similar in pregnant and non-pregnant bitch, and the anestrus period occurs regardless of pregnancy status, being characterized by a prolonged period of ovarian inactivity.

#### REFERENCES

- Arikan, S., Yigit, A. A., & Kalender, H., 2009 Size Distribution of Luteal Cells During Pseudopregnancy in Domestic Cats. Reproduction in Domestic Animals, 44(5), 842– 845. doi: 10.1111/J.1439-0531.2008.01099.X
- Chastant, S., 2023 Lactation in domestic carnivores. Animal Frontiers, 13(3), 78–83. https://doi.org/10.1093/af/vfad027
- Ciupe, S., Pop, R., Bogdan Sidonia, A., & Berean, D. I., 2020 - Reproducție, terapie și inseminări artificiale la animalele domestice. Editura Colorama.
- Concannon, P., 2009 Endocrinologic Control of Normal Canine Ovarian Function. Reproduction in Domestic Animals, 44(s2), 3–15. https://doi.org/10.1111/j.1439-0531.2009.01414.x
- Concannon, P. W., 2011 Reproductive cycles of the domestic bitch. Animal Reproduction Science, 124(3–4), 200–210. https://doi.org/10.1016/j.anireprosci.2010.08.028
- Donald J. Meuten., 2017 Tumors in domestic animals (Fifth Edition). ISBN:9781119181200
- Drugociu, D., & Drugociu, S. D., 2015 Patologie genitală și a glandei mamare la animale (D. Drugociu & D. S. Drugociu, Eds.). Editura "Ion Ionescu de la Brad" Iași.
- England, G.C.W., Heimendahl, A. von., British Small Animal Veterinary Association., 2010 - BSAVA manual of canine and feline reproduction and neonatology. British Small Animal Veterinary Association.

- Feldman, E.C., Nelson, R.W., Richard W., 2004 Canine and feline endocrinology and reproduction. Saunders.
- Johnson, A. K., 2022 Normal feline reproduction: The queen. Journal of Feline Medicine and Surgery, 24(3), 204–211.
- https://doi.org/10.1177/1098612X221079706 Noakes, D.E., Parkinson, T.J., England, G.C.W., 2019 - Veterinary Reproduction and Obstetrics, in: Veterinary Reproduction and Obstetrics. Elsevier,
- Pretzer, S. D., 2008 Canine embryonic and fetal development., Theriogenology, 70(3):300-3, https://doi.org/https://doi.org/10.101/j.theriogenolo gv.2008.04.029
- Raskin, R. E., & Meyer, D., 2016. Canine and Feline Cytology: A Color Atlas and Interpretation Guide (Third Edition). Elsevier Health Sciences.
- Rehm, S., Stanislaus, D. J., & Williams, A. M., 2007 -Estrous cycle-dependent histology and review of sex steroid receptor expression in dog reproductive tissues and mammary gland and associated hormone levels. Birth Defects Research Part B: Developmental and Reproductive Toxicology, 80(3), 233–245. https://doi.org/10.1002/bdrb.20121
- Reynaud, K., Saint-Dizier, M., Fontbonne, A., Thoumire, S., & Chastant-Maillard, S., 2020 -Follicle growth, oocyte maturation, embryo development, and reproductive biotechnologies in dog and cat, Clinical Theriogenology, 12(3), 189-203
- Roberts SJR., 1986 Physiology of female reproduction. In: Veterinary Obstetrics and Genital Diseases, Theriogenology, David and Charles, Inc; 1986. p.398- 433
- Roșca, P., 2005 Ginecologia animalelor de companie. Piometrul la cățea și pisică. Tehnopress, Iași.
- Runceanu, L.Gh., Cotea, C. v., Drugociu, D.Gh., Roșca, P., 2007 - Reproducție, Obstetrică și Ginecologie Veterinară. Ion Ionescu de la Brad, Iasi.
- Silver, I. A., 1966 The Anatomy of the Mammary Gland of the Dog and Cat. J. Small Anim. Pract, 7, 689– 696.https://doi.org/10.1111/j.1748-5827.1966.tb04394.x
- Sorenmo, K. U., Rasotto, R., Zappulli, V., & Goldschmidt, M. H., 2011 - Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. Veterinary Pathology, 48(1), 85–97. https://doi.org/10.1177/0300985810389480
- Vansandt, L. M., 2022, Feline Estrous Cycle. In Feline Reproduction (pp. 11–22). CABI. https://doi.org/10.1079/9781789247107.0002
- Zambelli, D., & Cunto, M., 2005 Vaginal and cervical modifications during the estrus cycle in the domestic cat. Theriogenology, 64(3), 679-684

# RESEARCH ON THE DEVELOPMENT AND THERAPY OF PERSISTENT CORPUS LUTEUM IN COWS

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#### Abstract

Pathological puerperium can mainly cause significant economic damage through the high losses that accumulate throughout the herd and especially through the high percentage of sterility and infecundity. According to literature data, it is shown that this infertility ratio can range quite widely between 5.0-30.0% of the total bull herd. A criterion in the occurrence of this gynecological disease is the feed, which can vary in terms of quantity and quality, the farming regime to which the cows are subjected and not especially environmental factors (lack of body hygiene, low temperatures during the winter season and prolonged housing). In both research years, the incidence of cows diagnosed with persistent corpus luteum was high at 7.1% in 2021 and 9.9% in 2022 in the non-lactating cows group and after a period of 90 days after parturition. In 2021 the persistent luteal corpus luteum (CLP) in April did not register any cases, the most significant values were recorded in the months: October (1.9%), November (2.5%), December (3.5%) and the highest level of (3.9%) being reached in January. In 2022 the recorded values of persistent corpus luteum were close compared to the first year of investigation, peaking in January at 3.1%. According to the number of lactations, in 2021, 19.3% females, in 1st lactation only 1.5% were diagnosed with this gynecological condition. Also, the highest level of 4.4% was recorded in cows in their 6th lactation and above.

Key words: cow, persistent corpus luteum, lactation number, pathological puerperium

#### **INTRODUCTION**

Today, one of the most important farm animals is the cow, because of the milk yields it produces. Cattle ensure that all human requirements for animal products are met, in particular through the quality and quantity of meat and the level of milk production (1, 2, 5, 9).

Persistent corpus luteum is an endocrine disorder described only in cows, with a key role in the induction of infecundity (3, 4). The name persistent corpus luteum in practice is attributed to that luteal formation which retains its function and size, especially without regressing beyond physiological values (6, 11).

The incidence of this condition can range from 2.0 to 15.0% of infertility cases. The highest incidence is in cattle with high milk yields, especially after parturition during the winter season (7, 8).

This condition can originate from the gestational luteal body or the progestational luteal body. Persistent corpus luteum can result from the gestational luteal body due to malnutrition or

unbalanced diet as well as in the case of endometrial inflammation (10).

#### MATERIAL AND METHOD

In this study, we set out to analyse the influence of various gynaecological diseases on the reproductive cyclogram of cattle recorded on the farm, i.e. the causes that can lead to the appearance and onset of genital diseases.

One of the goals we set out to achieve was to find some effective methods of treatment in order to achieve the best possible results. The research was carried out between January 2021 and December 2022 at the unit in the north-east of Moldova.

One of the goals we set out to achieve was to find some effective methods of treatment in order to achieve the best possible results. The research was carried out between January 2021 and December 2022 at the unit in the north-east of Moldova.

The total cattle herd for both years of the study was 428 lactating cows of the Hostain and Red-washed breeds.

The research began with the general clinical examination which consisted of collecting anamnestic information from the gynaecological records on the health status of the female, how the first heat after parturition was conducted, the degree of intensity of the heat, the number of artificial inseminations performed for a gestation, how previous parturitions were conducted, whether the conceived product was full term or whether it was live or dead, and whether complications occurred after parturition.

The diagnosis of persistent corpus luteum was established with certainty on the basis of anamnestic data from the farm register, clinical data (absence of oestrus) and last but not least by gynaecological examination.

To establish the diagnosis of persistent corpus luteum, the operator equips himself appropriately as for the transrectal examination, performing the transrectal examination when he palpates the ovary which is slightly enlarged in volume and identifies the corpus luteum with a spongy appearance, with a diameter of about 2.5 to 3 cm. This examination is repeated at 8-day intervals, performing 3 consecutive examinations every 21-24 days. The corpus luteum has 3 phases: the organizing phase, the efflorescence phase and the regression phase.

In some females, in the absence of prostaglandin F2 alpha secretion, the CL after forming and developing does not regress, and on transrectal examination performed for three hours at approximately 10-day intervals maintains its consistency, shape, position and size on the same ovary.

#### **RESULTS AND DISCUSSIONS**

On the farm, especially in females with high milk production, the incidence of corpus luteum is common.

Table 1 shows the incidence of persistent corpus luteum in two groups of females, i.e. non-lactating cows that were diagnosed with CLP about 90 days after parturition and non-lactating cows that also showed CLP 90 days after parturition.

In both years of the study, the percentage of females showing persistent corpus luteum was high at 7.1% in 2021 and 9.9% in 2022 in the group of unweaned females and after 90 days post-partum (Fig. 1).

#### Table 1

incluence of persistent corpus luteum in 2021-2022									
Period		Year		Year		Media			
	2021		2022						
Number of cows calved		202		226		214			
	Nr.	%	Nr.	%					
Persistent luteal body in females less than 90	18	9,1	16	7,1	34	16			
days postpartum									
Persistent luteal body in unweaned females	26	12,8	20	9,9	46	23			
over 90 days postpartum									
Total	34	21,7	36	15,9	80	40			

vidence of nonsistant communa luterum in 2021-202



According to the literature, after parturition, the heat resumes physiologically at about 21-45 days. On the farm, this criterion varies according to the age of the female, the conditions of maintenance and exploitation, the general condition of the genital apparatus in particular, the rate of uterine involution, the level of milk production per lactation, balanced feeding in relation to the physiological condition of the female and, last but not least, environmental factors. For the owner, if this interval is exceeded, it becomes a major problem, as he can no longer achieve his goal of producing one calf per year, and calving-interval values will be very high. The accumulated data on the incidence of persistent corpus luteum over the two study years (2021-2022) are presented in Table 2. In both study years the values of persistent corpus luteum were higher in the cold months starting in October.

Table 2

Year	20	21	2022		
Month	Nr.	%	Nr.	%	
January	8	3,9	7	3,1	
February	3	1,4	3	1,3	
March	2	0,9	1	0,4	
April	0	0,0	0	0,0	
May	1	0,5	1	0,4	
June	2	0,9	1	0,4	
July	3	1,4	3	1,3	
August	2	0,9	2	0,9	
September	3	1,4	3	1,3	
October	4	1,9	4	1,8	
November	5	2.5	5	2.2	

7

3.5

6

Monthly dynamics in source diagnosed with	nonsistant communa lutour in 2021-20	177
wonting ugnamics in cows diagnosed with	persistent corpus futeum m 2021-20	122

In 2021 the persistent luteal body in April did not register any case, in May one case was diagnosed (0.5%) and the highest values were registered in the months: October (1.9%), November (2.5%), December (3.5%) and in January the highest level of (3.9%) of the total herd of females monitored (fig. 2).

December

In the cold season there were more significant values of females diagnosed with persistent corpus luteum due to deficient rations, poor husbandry conditions and females with high milk yields were not given a specific feed intake according to the milk yield obtained.

2,6



Fig. 2 Incidence of persistent corpus luteum in 2021-2022

In 2022 the recorded values of persistent corpus luteum were similar, with a high level also in the cold season, and in January the highest percentage of 3.1 was obtained by 0.8% compared to the previous year.

In relation to the number of lactations, in 2021, out of 39 females (19.3) in 1st lactation only 1.5% were diagnosed with this gynecological condition. Table 3 shows the obtained values of persistent luteal body in the years (2021-2022) according to

the number of lactations. Also, the highest level of 4.4% was recorded in cows in their 6th lactation and above (Fig. 3).

Table 3

inclucing of corpus futurin by number of factations in 2021-2022										
	Year 2021				Year 2022					
	Peaceful		CLP cows		Peaceful cows		CLP	cows		
Lactation	cows									
	Nr.	%	Nr.	%	Nr.	%	Nr.	%		
Ι	39	19,3	3	1,5	43	19,0	3	1,3		
II	42	20,8	4	1,9	47	20,7	4	1,8		
III	34	16,8	5	2,5	36	15,9	5	2,2		
IV	35	17,3	6	3,0	38	16,9	6	2,6		
V	29	14,4	7	3,5	33	14,7	7	3,1		
a-VI-a and over a VI-a	23	11,4	9	4,4	29	12,8	11	4,8		
Total	202	100	34	16,8	226	100	36	15,9		

Incidence of corpus luteum by number of lactations in 2021-2022



Fig. 3 Incidence of persistent corpus luteum as a function of number of lactations

In 2021, the lowest value of 1.5% was reached in emales at 1st lactation and the highest share of 4.4% in females above 6th and above 6th lactation (Figure 7.3).

The frequency of the persistent corpus luteum in relation to the milk yield obtained per lactation increases progressively.

Thus, in 2021 in cows with a minimum milk production between 5000-5500 litres milk/lactation the lowest CLP value of 1.0% of the herd of 202 cows was recorded, and the highest share of 4.4% in females with milk/lactation productions between 7001-7500 litres.

Similar values of this gynaecological condition were also recorded in 2022 with the highest level of 4.0% in females with milk

production/lactation between 65001- 7000 litres (Fig. 4).

Table 4 shows the luteal body values obtained in the two years of the study (2021-2022).

#### Table 4

	Year 2021				Year 2022			
Litres milk/lactation	Peac	ceful	CL	CLP cows			CLP cows	
	со	WS			Pea	ceful		
						cows		
	Nr.	%	Nr.	%	Nr.	%	Nr.	%
5000-5500	19	9,4	2	1,0	21	9,3	2	0,9
5501-6000	24	11,9	2	1,0	28	12,5	2	0,9
6001-6500	30	14,8	3	1,5	34	15,0	3	1,3
6501-7000	34	16,8	8	3,9	39	17,2	9	4,0
7001-7500	36	17,8	9	4,4	40	17,6	10	3,6
7500-8000	33	16,4	6	3,0	36	15,9	5	2,2
>8001	26	12,9	4	2,0	28	12,5	5	2,2
Total	202	100	34	16.8	226	100	36	15.9

Incidence of persistent corpus luteum as a function of milk/lactation level



Fig. 4 Dynamics of persistent corpus luteum as a function of milk/lactation level

Prophylactic treatment in cattle with this gynaecological condition consists of removing stress factors, feeding them balanced rations in relation to their physiological condition, feeding them high quality feed and ensuring that the females are moved throughout the year.

Curative treatment consisted of administering substances with a rapid action on this gynaecological condition. In this unit, for the treatment of females diagnosed with persistent corpus luteum, PGF Veyx, injectable sol. is administered intramuscularly in a dose of 5 ml. PGF Veyx is also used in cows for parturition induction or synchronisation of heat. In 2021 following the treatment administered out of the total herd of cows calved i.e. out of 202 heads, 34 females were diagnosed with persistent corpus luteum (16.8%), of these 14.3% responded positively (Table 5).

In 2022, out of 226 calved cows, 15.9% females were diagnosed with persistent corpus luteum and 15.0% were reproductively recovered (fig. 5).

#### Table 5

Therapy of cows with persistent corpus luteum on the farm

Year	Peaceful cows	Cows diag	nosed with		
		persistent cc	npus iuteum		
		Nr	%	Nr	%
2021	202	34	16,8	29	14,3
2022	226	36	15,9	34	15,0



Fig. 5 Farm CLP therapy

### CONCLUSIONS

1.The monthly incidence of persistent corpus luteum showed close values in both years of the study. In April 2021, no cases of persistent corpus luteum were diagnosed and the highest value of 4.0% was recorded in January. In 2022, also in April, no females showed this gynaecological condition and in January, out of all surveyed cattle 3.9% were diagnosed with persistent corpus luteum.

2. In relation to the number of lactations, in 2021 the lowest incidence of persistent corpus luteum of 1.5% was diagnosed in females in 1st lactation and the highest of 4.4% in cows in 1st-6th lactation and above. In the year 2022, similar values were found in the same categories of cows with the highest value of 4.8% in females in the 6th and beyond lactation.

3. In relation to the level of milk production/lactation, in both years of observation the persistent luteal body showed an average value of 0.95% in cows with milk production between 5000-5500l, progressively increasing to 4.00% in those with milk production between 7001-7500l.

4.Following treatment of females diagnosed with persistent corpus luteum, 14.3% responded positively in 2021 and 15.0% in 2022.

#### REFERENCES

- Abdela N., Ahmed W. M., 2016 Risk factors and economic impact of dystocia in dairy cows: A Systematic Review. Journal of Reproduction and Infertility 7 (2): 63-74.
- 2. Arthur G.N., Noakes D. E., Pearson H. 2009 -Veterinary Reproduction and Obstetrics, Saunders Elsevier Publishers, 9th ed.
- 3. Ball P.J.H., Peters A.R. 2007 Reproduction in Cattle, Blackwell Publishing Ltd., Oxford, UK

- Drugociu Dan, Drugociu Dana Simona 2015 -Genital and mammary gland pathology in animals, Ed. Ion Ionescu de la Brad Iasi
- Groza Ioan Ștefan Coordinator, Cenariu Mihai, Ciupe Simona, Ivan Camelia, Morar Iancu, Pall Emoke, Pop Raul, Șonea Alexandru, Voloseniuc Mihai Sorin 2019, Physiology And Pathology Of The Post-Partum Period In Domestic Animals, Ed. Academiei Române, Bucuresti.
- 6. Hopper R.M. 2014 Bovine Reproduction, Wiley Blackwell Publishing, USA
- 7. Kahn Cynthia M., Line S. 2010 The Merck Veterinary Manual, Elsevier Health Publishing
- Nechifor F., Drugociu D., Roşca P., Ciornei Şt. 2016 - Elements of pathology in the diagnosis and therapy of dystocia in cows, Ion Ionescu de la Brad lasi Publishing House,
- 9. Nechifor Florin, Roşca Petru, 2020 Physiology of the puerperal period and its monitoring in cows. 2nd edition. Ed. Ion Ionescu de la Brad Iasi
- Noakes D, Parkinson T, Timothy J., England G., Gary W. 2019 -Veterinary Reproduction and Obstetrics 10 th Edition, Elsevier, China, pg. 148-153
- 11. Runceanu L.Gh., Cotea C. 2007 Veterinary Reproduction, Obstetrics and Gynaecology, Ion Ionescu de la Brad Publishing House, Ed.II, Iasi

# OBSERVATIONS ON THE DEVELOPMENT OF FETAL ATTACHMENT RETENTION IN INTENSIVELY BREEDING COWS

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## Abstract

The evolution of the puerperium is conditioned by the course of parturition - eutopic or dystocic, but also by possible placental retention or uterine atony and not rarely by infection with bacterial germs. After parturition, energy consumption increases with uterine involution, the volume of milk production and the needs determined by local and general self-defence. The puerperal period is one of the most important stages in the reproductive cycle of cows. Early and correct diagnosis of conditions during this period can lead to appropriate and more effective treatment regimens. Research in the literature indicates that special attention should be paid to high milk-producing females, especially in the intensive breeding system, as it is known that some diseases can easily develop in the genital tract during the puerperal period. An important aspect of reproduction in cows is sterility or inability to reproduce due to incurable lesions of the genital tract following dystocic parturitions. The aim of this paper is to complete the existing research and studies in the literature with some major aspects of reproductive pathology in cows, namely to highlight some of the factors that can generate the anaesthesia syndrome. In the first year of observation (2021), the incidence of fetal adnexal retention was 12.4% of the 242 cows monitored, and in 2022 out of a total of 270 cows, 9.6% of females were diagnosed with this condition. Analyzing the incidence of fetal appendix retention by calendar month, it was found that in 2021 no cases were reported in August, one case (0.4%) was recorded in June and July, and the highest level was recorded in February, 2.5%. In 2022, similar incidence of fetal appendage retention was recorded including the highest level of 1.8% being reported also in February. The incidence of fetal attachment retention in cows correlates with the level of milk production per lactation increasing progressively with the volume of milk production. Thus, in the year 2021 in cows with milk production ranging between 5000-5500 liters of milk/lactation the lowest level was obtained, i.e. 0.8% of fetal attachment retention.in the herd of 26 monitored females. Of the 45 cows with milk production between 7001-7500 l milk/lactation 3.3% of the females showed fetal attachment retention.

Key words: cow, fetal attachment retention, lactation number, puerperal disorders

The puerperal period is a key stage in a female's life, as it is dependent on the course of parturition and any post-partum disorders. Immediately after parturition there is an increase in plastic and energy consumption progressively with uterine involution and milk level/day (2,4).

In order to maintain and increase the level of milk production, which is a commodity on a farm, females should calve at intervals of not more than 12-13 months. Taking into account the average duration of gestation (285 days) and the duration of uterine involution, this means that the time interval for the female to remain pregnant after parturition is quite short (60-80 days) (5, 10). This period overlaps perfectly with the period when the lactation curve is rising. For cows parturition occurred in the spring season, the period of preparation for a new gestation coincides with the period of transition from stock feeding with canned feed to green meal feeding, which is stressful for the body. The level of milk production together with feed stress can negatively influence reproductive function, manifested by a lack of heat expression over a longer period of time (postpartum anaesthesia) and low fertility rates (6,7,). The evolution of the puerperal period in cows is the main segment in the breeding cycle in cows. This. The cyclogram includes monitoring of several stages such as: elimination of lochia, bacteriology of the puerperal uterus, postpartum uterine involution and resumption of ovarian activity (1,3).

In the intensive rearing system, the most common postpartum conditions in the cow are: fetal attachment retention (FAR), postpartum uterine atony, uterine prolapse and endometritis. Nutrition plays a major role in the resumption of ovarian activity after calving and through. .proteins synthesized and secreted by adipocytes (8,9).

## MATERIAL AND METHOD

To establish the diagnosis of placental retention and to keep a record of the consultation in the individual gynaecological records of the females as well as the consultation register. The electronic individual gynaecological records provide information on previous parturitions (eutopic or dystocic), puerperal conditions, number of artificial inseminations performed for a new gestation and other treatments performed antepartum, etc.

The history is an important step in establishing the diagnosis of placental retention and provides a range of information on: the breeding programme, the method of artificial insemination or artificial insemination; the forage of the females and the assessment of the quality of the forage, the quantities administered and the method of administration in relation to the physiological condition; the farming technology, movement, rest, microclimate, animal density and hygiene conditions; monitoring of activities to detect estrus, parturition and puerperium; data on lactation and mammary gland health; frequency of reproductive disorders and abortions; examination of vaginal discharge and reproductive indices.

## **RESULTS AND DISCUSSIONS**

In 2021, 242 cows were studied, with an incidence of fetal attachment retention of 12.4%. In the following year (2022) out of a total of 270 females monitored, 9.6% were diagnosed with fetal attachment retention.

Cases of fetal attachment retention in cows can be represented by: increased milk production, mammary gland disorders, too short a resting period, poor health status, inadequate quality of fodder fed during gestation, poor hygiene conditions.

Table 1

		Reter	ntion of			Ovariopathies					
Year	Cows calve	fetal appendages		fetal Uterine ppendages infections		Persi cor	stent pus	Hypot ova	rophic ries	Ova cag	rian Jes
	d					lute	eum				
		nr.	%	nr.	%	nr.	%	nr.	%	nr.	%
2021	242	30	12,4	56	23,1	36	14,8	31	12,8	24	9,9
2022	270	26	9,6	53	19,6	30	11,8	27	10,0	21	7,7
Total	512	56	-	109	-	66	-	58	-	45	-
Media	-	-	11,0	-	21,3	-	13,3	-	11,4	-	8,8

#### Dynamics of puerperal diseases in cows in 2021-2022

The most common postpartum disorders in cows are endometritis which occur in cases of uterine dynamics disorders expressed by atony or hypotonia following dystocic parturitions, plus the intervention of ascending infections occurring during operations to correct dystocia.

The literature states that the resumption of the uterine-ovarian cycle normally occurs at about 21-45 days, and is dependent on a number of factors such as: age of parturition, quality of feeding, general health of the female, husbandry regime, quality of the microclimate, level of milk production, season, etc. In our study, uterine infections were found to be much higher than fetal envelope retention, namely 23.1% in 2021 and 19.6% in 2022. The 3.5% lower value of uterine infection frequency recorded in the year 2022 is triggered by providing quality care to the parturition process and administering postpartum preventive therapy.

Analyzing the incidence of ovariopathies it is found that in the year 2021 out of the total 242 females taken in the study, 36 of them i.e. 14.8% were diagnosed with persistent corpus luteum, 31 females i.e. 12.8% were diagnosed with different degrees of ovarian hypotrophy and 24 females , which means 9.9% showed ovarian luteal cystic fibrosis (Table 1, Fig. 1).



Fig. 1 Frequency of puerperal conditions in the period 2021-2022

The In 2022 out of 270 cows monitored, 30 cows were diagnosed with persistent corpus luteum, representing 11.8% which is 3% lower than in 2021. In the same year there were also decreases in the values of ovarian hypotrophies of different degrees which were reported in 10% of the cases and luteal ovarian cystic fibrosis which were recorded in 7.7% of the cases.

Table 2 presents data on the frequency of fetal adnexal retention recorded in the form taken in the study in the years 2021-2022.

Analysis of these data shows very different values of the frequency of fetal adnexa retention both by calendar month and by season. In the year 2021 the only month in which no cases were recorded was August, while in June and July one case (0.4%) was recorded, in May, September, October and November two cases (0.8%), in January and March four cases (1.6%), the maximum value being recorded in February with 6 cases (2.5%) (Table 2 fig.2).

#### Table 2

Year	20	21	2022		
Month					
	Nr.	%	Nr.	%	
January	4	1,6	3	1,1	
February	6	2,5	5	1,8	
March	4	1,6	4	1,5	
April	3	1,2	2	0,8	
May	2	0,8	2	0,8	
lune	1	0,4	1	0,3	
luly	1	0,4	1	0,3	
August	0	0,0	1	0,3	
September	2	0,8	1	0,3	
October	2	0,8	1	0,3	
November	2	0,8	2	0,8	
December	3	1,2	3	1,1	
Total	30	100,0	26	100,0	

Monthly dynamics of placental retention in 2021-2022

As it results from the analyzed data we can see that the highest values in terms of fetal attachment retention were reported in December-February, so in the cold period of the year, a period when usually the rations administered are unbalanced in nutritional principles and of lower quality, temperatures can vary greatly and the level of daily milk production remains high.

The retention of fetal adnexa was also similar in 2022, with the highest level also in the cold season of the year and the month with the highest value was also February. (fig.2).


Fig. 2 Incidence of fetal appendage retention in 2021-2022

It is known that another factor involved in the incidence of fetal attachment retention in cows is the lactational rank. In this regard it was found that in 2021 out of 57 cows all at first lactation only 1.2% were diagnosed with placental retention (Table 3 Fig. 3). This value increases at 2nd lactation up to 1.6% to reach the maximum level of 3.3% at 6th lactation. Similar but somewhat lower values were also reported in 2022. For example, in cows in first and second lactation, the lowest values were recorded (0.7%) and the maximum value of 2.6% was also reported in the sixth lactation.

This can be explained by the fact that each lactation is preceded by gestation and parturition, events which bring about a series of changes in the genital apparatus requiring extensive postpartum restructuring.

One of the most important factors influencing the development and quality of the postpartum period in cows is the level of milk production.

Table 3

		Y	ear 2021		Year 2022					
	Partu	urient	Cow	s with	Partur	ient	Cows with			
Laction	COWS		placenta	I retention	cow	S	placental retention			
	Nr.	%	Nr.	%	Nr.	%	Nr.	%		
	57	23,5	3	1,2	61	22,6	2	0,7		
II	50	20,7	4	1,6	56	20,7	2	0,7		
III	43	17,7	5	2,1	48	17,8	4	1,5		
IV	39	16,2	5	2,1	44	16,3	5	1,3		
V	31	12,8	6	2,5	34	12,6	6	1,9		
VI and over	22	9,1	8	3,3	27	10,0	7	2,6		
VI										
Total	242	100	30	12,4	270	100	26	9,6		

Incidence of placental retention by number of lactations in 2021-2022

The influence of these factors on the evolution of the puerperal period was manifested by an increase in the incidence of fetal adnexa in proportion to the increase in milk production. For example, in 2021, out of the 26 cows studied with a milk production of 5000-5500 litres of milk per

lactation, only 0.8% showed fetal attachment retention, while in cows with a milk production between 7000 and 7500 litres of milk per lactation, this index reached a maximum of 3.3% (Table 4, Fig. 4).



Fig. 3 Incidence of fetal adnexal retention in relation to number of lactations in the years 2021-2022

Table 4 Dynamics of fetal attachment retention in cows as a function of milk production/lactation level in 2021-2022

	Year 2			Yea	r 2022						
Litres milk/lactation	Part	urient	Co	ws with	Part	urient	Cows with				
	co	ws	pla	acental	co	ows	placental				
			re	tention			retention				
	Nr.	%	Nr.	%	Nr.	%	Nr.	%			
5000-5500	26	10,7	2	0,8	29	10,7	2	0,7			
5501-6000	32	13,2	3	1,2	36	13,3	2	0,7			
6001-6500	36 14,9		4	1,6	40	14,8	3	1,1			
6501-7000	40	16,5	5	2,0	44	16,3	5	1,8			
7001-7500	45	18,6	8	3,3	50	18,5	8	3,0			
7500-8000	38	15,7	5	2,0	42	15,5	4	1,5			
>80001	25	10,4	3	1,2	29	10,9	2	0,7			
Total	242	100,0	30	12,4	270	100	26	9,6			



Fig. 4 Dynamics of fetal attachment retention in cows as a function of milk production/lactation level in 2021-2022

The season of the last part of gestation and parturition has an important influence on the development and quality of post partum restructurings, on the length of the puerperal period and on the timing of the resumption of the puerperal sexual cycle. The influence of the season is due both to the quality of the feed, which suffers in the cold season of the year as a result of poor preservation methods, and to the microclimate, which is more difficult to ensure at an optimal level for the comfort and exploitation of the animals.

In the case of our study the influence of the season was demonstrated by recording higher values, namely 3.3% cows with fetal attachment retention in the winter season and 4.5% in the spring season of 2021, the lowest values being recorded in the summer season 2.1%. (Tab. 5 fig.5)

Similar values were also found in 2022, namely proportions of 2.6% in the winter season

and 3.7% in the spring season.

Table 5

Incidence of placental retention by season in 2021-2022															
Sezons															
Spring			Summer			Autumn				Winter					
Part	urient	Cow	/s with	Parturient		Cows		Parturie		Cows		Parturient		Cows	
cows R.A.F.		cows		with		nt cows		with		cows		with			
			R.A.F.		A.F.			R.A.F.				R.A.F.			
nr	%	Nr	%	nr	%	nr	%	Nr	%	nr	%	nr	%	Nr	%
38	16,1	11	4,5	95	39,2	5	2,1	50	20,	6	2,5	59	24,	8	3,3
									6				3		
44	16,3	10	3,7	10	37,7	4	1,5	58	21,	5	1,8	66	24,	7	2,6
				2					4				4		



Fig. 5 Incidence of placental retention by season in 2021-2022

## **CONCLUSIONS**

1. The evolution and quality of the puerperal course is influenced by many factors that can lead both to the appearance of specific conditions at this delicate period in a parturient's life and to a delay in the resumption of the postpartum sexual cycle.

2. The influence of these factors was reflected in our study in the increased incidence of fetal adnexal retention and the incidence of puerperal infections in the females studied.

#### REFERENCES

- 1. Abdela N., Ahmed W. M., 2016. Risk factors and economic impact of dystocia in dairy cows: A Systematic Review. Journal of Reproduction and Infertility 7 (2): 63-74.
- Arthur G.N., Noakes D. E., Pearson H., 2009. 2. Veterinary Reproduction and Obstetrics, Ed. Saunders Elsevier, Ed.IX

- Beagley, J.C., K.J. Whitman, K.E. Baptiste, J. Scherzer, 2010. Physiology and treatment of retained fetal membranes in cattle. Journal of Veterinary Internal Medicine, 24:261-268
- 4. **Drillich, M., 2006.** An update on uterine infections in dairy cattle. Slov. Vet. Res., 43: 5–11.
- Groza Ioan Ștefan Coordonator, Cenariu Mihai, Ciupe Simona, Ivan Camelia, Morar Iancu, Pall Emoke, Pop Raul, Șonea Alexandru, Voloseniuc Mihai Sorin 2019. Physiology And Pathology Of The Post-Partum Period In Domestic Animals, Ed. Academiei Române, București.
- Nechifor F., Drugociu D., Roşca P., CiorneiŞt. 2016 - Elemente de patologie în diagnosticul şi terapia distociilor la vacă, Ed. Ion Ionescu de la Brad Iaşi,

- Noakes David E., Parkinson J. Timothy, England C. W. Gary, 2019. Veterinary Reproduction and Obstetrics 10<sup>th</sup> Edition, Elsevier, China, pg. 148-153.
- 8. **Pook S., Horner J., Milhollin R., 2009**. Dairy cattle reproductive manual. Ed. Extension, Missouri.
- Runceanu L., Drugociu D., Roşca P., Ciornei Şt., Nechifor F. 2014 – Fiziopatologie maternofetală veterinară, Ed. Ion Ionescu de la Brad Iași.
- Tudor L. 2007 Reproducţia, andrologia şi obstetrică animalelor domestice. Ed. Fundaţiei România de Mâine, Bucureşti
- 11. *Zaabel S. M., Hegab A.R., 2003.* Practical notes on veterinary ginaecology and andrology. Mansoura University.

# CONTROL OF COCCIDIOSIS OF FARM BREEDING SHEEP

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#### Abstract

Infection with *coccidia* is one of the most common and damaging sheep diseases, particularly young lambs and economically important diseases of sheep. Coccidiosis is caused by parasitic protozoa in the genus *Eimeria*. Coccidial infection is virtually universal in sheep and large numbers of oocysts may be found in the faeces of clinically normal animals. Usually it is presented at animals at 4-10 weeks.Lamb and kids infection had moderate morbidity and low mortality rate. Environmental contamination and resulting clinical disease is generally influenced by local weather conditions and the grazing management practices of the flock. In our paper, we present measures to control coccidiosis in sheep kept in frame breeding conditions.

Key words: coccidiosis, lambs, sheep, control

Breeding of sheep represents a significant branch of livestock production. The reason for this lies not only in tradition, but also because the production of wool and milk, as well as lamb meat, is a highly sought-after item on the world market. Based on research in the world, diseases of parasitic etiology dominate in sheep both in terms of prevalence and incidence, accompanied by significant morbidity and moderate mortality (Kusiluka and Kambarage,1996, Pavlovic et al., 2009, Bojkovski et al.,2010 Altaf and Hidayatu, 2014).

Coccidiosis in sheep is parasitic infection caused by protozoa in the genus *Eimeria* (Levine, 1985, Foreyt, 1987, Bangoura and Bardsley, 2020). Historically, some *Eimeria* spp were thought to be infectious and transmissible between sheep and goats, but the parasites are now considered hostspecific (McDougald,1979). Numerous data from world literature support the fact that coccidiosis is the most common parasitic disease of sheep in herds (O'Callaghan et al.,1987, Gregory, 1990, de Silva et al.,2008, Chartier and Paraud, 2012, Almeida 2013, Ibrahim and Afsa, 2013). All over the world, an extremely large number of older animals are infected with protozoa, which are involved in the etiology of this disease (Gregory and Catchpole,1989, de la Fuente et al.1993, Platzer et al.,2005, Wang et al.,2010). It is of even greater importance that the infection of the young that occurs already in the first months of life (Ozdal et al.,2009)

Based on existing data, the prevalence of coccidiosis is highest in environments where the most intensive keeping is in stable conditions () Unlike helminths, which need free grazing areas for biological development, protozoan infections are related to stable housing, where there is a permanent microclimate, especially temperature and humidity (Wright and Coop, 2007, bakunzi et al.,2010, Ibrahim and Afsa, 2013).

In addition, a large number of animals of different ages in a relatively small space leads to population pressure that favors the development of protozoa and thus infections. The main goal of the control program of sheep coccidiosis is to raise the health status of sheep in the Republic of Serbia (Pavlovic et al., 1995, 2013, 2019, 2021a, b).

By preventing the occurrence and spread of coccidiosis by undertaking certain biotechnical and health measures that have the role of reducing the prevalence of parasites, which achieves higher growth and better production results from sheep. The application of this integrated concept of cocidiosis control required systematic monitoring of infection on farms before and after the applied measures and required the involvement of all

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relevant entities, primarily veterinary services, starting from farms to animal workers.

# MATERIAL AND METHOD

Coccidiosis control measures were applied to nine test farms raising sheep in herds of more than 50 animals, from January 2014 to March 2015.

In order to establish the prevalence of parasitic infections and the biodiversity of the causative agent in the investigated sheep herds, samples of feces of all categories of animals were collected. On each of the farms, 30 samples of feces were sampled, 10 from each production category - lambs/kids at teats (from 0 to 28 days of age), rearing animals (before the first release) and breeding animals.

Colected faeces samples were examined using routine coprological methods (Soulsby,1977, Taylor,1995, Pavlović and Anđelić-Buzadžić, 2010). Despite the general relationship between clinical coccidiosis and oocyst excretion, no threshold for treatment has been established, though a fecal oocyst count of >20,000 OPG of a pathogenic species is considered characteristic of coccidiosis in lambs. Determination of occidia we performed by morphological characteristic (Levine 1985, Foreyt, 1987, Eckert et al.,1995).

### **RESULTS AND DISCUSSION**

Our examination revealed coccidiosis in 139 animals (23.61%).

Lambs most often showed clinical symptoms of the disease and they were present in 39.37% of the affected individuals. There was no gender difference in the prevalence and incidence of coccidiosis.

In adult animals, the results showed that 57.70% of sheep were infected and had subclinical coccidiosis. Adult female sheep were significantly (P<0.05) more infected (82.2%) than adult rams (40%).

In the overall finding, *E. ovoidalis* was the most represented species, found with 87.1%, followed by *E. faueri* (63%), *E. ovina* (53%), *E. granulosa* (31%), *E. parva*, (25%), *E. intricata* (19%) and *E. pallida* (8%),

Infections with two or more Eimeria species were detected in 78%.The number of excreted oocysts was higher in lambs (18325+/-23383 OPG) than in adult sheep (2597.3+/-12373 OPG).

After diagnosing coccidiosis, all animals in the herd were treated with Toltrazuril (20 mg/kg, PO, once), whose metabolite toltrazuril-sulfone (ponazuril) significantly reduces the number of cysts Platzer et al.2005).

After the therapy, hygienic measures were carried out in the facilities where the sick animals were. All clinically affected lambs should be separated from their mothers and placed in a clean, disinfected pen with plenty of straw. Other animals should be moved to less contaminated areas of the facility to ensure food and water sources are free of feces.

Control management of coccidiosis is a delicate balance between ensuring that lambs are exposed to infection and thereby acquire immunity, and preventing them from developing clinical disease. Management plays a key role in prevention by ensuring that all areas used by sheep, especially lambs, are thoroughly cleaned and disinfected, ensuring that pens are not overcrowded and that kids are kept in small groups of the same age.

Management can help prevent disease by reducing the size of the number of individuals in the facility, thereby reducing fecal contamination from sheep or lambs. All feed and water troughs should be raised off the ground and faecal contamination prevented (Bojkovski et al.,2010, Altaf and Hidayatu, 2014, Bangoura and Bardsley, 2020). All troughs inside and out should be placed in well-drained areas. Regular transfer of lambs to other segments of the facility prevents excessive accumulation of oocysts

After that, a control examination was performed in the herds. The total number of excreted oocysts in lambs was 11892+/- 10584 OPG, while in sheep it was 10017+/-9747 OPG.

These measures achieved the goal of controlling coccidiosis in sheep/lambs in two ways - by creating primary immunity in lambs after subclinical infection, which achieves lifelong immunization that prevents clinically manifest diseases, and by preventive therapy that is used when they are due to population pressure and environmental factors (Gregory and Catchpole, 1989). conditions created predisposing conditions for the breakthrough of immunity and the emergence of clinically manifest infections. This refers primarily to the winter period, when a critical amount of oocysts per unit area is reached in the pens and the possibility of infection.

The second is timed and targeted preventive treatment. Because occurrence of coccidiosis under these management systems often becomes so predictable, coccidiostats should be administered prophylactically for 28 consecutive days beginning a few days after lambs are introduced into the environment (Platzer et al.2005).

#### CONCLUSIONS

Sheep coccidiosis was of great importance to health status of lambs and its performances. Lambs infection had moderate morbidity and low mortality rate. Consequence is significant increase of lamb accrescense, its weakens and less growth.The best preventive measure a lamb producer can take is to use a feed with a coccidiostat added. With careful management and sound preventive measures, the losses associated with this disease can be reduced to minimal levels. By preventing the occurrence and spread of coccidiosis by undertaking certain biotechnical and health measures that have the role of reducing the prevalence of parasites, which achieves higher growth and better production results from sheep. The application of this integrated concept of parasite infection control required systematic monitoring of infection on farms before and after the applied measures.

#### ACKNOWLEGMENTS

The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030).

#### REFERENCES

- Almeida J.D.M. 2013 Infection due to *Eimeria* spp. in sheep in the municipality of Colinas, state of Tocantins. Med Vet (UFRPE) 7:33–36.
- Altaf A.R., Hidayatu A. 2014 Study of some potential risk factors associated with coccidia in sheep. J.Agr. Vet. Sci., 65:11-13.
- Bangoura B., Bardsley K.D. 2020 Ruminant Coccidiosis. Vet Clin North Am Food Anim Pract. 36(1):187-203.
- Bojkovski J., Relić R., Hristov S., Stanković B., Savić B., Pavlović I., Petrujkić T. 2010 - Influence of biological and chemical contaminants on health status of small ruminants. - Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 67 (2):37-39.
- Bakunzi F.R., Thwane S.N., Motsei L.E., Dzoma
  B.M. 2010 Diversity and seasonal occurrence of *Eimeria* species in a mixed flock of communally reared sheep and goats in Mafikeng in the North West Province, South Africa. J S Afr Vet Assoc 81:148–150.
- Chartier C., Paraud C. 2012 Coccidiosis due to Eimeria in sheep and goats, a review. Small Rumin Res.103(1):84–92.
   de la Fuente C., Cuquerella M., Carrera L., Alunda
- de la Fuente C., Cuquerella M., Carrera L., Alunda J.M. 1993 - Effect of subclinical coccidiosis in kids on subsequent trichostrongylid infection after weaning. Vet Parasitol. 45(3 4):177-183.

- da Silva F.R.C., de Souza J.D., Fialho C.G., Escopeli K.S., de Araújo F.A.P. 2008 -Identification of *Eimeria* species in sheep in Mostardas, southern Brazil. Vet Em Foco 6:16–20.
- Eckert J., Taylor M., Catchpole J., Licois D., Coudert P., Buclar H. 1995 - Identification of Eimeria species and strains. In: Biotechnology; Guidelines on Techniques in Coccidiosis Research, Brussels, Luxembourg, pp 103-119.
- Foreyt W.J. 1987 Coccidiosis in sheep and goats. Vet Hum Toxicol.29:60-64.
- Gregory MW. 1990 Pathology of coccidial infections. Boca Raton Coccidiosis of man anddomestic animals. Florida: CRC Press, Inc. pp. 235–61.
- **Gregory M.W., Catchpole J.** 1989 Ovine coccidiosis: heavy infection in young lambs increases resistance without causing disease. Vet. Rec., 124: 458-461.
- Ibrahim M.M., Afsa A.A.S. 2013 Natural coinfection a and species composition of *Eimeria* in sheep in Al-Baha area, Saudi Arabia. Egypt Acad J Biol Sci 5:49–58.
- Khodakaram-Tafti A., Mansourian M. 2008 -Pathologic lesions of naturally occurring coccidiosis in sheep and goats. Comp. Clin. Pathol., 17: 87-91.
- Kusiluka L., Kambarage D. 1996 Disease of small ruminants, Easter Bush, Scotland. pp 87-90.
- Kyriánová I.A., Vadlejch J., Langrová I. 2017 -Eimeriosis seasonal dynamics patterns at an organic sheep farm in the Czech Republic. Sci Agric Bohem 48:70–75.
- Levine N.D. 1985 Phylum II. Apicomplexa. In: Lee J.J., Hunter S.H., Bovee E.C. (eds), An Illustrated Guide to the Protozoa, Allen Press, Lawrence. KS., pp 322-374.
- McDougald L.R. 1979 Attempted cross-transmission of coccidia between sheep and goats and description of Eimeria ovinoidalis sp. n. J Protozool. 26(1):109-113.
- O'Callaghan M.G., O'Donoghue P.J., Moore E. 1987 -Coccidia in sheep in South Australia. Vet Parasitol, 24(3–4):175–83.
- Ozdal N., Tanritanir P., Goz Y., Deger S., Kozat S. 2009 - Parasitic protozoans (*Eimeria, Giardia*, and *Cryptosporidium*) in lambs with diarrhoea in the Van province (Turkey). Bull Vet Inst Pulawy 53:47–51.
- Pavlović I., Anđelić-Buzadžić G. 2010 Osnovi dijagnostike parazitskih bolesti životinja za studente visoke poljoprivredne škole strukovnih studija u Šapcu studijski program: strukovna veterina. Naučni institut za veterinarstvo Srbije, Beograd.
  - Pavlović I., Kulišić Z., Nešić D., Romanić S. 1995 -Endoparasites of sheep and goats in Prizren district Proceedeing of 3<sup>rd</sup> International Conference of Sheep and Goats Production, Ohrid, Macedonia, 106-110.
  - Pavlović I., Ivanović S., Žujović M. 2009 Coccidiosis of goats and its role and importance of goat production. Proceeding of IV Balkan Conference of Animal Science BALNIMALCON 2009, Challanges of the Balkan Animal industry and the Role of science and Cooperation, Stara Zagora, Bulgaria, 393-395.

- Pavlović I., Ivanović S., Bojkovski J., Kulišić Z., Savić B., Tambur Z. 2013 - Eimeriosis of small ruminants in Belgrade area. Proceeding of XIII Middle European Buiatrics Congress, Belgrade, 480- 483.
- Pavlović I., Ivanović S., Hadžić I., Petrović P.M., Caro Petrović V., Bojkovski J., Pavlović M., Zvekić D. 2019 - Zdravstvena zaštita malih preživara u poluintenzivnoj proizvodnji. -Nacionalni naučni skup sa međunarodnim učešćem Održiva poljoprivredna proizvodnja uloga poljoprivrede u zaštiti životne sredine, Bačka Topola, Zbornik radova,165-172.
- Pavlović, I., Radović, B., Milanović, V., Caro-Petrović, V., Bojkovski, J., Relić, R., Mladenović, V., Zdravković, N., Becskei, Z. 2021a - Protosan infection of small ruminants in north part of Serbia, with emphasis to North Kosovo. - Lucrări Ştiinţifice Medicină Veterinară Timişoara, LIV (3):125-133.
- Pavlović I., Caro-Petrović V., Csordás F., Minić S., Zdravković N., Bojkovski J., Stefanović V. 2021b - Coccidiosis in lambs in northern Serbia (Vojvodina). Proceeding of 3rd International Symposium: Modern Trends in Agricultural Production Rural Development and Environmental Protection, Vrnjacka Banja, Serbia, 342-347.
- Platzer B., Prosl H., Cieslicki M., Joachim A. 2005 -Epidemiology of *Eimeria* infections in an Austrian milking sheep flock and control with diclazuril. Vet Parasitol 129:1–9
- Reginsson K., Richter S.H. 1997 Coccidia of the genus *Eimeria* in sheep in Iceland. Icel Agric Sci 11:99–106
- **Soulsby E.J.L.** 1977 Helminths, arthropods and protozoa of domesticated animals, and Cassell Co, London, pp.702.
- Taylor M. 1995 Diagnosis and control of coccidiosis in sheep. In Practice., 17: 172-177.
- Vasilková Z., Krupicer I, Legáth J., Kovalkovicova N., Petko B. 2004 - Coccidiosis of small ruminants in various regions of Slovakia. Acta Parasitol 49:272–275.
- Wang C.R., Xiao J.Y., Chen A.H., Chen J., Wang Y, Gao J.F., Zhu X.Q. 2010 - Prevalence of coccidial infection in sheep and goats in northeastern China. Vet Parasitol 174:213– 217.
- Wright S.E., Coop R. 2007 Cryptosporidiosis and coccidiosis. In: Diseases of sheep, 4th edn, Blackwell Publishing, Oxford, UK, pp 179-185.