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## CALVES AND DAIRY COWS' HEALTH AND NUTRITION IN INTENSIVE BREEDING

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### Summary

An intensive cattle breeding encompasses a whole range of procedures that should enable continuous production and optimal utilization of production capacities. In such conditions, newborn calves must adapt to a variety of environmental factors, including housing, care, and feeding conditions. Inappropriate rearing conditions favour the emergence of gastrointestinal and respiratory diseases, as the most common health problems for calves. The same factors, and especially the unbalanced diet, have a major impact on the health of dairy cows and the incidence of diseases such as ketosis, fatty liver syndrome, puerperal paresis, rumen acidosis, and laminitis. This review paper is based on monitoring results of the health of calves and adult cows on dairy farms in Serbia, from 2011 to 2019. Also, some procedures in nutrition that can prevent the occurrence of metabolic diseases of cows are discussed.

**Keywords:** calves, cows, diseases, nutrition

High cattle production is based on the successful breeding of calves. Rearing conditions should allow calves to meet their basic living needs, such as optimal air quality, sufficient space for movement, rest and adequate nutrition and watering (5, 20, 27). If life needs are not met, the welfare of calves is questioned. Consequences of impaired welfare are usually manifested as changes in the health status of calves i.e. the occurrence of various diseases, and eventually the death (19). In the first place, losses in calves are caused by diseases of the digestive system and respiratory organs (16, 25). On dairy farms, losses are most often present in calves' transition from one to another breeding stage (8).

Welfare in terms of accommodation is most often compromised by inadequate ventilation, air velocity, and temperature, as well as exposure to pathogenic microorganisms and parasites (21, 22, 23, 24). Besides data on housing conditions, for the complete picture of calf welfare status certain procedures such

as feeding and watering management are taken into account (23). In high milk-yielding cows, metabolic disorders such as ketosis, fatty liver syndrome, puerperal paresis, rumen acidosis, and laminitis have been influenced by many factors, including unbalanced nutrition, inadequate rearing conditions and poor care (13, 14).

This paper is based on the results of monitoring the health status of dairy herds in Serbia for the period 2011-2019. Also, given the high proportion of nutrition on the occurrence of diseases of calves and cows, some methods that can prevent the occurrence of these diseases in intensive breeding conditions have been considered.

### **Health status of calves**

Calves are susceptible to cardio-respiratory and metabolic disorders that can be diagnosed as early as the first weeks of life. The reason for this is that the organism of the newborn calf is not fully adapted to the life in modern cattle production systems, and the adaptation must be done very quickly (6, 7).

The vitality of calves can be affected by errors in the gestation period, especially the nutrition of cows in the last third period of pregnancy. The need for optimal amounts of  $\beta$ -carotene should be especially emphasized in the diet of pregnant cows since calf diarrhea often occurs in herds where the concentration of  $\beta$ -carotene in the blood serum of highly pregnant cows was very low (8, 18). It is well known that timely and adequate intake of colostrum is of great importance for the survival of calves and their further progress. Through colostrum, newborn calves are supplied with nutrients (proteins, amino acids, fatty acids, lactose, vitamins, macro- and microelements), as well as immunoglobulins, hormones, enzymes, and other substances (1, 17). The possibility of supplying calves with colostrum derived from cows with mastitis or with colostrum containing any kind of toxins should be completely excluded (12).

Gastrointestinal disorders are considered to be the most significant problem in calf rearing, especially in the first month of life. Diarrhea is an etiologically complex syndrome, which arises as a result of an interaction of the environment, the diet, and several different infectious agents, among which the dominant are strains of *E. coli* and clostridia (2).

Gastrointestinal diseases of viral etiology (rotaviruses,) occur when the effect of virulence of a certain strain of the virus suppresses local intestinal immunity and reduces the intestinal bacterial flora. Viruses are not considered to be the primary causes of disease, even when isolated from the feces of calves with diarrhea. The presence of enteropathogenic strains of *E. coli* promotes their pathogenicity, and inadequate hygienic conditions and irregular manure removal favor the retention of pathogenic microorganisms from feces in facilities for animals (2).

The synergistic action of bacteria and viruses is important for the occurrence of respiratory diseases in calves, especially in an overcrowded barn where the microclimate is bad (i.e. low temperature and high humidity, high concentration of ammonia, draft), as well as in animals with weakened immunity. Respiratory tract infection is a constant problem with seasonal outbreaks, especially on farms where hygienic measures are poorly implemented. Milder forms of respiratory tract infection have been observed in calves that resided in open and semi-open areas (23). From the nasal swabs of calves, *Pasteurella multocida*, *Mannheimia haemolytica* (*Pasteurella haemolytica*), *Trueperella pyogenes*, *Haemophilus sp.*, and *Klebsiella pneumoniae* were isolated (2).

Of the parasitic diseases in intensive cattle production, problems are protozoan infections and ectoparasites. Inadequate hygienic and microclimatic conditions, as well as the high population density also affect the retention of parasites and their developmental forms in the stable, as well as the spread and occurrence of diseases.

Protozoan infections are very common in calves, and most commonly coccidiosis and cryptosporidiosis appear. Infections with helminths rarely occur at this age, and in intensive (indoor) keeping they are almost eliminated (15).

Ectoparasitic infections are dominated by scabies, which is caused by *Sarcoptes scabiei var. bovis*, *Psoroptes equi var. bovis* and *Chorioptes bovis var. bovis* (15).

### **Health status of dairy cows**

In dairy cows, metabolic disorders (ketosis, fatty liver syndrome, puerperal paresis, and rumen acidosis), and laminitis are the most often health problems observed. Ketosis is a metabolic disorder that occurs in high-productive cows, usually in the third or fourth lactation, during the first weeks after calving, or in early lactation. Several factors contribute to the onset of ketosis, but the main reason, in addition to physiologically low glycemia in ruminants, is the insufficient intake of energy in the ration needed for the early stage of lactation. Blood glucose is the most important source of energy for a cow. However, glucose is easily and quickly fermented in the rumen. When there is not enough energy, fat reserves are mobilized and excess ketone bodies accumulate in quantities that can cause disturbances in the body with signs of ketosis: loss of appetite, weight loss, decrease in milk production, and in more severe cases nerve symptoms (18).

The task of dairy cows feeding is to provide, above all, good health and proper body condition of the animal, lactation lasting about 300 days, as well as a larger number of lactations during exploitation. Furthermore, goals are to get the maximum amount of milk of optimal chemical composition and the birth of a healthy and vital calf every year. In order to respond to such requirement, a high-productive cow must be provided with housing conditions, care, health protection and, above all, nutrition maximally adapted to the needs of its organism (a meal adapted to the

production of milk), and brought to the limit of ideal, within the possibilities of modern cattle breeding (18). Mistakes that occur in the last third of pregnancy become clearly noticeable and pronounced during the puerperium.

Ketosis is a disorder of carbohydrate and fat metabolism, in which the  $\beta$ -oxidation of fatty acids is blocked, and the process is stopped at the level of acetyl-CoA. Oxaloacetate is a crucial crossroads of many pathways in intermediate metabolism, as well as in glucose resynthesis and fatty acid breakdown. Since the process of glucose synthesis is maximally activated, the largest amount of oxaloacetate is "captured" and "carried out" by gluconeogenesis. Therefore, a larger amount of acetyl-CoA accumulates, which cannot be completely oxidized, and two molecules of acetyl-CoA merge and the synthesis of the first ketone body, acetoacetic acid, and from it  $\beta$ -oxybutyric acid and acetone. The synthesis of ketone bodies, within physiological limits, also takes place in healthy animals with an intensity of 25  $\mu\text{g} / \text{h}$ . Most tissues are able to use ketone bodies as an energy source, and the degree of their breakdown is proportional to the concentration in the blood up to a level of 3.44 mmol / L. Ketone bodies accumulate in the blood, and then are excreted in urine and milk, and to a lesser extent through the lungs. By excreting ketone bodies, an organism that is already in an energy deficiency, additionally loses a large amount of energy. About 75% of the energy value of fat is still bound in ketone bodies (18).

Summarizing the above considerations, it is necessary to focus on nutrition, which is important in the prophylaxis and treatment of this metabolic disorder. Subclinical ketosis can be monitored by regular monitoring of metabolic profile parameters, which include some analyzes to assess the functional status of liver cells (glucose concentration, AST activity, total bilirubin, and albumin) (11, 18). Extremely high needs of cows with high milk production condition the use of a larger amount of concentrated nutrients with a consequent reduction in the content of crude feeding stuff. In order for the meal to meet the nutritional requirements of the animal in production, it is recommended to avoid bulky foods with a high water content (silage with less than 25% SM, cellulose nutrients). Due to the excessive volume of such nutrients, e.g. a small amount of energy and nutrients per unit mass, the cow is not able to consume a sufficient amount to meet basic needs. The feed of choice is good and quality hay (from meadow, legumes, or mixtures) that can be given at will, and the concentrated part of the meal, adjusted to the production status, should be divided into several parts, in order to achieve a more even arrival of carbohydrates in the rumen and their decomposition under the action of microflora, and avoided or significantly reduced the possibility of rumen acidosis with all the consequences. This way of feeding avoids the occurrence of "peaks" of glycemia, which is inevitable when feeding high-productive cows fed mostly concentrated nutrients (18).

A special problem in feeding stuffs planning is the harmonization of the ratio of certain types of carbohydrates that animals eat. It is necessary to emphasize the importance of cellulose, but also starch as a precursor of propionic acid, as a key mediator of gluconeogenesis, while the participation of easily digestible

carbohydrates, although they represent "instant energy", carries a constant risk of metabolic disorders. The parameter that should be taken into account is the daily consumption of food, which is especially a problem in conditions of stress and drastic physiological changes during late pregnancy, parturition, and early lactation. General, feeding stuff should be tasty and tempting for the cow, with preserved organoleptic properties and safe. The meal control mechanism includes short-term control (pH of rumen content, amount of volatile fatty acids and levels of some hormones such as insulin, glucagon, and gastrin) and long-term control of consumption, physiological state, nitrogen status, environmental factors, photoperiodicity and seasonal differences, production rate, and total energy needs. Also, important are the bodyweight of cows, genetic basis, health status, competitive instincts of higher priority (thermoregulation), as well as factors related to the taste and attractiveness of meals (9, 18). In conditions of abundant unbalanced nutrition in the prepartum period, excess nutrients are deposited in body depots and form the so-called "fatty liver syndrome". In the conditions of energy deficit and consequently accelerated mobilization of fat from the depot, which is a common occurrence in fattened cows, the accumulation of free fatty acids in the blood and their deposition in the liver is too fast. The high concentration of free fatty acids in the blood causes a decrease in appetite, e.g. animals react by reducing consumption, which then leads to an energy deficit when cows lose weight abruptly with a possible even lethal outcome. Accumulation of fatty acids in the liver occurs in the form of fatty infiltration or degeneration of hepatocytes. With such an additional load, the liver loses its functional activity, which contributes to the occurrence of ketosis; reparation and restitution processes in hepatocytes are difficult because complete recovery is achieved only when the liver gets rid of excess fat, which is a slow and long process (9, 18). The fatty liver syndrome is, therefore, an example of an energy imbalance caused by excessive food/energy consumption and increased fat deposition in hepatocytes, but also in other tissues such as subcutaneous tissue, so the term "fatty cow syndrome" is often used. When a cow receives high-energy meals during the last lactation period and the dry period, fat is stored. This condition also affects the appearance of heavy calving, placental abruption, or even the appearance of metritis. When there is a loss of appetite, there is an accelerated mobilization of fat from the body's reserves and consequently an increased formation of ketone bodies. Common to all animals fed with these diets is that they have fertility problems, which is reflected in long service periods and reduced conception rate. In herds with long intervals between calving, cows are usually too fatty at the time of calving. Ketosis is one of the secondary diseases that occur in such conditions. As a measure of the detection of subclinical ketosis, it is necessary to conduct the metabolic profile testing at least twice a year in cows in high pregnancy and early lactation (10, 11, 18, 26). The goals to be pursued to prevent fatty liver syndrome are to provide adequate, not overly energy-rich meals, to prepare rumen microorganisms for the meals that will follow in lactation, giving dried cows 2-3 kg of these nutrients two weeks before calving. After calving, the concentrate should be increased by 1 kg/day, until the



peak of production is reached. It is necessary to give 2-4 kg of hay per day, to reduce the fluctuation of microorganisms, which can occur during a change in the concentration of meals as a consequence of high needs in the early period of lactation. It is desirable to maintain the interval between calving in the period from 12 to 13 months, ie. years/calf, to separate dried cows from lactating cows to reduce the possibility of "fatty liver syndrome" and to maintain high lactation (18). In cows fed feeding stuff with a high content of easily soluble proteins (17% protein with 75% solubility), there may be a need for more insemination and prolongation of the cycle between calving, which is also called "infertility syndrome". As a consequence of this nutrition, there is a high content of ammonia in the rumen, which is excreted through urine or milk. Excess ammonia ion is toxic to the embryo and prevents implantation and fetal development. Therefore, it is necessary to avoid a diet with high amounts of protein, especially easily soluble proteins, and to provide the necessary amounts of energy with a harmonized ratio between protein and energy (9, 18). The needs of microorganisms in amino acids and energy, as well as the pH range in the rumen (which is physiological from 5.5 to 6.8), should be taken into account when determining the best meal in high-milk lactating cows. Acidosis caused by the accumulation of excess lactic acid is a fermentative disorder that manifests itself in several forms, depending on the amount of imbalance. Rumen acidosis occurs when eating highly soluble carbohydrate nutrients, ie. when hay decomposes rapidly in the rumen under the influence of microorganisms. The drop in pH from 6.8 to about 5 in the rumen inhibits certain groups of microorganisms (protozoa and Gram-negative bacteria) and accelerates the development of those bacteria that require an acidic environment. Thus e.g. the presence of a large number of *Streptococcus bovis* and *Lactobacillus sp.*, microorganisms that produce lactic acid and further reduce acidity. If the animal is on this diet for a long time, acidosis can lead to damage to the mucous membrane of the rumen, which allows the bacteria to develop ruminitis. Complications such as peritonitis, liver damage caused by the transfer of infectious agents into the bloodstream, and consequently laminitis occurs. Less important symptoms can occur as a simple rejection of food, which later continues into overeating, which can lead to the atony and accumulation of excess fluid in the rumen. This, together with excess fluid in the rumen, leads to dehydration, decreased appetite, decreased milk production, decreased rumen contractions, occasionally dry dung, which can continue into diarrhea. Prevention involves a balance between microorganisms, which can be achieved by eating certain amounts of feeds given at regular intervals, avoiding sudden changes towards high-energy meals. Adequate amounts of concentrate and quality bulky food are necessary to achieve these goals (18). As a consequence of disturbances in the circulation of mineral substances (either insufficient intake or increased loss), there is the appearance of puerperal paresis, ie. hypocalcemia or milk fever. In cows with puerperal paresis, the decrease in calcium levels (hypocalcemia) manifests itself with general weakness, "fall from the legs", sometimes coma and death, if this disorder is not treated on time. In cows with puerperal paresis, disorders in the form of indigestion and reduced absorption

of nutrients are also manifested. Factors that contribute to the occurrence of hypocalcemia are: reduced ability in older cows to quickly mobilize calcium from the bones or resorption from the small intestine, and the decrease in appetite that occurs after calving further deepens this problem (18). Imbalance of mineral substances immediately after calving, in older high-productive cows, usually leads to so-called typical hypocalcemia, but often to atypical puerperal paresis in which a decrease in phosphorus levels is dominant, and/or even less often to "downer cow syndrome". Prophylaxis and recommendations in this regard also refer to increased attention in the way of rearing and feeding of dry cows. Therefore, up to three weeks before calving, highly pregnant cows should be gradually translated and accustomed to a lactating cow meal, in order for the rumen microorganisms to "prepare and develop" for the conditions of a new meal, and to add the same ratio or even more phosphorus to the pre-drying meal than calcium, in order to timely initiate the mobilization of calcium from reserves (9). It is also possible to prepare an acid or anionic meal. Such a meal reduces the occurrence of milk fever through a certain acidification that maximizes the ionization of calcium in the intestines and improves its absorption, and probably improves the ability to transport and absorb calcium from the bones. Another measure that can be taken is to add vitamin D to the meal, if the term of calving is known. Adding vitamin D in frequent doses is not recommended, especially not an injectable preparation, because vitamin D has a structure and catabolism similar to steroid hormones, which can be bad for hepatocytes, which are otherwise significantly damaged in high-productive cows fed unbalanced meals (18). Feeding stuffs with a low amount of calcium a couple of weeks before calving prevent the occurrence of puerperal paresis by prepartal activation of the skeletal system and intestinal tract. It is recommended to use a meal with less than 80 g Ca, 60 g P and about 35,000 IU of vitamin D two weeks before calving. It is very important to solve the issue of choosing nutrients poor in calcium and composing an adequate meal that meets the other needs of the animal in the last weeks of the dry period. Potatoes and potato by-products, other root-tuberous nutrients, beer trope, corn germ meal, bran and fodder flour are used as nutrition of choice. Immediately after calving, when it is necessary to offer the animals a meal rich in calcium, fodder beet noodles, hay and silage (grasses, leguminous) can be considered, as well as the addition of mineral nutrients as a source of calcium. A practical disadvantage of this nutrition is a need to gradually change the feeding stuff after calving because sudden changes in the diet can cause a disorder of microorganisms in ruminants and lead to indigestion. Practical experience shows that on some farms there are cases of puerperal paresis without severe hypocalcemia, but with severe hypomagnesaemia. Magnesium deficiency is shown to be a significant etiological factor in the development of puerperal paresis, disrupting calcium homeostasis, because it reduces the degree of calcium mobilization after calving; since the mobilization of magnesium from the depot is much slower than calcium mobilization, symptoms resembling grazing tetany often occur (9, 10, 18).

Laminitis is an aseptic inflammation of the corium of the toe. In addition to the mechanical overload of the toe, toxic causes and the occurrence on an allergic basis are also mentioned as the causes of this disease (3, 4, 24). Prolonged feeding of easily digestible concentrated food, creation of acidosis in the rumen, sudden change of food, especially nutrition of green barley, oats, freshly mowed young legumes and nutrition of moldy food, can lead to the appearance of laminitis. Laminitis is often the result of the influence of a large number of factors, such as metabolic and digestive disorders, stress, mastitis, metritis, a bed without or with very little mattress, inability to move, overweight, and poor nutrition management. A meal that leads to acidosis also leads to laminitis, which is very difficult to correct when the largest percentage of nutrients in a meal consists of carbohydrates. It is believed that vasoactive substances (histamine) that enter the bloodstream from the rumen lead to damage to the corium of the toe. A metabolic disorder occurs due to low pH content of the rumen and pathophysiological disorders occur in the chain, which eventually results in ischemia of the corium and clinically manifested laminitis (delayed step due to pain, and sometimes forced lying). In addition to histamine and bacterial endotoxins, lactic acid and other biologically active substances contribute to the development of this disease. The amount of concentrated food that a cow ingests with a meal, occasionally low pH of rumen content and the appearance of locomotor disorders, have a common etiopathogenetic background (3, 18).

### **Conclusions**

Intensive cattle breeding requires continuous monitoring of the health condition of the herd and adequate application of prophylactic, hygienic, and zootechnical measures. Improving health has a positive effect on production results and on the welfare of calves and adults. Prophylactic measures to prevent disease in highly productive dairy cows include regular monitoring of the metabolic profile.

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## **SYSTEMATIC REVIEW OF THE ANAESTHETIC PROTOCOL IN BRACHYCEPHALIC DOGS**

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### **Summary**

The popularity of brachycephalic breeds has increased considerably over the past few years in Romania and Europe and as such the number of brachycephalic patients who require surgical treatment and general anaesthesia has increased as well. These breeds are predisposed to several pathologies which can have a significant negative impact on the anaesthetic management and on the surgical outcome. The frequency of post-operative complications is significantly increased in these breeds and the clinician needs to observe early the presence of any of these complications and be prepared to act immediately. Various studies analysing these breeds, have been conducted in an attempt to identify effective strategies to reduce the anaesthetic risks and the immediate post-operative complications. The present article incorporates the available literature regarding these conditions with an important implication for the anaesthetic management of the brachycephalic patient, the anaesthetic protocol and the post-operative complications frequently encountered. The incidence of intra and post-operative complications in the brachycephalic patient can be reduced when the other frequently encountered pathologies are considered and treated accordingly. Occasionally the severity of these pathologies leads to cancellation of the surgical procedure and the clinician should identify the brachycephalic patients which require treatment or further investigations before undergoing general anaesthesia. At the same time, the clinician should be prepared to closely monitor the brachycephalic patient from the moment of admission in the practice until the discharge.

**Keywords:** brachycephalic dogs, anaesthesia, anaesthetic risks

The popularity of brachycephalic breeds has increased considerably in the last few years in Romania and Europe, the French Bulldog being the UK's most popular dog breed in 2018 (1).

Brachycephaly is a genetic feature that has been promoted by the breeders and is characterized by premature ankylosis of the basicranium which leads to chondrodysplasia of the longitudinal axis of the skull and implicitly a skull that is shorter rostro-caudally, with a shortened nose (6, 15).

The following breeds belong to this category: Pug, English and French Bulldog, Pekingese, Maltese, Shih-Tzu, Chihuahua, Dwarf Pinscher, Yorkshire terrier, Cavalier King Charles Spaniels, Boxer, Boston terrier, Cane Corso, Dogue de Bordeaux, Japanese Chin (6, 15).

Due to this conformation, the brachycephalic breeds have stenotic nasal cavities, elongated soft palate and hypoplastic trachea. These changes subsequently predispose to severe respiratory pathologies such as laryngeal and soft palate oedema, inflammation, tonsil inversion, laryngeal collapse, brachycephalic airway syndrome or BOAS (brachycephalic obstructive airway syndrome) (7, 15).

The increase in the popularity of these breeds has implicitly led to an increase in the number of brachycephalic patients requiring anaesthesia in veterinary medicine. The multitude of pathologies to which these breeds are prone, makes the anaesthesia of brachycephalic patients complex. It is important to review pre-existing conditions with an impact on anaesthesia, and to carefully plan the pre, intra and postoperative period (6, 10).

### **Materials and methods**

#### *Pre-anaesthetic conditions in brachycephalic dogs*

Pre-existing conditions that might have an impact on the anaesthesia of the brachycephalic patient are BOAS (brachycephalic obstructive airway syndrome), oesophageal and gastrointestinal disorders, corneal ulcer, blood hypercoagulability and hypertension, although the significance of blood disorders has not yet been fully understood (6, 7, 10, 28).

#### *1) BOAS (brachycephalic airway syndrome)*

BOAS is characterized by dynamic or static airway obstruction associated with clinical signs such as dyspnoea, snoring, cyanosis, exercise intolerance and syncope (6, 7, 15).

Brachycephalic breeds with BOAS are prone to thermal stress due to anatomical abnormalities that prevent an efficient heat dissipation. At the same time, the panting associated with hyperthermia can further complicate the obstruction of the respiratory tract in these patients, so maintaining a normal body temperature throughout hospitalization is essential (6).

Patients with BOAS have an increased risk of post-obstructive pulmonary oedema due to the effect of negative intrathoracic pressure on fluid distribution, as well as hypoxia and the hyperadrenergic status of these dogs. It is important to immediately identify this complication and treat it by maintaining airway permeability, supplementation with oxygen and mechanical ventilation if necessary (27).

#### *2) Gastrointestinal pathologies*

Although not always reported clinically, brachycephalic breeds may have associated gastrointestinal pathologies such as regurgitation, vomiting, gastroesophageal reflux, hiatal hernia, gastritis, esophagitis, pyloric hyperplasia (6, 9, 15, 22). Poncet et al. (21) identified a correlation between the severity of respiratory symptoms and the severity of gastrointestinal symptoms in French Bulldogs, male and large dogs (22). It is therefore important to identify and treat any pre-existing gastrointestinal pathology before an anaesthetic procedure, to reduce postoperative complications (6, 8, 21).



### 3) *Corneal ulcers*

Brachycephalic breeds are prone to corneal ulcers, a consequence of their conformation, exophthalmia, due to a reduction in corneal sensitivity (2, 19). It is important not to neglect this aspect during general anaesthesia and frequent lubrication of the eyes is essential to avoid postoperative corneal ulcers (6).

### 4) *Blood hypercoagulability and hypertension*

So far the clinical significance of these alterations has not been fully understood, Hoarea and Mellema (11) and Crane et al. (4) reported an increase in the hypercoagulability severity directly proportional with the increase in BOAS severity but currently there is not enough scientific evidence to justify the use of anticoagulant medications (5, 11, 12).

In human medicine, obstructive sleep apnoea syndrome (OSAS) is associated with hyperglycaemia, insulin resistance, dyslipidaemia, and a high mortality rate secondary to cardiovascular and thromboembolic pathologies (3, 13, 23, 26). Due to the fact that BOAS has similar symptoms with OSAS, it is assumed that the metabolic and inflammatory status of dogs with this syndrome could have alterations similar to those identified in human patients (6).

In 2019, the consequences of upper airway obstruction on the metabolic profile of these dogs were studied: Gianella et al. (8) evaluated 30 brachycephalic dogs without being able to identify a significant correlation between clinical signs, anatomical alterations, C-reactive protein and the metabolic profile of these dogs (9). However, further studies evaluating these aspects are needed in order to validate these conclusions as the above study presented several limitations.

### *Anaesthesia in brachycephalic dogs*

Prior to planning a surgical procedure, relevant information about the patient's health and a thorough clinical examination are essential for any patient undergoing general anaesthesia. In the case of brachycephalic patients, the clinician must assess the severity of clinical signs such as cyanosis, the presence of syncope, snoring, stertor/ stridor, heat/ exercise intolerance, the presence and frequency of vomiting or regurgitation (6). When present, these symptoms may indicate a secondary pathology and the need for further investigation before planning a surgical procedure (4, 6, 21, 28).

Often brachycephalic dog owners will report regurgitation or chronic vomiting, signs that indicate gastrointestinal or oesophageal pathologies. These pathologies may predispose the patient to intra- or post-operative regurgitation, aspiration pneumonia, airway obstruction and intubation difficulties (6).

It has been observed that the risk of regurgitation in the postoperative period is reduced when gastrointestinal pathologies are identified and treated appropriately before surgery (4, 8, 21). In an attempt to reduce regurgitation in anesthetized patients, the administration of 1 mg/ kg Omeprazole is recommended at least 4 hours before the induction of general anaesthesia (6, 20).

In 2019 Tarricone et al. (25) developed a questionnaire for patients scheduled for BOAS correction surgery. This questionnaire called Modified BRisk score (Brachycephalic Risk Score) takes into consideration the age, previous surgeries, the patient's physical condition, the severity of the respiratory symptoms, the rectal temperature and the planned procedure. To each of these elements will be assigned a score that summed together will classify the patient from the preoperative period as a patient with low, moderate or high risk to develop major complications associated even with death. The implementation of this questionnaire in the pre-anaesthetic period allows the clinician to have a more realistic discussion with the brachycephalic owner regarding any possible complications that may occur, but also allows him to take the necessary in order to control and reduce as much as possible the anticipated risk (28).

Another method available for the clinician to identify the patient at risk to develop postoperative complications is to assess the patient during and after 3 minutes of exercise (24). Failure to tolerate the test will classify the patient as high-risk and the surgical procedure should be post-poned until the animal has an ideal weight and an improved physical condition (6).

#### *Premedication and induction*

Although there is no perfect premedication, the selection of the premedication protocol must be based on the patient's temperament, the presence and severity of other pathologies, the type of surgical procedure, the different categories of drugs available and the clinician's familiarity with them (6).

In Table 1 are described the recommended medications and doses for premedication, induction of general anaesthesia, and postoperative sedation (6).

Acepromazine (ACP) is often used in combination with an opioid. Because it causes relaxation of the pharyngeal musculature, it is important to consider the possibility of worsening respiratory symptoms following the use of acepromazine and the patient should be closely monitored (6). It has been observed that some boxers may develop deep sedation, bradycardia and hypotension following the administration of ACP, and therefore the usage of a lower dose in this breed is advised (less than 0.01 mg/ kg) (17).

Medetomidine and Dexmedetomidine are  $\alpha$ -2 agonists that will cause deep sedation hence reducing the tone of the pharyngeal muscles. In the brachycephalic breeds these effects can worsen the symptoms of airway obstruction and these patients are at risk of developing complications from the preoperative period (6). It is therefore recommended to permanently monitor the brachycephalic patient immediately following the administration of the premedication drugs (6, 16, 17).

Preoxygenation achieved by mean of an oxygen mask for a period of 3 minutes before the induction of general anaesthesia might prevent early oxygen desaturation, especially in brachycephalic breeds where desaturation can occur faster than in other breeds (6). At the same time, not all canine patients will tolerate the presence of a mask and sometimes the stress associated with preoxygenation can worsen airway obstruction and increase further the patient's oxygen

requirements (6). It is therefore essential to assess the patient and his temperament to decide whether preoxygenation will be beneficial or contraindicated.

Table 1

**Common drugs for premedication, induction of general anaesthesia and sedation**

Acepromazine	Premedication: 5-20 µg/ kg Post- operative sedation 5-10 µ/ kg q 3-6 h	IM or IV IV
Medetomidine	Premedication: 1-10 µg/ kg Post- operative sedation: 1-3 µg/ kg IV q 1 h	IM or IV IV
Dexmedetomidine	Premedication: 1-5 µg/ kg Post-operative sedation 0.5-1 µg/ kg IV q 1 h	IV or IM IV
Methadone	0.1- 0.3 mg/ kg	IV or IM
Buprenorphine	0.02 mg/ kg	IV or IM
Butorphanol	- 0.3 mg/ kg	IV or IM
Propofol	1- 4 mg/ kg (given to effect)	IV
Alfaxalone	2 mg/ kg (given slow to effect)	IV

Because hypothermia causes tremors and thus increases oxygen demand by up to 400% (16) and hyperthermia may worsen respiratory stress, it is essential to try to maintain a normal body temperature throughout the hospitalization of the brachycephalic patient (6).

Endotracheal intubation of the brachycephalic patient may be difficult. It is recommended to prepare a variety of endotracheal tubes, laryngoscope, light source, suction tube in case the patient regurgitates, as well as the instruments necessary to perform emergency tracheostomy (6).

Due to tracheal hypoplasia and intubation with a small endotracheal tube compared to the patient's size, respiratory effort and airway resistance will increase so that the brachycephalic breeds are prone to hypoventilation during anaesthesia (6). When hypoventilation is severe, assisted mechanical pulmonary ventilation (PPV) is required. Capnographic monitoring allows the detection of hypoventilation, and the intervention of the clinician is recommended when ETCO<sub>2</sub> exceeds 55 mmHg in stable patients (6).

Intraoperative and postoperative analgesia are essential in any patient, but even more important in brachycephalic breeds, especially in patients who have underwent upper airway interventions.

The clinician must anticipate the level of postoperative pain and decide whether a nonsteroidal anti-inflammatory medication is sufficient or the addition of opioids, paracetamol, local anaesthesia, ketamine are needed to control pain and

avoid postoperative complications (6). Numerous questionnaires are available for the evaluation of postoperative pain and the administration of an analgesic must be justified.

*Post-operative period*

Over the last few years, the number of studies describing the most common post-operative complications encountered in brachycephalic dogs has increased.

Brachycephalic dogs and especially the patients undergoing airways surgery are at higher risks of developing post-operative complications than other breeds. This might be due to various factors such as post-operative oedema, active bleeding or blood clots obstructing the airways, reflux, or regurgitation (6). A study including 248 dogs treated surgically for brachycephalic airway syndrome (BAS) identified that the age, concurrent airway pathology and emergency presentation are predictive for post-operative respiratory complications (14).

The most common encountered complications are emergency reintubation, temporary or permanent tracheostomy, sedation in the post-operative period and regurgitation (6).

The clinician should be prepared for emergency reintubation in the immediate post-operative period and the owner should be made aware of this risk before the surgical procedure. Laryngoscope, endotracheal tube identical in size as the tube used during anaesthesia or even smaller, drugs to re-anaesthetize and a tracheostomy kit should be available, especially for patients that undergo BOAS surgery (6).

Temporary or permanent tracheostomy requires intensive nursing, significant financial resources and is associated with high complication rate (6, 25). A study identified that obstruction and dislodgement of the tracheostomy tube as the most common complications associated to the procedure, while pneumomediastinum, pneumothorax and pyothorax have been described elsewhere (25, 18). Despite the complications described, the mortality rate associated with the placement of a temporary tracheostomy tube has been found to be low in one study (25).

Recovery period is important for these breeds and all efforts should be made to help these patients maintain airway patency until they are alert enough to do this without support. The dog should be positioned in sternal recumbency, with neck extended, head elevated, and the tongue drawn rostrally out of the mouth (6).

Sedation might be required in the post-operative period in some patients that are very stressed and barking or panting excessively (6).

If severe laryngeal oedema occurs, nebulization with 1 mg of epinephrine diluted in 2 to 3 ml of sterile saline, administered for 10 minutes every 6 hours during 24 hours may be used without causing adverse effects (6). Care should be taken during nebulization as some patients will not tolerate the physical restraint, face masks or noise and this procedure might be too stressful (6)

The incidence of postoperative regurgitation and subsequent aspiration pneumonia was found to be higher in dogs that had history of regurgitation in a study which included 84 dogs (4).

### **Conclusions**

Given the anatomic abnormalities and concurrent pathologies, anaesthesia might pose greater risk to brachycephalic dogs versus other dog breeds. These risks are likely to be higher in the post-operative period rather than during anaesthesia.

A study identified a higher rate of complications in brachycephalic dogs during the intra-anaesthetic and post-anaesthetic periods with increasing duration of anaesthesia, invasive procedures compared to routine procedures and in higher ASA status (1). Brachycephalic dogs were 1.57 times as likely to have intra-anaesthetic complications and 4.33 times as likely to have post-anaesthetic complications as were non-brachycephalic dogs in the same study (17).

A thorough history, clinical examination and methods such as Modified BRisk score, 3—minute trot test may help clinicians identify high-risks patients before anaesthesia and decide if postponing the procedure will be beneficial for the patient while addressing concurrent diseases.

The clinician should always aim at reducing hospitalization time and the stress associated with hospitalization in the brachycephalic patient.

Administration of gastro protectant, anti-emetic or prokinetic drugs in the pre-operative period should be considered in patients with evidence of gastrointestinal disease or history of regurgitation or vomiting.

The patient might benefit from preoxygenation while a multimodal analgesic plan should always be provided together with conditions to maintain normothermia and reduce hospitalization stress.

Anesthetized brachycephalic dogs should remain intubated until they are awake. They should be positioned carefully to minimize the risk of regurgitation during anaesthesia and to maintain airway patency during recovery.

Close monitoring during recovery is essential to reduce the post-operative complications and in some cases sedation, additional analgesia or anti-inflammatory drugs are needed to enhance recovery.

The aetiology of peri anaesthetic complications in brachycephalic breeds is likely multifactorial, and further studies are needed to identify more anaesthetic protocols that can reduce the risks of complications in these patients.

Efforts should be made to improve the management of associated diseases and to provide closer monitoring of the brachycephalic patient in the pre- and post-operative period.

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## CT APPEARANCE AND SURGICAL TREATMENT OF A HANSEN TYPE 1 (HT1) INTERVERTEBRAL DISC HERNIATION IN THE THORACO-LUMBAR REGION IN A PEKINEZ: CASE REPORT

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### Summary

A toy breed dog was presented non ambulatory at our hospital following a fall from a height, that occurred approximately 36 hours before the presentation. The neurologic examination revealed paraplegia and the presence of a myelopathy at the level of T3-L3 was suspected. Spinal radiographs revealed a narrowed disc space corresponding to L3-L4 and calcified disc material within the canal at this level. Computer tomography confirmed the presence of a Hansen type 1 (HT1) intervertebral disc herniation in the thoraco-lumbar region at the level of L3-L4. Decompressive surgery was considered appropriate given the dog's presentation and a left sided hemilaminectomy was performed. The dog presented ambulatory 14 days after the surgical treatment. The present report describes the CT appearance and the surgical outcome of a Hansen type 1 (HT1) intervertebral disc herniation in the thoraco-lumbar region in a toy breed dog following hemilaminectomy.

**Keywords:** Thoraco-Lumbar Myelopathy, Acute type 1-disc hernia, Hansen type 1, HT1, Hemilaminectomy in dog

Intervertebral disc herniation is one of the most common causes of myelopathy in dogs, the acute herniation being considered the most common spinal emergency in the canine species (3, 9).

The intervertebral disc has a central component, the nucleus pulposus which is surrounded by a peripheral component called the annulus fibrosus. Degenerative changes of these two components of the disc material can cause its herniation in the spinal canal that can result in compression and / or concussion of the spinal cord. An acute herniation of a non- degenerative disc material is also possible and two types of disc herniation have been described:

- Acute non- compressive nucleus pulposus extrusion (ANNPE)
- Hydrated nucleus pulposus extrusion (HNPE, AHNPE) (3, 7).

Hansen and Olsson classified hernias of the intervertebral disc into three categories:

1. Hansen type 1 (HT1) or acute extrusion of the intervertebral disc,
2. Hansen type 2 (HT2) or chronic intervertebral disc protrusion,
3. Hansen type 3 (HT3) or Acute Non-Compressive Nucleus Pulposus Extrusion (ANNPE) (6, 13).



This article illustrates the diagnostic approach and the surgical management of a type 1 acute disc disease in a dog that presented to our hospital for acute spinal pain.

### Materials and methods

#### *History and clinical examination:*

A 7-year-old female, entire Pekingese showed up at our hospital being non-ambulatory. The owner reported that these signs started gradually about 36 hours ago after a fall from height. The patient is up to date with the vaccination, dewormed internally and externally according to the age and so far, has been clinically healthy. At the time of presentation, the patient had the vital signs within the normal limits T: 38.1°C, HR: 84 bpm, RR: 36rpm.

During the general and neurological examination, it was observed that the patient had bilateral proprioceptive and motor deficits in the hind limbs being paraplegic. Deep pain sensation was still present in the pelvic limbs. The identified clinical signs led to the suspicion that the patient suffers of a myelopathy in the thoracolumbar region, more precisely in the T3-L3 region:

Following the clinical examination, we requested a radiological examination of the spine performed under moderate sedation using 0.02 mg/kg medetomidine and 0.2 mg/kg butorphanol both given IV. In Table 1 are available some of the most preferred para-clinical methods used in neuro-diagnostic.

Table 1

#### **List of recommended diagnostic tests after Sathya S. BVSc, MVSc, MvetSc, Clinical Advisor 3, 2017: 565-567**

Radiographic examination
Myelography
Computer tomography, with or without contrast administered intra-venously
Cytologic exam of the CSF
Computer tomography combined with myelography

The radiographic examination confirmed the presence of opacity at the level of the intervertebral hole between the lumbar vertebrae 3 and 4 (L3-L4) as well as narrowing of this intervertebral space. These changes suggest the presence of a type 1 disc herniation (12). At the same time, the radiographic examination allowed the exclusion of some pathologies of the spine such as vertebral fractures, dislocations or intervertebral subluxations. The list of differential diagnosis is available in Table 2.

To confirm the diagnosis and to accurately determine the position of the extruded nucleus relative to the spinal cord, the patient underwent a simple CT scan of the thoracolumbar segments of the spine on the same day.

Table 2

**List of differential diagnoses for myelopathies in the thoracolumbar region  
by Sathya S. BVSc, MVSc, MvetSc, Clinical Advisor 3, 2017: 565-567**

Vertebral fractures	Vertebral luxations/subluxations
Neoplasia	Degenerative Myelopathy
Intervertebral disc herniation	Syringomyelia
Myelitis	Discospondylitis

*Diagnosis:*

CT examination revealed a compressed mineralized mass relative to the spinal cord, located in the left ventrolateral portion of the spinal canal near the intervertebral hole at the L3-L4 level.

The CT report confirmed the presence of a Hansen type 1 inter-vertebral disc herniation (HT1), the disc material occupying over 50% of the vertebral canal (Fig. 1). This type of hernia is characterized by the fact that the disc material extrudes in the ventral direction, invading the left dorso-lateral floor of the spinal canal and also causing a severe spinal cord compression (Fig. 2).



Fig. 1. CT image in the transverse plane: The extruded nucleus exceeds half the diameter of the vertebral canal

*Treatment:*

Considering the severity of the neurological signs and the identification of the acute compression of the spinal cord, the next day, the patient underwent hemilaminectomy surgery on the left side with the aim to perform the decompression of the spinal cord and the removal of disc material from the L3-L4 level.

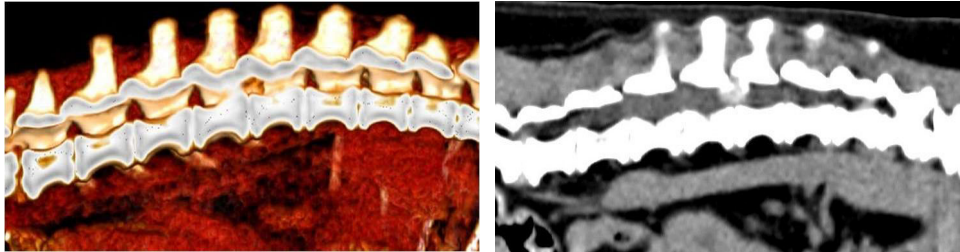


Fig. 2. CT image in sagittal plane in which we can observe the presence of the disc material in the vertebral canal at the level of the intervertebral hole L3-L4

Medetomidine 0.02 mg/kg IV, Ketamine 5 mg/kg IV and Buprenofine 0.2 mg/kg IV was used as premedication, after which the patient was induced using Propofol 2-3 mg/kg IV and intubated using an endotracheal tube of ID 4.5. Anaesthesia was maintained with Isoflurane in Oxygen using a semi-open respiratory circuit. The analgesia and anti-inflammatory protocol included intraoperative administration of 10 mg/kg Paracetamol IV as well as the administration of 0.2 mg/kg Meloxicam IV. Perioperative prophylactic antibiotherapy was performed with a third-generation cephalosporin antibiotic (Ceftriaxone): 30 mg/kg IV administered 30 minutes prior the first incision and repeated once after 90 minutes.

Patient positioning was performed in oblique sterno-abdominal recumbency, tilted slightly to the right to facilitate surgical access and visualization of the spinal canal on the left side.

An approximately 8 cm longitudinal incision was made, para-lateral to the spinous processes to avoid excessive tension on the final suture. Using a combination of blunt and sharp dissection, the access to the lateral ventro wall of the spine was continued, releasing the structures adjacent to the caudal and cranial joint processes of the lumbar vertebrae 3, respectively 4.

Tissue dissection and debridement continued to the ventral level of the accessory process, which is an approximate landmark of the ventral floor of the spinal canal. The access was facilitated by using classic distractors but also Gelpi self-retaining retractors. Different sizes Rongeurs were used to remove the articular processes.

The bony tissue of the dorso-lateral vertebral wall was partially removed to the level of the internal cortex using a high speed drill (40000rpm) to create an oval window with a length of approximately 10 mm and then with the help of spatulas and dental curettes of different sizes the inner cortex and periosteal tissue were removed.

Soon after, we can see how an enormous amount of disc material was exposed in the hemilaminectomy window due to the decompression of the spinal canal performed at that level (Fig. 3).

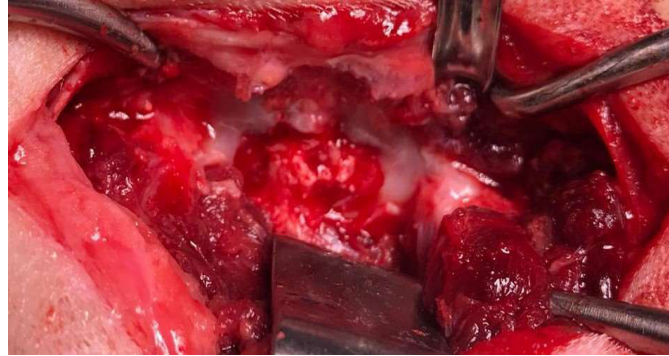


Fig. 3. Presence of the exteriorized nucleus pulposus in the hemilaminectomy window

Using spatulas and dental currettes but also by using the surgical suction, we removed the disc material visible on the outside and then carefully checked the epidural space both dorsally and ventrally to make sure that the compressive source was completely removed from the epidural space (Fig. 4).

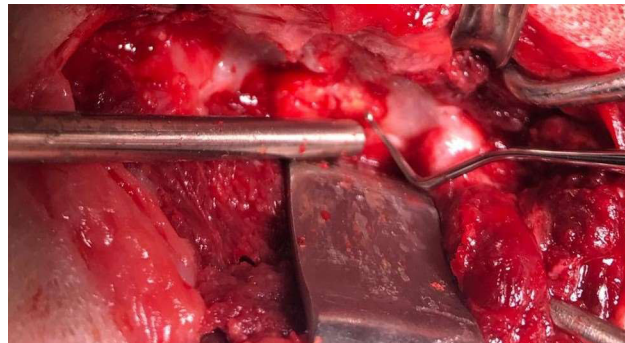


Fig. 4. Removal of the disc material using spatula and suction

Visual examination of the spinal cord confirmed the continuity of the dura at that level and a normal pearly white colour without visible changes caused by compression.

The wound was flushed with warm saline after which the soft tissues were appositioned and sutured together, to avoid leaving any dead space and to reduce the chance of seroma formation that could later be a possible cause of a compression in the spinal cord. The suture material used for both the thoraco-lumbar fascia and the skin was of 2m (3/0 USP) monofilament.

The patient was hospitalized at the 24 hours service in the university hospital in order to benefit from continuous monitoring in the immediate postoperative period but also during the night.

The postoperative analgesic and anti-inflammatory protocol included:

- Paracetamol 10 mg/kg/8h PO
- Gabapentin 10 mg/kg/8h PO
- Meloxicam 0.1 mg/kg/24h PO
- Tramadol 2 mg/kg/12h PO

### **Results and discussions**

Less than 24 hours after the surgery, the patient was ambulatory, with a normal appetite, normal urination and defecation, being very lively (Fig. 5). Neurological examination revealed almost normal proprioception and functionality just 12 hours after surgery (Fig. 6).

The patient was discharged 48 hours after surgery, the analgesic treatment being extended for a period of 7 days.

Strict recommendations were made regarding physical activity, namely:

Week 1: Controlled walks on a leash for 10 minutes 3 times a day

Week 2: 15 minutes 3 times a day.

Postoperative rechecks were performed 7 and 14 days after the surgical intervention when it was noted that the patient recovered functionally complete (Fig. 7).



Fig. 5. The Patient 24 hours after surgery



Fig. 6. Evaluation of the patient's proprioception at 12 hours after the intervention

Intervertebral disc herniation is one of the most common causes of myelopathy in dogs (9) and it can be treated both medically and surgically, the presence and severity of neurological signs being the discriminating factor in choosing therapy (2).

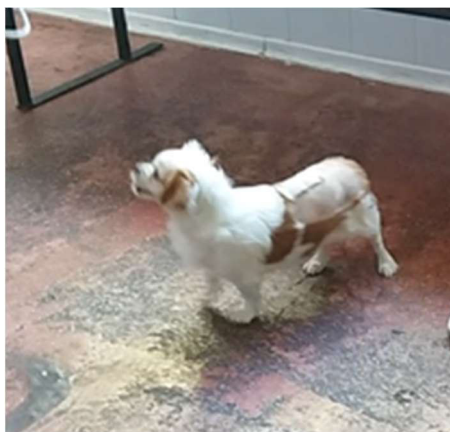


Fig. 7. 14 days after the surgery the patient was walking perfectly normal

The intervertebral disc has a central component called the nucleus pulposus which is surrounded by a peripheral component called the fibrous annulus. Degeneration of the peripheral component of the disc material can cause its

herniation in the spinal canal that can result in compression and / or concussion in the spinal cord (7).

Hansen and Olsson classified hernias of the intervertebral disc into three categories, namely:

Hansen type 1 (HT1) or acute extrusion of the intervertebral disc

Hansen type 2 (HT2) or chronic intervertebral disc protrusion

Hansen type 3 (HT3) or Acute Non-Compressive Nucleus Pulposus Extrusion (ANNPE) (6, 13).

In a study that compared the medical with the surgical treatment, it was stated that in dogs that are non-ambulatory with at least one limb, surgery provided superior results (89% vs. 45%) in terms of resumption of functionality and time necessary for recovery (days vs. weeks) (9).

Another comparative study published in 2017 showed that both medical management (18 cases) and surgical management (16 cases) provided positive results in the treatment of cervical intervertebral disc herniations, thus emphasizing that both therapies should be considered for this pathology (2).

In paraplegic dogs the presence of deep pain sensation is the most important factor in terms of prognosis and evolution during the recovery period (10).

In a study that evaluated the long-term outcome of 87 dogs with thoracolumbar injuries, 92% of dogs with present pain sensation regained their ability to walk after the treatment, while only 69% of dogs in which the painful sensation was absent or altered, resumed their functional abilities after treatment (10).

Assessing the presence of deep pain sensitivity was considered a fairly subjective quantitative clinical indicator that may differ from patient to patient but also from one examiner to another. Another aspect considered important in the patient's prognosis was the evaluation of the time interval from the detection of the absence of deep pain sensation. This has been considered impossible to assess with certainty by many clinicians (5).

Some studies suggested that the chances of functional recovery in patients suffering from acute spinal cord compression in the thoraco-lumbar region are better the sooner the decompression surgery is performed (4).

The mean functional recovery in paraplegic patients with present deep pain sensation was 80 to 90% and approximately 50% (0-70%) in paraplegic patients with absent deep pain sensation (1, 11).

Hemilaminectomy is the most frequently used surgical technique for decompressing the spinal cord in dogs and cats, being also the most indicated technique in spinal cord compressions due to intervertebral disc herniation in dogs (14).

Starting from the classic hemilaminectomy, other alternative procedures were developed, such as pediculectomy and mini-hemilaminectomy.

• Pediculectomy is performed on the vertebral pedicle and is characterized by the fact that it preserves certain anatomical structures encountered in the classic

hemilaminectomy procedure but also by the fact that it is a much faster technique due to reduced hemorrhage.

- Mini-hemilaminectomy is performed in the same place as the classic hemilaminectomy but it preserves the joint processes, the window being much smaller in diameter

The disadvantage of these two techniques is the limited access and exposure of the spinal cord. In the absence of sufficiently extensive access, the risk that the disc material present in the spinal canal will not be completely removed is increased (8).

At the same time, the presence of residual disc material was reported even after the hemilaminectomy procedure, but the risk appears to be lower compared to the other two methods (8).

### **Conclusions**

Intervertebral disc herniation is the most common cause of myelopathy in dogs, causing neurological dysfunction whose severity depends on the type of hernia and the level of compression caused in the spinal cord.

The classification of disc herniations according to the model proposed by Hansen and Olsson is fundamental in choosing a correct treatment plan.

For an accurate diagnosis and classification of this pathology, it is important to combine the clinical examination and the use of diagnostic imaging methods presented in this article, both having an essential importance in identifying the disc pathologies.

The perception of the deep pain sensation by the patients with myelopathies caused by spinal cord compressions represents a fundamental element that dictates the prognosis and the neurological recovery. In other words, when discussing the prognosis with the patient's owner or another clinician, it is important to remember that paraplegic patients with present deep pain sensation are considered candidates for spinal cord decompression surgery, having a better neurological recovery rate when compared with paraplegic patients whose deep pain sensation is abolished or absent.

Even if it is impossible to calculate exactly for how long the deep pain sensation has been affected, it is advisable to understand that the longer we delay the decompressive surgery, the lower the chances of recovery are.

Studies have shown that both medical and surgical treatment can have good clinical results in terms of recovery time and return to function.

There are many techniques available for the surgical treatment of myelopathies caused by herniated disc in the thoraco-lumbar region, hemilaminectomy being the one that has given the best results to date. That being said, the hemilaminectomy is currently the most appropriate surgical technique in the surgical treatment of compressive myelopathies in the thoraco-lumbar region.



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## RESEARCH ON THE EFFECT OF SUPPLEMENTING FEED WITH SEA BOWL POWDER (*HIPPOPHAE RHAMNOIDES*) ON PRODUCTIVE PERFORMANCE OF BROILER CHICKENS

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### Summary

The aim of this study is to highlight the effect of introducing natural products into animal feed, rich in biologically active nutrients. Five-day-old broiler chickens from the Ross 308 hybrid were divided into two groups of 25 individuals each, in compartments with the ground breeding system. The control group (the one without the addition of sea buckthorn) and the experimental group (with the addition of sea buckthorn). Significant differences ( $p < 0.05$ ) are recorded in terms of the initial average weights of the two batches of chickens. The average body mass at the end of the experimental period of 1684.4 g for the group with sea buckthorn supplement, relatively close to that of the control group (1722.8g).

**Keywords:** Broiler chickens, sea buckthorn

Reducing the use of synthetic chemicals and increasing the productive performance of broiler chickens is an area of permanent interest (1, 2, 3, 4, 7, 9).

Sea buckthorn is one of the easily accessible and very popular natural remedies worldwide. Native to China, Russia and Mongolia, sea buckthorn is widespread in Romania.

Due to the nutrients it contains, sea buckthorn fights avitaminosis and is an excellent detoxifier (6, 8).

Sea buckthorn is a vascular and nervous trophic and also has an extremely important and active role as a coronary protector. Sea buckthorn juice contains an oil that, administered to the heart, reduces or eliminates tachycardia and anxiety (5, 11, 16).

Unlike other plants, not only fruits but also bark and root are used in sea buckthorn bush. Wet seeds and shells are dried, sprayed and used as such, as a vitamin.

### Materials and methods

The experiment took place in the Clinics of the Faculty of Veterinary Medicine in Timisoara.

Five-day-old broiler chickens from the Ross 308 hybrid were purchased. This hybrid is most frequently grown on farms in Europe because it optimally combines

extraordinary performance with adaptation to bioclimatic and growing conditions in Romania, being the most recommended for the household growth system.

The chicks were divided into two groups of 25 individuals each, in compartments with the ground breeding system. The control group (the one without the addition of sea buckthorn) and the experimental group (with the addition of sea buckthorn) were thus constituted. In the growth compartments they were given water at will. The feeding system was ad libitum (5, 11, 12, 13, 14).

The chicks were weighed weekly and after each weighing the space for the chicks was changed by rotation to prevent external influences (proximity to the window, door, heat source, etc.). The temperature in the shelter was constantly monitored.

The fodder administered to the chicks was weighed daily. Along with the weighing of the chicks, the possible unconsumed remains were taken into account.

Although the animals received the same forage assortment corresponding to the category and age of the chicks, the differences consisted in the addition of 1% of the dried sea buckthorn powder. After complete homogenization, the feed was placed in bags which were marked with the color corresponding to the batch.

### **Results and discussions**

Assessment of the productive performance of broiler chickens is presented now.

During the experimental period, five weighings were performed at one week intervals. The chicks were weighed individually for each lot (Fig. 1, Fig. 2).



Fig. 1. Weighing the chicks from the control group



Fig. 2. Weighing the chicks from the sea buckthorn group

The results of the individual weighings of the chicks in each batch together with the average weight are given in Tables 1 and 2.

Table 1

**The values of the individual weights, the average weight and the statistical calculation of the chicks from the control group**

No.	Weighing 1 (g)	Weighing 2 (g)	Weighing 3 (g)	Weighing 4 (g)	Weighing 5 (g)
1	270	590	1220	800	2370
2	170	400	780	1060	1450
3	300	590	1130	1740	2120
4	140	240	1180	1280	2310
5	190	510	640	1380	1350
6	280	370	1310	1930	1830
7	290	580	900	2040	1490
8	150	400	1060	1440	1430
9	170	370	1370	1050	1130
10	240	260	740	1220	1540
11	250	390	950	1200	2060
12	180	480	1270	860	1950
13	170	400	870	970	1550
14	230	500	970	1350	1830
15	240	380	830	1070	1800
16	130	650	770	1430	2640
17	250	530	1240	1850	1150
18	170	280	1060	1760	1140
19	130	250	530	1750	1630
20	140	310	450	1200	1650
21	140	560	580	1500	2280
22	160	420	740	1400	1250
23	240	540	870	1620	1600
Mean	201.3043478	434.7826087	933.0434783	1386.956522	1719.565217
Standard Error	10.19803903	10.19803903	10.19803903	10.19803903	10.19803903
Standard Deviation	50.99019514	50.99019514	50.99019514	50.99019514	50.99019514
Minimum	130	240	450	800	1130

Maximum	300	650	1370	2040	2640
Confidence Level (95.0%)	21.04771808	22.04771808	23.04771808	24.04771808	25.04771808
CV%	25%	12%	5%	4%	3%

Table 2

**The values of the individual weighings, the average weight and the statistical calculation of the chicks from the sea buckthorn group**

No.	Weighing 1 (g)	Weighing 2 (g)	Weighing 3 (g)	Weighing 4 (g)	Weighing 5 (g)
1	170	600	660	2060	1550
2	260	500	930	1500	1370
3	280	270	940	1340	1860
4	140	490	780	1390	1530
5	150	400	450	1950	1930
6	180	420	940	1130	1840
7	140	420	780	1210	1800
8	120	410	870	830	1630
9	150	170	920	1510	1220
10	170	280	1270	1190	2570
11	140	480	1005	900	1410
12	110	390	850	1460	1870
13	230	280	970	1450	1930
14	200	690	710	1500	1730
15	120	340	670	1480	1060
16	190	410	950	1130	1860
17	160	330	960	1350	1360
18	160	380	830	1330	1470
19	160	340	900	1530	800
20	140	330	860	1060	1590
21	140	360	760	1460	1770
22	220	270	1210	1070	1610
23	110	340	1400	1390	2600

24	290	340	660	1400	1300
25	120	570	1004	1320	1450
Mean	170	392.4	891.16	1357.6	1684.4
Standard Error	10.19803903	10.19803903	10.19803903	10.19803903	10.1980390
Standard Deviation	50.99019514	50.99019514	50.99019514	50.99019514	50.99019514
Minimum	110	170	450	830	1060
Maximum	290	690	1400	2060	2600
Confidence Level (95.0%)	21.04771808	22.04771808	23.04771808	24.04771808	25.04771808
CV%	30%	13%	6%	4%	3%

Analyzing the data presented in Tables 1 and 2 it is found that at the first weighing the average weight of the control group is higher than that recorded in the sea buckthorn group, so that the difference between the lots is 15.42%.

At the end of the experimental period, at the last weighing, this difference was 38.4g as an average value. This value represents a percentage of 2.23%, which demonstrates that during the experimental period the chickens from the group fed sea buckthorn supplement had a higher growth rate; the initial difference was reduced to 2.23%. Improving the growth rate can be attributed to the beneficial effect that supplementing the ration with sea buckthorn powder has on the chicks. Sea buckthorn powder comes from hand-picked grains at the optimum time. The transport was done quickly and the drying in artificial conditions, strictly controlled. As the temperature was not too high and the oil was not extracted the bioactive substances from sea buckthorn were kept.

The average weights of the chicks of the two groups were compared after each week of the experiment. The results are presented in the form of Box Plot diagrams (Fig. 3).

The statistical calculation shows that significant differences ( $p < 0.05$ ) are recorded in terms of the initial average weights of the two batches of chickens.

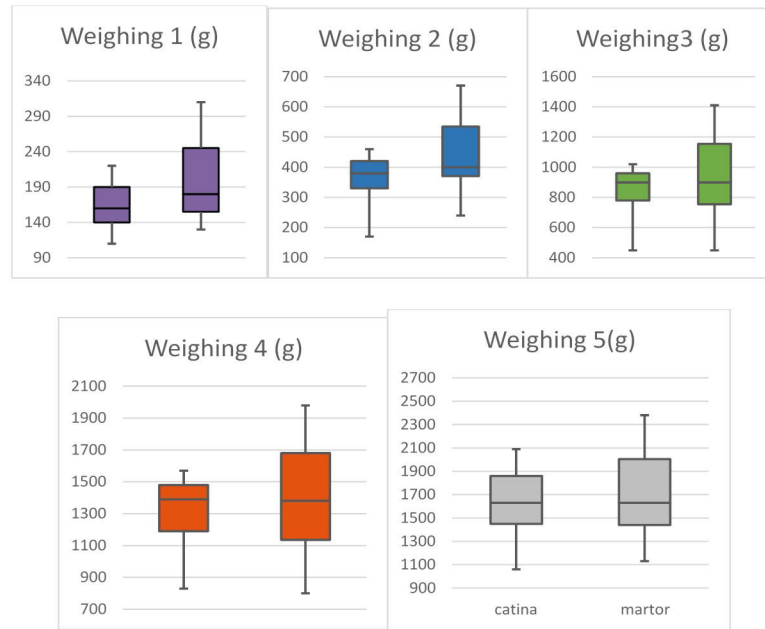


Fig. 3. Box Plot diagrams regarding the comparison of the average weight of the two lots (Sea buckthorn on the left side and the control on the right side of each diagram)

### Conclusions

The acceleration of the growth rate can be attributed to the addition of sea buckthorn which, although introduced in the ration in a very small proportion, had beneficial effects on the chicks in the group fed with supplement so that they recovered from the difference. The average body mass at the end of the experimental period of 1684.4g for the group with sea buckthorn supplement, relatively close to that of the control group (1722.8g).

The total amount of feed consumed by the control group is approximately 3 kg higher than the group of sea buckthorn powder, at the same time, the increase in body mass for the entire group of 25 chickens registers relatively close values between the two groups: 38.08 in the control group compared to 37.86.

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16. \*\*\*[https://ro.wikipedia.org/wiki/Cătină\\_albă#/media/Fișier:Hippophae\\_rhamnoides\\_Sturm41.jpg](https://ro.wikipedia.org/wiki/Cătină_albă#/media/Fișier:Hippophae_rhamnoides_Sturm41.jpg)



## CONSIDERATIONS ON THE FIBER AND PROTEIN CONTENT INTERINFLUENCE IN OATS USED AS FEEDSTUFF IN ANIMAL DIETS

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### Summary

Oats is a valuable fodder plant but with limitations in its use in animal feeding due to the high content of crude fiber (cellulose). In this regard, this study aimed to assess the interinfluence of fiber and protein content in oats. 65 samples of autumn oats collected from Timis County, were analyzed. The samples were analyzed by standardized methods, adapted by the manufacturer of laboratory equipment. For the crude fibers an average of 12.5103% was obtained, with variations between 6.97 and 22.25%, and for the crude protein the average value was 13.6850%, with a minimum of 9.14% and a maximum of 15.62%. The statistical evaluation of the data revealed the direct influence of the fiber content on the protein component, in the sense of decreasing the protein value in the samples that had high crude fiber content. The results of this study underline the importance of the raw chemical analysis of raw feed materials in order to assess their nutritional value and to establish the percentage of inclusion in animal diets.

**Keywords:** oats, chemical composition, crude fiber, crude protein

Oats (*Avena sativa* L.) is a major cereal used worldwide, after corn, rice, wheat, barley and sorghum (9). Dual use, as food for human consumption and as feed in animal nutrition, makes oats a valuable cereal in some areas (10). In Romania, it is mostly used as a fodder plant, especially for horses and breeding animals (17). Due to the presence of hulls, oat grains contain more fiber, respectively cellulose, than other cereals, and their nutritional value, for animal nutrition, tends to be lower (13). Dehulled oats have a better energy density and nutritional value, but the dehulling process is not a common practice in animal feeding.

Oats is used in animal feeding especially as an energy source. However, the chemical composition can vary greatly, depending on the variety, climate, soil and practice of fertilizer use (16). The nutritional value of oats is largely determined by the percentage of hulls, which varies from 20 to 30% of grain weight (8). Oats are richer in protein than corn (8-15% DM), but less so than wheat and barley (3).

Unlike other cereals, oats have a high fiber content due to the presence of hulls. The presence of hulls and high fiber content make the oats inferior to other

cereals used for feeding high-yielding animals, both due to their volume and lower digestibility (6). Oats remain a valuable fodder, for example for young ruminants, poultry, sows and horses. In some cases, processing can help improve their nutritional value (16).

The aim of this study was to determine the fiber and protein content of spring oats harvested from western Romania and to analyze the influence of fiber on the protein percentage of whole, unhulled grains. The interinfluence of the chemical parameters from oat grains is important in establishing the nutritional value and the degree of inclusion of this cereal in the feed recipes intended, especially for monogastric animals.

### **Materials and methods**

To carry out this study, 65 samples of autumn oats collected from Timis County, were analyzed. The samples were processed within the Laboratory of physico-chemical feed analysis, of the discipline of Animal Nutrition and Agronomy, Faculty of Veterinary Medicine Timisoara.

The Kjeldahl method and the FOSS 8400-8420 equipment were used to determine the crude protein, respecting the manufacturer's application notes (1). The Kjeldahl method allows the determination of the crude protein content of the samples on the basis of the nitrogen content. The test sample ( $1 \pm 0.2$  g) is mineralized with 98% sulfuric acid in the presence of a catalyst and boiled at 450°C for 45 minutes. The ammonia released from the reaction is distilled in a solution of excess boric acid, followed by titration with a solution of 0.1 N hydrochloric acid. The nitrogen captured is multiplied by the conversion factor - 6.25 - and expressed in crude protein equivalent. The FOSS 8420 analyzer is an automatic device that, by entering the data and the weight of the sample, generates the automatic result, expressed in % of crude protein.

For the determination of the crude fiber (cellulose), the standardized method was used, adapted by the company FOSS, by using the FOSS 2010 device, automatically set to the analysis method (1). Each sample was weighed  $1 \pm 0.2$  g. The sample was degreased with acetone by three successive washes in the cold unit of the equipment. After degreasing, the samples were introduced into the work unit and boiled with 12.5% sulfuric acid for 30 minutes at the boiling point of the acid. Then, the samples were washed with distilled water and boiled, again for 30 minutes, with 12.5% potassium hydroxide, at the boiling point of this reagent. The samples were extracted from the equipment and placed in the furnace oven at 525°C for 3 hours. After cooling, the samples were reweighed and the percentages of fiber contained in oats samples were calculated using the following formula:

$$CB\% = \frac{mc}{mi} \times 100$$

where: CB% - crude fiber (cellulose)

mc - mass resulting from calcination  
 mi - the initial mass of the sample taken.

The obtained results were statistically analyzed using Excel Data Analysis. Statistical correlations and descriptive statistics were made, and the data were analyzed by calculating the t-Test and the positive or negative correlations between the parameters. Statistical data were expressed in tables and diagrams.

### Results and discussions

In the chemical analysis of the fiber and protein content, various results were obtained, summarized in the table below:

Table 1

**Crude fiber and crude protein content in oats**

Parameter	Mean %	Min. %	Max. %	SD	CL (95.0%)
Crude fiber	12.5103	6.97	22.25	4.4331	1.0984
Crude protein	13.6850	9.14	15.62	1.4046	0.3480

From the data analysis it can be seen that, in the case of fibers, values between 6.97% and 22.25% were obtained. The statistical analysis of the obtained values indicates significant differences between the percentages obtained for the 65 analyzed samples (see Table 1).

In the case of protein content, the values obtained were between 9.14% and 15.62%. In the case of this chemical parameter, the differences between the values obtained were smaller in comparison with the fiber content (see Table 1 and Fig. 1).

The diagram from Fig. 1 shows graphically the evolution of the values obtained from the chemical analysis of fibers and proteins from the analyzed oats samples. It can be observed very clearly the significant differences identified between the values obtained for the fibers and the smaller difference between the values obtained for the crude proteins.

Statistical data processing allowed the analysis of positive and negative correlations between the values obtained for fiber and protein of each sample. Thus, in the graph from Fig. 2 it can be seen how the two chemical parameters are influenced each one. For the samples that showed high values at the fiber content (n = 21) it is observed how the value for the crude protein is directly correlated, presenting lower values. Also, for the samples in which higher values of crude protein were obtained (n = 44), values directly correlated with the percentage of fibers were obtained, these being lower.

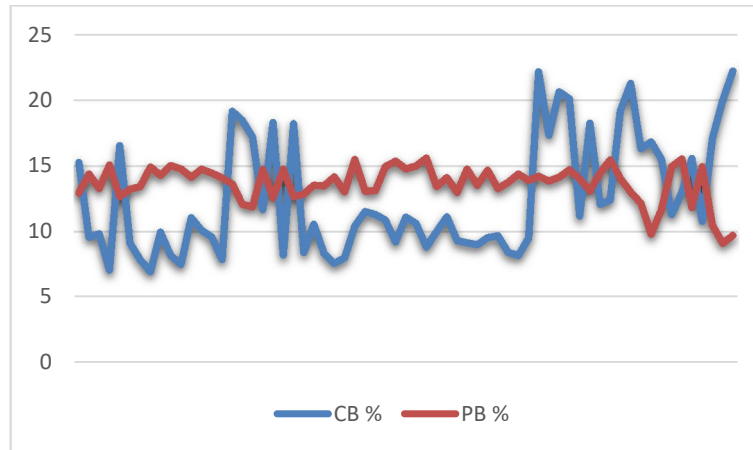


Fig. 1. Variation of crude fiber (CB) and crude protein (PB) content in oats

Following this study, the direct correlations between the fiber content and the protein content for the analyzed oats samples can be observed. These data underline the importance of performing the chemical analysis of the feed material for each batch harvested. Given that oats are used in animal feed as an energy and protein source, this study shows that the nutritional value of oats decreases if the feed has a high fiber content.

It can be seen that the values of fiber content also presented percentages of over 20%, much higher than the average from the literature - 13.9% (12, 18). This entails a reduction in the nutritional value of the oats, regardless of the animal species for which it is intended. The fiber content is also necessary to be known in order to establish the percentage of inclusion in the feed rations. Given that monogastric animals have a lower digestibility of cellulose, the fiber content of feed materials used in their diet is very important. It is known that the harder a feed is to digest, the lower the growth yield (5, 14, 15). Thus, the use of feed with high fiber content, in growing animals and those raised in intensive system, for high yields, would reduce the increase in body mass of animals. The use of oats in monogastric animal feed must be done carefully, precisely because of this reason, presented above. Without a precise chemical analysis, the degree of inclusion of oats in the diet of monogastrics could cause problems in terms of productive yield (2).

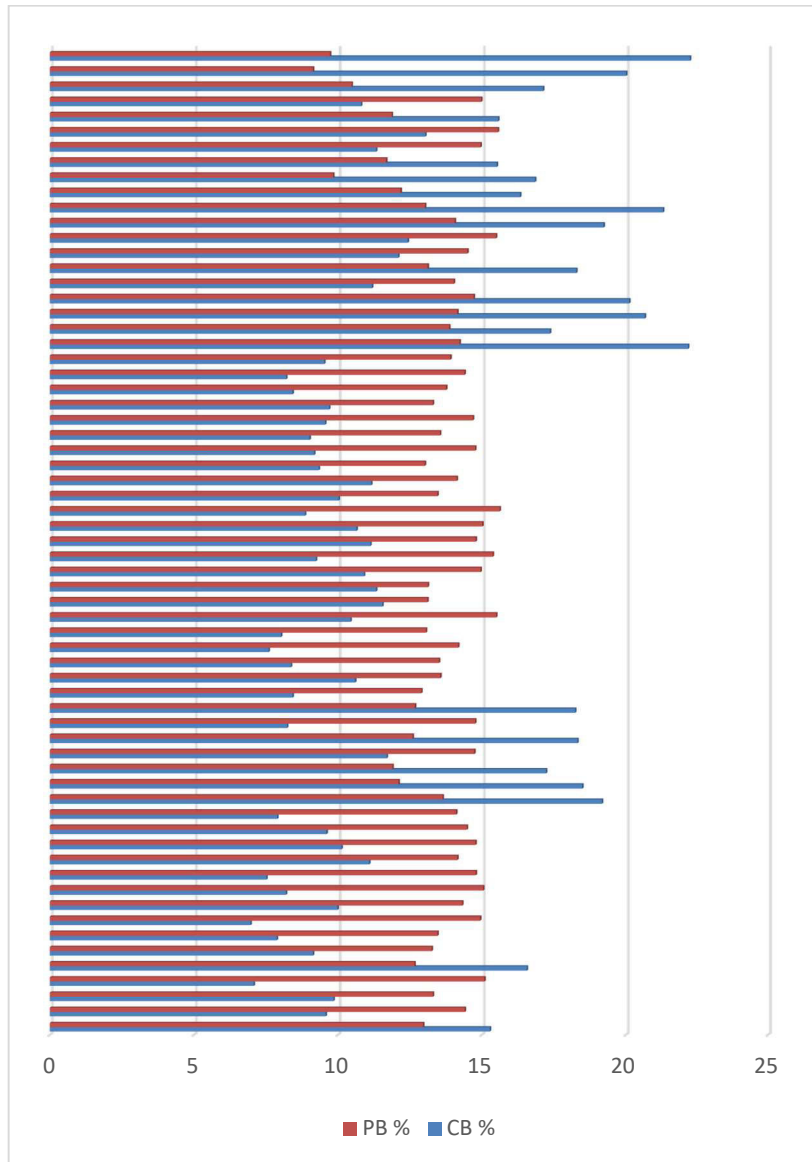


Fig. 2. Direct correlations between fiber (CB%) and protein (PB%) in oats

The fact that the percentage of fiber in oats directly influences its protein content, entails other negative effects. Failure to meet the protein requirements of

the animals determines the reduction of the growth increase, the yield of the productions, as well as the quality of the products used for food purposes.

The results of this study are also supported by the research of other authors in the field. Svihus and Gullord (12), analyzing the chemical composition of the main cereals used in animal feed and the influence of components on the nutritional value of broiler diets, confirmed that the nutritional value of wheat, barley or oats varies considerably from one batch of cereals to another. For oats, the fat and fiber content of oats has been identified as the main cause of the change in nutritional value (12).

On the other hand, the participation of a limited percentage of fiber in animal nutrition is necessary in order to improve intestinal transit and nutrient absorption. In this sense, González-Alvarado et al. (5) tested the effect of including whole (unhulled) oats in the diet of broiler chickens and pointed out that the type of fiber used in the diet affects the performance and digestive processes of broilers. The inclusion of oats fibers improves growth performance, N retention and digestibility of nutrients in broilers, regardless of age. The beneficial effects of including oats fibers on the productivity of broilers were primarily due to its effect on digestibility and absorbed nutrients (5).

In another study, Scholey et al. (11) show that diets containing whole oats (unhulled) can be used in poultry feed without having a detrimental effect on bird weight, with a positive influence on food intake, but with the reduction of available metabolizable energy, which can generally reduce, the economic advantages in using diets that contain whole oats. Amino acid digestibility was improved using these diets compared to diets containing an equivalent percentage of husked oats (11).

Moreover, Hodgkinson et al. (7) conducted a study comparing the digestibility of protein in corn, oat and alfalfa diets in pigs. The apparent digestibility of protein in the faeces of pigs fed oat-based diets was lower than in corn-based diets, and in those based on alfalfa flour, protein digestibility was much lower than in the case of oats or corn.

The results of this study, supported by the results of other researchers in the field, confirm the importance of determining the total chemical content in order to establish the nutritional value of feed materials. Monogastric animals, having a physiological limitation in terms of the degree of digestion of insoluble fibers, represent a special category that must be given special attention to nutrition. Thus, the percentage of inclusion of oats in the diet of monogastric animals depends on the nutritional value and the content of insoluble fiber.

According to this study, oats are a valuable feed for animals, with a high protein content, which could be used successfully even in intensive breeding. The rather high percentage of insoluble fiber, compared to other cereals used in animal diets, makes oats less used, especially in the diet of pigs and birds. In the future, the development of naked oats crops could be a solution in capitalizing on oats as feed material.

### **Conclusions**

The high protein content of the analyzed oats emphasizes the fodder value of this cereal for animal feeding.

The direct correlations obtained between the fiber content (cellulose) and the protein content, in the sense of the negative influence of fiber on the valuable chemical component - protein - highlights the influence of fiber on the nutritional value of oat-based diets.

The interinfluence of the analyzed chemical compounds demonstrates the importance of the chemical analysis of raw feed materials in order to formulate diet recipes for animals.

### **Acknowledgement**

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## PREDICTION OF NUTRITIONAL VALUE OF SUNFLOWER SEEDS FOR MONOGASTRIC ANIMAL NUTRITION

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### Summary

Sunflower seeds are feedstuffs with high energy and nutritional content that can be used successfully in all animal species feeding. Considering the possibility of variation of the nutritive compounds from the seeds, this study aimed to evaluate the chemical analysis of the sunflower seeds, harvested from Timis county, as well as the estimation of the nutritional value by using the predictive equations. In this regard, 20 samples of sunflower seeds were analyzed. The basic chemical compounds, dry matter (DM%), total ash (Ash%), crude protein (CP%), ether extract (EE%), crude fiber (CF%) and nitrogen-free extract (NfE%) were determined, obtaining various values and emphasizing the interinfluence between them. Starting from the chemical composition of the seeds and using theoretical digestibility coefficients, as well as predictive equations for the calculation of metabolizable energy (ME), the nutritional value of sunflower seeds for pigs and birds was established. It was observed that this feed raw material has a higher nutritional value for pigs (4373.0860 kcal / kg ME) than for birds (4207.9145 kcal / kg ME). The analysis of this study shows the need of knowing the raw chemical composition of feeds and the usefulness of using predictive equations to estimate the nutritional value in order to establish the percentage of use in animal feeding of different feedstuffs.

**Keywords:** sunflower seeds, pigs, poultry, nutritional value

Sunflower (*Helianthus* spp.) seeds are mainly used for oil production. Usually, in animal feeding is used the sunflower meal left after the oil extraction, representing a valorous feed for all animal species. It is also possible to use whole sunflower seeds in the diets of some animal species, including monogastric ones (5, 22). Sunflower seeds do not contain anti-nutritional factors, such as those found in other major oilseeds, including soybeans, flax seeds, cotton seeds, rapeseed. For this reason, it is considered a safe feed for all animal species (3).

In Romania, sunflower is one of the most widespread oil plants. Its seeds are used to obtain a high-spread oil, both in Romania and abroad. The high demand for sunflower oil, but also the multiple uses of this plant determine more farmers to cultivate it. Thus, the importance of sunflower cultivation is given by its widespread use in human nutrition, but also in animal feeding, having industrial and energy uses (25). Large-scale cultivation in Romania makes this plant with high potential as feed to be accessible to animal breeders. Thus, many animal breeders use sunflower seeds, either from their own culture or purchased at a lower price, for most animal

species. The use of whole sunflower seeds in animal diets is important both due to the pleasant taste that makes the feed mixture to be consumed with pleasure, and also due to the increased energy and nutritional value.

Sunflower seeds are valuable due to their oil content, which represents about 50% of the dry weight of the seeds. Sunflower oil is characterized by a high concentration of linoleic acid (62-75%), followed by oleic acid (16-27%). Saturated fatty acids do not represent more than 15% of the fatty acid content (9). The rest of the dry matter consists of protein (14-20%) and fiber (NDF 18-34%, ADF 15-25%, lignin 5-8%). Sunflower protein is less rich in lysine than soy protein, but has relatively large amounts of sulfur-containing amino acids (1.9 and 2.2% protein for cystine and methionine, respectively) (14, 20, 26).

In order to meet the nutritional requirements of the animals, it is necessary to know precisely the composition of the feed. Such information is vital when trying to achieve high levels of production by using balanced animal diets. This knowledge is also essential for the correct use of the various ingredients in the manufacture of valuable compound feed. Also, knowledge of the chemical composition and nutritional value of feed materials is fundamental in planning feed production on the farm, so that crop yields can be balanced with animal requirements (8).

Thus, the purpose of this study was to analyze the crude chemical composition of sunflower seeds from Romanian crops and to use the predictive equations in order to mathematically establishment of the nutritional value of feed for pigs and poultry.

### **Materials and methods**

In conducting this study, 20 samples of sunflower seeds were analyzed. The seeds were harvested from the Western plain, Timis county, Romania, in 2019.

The samples were processed in the Animal Nutrition Laboratory within the Faculty of Veterinary Medicine in Timisoara. To evaluate the crude chemical composition, the following parameters were analyzed: moisture (M%), dry matter (DM%), total ash (Ash%), crude protein (CP%), ether extract (EE%), crude fiber (CF%) and nitrogen-free extract (NfE%).

In the processing of the samples were used the standardized methods for chemical analysis of feeds (2) adapted by the manufacturer of laboratory equipment, through the application notes provided. The samples were grounded using the FOSS Cyclotec 1093 laboratory mill and weighed ( $1 \pm 0.2$  g) at the Shimadzu analytical balance, following the working procedure for each method.

The equipment used were produced by FOSS. Thus, for the analysis of the crude protein by the Kjeldahl method, the FOSS analyzer with the following components was used: Kjeltex 8400 Analyzer Unit and Kjeltex Sampler 8420, together with FOSS Tecator Auto Digester and FOSS Tecator Scrubber. The FOSS Soxtec 2055 equipment was used to determine the ether extract. The crude fiber analysis was performed using the FOSS Fibertec 2010 analyzer, with the FOSS Cold

Extraction Unit 1021. The calcination of the samples was performed with Nabertherm B150 furnace oven. The Binder oven was used to determine the dry matter and moisture, as well as the formula:  $100 - \text{SU}\% = \text{U}\%$ .

To estimate the nutritional value, digestible nutrients (%) were calculated using the values of the determined crude chemical composition and digestibility theoretical coefficients for pigs and poultry (according to Burlacu, 1983) (4), applying the formula:

$$D.N.\% = \frac{C.C. \times D.C.}{100}$$

D.N. = digestible nutrients;  
C.C. = crude composition;  
D.C. = digestible coefficient.

The nutritional value for monogastric animals is expressed, especially on the basis of metabolizable energy. Thus, the calculation of metabolizable energy (ME kcal / kg) was performed according to the formulas described by Milos et al., 1983 (16):

$$M.E. (kcal/kg)_{pig} = [(5.01 \times D.P.) + (8.93 \times D.E.E.) + (3.44 \times D.F.) + (4.08 \times D.NfE.)] \times 10$$

$$M.E. (kcal/kg)_{poultry} = [(4.26 \times D.P.) + (9.50 \times D.E.E.) + (4.23 \times D.F.) + (4.23 \times D.NfE.)] \times 10$$

D.P. = digestible protein,  
D.E.E = digestible ether extract,  
D.F. = digestible fiber,  
D.NfE. = digestible nitrogen-free extract

Statistical data processing was performed using Excel Data Analysis. Statistical correlations and descriptive statistics were processed, and the results were analyzed including by making positive or negative correlations between the data obtained. The data resulting from the statistical processing were expressed in tables and diagrams.

## Results and discussions

The results of the analysis for the crude chemical composition of the studied sunflower seeds samples are presented in Table 1.

Table 1

**Chemical composition and statistical data for sunflower seeds**

	<i>M%</i>	<i>DM%</i>	<i>Ash %</i>	<i>CP%</i>	<i>EE %</i>	<i>CF%</i>	<i>NfE%</i>
Mean	6.7035	93.2965	5.203	24.3715	34.4015	19.1155	16.9085
Minimum	5.36	91.76	2.98	18.28	28.93	16.45	10.21
Maximum	8.24	94.64	7.55	28.68	40.31	23.17	20.83
Standard Deviation	0.8081	0.8081	1.3486	3.3976	3.1658	1.7905	2.8463
Confidence Level(95.0%)	0.3782	0.3782	0.6311	1.5901	1.4816	0.8380	1.3321
Qd % for pig**				90	90	25	50
Qd % for poultry**				66	95	11	46

\* M-moisture; DM-dry matter; CP-crude protein; EE-ether extract; CF-crude fiber; NfE-nitrogen-free extract;

\*\* Qd-digestibility theoretical coefficient for pigs and poultry (dupa Burlacu, 1983)

For the moisture content, the average value registered was 6.7035%, and for the dry matter - 93.2965%. The variations in the values recorded for moisture and dry matter were insignificant (see Table 1).

Instead, for the total content of inorganic substances (ash) and for the organic ones (CP, EE, CF, NfE) the values obtained showed significant variations (see Table 1). Thus, the total mineral content (ash) presented an average of 5.203%, the minimum value being 2.98%, and the maximum 7.55%. The most significant variations were for the crude protein content, which showed an average of 24.3715%, with limits between 18.28 and 28.68%. Also, the ether extract content revealed significant differences, presenting minimum values of 28.93% and maximum values of 40.31%, with an average of 34.4015%. The fiber content (cellulose) showed lower variations than those recorded for protein and fat. For the fiber content, the average value obtained was 19.1155%, with a minimum of 16.45% and a maximum of 23.17%. The last parameter analyzed, nitrogen-free extract, also presented significant differences between the values obtained, with limits between 10.21 and 20.83%, presenting an average value of 16.9085% (see Table 1).

The statistical analysis of the data showed the interinfluence of the chemical components from the analyzed sunflower samples (Fig. 1).

Thus, in the graph from the Fig. 1, direct correlations between chemical compounds can be observed. Important correlations can be observed between the protein content and the ether extract. The higher the protein content, the lower the fat content, and vice versa were. The two components, being the main constituents that ensure the nutritional value of the feed, it is very important to know the interinfluence between them.

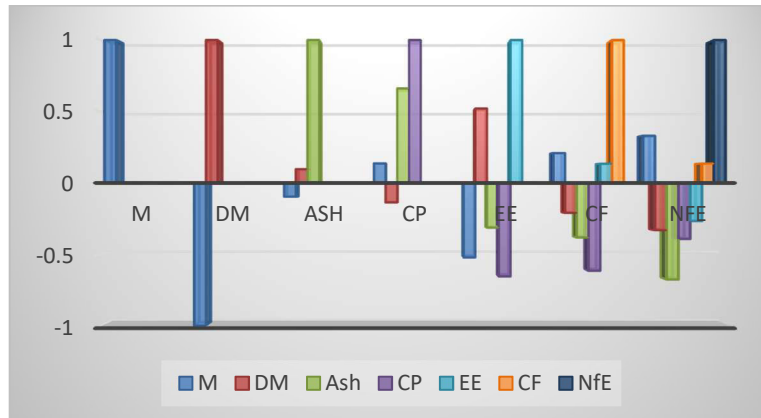


Fig. 1. Statistical correlations between chemical compounds identified in sunflower seeds

It can also be seen that the fiber content directly influences the protein content, in the sense of decreasing the second one. The direct influence seems to have the nitrogen-free extract on the protein and ether extract content. The higher it is (NfE), the lower the protein and lipid compounds are.

Thus, in the graph from the Fig. 1, direct correlations between chemical compounds can be observed. Important correlations can be observed between the protein content and the ether extract. The higher the protein content, the lower the fat content, and vice versa were. The two components, being the main constituents that ensure the nutritional value of the feed, it is very important to know the interinfluence between them. It can also be seen that the fiber content directly influences the protein content, in the sense of decreasing the second one. The direct influence seems to have the nitrogen-free extract on the protein and ether extract content. The higher it is (NfE), the lower the protein and lipid compounds are.

The predictive nutritional value of the analyzed sunflower seeds, both for pigs and birds, showed different values, depending on the chemical compounds analyzed (see Table 2).

Thus, as can be seen in Table 2, the same feed has a different nutritional value, expressed in the content of metabolizable energy, depending on the species for which it is intended. This is due to the different digestibility coefficients for pigs and poultry, shown in Table 1.

Table 2

**Predicted metabolizable energy and statistical data for sunflower seeds**

	<i>ME pig Kcal/kg</i>	<i>ME poultry Kcal/kg</i>
Mean	<b>4373.0860</b>	<b>4207.9145</b>
Minimum	<b>4079.20</b>	<b>3803.94</b>
Maximum	<b>4693.55</b>	<b>4611.83</b>
Standard Deviation	155.8833	212.9292
Confidence Level(95.0%)	72.9556	99.6539

Table 2 shows the average values for predictive metabolizable energy, these being 4373.0860 kcal/kg for pigs and 4207.9145 kcal/kg for poultry. It can be observed that between the values obtained for the metabolizable energy in poultry there were higher variations (3803.94 - 4611.83 kcal/kg) than for the one calculated for pigs (4079.20 - 4693.55 kcal/kg).

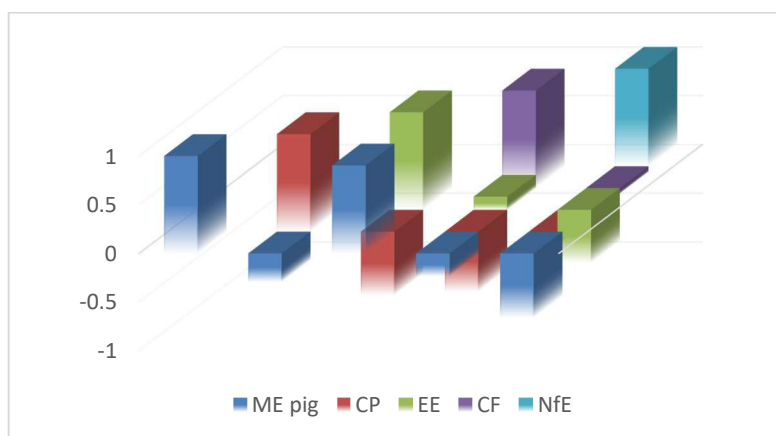


Fig. 2. Statistical correlations between metabolizable energy for pig and chemical compounds identified in sunflower seeds

Figures 2 and 3 show the statistical correlations between the values of predictive metabolizable energy in pigs and poultry and of the analyzed chemical compounds. A direct influence can be observed between ME and EE, in the sense of increasing the value of ME directly proportional to the increase of the ether extract. This is due to the high calorific value of fats (2.25 times higher than other chemical compounds). The protein content has a direct influence on the ME value in pigs and

poultry, in the sense of reducing it. This is due to the direct and competing interinfluence between the protein and lipid content. If the protein factor increases, the lipid factor decreases, this directly influencing the caloric value of the analyzed feed.

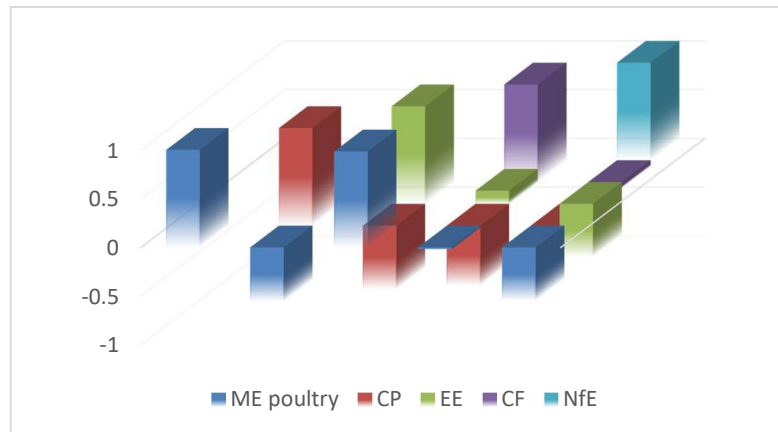


Fig. 3. Statistical correlations between metabolizable energy for poultry and chemical compounds identified in sunflower seeds

Instead, a negative influence of fiber content on the ME value can be observed. The higher the fiber content, the lower the ME value, due to the low digestibility coefficients for CF, both in pigs (Qd for CF - 25%), but especially in poultry (Qd for CF - 11%). Also, in Fig. 2 and 3 it can be observed that an increased content in NfE of feed materials entails a reduction of their nutritional value, respectively of ME, due to the negative influence on all other compounds. The higher the NfE, the lower the CP, EE, CF and, implicitly, ME values were. However, the negative influence that NfE exerts on the value of ME is lower compared to the influence that CF has on ME.

The results of this study prove the strong influence that some chemical compounds have on others, as well as they are on the nutritional value of feed. The variation of chemical compounds in feed directly influences the nutritional value of feed. Thus, the need for chemical evaluation of feeds is indisputable, both in order to determine their nutritional value and to establish the percentage of inclusion in animal rations.

The variation in nutritional value highlighted in this study, and due to the variation of the chemical compounds analyzed, may influence the degree of compliance with the nutritional requirements for different animal species. For monogastric animals, where the energy value of the ration ensures the degree of satiety, the increase of body mass and their productions, the determination of ME in

feed is mandatory.

Through this study it was shown that, starting from the analysis of the crude chemical composition of feed materials, the nutritional value of animal feeds can be predicted, in order to ensure their nutritional requirements, to prevent some deficiencies, but also the surplus of nutrients that would reduce the economic value of animal production, thus increasing the profitability of animal husbandry (11).

Given that in Romania many animal breeders use whole sunflower seeds, from their own culture, in animal feeding, especially in extensive breeding, the results of this study can be used even in formulating diets for pigs or poultry by breeders.

The diets for monogastric animals extensively raised are mainly formulated using various cereals and oilseeds as main ingredients, due to their availability and relatively lower costs. There is a great variation in the nutritional values of feed materials, creating the need to develop routine evaluation techniques to detect these variations in the formulation of balanced diets and to obtain optimal productive performance in animals (11).

Researches of other authors has shown that information on feed quality is essential, as feed represents 65-70% of the total cost involved in the production of monogastric animals (23). The goal of any commercial farm is to formulate animal diets that combine ingredients at the lowest cost to be cost-effective. However, the chemical composition and nutritional content vary greatly between crops, depending on the fodder hybrid, its type (12, 13), the harvest period, the conditions and methods of processing (10).

Sunflower seeds have a high oil content and can be a valuable energy food for pigs, but their fiber content limits their use in growing animals. Knowledge of chemical composition and nutritional value is essential, because the rates of inclusion of sunflower seeds, recommended by various researchers, based on the results obtained by them in their studies, range from 8% to 10% in diets for growth and finishing pigs and up to 25% for sows (1, 19, 24).

In different studies on animals it has been observed that the inclusion of whole sunflower seeds in pigs' diets can result in a slight increase in protein digestibility (5). On the other hand, the results of other studies have shown a decrease in food consumption and weight gain with increasing levels of inclusion of whole sunflower seeds at all stages (weaning, growing, finishing) (6).

Sunflower seeds are also valuable energy feeds for poultry. They can be used raw or hulled and have been shown to be included in the diets of poultry up to 50% (7, 18). During the finishing period for broilers, the inclusion of sunflower seeds can reach up to 40%, without having negative effects on weight gain, feed intake or feed conversion efficiency (17). Sunflower seeds have been found to increase feed efficiency due to lower feed intake and better or similar weight gain, which can lead to lower feed costs (7, 21).

Unlike soybeans, sunflower seeds do not require prior heat treatment and are a cheaper source of energy than other oilseeds (15).

All these studies performed on animals emphasize the importance of



knowing the chemical composition of feed included in diets, in order to predict the productive effects of the ration and to prevent economic lost. Thus, the results of this study support the need for chemical analysis of feed materials and facilitate, by giving an example, the understanding of methods for applying calculation formulas in predicting the nutritional value of feeds used in monogastric animal nutrition. Starting from the crude chemical composition and the correct use of the predictive equations, the nutritional value of the fodder can be estimated, facilitating the establishment of the percentage of inclusion in the rations, facilitating the feeding process and increasing the animal productions.

### **Conclusions**

The chemical analysis of sunflower seeds showed the variation of chemical compounds and their interinfluence, especially for the content in crude protein, ether extract and crude fiber.

Using the theoretical digestibility coefficients of the chemical compounds from sunflower seeds for pigs and poultry and the predictive equations for calculating the metabolizable energy, it was possible to establish the nutritional value of the tested feed for monogastric animals.

The direct influence of chemical compounds on the energy value identified in this study, emphasizes the importance of knowing the methods of estimating the nutritional value of sunflower seeds in order to include them in animal diets.

The use of mathematical prediction in estimating the nutritional value of feeds facilitates the process of formulating animal diets, reduces the economic injury associated with inefficient valorisation of food and, implicitly, increases animal production.

### **Acknowledgement**

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## INVESTIGATION ON THE QUALITY AND POLLUTION WITH FUNGI AND MYCOTOXINS OF FODDERS USED IN A BEEF CATTLE FARM FROM BRAȘOV COUNTY

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### Summary

The study was conducted on a Charolais beef cattle farm with a herd consisting of 105 cows and 3 bulls, situated in Brașov county, through visual observations and using the information received from the farm employees and farm registers. Were taken under observation also the breeding technology, housing, welfare conditions and beef cattle feeding. A number of 4 samples of fodders were collected, representing all the fodders used in feed for these categories of beef cattle, both components and mixtures administered to the animals and analyzed within Laboratory of Animal Nutrition from the Faculty of Veterinary Medicine Cluj-Napoca. An organoleptic examination was performed, followed by gross chemical composition, mycological assessment and total aflatoxin content. The results indicated an adequate quality of forage in terms of organoleptic and crude chemical composition. Following the mycological examination the presence of *Penicillium* and *Fusarium* genera were observed. The total aflatoxin values were within normal limits required by the European Legislation, with a maximum value for the total aflatoxins of 7.65 ppb.

**Keywords:** cattle, chemical composition, fungi, mycotoxins

Charolaise is a breed of beef cows par excellence, which stands out both for the qualities of the special organoleptic meat (8), is dietary and non-fat with a fat percentage of only 10% compared to other breeds in which the percentage is 15-30% as well as by their docile character, easy to grow and maintain (3, 4). The best use of fodder, especially roughages fodder could be observed in this breed. In fact, their ingestion capacity is superior to other breeds, and their efficient metabolism allows this capitalization of roughages (straw, grass on pasture, hay), which are also the most economical. The average daily gain is 1100-1400 grams, in fattening conditions. The dressing percentage in young animals is 62-67%, with 80% of the meat in the carcass. Diseases caused by the consumption of contaminated feed and food are becoming an increasingly important problem worldwide. The most important factor involved in food and feed contamination is represented by microorganisms and among them, especially fungi. They make secondary metabolites with toxic properties called mycotoxins (13). Most mycotoxins are ubiquitously encountered and can cause serious health disorders (such as neurological disorders and even tumours) in both animals and humans (2).

Mycotoxins are chemicals produced by certain species of moulds: *Aspergillus*, *Fusarium*, *Penicillium*, etc. They can also be highlighted in their spores

or in the substrate on which the fungi grow. There is a wide variety of mycotoxins, but not all of them are important for human food safety. The most important mycotoxins with significant risks to human food safety are: aflatoxins, fumonisins, ochratoxins, deoxynivalenol and zearalenone (7). Aflatoxins are a group of about 20 metabolic products of fungi of the genus *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nominus*). The toxicity of aflatoxins evolves both acutely and chronically, aflatoxin B1 being one of the most known hepatocarcinogenic substances (15). Chronic hepatic exposure to aflatoxins can cause liver cancer, chronic hepatitis, jaundice, hepatomegaly, cirrhosis and can also affect the immune system (14).

### **Materials and methods**

The study was carried out between October 2018 and June 2019, in a beef cattle farm in Brașov County and samples were processed within the Laboratory of Animal Nutrition from the Faculty of Veterinary Medicine Cluj-Napoca. A number of 4 fodder samples collected from the farm were investigated, fodder used to feed a number of 108 Charolais beef cattle.

The quality of the collected samples was assessed by both organoleptic, chemical and by cultural examination. Pollution of the mycotoxin samples was also monitored, and the total aflatoxin concentration was analyzed. Determination of the total colony number of yeasts (colony forming units CFU/g) and molds consists in the preparation of Petri dishes, in which a selective culture medium (Sabouraud medium) and a determined amount of the initial dilution were poured, in the case of the products used. The preparation of other dishes under the same conditions using decimal dilutions from the sample to be analyzed or from the initial dilution was performed, followed by an aerobic incubation of the dishes at 25°C for 5 days.

To perform laboratory tests, was used the RIDASCREEN®FAST Aflatoxin test, a competitive enzyme-linked immunosorbent assay for the quantitative determination of aflatoxins in cereals and feed. The samples were processed and analyzed in strict hygienic conditions, respecting the legislation in force so that the results are as conclusive as possible.

The analysis of the rations administered to the cattle was carried out with the program HYBRIMIN Futter 2018 (Germany).

### **Results and discussion**

The organoleptic examination of the fodder used in the animal ration revealed that they were of good and very good quality. Natural hay presented plant content from the spontaneous flora of natural meadows and no toxic plants have been detected in its structure. Lucerne is the one that showed a high degree of shaking. Following the analysis of the chemical composition of the alfalfa hay sample, the percentage of protein is above average being 18.55%, while an average value of this type of feed is 17%. Low values were obtained in fat 1.53%, the average

value 2.3%. Corn had values below the average values for all components, the biggest difference being found in raw cellulose, being lower except for the fat which had values with a percentage above average (average value 3.7). For the hay sample the values did not deviate (Table 1).

Table1

### Raw chemical composition %

Sample no.	Sample type	D.M.*	Crude protein	Crude fat	Crude cellulose	NFE**	Crude ash
1.	Corn kernel	83.12	6.34	4.58	9.21	61.52	1.47
		100	7.62	5.51	11.08	74.03	1.76
2.	Alfa alfa hay	91.44	16.97	1.40	29.13	35.59	8.35
		100	18.55	1.53	31.85	38.92	9.13
3.	Natural hay	92.42	6.49	5.18	33.84	38.0	8.91
		100	7.02	5.60	36.61	41.13	9.64
4.	Straw	93.53	2.48	1.04	42.33	42.93	3.55
		100	2.65	1.14	46.35	45.89	3.97

\*- dry matter; \*\*-nitrogen free extract

Following the mycological examination performed on the 4 feed samples, it was found the presence of *Penicillium* fungi genera in 3 of them: natural hay, wheat straw, corn kernel. At the alfalfa hay sample the mycotic load (CFU / g) was below the allowed value of 5000 CFU/g (order 243/2011 ANSVSA) ([www.monitoruljuridic.ro/](http://www.monitoruljuridic.ro/)), at the corn kernel it was over 45 times higher (222,564 CFU/g), for natural hay more than 23 times (115,281 CFU/g), wheat straw 17 times higher than admitted value (87,563 NTF/g) (Table 2).

Table 2

### The types of mycetes and the fungal load of feed samples

Sample	Identified mycetes genera	CFU*/g	Specifications (admitted value 5000 CFU/characteristics)
Alfa alfa hay	<i>Penicillium spp.</i>	3754	According to the Order No. 243
Natural hay	<i>Penicillium spp.</i>	115.281	nonconforming
Wheat straws	<i>Penicillium spp.</i>	87.563	nonconforming
Corn kernel	<i>Penicillium spp.</i> <i>Fusarium spp.</i>	227.564	nonconforming

\*colony forming units

Several authors (1, 5, 6, 9, 10, 11, 12) present the importance of humidity control in the prevention or limitation of pollution of fodder by fungi.

Maximizing feed flow in a livestock unit by reducing storage times, limits fungal contamination. Moisture is the most important factor in determining whether and how fast will grow the mycetes in fodder.

Aspects of mycetes cultures on Sabouraud solid medium are presented in Fig. 1 and Fig. 2.



Fig. 1. *Penicillium spp.* and *Fusarium spp.* colonies



Fig. 2. *Penicillium spp.* ad *Fusarium spp.* colonies

The determination of aflatoxin was performed from the 4 collected samples. Thus, aflatoxin was detected in all samples examined, but no sample exceeded the maximum values allowed in the feed of beef cows. The values of total aflatoxins in the 4 feed samples being: 3.40 ppb - corn kernel; 5.92 ppb - Alfalfa hay; 7.65 ppb - Natural hay; 5.65 ppb - wheat straw. None of the samples exceeded the maximum limit allowed by European Legislation, this being 20 ppb. The results showed a mildly moderate contamination of the collected samples which indicates an inadequate processing and storage of the fodder administered to cattle and is a major risk factor for diseases of the reproductive system and general health. We analyzed the compositions of the rations both for the young fattening category and for the adult cattle. We found that the ration for the young fattening animals had a metabolizable energy deficit of 9.24 MJ, so we balanced the ration in terms of energy with the same nutrients. The same problem was observed in the case of the ration for adult cows, where there was a net energy deficit of milk of 14.82 MJ, balancing the ration in this case as well.

### Conclusions

Following the micological examination, all samples were contaminated with mycetes, three of them exceeded the maximum admitted level regarding the micetes load. Aflatoxin was detected in all analysed samples, but none of them exceeded the maximum admitted value permitted by the regulation. In this case we recommend the use of mycotoxin inhibitors.

We also recommend complete and correct rations to optimize the feeding of cattle on the Charolaise cow farm as well as improving animal husbandry conditions.

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## **NEONATAL DEVELOPMENT OF NEWBORN DOGS, FED WITH NATURAL DOGMILK IN COMPARISON WITH THOSE FED WITH COMPLEMENTARY FORMULAS**

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### **Summary**

The neonatal period is a short but decisive period in the development of newborn puppies and the type of nourishment and their environment has a great impact upon it. The purpose of this study is to pinpoint the essential part of the nutrition during this period and to emphasize the fact that human intervention can influence in this stage the development, morbidity and mortality rate of dogs. The study included 4 different litters referring to the nutritional conditions, environmental features and specific care. The newborns were monitored from birth to weaning. The following parameters were encountered: growth rate, surrounding temperature, body temperature, sucking reflex and involuntary muscular contractions. If bodyweight loss or even standstill was established, the improvement of environmental conditions were not a remedy, unless a nutritional plan for the addition of milk was developed for each case. Through a proper nourishment intake and a comfortable environment, bodyweight was gained to normal or similar outcome.

**Keywords:** nutrition, neonatal period, complementary formula, growth rate

The knowledge regarding the puppy needs during the neonatal period has increased in the last years. The crucial role of proper nutritional support for sustaining health and preventing morbidity and mortality during this period is recognized. The first 36 hours postpartum are critical due to the parturition and sudden changes in the environment that a puppy experiences. They are particularly physiologically stressful. Therefore, efforts must be made to minimize stress and changes in the environment (2). There are three factors that are critical during nursing, namely the nutrition of the female during pregnancy and lactation, the behavior and health of the female and ensuring a quiet, isolated and comfortable space that allows the maintenance of an optimal temperature and humidity and that provides security and privacy for the bitch. Compared to the young of other species, newborn puppies are relatively immature at the time of calving and that their skeletons have a low degree of mineralization (12). Large breed puppies are much more susceptible to malnutrition and the development of orthopedic diseases throughout the growing period, because the stage of development is further back than small breed puppies (3). An optimal nutritional plan starts from the moment of parturition and lasts for the whole life and ensures over time an increased level of quality of life, prolongation of life expectancy and thus a satisfactory relationship of the owner with the pet. Puppies are completely dependent on the mother until weaning. The mortality rate can reach

40% in the neonatal period. Birth weight varies due to large differences between dog breeds, and range from 1% of female weight for giant breeds to 6.5% for Cihuahua (16). Most orphaned puppies can be successfully raised if they receive proper care and nutrition. However, the case where the puppies are deprived of colostrum is a special case that must be treated as such. Orphaned puppies will be rigorously evaluated at first sight and the reason why they are abandoned by the female will be sought (7). Colostrum maintains not only specialized nutrition but also passive immunity, represented by immunoglobulins and bioactive factors (18). In some species, passive immunity is acquired in the uterus (man, rat, rabbit, guinea pig) to a large extent, unlike puppies, which acquire most of the passive immunity through the consumption of colostrum. Nutritionally, colostrum differs greatly from the milk (4). Is important not only the nutritional quality of colostrum but also the quantity (17). As in other mammals, colostrum has a different composition from mature milk to meet the needs of immediate development of the puppies, in the first 24-72 hours, after which the composition of milk stabilizes and turns into mature milk (5). Due to the high percentage of dry matter in the colostrum, it has a viscous, sticky consistency, making it difficult for puppies to suck (14). Dogs have a placenta of the endotheliochorial type that allows the passage of 10-20% of passive transplacental immunity (11). Complementary formulas replace mother's milk until the introduction of semi-solid food. It is very important to have a composition similar to bitch's milk because if this composition is not followed, diarrhea and other digestive disorders occur that disrupt development and compromise growth. There is a wide range of commercial products that replace mother's milk. They are based on modified cow's milk, to simulate the composition of bitch's milk. There are also home-made replacement recipes, most of which are based on cow's or goat's milk, and involve the addition of eggs (13).

In the neonatal period, the activities of puppies are limited to sucking and sleeping precisely to support the rapid development they suffer. For this reason we wanted to research how puppies develop in natural conditions, by providing the nutritional needs with breast milk compared to providing this needs by supplementing with complementary formulas.

### **Materials and methods**

The research was carried out on four different litters, between 2016-2019, in order to better reveal the development of the puppies in relation to the nutritional, environmental and care conditions.

Litter number 1 was represented by the Russian Black Terrier breed, the first female litter, consisting of 9 puppies: 4 males and 5 females. The second litter studied belonged to the same female Russian Black Terrier, two years later and consisted of 8 puppies: 5 males and 3 females. The third litter belonged to a Fox Terrier breed female, at her first gestation, and consisted of 2 males and one female. The last litter studied was a Fox Terrier mixed breed litter consisting of 5 females.

The puppies were monitored from birth to weaning except for one litter that was supervised from the seventh postpartum day until weaning. For the examination of the puppies, the parameters considered after the clinical examination were body weight, body temperature and level of vitality.

The clinical examination was performed on day 1, 4, 7 and 14 at three of the nests, and at nest number 4 on days 7, 10, 13 and 20. However, the puppies were monitored daily by breeders. In this research we used two complementary formulas in the form of soluble powder. The supplementation with these formulas was carried out exactly following the manufacturer's instructions for reconstitution and was administered using nurser bottles specially designed for puppies or nurser bottles for human use with small nipple, suitable as a size for the puppies in question. First complementary formula is a high quality formula, recommended for mother's milk replacement or for the transition period before weaning. According to the manufacturer, the formula has the following qualities: is designed for the digestive tract of puppies, closely monitors the composition of mother's milk, has an increased digestibility, has a high solubility, is easy to use. Ingredients: skim milk powder, vegetable oil, casein powder, whey powder, wheat starch, dicalcium phosphate, antioxidant. For reconstitution: one part powder was mixed with two parts water at 50°C. It was administered to puppies only after the temperature has dropped to 37-38°C. The second formula ensures a harmonious development, has a high level of protein and energy, has an increased digestibility, has a percentage of lactose close to that of breast milk, does not contain starch, is easy to dissolve, and is enriched with polyunsaturated fatty acids. As ingredients, contains milk protein, animal fat, whey protein, soybean oil, coconut oil, fish oil, minerals, fructose-oligo-saccharides 0.48%, and flavors. For reconstitution: about one measure in 20 ml of water.

### **Results and discussion**

Following the study conducted to determine the nutritional needs of puppies in the postpartum period, as well as to determine the influence of female health on the amount of milk it produces and to determine the influence of the environment on the health and development of puppies we achieved the following results.

Regarding a natural diet, exclusively with breast milk, we have registered the well-known advantages. Thus, we obtained the best growth increase both in the large litter of big puppies and in the medium litter of small puppies. In both cases the females were healthy, being fed according to age and waist, both before pregnancy and throughout the gestation period.

Regarding the first litter studied, it was necessary to supplement the ration of mother's milk, insufficient in quantity, with complementary formulas. By manually milking the bitch from litter 1 (Table 1), it was obtained about 65 ml. The growth rate of the puppies was within normal limits, with slightly lower values than in litter 2 (the same breed) which was fed exclusively maternally (Table 2).

Litter 1 and litter 2 came from the same female, two years apart. Although the female was in the same environment, the same breeder and optimal feeding and environment conditions were created for her during both gestation and lactation, and the two litters were almost equal, in the case of nest 1 the female did not managed to provide the necessary quantity of milk and supplemented the puppies' food. The variable factors in these two situations were the experience of the bitch and the amount of breast milk produced.

Table 1

**Body weight and temperature of puppies from litter 1**

Puppies	Body weight [g]				Body temperature [°C]			
	Day 1	Day 4	Day 7	Day 14	Day 1	Day 4	Day 7	Day 14
Male 1	423	433	464	719	34.6	34.5	35	36
Male 2	714	724	769	1017	35.3	35.4	35.6	36.1
Male 3	548	551	582	894	35.1	34.8	35.4	35.9
Male 4	564	575	591	840	35	34.6	35	36.2
Female 1	490	497	519	777	34.8	35.2	35.6	36
Female 2	510	521	554	874	34.9	35.4	35.6	35.9
Female 3	507	520	562	896	35	34.8	35.7	35.9
Female 4	411	421	449	717	34.6	35	35.2	36
Female 5	470	478	503	787	34.9	35	35.4	36.1

The different seasons in which the puppies were born (winter-summer) could be considered, but this aspect was not taken into account, given that the female lives in a constant environment, not influenced by external environmental conditions. All this signals the existence of factors independent of the environment, such as: the experience of the bitch and the adaptation of her body to such a situation.

The influence of the environment and the maintenance of the bitch by the breeder, respectively its absence, are obvious in the case of the forth litter. The female, who was not properly cared for before and during the pregnancy, failed to provide the necessary amount of milk to the puppies, their condition deteriorating quite quickly in an unfavorable environment and with insufficient food. The body weight and temperature of the puppies belonging to the third litter are presented in table 3.

Both in pregnancy and lactation, females need nutritional supplements (9; 15). A previous study shows that beagle puppies consume 165-170 g of milk per day (12). The puppies included in our study consumed the complementary formulas *ad libidum*. Although the litter was adopted a few days after birth and placed in a comfortable environment, 2 of the puppies could not be saved. In this environment and with appropriate food, the condition of the female has improved, but the amount remained insufficient, being necessary to supplement the food of puppies with complementary formula. A 2011 study by Olav T.O. (17) showed that puppies

manage to consume 95% of the total food needed in 4, maximum 5 sucks, at the same time the same author insists that 1-2 sucks are not enough.

Table 2

### Body weight and temperature of puppies from litter 2

Puppies	Body weight [g]				Body temperature [°C]			
	Day 1	Day 4	Day 7	Day 14	Day 1	Day 4	Day 7	Day 14
Male 1	740	769	819	1450	34.6	34.7	35.1	35.4
Male 2	679	702	748	1281	34.7	34.7	35	35.2
Male 3	664	791	822	1455	34.5	34.8	34.9	35.7
Male 4	728	755	794	1118	34.6	34.7	35.1	35.5
Male 5	781	804	859	1324	34.6	35	35.1	35.5
Female 1	570	593	615	933	34.5	34.7	35	35.7
Female 2	514	538	571	924	34.7	34.6	35.1	35.4
Female 3	488	507	532	898	34.6	35.1	35	35.6

Table 3

### Body weight and temperature of puppies from litter 3

Puppies	Body weight [g]				Body temperature [°C]			
	Day 1	Day 4	Day 7	Day 14	Day 1	Day 4	Day 7	Day 14
Male 1	98	122	163	209	34.8	34.9	35.1	35.4
Male 2	90	117	152	215	34.9	35	35	35.2
Female 1	72	90	115	150	34.8	35.1	35.3	35.7

In litter number 4 (Table 4), the puppies received the complementary formula in 8-12 rounds per day. 6 rounds were not enough in their case, they manifested the feeling of hunger through crying and anxiety. At the end of the neonatal period, the weight and development of the puppies was in normal parameters, with average values.

A study performed on milk composition coming from lactating German Shepherd nursing females showed that differences observed between colostrum and normal milk composition after day 4 were insignificant (6). According to Fontaine, newborn puppies do not eat more than 10-20 ml of milk per feeding (8). Recent research showed that in order to minimize the impact of weaning on digestive stress, 4 meals per day should be given to the puppies. In the same time, more meals per day increase their faecal qualities (10) and the addition of probiotics improves the immune system (1).

Table 4

**Body weight and temperature of puppies from litter 4**

Puppies	Body weight [g]				Body temperature [°C]			
	Day 7	Day 10	Day 13	Day 20	Day 7	Day 10	Day 13	Day 20
Female 1	123	141	170	215	34.2	35.2	35.9	36.4
Female 2	174	193	221	287	34.5	34.9	35.7	36.2
Female 3	210	245	289	364	34.6	35.5	35.8	37.2
Female 4	114	137	172	254	34.2	35.2	34.9	36.8
Female 5	130	163	201	220	33.9	35	35.6	36.6

In the case of litter 1, the optimal quality mother's milk was insufficiently quantitative and was supplemented with milk formulas, resulting in a development in normal parameters. The best growth rate was obtained in the case of litter 2 and 3 in which the mother's milk was sufficiently quantitative, without the need for supplementation. Due to the unfavorable environmental conditions and inadequate care at litter 4, a mortality of 28% was registered in the first week of life. Although the environmental conditions improved in the case of this litter after 5 days from birth, the female's milk production remained insufficient and it was necessary to supplement the puppies' feed with complementary formulas. Obtaining an increase in growth and development in the normal parameters of litter 4 was not achieved by improving environmental conditions, being mandatory to supplement the food quantitatively.

**Conclusions**

After ensuring an adequate food intake and a comfortable environment, we obtained comparable weight gains, included in the breed parameters. The neonatal period is particularly important and any variation of the environment and an inadequate quantitative and qualitative intake of food has serious repercussions on the development, morbidity and mortality of the puppies.

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## ECTOPARASITES INFESTATION OF RED FOXES (*VULPES VULPES* L.) IN SERBIA

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### Summary

The result of entomological examination of 175 red foxes hunting in Serbia during 2016-2018 were presented. During our study infection with tick were occurred at 57.71% (101/175), fleas at 32.00% (56/175) and mange was observed in 21.71% (38/175) of the examined foxes. We identified following species: *Ixodes ricinus* 49.14%, *I. canisuga* 12.00%, *I. hexagonus* 6.28%, *I. persulcatus* 5.14%, *Rhipicephalus sanguineus* 10.87%, *Haemaphysalis punctata* 6.28%, *H. concinna* 4.57%, *Dermacentor marginatus* 42.85% and *D. reticulatus* 5.71%. At same examinations, four flea species we found at foxes. *Ctenocephalides felis felis* was the most abundant found at 21.14%, followed by *C. globiceps* 4.00%, *C. canis* 4.00% and *Pulex irritans* 2.85%. From mange were established two species: *Sarcoptes scabiei* var. *vulpes* we occurred at 18.85% and *Otoedres cynotis* we found at 2.85%. This is the first research of ectoparasites fauna of red foxes in Serbia.

**Keywords:** fleas, ticks, mange, foxes, Serbia

Natural and hunting grounds in the territory of Serbia are inhabited by with several common types of carnivores belonging to the *Canidae* family - foxes (*Vulpes vulpes* L.), golden jackal (*Canis aureus*) and wolf (*Canis lupus*). From this entire species, red fox is a widely distributed species, inhabiting the whole territory of the state. It is also the most abundant predatory species in our country (4). On the other hand, they can feed with dead or hunted animals, and thus play a role in the "cleaning", i.e. removal of animal remaining from the nature (31). For these reasons, the parasitic fauna of fox rich and diverse (18).

In most European countries, including Serbia, and as in many other parts of the world, research surveys been carried out to study of helminthes fauna of the red foxes (*Vulpes vulpes*). In contrast with ectoparasites limited data are available on the prevalence of ectoparasites on red fox populations. In Europe, the majority of data came from Austria, Germany, France, England, Hungary and Italy (2, 8, 13, 26, 30).

In our paper, we present the first research of ectoparasites fauna of foxes in Serbia.

### **Materials and methods**

We examined carcasses of 175 hunted animals which we collected in cooperation with local hunting organizations. Examination of hunted foxes and on the presence of ectoparasites during all years was carried out throughout the period of the hunting season, i.e., from November to March 2015-2016.

Each foxes examined carefully for the presence of ticks and fleas. The parasites collected in separate glass tubes containing 70% ethanol until final identification.

Foxes with extensive hyperkeratosis and the formation of thick crusts we examined to mange. Affected areas of skin from the collected animals were scraped with scalpel crusts for parasitological examination. The scrapings were placed in universal bottles containing 70% ethanol and transported to the laboratory. A portion was removed from the alcohol and subjected to KOH to recover parasites for microscopy.

From ear mange we examined cerumen collected from both ear canals to the depth of the eardrum by using cotton swabs. Cerumen samples filled with 10% NaOH solution, boiled three times, centrifuged at 350-xg and the sediment examined for the presence of mites under a light microscope.

Determinate of all ectoparasites were perform at Scientific Veterinary Institute of Serbia in Belgrade using morphological criteria. The tick species were determinate using keys by Kapustin (10), fleas using keys by Kalvelage and Münster (9) and mange using keys by Keiras (11).

### **Results and discussions**

During our study infection with tick we occurred at 57.71% (101/175), with adult fleas at 32.00% (56/175) and Sarcoptic mange was observed in 18.85% (33/175) and Ear canker mites (*Otoedres cynotis*) in 2.85% (5/175) of the examined foxes.

#### **Ticks**

Ticks occurred in foxes belong to the family of *Ixodidae*. We identified following species: *Ixodes ricinus* 49.14%, *I. canisuga* 12.00%, *I. hexagonus* 6.28%, *I. persulcatus* 5.14%, *Rhipicephalus sanguineus* 10.87%, *Haemaphysalis punctata* 6.28%, *H. concinna* 4.57%, *Dermacentor marginatus* 42.85% and *D. reticulatus* 5.71%. During our examination, we not observed ticks of larval stage.

The sex ratio within individual species showed a higher number of females in all *Ixodes* species, *Haemaphysalis* species, *R. sanguineus* and *D. marginatus*, and an equal number of male and female ticks in one species, *D. reticulatus*. Our results are similar to results of the research performed by Pavlović et al. (19), Tomanović et al. (32) and Stojanov et al. (31).

Our study revealed the highest prevalence of *Ixodes ricinus* and *Dermacentor marginatus* in hunted foxes, which predominated over other tick species. Moreover, our findings confirmed the results on the prevalence of these two species reported by Milutinović et al. (17), and Pavlović et al. (20). *I. ricinus* is the

most common tick species in Serbia and Western Balkans countries (Romania, Bulgaria, Macedonia and Montenegro) and established in dogs, cattle, sheep and goats (6, 15, 16, 17, 23, 24). Considerable geographical differences in the species composition of tick fauna would be observed in the Central European region.

The prevalence of *Haemaphysalis concinna* and *Dermacentor reticulatus* decreased westward in Hungary, Romania and a similar tendency could be observed in central Europe (13, 14, 26, 30).

### Fleas

At same examinations, four flea species we found at foxes. *Ctenocephalides felis felis* was the most abundant found at 21.14%, followed by *C. globiceps* 4.00%, *C. canis* 4.00% and *Pulex irritans* 2.85%. That species of fleas are common in foxes in numerous European countries like Hungary, Austria, France or Germany (2, 8, 12). At same time that fleas species are usually found at dogs and cats in the Western Balkans (Serbia, Macedonia, Republic Srpska (BiH) and Montenegro) as shown the research of Pavlović et al. (21, 23). Similar results were established in other European countries where *Ct. felis felis* present dominant fleas species (1, 3, 7, 26).

Cat fleas presented dominant species established at foxes during our examination. Overall cat flea *Ct. felis felis* abundance were highest in rural areas, whereas the presence of other pets sharing the abode was associated with higher overall flea abundance and *Ct. canis* or *P. irritans* abundance. This phenomenon might be explained by the urbanization of foxes as a consequence of population increase in Serbia and by the closer contact with animals living in the synanthropic environment in rural and suburban areas (23). Similar tendency was observed in Hungary, Austria and France (2, 8, 26, 27).

*Ct. canis* and *P. irritans* abundances were higher during the warm period of the year. Mean annual temperature was negatively correlated with overall, *Ct. canis* and *P. irritans* abundances, but positively related to *Ct. felis felis* abundance (5). Although the prevalence was generally higher during the summer months, no statistical differences were detectable when looking at the pattern between the four seasons. The preliminary results did not indicate any tendency for a relationship between climatic conditions and flea infestation rates (9, 21). In general, the abundance of adult cat fleas fluctuates with seasonal changes. The warm months of spring and summer give rise to the highest numbers, whereas few are found during the cold months of late fall and winter.

### Mange

Sarcoptic mange mite (*Sarcoptes scabiei* var. *vulpes*) infections we occurred at 18.85%. Our Results are similar to the majority of European data (14–25%) (8, 12, 26, 27), although in some countries considerably higher prevalence (60–67%) has also been recorded (29). *Sarcoptes* mange are endemic and highly prevalent in red foxes in Europe and infection may reduce the population of red fox by over 70%.

Ear canker mites (*O. cynotis*) we found at 2.85%. Limited data are available on the prevalence of this infestation in free-living red foxes in Europe; nevertheless, the prevalence of infection was low to medium (2–17%) in Austria, Hungary, Czech Republic and Russia (12, 25, 28, 30).

### Conclusion

Studies of fox ectoparasite fauna have shown the presence of several species, especially ticks, which are significant vectors of significant zoonotic diseases - anaplasmosis, ehrlichiosis, etc. They also pointed to the potential role of foxes, especially those living in suburban environments around large cities, in the spread of certain species of ectoparasites to dogs (primarily hunting and non-owner animals) and to green areas (hunting grounds, parks, picnics).

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## **FOOD FRAUD A NEW "CONCEPT" IN FOOD COUNTERFEITING**

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### **Summary**

Acts of food fraud by adding dangerous substances to replace real food and also pose a real danger to the health of the final consumer have become more common nowadays, leading to a significant worldwide scandal. Experts from the Food Fraud Database estimate that 10% of the food found on the markets of developed countries are counterfeit, some of them being found most frequently on their blacklist (chocolate, fish, meat, wine, olive oil, cheese, honey, saffron, vodka, etc.). Food fraud has caused a great deal of controversy around the world, leading to taking and strengthening measures to prevent it with regard to the food chain. Precisely to protect consumer health, corporations and food safety authorities consider that this issue must be taken seriously as long as for food markets easily to counterfeit are built, thus misleading the consumer and endangering his health. The food management and safety system was not designed to prevent the fraud of some food products. The prevention of food fraud can be done by introducing in the units of systems that can manage in addition to the physical, chemical and biological dangers also the possibility of food fraud, thus taking into account the patterns of fraud of a particular food product and the measures that may be subsequently taken to prevent the phenomenon of counterfeiting them and misleading the final consumer.

**Keywords:** Food fraud, Vulnerability Assessments, VACCP, counterfeit

Committing food fraud has become a hotly debated topic given that it is about food. By definition, food fraud is meant a fraudulent and intentional substitution, dilution or addition to products or raw materials, or misrepresentation of products or materials for the purpose of financial gain, by increasing the apparent value of the product or reducing the cost of production (14).

A few decades ago, in ready-made or ready-to-cook foods, the more expensive components that were part of them were replaced with cheaper ones, so the food that reached the consumer's table posed a real danger to his health. In 2013 in Great Britain a scandal broke out because in the composition of a lasagna product, beef was replaced with horse meat in 60% or in some cases even 100%. The same problem was identified by the inspectors of the control authority in Hungary, where hamburger beef was partially or completely replaced by horsemeat. Also in the category of counterfeit food are poor quality basic materials, cheaper repackaged products, changed shelf life, spoiled products that are processed, counterfeit original products, and the sale of non-biological products as organic products. According to experts, food counterfeiting is an increasing problem on the world food market. Nowadays, of the total number of counterfeit

items, about 10% are food products and of the total food sold between 1% and 3% are counterfeit. Globally, all sorts of methods are being tested, in order to sanction food counterfeiters (3).

According to specialists, reducing the amount of counterfeit products has a major role for consumers and companies in the food industry. These fakes are not only a significant economic damage, but also an invaluable risk to public health in terms of food security. If issues related to food safety and quality of agri-food products are regulated by both European and national legislation, for the authenticity of agri-food products, there is no regulated framework: there is no validated definition, conditions, characteristics, procedures, contraventions, sanctions. This shortcoming favours the potential counterfeiting, the fraud of agri-food products throughout the production-marketing chain.

In this context, inadequate and incomplete regulation, which could provide a favorable environment, has contributed to the problem of the presence on the market of non-authentic, counterfeit food, with multiple effects on both consumers and the state. Consumers may refuse foods that are not organoleptically and visually compliant, as well as products whose modified microbiological, physicochemical, organoleptic and/or toxicological characteristics, are altered. All these features of an adnomal product like meat can be easily highlighted after laboratory investigations, finally showing a decrease of the quality of the product (6).

Consumers have the right and must refuse products with oxidised surfaces, altered odor and color and a "spoiled" look, meat preparations that have a pseudofrozen appearance (subject to thawing), products whose properties certainly have changes compared to a similar one, which has benefited from compliant conditions, products that have doubts about the quality and method of information by labeling.

Buyers have the right to expect the food they eat to be safe and suitable for consumption. The diet has undergone major changes in the last two decades, which is why new production, preparation and distribution techniques have been developed and applied. There are currently several factors that dispute its primacy in the negative influence on the quality of meat and meat preparations: chasing after higher returns and - not infrequently - the unconsciousness of producers or traders, materialized by non-compliance with technologies and changes in manufacturing recipes. Counterfeiting is and will be remain a difficult phenomenon to remove from the domestic food market, as well as from foreign markets, as long as the control of goods is not perfected. The perception of transformations produced in meat, influenced by microbiological and physico-chemical factors is facilitated by the appreciation of the morphological structure, external characteristics and chemical composition of fresh meat, particularities highlighted including inspection, taste, smell and palpation.

Counterfeiting of food or other products - consists in the act of a person preparing counterfeit food or drink, altered or prohibited for consumption, harmful



to health, exposing, for sale, or selling such products, knowing that it is counterfeit or altered or prohibited for consumption (6). It is assimilated to the food or drink counterfeiting, the counterfeiting or substitution of other goods or products, if by counterfeiting or substitution they have become harmful to health. It is also assimilated with the counterfeiting of food the placing on public consumption of meat or meat products from slaughtering animals removed from veterinary control, if it has resulted in a person's illness.

On the Romanian meat market, currently, traders and producers of goods enjoy the freedom of action. In counterfeiting, food substitution is all the more dangerous as its effects are not limited to consumer fraud, but endanger their health and lives (1).

The successful attempt to deceive the buyers through such procedures represents, from a legal point of view, crimes that (if they are proved by veterinary forensic expertise) fall under the criminal law. In the case of foodstuffs, fraud involves "the addition of any natural or synthetic substance to products, for the purpose of modifying or conferring properties, which the products do not justify by their natural composition and by the rules of manufacture".

In veterinary forensic practice, a matter of acute interest is the possibility of identifying, before release for public consumption, unauthorized additions, counterfeits and substitutions of food of animal origin and - in particular - meat preparations: sausages, salami, minced meat with/without spices, small, (semi) preserves (7, 8).

The most dangerous and frequent frauds of this kind are committed in the private area of the sector and in the clandestine meat trade, introducing into the diet of consumers:

- Spoiled meat, whose condition is camouflaged by transformation and processing in the manufacturing process, by the addition of odorants, spices, preservatives and dyes.
- Meat from species for which the origin (horse, nutria, etc.) must be explicitly and clearly visible and mentioned.
- Meat from inedible species (fox, cat, dog, etc.).
- Meat from dead animals, slaughtered in agony or in particular physiological conditions, making them unsuitable for slaughter and consumption.
- Meat from sick animals, the slaughter of which is prohibited by law.

The main forms of fraud are adulteration, counterfeiting, health fraud and substitution. Frauds are anthropogenic actions, intentions and acts in bad faith committed in order to obtain undeserved, illicit advantages, most often accompanied by harmful effects on consumers/users. The immoral and illicit nature of these actions determines the consideration of frauds as antisocial acts, for which the law provides severe sanctions (5, 9).

The production of goods and the trade are areas where the phenomenon of fraud is very common, the result of these fraudulent operations being adulterations, counterfeits and substitutions.

More and more foods are being counterfeited lately, from spices and dairy products to frozen fish and honey. Counterfeit foods can be extremely harmful to health.

The top 10 products with the highest risk of fraud in the food sector are: olive oil, fish, organic food, milk, cereals, spices (such as saffron and chilli powder), maple honey and syrup, tea and coffee, alcoholic beverages (wine and spirits), certain fruit juices.

#### **Olive oil and olives**

Obtaining quality extra virgin olive oil takes a long time and it is expensive. On the other hand, its counterfeiting is fast, cheap and lucrative. And today, the issue of olive oil scams is affecting the whole world. Experts say that about 50% of olive oil sold in the US is counterfeit. Citizens actually consume a toxic product, combined with rapeseed oil, soybean oil and other inferior oils, and for color and flavor these oils have added beta-carotene and chlorophyll.

Considered as one of the premium oils with beneficial properties for cardiovascular health, the extra virgin olive oil is one of the most counterfeited foods today.

#### **Fish and fish products**

Salmon with the most nutritional properties is wild, a rarity in Romanian stores. The easiest way to counterfeit fish is to sell cheap species as salmon or other expensive fish.

Code species can be easily substituted among each other, with cheap ones being valuable. Sea trout - more expensive salmon, can be replaced with cheaper rainbow trout. In the case of smoked salmon, the valuable Pacific salmon is replaced by trout, from Norwegian and Scottish farms.

Crustaceans can be imitated by SURIMI (Novel food) products. Obtained from cheaper marine fish mixed with milk, caviar, food additives (dyes, gelling agents, emulsifiers) and flavors.

Sometimes, the fish thawed and kept in ice is sold as fresh fish, the identification of the fake being done by sensory testing and by laboratory analysis.

Black caviar (black ikra), but also caviar of Manchuria (red ikra), are counterfeited by replacing them with fake black roe obtained from mixtures of fat and fish meat with other food raw materials colored with synthetic dyes (E-s). Pike eggs, before being used in preparations, will be carefully examined so as not to be contaminated with tapeworm larvae. Generally, high-priced products are chosen, and cheaper surrogates are placed on the market that, in addition to being crowded with harmful E's, may also contain other dangerous substances, such as antibiotics or heavy metals. These "copies" of the original products not only endanger the health of consumers, but also bankrupt those who manufacture and sell the genuine product, usually much more expensive due to production costs.

#### **Honey**

Honey is currently the most counterfeit product, and not only on the Romanian market. It is considered that a fairly high percentage of honey on the

market is counterfeit due to the desire of producers to profit, who resort to all kinds of practices that are harmful to consumer health precisely because there is no legislation to prevent them to resort to the falsification of bee products. For example, the USA has been fighting for many years with an invasion of Chinese surrogates that no longer have anything in common with the original product, pure honey. Honey sold as acacia (expensive assortment) can actually be polyfloral or rapeseed honey, with fewer benefits. In addition, for the multiplication of honey, many producers add industrial glucose syrup, which decreases the nutritional value of the product. Another method of counterfeiting honey is to replace it 90% with sugar. This method is most often applied to acacia honey or lime because the flowers are boiled, and after obtaining the aroma, sugar is added to the water that resulted from boiling with the desired flowers. The "honey" obtained far exceeds the aromas of natural honey and is much transparent. In order to know if the honey was forged or not, we must take into account the simplest and safest method, more precisely the method of its crystallization. Each type of honey can crystallize in a shorter or longer period of time. For example, the honey that crystallizes the fastest is rapeseed honey (after 2-4 days after centrifugation), the other varieties of honey crystallize in a longer time (20-45 days), except for acacia honey, which can change shape (thickened due to low temperature), but returns to the natural shape (thinner), after remaining a few days at room temperature. Acacia honey crystallizes after about 10-18 months (13).

Another example offered by the literature is the counterfeiting of Italian products and the so-called "Italian sounding" (products presented under a brand with Italian resonance, but coming from other countries) are two constantly growing phenomena. In this way, the consumer is knowingly fooled. It has been estimated that the estimated turnover of imitations of "Made in Italy" products is approximately 60 billion Euros worldwide. Or buy a good-looking "Prosciutto italiano" that displays the Italian flag and the slogan "gustoso" on the label - to find out, if you have the patience to read the fine print, that the food comes from Germany. "Eating Italian means history, culture and tradition," said Luca Gentile, director of the ICE Bucharest Agency. "Italianized" products are also present in Romanian supermarkets: at the seminar a "Report on the finding of cases of Italian sounding in Romania" was presented, a research conducted by the Italian Chamber of Commerce for Romania among large retailers. In 9 hypermarkets, 32 products were found that imitated the style of Italian products - of which 17 dairy products, 5 boxes of pasta, 4 salamis and 6 cans. Spink and Moyer, define 'food fraud' as a collective term used to include the substitution, replacement, addition, handling, or the wrongful presentation of food, food ingredients or food packaging; or false or misleading statements about a product in order to obtain an economic gain.

In the US, food fraud is called "EMA-Economic Motivated Adulteration" and is defined as the fraudulent substitution or addition of a substance to a food in

order to increase or decrease its apparent value, production costs, in order to obtain an economic gain (4).

"Food fraud" means, as defined in a report in the US Journal of Food Science, "a collective term that includes cases of deliberate substitution, addition, handling, distortion and mislabeling of food, food ingredients or packaging," or false or misleading statements about a product in order to make money.

Food fraud is the act of deceiving buyers of food or food ingredients, regardless of their position in the agri-food chain (consumer, distributor, processor or importer), in order to obtain economic gains (10).

Fraud involves:

- Total or partial replacement of a food component with a cheaper one;
- The addition of small amounts of a natural or synthetic substance to mask the use of a lower quality food component or giving some properties which the products do not justify by their natural composition and manufacturing rules, or the excessive use of food components, packaging (e.g. ice)
- Intentional removal or omission of an authentic valuable constituent from an agri-food product;
- False or non-declarations related to the geographical, botanical origin, species or variety, of the food production process;
- Introduction of spoiled, smuggled or smuggled food in the agri-food circuit (Table 1).

GFSI Types of Food Fraud	Definition of SSAFE	Examples of GFSI/FFTT	General typer of food fraude de
<b>Dilution</b>	The process of mixing a high value liquid ingredient with a lower value liquid.	<ul style="list-style-type: none"> <li>• Products watered (irrigated) with drinking / safe water</li> <li>• Olive oil diluted with potentially toxic tea tree oil</li> </ul>	Counterfeit
<b>Substitution</b>	The process of replacing an ingredient or a part of the high-value product with another ingredient or a part of the product with a lower value.	<ul style="list-style-type: none"> <li>• Sunflower oil partially replaced with mineral oil</li> <li>• Hydrolyzed proteins from milk</li> </ul>	Counterfeit or Replacement
<b>Concealment</b>	The process of replacing an ingredient or a part of the high-value product with another ingredient or a part of the product with a lower value.	<ul style="list-style-type: none"> <li>• Pork injected with hormones to hide the disease</li> <li>• Harmful food coloring applied to fresh fruit to cover defects</li> </ul>	Counterfeit or Replacement
<b>Unapproved improvements</b>	The process of adding unknown and undeclared materials to food to improve their characteristics quality.	<ul style="list-style-type: none"> <li>• Melamine added to improve protein value</li> <li>• Use of unauthorized additives (Sudan dyes in spices)</li> </ul>	Counterfeit or Replacement
<b>Incorrect labeling</b>	The process of introducing false statements on the packaging for economic gain.	<ul style="list-style-type: none"> <li>• Expiration, provenance (uncertain origin)</li> <li>• Toxic Japanese anise marked as Chinese anise</li> <li>• Incorrectly labeled recycled cooking oil</li> </ul>	Replacement
<b>Production for gray market / theft / diversion</b>	Outside the scope of the SSAFE method.	<ul style="list-style-type: none"> <li>• Sale of excess unrepaiored product.</li> <li>• Product allocated for the US market that appears in Korea</li> </ul>	Overtaking, stealing or diversion
<b>Counterfeit</b>	The process of copying the brand, the packaging concept, the recipe, the processing method, etc.	<ul style="list-style-type: none"> <li>• Imitations of popular foods that are not produced with acceptable safety guarantees</li> <li>• Counterfeit chocolate bars</li> </ul>	Counterfeit
<p>Note:                      GFSI – Global Food Safety Initiative                      SSAFE – Safe Secure and Affordable Food For Everyone                      GFSI/FFTT – Global Food Safety Initiative: Food Fraud Think Tank</p>			

Thus, the concept of food authenticity has emerged is represented by the certain, undoubted origin and compliance with the standards and norms in force and with the documents on the label that accompanies the food. The interest in demonstrating the authenticity of a food is manifested for several reasons, including the elimination of unfair competition, the elimination of tax evasion due to the intentional omission of the declaration on the product label of certain ingredients, ensuring food safety. The criteria that define the authenticity of a food are numerous and they vary from one product to another, the most important being: geographical origin, botanical or animal origin (species/breed from which the raw material comes), the category of raw material (conventional, organic, environment-friendly, genetically modified), processing and preservation technology, year of production. Another concept refers to food integrity (or food chain integrity; in English Food Integrity) is a new concept and not yet very clearly outlined, emerged in response to major changes in the food system in recent decades.

Following the recent scandals over food fraud, the European Commission adopted on the 6<sup>th</sup> of May 2013 in Brussels a package of measures - *Smarter Rules for Safer Foods* - aimed at strengthening control over the application of health and safety standards for the whole chain. agri-food, so-called traceability. In this context, the European Commission has developed an IT tool dedicated to facilitating the exchange of information between national authorities, in order to prevent and combat situations that violate the cross-border dimension in Europe. This is the Administrative Assistance and Cooperation (AAC) system (12).

In this context, the Commission has developed an action plan to strengthen controls in the food supply chain. One of these measures was to set up a pan-European mechanism to ensure the rapid exchange of information between national authorities and the Commission on suspected cases of food fraud. This is how the European Food Fraud Network (NFF) was set up, which has been in operation since July 2013. Since its creation, the Commission has seen a significant increase in the number of exchanges of information between Member States, from 30 in 2013 to 90 in 2015, up to 180 cases of suspected fraud were analyzed (3).

The activity report of the FFN for 2014 shows that the exchanges of information on cases of fraud refer mainly to the mislabeling of products (date, addition of water or other ingredients) and to forged certificates or documents. According to the European Commission, the new WSS system will ensure the much more efficient operation of the European Food Fraud Network (2).

In the USA, the competent authorities in charge of consumer protection and food safety have imposed a series of legislative regulations on food safety, regulations that have been imposed on economic operators in the food field, but also on those dealing with food exports. Based on the information provided, economic operators need to implement a new management system on the vulnerability of food products that can be easily counterfeited or replaced, identify

vulnerable parts of food manufacturing processes and implement strategies to minimize them, by constantly monitoring and verifying this system. Unlike HACCP, the VACCP system is much more difficult to implement, the vulnerability of a product to be counterfeited being much more difficult to control (14).

**Why "vulnerability" and not "risk"?**

A vulnerability assessment is slightly different from a risk assessment; the risk is something that has happened before and can happen again, it can be quantified using existing data. A vulnerability is a "weakness" that can be exploited by someone who wants to take advantage or intends to do harm. A vulnerability can lead to a risk. Because food fraud is difficult to estimate and quantify, we use the word vulnerability and not the term risk. In addition, the use of the word "vulnerability" helps to minimize confusion in the food industry, where food safety risk assessments are routinely performed and well understood.

**Why make a vulnerability assessment?**

To protect consumers from possible counterfeiting, we need to know which foods are vulnerable to food fraud and pose significant risks to consumers. Foods that are counterfeit, modified or diluted cause direct risks to consumers through biological, chemical, allergenic contaminants or improper handling of the food during any replacement of any component with a similar composition or structure. There are also indirect risks from due to the addition of products used in the falsification of foods that are devoid of nutrients and cause chronic disease, or are carcinogenic. In order to meet regulatory requirements: the addition, replacement or dilution of an ingredient indicates a defect in the traceability of the food, which means non-compliance with the requirements of food safety regulations. To prevent financial loss: product withdrawals, recalls, prosecutions and civil proceedings usually occur from fraudulent food modification.

We are currently working on a possible implementation of a VACCP plan and taking measures to prevent and reduce food fraud. A vulnerability assessment is simply a record of what has been taken into account and how the vulnerability of raw materials or finished products has been estimated. The purpose of an assessment of the vulnerability of a food product is to understand the probability that food fraud will affect that product which may have a negative impact on the health of the consumer but also on the brand of the product. This VACCP system gives priority to strategies to prevent the occurrence of food fraud and reduce risks if they occur. The assessment of the vulnerability of food fraud is similar to a conventional risk assessment, which is based on the probability of the occurrence of the risk in relation to the consequences. In a vulnerability assessment for food fraud, the likelihood of occurrence and the consequences of food fraud occurring are represented on a risk matrix to obtain the overall vulnerability. This vulnerability assessment can be performed on raw materials, ingredients, products intermediates or finished consumer goods. It is recommended for all units in the food industry, including those operating under the GFSI (Global Food Safety

Initiative) food safety standards, including the British Retail Consortium (BRC) Food Safety Standard, FSSC 22000 and SQF (14).

To better understand the likelihood of food fraud affecting ingredients or products, it will be necessary to investigate the sensitivity of the “material” that can be replaced in the composition of a product, namely:

- ✓ Is it frequently affected by food fraud?
- ✓ Are there emerging issues that could increase the risk of food fraud for this material in the future?

Once we understand what is the sensitive point of food fraud for a particular food that can be easily counterfeited, we must consider the issues regarding the supply chain of raw materials and auxiliaries purchased, as well as the company's procurement policies. The economic operator will have to think about all the possibilities by which one of his products can be easily counterfeited and that the other components that are to enter the composition of the product or are verified and their authenticity is attested. The results it obtains will be compared with the risk matrix. If one of the components of a food product falls within the red zone of the risk matrix, it must be considered vulnerable and measures must be taken to prevent, detect and mitigate the food fraud of that food product (11) (Fig. 1).

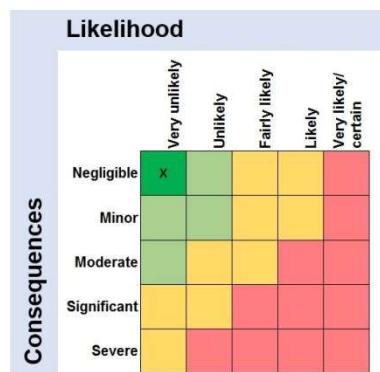


Fig. 1. Vulnerability Assessment Matrix – likelihood versus consequences  
 Red area – high risk, urgent action is required and regular monitoring may be needed, Yellow area – medium risk, action is needed with occasional monitoring to mitigate the risk, Green area – low risk

### Results obtained after the evaluation

A full assessment of vulnerability to food fraud should include the following:

- ✓ Product name: product or food ingredient;



- ✓ Date of evaluation;
- ✓ Date on which the evaluation is to be reviewed (usually one year from the date of the first evaluation);
- ✓ The name of the person (s) involved in the verification of the vulnerability plan and its evaluation the number of pages and other document control functions;
- ✓ Information on the scope that reflects the requirements of the standard, food safety management system;
- ✓ Estimating the probability that food fraud will affect the material
- ✓ Estimating the consequences of food fraud on materials
- ✓ A risk matrix with results marked in the relevant box
- ✓ A conclusion about the general vulnerability of the product, the trust, etc.
- ✓ Information showing how the estimates of probability and consequences were reached.

### **Conclusions**

Food fraud poses a significant risk and it is important that the food industry takes action worldwide.

Food law interferes with the main branches of law: criminal law, administrative law, international law, commercial law, civil law, financial law. Thus we can see that there is a new branch of law entitled "Food Law" which, unlike the others, has a certain specificity, in the sense that it consists of a set of rules, based on scientific and hygiene considerations, which establishes a certain order that protects the health of the consumer.

Food fraud is the collective term that includes the intentional substitution, intentional addition, counterfeiting or distortion of food/feed, food/feed ingredients or food/feed packaging, labeling, product information or false or misleading statements about a product for economic gain, which may have an impact on the health of consumers.

Counterfeiting is and will remain a difficult phenomenon to remove from the domestic food market, as well as from foreign markets, as long as the control of goods is not perfected.

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## DYNAMICS OF CARDIO-RESPIRATORY PARAMETERS IN SURGICAL INTERVENTIONS WITH A MINIMUM TWO-HOUR LENGTH

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### Summary

Essential elements for a perioperative evolution without complications are the existence of an anaesthetic plan and continuous monitoring. The use of anticholinergic drugs in premedication is questionable. Review of medical records of hemodynamic parameters monitored two-hour during the intraoperative period in 146 patients, did not highlight the need for anticholinergic medication use. In case of surgical interventions carried out under balanced anesthesia, the use of anticholinergics to improve hemodynamic parameters is not necessary nor indicated.

**Keywords:** hemodynamic parameters, two-hours operations, dog

The correct and tailored elaboration of an anaesthetic plan are essential elements for a case perioperative evolution without complications. The reasons for including anticholinergic medication, more specifically of the muscarinic antagonist group (atropine, glycopyrrolate) (3), in premedication are multiple. Atropine is clinically used alongside anaesthetic premedication to manage bradycardia associated with the use of anaesthetics drugs or as a result of medical procedures such as bronchoscopy, and excessive bronchial secretions associated with use of anaesthetics and opioids (4, 10, 11, 13).

In recent years, however, the use of anticholinergic products in premedication has been questioned. Currently in veterinary practices in the UK, anticholinergics are much less used in premedication, being a tendency to be used only when the situation imposes it. In the US are still commonly used (5). In our country even if there are no data on the extent of their use in anaesthesia, it raises the question whether or not their integration into daily routine, as practiced by some veterinarians, is justified. The decision to include anticholinergics in premedication should be based on the species, take into account the drugs to be used for anaesthesia, the possibility of bradycardia or vagal reflexes occurrence, and not least of the availability of intra-anaesthetic monitoring (4). Even if after the use of antimuscarinic agents, the ileus and vomiting are rare in the case of small animals (11) it should not be forgotten two important aspects. First, that atropine overdose causes a "central cholinergic effect" manifested by fluctuations between hyperexcitability and depression (4) and secondly, the fact that atropine increases

intraocular pressure and reduces the production of tears, therefore its use is contraindicated in patients with glaucoma and keratoconjunctivitis sicca (11).

The aim of this paper work was the comparative analysis of respiratory and hemodynamic changes induced by different anaesthetic drugs combination used in Surgery Clinic of F.M.V. Timișoara, alongside the identification the situations in which would have been imposed antimuscarinic use in premedication.

### **Materials and methods**

Medical records from January 2012 to June 2018 were reviewed and canine cases, ASA II or III, in which were carried out surgical interventions on both hard and soft tissues, and intraoperative monitoring was extended over two hours were included into the study.

The analysed parameters were heart rate (HR), respiratory rate (RR), oxygen arterial saturation of haemoglobin (SaO<sub>2</sub>), systolic (SAP), diastolic (DAP) and mean blood pressures (MAP). Patient monitoring was performed using Cardell 9405 Monitor and PO100 Pulsoximeter.

For data processing, the values of these parameters were taken into account at 30 minutes intervals, starting with the onset of the operation. Statistical analysis was done after grouping patients into three age groups (patients ≤ 1 year, > 1 year and ≤ 10 years and > 10 years), differentiated by gender (male and female), and finally differentiated by types of operations (soft versus hard tissue surgeries).

To assess statistical significance the program Minitab (one way Anova) was used for the comparison of age groups, and T-Student test for comparing gender and types of surgeries groups. Significant differences were considered for p-values ≤ 0.05 and very significant for p ≤ 0.001. The resulting data were expressed as mean ± standard deviation.

### **Results and discussions**

The inclusion criteria in this study met a number of 146 cases (Table 1) to which 24 different anesthetic combinations were used including phenothiazines, benzodiazepines, alpha-two adrenergic agonists, cyclohexamines, alkylphenol derivatives, opioids and halogenated anesthetic gases, without any anticholinergic medication.

Mean HR values did not record significant differences between age categories. In all groups the initial values are higher than those subsequently recorded during the monitoring period, in age group under one year being observed the highest average value (Fig. 1) phenomenon not surprising. In dogs or cats it is recommended to administer atropine or glycopyrolate if the heart rate decreases below 90 beats/minute, for elderly patients being preferred glycopyrolate (14). In any case, when a bradyarrhythmia occurs before the administration of atropine or any other antiarrhythmic drugs, should be eliminated other possible triggering causes for

unusual heart rhythm, for example, too deep level of anesthesia, hypoventilation, hypercapnia or hypoxemia, on which it will act (1).

Table 1

**Casistry included in the study (number of cases)**

		Age category			TOTAL
		≤ 1 year	>1 year and ≤ 10 years	>10 years	
Soft tissue surgeries	♂	5	21	8	34
	♀	7	46	14	67
TOTAL		12	67	22	101
Hard tissue surgeries	♂	8	16	5	29
	♀	9	7	-	16
TOTAL		17	23	5	45

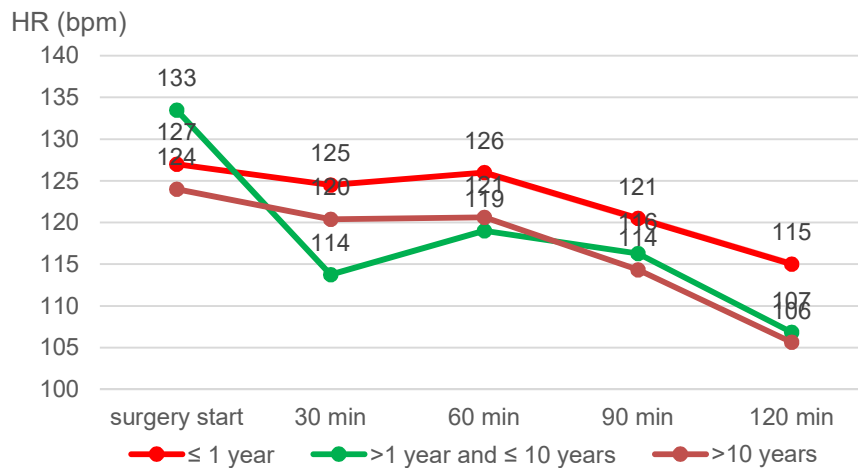


Fig.1. Heart rate curves for the three age categories

The values of SaO<sub>2</sub> were in all age groups in the limit considered normal, over 98%. Transient decreases, without reaching the threshold of hypoxemia, were recorded in animals belonging to the second age category (97,92±0,84% at the beginning of the operation, 97,97±0,81% at 30 minutes), being statistically significant (p = 0.005) only at the onset of surgery.

Blood pressure values in all age categories were within the limits considered

normal under anesthesia close to the level exhibited by the patient prior to anesthesia. Stabilization of blood pressure takes approximately half an hour and insignificant transiently oscillations throughout the monitoring period were recorded (Fig. 2).

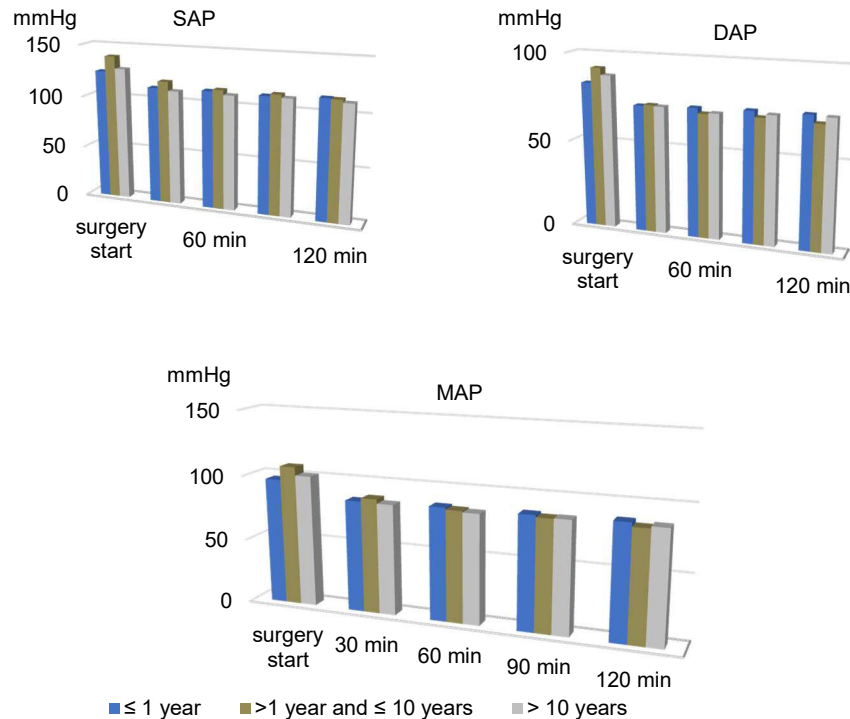


Fig. 2. Dynamics of blood pressure values by age categories

Generally lower values in patients over 10 years of age are compensated by the HR increase, which was in some periods above that recorded in second age group (Fig. 1). On the other hand, the values of HR in this age category can also be explained by the fact that represents one physiological compensatory mechanism in response to bleeding and to vasodilation induced by anesthetic. In the face of hypotension age diminishes autonomic responses, so geriatric animal increases its cardiac output by increasing HR, stroke volume and left ventricular end diastolic volume (7). Some drugs, particularly alpha-2 adrenergic agonists and high-dose of opioids, can cause drug-induced vagal bradycardia (4). Therefore, atropine is used to prevent bradycardia caused by the administration of alpha-2 adrenergic agonists in dogs and thus counteract cardiac output reduction produced by these drugs (8,

9). In advanced geriatric animals, alfa-2 adrenergic agonist will be used with caution because of numerous side effects and patients' diminished cardiovascular reserve. So, all these things were taken into account when anesthetic combination was chosen for this category of patients.

In terms of differences between genders, there were no significant differences between SaO<sub>2</sub>, RR and HR values. It was noted that blood pressure values are significantly higher in males in the second part of the monitoring period (Fig. 3).

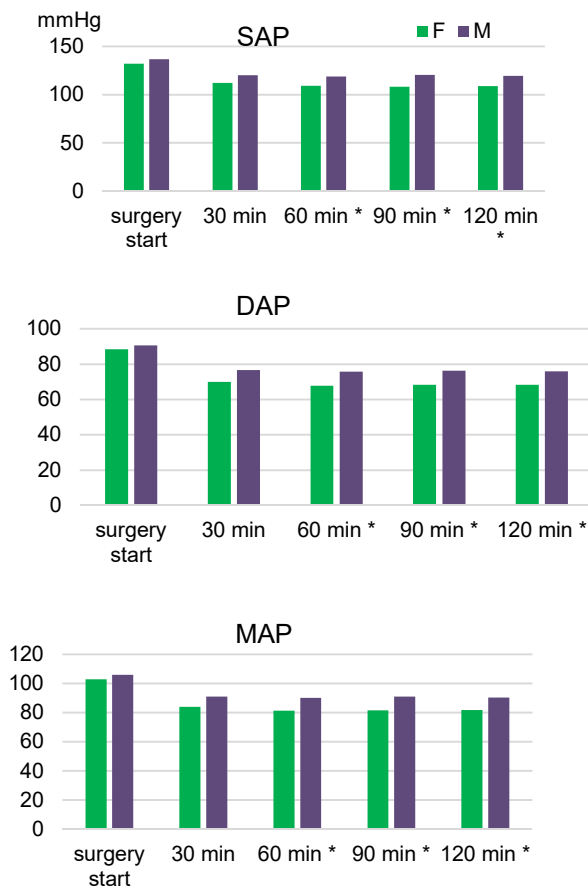


Fig. 3. Comparative representation of blood pressure values (SAP, DAP and MAP) in the two sexes

This data could be explained only if males would belong to small breeds and females were framed in large dog breeds, which cannot be confirmed in this study. There are numerous researches confirming the differences in blood pressure related to the size of breeds (2) and studies indicating that there are no differences in blood pressure dynamics between males and females belonging to the same breed (6, 12).

When the patients sharing criterion was the type of operation, it was observed that with regard to HR did not result significant differences, except for the minute 30, when the mean values in surgeries involving hard tissue were significantly higher. Tachycardia can be a response to surgical stimulation and, in combination with other factors, may indicate inadequate anesthetic level and/or insufficient analgesia. In any case, the response to surgical stimulation is normal (16), but it must be controlled. Despite the fact that handling of long bones during the treatment of fractures, traction on the peripheral nerves, or traction on the abdominal organs can produce vagal stimulation with pronounced bradycardia (15), in our study this was not observed. The reason may be the large time interval between observations, respectively analyzed data. We chose to analyze only these values, because we focused on tendency not on absolute values of one parameter.

SaO<sub>2</sub> values increases towards the end of the monitoring period, without significant differences between the first and last moment of monitoring (Fig. 4). Absolute values denotes a good blood oxygenation, mainly explained by the fact that in these patients, with the exception of six cases, anaesthesia was maintained by inhalation and therefore oxygen was supplied in high concentrations throughout the operation.

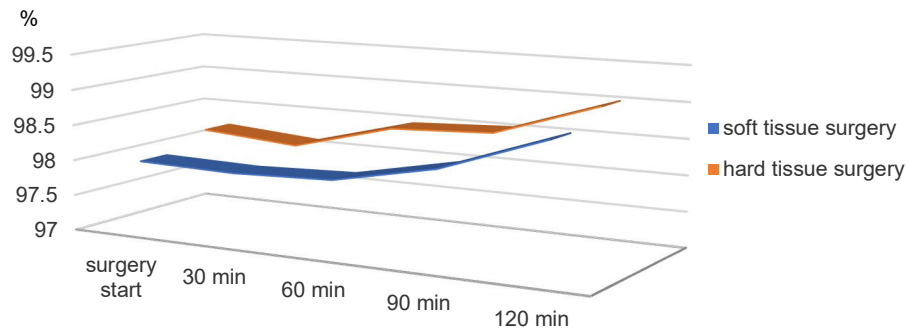


Fig. 4. Comparative (soft versus hard tissue surgery) curves for SaO<sub>2</sub>

The RR correlates with the HR, being increased in orthopedic interventions. Overall, however, it remains constant, close from baseline recorded prior to



anesthesia and without significant statistical differences between the two types of surgical interventions.

Both systolic and diastolic blood pressures have higher average levels in orthopaedic surgery, despite the fact that majority of these patients (those subjected to fracture repair) are often hypovolemic due to occult blood loss. The fact that also in the case of interventions on soft tissues the blood pressure values are maintained within normal levels, denote that intraoperative fluids manages to compensate the comparatively higher volemic loss (in those with malignant soft tissue tumors resection) and the fact that the chosen anaesthetic combinations influences minimally autonomic responses and cardiovascular adaptation capacity. Overall, blood pressure values are higher at the onset of surgery and in the first 30 minutes. At any time of monitoring, no significant differences between the two types of interventions were recorded.

### Conclusions

Although there are various combination of drugs used for anesthesia in the Surgery Clinic of FMV Timișoara, the dynamics of cardio-vascular function reveals that anesthetic protocols are adapted to the procedure and the patient.

All monitored parameters fall within the limits considered safe for this species under anesthesia conditions.

In case of surgical interventions carried out under general anesthesia, as practiced in the surgery clinic, the use of anticholinergics to improve hemodynamic parameters is not necessary nor indicated.

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## **SURGICAL REPAIR OF A STRANGULATED UMBILICAL HERNIA IN A 6-MONTH-OLD LARGE WHITE PIG USING V-LOC BARBED SUTURE: CASE REPORT**

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### **Summary**

Umbilical hernia in pigs is a rather common condition, often evolving without complications. In certain cases however, complications do occur, leading to changes in the animals' general condition. Although most cases evolve without complications, young pigs with umbilical hernia are either sold cheaper or culled. This paper reports the treatment and outcome of a client-owned underdeveloped 6-month-old Large White female pig with a large strangulated umbilical hernia, which was repaired using V-Loc™ barbed suture.

**Keywords:** piglet, strangulated umbilical hernia, barbed suture

A hernia represents an ectopic displacement of organs or tissues through a physiological or pathological opening of an adjacent wall (8, 10, 11, 26), and is comprised of a hernia sac (26, 29), ring (26, 29), content (26, 29) and layer (10). Different criteria are used to classify and name a hernia, such as etiology, region, content, response to reduction, size and evolution (10, 11, 26). While with simple hernias, patients may not show any clinical signs, complicated hernias are considered to be surgical emergencies as the general state of the patient is altered (8). In the latter, clinical signs are both organ-specific, as well as non-specific signs (e.g., refusal to walk, anorexia, vomiting, weight loss, etc.) (10).

In umbilical hernias, abdominal organs herniate via the umbilical ring (8). These hernias are, in most cases, congenital and are often associated with other anomalies; hence a careful examination of these patients is recommended (8, 10, 18, 26, 29). In fetuses, the umbilical ring allows passage for the umbilical cord, but after birth it normally closes, as the components of the umbilical cord transform into the falciform ligament and umbilical ligament of the urinary bladder (10). Complicated hernias may be strangulated – occlusion is secondary to small size of the umbilical ring or incarcerated – occlusion is as the level of the hernia sac; these hernias are typically firm and painful (10).

Diagnosis of umbilical hernia is based on clinical signs. When dealing with complicated umbilical hernias, imaging diagnostics is recommended in order to identify possible complications associated with trauma (29).

Surgical treatment – herniorrhaphy - consists in replacing displaced organs into their anatomical position, removal of irreversibly damaged tissues and closure of the defect (8). However, there are various surgical options that mostly depend on the type of hernia. In small, reducible hernias the skin incision is longitudinally made on the swelling with repositioning of the hernia sac and its contents inside the abdomen (8, 10, 11).

With non-reducible hernias, kelotomy – opening of the hernia sac – is performed and the umbilical ring is enlarged or adhesions are removed. If there is excess hernia sac, it is resected after placing sutures at its base. If a large umbilical hernia is reducible, the contents are replaced in the abdomen by twisting the hernia sac (10, 11).

Closure of the abdomen can be made with horizontal mattress sutures (10, 11), simple continuous suture (8) or purse-string suture (10, 11).

Large umbilical defects can be repaired using implants (10, 11, 26), or by using the Olivkov method – utilizing the hernia sac to cover the large defect (10, 11). Subcutaneous tissue and skin are closed routinely.

Castration is recommended in patients with congenital umbilical hernias (26). One study showed that the CAPN9 gene could be involved in umbilical hernias in pigs (13).

Umbilical hernias are quite common in pigs, ranging from 0.78% to 6.7% (3, 14, 15, 20, 22), and pose economic disadvantages, due to the fact that such animals may have lower performances and market value (3, 17, 27), hence these are either sold at a lower price or are being culled (3, 14).

### **Materials and methods**

Our patient was a 6-month-old Large White female piglet, weighing only 10 kg, which was presented for a swelling in the umbilical region, which she had since birth, and a 3-day-old altered general state. Due to its smaller size and body weight, the owners kept the piglet as a family pet, instead of culling her, as indicated by their local veterinarian. For the past 3 days she was anorexic, hadn't defecated and was adynamic and reluctant to play.

On presentation, the piglet was reluctant to move, to stand for longer than 1 minute, she was apathetic and hypothermic – 37.9°C. The swelling in the umbilical region was firm on palpation, painful, non-reducible; the skin covering the swelling was pink-reddish, with a large erosion on its most ventral aspect. The size of the swelling,

compared to the overall size of the patient, was large, measuring 6.5 cm in height, 5 cm wide and 6 cm long (Fig. 1). No paraclinical examinations were accepted by the owner, therefore a diagnosis of strangulated umbilical hernia was established. As this was a complicated hernia, with the owner's accept the piglet was taken into emergency surgery.

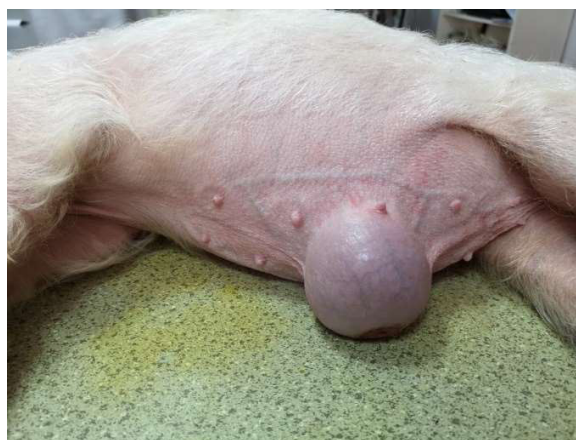


Fig. 1. The patient in lateral recumbence, revealing the large umbilical hernia

Preparation of the surgical site was classic as for abdominal surgery. Antibiotic therapy began 30 min prior to anaesthesia, continued for 10 days postoperative, and consisted in intramuscular administration of Ampiplus 1000 mg/500 mg (1000 mg/ml ampicillin and 500 mg/ml sulbactam) – Antibiotice S.A. at a dose of 10 mg/5 mg/kg, t.i.d.

Anaesthesia consisted of premedication using a combination of Xylazine (2 mg/kg IM) and Ketamine (20 mg/kg IM) followed by induction with Propofol (1 mg/kg IV), and maintenance on Isoflurane 2-3 % vaporized in oxygen using intermittent positive pressure ventilation via a facemask throughout the surgery.

The incision was performed directly on the swelling. The umbilical ring was very narrow and we had to incise it in order to gain proper access inside the abdominal cavity; the final incision was 10 cm long. Kelotomy was also performed and revealed adhesences between small intestinal loops and the hernia sac. The herniated intestinal loops were partially necrotic and very friable; upon attempting to dissect the adhesences, some necrotic portions of the intestinal loops were ruptured, but no

intestinal content was spilled inside the abdominal cavity, as this was isolated using sterile pads (Fig. 2).



Fig. 2. A segment of non-viable small intestine that ruptured whilst dissecting the adhesences between it and the hernia sac; non-crushing intestinal forceps were placed in order to occlude the lumen

The entire abdomen was inspected and no other abnormalities were identified. The non-viable portions of the small intestine were isolated and a segment of approximately 10 cm of small intestine was resected. Non-crushing intestinal forceps were used to occlude both ends of the healthy intestinal segments and crushing forceps were placed closer to the affected segment. The blood vessels that supply the affected segment were double ligated, and transection was performed between the double ligatures and was perpendicular on the small intestine. Everting mucosa was severed and the intestinal ends were aspirated of any chime or debris. Anastomosis was performed using a Cushing pattern, using 3-0 Vicryl – Ethicon; the same suture material was used to ligate the blood vessels and to appose the mesentery (Fig. 3).



Fig. 3. Aspect of the enteroanastomosis - Cushing pattern

After completing the anastomosis, we checked for leakage by distending the lumen with sterile saline, after digitally occluding the intestinal lumen adjacent to the anastomosis site; one leakage site was identified between the entry points of the Cushing suture, and was closed using a simple interrupted knot. The anastomosis site was then lavaged with warm sterile saline and placed back into the abdomen.

The edges of the umbilical ring were resected, alongside any remaining adhesions. Closure of the muscle layer and *linea alba* was done using 2-0 V-Loc™ unidirectional barbed suture – Covidien (Fig. 4a). Suturing time was 3 min and began by passing the needle through the prefabricated loop, continuing in a simple continuous pattern, and ending the suture without tying a knot, but by simply cutting the material flush with the tissue (Fig. 4b and 4c). Closure of the subcutaneous layer was done in an inverting pattern – 4 min, and the skin was closed in simple interrupted sutures – approximately 6 min.

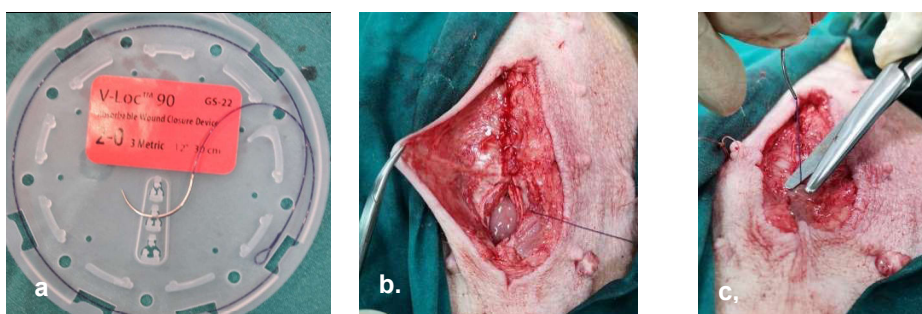


Fig. 4. a. 2-0 V-Loc™ unidirectional barbed suture – Covidien; b. simple continuous pattern of the muscle layer and *linea alba*; c. end of the suture, with cutting the suture material flush with the tissue

Recovery from anaesthesia was uneventful and for the next 10 days the patient was managed at home. Semi-fluid food was placed *ad libitum* as soon as she fully recovered from anaesthesia; the patient began eating the next day. After 2 days of semi-fluid food, the piglet was gradually returned onto her normal feed. At 10 days postoperative we presented at the owner's home for follow-up and to remove the skin sutures (Fig. 5); at the time of the follow-up, the piglet had gained 0.8 kg body weight. No issues were reported by the owner, nor were observed at the time of the follow-up.



Fig. 5. Postoperative aspect of the healed surgical site at 10 days



### **Results and discussions**

Barbed sutures were studied in veterinary surgery in multiple studies, covering different branches – orthopaedics (4, 5, 12, 16, 19, 28), gynaecology (7, 9), while other authors examined tissue reactions to this new type of suture material (1, 2, 6, 21, 25).

Unfortunately, *in vivo* studies are few, and are needed in order to establish in which situations barbed sutures may be considered as alternatives to conventional suture materials. *In vivo* studies published by the present authors have shown promising results (23, 24).

Barbed sutures pose the disadvantage of a higher purchasing price, as compared to conventional sutures, and may not be profitable in farm animals, except those of high value, since animals with defects such as umbilical hernias are culled or sold at lower prices (3). In our case, the owner agreed on treating the piglet using a barbed suture material, regardless of the cost, since the piglet had an emotional significance for the owner and the owner's family. Nevertheless, she did not agree on neutering the piglet, but understood the importance of not breeding it, and agreed on it.

Enterectomy and anastomosis were accomplished using conventional materials and the same techniques as used in small animal surgery, and were uneventful. Closure of the abdomen was facile and fast using the V-Loc™ barbed suture; apposition and tension in the surgical wound were better, as compared to conventional suture materials, with no need of an assistant to maintain tension in the wound. The uniformly distributed barbs alongside the thread ensured an adequate anchoring in the tissues and proper tension in the surgical wound, and require no knot tying (30).

Although the piglet gained considerable weight in the immediate postoperative period, it remained of considerable smaller size and body weight, as compared to the other piglets in the family household.

### **Conclusions**

In this particular clinical case, V-Loc™ barbed suture showed optimal performances in repairing a large strangulated umbilical hernia. No complications were observed during the follow-up. In the 13 months postoperative, no complications were reported by the owner.

We recommend the use of barbed suture materials while repairing large defects, in which tension is great and apposition of the wound edges may pose difficult, and with a risk of dehiscence.

Further studies, especially *in vivo*, are still needed to assess and identify situations where the use of barbed sutures is indicated.

### Acknowledgement

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## BACTERIOLOGICAL AND THERAPEUTICAL RESEARCH IN DOG'S OTITIS EXTERNA

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### Summary

In the paper are presented the results obtained after the investigations carried out on a number of 56 dogs, that were examined clinically in order to detect the lesions that denote diseases of the external auditory canal, following the bacterial flora involved in the pathological process. The investigations carried out with the help of the bacteriological examination allowed to isolate from the auricular secretions of the investigated dogs two species of staphylococci: *Staphylococcus pseudintermedius* and *Staphylococcus aureus*. The two isolated species could be differentiated relatively easily, based on specific morphological characteristics and the fact that *Staphylococcus pseudintermedius* can be isolated only from dogs. On the usual media, *Staphylococcus aureus* strains produced golden-yellow colonies, and *Staphylococcus pseudintermedius* strains produced creamy, bulky, glossy and unpigmented colonies with a whitish tint. The research done to establish the therapy, had as study object the sensitivity test against 10 antimicrobials. The staphylococcal strains studied had variable behavior, following the *in vitro* interaction with the 10 antimicrobials for which antibiograms were performed. The results of the antibiogram show that the antimicrobials introduced relatively recently in the therapy of dogs, have been found to be sensitive to most staphylococcal strains isolated from dogs. The maximum susceptibility (96.42%) was obtained with respect to ciprofloxacin. Maximum resistance was obtained from doxycycline, while no strain was resistant to cefaclor.  
**Keywords:** dogs, otitis externa, *S. pseudintermedius*, antibiogram, sensitivity/resistance

Otitis externa plays an important role in canine pathology, being one of the most common reasons why dogs are presented in the veterinary office. The importance of this condition derives from the fact that it affects a large number of dogs, while raising special problems in terms of therapy.

Otitis externa is defined as an acute or chronic inflammation of the epithelium of the external auditory canal that may include the auricle. This condition is characterized by erythema and accentuation of scaly epithelium with gradual variations in pain and itching (4).

The conditions that lead to the disease result from the combination of dynamic changes that affect the anatomical, physiological and microbiological status of the external auditory canal. Among the predisposing factors of otitis externa in dogs are: conformational variations in breeds, predisposition; increase in the number

of ceruminous glands; high, abnormal and chronic moisture of the external auditory canal (5, 6).

According to studies, the incidence rate of otitis externa is around 12.6% in dogs with dangling ears and only 5% in dogs with erect ears (2).

One of the most common causes associated with otitis externa in dogs is staph infection (3).

In medical-veterinary practice with a fairly approximate value, it is considered that *S. intermedius* is the etiological agent isolated from ear secretions in dogs (7, 9).

Success in the management of otitis externa depends on the understanding of the primary, predisposing and perpetuating factors involved in the pathogenesis process (1).

Otitis externa is a complicated condition, difficult to diagnose and treat, due to the unique physiology of the ear canal and the common pathophysiology of otitis externa, regardless of the cause (8).

### **Materials and methods**

The research was performed on a number of 56 dogs that presented at the clinical examination of the outer ear pruritus, frequent shaking of the head, pain on palpation of the ear, abundant secretion of different consistency, pungent odor and erythema.

Samples for bacteriological examination were taken using sterile cotton swabs attached to a wooden rod, after a preliminary examination of the external auditory canal using an otoscope.

After collection, the samples were placed in sterile tubes. If the bacteriological examinations and the processing of the samples could not be performed immediately, the samples were kept in the refrigerator for 24-48 hours, until their processing in the laboratory of the Infectious Diseases and Preventive Medicine laboratory, within FMV Timișoara.

For etiological diagnosis, to be able to identify the existence of bacteria, the inoculations were performed on agar and nutrient broth, after which, for 24 hours, they were left on the thermostat at 37°C, to be processed and interpreted.

Antibiograms were also performed for 56 strains isolated from dogs undergoing clinical examination, diagnosed with otitis externa, using 10 antimicrobials that belonged to several classes of substances (Table 1).

Antibiotic behavior was tested by the disc-diffusimetric method (Kirby-Bauer method), using for this purpose the Mueller-Hinton medium and antibiotic biodiscs provided by the manufacturing companies.

Table 1

**List of antimicrobials for which the antibiogram was performed**

No.	Antimicrobial used	Abbreviation - tablet concentration
1.	Ciprofloxacin	CIP - 5 $\mu$ g
2.	Gentamicin	GM -10 $\mu$ g
3.	Tetracycline	TE - 30 $\mu$ g
4.	Kanamycin	K -30 $\mu$ g
5.	Doxycycline	DO-30 $\mu$ g
6.	Erytromycin	E-15UI
7.	Lincomycin	MY - 10 $\mu$ g
8.	Cefaclor	CEC-30 $\mu$ g
9.	Ampicillin	AMP - 10 $\mu$ g
10.	Amoxicillin	AMC- 30 $\mu$ g

**Results and discussions**

Research shows that the diagnosis of otitis externa in dogs is easy to establish based on clinical examination of the outer ear. In all cases of otitis externa in the dogs studied, staphylococci were also incriminated, the prevalence of staphylococcal infections in dogs with otitis externa was 100%, given that the bacteriological examination isolated and identified 56 positive samples, out of 56 examined samples.

Bacteriological examination revealed that in cases of otitis externa in dogs are involved staphylococci, which we initially considered to be *Staphylococcus pseudintermedius* (50 samples), but which later, some strains turned out to be *Staphylococcus aureus* (6 samples).

On common media, *Staphylococcus aureus* strains produced golden-yellow colonies (Fig. 1), and *Staphylococcus pseudintermedius* strains produced creamy, convex, glossy, unpigmented colonies with a whitish tint (Fig. 2).

From all isolated strains, smears were performed that revealed the presence of irregular piles of Gram-positive staphylococci (Fig. 3).

Given that 6 of the 56 strains of staphylococci isolated from atrial secretions in dogs with otitis externa were *S. aureus*, which represents 10.71%, we consider that these results are similar to the data in the literature, in which it is stated that 10% strains of *S. aureus* can be isolated from skin conditions in dogs, the difference being *S. pseudintermedius* (7).

Antibiotic susceptibility testing was performed in order to determine the therapeutic conduct of the clinical cases presented at the veterinary office, highlighted in the present study.

The results obtained after performing the antibiogram were processed, interpreted and are presented below.

In Fig. 4 the result of an antibiogram after 24 hours of incubation is presented.



Fig. 1. Cultural aspects of *S. aureus*

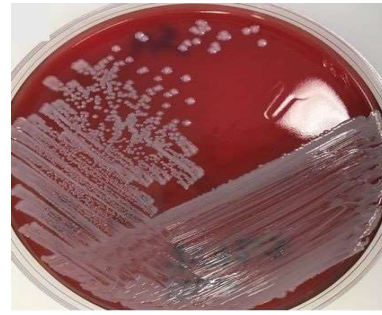


Fig. 2. Culture of *S. pseudintermedius*

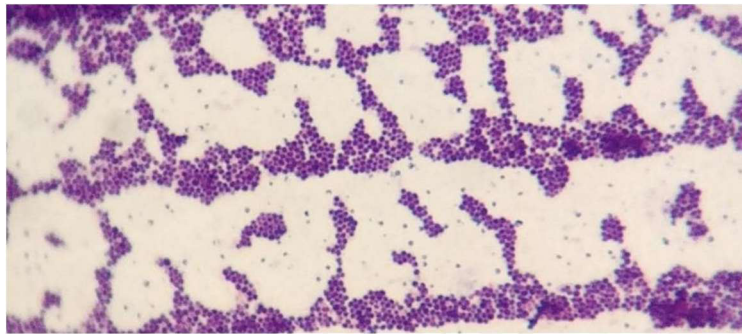


Fig. 3. Staphylococci in smear, stained by Gram method



Fig. 4. Antibiogram



The results obtained from the antibiotic susceptibility test for 56 strains of staphylococci isolated from dogs are shown as absolute values in Table 2, and in Table 3 the results are shown as relative values.

Table 2  
**Antimicrobial sensitivity in 56 staphylococcal strains isolated from dogs with otitis externa**

Crt.no	Antimicrobial name	Category	No. strains
1.	Ciprofloxacin	Sensitive	54
		Intermediate	0
		Resistant	2
2.	Gentamicin	Sensitive	52
		Intermediate	1
		Resistant	3
3.	Cefaclor	Sensitive	51
		Intermediate	5
		Resistant	0
4.	Kanamycin	Sensitive	49
		Intermediate	0
		Resistant	7
5.	Ampicillin	Sensitive	46
		Intermediate	4
		Resistant	6
6.	Lincomycin	Sensitive	38
		Intermediate	10
		Resistant	8
7.	Erythromycin	Sensitive	26
		Intermediate	29
		Resistant	1
8.	Amoxicillin	Sensitive	28
		Intermediate	3
		Resistant	25
9.	Doxycycline	Sensitive	17
		Intermediate	9
		Resistant	30
10.	Tetracycline	Sensitive	15
		Intermediate	13
		Resistant	28

Analyzing the results obtained, shown in Table 2, it is observed that compared to the less frequently used antimicrobials (ciprofloxacin, gentamicin, cefaclor), or recently introduced in pet therapy, most staphylococcal strains, isolated from dogs with otitis externa were proved to be very sensitive.

The results obtained in the present study were similar to those obtained by Kicuchi et al. (5). They were resistant to tetracyclines, to 60% of the isolated strains, to 32% resistance to erythromycin, and to lincomycin 25% of the isolated strains were resistant (8).

Table 3

**Distribution of antibiotic susceptibility to staphylococcal strains isolated from dogs with otitis externa**

Antimicrobial used	The response of staphylococcal strains to antimicrobials		
	Sensitive %	Intermediate %	Resistant %
<b>Ciprofoxacin</b>	96.42	0	3.57
<b>Gentamicin</b>	92.85	1.78	5.35
<b>Cefaclor</b>	91.07	8.92	0
<b>Kanamycin</b>	87.5	0	12.5
<b>Ampicillin</b>	82.14	7.14	10.71
<b>Lincomycin</b>	67.85	17.85	14.28
<b>Erytromycin</b>	46.42	51.78	1.78
<b>Amoxicillin</b>	50.00	5.35	44.65
<b>Doxycycline</b>	30.35	16.07	53.57
<b>Tetracycline</b>	26.78	23.22	50.00

We consider that the sensitivity to antimicrobial substances, for staphylococci isolated from otitis externa in dogs, is due to the fact that staphylococcal strains that were not previously exposed to antibiotics were sensitive to these substances, instead, strains isolated from dogs with previous antimicrobial treatments, subjected to pressure on antibiotics, due to therapy, may present the phenomenon of resistance.

In Fig. 5 and 6 show the antimicrobial behavior (Fig. 5 shows sensitivity, Fig. 6 shows resistance) for 56 strains of staphylococci isolated from dogs.

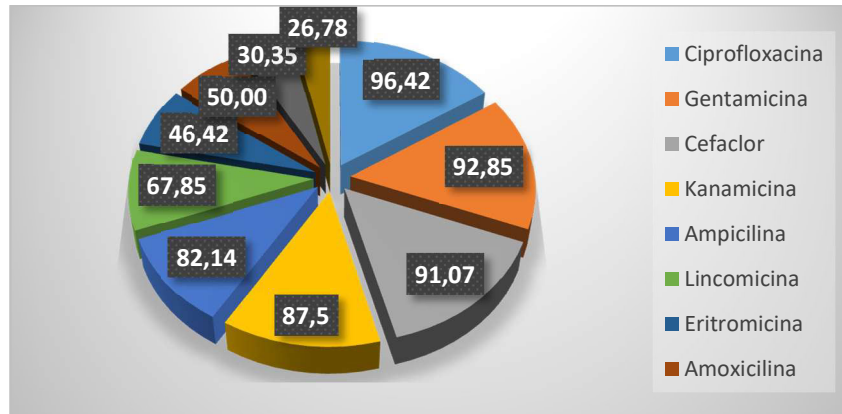


Fig. 5. Distribution of antimicrobial sensitivity

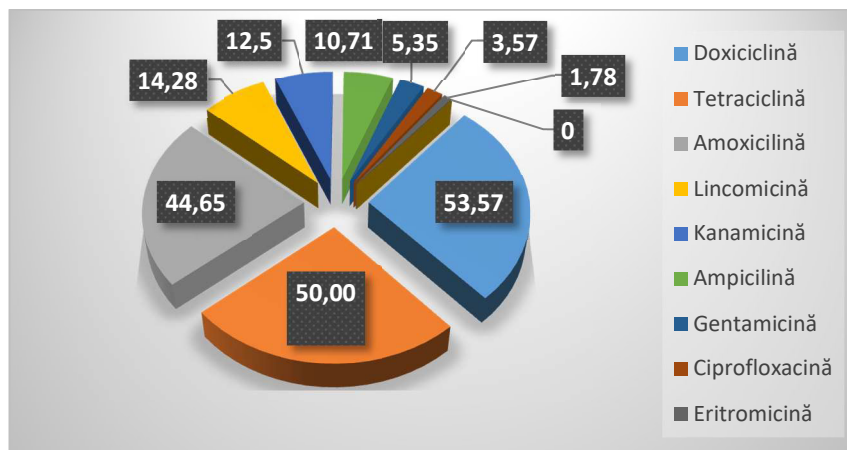


Fig. 6. Distribution of antimicrobial resistance

From the analysis of the data presented in Fig. 5, it results that for the antimicrobial therapy of otitis externa in dogs is recommended as a first intervention antimicrobial one of the 4 antimicrobials (ciprofloxacin, gentamicin, cefaclor and kanamycin) whose sensitivity was over 85%.

From the analysis of the data presented in Fig. 6, it results that for the antimicrobial therapy of otitis externa in dogs it is desirable to avoid antimicrobials with high resistance, such as one of the 3 antimicrobials. (doxycycline, tetracycline, amoxicillin) for which an increased resistance was found, with values between 44-54%.

### Conclusions

The results of the present study show that staphylococci were isolated from dogs with otitis externa in all 56 samples taken.

Bacteriological examination allowed the isolation of two species of staphylococci from the ear secretions of the dogs investigated: *Staphylococcus pseudintermedius* and *Staphylococcus aureus*. The two isolated species could be differentiated relatively easily, based on specific morphological characteristics and the fact that *Staphylococcus pseudintermedius* can be isolated only from dogs.

The staphylococcal strains studied had variable behavior, following the in vitro interaction with the 10 antimicrobials for which antibiograms were performed.

The results of the antibiogram show that antimicrobials introduced relatively recently in the treatment of diseases in dogs, have been shown to be sensitive to most strains of staphylococci isolated from dogs.

Maximum susceptibility (96.42%) was obtained with ciprofloxacin.

Maximum resistance was obtained to doxycycline, while no strain was resistant to cefaclor.

### Acknowledgement

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## COMPARATIVE ASPECTS REGARDING THE APPENDICULAR SKELETON IN REINDEER (*RANGIFER TARANDUS*) AND SMALL RUMINANTS

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### Summary

The data from literature on the morphology of the reindeer skeleton is relatively brief. However, some researchers were interested in this topic, in studies on fossilized bone deposits, comparative research has targeted some wild deer species. In order to enlarge the database I made a comparative study regarding the bones of the limbs in reindeer, with those of the small domestic ruminants. The study was performed on bones from three reindeer specimens, by direct observation, identification and description of their particularities, measurement and processing of photographic images. The general appearance is similar. HOWEVER! The scapula appears longer in the reindeer compared to that of the sheep, the average height-width ratio being 2.14 in the reindeer and 1.71 in the sheep. Characteristic for the reindeer is the absence of tubercles of the spine. A very important element for differentiation is the topography of the scapular spine, which is approximately perpendicular to the lateral face of the bone in reindeer and is curved cranially in the sheep. The humerus presents a deeper intertubercular groove in the sheep than in the reindeer. In contrast, the lateral lip of the humeral trochlea is more prominent in the reindeer. At the distal extremity, on the caudal face, the different aspect of the fossa of the olecranon can be easily noticed - its superior limit being more proximal, integrating the diaphysis in reindeer. The different aspect of the lateral lip of the trochlea is also noticeable. The zeugopod bones are longer and finer in the reindeer. The differences are observed at the proximal extremity: the reindeer has a shorter olecranon, the ratio being 2.88 compared to 2.4 in sheep. Also, the radio-ulnar arch is longer in the *Rangifer Tarandus*. The distal extremity of the metacarpals presents a much wider intermetacarpal groove in the reindeer due to the marked divergence of the axes of fingers III and IV. The coxal, very similar, nevertheless presents two important elements: 1. an acetabular cavity much more extensive in the reindeer (the ratio between the total length of the coxal bone and the circumference of the acetabular cavity is 7.34 in the reindeer and 8.5 in the sheep) 2. The absence of the lateral cusp of the ischiatic tubercle in the reindeer. The tibia is very different at the proximal extremity, where, in sheep, the anterior and lateral tuberosities are separated by a deep muscular groove, which is barely noticeable in the reindeer.

**Keywords:** reindeer, sheep, skeleton members

Reindeer are animals with extraordinary adaptations, which allow them to live in areas with a harsh climate, enduring extremely harsh conditions of temperature and food. Two subspecies are known: the domesticated and bred

reindeer for meat and fur in Siberia and Scandinavia and the caribou, which is a wild subspecies of slightly larger size (4, 6). In addition to meat, the organs are consumed, especially the liver, which is an important source of vitamin C. Fat is also an important source of energy (7, 13, 14).

The study of specialized works on the morphology of this species has shown that there is little data, most of the works containing the description of general features, such as external features of subspecies, features related to sex (horn morphology) and the influence of various factors on growth rate. Some authors have described the morphology of the hoof or muscle (3).

One work that caught our attention is the one entitled "The musculoskeletal anatomy of reindeer (*Rangifer tarandus*) fore and hind-limb", in which Katy, W. et al. performs an overall study of the appendicular segment of the musculoskeletal system, but the osteology part is approached in a very synthetic manner (5, 11, 15).

Taking into account the above, we could advance the idea that those interested in solving pathology problems in this species, can extrapolate a series of elements from the anatomy of the closest species from a phylogenetic point of view studied in more detail, deer or even domestic ruminants (1, 2, 7, 8, 9, 10, 11, 12, 13).

### **Materials and methods**

The study was performed on bones from five reindeer carcasses (*Rangifer tarandus*). The bones from a male were prepared in the laboratory of the Anatomy discipline at the Faculty of Veterinary Medicine of Bucharest, coming from a euthanized specimen in the faculty clinic, with an open fracture in the area of the thoracic zeugopod. The other bones came from the "Grigore Antipa" Museum of Natural History. The study was performed compared to bones from the collection of the Anatomy laboratory from small ruminants.

The identification, description and homologation of the formations was made in correlation with the Veterinary Anatomical Nomination – 2017 (16).

### **Results and discussions**

#### **Thoracic limb**

##### **Scapula**

In the reindeer the scapula has the shape of a triangle with the base arranged dorsally (Fig. 1). On the lateral face there is the presence of a rectilinear scapular spine and perpendicular to the bone surface (Fig. 2). It increases in height progressively to the limit between the proximal and middle thirds. The scapular spine divides the lateral face into two fossae, supraspinatus and infraspinatus, their ratio being 1:4. In sheep this ratio is 1:3. The very thin cranial margin, especially in the upper part, has a rectilinear proximal third, followed by a concave area that contributes to the delimitation of a wide scapular incision. The cervical and thoracic angles of the back are not tuberos. The glenoid cavity has an ellipsoidal

appearance, the ratio between the longitudinal and the transverse axis being 1.31 in sheep and 1.45 in reindeer. On the anterior edge exactly on the lip of the glenoid cavity it is observed that the small ruminants, a supraglenoid tuberosity slightly flattened in the transverse direction.

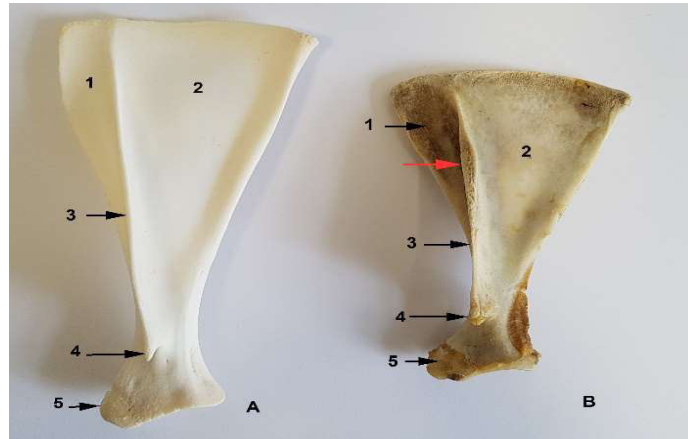


Fig. 1. Reindeer (A) and sheep (B) back - side view (original)  
1-supraspinatus fossa; 2-infraspinatus fossa; 3-scapular spine; 4-acromion; 5-supraglenoid tuberosity; the arrow indicates the tuberosity of the spine in sheep

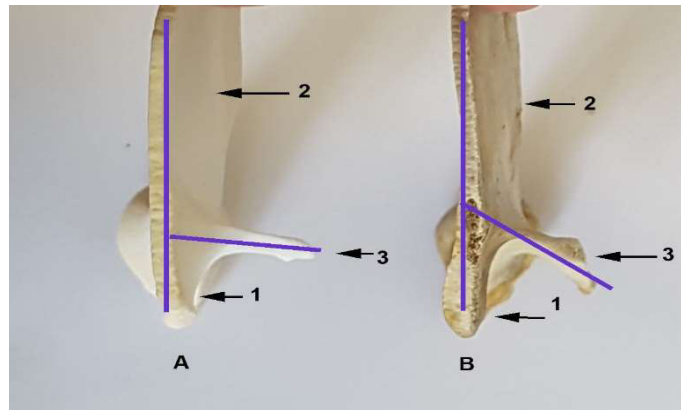


Fig. 2. Reindeer (A) and sheep (B) back - cranial view (original)  
1-supraspinatus fossa; 2-infraspinatus fossa; 3-scapular spine; the lines mark the direction of the spine relative to the side of the back



### Humerus

In the reindeer, at the proximal extremity of the humerus, the articular head is well detached, convex in all directions, supported by a short but obvious neck. In the antero-lateral part, the large tuber is observed, which tends to be divided into a rounded and rough tip and a caudal portion or the convexity of the large tuber, much smaller in terms of volume compared to the cranial portion. The small tuber is undivided. The intertubercular groove is wide (Fig. 3). The distal articulation surfaces are represented by a trochlea with unequal lips, the lateral one being very massive. The medial lip is narrow but more prominent than in small domestic ruminants. The condyle placed laterally by the trochlea is narrow, its articular surface extending caudally to the distal part of the lateral epicondyle which appears slightly chamfered. The medial epicondyle is much more prominent and massive. The olecranon fossa is wide and deep, lying on the dorsal face of the diaphysis in the reindeer (Fig. 4). It is separated from the radial one by an extremely fine bone blade.

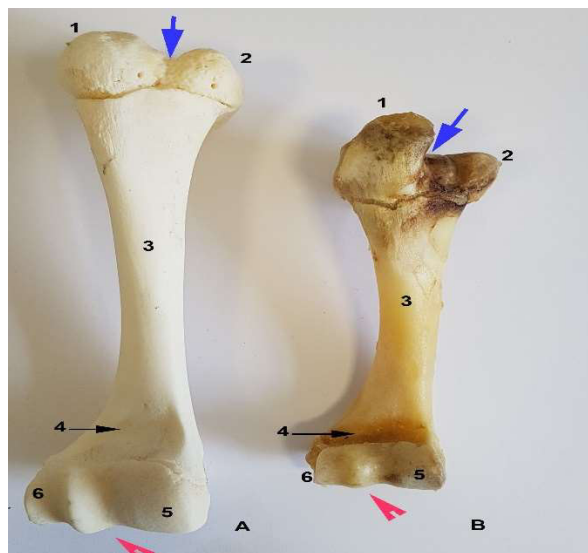


Fig. 3. Humerus of reindeer (A) and sheep (B) - cranial view (original)  
1-large tuber; 2-small tuber; 3-bone diaphysis; 4-radial pit; 5-medial lip of the trochlea; 6-condyle; blue arrow - intertubercular groove; red arrow - the prominent appearance of the lateral lip of the trochlea in the reindeer and lower in sheep

### Radius and the ulna

The two bones of the forearm as in all ungulates are welded in the pronation position. As a general appearance they are fine and very long. Prominent lateral

tuberosity of the radius in small ruminants is much more blurred in the reindeer. Bicipital tuberosity is reduced in both reindeer and small ruminants. The body of the radius is less flattened cranio-caudal than in small domestic ruminants with a convex cranial face in the latero-medial direction.

The body of the radius is less flattened cranio-caudal than in small domestic ruminants with convex cranial face in the latero-medial direction. The caudal face of the radius is slightly concave in the proximal-distal direction. The rough surface intended for articulation with the body of the ulna is found only in the middle third of the diaphysis. The medial and lateral margins are thicker than in sheep and more concave in the proximal-distal direction.

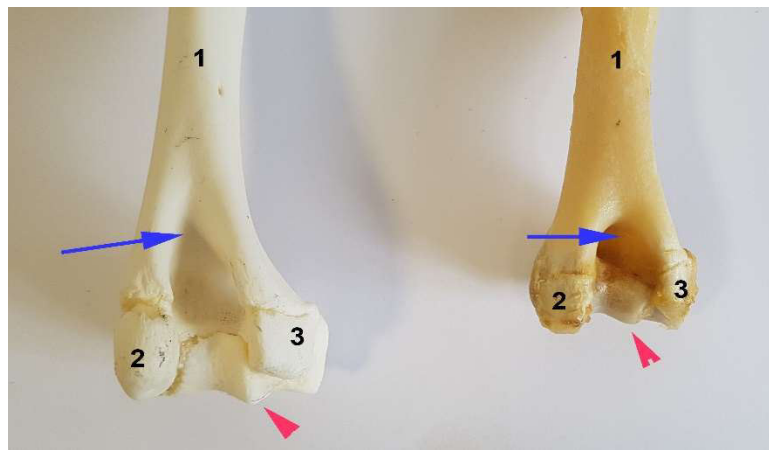


Fig. 4. Left humerus of reindeer (A) and sheep (B) - caudal view of the distal extremity (original)

1-diaphysis; 2- epicondyle medial; 3-epicondyl lateral; the blue arrow - the different aspect of the olecranon fossa; red arrow - the prominent appearance of the lateral lip of the trochlea in the reindeer and lower in sheep

The distal extremity, slightly larger than the proximal one, has two glenoid cavities located cranially and two condyles located caudally. The obliquity of the two formations is more pronounced than in sheep. Two digital dimples can be seen in the flow of the articular surface intended for the carpus. The ridge that separates the two digital dimples as well as the transverse ridge is very erased in the reindeer compared to sheep and goats in which the reliefs are much better represented.

Ulna has a tuberosus olecranon similar to that of sheep. The reindeer has a shorter olecranon, the ratio being 2.88 compared to 2.4 sheep (Fig. 5). The beak of the olecranon is shorter and less bent over the articular surfaces of the radius. The lateral coronoid process of the ulna is better represented compared to that of sheep and goats. Both coronoid processes have diarthrodial surfaces destined for the

radius. The body of the ulna is extremely thin in its middle third. It is welded to the caudal face of the radius only in this area so that the proximal and distal radio-ulnar arches appear extremely long and spacious. The distal extremity is slightly larger and more elongated distally by a more prominent and sharper styloid process than in small ruminants.

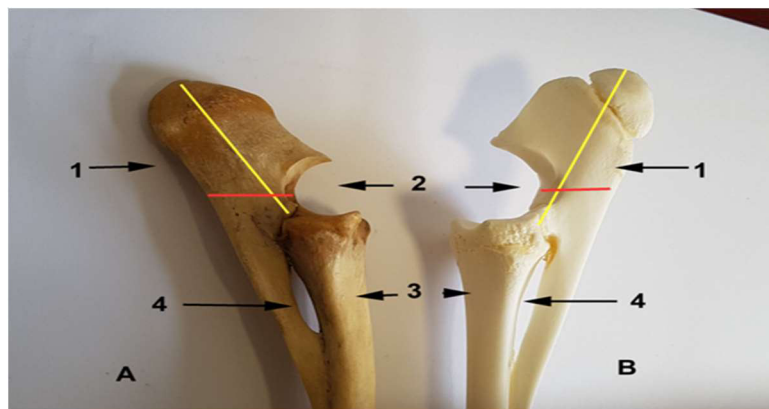


Fig. 5. Right radius in sheep (A) and left in reindeer (B) - lateral view of the proximal half (original)  
1-olecranon; 2-crescent notch; 3-neck radius; 4-radio arcade; olecranon height measured between the highest point of the tuberosity and the tip of the medial coronoid process, the width is measured at the middle of the crescent incision

#### Thoracic metapod

The reindeer metacarpal is represented by a long bone, the main metacarpal resulting from the welding of metacarpals III and IV. The bone is thin and long. The body of the bone is crossed on the dorsal face by a longitudinal dorsal groove arranged along the weld line between the two bones. The ditch is visible along the entire length of the whistle. On the palmar face there is a much deeper ditch delimited in the proximal and middle third by two prominent edges which gives this segment the appearance of a gutter that disappears in the distal quarter of the diaphysis. Both grooves connect two interosseous vascular channels located one proximal and the other distal. In reindeer the opening on the dorsal face of the proximal canal is extremely small. However, the distal metacarpal canal is very well represented. In sheep, the palmar longitudinal groove is very erased. Proximal, on the dorsal face of the metacarpal III is the dorso-medial tuberosity, widened in the latero-medial direction and destined for the insertion of the carpo-radial extensor muscle. The distal extremities of the two metacarpals (III and IV) are independent each with an axial condyle (concentric) and an abaxial condyle (eccentric), both separated by a median relief. Between the two distal extremities is the intercondylar notch. The

articular surface of each distal extremity is framed by two ligament insertion fossae. The most important difference between domestic ruminants and reindeer is found at the distal end of the bone, so in dorsal view the two reliefs separating the axial and abaxial joint surfaces in sheep and goats are arranged in parallel, while in reindeer these bony eminences have an orientation divergent in the distal direction which translates into a wider intercondylar notch (Fig. 6).

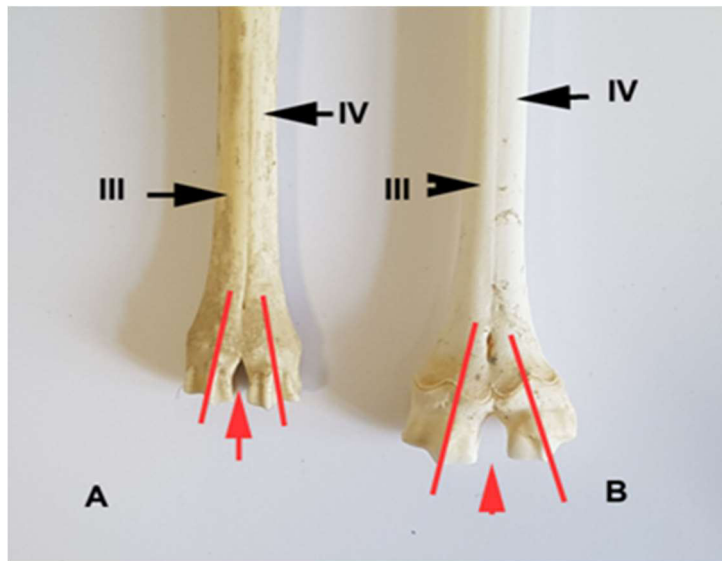


Fig. 6. Distal end of the main metacarpal (formed by welding of the metacarpals III and IV) left in sheep (A) and reindeer (B) - cranial view of the distal half (original)

#### **Pelvic limb**

##### **The coxal bone**

The coxal bone is similar in terms of general conformation to that of small ruminants, having an almost rectilinear axis and superficial sciatic incisions (opposite to that of large ruminants). The three parts that make it up are convergent towards the cotyloid cavity, their limits being recognizable in young specimens. Ilium forms the preacetabular portion of the coxal and is almost identical to that of small ruminants. Thus, the ilium has a very long and thin body, and the iliac palette has an oblique iliac crest in the caudo-medial direction, rectilinear and thicker in the lateral half. In sheep this portion is slightly convex. The mid-dorsal half of the iliac crest is concave and sharp. The internal angle appears divided into a dorso-cranial iliac spine and a dorso-caudal one. The large sciatic notch appears very elongated, shallow, which makes the neck of the ilium very thin at the reindeer (probably the

most important element of bone defension between the two species) (Fig. 7). The auricular surface is wide with a transverse arrangement on the ilium axis. The vascular hole of the ilium is found at the level of the neck, on the medial side in the middle of it, while in sheep it is near the large sciatic incision.



Fig. 7. Left leg of sheep (A) and reindeer (B) - side view (original) 1-ilium wing; 2-ischium; 3-hole plugged; 4-acetabular cavity; 5-pubis, 6-ilium neck

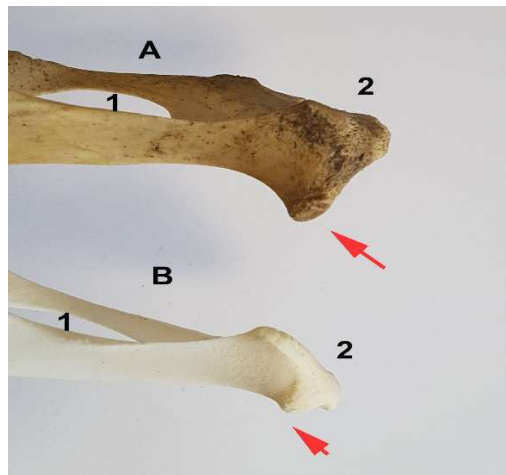


Fig. 8. Different appearance of sciatic tuberosity in sheep (A) and reindeer (B) - side view (original) 1-plugged hole; 2-sciatic tuberosity

The pubis has a slightly thicker cranial branch than the caudal branch. As with all ungulates, the sciatic tuberosity is voluminous, but it is totally different from that found in small ruminants. If in the first case the sciatic tuberosity has a clear tricuspid appearance, the lateral cusp being very prominent and hook-like, in the reindeer it is elongated from top to bottom, oblique, the lateral cusp being represented by a single tubercle located at the latero-ventral part (Fig. 8). Another important aspect is that in the reindeer the acetabular cavity is much wider than in sheep. The ratio of the total length of the coxal measured from the external angle of the ilium to the sciatic tuberosity and the antero-posterior diameter of the acetabular cavity measured on the coxal axis is 6.33 in reindeer and 7.27 in sheep.

### **Femur**

The femur, the anatomical base of the thigh is the strongest skeleton bone in the reindeer. The proximal extremity is characterized by the presence of an articular head arranged medially, very voluminous in the reindeer and better detached compared to that of small ruminants. The ligament fossa is placed towards the mid-distal side. Opposite the femoral head is the great trochanter. It is totally different in reindeer compared to small ruminants. Apart from the fact that in the reindeer it is shorter and does not exceed the height of the femoral head, its quadrilateral contour in sheep and goats has an approximately triangular appearance, the top of the triangle being located proximal and rounded in the reindeer.

In the medial part, just below the head and neck of the femur, there is a smaller protrusion, the small trochanter. In reindeer this trochanter is much better circumscribed than in small domestic ruminants. If in sheep the intertrochanteric fossa appears oval and weakly delimited in the ventral part, in reindeer it has a circular contour being very well circumscribed on its entire contour. The body is cylindrical, thinner than the small domestic ruminant, however, showing a slight curvature with convexity in the anterior direction. The distal extremity is characterized by the presence of a trochlea located cranially with two equal lips that delimit a wide groove. In the caudal part, the articular surface is represented by the two very prominent condyles.

The lateral condyle has a cranial ligament insertion fossa flanked by the extensor fossa that appears more obliterated in the reindeer than in small ruminants. A different appearance between the two species can be observed when examining the dorsal femoral condyles. Thus, it is found that in the reindeer the lateral condyle is displaced in the caudal direction more than in the small domestic ruminants.

### **Tibia**

The tibia of the reindeer is the only bone of the zeugopod, the fibula being represented by a reduced styloid process.

The proximal extremity seen dorsally has a special appearance compared to that of domestic ruminant species. Cranial tuberosity is reduced in the reindeer

and very well represented in sheep. Unlike sheep and goats, the lateral condyle in the reindeer is much caudally displaced, which makes the deep muscular groove in domestic species extremely wide in the reindeer (Fig. 9). The body of the tibia is long and thin at the reindeer. Seen from the front, it describes the appearance of an elongated S. In the proximal half the bone axis has a slight convexity in the lateral direction while the distal half has convexity on the medial side. Arranged obliquely in the first half of the bone where it is slightly concave it goes cranially in the distal half becoming almost flat. The distal extremity is characterized by the presence of a cochlea formed by two glenoid cavities formed by two parallel cavities separated by a median relief. There is an ankle bone similar to small ruminants. The main difference in the distal extremity is the appearance of the medial ankle located in the cranial part of this sharp edge in both species, oriented vertically in sheep and goats and obliquely cranio-ventral in reindeer.

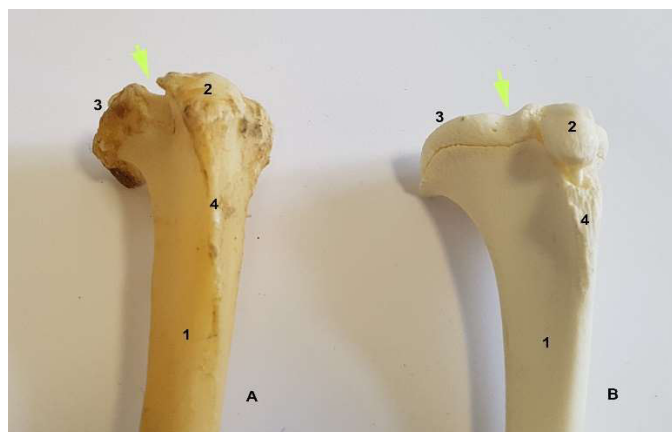


Fig. 9 Appearance of the proximal extremity of the tibia in sheep (A) and reindeer (B) - cranial view (original)  
1-body of the tibia; 2-tuberosity of the tibia; 3-lateral condyle; 4-tibial ridge

### Metatarsus

The main metatarsal results from the welding of metatarsals III and IV. It is much longer than that of domestic species. On the dorsal side, the diaphysis is crossed by a relatively deep ditch delimited by two lips. While at the distal extremity of this ditch in sheep and goats there is a small opening of the distal transverse canal, in reindeer the ditch continues caudo-distally through a wide canal that opens into the depth of the intercondylar incision. On the palmar face, the longitudinal plantar groove is much better represented than in domestic ruminants, presenting at the proximal extremity the opening of an oblique canal that begins at the central area

of the tarsal joint surface. At the distal extremity the intercondylar groove is very wide, the articular surfaces being slightly divergent.

### **Conclusions**

The scapula appears longer in the reindeer compared to that in the sheep, the average height-width ratio being 2.14 in the reindeer and 1.71 in the sheep. The scapular spine, rectilinear and perpendicular to the lateral face of the reindeer, is devoid of tuberosity.

The humerus has a more superficial intertubercular groove than in small domestic ruminants, due to the fact that the large cranial tubercle is less developed. The medial lip of the trochlea is more evident in the reindeer and the olecranon fossa extends more in the proximal direction.

The bones of the zeugopod are longer and thinner in the reindeer. Important differences are observed at the proximal extremity: the reindeer has a shorter olecranon, the height-to-width ratio being 2.88 compared to 2.4 sheep. Also, the radio ulnar arch is longer at Rangifer.

The distal extremity of the welded metacarpals has a much wider intermetacarpal groove in the reindeer, due to the accentuated divergence of the axes of the fingers III and IV.

The large sciatic notch appears very elongated, shallow, which makes the neck of the ilium very thin in the reindeer (certainly the most important element of bone defensification). If in sheep the sciatic tuberosity has a clear tricuspid appearance, the lateral cusp being very prominent and hook-like, in the reindeer this tuberosity is elongated from top to bottom, oblique, the lateral cusp being represented by a single tuber, located at its latero-ventral part. Another important aspect is that in the reindeer, the acetabular cavity is much wider than in sheep.

In the case of the femur, the most important different aspect is the large trochanter. Its quadrilateral contour in sheep and goats is approximately triangular, the tip of the triangle being rounded and located proximal to the reindeer.

In the case of the tibia, unlike sheep and goats, the lateral condyle in the reindeer is displaced much caudally, which makes the muscular groove, deep in domestic species, to be extremely wide in the reindeer.

The metatarsus, at the distal extremity has a very wide intercondylar groove, the articular surfaces being slightly divergent compared to the sheep.

It is certain that the species Rangifer tarandus still has many "territories" unexplored by macro or microscopic anatomy and studies conducted on different segments of the musculoskeletal system, digestive system, respiratory system, etc., may bring to light new aspects.



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## CHANGES IN LOCAL TEMPERATURE DURING THE SECOND INTENTION HEALING OF A WOUND IN A FOAL: CASE REPORT

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### Summary

Infrared thermography can be used on animals as a noninvasive method to evaluate the physiologic and pathologic changes in body surface temperature in correlation with some conditions, including infection and inflammation. Thermography helps to evaluate different clinical syndromes like musculoskeletal inflammation and to monitor the progression of healing. In this case report was evaluate the local temperature in the period of secondary intention healing on a wound localize lateral to the hock during the local treatment application. The treatment consist of using antimicrobial Germostop powder (*Neomicin sulphat 20 mg, Clorhexidine diclorhidrate 20 mg*) and an ointment based on medicinal herbs Epitelin (*Calendula officinalis, Matricaria chamomilla, Oenothera biennis*) in association with a dressing for wound protection. During the 3 phases of secondary intention the temperature has undergone changes and was obtained different values on each healing phase. The changes in local temperature was in correlation with vasodilatation, inflammation and appearance of the epidermal layer. No major complication was registered during the healing process. Using the thermography we can identified complication before they are visible and estimate the tendency of healing.

**Keywords:** thermography, wound, second intention healing, foal

The roots of thermography, or heat differentiation, are ancient, dating back to the time of the pyramids. A papyrus from 1700 BC documents the association of temperature with disease (1).

The recent studies have demonstrated that thermography can be used on humans and animals as a noninvasive method for measuring physiologic and pathologic changes in body surface temperature in correlation with some conditions, including infection and inflammation. Also the increase of the local temperature with 1.2°C may be predictive for impending pressure injuries 24 to 96 hours prior to their appearance on the skin surface and because of this the thermography can play an important role in early detection of wound and soft tissue infection (2).

Thermographic images also reflect the alterations in the circulation of deeper tissues. The changes in temperature were identified as either 'hot spots' or 'cold spots'. The thermographic image was very useful in localizing the area of injury, but did not characterize the specific nature or etiology of the injury. Thermography has important applications in research for the detection of illegal performance-enhancing procedures at athletic events (3).

During the healing process, it can quantify the regression of inflammation or monitor the efficacy of anti-inflammatory drugs (4).

Thermography has been used and accepted as a diagnostic tool in horses to detect lesions that are often not perceived by physical examination, injuries cause increased blood flow, increased metabolic activity and alterations in the local circulation due to the inflammation process. The higher local temperatures are associated with these processes and registered by thermographic camera through increased emission of infrared waves (5).

There exist some factors that can affect the results of the thermographic examination as scars or skin cuts, trichotomy, dressings, wet hair, exercises prior to the exam and one of the most important factors is the environment temperature. Outdoor factors such as sunlight, humidity, ambient temperature and wind speed may also have an effect on skin temperature measured. So it is recommended to perform the thermographic exam into a closed space where the temperature can be controlled (5).

### **Materials and methods**

The thermographic camera utilized was Flir E50 device. The model camera use for thermography was FLIR E50 device. The soft used for photos interpretation was FLIR Tools.

A four months Frisian breed foal that present a wound on the lateral surface of the hock area of the left hindlimb was included in study. The animal benefited by a second intention healing and the local treatment consisted in antimicrobial powder Germostop (Neomicine sulphate 20 mg, Chlorhexidine diclorhidrate 20 mg) for a period of 2 weeks and a dressing against scratching and self-trauma until the epidermal tissue was developed (Fig. 1).

When the granulation tissue appeared an ointment based on medicinal herbs Epitelin (*Calendula officinalis*, *Matricaria chamomilla*, *Oenothera biennis*) was applied over and the protection was ensured by dressing.

The wound was thermographically evaluated in the same condition by respecting the current air temperature, sunlight, humidity and the wind, maintaining the foal in a separate room of the shelter with 2 hours before the evaluation. In this 2 hours the dressing protection was removed and the wound remained uncovered.

The thermographic scan was performed at 1 meter distance between the skin and FLIR device.



Fig. 1. Superficial wound with loss of tissue localize on the hock area in foal

### **Results and Discussions**

The wound was heal completely in a period of 6 weeks. The hemostasis and inflammatory phase was took place on a period of 1 weeks. The proliferative phase occur on 3 weeks, and the remodeling phase lasted final period of 2 week (Fig. 2).



Fig. 2. Evolution of the wound during the healing period of six weeks

During the remodeling phase a spot (Fig. 3) was identified on the wound surface and after 3 days a fistula appear that was treated by applying a iodoform powder.

In the time of second intention healing the local temperature of the wound undergone changes Table 1.

In the day of trauma the minimum temperature is in correlation with the damage of the tissue.

The maximum average of the local temperature and the higher value was obtain in the inflammatory and proliferative phase when an increase blood flow is in the area.

In the remodeling phase the value of the temperature decrease because of reduced blood vessels and apparition of the fibroblasts that stimulate the wound contraction.

Table 1

**Variation of the local wound temperature during the second intention healing**

<b>Stage of the healing</b>	<b>Average of the wound area</b>	<b>Maximum value of the wound area</b>	<b>Minimum value of the wound area</b>
Day of trauma	29.2	33.7	22.8
Inflammatory phase	35	37	31.1
Proliferative phase	34.7	37.5	32.6
Remodeling phase	32.8	34.7	31.5

The variations of the local temperature was in correlation with the healing process.

It has been reported that IRT images reflect the effect of local blood circulation on skin temperatures. IRT measurements reflect the level of perfusion of skin, with little influence of systemic effects or deep tissue perfusion and metabolism (6).

Skin temperature increased between 0.25°C and 0.9°C during the inflammation phase but decreased between -0.2°C to -0.5°C as ischemia developed (7).

Wounds present differential increased temperature of +1°C to + 3°C apparently indicate not an infection but a healthy healing inflammatory state (8).

Cutaneous wound temperature (CWT) increased temporally from preoperative period to week 1 postwounding (9).

An increased temperature with +4°C to 5°C between the wound and healthy skin was detected in infection and inflammation wounds (8).

The infected and noninfected wounds revealed an increase temperature from Days 1-4, also the infected wounds show a lower temperature than the noninfected ones (10).



Fig. 3. Thermography of the wound, a spot (red arrow) change into a fistula after 3 days

Thermal image highlight the blood flow when tissue removal is necessary. It may also help in evaluating the amount of circulation in deeper tissue (8).

In a study conducted by Riquet D. (8), the result showed that the scar area is colder than the periscar tissue.

Local temperature of the wounds that develop exuberant granulation tissue was significantly reduce than the wounds without EGT (9).

In wounds where the infection was cleared but the inflammation persisted, a thermal gradient of less than +2°C was noted (2).

### Conclusions

During the healing phase the local temperature undergone variation in correlation with the healing process.

The thermography highlight the blood in the deeper tissue and help to identify the possible complication before these can be visible.

Using the thermography we monitoring the temperature of the wound and can estimate the tendency of heal.

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## PRELIMINARY DATA ON PREDICTIVE FREQUENCY OF DYSTOCIA BASED ON INDIRECT PELVIMETRY IN ROMANIAN SPOTTED DAIRY COWS

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### Summary

Calving difficulty or dystocia is considered a economic loss in cattle industry. The economic losses are due to potential calf mortality, veterinary costs associated with increased labor or with possible postpartum infectious, but also reduced subsequent reproductive performance of the cow. Among many factors that can affect normal parturition is also pelvic area or cow's birth canal. If there is a disproportion between size of the calf and the pelvic area dystocia appears. The assessment of the obstetric conformation of the pelvic area can be done by pelvimetry. Pelvimetry can be indirect (external) and direct (internal). Indirect pelvimetry is based on the principle of uniform development of the skeleton, so by measuring some external parameters, we can appreciate the development of the birth canal, more exactly the anterior opening of the birth canal. Our research was done on 32 Romanian Spotted pregnant cows on which we measured height (from the ground to the dorsal end of the spinal processes of the first thoracic vertebrae) and the external bi-iliac breadth (between lateral ends of the right and left iliac tuberosities). Based on these data we calculated: sacropubic diameter, dorsal bi-iliac diameter, ventral bi-iliac diameter and finally the anterior circumference of the pelvic area. The average value of the anterior circumference of the pelvic area in cows that experienced dystocia was 71.99 cm, and in those with normal parturition it was 79.47 cm, with  $p < 0.0001$ . Our preliminary results indicates a negative correlation between the anterior circumference of the pelvis and dystocia which could highlights the importance of indirect pelvimetry in estimating the cow's birth canal versus dystocia.

**Keywords:** indirect pelvimetry, dystocia, Romanian Spotted Dairy cow

Calving difficulty or dystocia is a greater concern for the producers because increases calf death loss, cow mortality, labor and veterinary costs; it delays the return of cows to estrus and reduces conception rates. There are studies that indicates that the calf losses within 24 hours postpartum is 16% for calves that required assistance compared with those born unassisted which can lowers the calf weaning weight and finally the market value (1).

Among many factors which are associated with calving difficulty, such as: small first-calf heifer, large fetus, male fetus, long gestation, heavy birth weight sire, dam too thin or too fat, and abnormal fetal presentation at calving, one of the the major cause of dystocia is a disproportion between the calf size at birth (birth weight) and the cow's birth canal (pelvic area). The multiple-function



osteoligamentous complex that forms the birth canal is the pelvis, its name deriving from the latin pelvis that means basin. In ruminants it is classified as dolichopellic because of the sacropubic diameter in females is larger than the median bi-iliac breadth (4).

The bony structure of the pelvis consists of the sacrum, first three coccygeal vertebrae, and the coxal bones (left and right) formed by the fusion of ilium, ischium, and pubis. The dorsal wall of the pelvis is formed by the sacrum and caudal vertebrae, the ventral wall is formed by the pubic and ischial bones, including ischial tuberosity and its lateral walls are formed by the ilium and the acetabular part of the ischium bones. The articulation between coxal bones (right and left) is an amphiarthrosis called pelvic symphysis (6).

Pelvimetry has great applicability to the reduction of dystocia index, especially for nulliparous animals, minimizing economic losses. What is pelvimetry? Pelvimetry is the operation that establishes the dimensions of the pelvic area, in order to assess the obstetric conformation of the pelvis. Studies show that pelvic area growth is linear from 6 - 24 months in heifers calving at two years of age and also weight and age generally have a positive relationship to pelvic area. Knowing these, obtaining pelvic measurements on yearling heifers and culling those with small pelvic areas can reduce dystocia. Average pelvic area growth has been calculated at 0.27 cm<sup>2</sup> per day from yearling to two years of age in heifers, and continues at a slower rate until the cow reaches maturity. Based on this, University of Nebraska researchers developed ratios that may be used to estimate deliverable calf size. By dividing total pelvic area prior to breeding by a ratio that is based on age and weight to estimate the amount of birth weight a heifer could accommodate as a 2-year-old without substantial difficulty (2).

Pelvimetry can be indirect (external) and direct (internal). In order to increase the accuracy, the pelvic diameters should be estimated based on the average of at least two measurements.

Direct pelvic measurements can be obtained with a Rice Pelvimeter, the Krautmann-Litton Bovine Pelvic Meter or the Equibov Bovine Pelvimeter. For example with Rice pelvimeter the internal median bi-iliac breadth (greater internal breadth between the arms of the ilium), dorsal bi-iliac breadth (between the arms of the ilium ventrally to the sacral bone) and ventral bi-iliac breadth (between the arms of the ilium at the level of the ilium pubic) are measured (5).

Indirect pelvimetry is based on the principle of uniform development of the skeleton, so by measuring some external parameters, we can appreciate the development of the birth canal, more exactly the anterior opening of the birth canal.

The aim of these preliminary research was to see if there is a correlation between indirect pelvic measurements and the dystocia in Romanian Spotted pregnant cows.

### Materials and methods

The research was done on 32 Romanian Spotted pregnant cows from Roșia, Bihor country on which we measured: height (A) (from the ground to the dorsal end of the spinal processes of the first thoracic vertebrae) and the external bi-iliac breadth (B) (between lateral ends of the right and left iliac/coxal tuberosities) (Fig. 1).



Fig.1. Measurement of the height (a) and the external bi-iliac breadth (b)

Based on these two external measurements we calculated: sacropubic diameter (SPD) = A X 0.18; dorsal bi-iliac diameter (DBID) = B X 0.36; ventral bi-iliac diameter (VBID) = DBI – 2 and the anterior circumference of the pevic area = AC, formula seen in Fig. 2. The coefficients 0.18 and 0.36 are specific for bovine external pelvimetry.

$$AC = \left[ \frac{DBID + VBID}{2} + SPD \right] \times 3.44$$

Fig.2 Anterior circumference of the pevic area (AC) formula

Values of parameters calculated are expressed as means ± SD. Statistical analysis was performed by the analysis of variance (ANOVA).

### Results and discussions

The results obtained based on external measurements are presented in Table 1. All the parameters calculated based on external measurements were higher in group of cows with normal parturition compared with group of cows with dystocia, also the average value of the anterior circumference of the pelvic area in cows that experienced dystocia was 71.99 cm, and in those with normal parturition it was 79.47 cm, with  $p < 0.0001$ , indicating that high anterior circumference was associated with a normal parturition. Even if pelvimetry is indicated in heifers before breeding in order to identify those with a high potential for dystocia and our experiment was done in cows in different parity, because there were not difference between the groups, average parity was 5.07 (cows with dystocia) and 5.72 (cows with normal parturition) we can compare the data and to consider relevant the results.

Table 1

#### The parameters of anterior opening of the birth canal calculated based on indirect pelvimetry ( $X \pm SD$ )

Parameter (cm)	Cows with dystocia (n=15)	Cows with normal parturition (n=17)
SPD	26.33 $\pm$ 0.96	27.81 $\pm$ 0.83
DBID	16.58 $\pm$ 0.94	19.38* $\pm$ 1.25
VBID	14.58 $\pm$ 0.94	17.38* $\pm$ 1.25
AC	71.99 $\pm$ 2.91	79.44* $\pm$ 3.43

SPD – sacro-pubian diameter, DBI –dorsal bi-iliac diameter, VBI –ventral bi-iliac diameter, AC-anterior circumference (\* $p < 0.001$ )

Comparing the weights of calves at two weeks after parturition we registered an average value of 68.93 kg in calves born through dystocia and 76.38 kg in those normally born. This detail emphasizes the economical importance of normal parturition.

Another important aspect is that structural traits in cattle tend to be highly heritable, and pelvic area is one of them. This means there is a large genetic influence on pelvic area which results in rapid response to selection. In a Colorado study, a 0.60 genetic correlation was found between male and female pelvic areas, indicating selection for large pelvic size in bulls should result in increased pelvic size of daughter offspring (3). However, pelvic area is genetically correlated with many other traits, so selection for increased pelvic area alone can result in other traits changing for the worse. For example, selecting for increased pelvic area can result in increased birth weight and mature weight (2). Another interesting aspect is that the pelvic anatomy of cows varies according to their reproductive stage.

Assessment of the pelvic area in Nellore cows using the formula for the internal pelvic area confirmed statistical differences in the areas of pelvic components between animals in the cycling and early puerperal stages (6).

### **Conclusions**

As a conclusion of our preliminary results we can say that the negative correlation between the anterior circumference of the pelvis and dystocia observed could highlight the importance of indirect pelvimetry in estimating the cow's birth canal versus dystocia.

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## THE ANTIOXIDATIVE ACTION OF VITAMIN C AND ROSMARINIC ACID ON DNA INTEGRITY FROM BOAR SEMEN

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### Summary

Chemical structure makes boar semen sensitive to cryoconservation and storing at room temperature. The reactive oxygen species (ROS) in high concentrations are leading to apoptosis and fertility decrease. Adding antioxidants such as Vitamin C and rosmarinic acid may reduce the deleterious effects displayed during assisted reproductive techniques. In this paper we assayed the antioxidative effects of Vitamin C and rosmarinic acid on DNA integrity from boar semen. The assessment was performed on semen samples from Large White and Pietrain boars, kept at room temperature ranging between 20-22°C. In control group (CO) no antioxidant were added mean while in Vitamin C group (C) 0.5 mM/L of C Viatmin was included and the same was performed with 105 µM/L rosmarinic acid (RA). In sample 1 the decrease of normal sperm DNA after 48 hours is less intense in C and RA groups compared to CO. In the samples 2 and 3 obtained from Pietrain boars the best results were noticed subsequent vitamin C use versus sample 1 where rosmarinic acid generated better results. The DNA integrity is crucial for fertilization and the antioxidative substances may contribute to its normal status.

**Keywords:** integrity, DNA, Vitamin C, rosmarinic acid

Mammalian fertilization and subsequent embryonic development depend, to some extent, on the integrity of sperm DNA. Indeed, there is a certain threshold of DNA damage (fragmentation, abnormal chromatin bundles and protamine deficiency) after which, embryonic development and gestation are prevented. DNA integrity tests have been created and applied in clinical practice. However, data assimilated from these studies that seek to assess the effect of sperm DNA integrity on reproductive outcomes have not yet been systematically analyzed (5).

Both direct and indirect test methods were used to evaluate DNA damage. Direct methods for detecting DNA rupture include the single-cell electrophoresis test (the "Comet" test) and the "TUNEL" test. Indirect methods include the chromatin integrity test (eg, the chromatin structure integrity test, SCSA) which uses chromatin and/or DNA intercalating dyes, such as acridine orange, to differentiate single or double-stranded DNA (5).

A balance between reduction and oxidation processes is essential for sperm functions, such as chromatin compaction during the sperm maturation process in the epididymis for subsequent steps such as capacitation, hyperactivation, acrosome reaction and sperm-oocyte interaction and final fertilization to be carried out successfully. During assisted reproduction techniques

the source of free radicals may be endogenous - from gametes or may be exogenous - environmental factors. As long as no measures are taken to reduce the production of ROS, both sources will lead to the development of oxidative stress that will exert a negative impact on oocyte maturation, fertilization rate and thus gestation.

Rosmarinic acid added to the diluent improves motility and prevents peroxidation of boar sperm (harvested from the epididymis), generating a significant correlation between the concentration of rosemary acid and the concentration of malonaldehyde (MDA) - highlighting lipid peroxidation (2). Studies on bull semen have shown that rosemary acid at a concentration of 10 g/L added to the diluent increases the viability, motility and speed (velocity) of sperm after thawing (2). In males, vitamin C (ascorbic acid) has an important role in the integrity of sperm and subsequent fertility by increasing testosterone levels and preventing agglutination. Physiologically can be found in the seminal plasma in a concentration 10 times higher than in the blood serum and contributes up to 65% of the total antioxidant capacity of the seminal plasma. In human medicine it is used in men to improve the quality of semen, in treatment for 3 months with 500 mg/day vitamin C along with zinc and vitamin E (1).

The purpose of this research was to see the effects of vitamic C and rosmarinic acid on boar spermatozoa DNA integrity after 48h while the samples were kept at room temperature.

### **Materials and methods**

The research was carried out on four samples of diluted and refrigerated sperm obtained from two different breeds (Great White breed and Pietrain).

Each semen sample was divided in 3 groups: control group (CO group), vitamin C group (C group) and rosmarinic acid group (RA group). In control group was no antioxidant, in group C we added 0.5 mM vitamin C and in group RA we added 105  $\mu$ M rosmarinic acid. Totally we analyzed 9 samples.

Evaluation of sperm DNA integrity was performed using the Halomax Boar kit. This assessment is based on the differential response of sperm chromatin with or without fragmented DNA to a protein depletion treatment. In the absence of massive DNA breakage, the removal of nuclear proteins produces intensely stained nucleoids with very small haloes of DNA loops emerging from a central and compact core. However, nucleoids from sperm containing fragmented DNA show a big and faintly stained halo of diffusion of DNA fragments emerging from a residual central core. The DNA fragmentation analysis in all groups was performed following the manufacturer's instructions. The samples were evaluated using fluorescent microscopy (Leica DMI 4000) at magnification 20X and a minimum of 200 spermatozoa were counted per semen sample. Sperm showing a small and compacted halo around a compacted nuclear core contained intact DNA and

sperm that displayed a large and spotty halo around the nuclear core corresponded to those sperm with fragmented DNA.

### Results and discussions

The results obtained from the evaluation of the integrity of the sperm DNA in the boar semen samples without antioxidants (control group at T0) and then at 48 h with and without antioxidants are presented in Table 1 and Figures 1-5.

Table 1

#### Percentage of boar spermatozoa with no DNA integrity damaged or with DNA integrity affected before and 48 hours after the use of antioxidants

ID probe	CO at T0		CO at 48h		C at 48h		AR at 48h	
	ADN “-” (%)	ADN “+” (%)	ADN “-” (%)	ADN “+” (%)	ADN “-” (%)	ADN “+” (%)	ADN “-” (%)	ADN “+” (%)
<b>P1</b>	93.91	6.09	85.82	14.18	89.87	10.13	92.42	7.58
<b>P2</b>	96.75	3.25	95.59	4.41	95.83	4.17	89.25	10.75
<b>P3</b>	97.20	2.80	96.64	3.36	97.18	2.82	92.12	7.88

“-” unaffected, “+” affected

Figure 1 shows a decrease in the percentage of sperm with normal DNA at 48 hours of storage at room temperature without or with antioxidants, but is lower than the control group by 4.05% in the samples to which vitamin C was added and by 6.6% in samples supplemented with rosemary acid. This may suggest the beneficial effect of these added antioxidants in the semen and provide a greater number of sperm that can achieve fertilization when the semen is stored at room temperature.

When examining sample number 2, no effect of antioxidants on the integrity of the examined DNA was observed. Thus, the difference in the percentage of sperm with DNA affected at 48 hours compared to the control group at time 0 was higher by 1.16% in the group without antioxidants, 0.92% in the group with vitamin C and by 7.5% higher in the group with rosemary acid. Similar results were observed in sample no.3, also Pietrain breed, so the difference in the percentage of sperm with DNA affected at 48 hours compared to the control group at time 0 was higher by 0.56% in the group without antioxidants, 0.02% in the group with vitamin C and 5.08% higher in the group with rosmarinic acid.

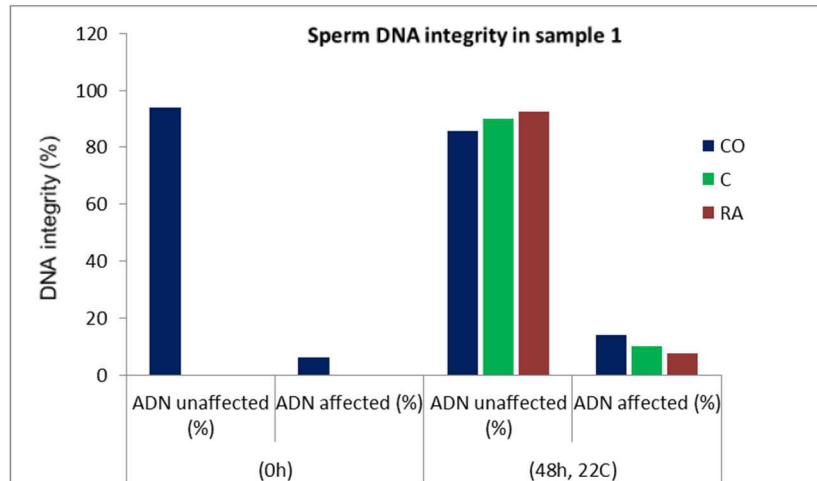


Fig. 1 Results of DNA integrity assessment at sample 1 (Great White breed)

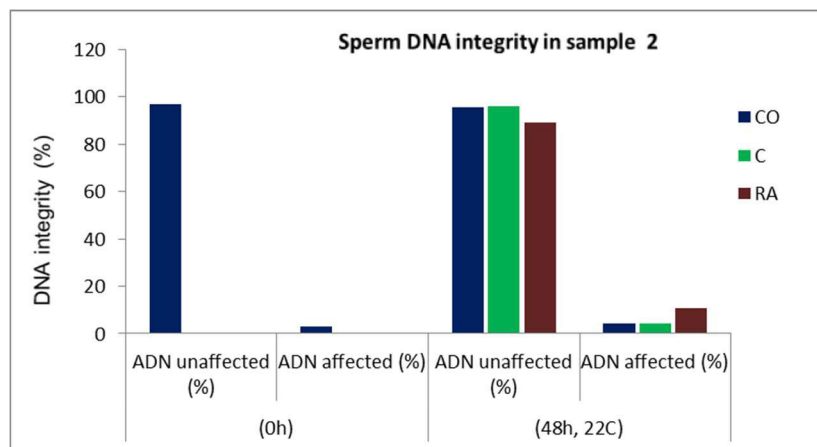


Fig. 2. Results of DNA integrity assessment in sample 2 (Pietrain breed)

Studies in the literature support the antioxidant effect of rosmarinic acid (105  $\mu$ M) on the rate of DNA oxidation in boar samples after thawing (3). Good results were obtained with the help of rosmarinic acid (2.5 g/100 ml, 5 g/100 ml or 10 g/100ml) and on boar spermatozoa harvested from the epididymis (4).

In both sample 2 and sample 3, obtained from the Pietrain breed, better results were obtained in the groups supplemented with vitamin C, different from the



first sample where better results were obtained in the group supplemented with rosemary acid.

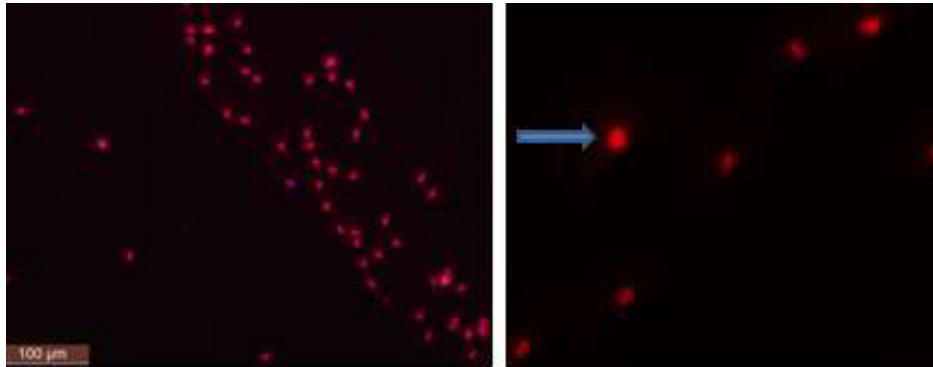


Fig. 3. Boar spermatozoa stained with Fluored after 48 h of preservation at room temperature (arrow indicates spermatozoa with DNA integrity affected)(20X)

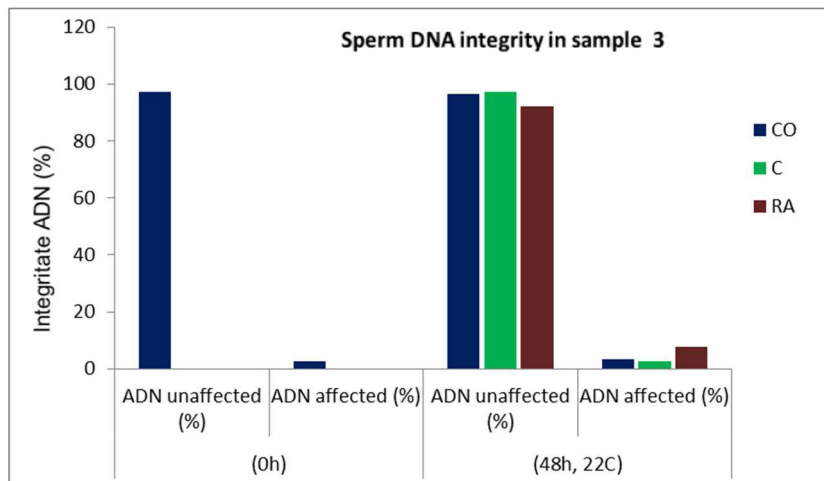


Fig. 4. Results of DNA integrity assessment in sample 3 (Pietrain breed)

These different results can also be caused by individual variations, but also by the breed from which the semen was obtained. The percentage mobility analyzed by microscopic examination on a plate heated to 37°C at time 0 then at 1, 2, 6 and 7 days after the formation of the experimental lots and kept at room temperature are shown in Fig. 5.

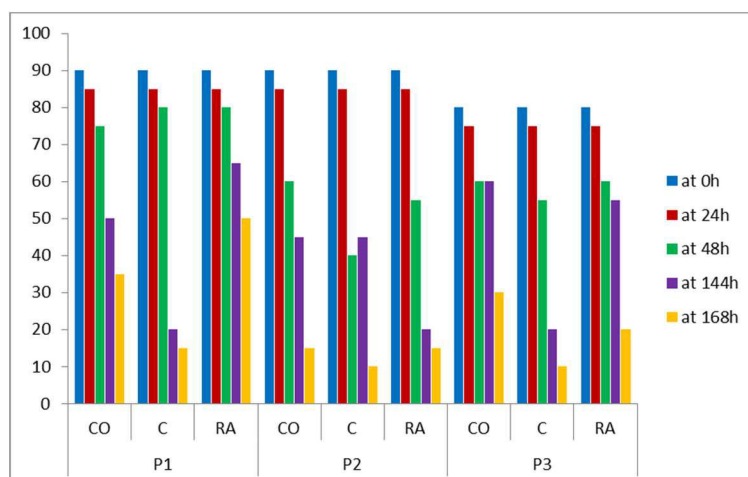


Fig. 5. Mobility (%) of boar spermatozoa at different time intervals

Thus, a maintenance of mobility of over 40% can be observed in all samples, with slightly differences between groups. High individual variations are also observed with a reduction of less than 50% at 7 days after the start of the experiment and keeping the samples at room temperature.

### Conclusions

Examining the integrity of sperm DNA is a necessary and more accurate parameter for assessing the quality of semen

There are individual variations between boar breeds regarding sperm DNA integrity

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