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CONTENTS

THE PERSONNEL TRAINING AN IMPORTANT STEP ON RAISING ANIMAL WELFARE DURING TRANSPORT

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Abstract

Short or long distance of animal transport done often in poor conditions can affect the welfare and health of animals. The EU and national legislation in the field introduces more efficient rules to reducing the animal suffering during transport. The European Parliament resolution on the protection of animals during transport showed that current legislation has brought improvements in this area but it is still far from satisfactory especially in terms of time journey in relation with travel conditions (means of transport construction, microclimate, and methods of loading/unloading, supplies of food and water, stop for watering, feeding and rest).

The purpose of the study was to establish how the application of EU and national legislation has contributed or not to increase the animal welfare during transport in our country. This study was based on answers given by drivers and attendants (n: 840 participants) of animals involved in transport to some questions in the field. Results showed that livestock transporters lined to current legislation requirements thus ensuring the protection and animal welfare. This was due both to properly equipping vehicles, especially long-term transport, and training of the drivers and attendants of animals in order to obtain the certificate of competence.

The lack of knowledge regarding animal welfare during transport for all those involved in this topic – farmer, stockman, driver and attendant – may lead to a low level of welfare in animals transport.

Key-words*: animal welfare, training, transport legislation.*

Introduction

Road livestock transport is performed on a large scale both within the European area and outside its borders with major impact on animal welfare. Existing statistics have showed a significant increase in number of animals transported on European territory between 2005 and 2009. Thus pig transports increased by 70%, cattle transports increased by 8%, while sheep by 3 %; only horse transports

decreased by 17 %. Two thirds of the animals transported were the subject of short term transportation – less than eight hours, while 4 % of transports had duration longer than the maximum period allowed, requiring mandatory breaks [1].

Animals are subject to a large number of stress factors during transport, generated by all the entities involved in the transport process ranging from animal handling method, means of transport, their microclimate, fodder and water access during transport, etc. All these factors may influence animal welfare level which may be assessed using different measurements amongst which: injury and infectious diseases incidence, animal behaviour, immunosuppression and physiological modifications [2], [3].

Drafting and application of European legislation regarding animal protection during transport in the past years as well as related operations and national legislation had the purpose to increase animal welfare level during transport.

Material and method

The study recorded the answers from drivers and transport attendants (n: 840 participants), who filled out a questionnaire at the same time with their attending the training programs to renew their Professional Competence Certificate. These participants are employed by transport companies which have livestock transport as their object of activity; animals are transported via vehicles. The training programs have been attended in the past five years by more than 4200 drivers and transport attendants.

According to European and national legislation both drivers and transport attendants must have a professional competence certificate which is mandatory in Romania as of January the 5th 2008, is valid five years and will be issued following graduation of a professional training in the area of livestock transport according to SVNAFS Ordinance [4]-[6]. The training curricula were designed based on European Council's Regulations, regarding animal protection during transport and other related activities [6].

In order to establish the manner in which application of current legislation regarding animal protection during transport has led to an improvement to animal welfare, drivers and transport attendants employed to transport livestock by means or road vehicles completed a 28 item questionnaire. Among others, the questions referred to: usefulness of knowledge acquired following training participation, means of transport characteristics, distance travelled, loading surface, injury and mortality rate upon arrival at destination, unloading time and traffic inspections.

Data obtained were statistically processed (ANOVA) and thus we were able to highlight the efficiency in applying current legislation and regulations on animal welfare during transport.

Results and discussions

The requirement of animal transportation that has resulted from the answers given by the participants was on average of 63.45% for chickens, 16.5% for pigs, 18.8% for cattle and sheep and 1.25% for other species (Fig. 1).

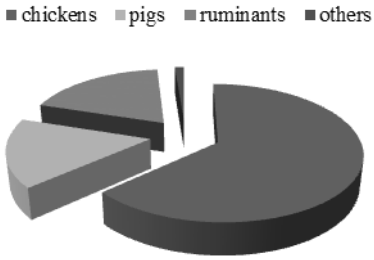

Fig.1. *The proportion of species transported (%) resulting from the study*

Following completion of questionnaires, we have concluded that the majority of journey during study time were short term (71.32 %), while the long term represented 28.68 % and were achieved both across Europe and to non-European countries.

The vehicles used during animal transport fulfilled the necessary physiological conditions required by the animals transported, both for long and short duration transport, as many of them were replaced or improved compared to the period prior to the study (Fig. 2).

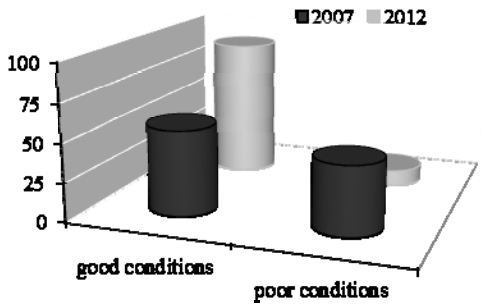

Fig. 2. *Ensuring the transport conditions for transported animals (%) in the short transport*

Moreover, in the long transport road vehicles were equipped with temperature monitoring and recording systems, artificial ventilation system and appropriate navigation system Global Positioning System (GPS).

Most of law breaches reported in long transport over the time were: the transport of unfit animals; the ceiling height; exceeding the loading density; failure to provide water on the vehicle; the use of vehicles that fail to meet the legislative standards [7].

Animal welfare during transport was ensured by abiding by legislative norms and requirements, fact rendered visible by the answers rate related to animal loading surface. This was ensured in short term transport (Fig. 3) to the maximum

for 63.8% of the animals transported ($p \geq 0.05$); to the minimum for 32.6% of the animals transported while for 3.6% of the animals transported this was not ensured ($p \leq 0.05$).

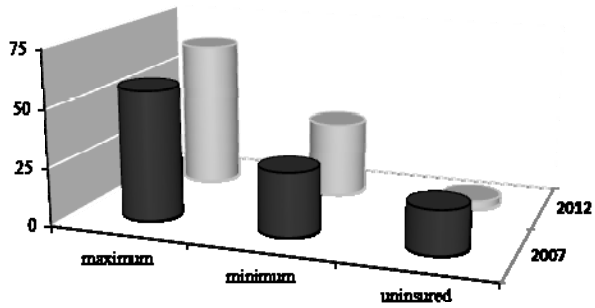

Fig. 3. *The loading surface variation in short transport (%) before 2007 and after five years application of current legislation*

The study results have shown an improvement in ensuring the appropriate loading surface to the animals transported compared to the period prior to implementing the specific legislation and drivers' participation in the professional training programs, due to the fact that they understood and became aware of the importance of ensuring this surface which may influence animal welfare during transport.

In our study, during the period when the Regulations were applied animal welfare level has improved considerably with respect to: transport means, ensuring loading surface, travel duration and environmental conditions.

As a result of the knowledge acquired, 81,3% of respondents answered affirmatively the question related to the decrease in animal injury and mortality rate upon arrival at destination thus confirming once more the importance of attending the programmes. Many of the participants by the answers given have confirmed that they have understood the impact of transport on animal welfare.

We have considered as extremely important the answer of 92% of the participants, who stated that these trainings have been useful for their activity from then onwards while only 8% of the participants have declared that the trainings did not help them in any way ($p \leq 0.001$).

Zanardi *et al.* (2007) have showed that the poor welfare is often due to lack of education, which has also resulted from our study. The animal transportation may have significant consequences regarding animal welfare, that ranging from pain, restlessness and fear to high levels of stress, due to a difference in attitude towards animals from the stakeholder – from farmer, Transport Company, driver, to those that handle the animals. Most of the times this happens due to lack of knowledge regarding the characteristics of transport means, loading surface, loading methods, all the more reasons to train all parties involved.

Conclusions

Our study showed that it is equally important the person that handles animals during loading/unloading to be trained to guarantee a high level of animal welfare.

Also the codes of good practice in this topic can have significant effects on animal welfare during transport.

Although mandatory by applicable current legislation, organizing the programmes to train drivers and transport attendants proved to be a necessity in order to ensure animal welfare during transport.

Both in the case of drivers and that of other people involved lack of knowledge regarding animal protection and welfare during transport may lead to a low level of welfare in animals transported, due to their behavior in traffic, together with other transport or transporter related causes.

To guarantee a high level of animal welfare during transport it is as important training the persons that handle animals during loading/unloading.

Acknowledgment

The authors wish to thank the Centre for Training in Road Transport (FATII-ARTRI) for their support in conducting this study.

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METABOLIC DISEASE OF BOVINS AND RISKS FACTOR

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Abstract

Most of the metabolic diseases of dairy cows – milk fever, ketosis, retained placenta (RP), and displacement of the abomasum – occur within the first two wk of lactation. In addition to metabolic disease, the majority of infectious disease experienced by the dairy cow, especially mastitis, but also diseases such as Johne's disease and Salmonellosis, become clinically apparent during the first two wk of lactation. Metabolic disease is the most commonly recognized disease on dairy farms. While the pathogenesis is well known, metabolic disorders continue to occur. Metabolic diseases are associated with one disease predisposing to another. Evidence suggests that metabolic disease affects host defense, and therefore, impacts the common infectious diseases of dairy cows. Risk for metabolic disease is affected by dietary formulation but is modified by cow behavior and intake. Regardless of dietary formulation, the cow and management factors on a given farm may determine the impact of metabolic disease.

Key words: *metabolic diseases, dairy cow, management factors.*

Introduction

Metabolic diseases are those associated with the chemical processes necessary for maintenance of life. In cattle, metabolic diseases include errors in electrolyte / mineral metabolism, of which parturient hypocalcemia (milk fever) is most common, or errors associated with energy metabolism, including ketosis and displaced abomasum. This review will make two assumptions before reviewing studies correlating metabolic disease with changes in infectious disease resistance. One of those assumptions is that mammary gland infections are less likely in animals with a *strong* immune system and that periparturient immune suppression exists and predisposes cows to mastitis and other infectious disease. Metabolic diseases are associated in that the occurrence of one increases the risk of another. These associations tend to leverage the impact of disease on the animal (Correa *et al*., 1993).

Parturient hypocalcemia and ketosis can present in either clinical or subclinical states. Clinical disease implies that cows exhibit physical abnormalities.

Subclinical disease is one where cows do not exhibit clinical signs, but the biochemical condition is present. Most producers have been content to estimate the impact of metabolic disease as a function of occurrence of clinical disease. While clinical disease occurs at a modest rate, subclinical disease has become recognized as common.

Occurrence of Metabolic Disease

Clinical parturient hypocalcemia affects an average of 6% of cows and has been associated with a 3-fold increased risk of dystocia, retained placenta, and displaced abomasum, and a nearly 9-fold increased risk for clinical ketosis and mastitis (Curtisetal., 1983; Keltonatal., 1998). Subclinical hypocalcemia, defined as plasma calcium of 5.5-8.0 mg/dl within 48 hours of parturition, has been preliminarily reported to occur in 25.3, 43.9, and 57.8% of lactation 1, 2, and 3+ cows (Reinhardt *et al.*, 2004).

Clinical ketosis is estimated to affect about 6% of cows (Kelton *et al.*, 1998). However, subclinical ketosis, defined by postpartum serum beta hydroxybutyrate, affected 59% of cows (Duffield *et al.*, 1998). Ketosis is associated with a decrease in milk production and increased risk of other postpartum diseases (Rajala-Schultz *et al.*, 1999). It is known that the risk of displaced abomasum is increased as a consequence of subclinical ketosis in lactation (Geishauser *et al.*, 1997) or in the 2 weeks leading up to calving (LeBlanc *et al.*, 2005).

These data may be interpreted several ways. They do suggest that there are a high proportion of cows very near "the edge" of clinical disease. This further suggests that any limited stressor, acting to tip the balance in favor of disease, may cause a very considerable proportion of cows to be clinically affected.

In the most parsimonious terms, metabolic disease, both electrolyte related and energy related, may be considered a problem associated with diet formulation, diet consumption, and/or individual (i.e., genetic) factors. Of these, diet consumption is probably the most variable. Therefore, if a single risk factor "root cause" of metabolic disease is to be considered, that "root cause" would focus on the factors associated with dry matter intake (DMI) in late gestation/early lactation cows. This is particularly and directly the case for the energy related diseases.

Energy Associated Disease

Ketosis, fatty liver disease, and displaced abomasum are the common energy related metabolic diseases. Energy related disease is generally thought to occur as a result of excessive lipolysis (fat breakdown) that leads to ketosis/fatty liver. Lipolysis is stimulated when energy output exceeds intake. Endocrine drivers of lipolysis include decreased insulin (low insulin allows lipolysis to continue), increased glucagon (which increases lipolysis), increased glucocorticosteriods (cortisol – which increases lipolysis), and catecholamines (epinephrine/norepinephrine – the so called "fight or flight" hormones that are powerful lipolytics). While some of these mediators are beyond direct control, the glucocorticosteriods and

catecholamines are important mediators that are, to at least a partial degree, dictated by and within control of management.

Energy related disease occurs as a *consequence* of energy distress. Energy distress can be pictured as a non-adaptive or inappropriate cow response to negative energy balance. Since all cows are expected to go through a period of acute negative energy balance postpartum, the key to health is really how the cow responds to the total environmental stress. Negative energy balance occurs prior to calving, and lipid mobilization pre-partum is extremely rapid (Goff and Horst, 1997). Therefore, energy distress is initiated before calving. Classically, much focus has been placed on improving energy intake of cows through activities aimed at increasing voluntary DMI. The importance of maximizing dry period DMI has been recently questioned, and there has been some thought that stabilizing dry period DMI may be of principle concern (Grummer *et al*., 2004). Irregardless of whether maximizing or stabilizing DMI is found to be of primary importance, factors that contribute to acutely decreased DMI must still be identified and controlled.

Risk Factors for Altered DMI

Body condition, social interaction, and concurrent disease are a few of the many factors affecting DMI. It is well known that over-conditioned cows [body condition score **(BCS)** • 4.0] have a greater decline in DMI around calving, putting them in a position of susceptibility to energy related disease. It has been suggested that adipose cells of over-conditioned cows are more sensitive to signals to initiate fat breakdown, and fat cows may exhibit insulin resistance. Over-conditioned cows tend to have increased fat breakdown, increased liver lipid concentration, and a shift toward ketogenesis. It appears that cows near calving with BCS 4.0 have a marked propensity toward lipid mobilization, and cows with BCS 3.0 have little propensity to mobilize fat (Duffield *et al*., 1999). Therefore, the recommendation that late dry cows be in a BCS range of 3.25 to 3.75 probably represents a good tradeoff between subsequent milk production and risk of metabolic disease. However, careful managers may be able to maintain health and gain high production in cows with greater BCS if environmental conditions are optimal and energy distress is avoided (Contreras *et al*., 2004).

Social (or grouping) stress can result in alterations of cow behavior and may affect energy balance. The effects may be mediated through decreased feed intake or through the stress induced lipolysis pathways. Pen moves result in observed social disorder for 2 days, with a milk yield depression of 2 to 5% for the average cow (Hasegawa *et al*., 1997). While this is a modest effect, social stress can effect the non-dominant cow to a much greater degree. Dominate cows (usually older, larger, more senior, and gaining weight) are largely unaffected by a group change. However, non-dominate cows (typically younger, smaller body size, and/or cows losing weight) may be targets of aggressive social behavior, with resulting less opportunity for feed and rest. Clinical ketosis and fat infiltration of the liver in late pregnant cows has been observed following feed restriction of 30 to 50% or fasting

for 4 to 6 days (Gerloff and Herdt, 1984). Therefore, coupling the natural decline in DMI with social stress lasting more than two days, especially in non-dominate animals entering a marginal housing situation, a significant proportion of animals could be placed in acute negative energy balance leading to energy distress and clinical disease.

Social effects are accentuated in larger cow groups/herds, so they assume more importance as herds grow in size. The ability to measure cow interaction, and the effect it has on feeding behavior, is only beginning to be addressed. Social interaction is dependent on the constitution of the group, as well as housing, feeding, and other environmental factors. Therefore, the relationships can become complex and difficult to predict. In general, minimizing re-grouping at key times has been under investigation. These times include the period of 5 days prior to calving and 1 to 10 days after calving (Cook and Nordlund, 2004).

Relationships of Energy, Disease, and Host Defense

Three other related diseases, retained placenta, endometritis, and mastitis, are prevalent conditions that have been putatively associated with energy deficiency in cows. Endometritis and mastitis affect 17% and 13 to 45% of lactations, respectively, and are infectious in origin, but the bacterial agents are considered opportunists so that these diseases are largely determined by cow defense (Hoganetal., 1989; Epperson *et al*., 1993; USD A, 1996; LeBlanc *et al*., 2002). Neutrophils are very important in bacterial defense, and it was shown that neutrophil function declines in late gestation, reaching a nadir near calving (Kehrli *et al*., 1989). Additionally, neutrophils are important in placental release, and cows with retained placenta had a deficiency in neutrophil function in the prepartum period (Kimura *et al*., 2002). Ketone bodies appear to decrease neutrophil response (McMurray *et al*., 1990; Sartorelli et al., 1999). Cows that exhibited hepatic lipidosis, a lesion consistent with energy distress, took longer to clear experimental intramammary infection and had blunted response to vaccination (Hill *et al*., 1985; Wentiketal., 1997). In addition, *invivo* work suggests that improvements in energy balance in late gestation tended to decrease retained placenta (Duffield *et al*., 2002). While it is unclear how negative energy balance affects host defense, it is important to recognize that diseases of the mammary gland and uterus may be associated with energy distress. Energy balance should be considered a potential contributor to these energy related diseases if antioxidant vitamins and minerals are adequate.

Summary

Metabolic diseases are interrelated, so that one disease increases risk for another. The energy associated diseases include ketosis, displaced abomasum, fatty liver, retained placenta, metritis, and possibly mastitis.

Providing an environment for an adaptive cow response will remain key to health. Dairy advisors must take an active role in promoting quantitative monitoring to assist the producer. In addition to tracking average DMI, monitoring

energy balance using milk or blood NEFA or ketone assays may be essential, and may provide an early warning of problems to come. Since disease represents failures (those cows who could not negotiate stress), analysis of disease incidence records must be conducted and compared to known risk factors, including BCS, DMI, pen moves, and concurrent disease. These areas are obvious points where nutritionists and veterinarians can interact in a cooperative relationship.

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FUNCTIONAL FOODS – A NEW CHALLENGE FOR FOOD INDUSTRY MANUFACTURES

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Abstract

The aim of the research was to investigate the consumer awareness regarding Functional Foods for a new approach regarding producers in Food Industry. Lately, the consumers are becoming more aware regarding a healthy nutrition, food quality, or food components that can bring a benefit for general health, either general well being or either improving health for some particular diseases. On a particular note, it is generally recognized that calcium and probiotics (milk or dairy products) are interconnected with bone health or digestive health, or the fish rich in omega -3 fatty acids will reduce risk of heart disease.

Key words*: Functional foods, healthy nutrition, food quality, general health.*

Introduction

One of the most concluding definition regarding Functional foods belongs to Diplock *et al* (1999) and it states: "A food can be regarded as functional if it has beneficial effects on target functions in the body beyond nutritional effects in a way that is relevant to health and well-being and/or the reduction of disease."

Today is a wide concern regarding a good health and a healthy nutrition based on nutrients that can provide the body more than a simple nutrition. Health is one of the most frequent choices regarding foods in European countries (Lappalainen *et al* 1998). Functional foods can be natural (fruits rich in fiber and antioxidants, oily fish with high levels of omega 3-fatty acids), added to minimally processed foods (orange juice with soluble fiber, margarines containing plant stanols), achieved through breeding techniques or through customizing animal's diet (cows fed with high selenium diet to produce organo – selenium enriched milk) (Thompson and Moughan, 2008).

From a historical point of view Hippocrates in 400 BC is one of the pioneers. 'Let food be your medicine and medicine be your food.' The modern concept of functional foods belongs to Japanese in the 1980's linked with old Asian philosophy food and overall health. Thus, an epidemiological evidence diet and health exist since 1950's. New types of foods designed to promote health or to reduce the risk of diseases, known as functional foods, have been entering the

market since the 1990's. Based on manufactures point of view, the industry is offering a lot of opportunities, thus consumers expectation is paying an important role (Thompson and Moughan, 2008). Different generations have been identified as having different attitudes and behaviours which result in specific patterns of functional food consumption (Duff 2006).

According to Berry (2006), consumers are becoming more aware of the effect that certain foods or food components may have on their risk of developing specific diseases (examples: calcium promote bone health, dietary fiber may reduce risk of cancer, omega -3 fattty acids will reduce risk of heart disease or probiotics will help digestive health).

The topic of research will be to investigate if there is an appropriate consumers culture linked with functional foods and if it provides the interest for consuming these types of foods.

The Rationale of the Research will cover the gap in literature and will try to identify a new opportunity for food industry producers.

The Method of the Research is an exploratory method based on literature review analysis.

Material and methods

Methodology used is an exploratory one based on previous literature review analysis. According to Niva and Mäkaelä (2005), qualitative and quantitative consumer studies on functional foods tend to focus on different aspects of the phenomenon. Many qualitative studies have focused on the meanings and interpretations of functional foods among consumers and indicated that the acceptability of new foods is a complex issue with a multitude of aspects. In contrast to the qualitative approaches, quantitative studies often focus on attitudes towards specific products or product types with the aim of finding out what kinds of products, added ingredients, tastes, health claims or combinations of these would most appeal to consumers (Poulsen, 1999; IFIC, 2000; NIN, 2002; van Kleef *et al*., 2002; Bech-Larsen and Grunert, 2003; Urala *et al*., 2003).Therefore qualitative studies have largely focused on consumers' interpretations of functional foods, quantitative approaches have concentrated on factors that may explain differences in the acceptability of functional foods Niva and Mäkaelä (2005).

Results and discussions

Research findings are based on literature review analysis. In modern Western societies, health is one of the central values and even a purpose in itself. Also government policies focus on health promotion and preventive measures against illnesses. For many, health has become a life-long project of keeping well and fit, including self-control and continuous work towards better health (Burrows *et al*., 1995; Petersen and Lupton, 1996). According to Burgarolas *et al*. (2006), looks like the consumers most likely to purchase functional foods are women and those who place a high level of importance on health and nutrition.

In Europe, relatively few studies have investigated the role of socio-demographic factors in the acceptability of functional foods, even though a multitude of studies indicate that citizens' views about food and health as well as their eating patterns are related to age, gender, socio-economic status and phase of life. The 'ACNielsen Functional Foods and Organics Consumer Behaviors and Attitudes Survey' (2005) found that high-fibre products were the most common functional food purchased worldwide, followed by iodine-fortified salt, cholesterol-reducing margarines and fortified fruit juices (Table 1).

Table 1

Frequency of the purchase for particular functional food categories in different regions. Adapted from (ACNielsen Functional Foods and Organics Consumer Behaviors and Attitudes survey, November 2005)

Functional product purchased regularly	**Asia/Pacific**	**Europe**	**North America**	**Global average**
	%	**%**	**%**	**%**
Whole grain, high-fibre products	37	38	55	40
Iodine-enhanced cooking salt	32	30	24	32
Cholesterol-reducing oils and margarines	28	27	41	31
Fruit juices with added supplements/vitamins	32	26	32	30
Yoghurts with acidophilus cultures/probiotics	30	20	22	25
Milk with added supplements/vitamins	25	12	23	19
Bread with added supplements/vitamins	24	10	25	18
Fermented drinks containing 'good' bacteria	21	14	4	17
Soy milk	27	6	10	14

There are a number of trends regarding purchasing of functional foods, most of them linked and appears to be relevant on a global scale the desire for individualized nutrition, the need to control body weight, and the use of foods rather than pharmaceuticals to positively influence mood and mental health (Mellentin 2007; French 2006; Kern 2006).

The rise of functional foods can be seen as part of the rapid developments in medicine and life sciences that study the interconnections between nutrition and health, or more specifically, between food components and risks of diseases. At the same time, technical advances in food engineering and manufacturing have opened up possibilities in developing products with new technologies and enriching foods with new ingredients. (van Kleef *et al*., 2002; Verschuren, 2002.)

According to Niva and Mäkaelä (2005), the appropriation of functional foods in terms of acceptability is a multifaceted phenomenon. It is possible to discern several aspects: personal experiences of functional foods and opinions of their quality and safety, but also concerns about the consequences of functional foods for our eating practices as well as assessments of the need for control and scientific substantiation of products and their health claims. The importance of these factors varies amongst consumers.

Conclusions

It is clearly that a number of opportunities exists for consumers and manufacturers within the functional food sector, but there are a number of issues that must be resolved if the industry is to continue to grow. Some important challenges regard consumer awareness, understanding and acceptance. Manufacturers who are not currently operating within the functional foods market identified price, lack of consumer awareness and lack of scientific evidence as the key issues they were concerned about when contemplating entering this sector (Thompson and Moughan, 2008).

Collaboration between scientists and health professionals, regulatory authorities, manufacturers and retailers will be appropriate for developing this opportunity for food industry.

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STUDIES ON SMALL RUMINANT RUMINAL MICROFLORA

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Abstract

We have conducted studies on ruminal microflora content, from small ruminants in different feeding conditions to track how this food conditions influence the development of these populations of microorganisms, being known that disruption of the microflora entails inability to capitalize animal optimal food intake.

For these experiments we used Spanca breed adult rams, having an average weight of 50 kg, divided into two groups.

Group 1 comprised rams that were fed with natural hay ad libitum and group 2 included rams fed with concentrated feed in proportion of 80% and natural hay in proportion of 20%.

After that we proceeded the determination of total number of bacteria in rumen juice and established their tinctorial affinity using Gram stain technique.

The research carried out on sheep ruminal microflora showed oscillation in the number of bacteria per milliliter of rumen content, as well as their dyeing affinity. This oscillation was determined by the composition of feed rations administered to two groups of rams in the study and the physiological state of the animals.

Key words: *ruminal microflora, microorganisms, small ruminants.*

Introduction

Rumen microbial population depends on the food consumed and it is impossible to generalize the relationship between microflora and age. It is significant that in the young animal predominant is the lactic acid producing flora, and in time, after the consumption of solid food occur changes in the microbial population that becomes more diverse, producing volatile fatty acids.

Ruminal population at very young animal is probably derived from the contents of the clot recirculation (being a large number of lactobacilli, streptococci and coliforms), (Yáñez Ruiz, DR *et al*., 2004).

Bacteria using lactate found in greater numbers in youth and are facultative anaerobes. However we find the cellulosal bacteria in the rumen from the age of 7

days, which indicates that the bacteria found in 1-3 weeks young animals are different from those found in the adult animal (Giraldo, *et al*., 2008).

Also the ureea ruminal secretion from the saliva influences the microbial activity . The absorbtion speed of the microbial fermentation products influences the microbial growth and also the physical state of the feed, the time and speed of feed administration(Thauer, R.K. şi colab., 2008).

Type of food has an impact not only its rough composition by carbohydrates and proteins but also the content of microelements. These factors influence the development of symbiont populations constant both in numbers and in terms of variability existing species. Changing biotype optimal conditions will lead to changes in the flora and fauna but tend to stability and recovery (Cantalapiedra-Hijar G., *et al*., 2009).

Materials and method

For this study the biological material was freshly harvested ruminal contents from rams maintained under the same conditions and fed rations balanced microclimate throughout the examination.

Animals used in the experiment were rams Spancă breed adults with an average weight of 50 kg; divided into two groups of 4 animals.

Lot 1 consisted of 4 rams that were fed with natural hay ad libitum.

Group 2 consisted of 4 rams that were fed with feed concentrates 80% and 20% natural hay to stimulate rumen motility.

Harvesting ruminal juice technique through catheterization method

Catheterization was performed using a probe inserted through the animal's mouth to the rumen, removing the juice needed. The method has the advantage that it leaves no sequelae and does not injure the animal.

The method further has the advantage that captures flora and fauna in full activity, being able to grasp a difference depending on feed administered.

Sampling was carried out in the morning, before the rams received their ratio, after a diet for 12 hours, during which time water has been ad libitum.

Harvesting was performed using a probe equipped with a 200 ml syringe used for the aspiration of ruminal contents. Harvested juice was collected in isothermic containers and stored at 38°C .

Before using the juice was filtered through two layers of gauze to stop impurities (roughage).

Determining the total number of germs

For this determination the cell-dish culture was used. The method consists in seeding bacteria from the full ruminal juice and various dilutions and counting the colonies grown on agar after 24 hours.

Materials used: ruminal juice freshly harvested and filtered, sterile tubes with each 4.5 ml broth culture, medium Petri dishes with culture medium (agar),

graduated pipette thermostat. After 24 hours were counted the colonies in dilutions of 10-7 and 10-8.

Number of colonies resulting in Petri dishes was multiplied by the respective dilution.

Determination of bacteria after dyeing affinity of freshly harvested ruminal fluid

For ruminal juice of freshly harvested and filtered, smears were made which were then stained by the Gram method.

Bacteria were counted per smear Gram negative and Gram positive categories and within each category they were grouped by size into small bacteria (0.5 to 2 μ), medium (2-4 μ), and large (> 4 μ). The data are presented in tabular form for each batch of animals.

Results and discussion

The present study aimed to what extent the composition of feed intake may influence the number of bacteria in the rumen contents. We determined the total number of bacteria in the rumen contents of the two groups of rams in the study - fed different forages, the data obtained were pooled separately (Table 1, Fig. 1).

Table 1

The total number of germs / ml ruminal contents from rams fed natural hay (lot 1)

Animal number	The dilution of the count	Number of colonies on dish	Total number of germs
1	10^{-7}	50	500.000.000
2	10^{-7}	37	370.000.000
3	10^{-7}	38	380.000.000
4	10^{-7}	20	200.000.000

Analyzing the data in table 1 shows that the number of bacteria in rumen contents from rams fed natural hay recorded different values, with variations quite broad limits depending on the individual and its physiological condition.

The highest values recorded were 50 x 10^7 bacteria / ml of ruminal contents and lowest values, 20 x 10^7 bacteria / ml, achieving a lot average of 36,3 x 10^7 bacteria / ml.

Interesting data were obtained in animals fed with concentrated feed (80% of daily intake).

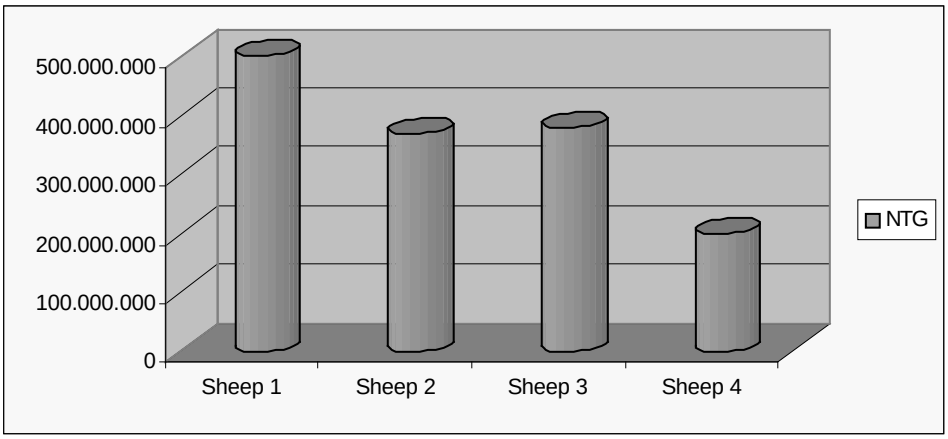

Fig. 1. *Graphical representation of TNG in ruminal contents from rams fed with natural hay*

It was found that the number of bacteria in the rumen content was much higher than the number obtained in the group fed exclusively with natural hay (Table 2, Fig. 2).

Table 2

The total number of germs / ml ruminal contents from rams fed with concentrate feed (lot 2)

Crt no.	Dilution	No of colonies	TNG
1	10^{-9}	64	64.000.000.000
2	10^{-9}	51	51.000.000.000
3	10^{-9}	47	47.000.000.000
4	10^{-9}	43	43.000.000.000

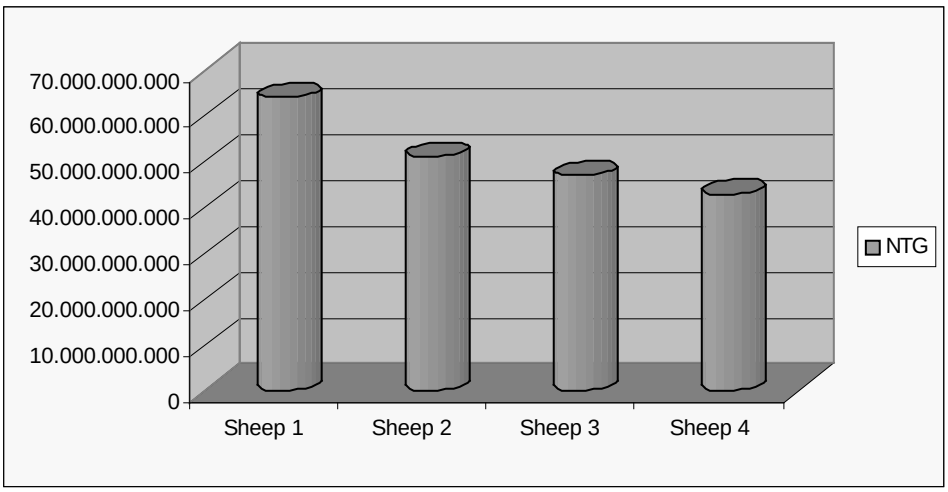

Fig. 2. *Graphical representation of TNG in ruminal contents from rams fed with concentrate feed*

After germ count in group 2 rumen bacterial count averaged 51 x 10^9/ ml content, with variations ranging from 43 x 10^9/ml and 64 x 10^9/ml.

A bacterial classification was obtained from ruminal contents under Gram stain, identified and counted in the smear setting 3 groups:

- Large bacteria (> 4 μ)
- Medium bacteria(2-4 μ)
- Small bacteria (0.5 to 2 μ).

The results were highlighted as follows (table 3, fig. 3):

Table 3

The number of bacteria in rumen contents after tinctorial affinity from rams fed with natural hay (lot 1)

Crt. No.	Determ. No.	Average number of bacteria					
		Large bacteria		Medium bacteria		Small bacteria	
		G+	G-	G+	G-	G+	G-
1.	2	12	35	11	10	2	4
2.	2	14	42	13	7	4	3
3.	2	9	37	12	9	3	5
4.	2	11	42	4	6	8	9

It is noted from Table 3 that the group of rams fed exclusively with natural hay (lot 1) predominates large bacteria in ruminal contents , especially Gram-negative highly possible cellulolytic. At the other batch of rams fed concentrated feed there is a situation contrary to the group of rams fed exclusively with natural hay (Table 4, Fig. 4).

This predominance of small bacteria in rumen contents from rams fed concentrates only likely to be due to lack in feed ration of fibrous feed and excess of short-chain sugars (starch), protein and fat provided by ingested concentrated animal feed.

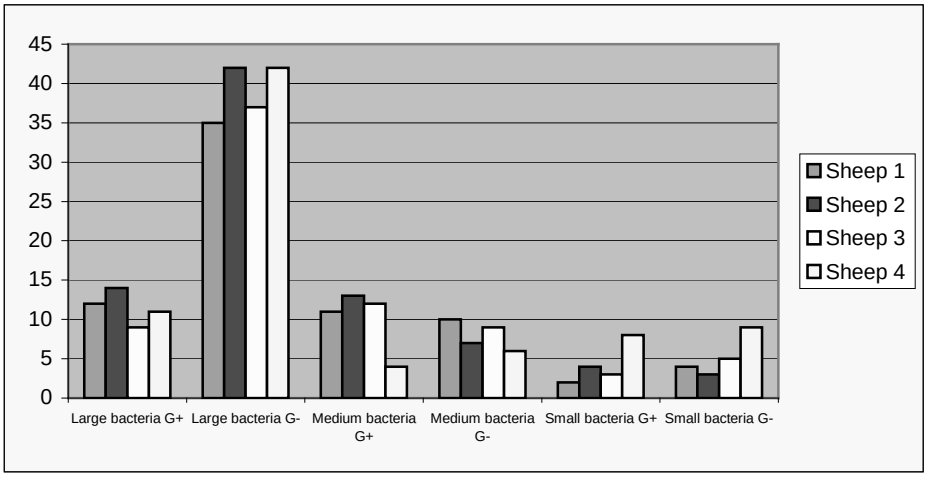

Fig. 3. *Graphical representation of the number of bacteria in rumen contents after tinctorial affinity from rams fed natural hay*

Table 4

The number of bacteria in rumen contents after tinctorial affinity from rams fed concentrated feed (lot 2)

Crt. no.	Dererm. No.	Average number of bacteria					
		Large bacteria		Medium bacteria		Small bacteria	
		G+	G-	G+	G-	G+	G-
1.	2	20	12	8	17	25	12
2.	2	14	17	4	12	30	13
3.	2	11	16	2	10	41	8
4.	2	9	19	5	12	39	16

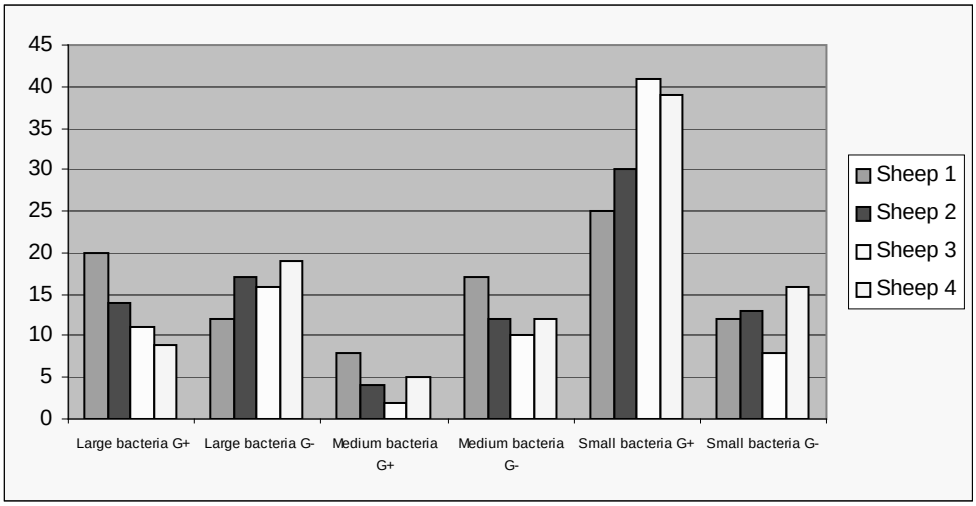

Fig. 4. *Graphical representation of the number of bacteria in rumen contents after tinctorial affinity from rams fed concentrated feed*

Conclusions

1. The total number of bacteria in rumen contents from rams fed exclusively with natural hay averaged 36.3 x 107/ml content to 51 x 109/ml content in rams fed with concentrates 80% of the ration.

2. It was thus found that the number of bacteria in the rumen content was much higher than the number of bacteria in the group fed solely with natural hay.

3. With the increase in the percentage of concentrate fodder ration (80%), large decrease in number of the bacteria, growing the small number of bacteria, particularly Gram-positive bacteria.

4. This predominance of small bacteria in rumen contents from rams fed only concentrates would likely be due to excess short-chain sugars (starch), developed by feeding hay, but the high percentage of protein and fat provided by concentrated feed ingested by animals.

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STUDY ON EFFECT PROBIOTIC BIACTON IN POULTRY GROWTH FOR MEAT PRODUCTION

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Abstract

This study is a test on commercial probiotic Biacton introduced in drinking water to determine their influence on production performance of chickens meat.

Testing was done on two batches of ROSS 308 broiler, in the control group (L1) composed of 16480 chickens and the experimental group (L2) consisting of 16560 chickens. There have been pursued: the evolution of body weight, weight gain, mortality rate and specific consumption.

The Biacton products led to significantly better results obtained in the experimental group compared with controls as follows:.

Medium-weight was 2672 g in group L2, with 13,84% more than the group L1(2347) g;

– *Average daily growth between 0-41 days was 63,6 g chicken in the experimental group 13,2% more than in the control group (55.8 g / chicken).*

– *Losses through mortality were 4,1% at L1, up 0,7% from the group L2 (3,4%);*

– *Specific consumption values 1.710g./chicken had the experimental group and control group respectively at 1,792 g./chicken.*

It demonstrated such beneficial effect of probiotic tested on some production parameters in broilers.

Key words: *probiotic, Biacton, performance, chicken broiler.*

Introduction

Antibiotics were first used growth promoters in animal nutrition and biostimulator their effect is demonstrated experimentally since 1946. Research has explained the positive impact of maintaining their health, increasing weight gain and feed utilization index.

Besides these beneficial effects have been noted frequently antibiotic resistance phenomena in particular located in the digestive bacteria. E coli have an R factor plasmid containing antibiotic resistance markers and micro characters

conferring multiple resistance to antibiotics. Because this R factor is transferable by conjugation to other bacteria, irrational use of antibiotics in farm animal feeds produced a significant increase in frequency of bacteria resistant to multiple antibiotics.

In these circumstances the European Union banned the use of antibiotics as growth promoter and suggested finding classes of products to replace antibiotics (acids, enzymes, probiotics) (1).

Materials and methods

To establish the effect of probiotic Biacton on broilers were used broiler hybrid belonging ROSS 308 controls divided into two groups (L1) and experimental (L2) with 16480 respectively 16560 chickens per batch.

Each group was individually studied and body weight was determined at the age of 1 day, 7 days, 14 days, 21 days, 28 days, 35 days and 42 days when the study ended.

Objectives in this experiment were:

- Evolution of body weight;
- Dynamic weight gain;
- Evolution of the percentage of mortality;
- Specific consumption dynamics;

The Biacton probiotic has been introduced in drinking water 500ml/1000 l water during all growth period.

Results and discussion

In Table 1 and Figures 1, 2, 3, 4 are presented the dynamic weight, average daily gain, mortality rate and specific consumption trends.

Table 1

The results of weight average daily gain, mortality rate and specific consumption

Week	Body Weight (g)		Average daily gain (g)		Percentage of mortality (%)		Consumption Specific (Kg feed/kg gain)	
	Lot 1	Lot 2 exp	Lot1	Lot 2 exp	Lot 1	Lot 2 exp	Lot1	Lot2 exp
0	38	42	-	-	-	-	-	-
1	166	182	18.2	20	0,5	0.4	890	832
2	422	502	36.5	45.7	1.1	1	1070	1020
3	890	1012	66.8	72.8	2.3	1.6	1380	1261
4	1355	1573	66	84.2	3.0	2.8	1440	1420
5	1991	2267	90.8	80.1	3.7	3.1	1650	1610
6	2347	2672	50.8	57.8	4.1	3.4	1710	1792
Total	2347	2272	50.8	57.8	4,1	3.4	1710	1792

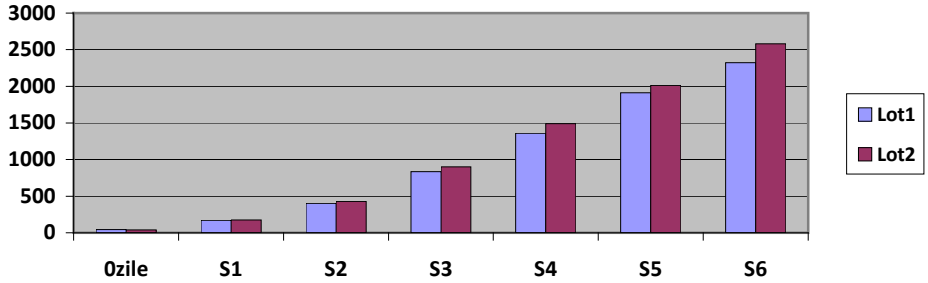

Fig. 1. *Trends in body weight (g)*

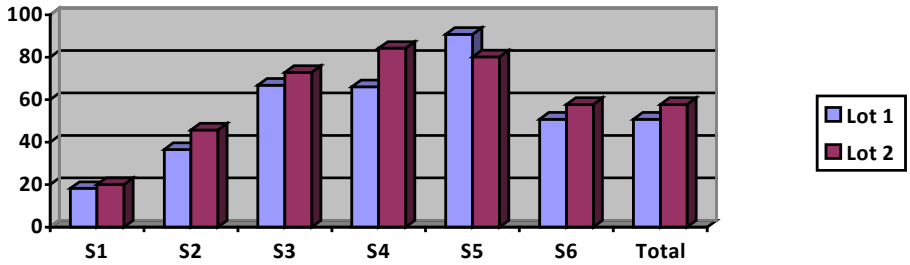

Fig. 2 *Evolution of average daily gain (g)*

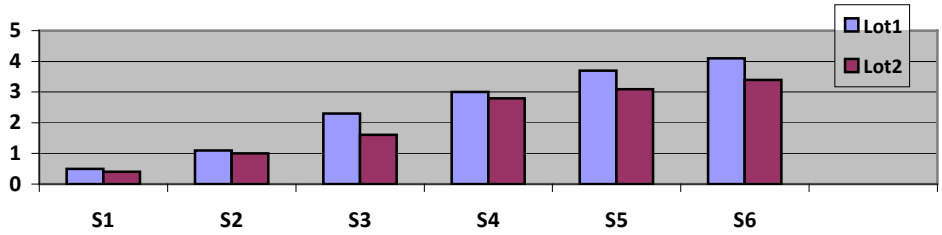

Fig. 3. *Evolution of the percentage of mortality (%)*

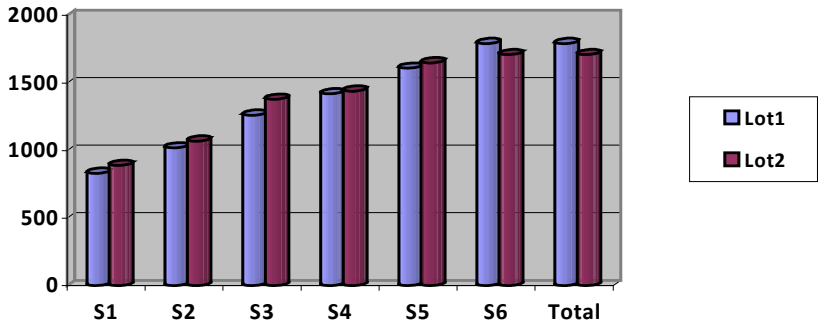

Fig. 4. *The evolution of specific consumption (kg feed / kg gain)*

Experimental group which was fed with mixed fodder used with added probiotic feed, had higher weights at all scales from the control group sample 182 g, 502 g, 1012 g, 1573 g, 1890 g, 2672 g to 166 g, 422 g 890 g , 1355 g, 1991 g, 2347 g.

Comparing the groups who received the probiotic in feed with the lot that has not received the probiotic it was noticed that this group had a total increase of 13.2% higher than the control group: 57.8 g to 50.8 g.

The mortality was 3.4 % in the experimental group and control at 4.1% This percentage is lower in the experimental group, the incidence of infections at Escherichia coli was reduced.

With regard to feed consumption in the two groups were recorded and processed and consumed quantities of the popular managed to delivery. Thus between 0 and 42 days, chickens in the control group had a consumption 1710g/kg 1792g/kg to the experimental group, with more than 82 g from the group that received the probiotic feed.

Conclusions

1. Broilers receiving feed containing two probiotic recorded higher body weight than those consuming normal feed at all scales of evidence.

2. Managing a fodder containing a mixture of probiotics from 0 to 42 days favors increased weight gain.

3. The mortality is lower in the experimental group due to lower incidence of bacterial diseases (E colli infections).

4. Feed consumption is lower in the experimental group compared to control group.

5. The values obtained showed beneficial effect of probiotic products used in chicken feed in conjunction with a control group that consumed the normal feed with no probiotic.

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IMPORTANCE OF CORONARY VASODILATORS IN DOGS WITH ISCHEMIC CARDIOMYOPATHY

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Abstract

The objective of this study was to identify the electrocardiographic changes to the older adults dogs with ischemic cardiomyopathy without signs of heart failure and to improve their clinical status, by administration of a coronary vasodilator. Visnadine was administered at a dose of 3 mg / kg of body weight, 2 times daily. This coronary vasodilator was added to the classical treatment initially established of these patients, respectively: vasodilator type of angiotensin converting enzyme inhibitors (Captopril), L-Carnitine, Catosal, vitamins (B complex and vitamin E), beta-blockers (Metoprolol).

Electrocardiographic disorders have been identified due to ectopic rhythm (ventricular extrasystoles, left and right), disorders caused by the intracardiac conduction disturbances (atrioventricular block and bundle branch block).

Visnadine, coronary vasodilator administration, improved the overall condition to the dogs of the experimental group (appearance appetite, normalization of heart rate and disappearance of syncope), compared with the control group, since the second week of administration.

From the 24th week, global clinical score was significantly degraded to the control group compared with coronary vasodilator treated group.

Key-words: *coronary vasodilator, ischemic cardiopathy, dog.*

Introduction

Under normal conditions, the heart muscle is dependent on the aerobic metabolism and therefore requires continuous supply of large quantities of oxygen through the coronary circulation. Immediate energy source for myocardial contraction and biochemical reactions taking place in the cell is ATP. Myocardial metabolism is directed to the production of ATP in the mitochondria, by aerobic oxidation of the substrate in the Krebs cycle, by acetyl coenzyme-A enzyme. The

substrates used in the myocardial metabolism to a large extent are free fatty acids, lactates, pyruvate and glucose (Whitney, 2000).

Ventricular myocardial oxygen consumption depends on a number of factors, of which three are most important and are represented by hemodynamic conditions:

- intraparietal voltage of left ventricular myocardium;
- contractile state of the myocardium;
- heart rate.

The other factors involved in myocardial oxygen consumption are:

- myocardial lusitropisme;
- myocardial basal metabolism;
- electrical activity;
- cardiac mechanical work.

The main means of adjusting blood flow to metabolic needs of the heart is achieved by changes in cardiac output. Cardiac output is the actual amount of blood pumped by each ventricle per unit of time. Compensatory mechanisms (tachycardia, hypertrophy, cardiac dilatation) that may increase cardiac ventricular performance are outdated at some point. Following pathophysiological changes install (tachyarrhythmia, cardiomyopathy), which causes reduced systolic ejection fraction supplementation and the decrease of cardiac output.

Decreased cardiac output and decreased entail coronary blood flow, reduced oxygenation of the heart. Anaerobic glycolysis cannot meet the energy needs of the heart, as it produces more milk than it can consume, thus installing the state of local acidosis. For a long period this state was causing hypoxia and myocardial ischemia (Fox *et al*., 1999, Borgarelli *et al*., 2007).

Our goal was to identify electrocardiographic changes of myocardial ischemia in older dogs without signs of heart failure and improve the clinical condition and prolong life by administering a coronary vasodilator, Visnadine added to the treatment initially established.

Materials and methods

The study was conducted on a total of 30 dogs, average age 12 years, who were electrocardiographic evaluated within the medical Veterinary Doctor's Vet Universe and our clinic. Electrocardiographic changes recorded specific myocardial ischemia: morphologic changes in the ST segment and T wave, microvoltage, ventricular extrasystoles, atrioventricular and bundle branch blocks. In these dogs it was confirmed the diagnosis of ischemic cardiopathy of senile heart without heart failure, by echocardiographic monitoring of changes in global and regional myocardial kinetics. They formed two groups: a group one, who included a classical treatment with a vasodilator type converting enzyme inhibitor (Enap or Captopril), L-Carnitine (Carnitall), vitamins (B complex and vitamin E) and beta-blockers (Metoprolol) in the case of ventricular extrasystoles. In the second group was added in addition to the initial treatment one coronary vasodilator (Visnadine). Visnadine is a pure herb extract *Amni visnaga*, with

antispasmodic, coronarodilatators, and diuretic properties and after administration increases blood levels of free fatty acids, enhancing energy metabolism.

The electrocardiography was performed using a device veterinary ECG VE type 100, using the standard method of the six derivatives bipolar limbs and working parameters were: speed 25 mm/sec and amplitude 10 mm/mV.

Results and discussion

The coronary vascular network has a very wide collateral blood achieved by arteriovenous anastomoses, veno-venous and intra coronary arteries. Following subendocardial capillary bed is itself rich in numerous anastomoses, which gives good protection against massive heart attacks. In the aged dog develops arteriosclerosis lesions by infiltrating into the extracellular space of amyloid deposits or by transforming the connective tissue into the hyaline tissue of the ventricular and atrial wall structure, or parietal microthrombus formation. These pathological processes lead the diffuse subendocardial infarctlets which produce myocardial ischaemia. Frequency of arteriosclerosis lesions found in adults dogs over 12 years, is on average 65% (Falk and Johnsson, 2000). Clinically, the animals exhibited dyspnea, fatigue and sleep walking, fainting, syncope, cardiac arrhythmias. In electrocardiographic terms, ischemia translates by morphology changes of the T-wave, ST interval, R-wave and the microvoltage or ventricular arrhythmias (extrasystoles, tachycardia, atrioventricular block, bundle branch block). In figure 1 is observed microvolt T-wave and QRS opposite polarity, respectively the direction of the T wave is the same as the terminal component of the QRS.

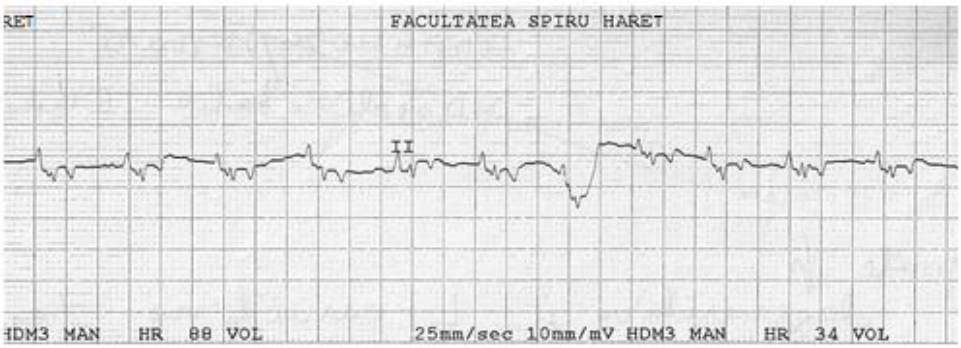

Fig. 1. *Microvoltage and T-wave inversion*

Another example to the subendocardial microischaemia of senile degenerative heart is shown in figure 2. One can observe wide QRS complex of about 0.08 sec. Wave T of negative polarity with the amplitude of 0.6 mV, ST-segment depression.

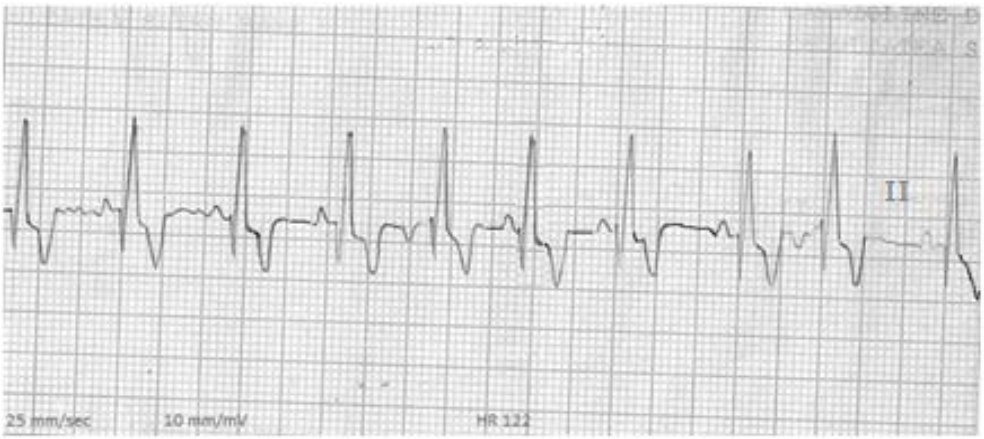

Fig. 2. *Microvoltage, wide QRS, reversed T wave, depression of ST-segment*

In figure 3, there is a broad and negative T wave with major depression in the ST-segment and deep Q wave, those elements characterizing the ischemic lesions.

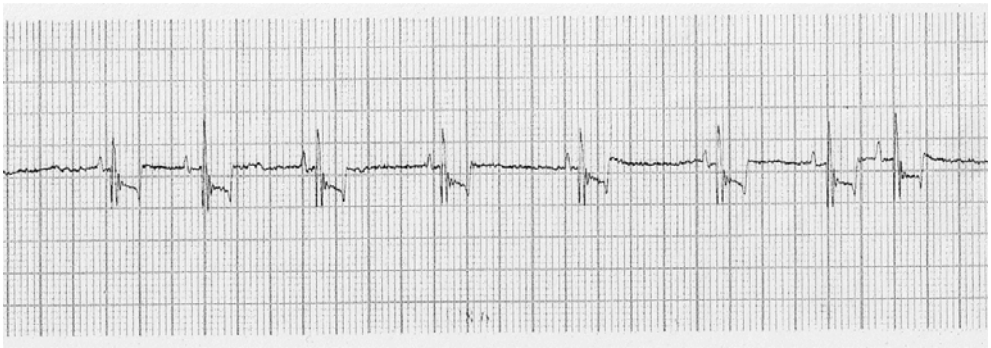

Fig. 3. *Microvoltage, ST-segment depression and deep Q wave*

In figure 4, we see atrioventricular block of grade 3 or completeness and supraventricular rhythm with atrioventricular dissociation. P waves are blocked and succeed with a sinus rate of 140 b / min and ventricles to contract at a junctional frequency 33 b/min. Atrioventricular blocks are the result of non-transmission of action potentials between the top of atrioventricular junction and the common trunk of arms. This bundle is due to inflammatory or degenerative processes at this level.

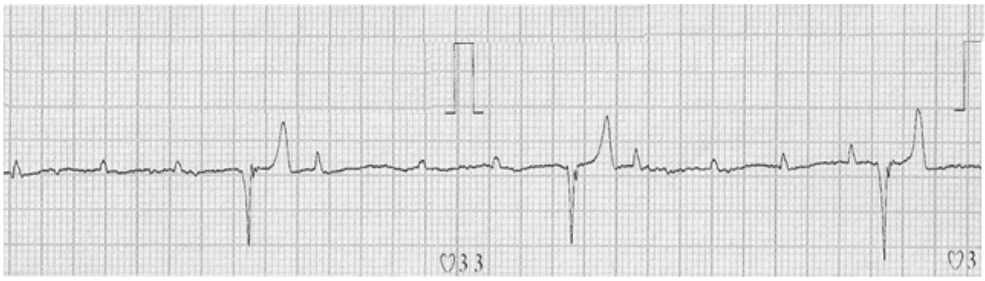

Fig. 4. *Atrioventricular block grade 3 and supraventricular rhythm with atrioventricular dissociation*

In figure 5, we see microvoltage with ventricular extrasystole produced by a reentry mechanism RR intervals' constant, wide QRS complex, T wave reverse polarity compared to the QRS complex.

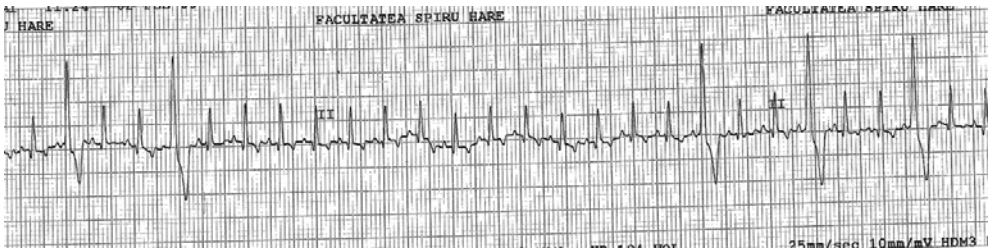

Fig. 5. *Microvoltage with ventricular extrasystole*

Branch block occurs as a result of cellular alterations by ischemia, degeneration or sclerosis, or as a result of aberrant intraventricular conduction, as in the case of dilated or hypertrophic cardiomyopathy (Fig. 6, 7).

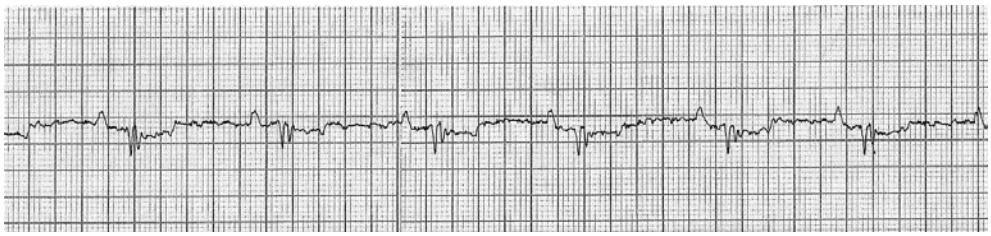

Fig. 6. *Microvoltage and right bundle branch block*

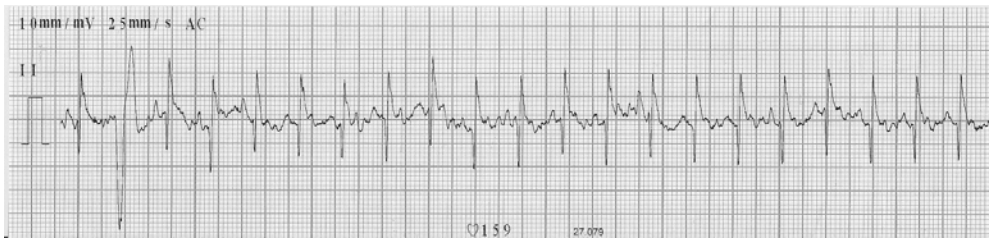

Fig. 7. *Microvoltage and left bundle branch block*

Coronary vasodilators can improve diffuse hypoxia of ischemic cardiomyopathy and can be administered with inhibitors of angiotensin converting enzyme (Enap, Captopril). We have used the product Visnadine (35 mg/cp) at a dose of 3 mg/kg of body weight, 2 times daily. Between these two vasodilators there is synergistic action: the inhibitors of generating angiotensin II have a poor dilating action on the coronary arteries, but decrease preload, afterload and heart frequency and Visnadine acting as antispasmodic effects on smooth fibers have coronarodilatators effect.

Visnadine spasmolytic effect is due to the release of a vascular endothelial relaxing factor in the nitric oxide form, in addition to the active channels K myocytes.

Poor cardiac perfusion of persistent myocardial ischemia, results in reduction of coronary blood flow and the occurrence of abnormal contractility manifested by hipovoltage, the excitability, manifested by ventricular ectopic outbreaks and cardiac conduction, such as atrioventricular blocks and blocks branch. In the case of ventricular extrasystole treated with beta-blockers (metoprolol), and in atrioventricular block prednisolone treated with 1-2 mg/kg /day, Ephedrine 20 mg/ kg/day (or theophylline) was added and Visnadine administration, which has enhanced than the clinical condition of patients.

Duration of experiment was 24 weeks. After one week of additional treatment with Visnadine, it was observed on the animals of experience lot an improvement in the general condition of their appearance appetite, normalization of heart rate and loss of syncope. From the 24th week, global clinical score was significantly degraded compared to the control group treated with coronary vasodilator.

Conclusions

1. Importance of ECG to identify cardiac arrhythmias and conduction disorders.
2. Improvement of animals condition and increasing life expectancy in older dogs suffering from ischemic heart disease treated with coronary vasodilator Visnadine type.

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MAMMARY TUMORS IN CATS, A MULTIDIRECTIONAL PERSPECTIVE, NEWS

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Abstract

We analyzed the frequency of mammary tumors in cats and determinants. We have found that an important factor is the pregnancy rate: cats with multiple gestation had a rate of only 10.42%, while the nulliparous stood at a high percentage (75.0%). Sterilization can be a trigger for tumor process: cats spayed represented 54.17% of the cases with mammary tumors, whereas the intact only had a percentage of 27.08%. The age was another factor involved in the development of mammary tumors in cats: most cases were reported in the age group 10-12 years (43.75%) and 7-9 years (35.42%). Few cases met the age group 1-3 years (6.25%).

Key words: *mammary tumors, cats, trigger factors.*

Introduction

In the past 20 years, little progress has been made to prolong survival time of cats with mammary tumors. Because stromal invasion, metastases are almost always present during the operation, and therefore gives a poor prognosis for severe cases. In the maintenance operation, 66% of the cats which were surgically excised, tumor had recurrence at the site of the operation. Many studies show that the time from detection to the moment of death in cats is 10-12 months.

The most important factors influencing prognosis and survival time in cats with mammary tumors are tumor size, extent of surgery and histological grade. Some studies have reported the significance of lymph node metastasis in accurate prognosis. In one study, 22 (49%) of 45 cats with mammary tumors had metastasized to regional lymph nodes. Lymph nodes were clinically palpable in 10 cases (21%). (36) This means that it is more rational to realize a radical mastectomy, including removal of regional lymph node (inguinal) in all cats. Because positioning axillary lymph node should be removed only if it is enlarged or cytology positive for tumor cells.

Studies took into account several criteria, namely patient age, number of gestation taken hormonal treatments performed; such tumors were classified according to the type histomorfologic detected by laboratory testing.

Materials and methods

The study was conducted between 2012-2014 on a number of 48 cats with mammary tumors, at the Veterinary Clinic "Spiru Haret" and Veterinary Office SC TETRAVET S.R.L in Bucharest. Histopathological examinations were performed in a histopathology laboratory at the „Victor Babeş" Hospital. Biopsy examination is welcome in any oncology treatment. In the absence of histological and cytological diagnosis is surgery risky and not recommended. Biopsy is indispensable of each case tumor lesions or possible tumor. An exception are breast tumors in carnivores, the histopathological diagnosis can be established without reserve post-surgically, if the relevant surgical methodology is based on the location of the tumor process. Before making a choice it must be determined the nature of the procedure biopsy tissue taken. Biopsy aims:

- detection of cancer cells in the primary tumor or metastases;
- determining the type of tumor histopathology;
- determining the degree of tumor histology (low, medium or high malignancy);
- determine the effectiveness of surgery by examining the edges of the tumor and determine the degree of infiltration.

Results and discussion

Mammary tumors are very common injuries in cats. Studies conducted during the two years led to the observation that the incidence of mammary neoplasms has been the highest in the age range between 10 and 12 years, then begins to decrease gradually as over 12 and under 7 years.

The incidence of mammary tumors in cats, depending on the age as following:

– 1-3 years: 3 cases (6.25%);
– 4-6 years 4 patients (8.33%);
– 7-9 years: 17 cases (35.42%); – 10 to 12 years: 21 cases (43.75%);
– > 12 years: 3 cases (6.25%); (see chart I)

The incidence of mammary tumors in cats by age

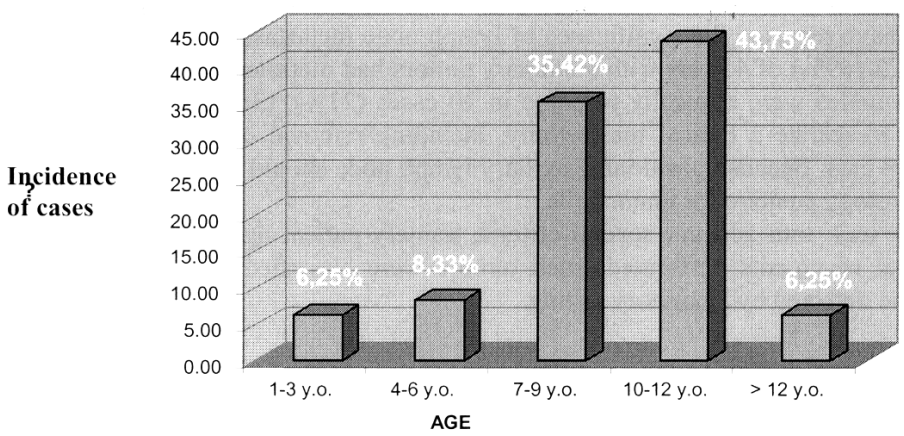


Chart I

This study took also into account the performance hormonal treatments yielding the following results:

– hormonal Treaties: 28 cases (58.34%);

– hormonal Untreated: 20 cases (41.67%). (see chart II)

The incidence of mammary tumors influenced by hormonal treatments (%)

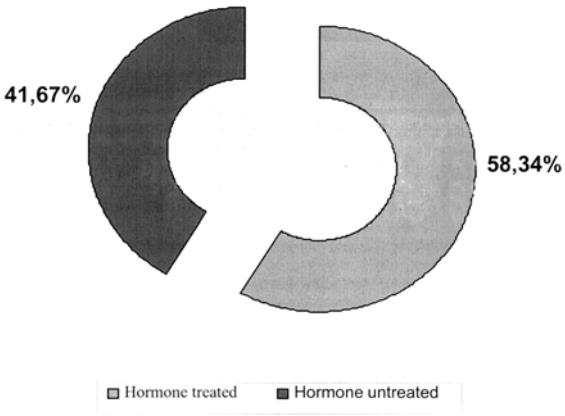


Chart II

Given that the mammary gland is an organ we performed studies on hormone-reproductive status of cats with mammary tumors and came to the following figures:

– Ovariohisterectomizate pre-mastectomy: 26 cases (54.17%);
– Ovariohisterectomizate during operation mastectomy 9 patients (18.75%);

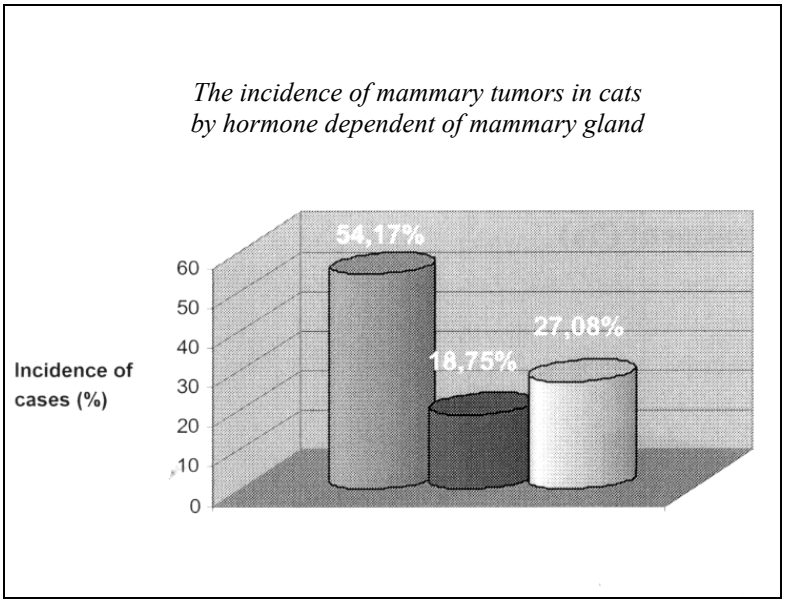
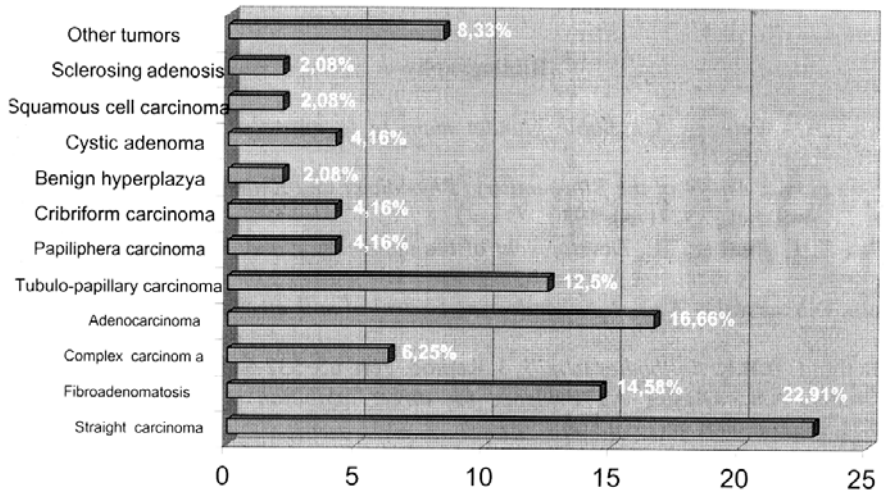


Chart III

The histopathological results have identified the following types of tumors:

– simple carcinoma..... 11 cases (22.91%);
– fibroadenomatosis ..... 7 cases (14.58%);
– complex carcinoma ..... 3 cases (6.25%);
– adenocarcinoma ..... 8 cases (16.66%)
– tubulo-papillary carcinoma ... 6 cases (12.50%);
– carcinoma papilifer ..... 2 cases (4.16%);
– cribriform carcinoma ..... 2 cases (4.16%);
– benign ..... 1 case (2.08%);
– cystic adenoma ..... 2 cases (4.16%);
– carcinoma scvamocelular .....1 case (2.08%);
– sclerosing adenosis ..... 1 case (2.08%);
– other tumor patients (8.33%). (see chart IV)

Incidence of mammary tumors in cats
in terms of histopathological way (%)



Case incidence

Chart IV

Conclusions

1. Mammary tumors in cats have a fairly high frequency, their incidence increasing from year to year.

2. Most cases were reported in the age group 10-12 years (43.75%) and 7-9 years (35.42%). Few cases met the age group 1-3 years (6.25%).

3. An important parameter is the pregnancy rate in the economy mammary tumors in cats. Cats with multiple gestation had a rate of only 10.42%, while the nulliparous stood at a high percentage (75.0%).

4. A certain balance was revealed in regard to the application of hormonal treatments, where the frequency of tumor diseases has stood at 58.34% and 41.71% untreated.

5. Sterilization is apparently not a factor in the occurrence of mammary tumors fren cats because cats spayed represented 54.17% of the cases with mammary tumors, whereas the intact only had a percentage of 27.08%.

6. From the histological point of view, the most common types of tumors were carcinomas solid (22.91%), adenocarcinomas (16.66%) and fibroadenomas (14.58%). Less represented were scvamocelulare carcinomas, benign hyperplasia, sclerosing adenosis, and each with a rate of 2.08%.

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THE AFFECTATION OF THE SANITARY SUSTAINABILITY UNDER BIOPHYSICAL AND BIOCHEMICAL EFFECTS

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Abstract

The article presents scientific investigations related to creating / developing the original management model of the flows (proposals for optimal and effective organization and management) and biophysical and biochemical effects on environmental sustainability, including the sanitary sustainability, in the infrastructures of modern society. We got to the conclusion that it is estimated that the aimed managerial model for elaboration is functionally overlapped on the bio-physico-chemical general model which is characterized by concentration, accumulation and amplification of processes and phenomena in the sanitary field.

Keywords: *sustainability, management, sanitary sustainability, biophysical structures, biochemical structures.*

Managerial structures for sustainability

The overall objective of the present study was divided into sub-goals that relate to analyzing and drawing conclusions following the steps below:

- defining and delimitation of sub-objectives;
- documentation, critical investigation of knowledge in the field; systematization of the damage sources;
- systematization of the ensuring and consolidation resources of the sustainability and the achievement of specific conclusions on analytical and logical-scientific bases;
- critical examination of the findings and the formulation of solutions, options, alternatives, etc. for overall sustainability ensurance, including sanitary sustainability;
- creating / developing the original management model of the flows (proposals for optimal and effective organization and management) and biophysical and biochemical effects on environmental sustainability, including the sanitary sustainability, in the infrastructures of modern society.

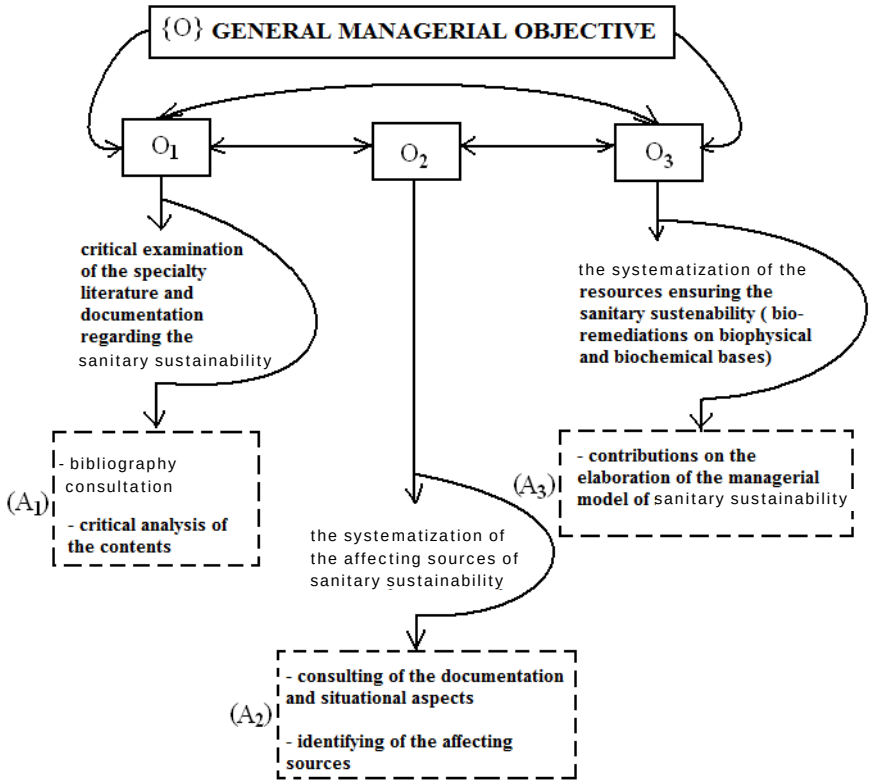

Fig. 1. *The general managerial objective {O}, the sub-objectives {O_1, O_2, O_3} and the activities {A_1, A_2, A_3} for the consolidations of the sanitary sustainability based on bio-remedies*

It is estimated that the aimed managerial model for elaboration is functionally overlapped on the bio-physico-chemical general model which is characterized by concentration, accumulation and amplification of processes and phenomena in the sanitary field (fig.2).

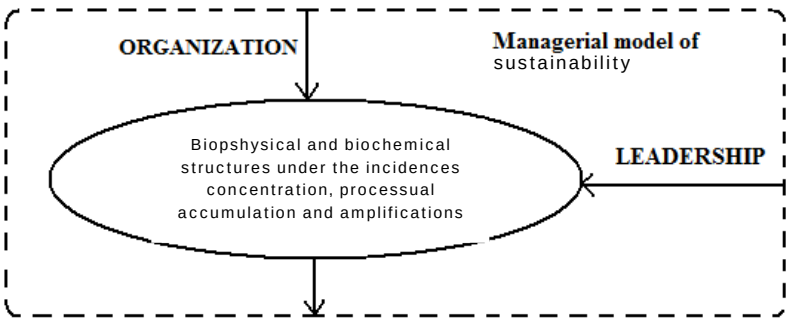

Fig. 2 – *Managerial model*

The disturbances from the bio-physical-chemical system (database, e.g., polution) impose additional functional requirements for the management of sustainability in the field.

Overall observation, with generalization possibilities, is the fact that the whole affecting process of insurance and consolidation of sustainability, is dominated by the phenomena and / or the processes of bioconcentration, bioaccumulation and biomagnification of inputs and outputs in the general bio-physicochemical system from the environment.

In this context, pollution is the damaging of the natural capital, consisting of contamination of air, water and soil with substances harmful to the living organisms (The Heritage Science Dictionary, 2010) [2]. (http://science.yourdictionary.com/pollution).

In our view, pollution is affecting the positive, conventional sustainability levels, including the sanitary sustenability (Figure 3).

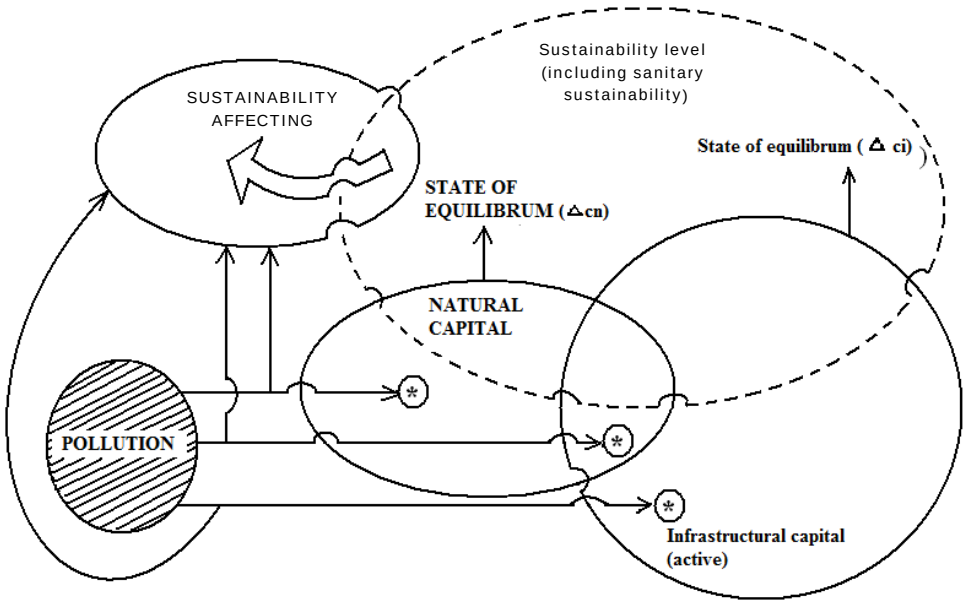

Fig. 3. *The disturbance of sustainability by polluting the natural and physical capital*

It is noticeable that nowadays, the infrastructure capitals increase, being found within the space of natural capitals (fig. 4).

They (the infrastructural capitals) produce impairments of sustainability, in terms where the limits (contours) of the natural general capital are fixed (quasi-fixed).

The extension (growth) of the tangible actives is made on account of the occupation, by degradation of the areas (spaces) related to natural capital.

Conversely, the increase of intangible assets (of knowledge) leads to overall sustainability, in which the knowledge based economy involves the development of

knowledge based society, arised when the requirement for a New Management and this, in turn, based on knowledge.

A low sustainability means damages of the functional capacities of the infrastructures when the relations of interdependencies of tangible assets are omitted from calculations.

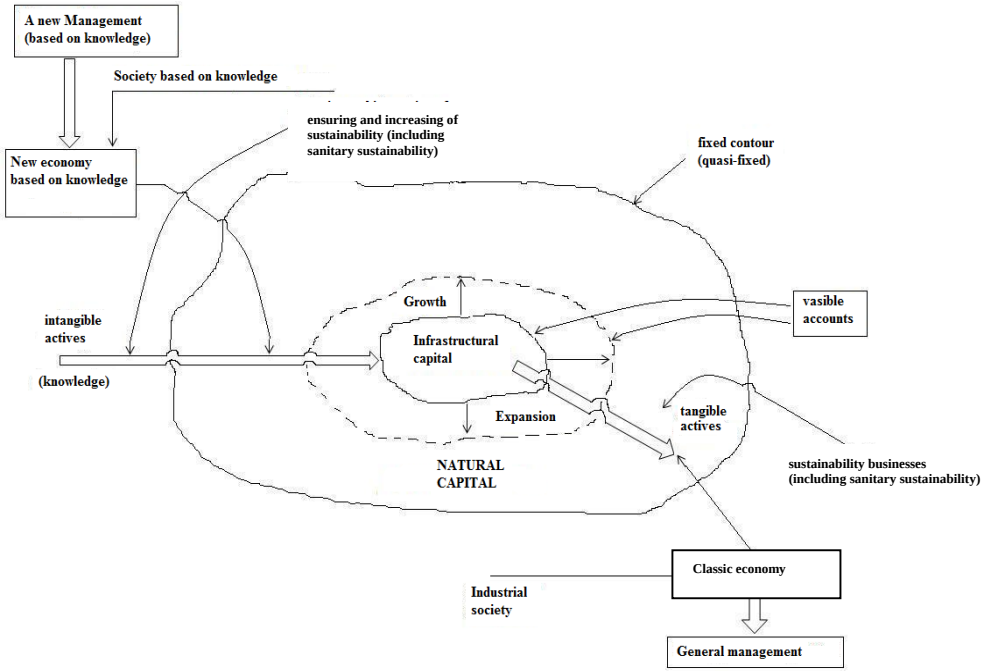

Fig. 4. *Intangible Assets and the ensuring of the sustainability of the natural and physical capital*

Management model for sustainability must face the need to provide solutions or alternative versions of organization and management to stop the occurrence of inadequate situations in the infrastructural capitals with tangible assets (e.g. in research laboratories for the biophysical and biochemical development).

Flows of biophysical, biochemical respectively affectants, tangent or retrieved in the process or phenomenon of pollution have dual or combined manifestations:

a) are the result of natural biophysical and biochemical processes;

b) are the result of human activities or;

c) are human-natural combinations.

For example, pollutants can be considered different compounds that have direct or indirect effects on living organisms damaging (eroding, altering) the energy flow distributions, varying the radiation levels or changing the physicochemical structures of living organisms or the structures of the natural environment. (Postolache, C., 2000).

Pollutant impairments derive from major classes of compounds: a) artificial b) inorganic or c) organic (already existing in nature).

Human activities (e.g. carrying out research and development activities) increases the flows of biophysical and biochemical factors and elements of wide diversity that have disruptive effects both inside and outside infrastructures (Figure 5).

We find that, in fact, basic and processual contents from biophysics and biochemistry are essential in the human body, meaning their physiological requirements for conventional favorable metabolic functioning of human / living organisms.

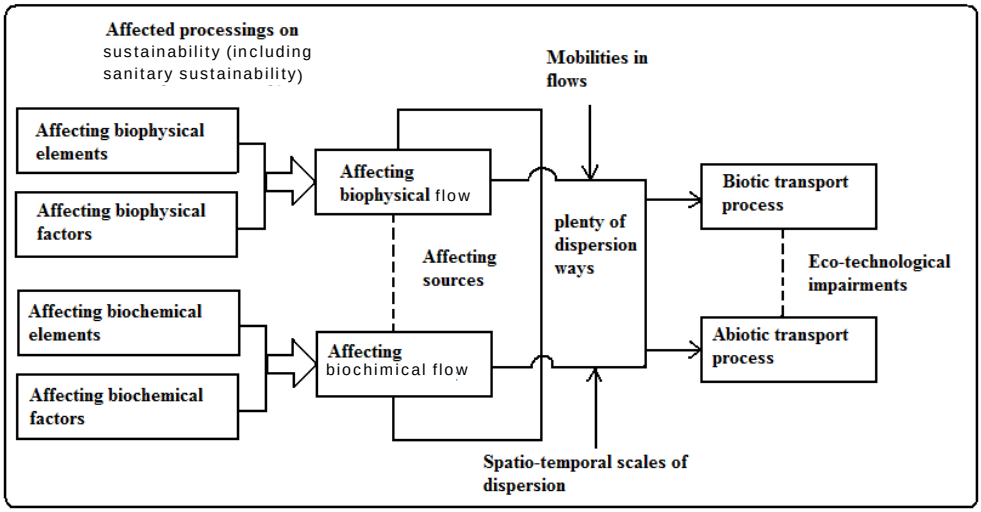

Fig. 5. *System of the affecting flows and processes on the sanitary sustainability in the studied area*

But overcoming concentration levels determine disrupts and exposures to toxicological effects for the organisms.

At individual level, biophysical and biochemical toxicity can cause damaging or physiological stress (Postolache, C., 2000) [1] (pp. 5-11).

The same, destructive stress is encountered after biophysical and biochemical disturbances given by lethal doses, and the physiological stress mainly lies in biochemical disturbances on molecular level (enzymatic disturbances, mutations, alterations in DNA structures etc.).

For example, in the hospital complex of the central sanitary entities in Arges Pitesti, during investigations for this paper, I found that biomass growth was slowed (2013) due to the high presence of concentrated chemical residues from 2 biochemistry laboratories.

The area observed was within a radius of 0.6 km from the laboratories, in municipal waste, where the containers were not properly handled for closure.

The appearance of grass and shrubs discoloration in the area confirms the disturbings in question. It was noted that occurred the so-called oxidative stress on plants and it was obtained the conclusion that, in fact, soil contamination with the

above mentioned substances led to phytotoxicity, increasing the acidity of the area, thus being recorded negative effects on plant metabolism.

By taking strict measures for waste management, the situation previously reported was no more negative.

Conclusions

The conclusions and consequences foreseen for the retaining, ownership and put into practice in terms of managerial concerns synthetically relate, according to the elements from the above case investigation, that the fundamentation of the control system mobility is necessary for: a) factors; b) substances and; c) operations from biophysical and biochemical processes.

Organization and management of functional and operational control ensures optimum human activities with tangible infrastructure (laboratories, research and development).

In this context, the optimality achieved by adjustments and guidelines lead to adequate conditions for sustainability conventionally scheduled and accepted as ensuring level in human communities.

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CASE STUDY CONCERNING CRIMINAL BY SUBSTITUTING FRAUD RELATED TO FOOD SAFETY

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Abstract

Besides others major chapters of veterinary forensics (forensic investigation and veterinary forensic expertise, thanatology, traumatology, asphyxiology, forensic toxicology, forensic graphology) forensic aspects correlated with food safety, unlike human forensics, have a huge importance, especially with developing the globalization process and technological blow-up in the field of food.

The purpose of this paper is to present, as case study, the most recent manifested crisis in the field of food safety and food security, crisis induced following criminal by substituting resort fraud of beef with horsemeat, mainly, or with meat from other animal species. In a first part of the paper, the criminal investigations ongoing in the country involved in this scandal have been shown, starting with Ireland, the first country where irregularities regarding the composition of some raw food materials or ready- to eat or finite food have been revealed, composition that wasn't complying with specifications inscribed on the labels attached to these products. Then, the results ad hoc undertaken criminal investigations and inquiries, at some national or international corporations and companies involved in the scandal are presented, as well as the notifications among national and international authorities having responsibilities in the food safety or food security or between these authorities and criminal prosecution authorities or criminal investigations authorities.

A crucial importance for gathering up all data, information and targeted samples to be able to elaborate the probatory and, then, to formulate the indictment, they had the results of testing performed on the collected samples, especially the tests concerning to detection equine DNA or from other animal species, in the food products labelled as containing only beef. The criminal investigations performed at those 11 corporations and companies, working in the field of producing or trading the raw food materials or ready-to-eat food, facilitated easier identification of meat origin or its supply which are at issue/litigation, as well as the modalities and places where criminal by substituting resort fraud took place. The implications of criminal offence correlated with public health, the involvement of horserace managers, the

implications of chemical agents used for horse euthanasia, the effects of horsemeat from horses affected by equine infectious anaemia on human health, the implications of horsemeat from horses treated with fenilbutazone entered in the food supplying chain on public health, the religious consequences correlated with Muslim or Jewish populations eating horsemeat or pork instead of beef. There are also presented the reactions of people and non-government organizations against criminal fraud committed, the reactions of corporations and concerned companies involved in the scandal, the reactions of food industry promoters, the approach of animal welfare and protection organizations, the official position of involved country governments and of European Commission or European Parliament, on behalf of European Union, the positions of mass media and press, the reactions of horserace managers, as well as of horse slaughterhouses managers. Having in view these aspects, the Community projects for defending against food criminal frauds (White Paper of Criminal Food Frauds and Financial Community Support for developing strategies and politics, as well as making available for food industry official supervisors tools for fraud earlier detection, implementation of tightening measures of veterinary inspections and controls and for constabulary measures, the sanctions applied after indictment formulation and as a result establishing the administrative offences or penal sanctions. The causes allowed or facilitated criminal substitution frauds were analysed, regarding deficiencies of traceability system, especially referring to food labelling, lack of conformity related to some definition in the concerned field, played out systems to ensure quality management and out of play specific standards, regulatory deficiencies and a permissive surveillance of food industry, especially of food supplying chain with food raw materials or finite food.

Key words: *forensic veterinary aspects, the crime of fraud by substitution.*

Introduction

Since the extension of globalization process and technological blow-up in the field of food industry is the main process of the world, the malefic aspects involves facilitating committing events which constitutes in fact legal faults, having contravention or penal implications. In this context, the possibilities to realize frauds on raw food materials, or on food, as they are, as finite food, ready to eat, experienced paroxysmal aspects, involving development of such forensic investigation technologies adequate to draft criminal evidence and to formulate the indictment. Within criminal fraud correlated with food safety or with food security, criminal substitution fraud is the frequent criminal fraud.

The importance of this paper consists in several essential aspects correlated with public health expressed as food safety and food security, underlying these notions as their ability do not cause food-borne-diseases or satisfying tastes and quality of the products.

The importance of the selected theme of the paper results also from the fact that in comparison with criminal acts related to animal health, those correlated with food safety and security are much more frequent, more difficult to be found, relieved and proved.

The third important aspect regards the dimensions of chosen case study implications, this food event being registered as the most recent and serious food manifest crisis, involving more than 20 big food processing companies, food suppliers and retailers, national authorities having responsibilities in the field of food safety or anti-fraud protection.

Materials and methods

To define the criminal by substituting resort fraud related to food safety, the information received during the second semester of veterinary forensics course given by prof.dr.Gheorghe Onțanu have been used.

For those aspects reffering to criminal substitution fraud of beef with horsemeat in raw food materials or finite food, as procedural approaches, the guidelines issued by dr. Traian Enache in the book "Full Forensic Veterinary Treaty" edited by ALT PRESS TOUR Publishing House.

For the most part of the paper, the information and public data, official declarations of the spokespersons representing national and Community authorities responsible with food safety and security, food processing companies, food suppliers and retailers, animal welfare and protection, food industry promoters, non-governmental organizations in the field, religious representatives, have been used.

As a method of scientific paper organization, its central part was structured, in order to comply with the schematic classical approach of a criminal investigation, starting with details presentation conducted to complete probatory, as well as the further criminal investigations, with the aim to be able to assign the legal guilt proven by probatory, to one or several natural persons or to legal persons, that is to say to formulate the indictment, having as final purpose to establish the amount of fault/guilt for each of those concerned/involved, directly or indirectly, in this delinquency/infraction and to allow to take rule a sentence.

Along the next chapter, the reactions of companies and firms doing business in the field of food sector, as raw materials processors and suppliers, or acting as retailers of ready- to- eat food products were registered and used to build probatory and formulate the indictment. These reactions consisted mainly in withdrawal from the market of the incriminated products and their testing for horsemeat, using ADN techniques, (PCR methods), cancellation the legal contracts with incriminated suppliers and official request to recover the damages and applying sanctions. At the same time, the reactions of national, Community and international institutions have

been taken into account, as well as the reactions of nongovernmental organizations, food industry representatives, were retained as evidence elements to clarify the detailed aspects of this criminal substitution fraud related to food safety. The implication of different structures and undertakings related to food industry sector, favouring the occurrence of this criminal action, have been scrutinized, to share the guiltiness, were carefully assessed and the result of these implications, in different aspects wee analyzed and solved. Taking into account all these aspects, recovery measures and preventive actions were proposed, discussed and applied or are going to be put in place, revamping from its foundations the food industry sector.

Results and discussions

The scandal of fraudulent substituting the beef with horsemeat in products prepared only from beef blew-up at the end of 2012, developed in Ireland and United Kingdom, and lasted the whole 2013 year, with consequences within food industry even in 2014.

The aspect was turned up on January, 15, 2013 when was reported that equine DNA was found in deep-freezed burgers made from beef and sold by various super-markets from Ireland and United Kingdom. The criminal investigations to build probatory and formulate the indictment were performed in most (22) of the Member State, directly or indirectly involved in this criminal actions, as well as in Russia and Azerbaijan, with some references to United States, as third countries.

To formulate the indictment, 21 companies, concerns or firms doing business in the field of food sector, as raw materials processors and suppliers, or acting as retailers of ready- to- eat food products were criminal investigated, and biological samples were collected by authorities responsible with public health, and laboratory veterinary forensic expertise was applied, using ADN techniques, with positive results for horsemeat, sometimes for donkey meat or pork.

The food companies and persons proved guilty to initiate and cause this infraction were established and were submitted to legal prosecutions, including jailing. All food companies indirectly involved and affected by this criminal substitution expressed public reactions against the alleged guilt. At the same time, the national authorities having responsibilities for food safety and security, are also empowered with anti-fraud activities; the European Commission and European Parliament were deeply involved in saving the situation and preserving the consumer confidence in national and Community authorities with aforementioned responsibilities.

Some horse slaughterhouses in France, as well as some horserace managers have been proved to facilitate this criminal act, when horses died or injured at horse-races.

The implications caused on public health, by use of horsemeat from animals treated with fenilbutazone, as well as from horses affected by infectious equine anaemia were assessed to establish the dimension of the fraud. The religious

implications induced on Muslim and Jewish populations eating horsemeat or pork have been taken into account.

Conclusios

1. The assessment of determined causes pointed out to produce and to facilitate this criminal infringement sourced that this is the result of serious deficiencies in traceability applied concept, especially regarding meat labelling and a premeditate criminal substitution of beef with horsemeat or pork.

2. Some deficiencies in the racehorses' passport, by intended non-enlistment of all treatments applied to these animals, facilitated illegal racehorses slaughtering and use of resulted meat in the food chain

3. Non application of a sort of a minimal official surveillance program at national level, for testing food products for their composition compliance with information entered on attached labels, facilitated the substitution of original labels horsemeat with a new one as beef.

4. Limited use of DNA techniques for detection of different kinds of meat within ready- to- eat or finite products

5. Weaknesses regarding the level and procedural aspects of food industry supervision by national and territorial authorities responsible with food safety and food security.

6. Weaknesses regarding the level and procedural aspects of slaughterhouses' and horseraces' supervision by veterinary authorities,

7. Too long supplying chain of food industry, allowing multiple transfer of food raw material among food industry operators

8. The several crisis occurred in the food sector, in the last 25 years, caused serious prejudices to supermarket trading system as retailer system, enhancing the reduction of the consumption.

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PROPOSALS FOR DEVELOPING THE VETERINARY FORENSICS DOMAIN IN ROMANIA

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Abstract

Veterinary forensics is indeed a new domain and a new foreseen competence of veterinary profession. Different from human forensics, that already has had a history, organization and specific operation procedures, making it compatible with more and more diversified level of the facts constituting contravention guilts or criminal offences entering under the incidence of Penal Code and of criminal pursuit and penal inquiry, based on Penal Procedure Code, veterinary forensics is officially recognized only as teaching matter in veterinary faculties.

The task of this paper is to perform a comparative analysis between two different sectors of forensics: human forensics domain and veterinary forensics domain, in order to point out common or similar approachable parts, but existing differences between two disciplines, as well, and to define, in consequence, the specific procedures, fields and modalities for veterinary forensics, with the aim to promote it as an official recognized specialization among the existing specializations in veterinary medicine.

This aspect implies official approaches or steps for drawing up the professional specialization standard and regulatory modifications and completions in veterinary profession legal framework.

Since the official recognition of this veterinary specialization will be obtained, it is compulsory to define organizational framework within the new specialization, in order to develop it. This implies institutional framework definition, both as forensic authority at national level and covering Romanian territory with public institutions recognized as having non-shared competences of veterinary forensics, taking into account the existing organizational system for human forensics.

Besides the definition of national and territorial veterinary forensic authorities, it is obviously necessary to ensure the organization and functionning of scientific structures in the field, as well as the assessment structures and for technical and juridical decision-taking bodies, similar to those working for human forensics.

The next step in the way of developing and promoting the veterinary forensics doctor specialization is taking over from human forensics the procedural framework that can be transferred into

veterinary forensics domain, as well as drawing up specific assessment procedures and criteria for the new veterinary specialization called veterinary forensics doctor, for the fields which are different from those of human forensics.

All these duties have to be realized in the context of expanding an academic program at the level of superior veterinary education, followed by post-university specialization course as veterinary forensics doctor, lasting at least six months, officially recognized by a specialized structure from the Ministry of Education and Research and finalized with a diploma, issued by the ministry and giving to graduates the competence of veterinary forensics doctor.

Veterinary forensics national authority has to implement an annual training program and establish relational system of the organizational framework, firstly with human forensics institutions at national and territorial level, but also partnerships with criminal pursuit and penal inquiry institutions, as well as with non-government organizations for animal welfare and protection.

All these are feasible, since veterinary forensics activities are already performed within Central Veterinary Institutes, especially at the Institute for Diagnosis and Animal Health and county sanitary veterinary and food safety directorates.

Key words: *veterinary forensics, assessment procedures.*

Introduction

Historically, veterinary forensics is derived from human forensics, since the modern approaches on animals has known very important changes, taking into account the development of animal welfare and animal protection concept that was the engine for veterinary forensics.

In the developed countries, veterinary forensics is an autonomous and very well defined specialization within the framework of veterinary qualifications, benefiting from specialized services of other professions.

In Romania, veterinary forensics is, indeed, a new domain, different from human forensics, which already has had a history, organization and specific operation procedures, making it compatible with more and more diversified level of the facts constituting contravention guilts or criminal offences.

Since the worldwide crisis related with animals and animal products and by-products (BSE crisis, growing hormone crisis, dioxins crisis, milk melamine crisis, foot and mouth disease crisis, horse meat adulteration crisis, animal protection issues, residues problem, stray dogs biting or even killing people, prohibited fighting between animals) are as much as important or probably overcame those manifest in human domain (cardiovascular death, degenerative diseases, HIV and immunosuppressant affections, human infectious hepatitis, obesity, neuronal

degenerative illness, traffic accident, suicides, family killing, hypertension and diabetes, starvation) raised the importance of veterinary forensics aspects, while those mentioned related to human health are not really subject of forensics, but mainly health problems.

Both these problems have an incredible social aspect, sometimes gaining political issues in debate.

In order to solve successfully these aspects, it is compulsory that veterinarians have to be involved in the process of medical and juridical expertise and veterinary forensics has to be recognized as a specialization of veterinary profession.

Even in the curricula of veterinary faculties from Romania there is a discipline of study called veterinary forensics, the graduated students of veterinary medicine faculties usually receive only basic specific information on the huge and variant fields of criminality against animals caused by people or by other animals, as well as regarding animal aggression against people in the responsibility of animal owner.

The curricula of human forensics and veterinary forensics disciplines of study are almost similar, with some differences which will be pointed out as a part of the aim of this paper, but the fields of practicing the knowledge acquired during the university stage are absolutely different as patients and specific criminal acts.

Materials and methods

There is a crucial difference between human forensics and veterinary forensics: while human forensics is recognized as a specialization among multiple specializations of human medicine, benefiting of all those necessary to be practiced as it is, there is, yet, no officially recognition of veterinary forensics, and aside from veterinary forensics discipline of study taught in veterinary faculties, there is nothing officially recognized related to this domain, and usually, the investigations and medical or juridical expertize of criminal events involving animals or animal products or by-products are done by specialists in criminology having other basic training than veterinarian.

This is in fact the purpose of the paper, to explain how is to be proceed to fulfil these objectives: the accomplishment of a comparative assessment between human and veterinary forensics, with the purpose to identify the existing differences which will be treated and managed in a specific manner, and evaluate the elements from human forensics which could be applied to complete veterinary forensics.

Based on the gaps found after performing comparative study, specific proposals to promote veterinary forensics as a specific capability and specialization within veterinary specializations have to be done, with all aspects ensuring its specific practice as a definite and separate proficiency of veterinary practice in the field of veterinary forensics.

To be able to perform these objectives, the framework legislation and regulatory deeds governing human forensics were taken into account, to identify how the proper way to build veterinary forensics basic elements is.

Documentary and official documents issued by some institutions performing genuine veterinary forensics pieces of expertise, but unofficially recognized as being veterinary forensics expertise, but medical-veterinary expertise, have been searched and evaluated, with the aim to use them as support to promote these institutions as veterinary forensics, too.

As working methods we shared the main aspects which are to be solved, as follows.

First of all, the aspects concerning institutional building (organization and functioning) existing in human forensics were compared with those existing in veterinary medicine and which could be a little restructured, to be compatible with specific veterinary forensics responsibilities and attributions. We took into account the basic legislation bringing under regulation the human forensics institutions organization and operation, as general rules.

At the same time, the sectorial legislation governing organization and functioning of some institutions from human and veterinary domains related with forensics were also taken into consideration.

Following this approach, the regulatory acts establishing the Ministry of Health organization and operation, correlated with human forensics, or properly referred to human forensics structures, were used, in comparison with legislation establishing the setting up, organization and operation of some institutions and organizations from veterinary domains, having specific veterinary forensics activities, but unofficially recognized as forensics activities, even if the results of these activities are usually used to complete medical or juridical expertizes and criminal investigations for criminal events involving animals, animal products or by-products or veterinary used products.

Talking about this aspect, we are discussing about the organization and functioning of National Sanitary Veterinary and Food Safety Authority versus Ministry of Health, the Institute for Diagnosis and Animal Health organization and functioning Regulation, under the authority of National Sanitary Veterinary and Food Safety Authority versus the organization and functioning Regulation of the National Institute for Human Forensics “Dr.Mina Minovici” under the competence of Ministry of Health, as well as the organization and functioning Regulation of the Romanian College of Physicians**,** versus the organization and functioning Regulation of the Romanian Veterinary College, especially for ethical, moral, deontological, civil and administrative responsibilities, including malpraxis assessment and sanctions.

Since the laboratory expertizes are done, for criminal events occurred in the fields of human forensics, by laboratories organized within the framework of National Institute for Human Forensics “Dr. Mina Minovici”, by institutes for human forensics from those 5 existing medical university centres, under the coordination of Ministry of Health and by county forensics services under the coordination of county public health directorates, the veterinary forensics

expertizes are performed by the Institute for Diagnosis and Animal Health, for criminal event involving live animals and animal protection, and by the Institute for Hygiene and Veterinary Public Health, as Central Reference Laboratories, as well as by specialized laboratories from veterinary university centres and veterinary laboratories organized within county sanitary veterinary and food safety directorates.

Legislation referring to National Reference Laboratories in the field of animal health and protection, also those having responsibilities for animal products and public health, has been assessed to underline how to promote National Reference Veterinary Forensics Laboratory for aspects related to animal health and protection and National Reference Veterinary Forensics Laboratory for cases occurred in the field of animal products and by-products and veterinary public health.

The very recent beef adulteration case is very present, as its consequences on traceability concept, especially for labelling tools and procedures applied for surveillance the food supplying channel.

In order to define procedural aspects of the new promoted specialization in veterinary profession, called veterinary forensics, the legislation regulating the organization of technical judicial and extra-judicial expertize activities, the Judicial Expertize Guide, the procedural norms regarding carrying out expertizes, findings and other forensic works, all have been carefully read and assessed, with the aim to identify the common aspects and those which could be transferred, as applicability, from human forensics to veterinary forensics.

The procedure was going on and regulatory framework referring to organization and functioning of taking of evidence system or probation/probatory system, as well as legislation setting up the execution of punishment, educational measures and other freedom privative or non-privative measures, disposed by judicial bodies, were used to make a balance between human forensics and veterinary forensics in the field of probatory system building.

As a completion of this approach, we studied, also, the legislation setting up some virtual structures created within human forensics system, charged with coordination of all aspects of human forensics and highest forum for decision making (Superior Forensics Commission), nominal composition of Commission for advising and control forensics activities performed and issued within the National Institute for Human Forensics “Dr.Mina Minovici”, nominal compositions of commissions for advising and control forensics activities issued by institutes for human forensics from those 5 existing medical university centres (Cluj-Napoca, Iaşi, Timişoara, Târgu Mureş and Craiova) and for functioning of these all commissions.

Besides all aspects presented till now, the last but not the least aspect to be solved is drawing up and promote the professional standard for veterinary forensics specialization, among the other specializations officially recognized in veterinary practice. It means a huge effort to draw up the patterns; to promote it through the bureaucracy involved, being listed by legislation recognizing specializations in different fields or professions.

The very detailed and complicated legislation governing this critical aspect have been identified, studied, correlated and selected, to retain only aspects of interest for completion our approach and fulfill the objectives of our proposal

Results and discussions

Making a comparison between curricula of human medicine and veterinary medicine, it is obviously important to notice there are few differences in the teaching process.

Aside from psychiatric investigations, use of lie detector, blood groups and Rh examinations, parental filiation determination, criminal graphology, intended suicide, the other main domains of forensics are common (general aspects on forensics-history, objectives, tools, framework procedures, thanatology, mechanical traumatology, physical, chemical and radiation traumatology, asphixology, criminal toxicology, use the animals in research and experimentation), but there are a huge number of infractions in the field of food safety, animal products adulteration, animal welfare and protection, where their investigation must be done by veterinarians participation at criminal investigations required by criminal prosecution authorities or by criminal enquiry bodies.

Regarding institutional building, there is an important difference between human forensics and veterinary forensics: the institutional framework for human forensics has a long history and knew more and more completing stages, being today almost completed, both at central level and territorial level.

By the other hand, even if there are some central institutions unofficially performing forensics activities, required by the events occurred in the field of animal health, animal welfare and protection or animal integrity, but also related to animal dispute, following a criminal act against an animal or a group of animals, these institutions are not officially recognized as performing forensics activities officially recognized, despite the fact that some official documents issued by these institutions are recognized by justice as basic documents for building the probatory, especially analysis bulletins based on necropsy and laboratory examinations of samples collected at necropsy.

This is the crucial aspect that is to be solved, to create institutional framework for the new specialization in veterinary practice and judicial practice.

Some institutional changes have to be done in the structure of National Sanitary Veterinary and Food Safety Authority and Romanian Veterinary College, as well as in the structure of two Central National Reference Institutes (Institute for Diagnosis and Animal Health and Institute for Food Hygiene and Veterinary Public Health) creating or adapting specific veterinary forensics structures that already exist as a misunderstood objective.

The third step that must be accomplished is to assign to the new created or adapted structures specific attributions and responsibilities for performing examinations in the field of criminal investigations, changing and completing the Organization and Functioning Regulations of these institutions and basic legislation governing their structure and operation.

The fourth step to reach the goals of this approach is to adapt procedural framework of human forensics to veterinary forensics, adding the forensics procedure already existing in the veterinary field to these new adapted procedures or extending human forensics procedure as common procedure for both human and veterinary forensics.

The fifth step to be accomplished is referring to activities aimed to create a new specialization among the already existing specializations, first stage being laying down the specific occupational standard and promoting it on the official list of occupational professional standard.

Conclusions

1. The criminal events related with animals and animal products and by-products are as much as important or probably overcame that manifest in human domain, raised the importance of veterinary forensics aspects, since those mentioned related to human health are not really subject of forensics, but mainly health problems.

2. From the legal point of view, these criminal events have to be solved by participation of the veterinarians at medical and judicial expertizes, being the only ones duely trained and competent to deal with animals and animal products and by-products.

3. Even there are no major differences regarding the curricula of forensics discipline during the faculty stage, there are important differences regarding institutional framework against the veterinary forensics, practically recognized only as teaching discipline in veterinary curricula.

4. At least five steps have to be covered to obtain the official recognition of veterinary forensic as a new specialization and to create institutional, procedural framework as well as specific responsibilities and attributions

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Minovici" Bucureşti, a componenţelor nominale ale comisiilor de avizare şi control al actelor medico-legale din cadrul institutelor de medicină legală din centrele medicale universitare Cluj-Napoca, Iaşi, Timişoara, Târgu Mureş şi Craiova, precum şi a modului de funcţionare a acestor comisii.

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PROPOSALS FOR TRACEABILITY CONCEPT IMPROVEMENT ON VETERINARY DOMAINS.

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Abstract

This paper has as main objective the improvement and completion of traceability concept, making some clarifications concerning its structure, its tools of traceability and elements of traceability caused by major crises occurred in the field of feed and food related to feed and food safety. To support the new elements proposed, to improve and completion the traceability concept definitions, all papers concerned, found on Internet on this subject, were studied; the diagram flows of the feedingstuffs and feed, animal holdings, food and foodstuffs establishments, also for distribution and commercial units from Romania were studied, and integrated as inputs. At the same time, all specific standards issued by international organizations and Community, international and national legislation have been taken into consideration.

The traceability concept is not restricted to the agricultural domains, but the pattern of this concept has been properly defined in various domains of agriculture, related to feed and food safety.

Known as empirical saying "from stable to table" or "de la fourche à la fourchette", these denominations are limited and do not express the nowadays real concept of traceability.

To explain it, we must take into account that we need feed to bread and rear animals, these animals are reared and breed in zoo or house yard holdings, most of them are slaughtered, some of these are intended for human and animal consumption, the others are processed for the same purposes and the third are used for industrial purposes, all being traded.

We propose to define these areas as ***chain-loop of traceability****: the feed, animal, food, industrial, commercial and finally, consumer domains of traceability, all these being defined as* ***chain of traceability*** *for agricultural products.*

In each chain-loop of traceability there are different steps: e.g.: for animal chain-loop there are newborns, suckling, weaned, young, growing or fattening animals. We propose to define these as ***stages of traceability****. Also, we propose to define the connected activities from*

*each stages of traceability as **phases of traceability**. In this respect, we propose to define the pathway to pursuit of all food products obtained from a single agriculture unit, from feed chain-loop to consumer chain-loop, as **pathway of traceability** and for a single product as **channel of traceability,** their traceability already being defined by Bolnot and Fleurynck as global traceability or partial/individual traceability.*

*When food products are pursued from a given stage to its origin, we are talking about **ascendant traceability** and from that point to the final consumers, **descendent traceability**. We propose to define the itinerary of one animal product along the flow of traceability as **administrative traceability**, and information and documents accompanying these products as **informative traceability** and to define the transport activities among subsequent chain-loops of traceability as **knots of traceability**. Also we propose to define the following only one product along the traceability channel as **line of traceability**. The pursuit of the products inside a chain-loop of traceability defines **internal traceability**, versus **external traceability**, outside of it. At the same time, we have some proposals to define the **tools of traceability** and the **elements of traceability**, to clear the structure of traceability concept*

These notions have been proposed especially after dioxin feed crisis, melamine milk crisis and very recently horsemeat fraud, facilitated by some "gaps" within traceability concept definition.

Keywords: *traceability, chain, stage, knots, channel, feed, food, safety.*

Introduction

Despite the concept of traceability is very recently established, in the last twelve years, the principles of pursuing something in time and space, with different purposes, is very old. In antiquity, pursuing something in time and space was sometimes correlated with public health, monitoring the movements of lepers or with religious activities – pursuit of Jesus imposed by Pilat as a social risk, either with political activities (pursuit the Roman cohorts by native insurgents) or with animal health-pursuit the wild animal herds to be killed to protect domestic animals.

Another capital tool of traceability consisted in animal identification, from antiquity, till now, using primitive or now, very sophisticated methods, followed by sanitary marking (12^{th} century) to identify corps and products derived from animals or people affected by major diseases, health certification for animals and plants or products derived from them (18^{th} century).

Moreover, the setting up of International Office of Epizootics is related with the pursuit of a transport of zebu from India to Brazil with stopping in Anvers

harbour and followed by a terrific pandemic evolution of Rinderpest in whole Europe in six months.

The concept of traceability is almost new, but its principles are very old, being used in all domains of agriculture, in feed and food industries, outspace activities, military field.

Feed and food crises occurred in a short period of time (BSE meat crisis, hormone meat crises, dioxin feed crisis, melatonin milk crisis, and recently horsemeat fraud crisis, revealed, every time the existence of some "gaps" in the definition of traceability concept. In this respect, our proposal for the improvement and completion of traceability concept definition has as main purpose to bring clarifications of its structure and removing the existing "gaps", getting thus the main tools for implementing feed and food safety.

Materials and methods

To support the new elements proposed, to improve and completion the traceability concept definitions, all papers concerned, found on Internet on this subject, were studied; the diagram flows of the feedingstuffs and feed, animal holdings, food and foodstuffs establishments, also for distribution and commercial units from Romania were studied, and integrated as inputs. At the same time, all specific standards issued by international organizations and Community, international and national legislation have been taken into consideration.

In order to identify the "gaps" in the definition of traceability concept, all existing definition and structure of the concept have been assessed.

At the same time, the production flows of different agricultural products, starting with feedingstuffs and animal feed, animal rearing stages, activities before and after animal slaughtering, industrial processing flows for main animal origin products, the chain-loop of distribution an trade of these products, and the habits of consumers related with each animal origin products and by-products, have been investigated and assessed.

Results and discussions

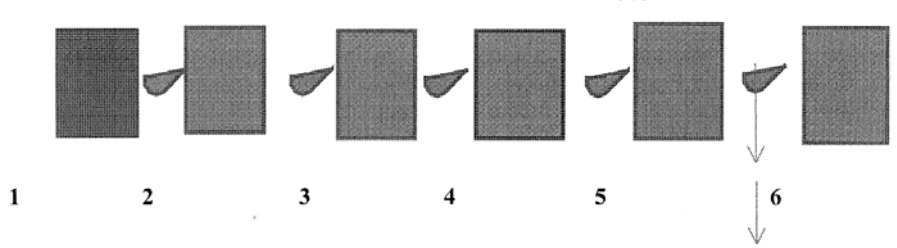
Starting with the definition of traceability concept, even in UK operated, from 18th century the "tracebacil system", used in England, to pursuit the people affected by syphilis, lepers, pulmonary tuberculosis, brucelosis with interdiction to live with healthy people and animals with tuberculosis, glanders or brucelosis, to be quarantinated and killed, the "tracebacil system" can not be assessed as first traceability system.

The concept of traceability was introduced much latter, by Reg.Council and the European Parliament no. 178/2002 establishing general principles of food law and setting up European Authority for Food Safety (EFSA), enforced by the famous food legislation ballot from 2004 (Council and European Parliament Regulations no. 852/2004/CE, 853/2004/CE, 854/2004/CE 882/2004/CE, 5/2005/CE and 931 /2011, the last governing the requirements for traceability for

animal origin food established by Reg. Council and European Parliament no. 178/2002.

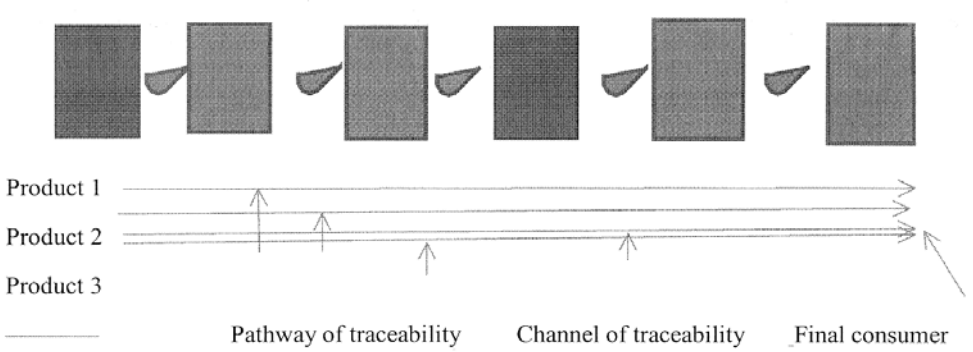
ISO 9000 – Quality management systems – Fundamentals and vocabulary, launched in 2000, at pt. 3.5.4., defines the traceability as the ability to cronologically refind, the application or emplacement of what we examine or we forsee by deduction.The most applicable definitions are done by ISO 22000:2005- Food safety management systems, and by Codex Alimentarius – CAC/GL 60-2006 – Principles for traceability/Product tracing as a tool within a food inspection and certification system. Despite very conclusive definitions and developments to establish the structure of traceability concept, taking into account the new practical aspects in the food safety sector, the concept can be improved defining new elements of its structure, which is the purpose of this paper.

The syntagms "from stable to table" or "de la fourche à la fourchette", express the limits of the concept at that time, excluding feed chain-loop. It is our first proposal to include feed chain-loop in the structure of traceability concept, as follows:



1= feed chain-loop 2 = animal chain loop 3 = slaughter chain-loop
4 = food chain-loop 5 = distribution and commercial chain-loop knot of traceability
6 = final consumer chain-loop

In this respect, we propose to define the way to pursuit of all food products obtained from a single agriculture unit, from feed chain-loop to consumer chain-loop, as **pathway of traceability** and for a single product as **channel of traceability,** their traceability already being defined by Bolnot and Fleurynck as global traceability or partial/individual traceability.



It is, in some extent, different from the definition given to *food chain* done by ISO 22005:2007 which involves in food chain only food, industrial, commercial and consumer chain-loops. We consider our proposal for traceability to be complete and more appropriate to reality.

To explain it, we must take into account that we need feed to bread and rear animals, these animals are reared and breed in zoo or house yard holdings, most of them are slaughtered (food-producing animals), others are used for sport or companionship, some of the products obtained from slaughtered animals are intended for human and animal consumption (as raw materials, the others are processed for the same purposes and the third are used for industrial purposes, all being traded.

We propose to define the pursuit of only one product along the traceability channel, from its logical origin to the final consumers, as **line of traceability** and the pursuit of all products obtained from an agricultural unit product (ex. an animal), from their logical origin to the final consumers, along the pathway of traceability, as **flow of traceability.**

We understand and propose to define the **logical origin** of a product starting with the feed domain; it means when talking about milk, the logical origin of milk is feed used by a cow to produce milk (the real origin is a dairy cow) and the **final consumer**, a human being or animal eating the milk or the place where a product is used (skins or leather for clothes).

These notions are very important, taking into account the recent horsemeat big scandal. It means, for instance, for milk, its traceability line consists in feed chain-loop (feed for feeding, minerals, feed supplements, etc), animal chain-loop (drugs, vaccines), food chain-loop (for raw milk)/industrial chain-loop (for processed milk), distribution and trade chain-loop, to the final consumer (human being, animals, cosmetic industry, etc.).

During the feed and food crises occurred in the last 30 years (starting with BSE crisis-after 1986), in order to detect the real point that caused a certain crisis, the involved and related products where pursued from the place that affected food safety, to its/their logical origin and to final consumers. Bolnot F.H. and Fleurynck Catherine defined *ascendant or "amonte" traceability* as being the following of a product/products, from a certain point to its logical origin and *descendant or "aval" traceability*, meaning the same thing but from a certain point to its final consumer. The point/chain-loop/stage (see below) from where the traceability of a product starts, either as ascendant traceability, to its logical origin or descendant traceability, to the final consumer is defined, by as, **origin of traceability**. It is quite different from logical origin but sometimes can be similar.

Inside a chain-loop of traceability, there are specific activities or processes to which a product is submitted (e.g. for food chain-loop of pork, there are pre-slaughtered examination, stunning, bleeding, evisceration, organs and carcases examination, skinning or scalding, drying, carcass identification, boning, packing, storing and freezing). We propose to define all these activities and processes as **stages of traceability**. When only several activities or processes, among all stages

of traceability inside a traceability chain-loop are involved, we propose to define them as **traceability segment** (inside a traceability chain-loop).

The clear definition of a stage of traceability and a segment of traceability, among the other stages of traceability within a chain-loop of traceability is very important, for certain product traceability and there is a direct relation between a stage of traceability and critical control points and control point defined for HACCP.

When talking about the traceability inside a chain-loop of traceability, along all stages of traceability of that chain-loop, we defined this sort of traceability as **internal traceability**, against **external traceability,** meaning the traceability of this/these product/s outside the concerned chain-loop of traceability.

In order to have and rear a cow, for instance, we need animal genetic materials provided from animal chain-loop, as well as, feed for feeding it, supplied by feed chain-loop, other materials for feeding and health or dietary supplements, purchased from chemical or pharmaceutical domain.

When the traceability of all these products, concurring it means and we define this approach as **conjunctive traceability**, because several products having different origins compete to obtain a single product – a cow.

When an animal is slaughtered, several products derived from the process: hair, skin, claws, meat, fat, organs, bones, bowels, endocrine glands, brain, tongue, etc. are obtained and each of them passes through food chain-loop (to be eaten as food or feed) or industrial chain-loop (to be processed obtaining derived processed products used as food or feed or other domain of traceability – cosmetic industry, pharmaceutical industry, etc.). We defined the traceability lines of these derived products as **disjunctive traceability**, because a lot of products derived from a certain origin – a slaughtered animal but each of them has its specific line of traceability, to different final consumers.

Usually, between different chains of traceability or even between stages or segments of traceability, there are transport activities. We propose to define the transport activities between chain-loop of traceability as **knots of traceability**. These are very important because, during the transport activities, some negative influences on a product may happen with alteration of its quality or safety.

Bolnot F.H. and Fleurynck Catherine defined *administrative traceability* as being the stages and processes through which a product passes from its logical origin to the final consumer. It means that administrative traceability contains all domains of traceability proposed by us. It is quite different from *commercial traceability*, pointed by ISO 22005:2007, point 3.6.

We propose to define all traceability chain-loops for product/products, meaning stages and processes through it/they pass from its logical origin to the final consumer, as **traceability elements.** It is, in some extent, different from *flowchart* defined by ISO 22000:2005, points 3.4 and 3.8 which are referring to a certain chain-loop of traceability and not to full traceability defined by us. Along the way of its traceability, a product is accompanied by documents (health

certificate), information (advertising, origin and producer), labelling and means of its identification (ear tag, for instance).

We propose to define them as **tools of traceability** or **logistic traceability** which is different from the term *traceability system* defined by ISO 22005:2007, point 3.12, which refers only to information accompanying a certain product, and it is in line with statements specified at point 3.6 of the same ISO, even if Bolnot F.H. and Fleurynck Catherine were spoken about *documentary traceability* or *informational traceability*.

Concerning the tools of traceability, they may be consisting in **marking** and **labelling system** (e.g. for feed and feedingstuffs, food and foodstuffs of animal origin or non-animal origin), **certification system** – conformity certification for feed and feedingstuffs or food and foodstuffs, health certification for live animals, animal origin and non-animal origin products, **stamping system** for animal origin products or for particular use products, **identification and registration system,** including **chip-reader system** for live animals, **codification system** for animal diseases and **bar-code system** animal origin derived products.

Results and disscussions

Food safety sector is one of the most sensitive components of world security strategy, creating, along the time, major crises with health, social, economic, financial and even political consequences. As a result, this domain is very well regulated and structured, in this process being involved a lot of international organisations and institutions, as well as national specific bodies.

This paper is an attempt to improve and complete the concept of traceability, establishing detailed elements and tools, making it more practical and more feasible, both for stakeholders and for technical and regulatory bodies.

Our proposals to improve the definitions and structures of traceability concept for food safety sector have been made taking into account the achievements obtained till now, avoiding parallelism and superposition with the existing ones.

Our proposals combine the existing definition and structure of food safety traceability with practical observations within food industry.

Conclusions

1. We assessed almost all official information and regulatory items existing in relation with food safety traceability, in order to be aware about the state of play in this field.

2. We’ve searched a lot of food safety industries, feed producing industry, animal husbandry, food processing sector, some industrial sectors using animal and non-animal food origin products, to find specific aspects which are not specified in existing standards or guidelines concerning food traceability.

3. We tried to propose some definitions and notions of food traceability, with the aim to complete the traceability concept for food and feed sector, removing important " gaps" existing in the present shape of food traceability.

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ASPECTS CONCERNING THE BREEDING OF LIMOUSIN CALVES IN ALTERNATIVE SYSTEM

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Abstract

The aim of the research was to monitor the growth process of the Limousin calves from birth to weaning (six months old). The research was performed on S.C.TAOS.S.R.L situated in Covasna. In this farm, the rearing of the calves was not made by a technological guide. The maintaining system was outdoor, during summer on pasture, and in the winter season, in free stabulation. The calves had been grown along with the mother cows, having free access to the paddock. The base of feeding was represented by semi-hay, corn silage, barley straw and cereals (barley, wheat and corn) and grazing is made on lowland meadow. The introduction of vegetal food into the calves ration is done at 3 weeks old. In the first period (first month old), the daily gain was 666.7 g at heifers and 800 g at calves. In the second period (from one month old until weaning), the daily gain was 1120 g at heifers and 1200 g at calves. The stress of weaning was present only to the young females; for ten days, these were restless, having the desire for sucking and the appetite for feed has decreased. Their bodyweight has decreased with 12%, the differences being significantly ($p \leq 0.05$).

Keywords: *Limousin calves, alternative system.*

Introduction

Limousin breed is originated in France and was formed over several centuries in the central and west central region around the city of Limoges in Limousin [2; 3]. Limousin breed was imported to Spain in 1965, Italy in 1968, Holland in 1969, Denmark in 1970 and the United Kingdom in 1971. In this moment, the breed is widespread in 70 countries [4; 6]. In Romania, the breed has been introduced since 2001 [7]. Unfortunately, Romanian farmers prefer to raise other meat breeds like Charolais and Blue White Belgium.

Limousin cattle are adapted to diverse climates and the widest range of management systems. This breed is efficient, the animals have moderate mature size and are excellent foragers walking long distances for food. Because it is a

rustic meat breed, the calving problems can occur at less than 6% [3]. The Limousin stamps its characteristics on other breeds when used in crossbreeding programs, especially its superior carcass characteristics [6].

Material and methods

The aim of the research was to monitor the growth process of the Limousin calves from birth to weaning (six months old).

The study was conducted on S.C.TAOS.S.R.L from Covasna country.

The study was conducted on a group of 28 calves from birth to 6 months of age when they were weaned.

The rearing of the calves was not made by a technological guide. The maintaining system was alternative mixed, during summer on pasture, and in the winter season, in free stabulation. The calves had been grown along with the mother cows, having free access to the paddock. The base of feeding was represented by semi-hay, corn silage, barley straw and cereals (barley, wheat and corn) and grazing is made on lowland meadow. The introduction of vegetal food into the calves ration is done at 3 weeks old.

The data were statistically processed using the variance analysis method.

Results and discussion

It was observed that the cows had an easy calving and did not require supervision. 83% of cows calved in the paddock. At calving, the heifers had 40 kg weight and the calves' 47 kg weight (table 1). Comparing with other breeds, Limousin calves had low birth weights, which lead to minimum calving problems when Limousin bulls are used over females of other breeds, in particular dairy cows [5].

In the basement of calving, calves stayed three days, after which they were moved with adult animals.

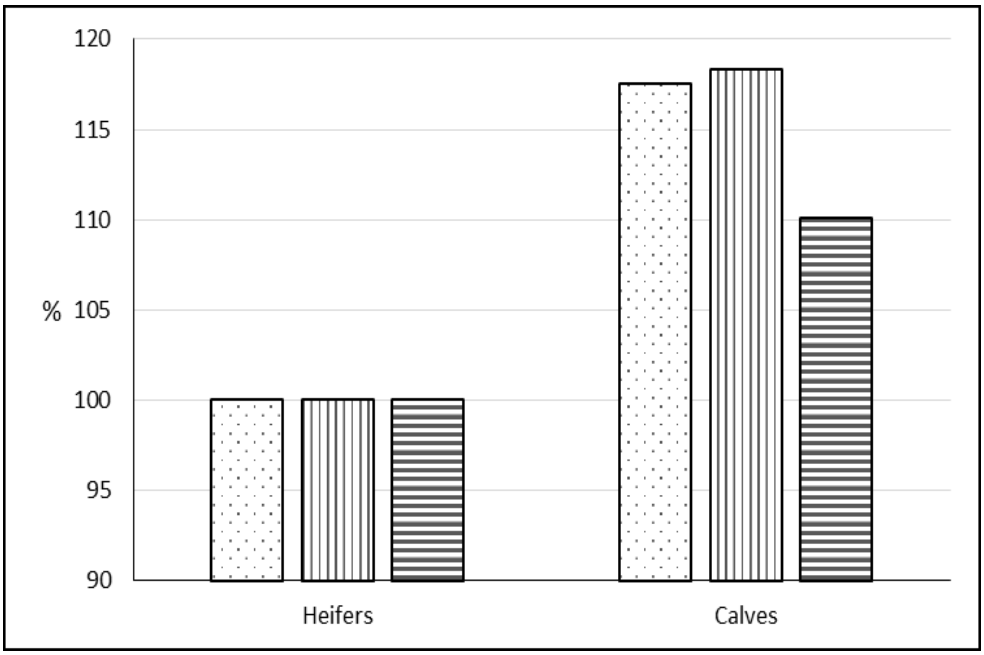
Table 1

Dynamic of weight (kg)

Sex	1 day	1 month	6 months
Heifers	40	60	228
Calves	47	71	251

At one month old, the heifers had 60 kg and the calves 71 kg, with a daily gain of 666.7 g and, respectively 800 g (graphic 1).

At six months old, the heifers had 228 kg and the calves 251 kg, with 10.1% more than the heifers.



Graphic 1. *Dynamic of weight (%)*

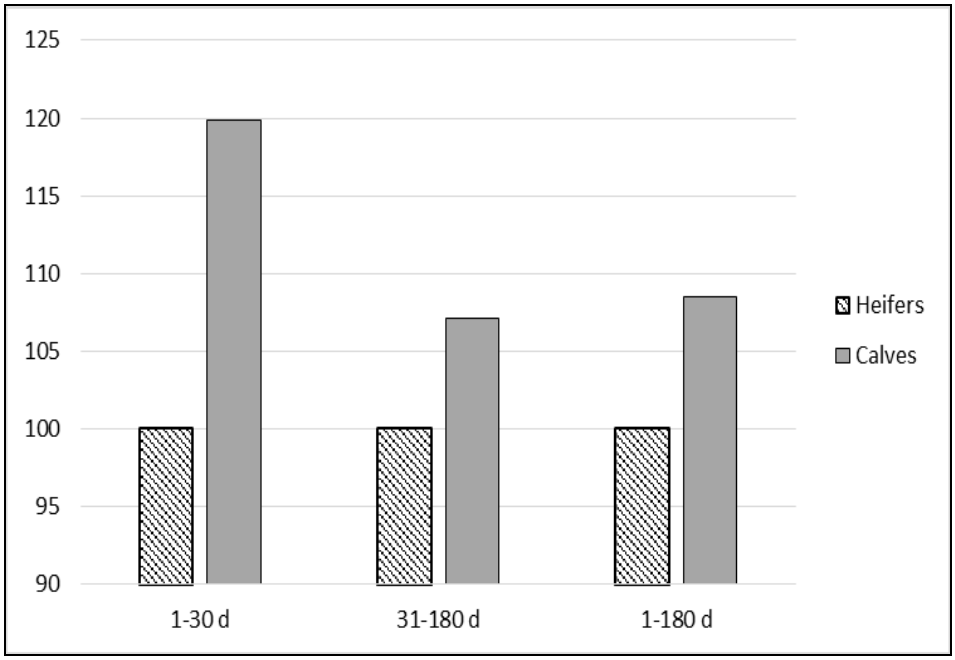
Data concerning the daily weight gain are shown in table 2.

Table 2

Daily weight gain (g)

	Period 1 1-30 days	Period 2 31-180 days	1-180 days
Heifers	666.7	1120	1044
Calves	800	1200	1133

In the first period, the daily weight gain was 666.7 g at heifers and 800 g at calves, with 19.9% more than females. The differences were significant ($p \leq 0.05$).



Graphic 2. *Daily weight gain (%)*

In the second period, the daily weight gain was 1120 g at heifers and 1200 g at calves, with 7.1% higher. The differences were not significant ($p \geq 0.05$).

From calving to weaning, the daily gain was 1044 g and, respectively 1133g, the differences being insignificant ($p \geq 0.05$).

In the literature it was shown that the live weight gain was 1300 g [1]; in the S.C.TAOS.S.R.L.farm the potential of this breed is not fully exploited.

After weaning, the weight has decreased with 5% at calves and 12% at heifers, the differences being significant ($p \leq 0.05$). It has been observed that the stress of weaning was present only to the young females; for ten days, these were restless, having the desire for sucking and the appetite for feed has decreased.

Conclusions and recommendations

1. The farm was located in a geographical area allowing the growth of cattle for meat.
2. The calves (males and females) had low weight at calving, enabling the crosses with all breeds, in particular dairy cows.
3. From calving to weaning, the daily gain was insignificant higher at calves, comparing with heifers.
4. The stress of weaning was present only to the young females; for ten days, these were restless, having the desire for sucking, the appetite and daily weight gain have decreased.
5. The raising of calves is done outdoor, in summer the feeding being done by grazing.

6. Limousin breed does not require specially equipped shelters, being resistant to environmental conditions.

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MORPHOPATOLOGICAL ASPECTS IN SOME EPITHELIAL TUMORS IN DOG AND CAT

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Abstract

The study presents the morphopathological aspects of epithelial tumors common in dog and cat, animals examined and undergone surgery at the Spiru Haret *Faculty of Veterinary Medicine clinic.*

The location of the tumoral process was observed macroscopically, evaluating its extension, the degree of local infiltration of the tumor and adjacent tissue, and involvement of adjacent lymph nodes and / or the presence of any metastases.

Macroscopic examination conducted targeted tumor consistency overall, but especially the section, then color, homogenous or heterogeneous aspect, outbreaks of necrotic and/or bleeding, cystic formations, nodular proliferation of soft, cartilaginous or bone consistency.

Histopathologically were diagnosed various hyperplastic proliferations some benign, others malignant with high metastatic potential. Identified tumors were papillomas, mammary adenocarcinomas (papillary simple, solid and cystic) and melanomas.

Key-words: *morphopathological diagnosis, tumours, dog and cat.*

Introduction

Companion animals cancer, mostly the canine, biologically resembles the human one, having cellular aspects in the malignan tumors of similar size.

At these species, the epidemiological studies are more numerous and of a special interest for human oncology (Baba Al. I., 2002; Dernell W., 2003; Moulton J.E., 1990).

In cats, the highest incidence has been in skin localised neoplasms, followed by lymphoid tissue, digestive tract and genital apparatus. All of these represented 76,5% of all the tumors (Balint Emilia, N. Manolescu 2009).

The cutaneous lymphoma is the most important malignant neoplasm in veterinary medicine. The incidence in dogs, cats and humans is rising, and on the

full understanding of the disease among species, have contributed new molecular and genetic studies.

In the cutaneous type tumors in descending order, the incidence is: basalioma, squamos carcinoma, lymphoma, perianal glands tumors and melanoma (Moulton J.E., 1990; Turrel J.M, Pool R.R., 1982).

In dogs, mastocytomas are present mostly in the cutaneous tissue. In most dogs, tumors are solitary, but in about 6%, the tumors are multiple (Moulton J.E., 1990).

Materials and methods

The research were conducted in the *Spiru Haret* Faculty of Veterinary Medicine clinic and in the *Spiru Haret* Faculty of Veterinary Medicine Histology Laboratory, on 20 subjects, dogs and cats, during 2013-2014.

The research purpose has followed the morphological aspects of some epithelial tumors frequently found in bitches and queens, animals examined and operated on at the *Spiru Haret* Faculty of Veterinary Medicine clinic.

The animals were clinically examined, in some cases also tested paraclinically and morphopathologically (macroscopic and histopathological).

The morphologic diagnostic was conducted through a rigorous macroscopical exam, followed by a microscopical exam (histopathological), preceded by sampling, fixing them and completing the other steps of proper inclusion, sectioning at 6μm and exposure to obtain permanent preparations.

Preparations obtained were stained by HE and trichromic Mallory, interpreted and microphotographed with Olympus microscope. Auxiliary were used PAS staining and toluidine blue staining (assay for metachromasy).

Results and discussion

From the collected samples, on macroscopic and microscopic examination, were identified and diagnosed mamary adenocarcinomas, papillomas and melanomas.

Most of the tumors morphologicallly examined, especially the malignant ones of big size, were invasive, adhered to the skin, some ulcerating and/ or haemoragical.

Associated to the malignant tumors, mostly adenocarcinomas, there was a lymphatic invasion affecting regional lymph nodes, and a sanguineous one affecting the parenchymal organs, especially kidneys and spleen (Fig. 1, 2).

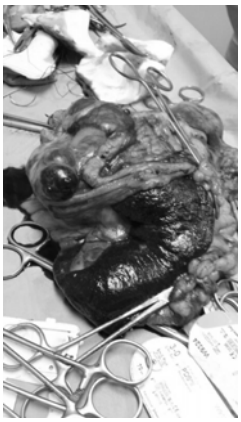

Fig. 1 – *Splenic metastases (macroscopic appearance)*

Fig. 2 – *Metastases of renal (macroscopic appearance)*

Morphological aspects of the mamary malignant tumors (mamary adenocarcinomas)

Morphologically, most of the examined mamary adenocarcinomas, particularly those of larger sizes, are invasive, have adhered to the skin, some of which ulcerated and/or bleeding.

Tumor growth was rapid, reaching a doubling of volume in a short time and with local invasion, infiltrating the surrounding normal tissue.

The skin was frequently ulcerated and hyperplastic proliferation of lymphatic vessels and lymph nodes invaded locally and adjacent mammary gland on the same side (Fig. 3).

In some cases, formations with tumor appearance are expansive, multinodular, ulcerated, skin and adjacent muscles adherent (Fig. 4). The primary hyperplasic formation has a multicentric disposition and a fast growth rate.

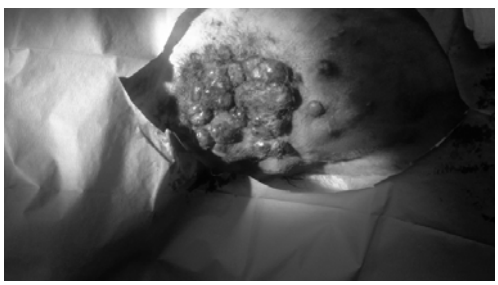

Fig. 3 – *Dog – Mammary tumor (macroscopic appearance)*

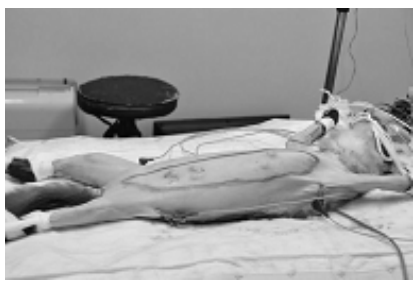

Fig. 4 – *Cat – Formations neoplastic mammary (macroscopic appearance)*

Of the samples, at microscopic examination were identified and diagnosed three types of malignant hyperplastic proliferation: solid adenocarcinoma, papillary and cystic (Fig. 5, 6, 7).

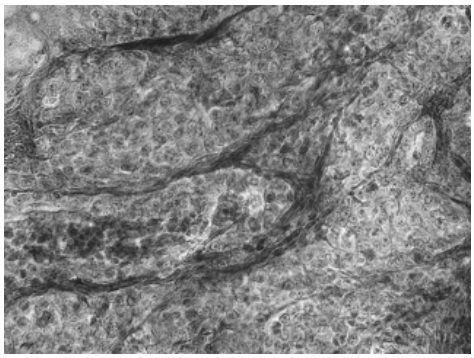

Fig. 5 – *Solid mammary adenocarcinoma; Col. Mallory trichromic; Ob 40x*

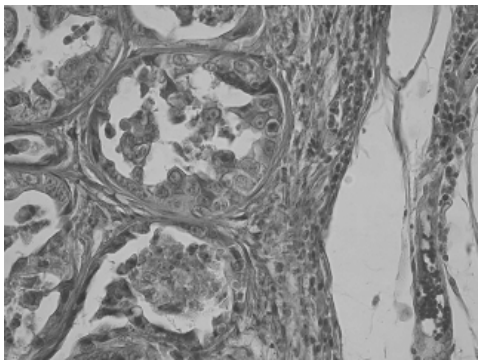

Fig. 6 – *Papillary mammary adenocarcinoma; Col. Mallory trichromic; Ob 40x*

In some malignant mamary tumors, connective tissue metaplasia was observed with the development of metaplasic connective tissue in the parenchyma of the mamary gland, following the stroma connective tissue transformation into cartilaginous tissue (Fig. 8).

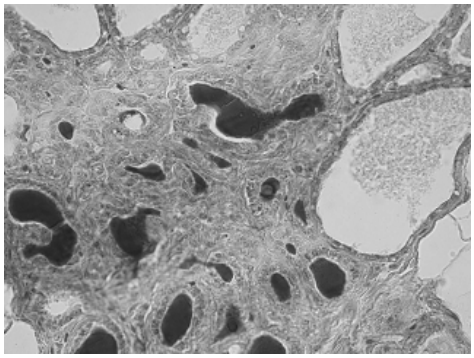

Fig. 7 – *Cystic mammary adenocarcinoma; Col. Mallory trichromic; Ob 40x*

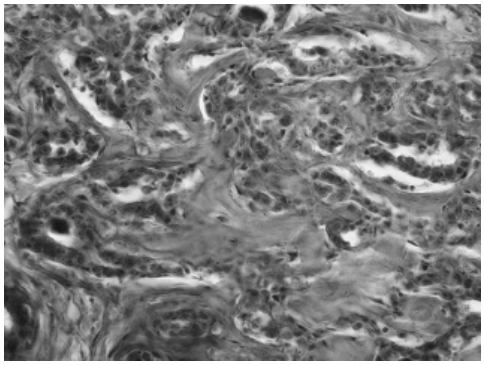

Fig. 8 – *Mammary adenocarcinoma – metaplasia connective; Col. Mallory trichromic; Ob 100x*

At lymphonodular levels, in initial stage was found the appeareance of limited tumoral proliferation with neoplasic hyperplastic cells outbreaks, accompanied by ectasia of the lymphatic, blood capillaries and lymphatic sinuses (Fig. 9, 10).

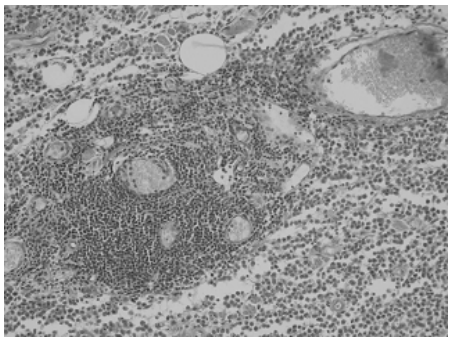

Fig. 9 – *Lymph node metastasis; Col. HE; Ob 20x*

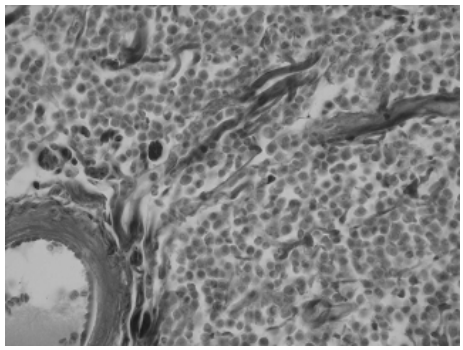

Fig. 10 – *Lymph node metastasis; Col. Mallory trichromic; Ob 40x*

In later stages, there is a proliferation of stromal connective tissue resulting in parenchymal limfonodular sclerosis as a result of blocking afferent and efferent lymphatic vessels entering and leaving the lymph nodes (Fig. 11).

At lymphonodular levels, the local invasion of hyperplastic cells, lead to a marked lymphocytic depletion of stromal connective tissue proliferation, followed by lymphonodulary sclerosis (Fig. 12).

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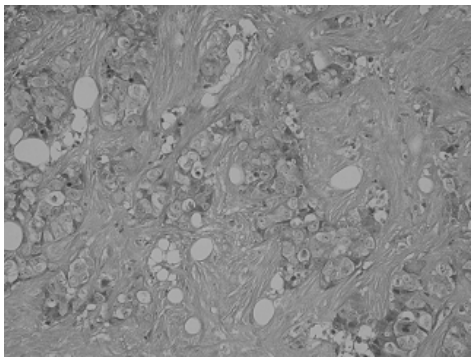

Fig. 11 – *Lymph node metastasis; Col. PAS; Ob 40x*

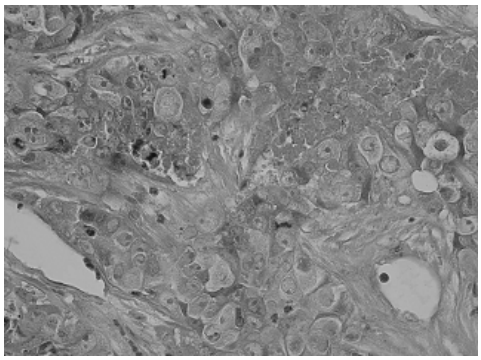

Fig. 12 – *Lymph node metastasis; Col. PAS; Ob 40x*

Metastases were observed in some parenchymal organs, especially the liver and lung, rarely in spleen and kidney. The image of tumor proliferation has nodular aspect, varying sizes and greasy on the section.

Microscopically, in the lung, there is a localized tumoral hyperplasia with microfocuses in the structure of the pulmonary parenchyma, with ectasia of the blood vessels (Fig. 13).

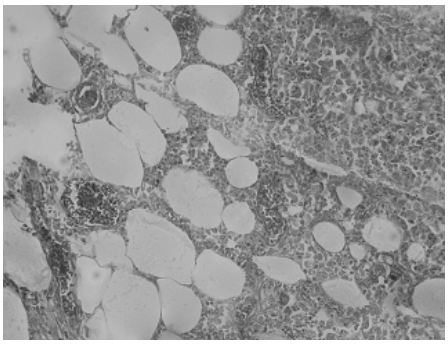

Fig. 13 – *Pulmonary metastasis; Col. Mallory trichromic; Ob 20x*

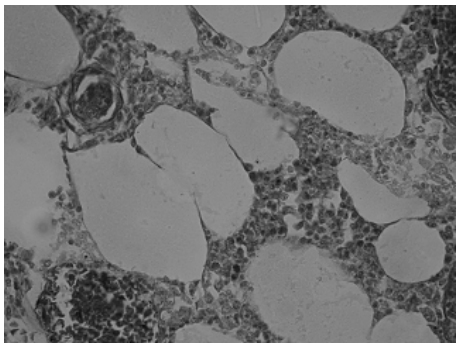

Fig. 14 – *Pulmonary metastasis; Col. HE; Ob 40x*

40x ob. exam indicates the presence of tumor cells in the lumen of blood capillaries and the occurrence of compensatory emphysema in the tumor periphery area, trying to fill hematosis lack of function in neoplastic focus (Fig. 14).

In the liver, the tumor cells are distributed among the cords of hepatocytes, being of different size and shapes (polygonal or oval) cell nuclei are oval, intensely hyperchromic with powderly nuclear chromatin with one or more nucleoli, which means a high protein synthesis activity (Fig. 15, 16).

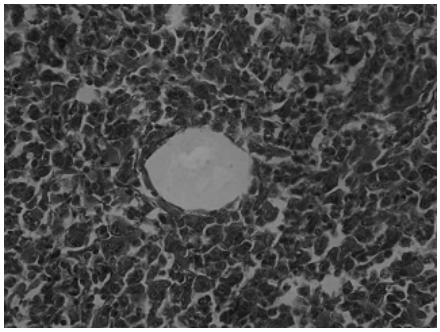

Fig. 15 – *Hepatic metastasis; Col. Mallory trichromic; Ob 20x*

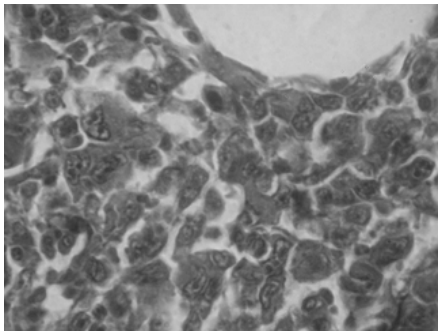

Fig. 16 – *Hepatic metastasis; Col. HE; Ob 40x*

Histological aspects of the epithelial malignant tumors (melanome)

Melanoma was identified in a single case, with the lowest incidence. As a result of microscopic examination of the sections taken, the neoplastic cell is large, polygonal, containing numerous cytoplasmic granules of melanin released into the connective-reticular stroma (Fig. 17, 18).

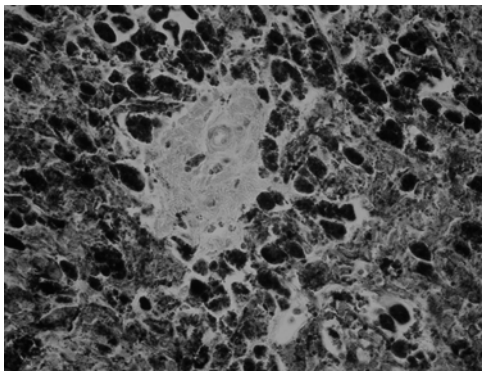

Fig. 17 – *Melanoma; Col. Mallory trichromic; Ob 20x*

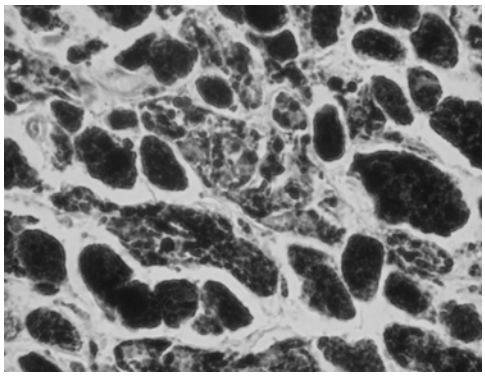

Fig. 18 – *Melanoma; Col. Mallory trichromic; Ob 40x*

To properly identify the type of tumor proliferation, metacromazine with toluidine blue test was performed, melanoma being difficult or even impossible to distinguish microscopically from mastocytoma with topographic staining.

The differential diagnosis is necessary and reveals directly and precisely the type of present intracytoplasmic granules. If melanoma cells present black granules, in mastocytoma`s coase will be red (Fig. 19).

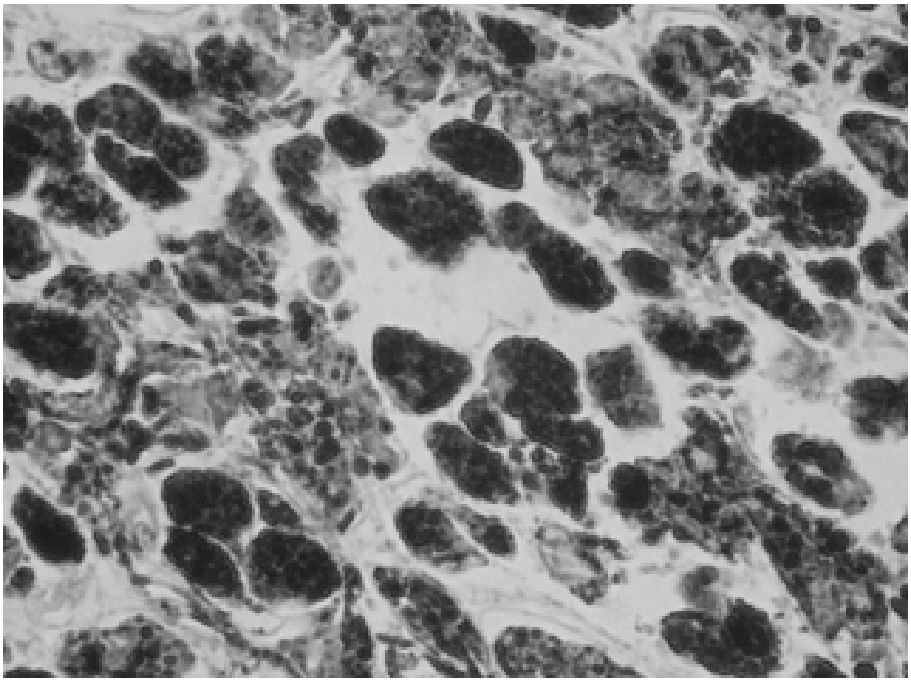

Fig. 19 - *Melanoma; Col. toluidine blue; Ob 100x*

Morphological aspects of benign epithelial tumors (papilloma)

Papillomas were present in older dogs, being sessile growths form of cauliflower appearance, unique in the skin, in sizes and shapes (Fig. 20).

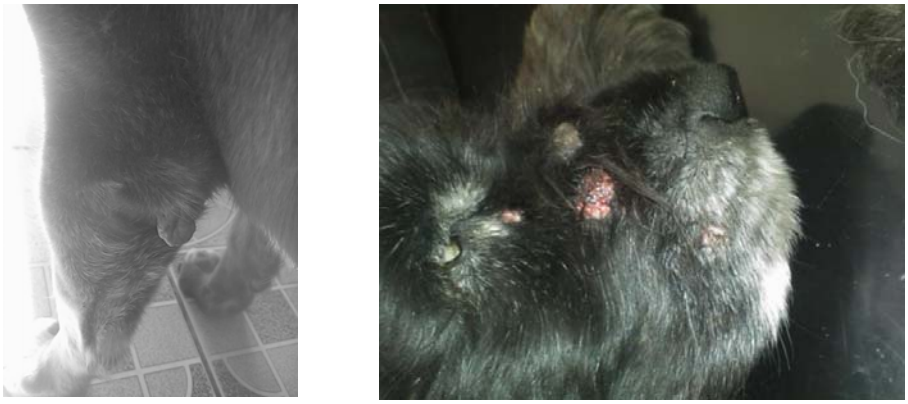

Fig. 20 – *Papilloma – dog - (macroscopic appearance)*

Microscopically, the presence of a connective-vascular rod is seen, wallpapered by hyperplastic epithelium (Fig. 21).

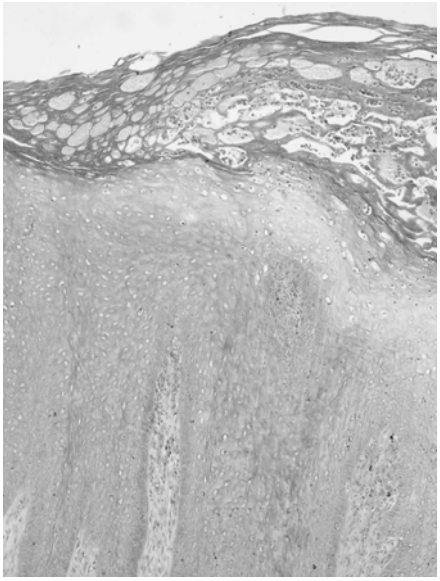

Fig. 21 – *Papilloma; Col. HE; Ob 10x*

Conclusions

Following research on macroscopic and histological aspects in some epithelial tumors in dogs and cats the following conclusions appear:

1. It has been found a significant morphopathological heterogeneousness regarding epithelial tumors found in both species, benign and malignant being identified, with different locations, on the surface epithelia and also on glandular levels.

2. From the total number of subjects were identified and diagnosed a number of benign proliferations and/or malignant mammary represented by adenocarcinomas melanomas and papillomas.

3. Mamary adenocarcinomas were developed, in the intralobular alveolar epithelium and also in the excretion ducts; also the mioepithelial cells as noninfiltrative and infiltrative structures, depending on type, contributed to it.

4. Certain malignant tumors of the mamary gland developed adaptive lesions represented by cartilaginous metaplasia of the conjunctiva, the body's adaptive response to neoplastic invasion.

5. High malignant potential tumors were papillary adenocarcinoma, which have shown a high degree of invasion and metastatic melanoma that in the animal survival time was reduced (2 months).

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CAUSES OF INFERTILITY IN CATS

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Abstract

To elucidate the diagnosis and for the effectiveness of prevention schemes causes of infertility were divided into two categories:

– External causes – related to the management of living conditions in the female

– Causes related to that individual or their partner (e.g. hormonal causes, infectious, congenital, etc.).

It is very important the management of the environment and observation (video or by staff) of living conditions of the patient.

Key words: *causes, infertility, cats.*

Introduction

Cats have become extremely popular pets and cat pedigree is most valuable, the many questions about their fertility produce insomnia to the owners, especially breeders. Infertility can arise from various causes, which can occur anywhere in the path of the mount at calving. Even before at least the mount, it was found that a number of external factors, through their influence on the hypothalamus, reduce a harmonious cooperation between the hypothalamus, pituitary gland and genital apparatus. Thus, the chance of the female becoming mother is compromised.

Materials and Methods

The study was done in the clinic FMV USH Bucharest on a total of 50 cats. When a cat is brought to the doctor with fertility problems, it must be accompanied by a long record of personal data sheet that includes:

– Name (officially registered and call);
– Date of birth, description, photo;
– Vaccinations, deworming, medical history (related treatments).

It's also important breeding registry, that must contain data on:

– Recording each side (with exact data on the age of each partner);
– Data on the female estrous cycles (duration, frequency, age of first estrus);
– Gestation concerned, the exact duration of each, treatments, diets, supplements administered during gestation;
– Number of kittens living and/or dead weight at birth and congenital defects.

Once completed this information, you have to make investigations which may be related to the problem for which the patient presented to the clinic. It makes a complete physical examination and blood and urine samples are collected for routine exams.

The complete history of the cattery data on conditions (light, ventilation, density of population, patient place the ladder) and note details on social behavior observed.

Elucidating both diagnosis and for the effectiveness of prevention schemes causes of infertility were divided into two categories:

– external causes – related to the management of living conditions in the female;

– Causes related to the individual concerned or its partner (e.g. hormonal causes, infectious, congenital, etc.).

Results and Discussion

The incidence of infertility due to environmental conditions

Management is very important for environmental factors and careful surveillance (video or by staff) of the living conditions of the patient. Fingerprints on metabolic and reproductive status can show:

– crowded, poor ventilation, frequent trips (to exhibitions, the doctor), noise;

– Aversion to certain members of the community followed by stress, isolation or conflict;

– light failure can cause fertility problems – generally need 14-16 hours / day at 100w, it says that "if you see easy to read a newspaper it is light enough";

– Nutrition – this is quite controversial, responsible for fertility problems are just very unbalanced rations (low in vitamin A, calcium, iodine);

– about breeding management is good to know that females have taken the male where he will stay 6-8 days. Obviously, the pair will be observed during the whole week.

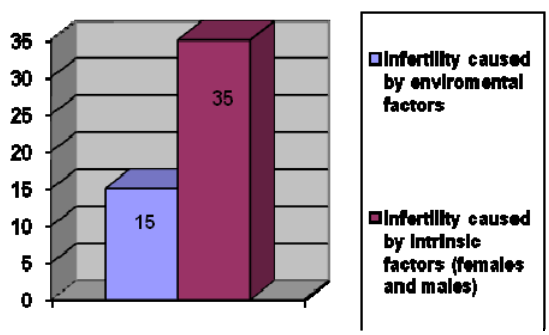

Chart I – *Statistical analysis of infertility*

In 15 of the animals subjected to observation suspected sterility was caused by external factors (in slices); also should be noted that the assessment is somewhat subjective and based only on the exclusion of the other parameters that can be measured.

The incidence of infertility caused by hormonal disorders

Some experts say the etiology is overrated, numerous abortions, fetal resorption or embryonic deaths being attributed to hormonal imbalances because of the lack of specific diagnoses.

Ovarian cysts are common and irrelevant as for the influence on pregnant cats; were discovered by ultrasound or by laparotomy numerous cysts (sometimes quite large) in females with reproductive performance satisfactory records.

Progesterone deficiency was, however, often criminalized. It seems that there is a regression of the corpora lutea of pregnancy before they realize placental unit, and this regression is correlated with progesterone too low to be able to maintain gestation; laparotomy performed at this stage showed that the embryos die, indeed, before implantation; in the subsequent pregnancy, females kept under observation received progesterone implants, allowing their insertion in early pregnancy normal development of pregnancy.

Progesterone values may be insufficient in the case of single fetuses, so they become viable, and often die at an early stage and are reabsorbed; this is the explanation for the appearance of a single kitten at birth which is unusual.

When you suspect that gestation failed due to progesterone deficiency in gestating follow these doses throughout pregnancy; values "necessary" should be:
end-to-estrus approximately 20ng/ml

- at 15-25 days postovulation-about 40-45ng/ml
- between 40-50 days postovulation-35-45ng/ml
- To 60 days postovulation-10ng/ml, before giving birth, progesterone levels decrease to approximately 5ng/ml.

Causes of failure of progesterone during pregnancy are multiple, involving stress, improper diet, thyroid disorders, hormonal therapies, etc.

Other times, the extension phase of the sexual cycle can compromise reproduction copy.

Prolonged anoestrus, for example, can be caused by hormonal impulses exogenous glucocorticoids or progestins (in this case anoestrus can take years when done improperly treatment in overdose or before puberty).

Prolonged estrus can also be a cause of breeding failure in this case "defendants" are manufacturers of estrogen excess (tumors, ovarian cysts); in this case, routine examinations and interventions (ultrasound, hormonal titrations, simple surgery) may solve the problem.

Other hormonal causes leading to infertility are quite rare in this species and envisage thyroid (hypothyroidism, thyroid hormone deficiency interferes negatively with the metabolism of sexual hormones) or adrenal (both hypo-and hyperadrenocorticism – Cushing's syndrome).

In our study were identified nine cats with abnormal hormone levels (1 case of Cushing's syndrome and 8 cases of progesterone deficiency).

The incidence of infertility cause by viral infectious diseases

It seems that the most important cause of early embryonic death (and resorption), especially in cats with pedigree who suffered a laborious selection is feline leukemia virus (FeLV).

It produces placental and uterine disorders and immunosuppression followed by endometritis permanently affecting the reproductive status of the female in question.

It is important, therefore, FeLV testing for all candidates to mount.

Also, feline rhinotracheitis virus (herpesvirus) and the infectious peritonitis (caused by a coronavirus) influences reproductive status in an irreversible manner (peritonitis is commonly diagnosed in young cats).

The incidence of infertility caused by bacterial diseases

Vaginal flora cat is not relevant to microbiological status uterus.

Therefore to the cats with fertility problems were collected pads in different phases of the sexual cycle, especially in estrus, when due to communication of the uterine cervix and vagina, one can get representative clues for the uterine flora.

From cats with breeding problems were isolated streptococcus ssp,staphylococcus ssp. (but they were also isolated from healthy cats).

The causes of endometriosis are complex, it creates actually a hormonal environment conducive bacterial infections.

Vaginal swabs are irrelevant, often the only method remaining uterine biopsy examination ecoguided or laparotomy.

A female with severe endometriosis turns into one temporary infertiliate with permanent sterility, while mild forms, treated correctly, can give hope to breeders and owners, as long as the female is not allowed to mount two estrous cycles after treatment.

Uterus as progesterone is dominant particularly sensitive to infection; exogenous progestins may exacerbate and complicate a preexisting endometriosis therefore empirical use of progesterone is totally contraindicated especially in genetically valuable females. The result leads most often to cystic endometrial hyperplasia-glandulo accompanied by endometrial secretion, with "contest" bacterial flora, turns purulent discharge (pyometra).

Infertility caused by genetic disease

In two of the cases were identified genetic causes clinically expressed by ovarian hypoplasia (1 case) and left uterine horn aplasia (1 case).

Acquired genetic disorders can often affect fertility, so despite therapeutic efforts, the female can not procreate.

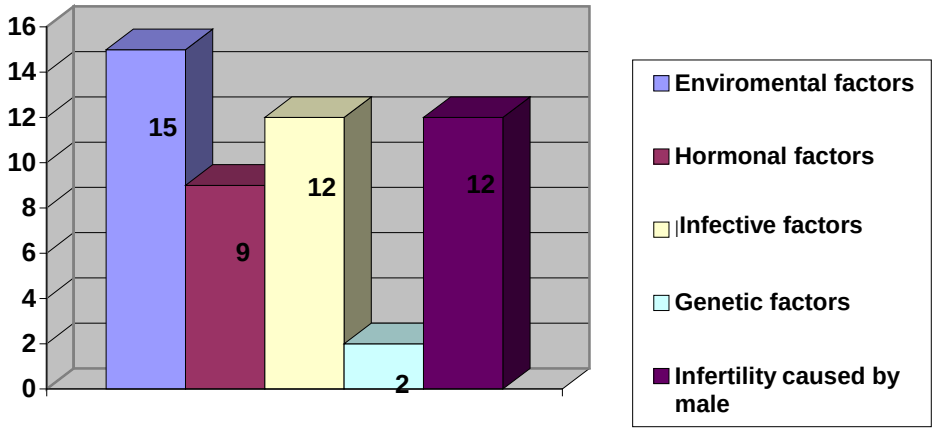

Chart II – *Statistical analysis of the causes of infertility*

Male Infertility

These causes are very common and a complete physical examination of the cat is very easy to do, it is recommended to take care of a few issues that can cause sterility:

• cryptorchidism

Testicular migration from tomcat is close to parturition. Rarely is delayed by a few months, so the final assessment is made at 5-6 months and obviously before monta. Where bilateral cryptorchidism is found, the male is infertile.

The monorhizi are generally fertile, but strongly recommended exclusion from mating of both categories, because the disease is transmitted genetically.

• Persistent frenulum that the free portion of the penis is attached to the slip Physiologically, it persists only during growth of remanence after one year of age or pain causing incomplete intromission followed by avoiding intercourse; the problem, once diagnosed, can be solved by a simple surgery.

• testicular hypoplasia – etiology genetics or infections caused by growth period (e.g. panleucopenia) – can adversely affect spermatogenesis

• Other causes with negative effect on spermatogenesis:

– Genital trauma (e.g. effects on semen quality, of a bite in the testicular and related wound may far exceed during wound healing of skin);

– Malnutrition, affects spermatogenesis reversible;

– Certain diets (as found sperm quality is negatively influenced by food containing liver, rich in vitamin A content of the liver causing testicular degeneration);

– Stress of captive conditions (especially affecting libido);

– Chronic debilitating;

– hypothyroidism.

Conclusions

1. Causes of sterility are very different, being almost equally divided between external causes related to the environment and causes related to male or infectious or hormonal factors.

2. Hormonal causes so much claimed unless otherwise Dovey (confirmed by objective methods of diagnosis) were overthrown by the infectious and maintenance ones (the latter so ignored and yet having so great an impact on fertility).

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COMPARISON OF THE HAEMODYNAMIC EFFECTS OF SEVOFLURANE ALONE SEVOFLURANE COMBINED WITH EPIDURALLY ADMINISTERED XYLAZINE FOR ANESTHESIA IN VENTILATED DOGS

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Abstract

Haemodynamic effects of 1.5 minimal alveolar concentration of sevoflurane alone (1.6% end-tidal) and 1.5 minimal alveolar concentration of sevoflurane (1.1% end-tidal concentration) combined with epidurally administered xylazine were compared during controlled ventilation in 10 dogs used on 2 occasions and randomly allocated to 2 groups.

Arterial blood pressure, cardiac index volume, left ventricular work, and pulmonary arterial pressure were significantly ($p<0.05$) higher in dogs of the xylazine-treated group before administration of xylazine.

After epidural administration of xylazine haemodynamic changes were not observed, and the aforementioned variables remained significantly higher than values in dogs of the sevoflurane only group.

Compared with sevoflurane (1.6%) alone, the reduction in sevoflurane end-tidal concentration (1.1%) associated with epidurally administered xylazine is beneficial in maintaining haemodynamic function.

Key words: *haemodynamic effects, sevoflurane, xylazine.*

Introduction

Cardiopulmonary function is considerably reduced in dose-dependent fashion in association with increased doses of inhalant anesthetic agents. Most inhalant anesthetic agents are compared at equipotent multiples of their minimal alveolar concentration (MAC).

In an effort to reduce the cardiopulmonary depression induced by inhalant anesthesic agents, sedatives, tranquilizers, or opioids are often administered systemically to reduce the end-tidal concentration of the inhalant anesthetic agent. However, the effects of these drugs on cardiopulmonary function often are

associated with a degree of depression similar to that attributable to the inhalant anesthetic agent alone (Haskins SC *et al*., 1986).

Xylazine administered by the epidural route induces segmental reduction in the MAC for halothane in dogs, and the technique provides useful postoperative analgesia. Little information is available about the haemodynamic effects of epidurally administered xylazine in combination with sevoflurane (Merin RG, Basch S, 1981; Valverde A. et all, 1989).

The purpose of the study reported here was to determine the effects of epidurally administered xylazine on selected haemodynamic variables in sevoflurane - anesthetized dogs and to compare the effects of similar anesthetic depths induced by halothane alone and those of sevoflurane combined with epidurally administered xylazine on these variables (Yaksh TL, Tudy TA, 1977).

Material and methods

Dogs and anesthesia

Ten healthy mixed-breed dogs (9 male, l female), weighing 25 to 31 kg, were used. The study was performed on 2 occasions, with 10 days between experiments, and dogs were assigned to each group in a randomized crossover fashion. Anesthesia was included with sevoflurane in 0_2 and nitrous oxide (1:2 ratio), using a face mask. The trachea was intubated and, thereafter, anesthesia was maintained with sevoflurane in 0_2, using a coaxial circuit system with an 0_2 flow of 3 L/ min. End-tidal sevoflurane and $C0_2$ concentrations were monitored, using an infrared gas analyzer calibrated before each experiment by use of a standardized calibration gas mixture designed for the analyzer. This calibration was verified each time, using a known sevoflurane concentration (2.97% sevoflurane in nitrogen). Mechanical ventilation was used, and the end-tidal $C0_2$ concentration was maintained between 30 and 40 mm of Hg. Rectal temperature was monitored electronically and was maintained between 38 and 39 C.

Instrumentation

End-tidal sevoflurane concentration between 1.5 and 2.0% was maintained, and a catheter was inserted percutaneously into the dorsal pedal artery attached to a length of sterile tubing and connected to a pressure transducer and recording system for determination of heart rate (HR), and systolic (SBP), diastolic (DBP), and mean blood (MBP) pressures, and for periodic arterial blood gas sample collections. A pulmonary artery thermistor catheter was threaded into the jugular vein until the tip of the catheter reached the pulmonary artery. This catheter was used to measure mean pulmonary arterial pressure (pap), and central venous pressure (CVP), and to determine cardiac output (CO). For each reading, 5 ml of iced 5% dextrose was injected through the proximal port of the catheter during expiration, and CO was the average of any 3 consecutive readings that did not differ from each other by > 10%.

EXPERIMENTAL GROUPS

Group 1 – After instrumentation, dogs were maintained at 1.6% end-tidal sevoflurane concentration, corresponding to 1.5 mac as determined in a previous study of the same dogs. After a period of equilibration of at least 30 minutes, baseline haemodynamic measurements were obtained (time 0) and measurements were made subsequently at 5, 10, 15, 30, 60, and 90 minutes.

Group 2 – A 16 – gauge needle was inserted into the lumbosacral space and a 19-gauge catheter was advanced through the needle approximately 2 to 3 cm cranially into the epidural space, at which point it was secured in place for subsequent administration of xylazine. After instrumentation, dogs were maintained at 1.1% end-tidal sevoflurane concentration, corresponding to 1.5 MAC of sevoflurane-xylazine combination for the hind limb, as determined in the previous study of the same dogs. Baseline (time 0) data were determined and xylazine at dosage of 0.1 mg/kg of body weight diluted in 0.26 ml of saline solution/kg was injected into the epidural space. The aforementioned variables were determined again at 5, 10, 15, 30, 60, and 90 minutes after xylazine administration.

The instrumentation and equilibration phases were completed in 60 to 90 minutes in all dogs, and administration of xylazine was completed in < 2 minutes. Therefore, little time difference (< 2 minutes) existed between collection of baseline data (time 0). After complete recovery from anesthesia and for up to 24 hours, dogs were monitored for appearance of adverse side effects, including pruritus, vomiting, respiratory depression, and inability to urinate.

Data analysis

Data obtained from each group were compared . Results within groups were analyzed and compared with baseline (time 0), using a General Linear Model . AP value < 0.05 was considered significant.

Results and discussion

Dogs remained eucapnic throughout the study, significant differences were not observed between groups. Side effects induced by epidurally administered xylazine were not evident in the group of dogs so treated. At all time intervals, SBP, DBP, MBP, PAP, CO were significantly higher in dogs of the xylazine treated group (Table 1). Significant differences were not found in HR and CVP between groups (Table 1).

Table 1

Haemodynamic effects of sevoflurane (S) and sevoflurane combined with epidurally administered xylazine (SX) in ten ventilated dogs anesthetized with 1.6% end-tidal and 1.1% end-tidal

Variable	Time (min)						
	0	5	10	15	30	60	90
HR (beats/min.)							
S	98	98	96	97	97	95	95
SX	86	92	84	90	82	80	80
CVP (mm of Hg)							
S	2.3	2.1	2.5	2.5	2.6	2.7	2.7
SX	3.1	3.9	3.3	3.3	3.5	3.7	3.6
PAP (mm of Hg)							
S	9.9	9.6	10.2	9.8	9.5	9.8	9.4
SX	10.6	11.8	11.5	11.6	11.5	11.4	11.5
SBP (mm of Hg)							
S	84	87	89	91	91	94	94
SX	111	117	111	113	114	113	114
DBP (mm of Hg)							
S	47	50	49	50	51	53	53
SX	62	70	62	59	58	58	59
MBP (mm of Hg)							
S	57	60	59	60	60	61	62
SX	73	82	74	73	71	69	71
CO (L/min.)							
S	1.86	1.92	1.95	2.03	2.10	1.94	1.97
SX	2.29	2.64	2.52	2.60	2.56	2.43	2.63

HR = heart rate; CVP = central venous pressure; PAP = pulmonary arterial pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; CO = cardiac output.

At the 15-, 30-, 60-, and 90-minute intervals, SBP, DBP and MBP increased significantly from baseline in dogs of the sevoflurane group (Table 1). Other significant changes were not observed in this group. At 5 minutes, DBP and MBP were significantly increased in dogs of the xylazine treated group (Table 1). Other variables were not significantly different, although slight increase was apparent in all variables at the 5-minute interval after epidural administration of xylazine, with return to baselin the 10-minute interval in most instances. Panting was also observed immediately after epidural administration of xylazine; this response lasted < 5 minutes.

Therefore, anesthetic depth compared between our 2 groups of dogs was only similar at the 60- and 90-minute intervals, and possibly at the 30-minute interval.

Epidurally administered xylazine has no effect on sympathetic fibers, unlike local anesthetics that can induce hypotension attributable to sympathetic blockade.

Haemodynamic effects induced by epidurally administered xylazine are the result of absorption of xylazine from the epidural space into the systemic circulation .The dose administered by us (0.1 mg/kg) did not appear to induce any major haemodynamic effects; changes were not observed in the first 30 minutes after administration.

Results of this study were in agreement with those of other studies, which indicated that haemodynamic function is less depressed at decreased MAC multiples. The administration of xylazine resulted in a temporary increase in all of the haemodynamic variables measured, but the increase was only signifîcant for DBP and MBP. The cause of these changes is believed to be administration of the xylazine solution at ambient temperature (20 to 22 C), because similar effects were not observed by the authors when the solution was warmed to body temperature.

In our dogs, lack of haemodynamic effects after epidural administration of xylazine makes it seem likely that haemodynamic function will be better maintained in spontaneously ventilating dogs at the same MAC of sevoflurane. The effects of various MAC multiples remains unanswered; however the mac used by us represents a clinically useful value for surgical procedures.

Conclusions

Epidurally administered xylazine does not adversely affect haemodynamic function in sevoflurane-anesthetized dogs, and the resultant reduction in sevoflurane requirement (1.6% vs 1.1%) depresses haemodynamic function less than does a similar anesthetic depth achieved with sevoflurane alone. Maintenance of near-normal haemodynamic function, long-lasting analgesia, and minimal side effects make the epidural administration of xylazine a safe, desirable technique for achieving pain relief during and after surgery.

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STUDIES ON MICROBIOLOGICAL SCREENING AND SELECTION OF SOME MCL-PHA PRODUCING BACTERIAL STRAINS

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Rezumat

Acizii poli(β-hidroxialcanoici) (PHA), cunoscuți şi sub denumirea de polihidroxialcanoați, formează o clasă de polimeri naturali care sunt produşi de o largă varietate de bacterii, sub formă de homo- sau copolimeri ai acizilor [R]-β-hidroxialcanoici, în funcție de sursa de carbon folosită pentru creşterea microorganismelor. Datorită proprietăților lor similare cu ale materialelor plastice convenționale, precum şi datorită biodegradabilității şi biocompatibilității lor, PHA prezintă interes ca alternative la polimerii sintetici, cu posibilități de aplicare în două mari direcții: materiale pentru ambalaje şi materiale biomedicale, utilizabile în suturile chirurgicale, sistemele de eliberare controlată a medicamentelor şi ingineria țesuturilor. Utilizarea unor substraturi de creştere adecvate şi alegerea judicioasă a microorganismului producător permit obținerea unei familii de astfel de polimeri, cu proprietăți corespunzătoare unui anumit domeniu de utilizare.

În acest context, scopul cercetărilor prezentate a fost acela de a realiza un screening microbiologic şi selecția unor microorganisme producătoare de MCL-PHA (PHA formați din monomeri ce conțin 6-14 atomi de carbon). Aceştia prezintă interes datorită posibilității de a fi utilizați în domeniul biomedical, în special în ingineria țesuturilor, unde o gamă largă de polimeri absorbabili sunt în prezent testați ca matrițe de regenerare. Studiile s-au realizat cu mai multe tulpini bacteriene, aparținând genurilor Pseudomonas, Alcaligenes şi Ralstonia. Criteriul de selecție a constat în evaluarea şi măsurarea fluorescenței apărute în lumina UV, după colorarea coloniilor bacteriene, cultivate pe mediu solid cu agar, cu coloranții benzofenoxazinici Nile Blue sau Nile Red. Aceşti coloranți au fost incluşi în mediul de cultură în concentrație de 0,5µg/mL şi microorganismele au crescut în prezența lor. Estimarea formării PHA

s-a realizat pe baza faptului că legarea coloranților lipofili sus-menționați de granulele intracelulare de PHA provoacă apariția unei fluorescențe specifice a coloniilor, când acestea sunt expuse radiațiilor UV. S-au efectuat de asemenea analize spectrofluorimetrice ale coloniilor crescute pe medii cu Nile Blue sau Nile Red.

Abstract

Poly(β-hydroxyalkanoic acid)s (PHA), also known as polyhydroxyalkanoates, form a class of natural polymers which are produced by a wide variety of bacteria, as homo- and copolymers of [R]-β-hydroxyalkanoic acids, depending on the C source used for microorganism growth. Due to their properties, similar to those of conventional plastics, to their biodegradability, and to their biocompatibility, PHA have attracted much interest as alternatives to synthetic polymers, applicable in two main directions: packaging materials and biomedical materials useful in surgical sutures, as drug carrier for long-term sustained release and for tissue engineering. Careful control of the starting materials and the choice of production organisms enable the production of a polyester family with tailored properties, according to their area of applications.

In this context, the purpose of our researches has been to carry out a micro-biological screening and selection of some MCL-PHA producing microorganisms (MCL-PHA are medium chain length PHA, formed of monomers containing 6-14 carbon atoms). MCL-PHA came into notice due to their potential use in biomedical area, especially in tissue engineering, where a wide range of absorbable polymers are tested as tissue scaffolds. The studies have been performed on several bacterial strains of Pseudomonas, Alcaligenes and Ralstonia spp. The selection criteria consisted in evaluation and measurement of the fluorescence appeared in UV light after staining the bacterial colonies, on agar plate, with the benzophenoxazine dyes Nile Blue or Nile Red. These dyes have been included in the culture medium in concentration of 0,5μg/mL and the microorganisms grown in their presence. The estimation of PHA formation has been done based on the fact that the lipophilic dyes bound to intracellular PHA granules cause a specific fluorescence of colonies when exposed to UV light. Spectrofluorimetric analysis of the stained colonies has also been performed.

Key words: *MCL-PHA, Pseudomonas, fluorescence, spectroscopy.*

Introduction

Polyhydroxyalkanoates (PHAs) are polyesters that function as intracellular storage compounds for carbon and energy in prokaryotic cells (Anderson and Dawes, 1990); a wide range of bacteria synthesize them when a carbon source is provided in excess and one essential growth nutrient is limited (Ramsay *et al*., 1990). PHA polyesters have been studied intensively in recent decades because their physical characteristics are similar to those of petrochemical polyesters, such as polypropylene, and possess biocompatible and biodegradable features; they are considered good candidates for biodegradable plastics (Chen G.-Q., 2009). Generally, PHAs are categorized into two groups based on the number of carbon atoms in the monomer units: short-chain-length (SCL) PHAs consist of 3–5 carbon atoms, and medium-chain-length (MCL) PHAs consist of 6–14 carbon atoms (Anderson and Dawes, 1990). SCL-PHAs possess higher melting temperatures and are stiffer than those of MCL-PHAs, while MCL-PHAs own properties of an elastomer with poor tensile strength and high extension to breakage (Poirier *et al*., 1995).

MCL-PHAs are polyesters that are synthesized and accumulated in a wide variety of Gram-negative bacteria, mainly pseudomonads. These biopolyesters are promising materials for various applications, because they have useful mechanical properties and are biodegradable and biocompatible. The versatile metabolic capacity of some *Pseudomonas spp.* enables them to synthesize MCL-PHAs that contain various functional substituents; these MCL-PHAs are of great interest because these functional groups can improve the physical properties of the polymers, allowing the creation of tailor-made products. Moreover, some functional substituents can be modified by chemical reactions to obtain more useful groups that can extend the potential applications of MCL-PHAs as environmentally friendly polymers and functional biomaterials for use in biomedical fields (Kim Y.B. et al., 1992; Kim O. *et al*., 1996; Hazer B. *et al*., 2012; Shrivastav *et al*., 2013). Although MCL-PHAs are water-insoluble, hydrophobic polymers, they can be degraded by microorganisms that produce extracellular MCL-PHA depolymerase. MCL-PHA-degraders are relatively uncommon in natural environments and, to date, only a limited number of MCL-PHA depolymerases have been investigated at the molecular level. All known MCL-PHA depolymerases share a highly significant similarity in amino acid sequences, as well as several enzymatic characteristics.

MCL-PHA biosynthesis in bacteria is closely linked to three different metabolic routes that generate MCL-PHA precursor molecules:

(i) de novo fatty acid biosynthesis pathway, which produces (R)-3-hydroxyacyl-CoA precursors from non-related carbon sources such as glucose and gluconate;

(ii) fatty acid degradation by β-oxidation, which is the main metabolic route of fatty acids;

(iii) chain elongation, in which acyl-CoA is extended with acetyl-CoA (Witholt and Kessler, 1999). These metabolic pathways produce various intermediate precursors of MCL-PHA, such as (R)-3-hydroxyacyl-acyl carrier protein (ACP), 2-trans-enoyl- CoA, (S)-3-hydroxyacyl-CoA, and 3-ketoacyl-CoA (Barker I. A., 2011; Baljeet S.. 2014).

Nile red is an oxazone form of Nile blue A, formed by the spontaneous oxidation of Nile blue A in aqueous solution or by refluxing Nile blue A with dilute sulfuric acid. It is poorly soluble in water but it dissolves in a wide variety of organic solvents, such as acetone. The dye is strongly fluorescent in all organic solvents, but is quenched in water (Greenspan *et al*., 1985). Many methods have been developed for detecting or quantifying intracellular PHA granules based on Nile red staining (Ostle and Holt, 1982; Degelau *et al*., 1995; Fouchet *et al*., 1995; Gorenflo *et al*., 1999; Kranz *et al.*, 1997; Spiekermann et al., 1999; Joseand J. *et al*., 2006). Nonetheless, these methods are not capable of analyzing or differentiating PHA compositions.

Methods and materials

Bacterial strains, media and growth conditions

According to literature the production of MCL-poly(3HA) is restricted to fluorescent *Pseudomonas* belonging to rRNA homology group 1 (Huisman *et al*, 1989). For this study we used four strains: *Pseudomonas fluorescens* ICCF 392, *Pseudomonas putida* ICCF 391, *Pseudomonas aeruginosa* ICCF 90 and *Ralstonia eutropha* ICCF 384. *Pseudomonas fluorescens* was isolated from rotten beech wood in the biotechnology department of ICCF. *Pseudomonas aeruginosa* was purchased from American Type Culture Collection. *Pseudomonas putida* strain comes from USAMV Bucharest collection. Selected strains were maintained in a vegetative stage on a solid medium (medium no. 44), having the following composition: yeast extract 1%, peptone 1%, glycerol 1% and 2% agar. The composition of the inoculum medium was: glucose 1%, corn extract 1.5%, KH_2PO_4 1%, NaCl 1%, $MgSO_4$ 0.05%. Inoculum culture was developed for 24 hours at 30°C in Erlenmeyer flasks of 500 ml capacity, containing 100 ml of medium, under continuous agitation on the rotating shaker at 220 rpm. Bioprocess medium had the following composition: $NaNH_4HPO_4x\ 4H_2O$ 0,35 g%, K_2HPO_4 0,75 g%, KH_2PO_4 0,37 g%, carbon source 0,25– 0,5 g %, trace element solution I 0,1mL%, trace element solution II 0,1 mL%. Trace element solution I contains 120g/L $MgSO_4x7H_2O$. Trace element solution II contains per liter, 2.78 g of $FeSO_4$ x $7H_2O$, 1.47 g of $CaCl_2x\ 2H_2O$, 1.98 g of $MnCl_2$ x $4H_2O$, 2.81 g of $CoSO_4x\ 7H_2O$, 0.17 g of $CuCl_2x\ 2H_2O$, and 0.29 g of $ZnSO_4x\ 7H_2O$ in 1 M HCl. The carbon source has been represented by citrate. Fermentation medium was supplemented at different times with a solution containing a carbon source (octanoate) which favors the production of MCL-Poly (3HA). Solution was added under sterile conditions in the fermentation medium, whose pH was in the range 7-7.2. One stock solution has

been made of sodium octanoate, 8.33% concentration. From these, 3 mL were added in the fermentation media, to a final concentration of octanoate of 0.25%.

Polymers biosynthesis was performed in 500 ml shaking flasks, containing 100 ml nutrient medium. This was inoculated with 10% inoculum culture. Fermentations were conducted for a period of 48 hours at 30°C on a rotary shaker. Regular measurements of bioprocess parameters were made: optical density, pH, dried biomass and PHA content.

Sample preparation and staining procedure

Briefly, direct smears were prepared from the cell suspension, after 48 hours of bioprocess. All the smears were air-dried and then heat-fixed by passing the slides through a flame several times, smear side up. Smears were first stained with Nile Red (80 µg/mL dissolved in dimethyl sulfoxide [DMSO]) for 10 min, rinsed with water, destained with acetic acid 10% and finally rinsed with water again. After staining the smears were examined at a fluorescent microscope.

Sample preparation and fluorescence spectroscopy

The PHA-accumulated cells were diluted with water until the absorbance measured at 600 nm was 1.0 or less. One milliliter of diluted cells was collected by centrifugation and discarded the supernatant as clear as possible. Ten microliters of Nile red stock solution (1 mg/ml of Nile red in acetone) was slowly added to the cell pellet and mixed with the pipette tip. After incubating at room temperature for 5 min, the stained cells were dried to remove trace amounts of acetone, resuspended in 1 ml of water, and. The relative amount of PHA within the cells, as indicated by the intensity of Nile red orange fluorescence, was measured using a spectrofluorometer. The stained cells were subjected to scanning for maximal emission wavelength from 530 to 700 nm at a fixed excited wavelength of 488 nm.

Results and discussions

The cultivation of the four strains of Pseudomonas in the medium containing sodium octanoate as a carbon source for the production of PHA was performed in order to follow the evolution of the fermentation process, both from the point of view of cell mass development, and from the point of view of the biosynthesis, accumulation of PHA and its composition.

The chart below illustrates the comparative time evolution of the cell mass growth using the E medium (Kim *et al*. 1996), well suited for bacterial cultivations; results from measurements of optical density at 550 nm are presented below.

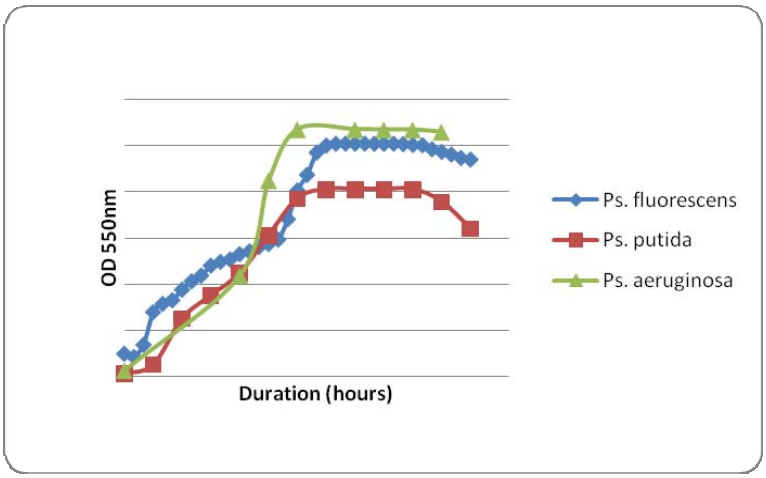

Fig. 1. *Growth curves of bacterial strain*

The results show a higher development of bacterial biomass during the fermentation process, for the strains, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, compared to the *Pseudomonas putida* strain. The evolutions of the cell mass of the three strains are distinct and the following: in the case of *Pseudomonas putida* strain, the stationary phase (characterized by the maximum concentration of biomass) is reached after 42 hours and it lasts for 18 hours; in the case of the *Pseudomonas fluorescens* strain, the stationary phase is reached after 46 hours and it lasts 10 hours; in the case of the *Pseudomonas aeruginosa* strain the stationary phase is reached after 36 hours and it lasts for 24 hours.

Wavelength scanning was performed as described above. All four PHA-accumulating bacteria exhibited significant fluorescence maximum.

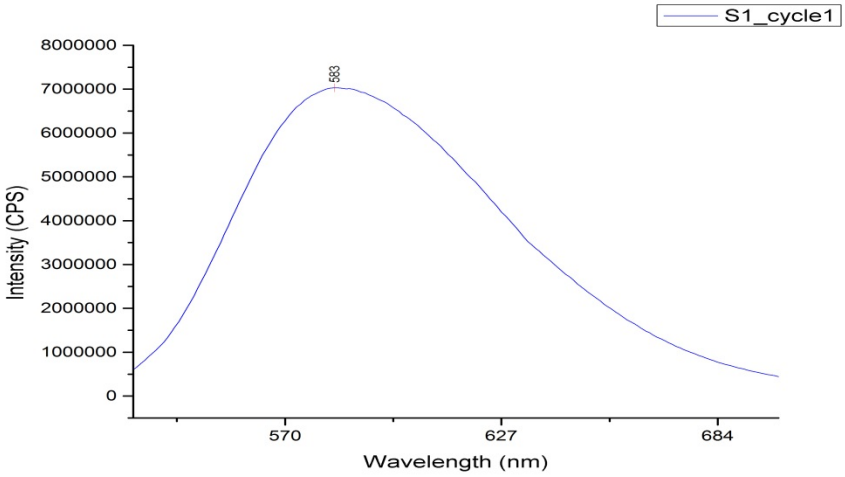

Fig. 2. *Fluorescence spectra of the bacteria Pseudomonas fluorescens grown on citrate and octanoate as carbon sources and stained with Nile red*

The graph from Figure 2 reveals a fluorescence maximum around 583 nm for the *Pseudomonas fluorescens* strain. The fluorescence intensity of the polyester granules stained with Nile red suggests that the bacterial cells possess significant PHA accumulation ability.

Fluorescence microscopy was performed as described above. All four bacteria strains exhibited significant accumulation of polyester granules stained with Nile red. The dye produces orange fluorescence on binding to polymer granules in the cell. The lipophilic dye Nile red can be directly added to the medium to stain colonies that store PHA, and at concentrations that do not inhibit cell growth. Thus, Nile red staining allows both the detection of PHA-producing strain from environmental samples and quantification of the polymer in liquid culture, resulting in a significant reduction of analysis time and labour.

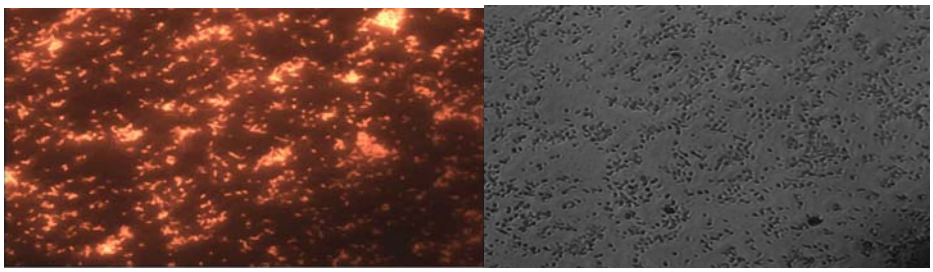

Fig. 3. Strain **Pseudomonas fluorescens** – *(left) Fluorescence intensity of polyester granules stained with Nile red; (right) Phase contrast microscopy*

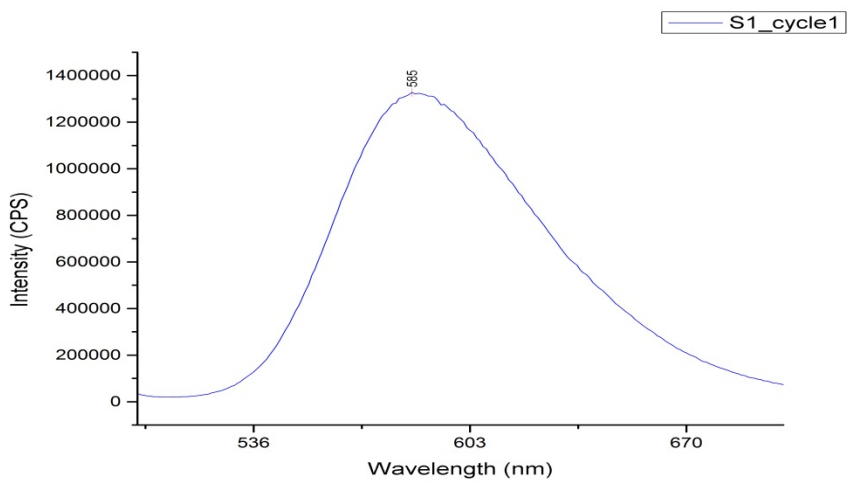

Fig. 4. *Fluorescence spectra of the bacteria* **Pseudomonas putida** *grown on citrate and octanoate as carbon sources and stained with Nile red*

The graph from Figure 4 reveals a fluorescence maximum around 585 nm for the *Pseudomonas putida* strain. The fluorescence intensity of the polyester granules stained with Nile red (Fig. 3) suggests that the bacterial cells possess significant PHA accumulation ability.

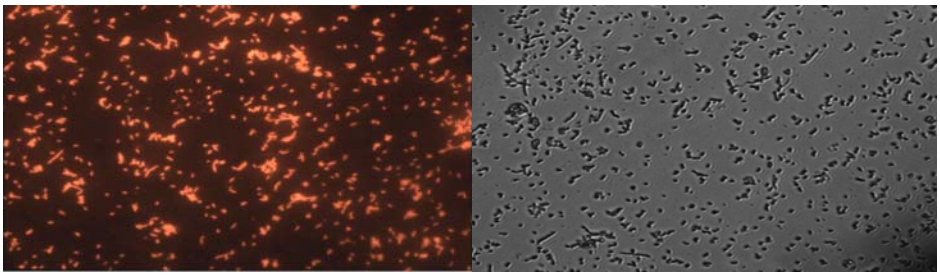

Fig. 5. *Strain* **Pseudomonas putida** – (left) *Fluorescence intensity of polyester granules stained with Nile red; (right) Phase contrast microscopy.*

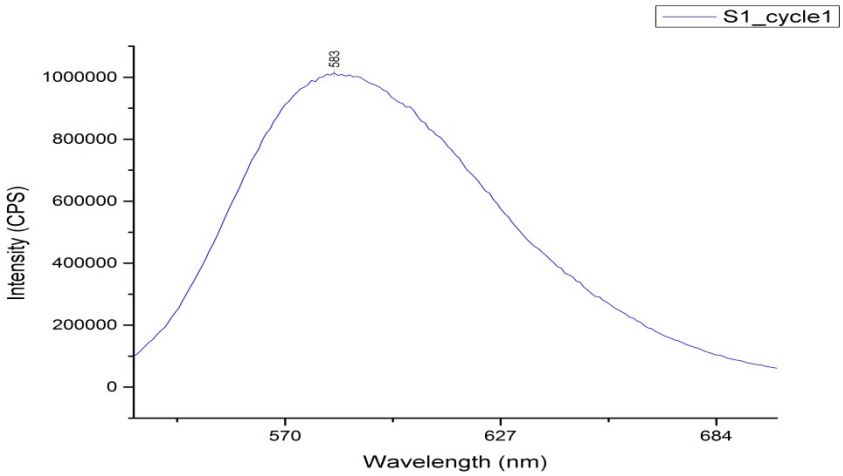

Fig. 6. *Fluorescence spectra of the bacteria* **Pseudomonas aeruginosa** *grown on citrate and octanoate as carbon sources and stained with Nile red*

The graph from Figure 6 reveals a fluorescence maximum around 583 nm for the *Pseudomonas aeruginosa* strain, but the fluorescence intensity of the polyester granules stained with Nile red is smaller than that of *Pseudomonas putida*, suggesting that this strain is less capable of PHA biosynthesis and/or accumulating.

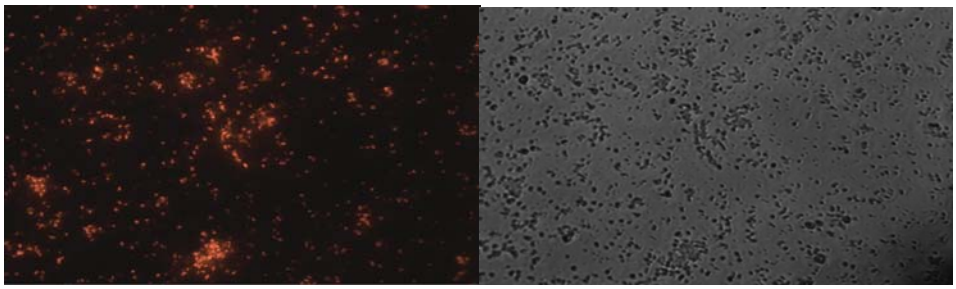

Fig. 7. *Strain* **Pseudomonas aeruginosa** *– (left) Fluorescence intensity of polyester granules stained with Nile red; (right) Phase contrast microscopy.*

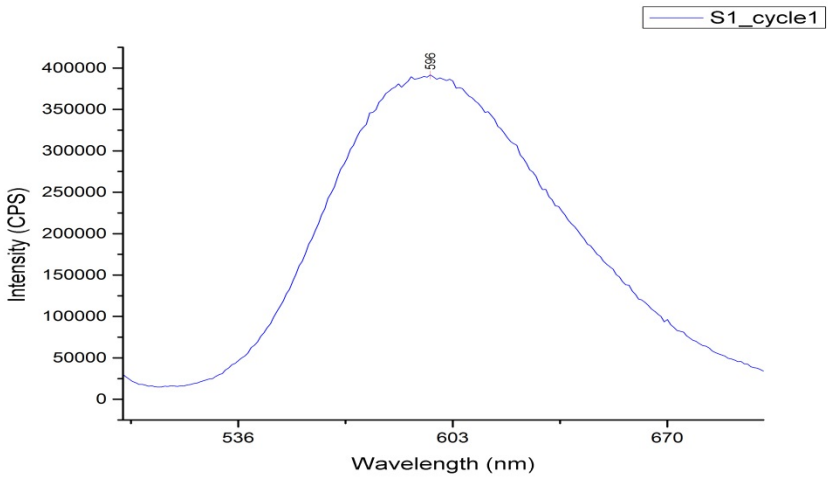

Fig. 8. *Fluorescence spectra of the bacteria* **Pseudomonas aeruginosa** *grown on citrate and octanoate as carbon sources and stained with Nile red*

The graph from Figure 8 reveals a fluorescence maximum around 596 nm for the *Ralstonia eutropha* strain. The fluorescence intensity of the polyester granules stained with Nile red suggests that the bacterial cells do not possess significant PHA accumulation ability.

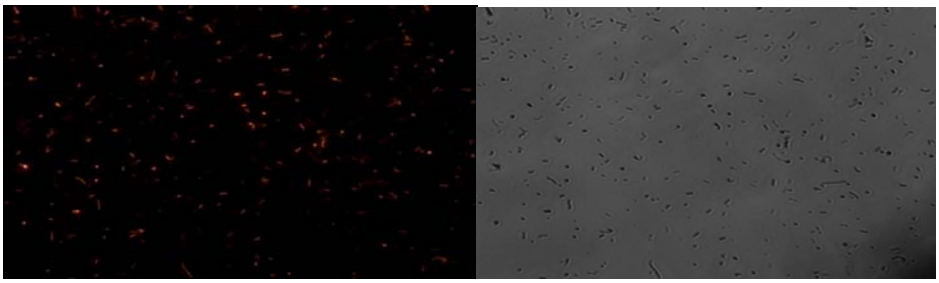

Fig. 9. *Strain* ***Ralstonia eutropha*** *– (left) Fluorescence intensity of polyester granules stained with Nile red; (right) Phase contrast microscopy*

The Figures 2, 4, 6, 8 show a difference in the fluorescence emission spectra between Nile red stained cells of the *Pseudomonas spp* and *Ralstonia eutropha*. When Nile red stained cells of Ralstonia eutropha with PHB homopolymer was excited at 485 nm, the fluorescence emission spectrum peaked at 596 nm. On the other hand, the fluorescence emission spectrum of *P. fluorescens*, *P. putida* and *P. aeruginosa* cells with MCL-PHA copolymer of C6 and C8 monomers exhibited a peak at 583 nm, 585 nm and 583 nm, respectively. There was an apparent difference (10nm) in emission maximum between the colonies with PHB homopolymer (*R. eutropha*) and the colonies with MCL-PHA copolymers (*P. fluorescens*, *P. putida*, *P. aeruginosa*). This can be explained by the fact that the maximum of emission peak is dependent on the structure of PHA polymers accumulated in bacterial cells.

Conclusions

The fluorescence behavior among PHA – accumulating bacteria could also be clearly classified into two groups, those with fluorescence maxima around at 585 nm and those at 595 nm. The bacteria strains of the 585 nm group, which included *P. fluorescens, P. putida* and *P. aeruginosa* are all MCL-PHAs producers according to previous reports (Timm and Steinbüchel, 1990). In addition, the strain of the 595 nm group, represented by *R. eutropha* is SCL-PHAs producers as previous reports have described (Timm and Steinbüchel, 1990; Lee, 1996).

Depending upon the relative hydrophobicity of the environment, the excitation and emission maxima of Nile red fluorescence can vary over a range of 60 nm; the fluorescence colors range from golden yellow (emission wavelength, >528 nm) to deep red (emission wavelength, >590 nm) (Greenspan *et al*., 1985; Dutta *et al*., 1996). The SCL-PHAs and MCL-PHAs are different in their chemical structure as regards the carbon side chain length, which also reflect the inherent hydrophobicity of the structure; longer carbon side chain, stronger hydrophobicity inherent. This may explain why Nile red staining elucidates different maximum emission wavelengths in SCL-PHAs and MCL-PHAs. This approach was excellent for detecting PHAs; however, there was a disadvantage that occurred in differentiating between lipids and PHAs. To overcome this drawback, a genotype-detecting approach would be required to further confirm Nile red staining results.

It can be concluded that this method combined with evaluation of the fluorescence of the grown bacteria on a mineral salt medium plate is capable of being applied to rapid differentiating SCL- and MCL-PHA producers isolated from environment.

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OSTRICH OIL REFINING THROUGH ADSORPTION ON ACTIVATED CLAY

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Rezumat

Pentru a putea fi folosit în preparate cosmetice şi în scopuri terapeutice uleiul de struţ trebuie să îndeplinească anumite standarde de calitate. Obţinerea unui ulei de struţ de calitate cosmetică sau farmaceutică, având indicele de peroxid < 2 mE/kg, indicele de aciditate < 0,1 mg KOH/g şi apa < 0,05%, presupune utilizarea în procesul de rafinare a uleiului de struţ a dezodorizării prin distilare cu antrenare cu aburi la temperaturi de 200-270ºC, la vid de 2-4 mm Hg şi/sau a distilării moleculare.

Scopul acestei lucrări a fost de a demonstra că se poate obţine un ulei de struţ de calitate farmaceutică în ceea ce priveste impurităţile, peroxizii, aciditatea liberă şi umiditatea fără să se utilizeze pentru îndepărtarea impurităţilor distilarea cu antrenare cu aburi la temperaturi de 200-270ºC, la vid înaintat şi/sau distilarea moleculară.

Pe baza cercetărilor efectuate în această lucrare am demonstrat că dacă utilizăm în procesul de decolorare uleiuri de struţ neutralizate, cu aciditatea liberă în limitele corespunzătoare, se poate menţine acest parametru constant dacă se foloseşte pentru decolorare alături de bentonită acidă şi o argilă bazică (care în apă are pH bazic). Procesul de decolorare decurge cu rezultate optime dacă se desfăşoară la 90ºC, sub vid de 2 mmHg, timp de 30 min, utilizând 2,5% bentonită acidă şi 2,5% argilă bazică (bentonită sodică sau veguum). Deoarece în aceste condiţii, din procesul de decolorare al uleiului rezultă un produs corespunzător pentru uz farmaceutic, din punct de vedere al celor trei parametri urmăriţi, dezodorizarea uleiului se poate face în condţtii mai blânde de temperatură, nefiind necesară îndepărtatea acizilor liberi. S-a reuşit obţinerea unui ulei fără miros, cu stabilitate de cel puţin 6 luni, prin efectuarea procesului de dezodorizare la 50ºC, timp de 5-7 ore, în rotavapor sub vid de 2 mm.

Abstract

In order to be used in cosmetic industry or for therapeutical purposes, ostrich oil must by compliant with certain quality standards. Obtaining an ostrich oil of cosmetic or pharmaceutical quality, having peroxide index < 2 mE/kg, acidity index < 0,1 mg KOH/g and water,0,05%, requires a process of refining using either deodorization, by stripping at temperatures of 200-270^{o}C, under 2-4 mm Hg vacuum and/or molecular distillation.

The aim of this paper is to demonstrate the possibility to obtain ostrich oil meeting the pharmaceutical standards (for peroxides, free acidity and humidity) without the use of stripping at 200-270^{o}C in high vacuum and/or by molecular distillation.

Based on the findings described in this paper, we proved that when using neutralized ostrich oil as starting material for bleaching, with free acidity in adequate limits, this parameter can be maintained, on condition of using bentonite together with a basic clay in the process of bleaching. The process is optimal if it takes place at 90^{o}C, under 2 mm Hg vacuum, for 30 minutes, using 2.5% bentonite and 2.5% basic clay (basic bentonite or veguum). Since under these conditions a product that meets the pharmaceutical requirements for the three above-mentioned parameters, it is possible to operate the deodorization at a lower temperature and without the removal of free acids by molecular distillation. Under these conditions, an odourless oil, with a shelf life of minimum 6 months is obtained, operating the deodorization at 50^{o}C for 5-7 h, under 2 mm Hg vacuum.

Key words: *ostrich oil, bleaching, deodorization, clay, bentonite.*

Introduction

Ostrich oil was used, since antiquity, by Egyptian, African and Roman civilizations for medical treatment and cosmetics. However, in the last decades its use extended globally in cosmetic and pharma industry, for two major reasons:

a. A large development of ostrich farms, providing meat and eggs for food industry, skin for leather industry and feathers for garments and decorations, generates important by-products, including ostrich fat that is processed into ostrich oil.

b. In the last decades, the therapeutic properties of ostrich oil have been highlighted on scientific basis, during researches performed on this subject *[Attarzadeh, 2013; Lunam, 2008; Mashtoub, 2013; Gavanji, 2013; López, 1999; Snowden, 1997; Whitehouse 1998; Yoganathan, 2003; Liu, 2013]*. Investigations on ostrich oil composition led to the identification of the compounds responsible for its beneficial effects, and in some cases, of the molecular mechanism that controls such effects. Many of the beneficial effects were correlated with the

presence of omega 3, 6 and 9 in the composition of ostrich oil [*Grompone, 2005*] (Table 1).

Thus, the anti-inflammatory actions of emu and ostrich oils was correlated with the content of oleic and linoleic acid [*Lunam, 2008*; *López, 1999; Snowden, 1997; Whitehouse 1998; Yoganathan, 2003*], as well as the ability to penetrate the skin; due to high content in oleic acid and a lipid content similar to the lipids naturally occurring in skin, it can also be used as a carrier for active pharmaceutical ingredients in drugs, and also for the preparation of cosmetics [*Gavanji, 2013*; *Liu, 2013*]. For the use in cosmetics and pharma industry, certain quality standards must be fulfilled; among other requirements, some parameters define the quality and affect dramatically the stability: peroxide index, free acidity and humidity. According to American Emu Association - AEA, Oil Standards, International Standards Emu Oil, the oil must have a peroxide index < 2 mE/kg, an acidity index < 0,1 mg KOH/g and water < 0,05% to fulfill the cosmetics and pharma quality. In order to meet these parameters, certain procedures of purification are applied [*AEA Oil Standards, 1998*].

Table 1

Major Fatty Acid Composition of emu and ostrich [Grompone, 2005]

Fatty Acid Composition	**Emu**	**Ostrich**
16:0 palmitic acid	21.4	34.9
16:1 palmitoleic acid	3.6	7.4
18:0 (stearic acid)	8.1	5.7
18:1n-9 (oleic acid)	43.6	30.5
18:2n-6 (linoleic acid)	20.6	16.0
18:3n-3 (acid alpha-linolenic acid)	1.5	2.1
saturated	29.5	40.6
MUFA (monounsaturated fatty acids)	47.2	37.9
PUFA (polyunsaturated fatty acids)	22.1	18.1

The crude oil, resulting from the extraction from ostrich fat, contains several impurities, such as: free fatty acids, peroxides, pigments, proteins, metals, etc. Each group of compounds affects its properties, rendering it inadequate for food, pharmaceuticals and cosmetics. For the removal of such impurities there is a need to refine the crude oil, for which purpose various chemical or physical treatments may be used, in order to obtain a product having the desired properties for its further uses.

The process of refining consists of various physical or chemical treatments (neutralization, drying, bleaching, winterization and deodorization) which are combined in several refining designs, providing an oil of a quality that meets the designated use. Each operation aims in eliminating a group of impurities, but generally, besides the main class eliminated, other classes are also partly associated [*Yoganathan, 2003; Liu, 2013; Grompone, 2005; Aea Oil Standards, 1998; Hernandez, 1995; Palanisamy, 2011; Konishi, 2005; Shahidi, 2005; Sikorski, 2002; Meireles, 2009; O'Brien, 2009; Sathivel, 2011]*.

Two main groups of technologies are applied for oil refining: chemical refining (alkaline) and physical refining. The classes differ in the manner of removing the free fatty acid from oil.

1. **Chemical refining** have the following main steps:

– Neutralization of crude oil with alkalies. This operation aims to eliminate free fatty acids, which are the main precursors of peroxidic and carboxylic compounds that generate the unpleasant smell and are causing the rancidity of oil. This is achieved using alkali solutions, at concentrations of 10-15%, which are added in a moderate excess. The saponified fatty acids are removed by centrifugation. The product is then washed with deionized water to remove residual saponified material, while the excess water is removed in a flash drier. Another approach to neutralize free fatty acids from tri-glyceridic oils is the treatment of crude oils with sodium silicate (or other silicates) and filtration of the resulting impurities; in such processes, additional washing with water is not required [*Hernandez, 2000*].

– Bleaching. This operation aims to remove undesired coloured compounds, such as some thermally degraded compounds, polymerized materials, chlorophyll, carotenoids. Usually, clay activated with acid is used in bleaching. During bleaching, other contaminants are removed as well. If acidic activated clay is combined with other adsorbents used for bleaching, the removal of peroxides and metals is achieved.

– Winterization (Fractioned crystallization at low temperature) – During several days at low temperature, the oil separated in two layers: one reach in stearic and other saturated fatty acids, and a liquid layer, which is enriched in triglycerides derived from unsaturated fatty acids. The saturated fractions can be removed by filtration or centrifugation, and thus, an oil that remains liquid at room temperature can be obtained.

– Deodorization. This operation aims to remove the odour, peroxides and carboxylic compounds resulting during bleaching. Sometimes, this operation removes also the free fatty acids. It is achieved by injection of overheated steam (177-209ºC) under high vacuum (2-4 mmHg). Usually, this is the last step in oil refining. After this operation, some antioxidant products, like BHT or α-tocopherol are added, providing additional stability over time.

2. **Physical refining**

Physical refining represents a modern alternative to separate the crude oil from the free fatty acids, by high temperature/low pressure distillation. Physical refining is also known as deodorization or neutralization by stripping, where free fatty acids and other volatile compounds are removed by stripping at certain temperature and pressure conditions. It has several advantages over chemical processing: improved yield, improved stability, lower cost equipment, less and/or

simplified operations. However, this process does not remove some of the proteins from the oil.

In Figure 1 are presented the steps of chemical refining using sodium hydroxide followed by water washing (a), sodium silicate refining (b), and physical refining (c).

Molecular distillation

More recently, in advanced purification of ostrich oil, but also of other animal oils, the molecular distillation is combined with other refining operations. The operation is rather expensive, and is used mainly in the enrichment of fish oil in omega 3 fatty acids.

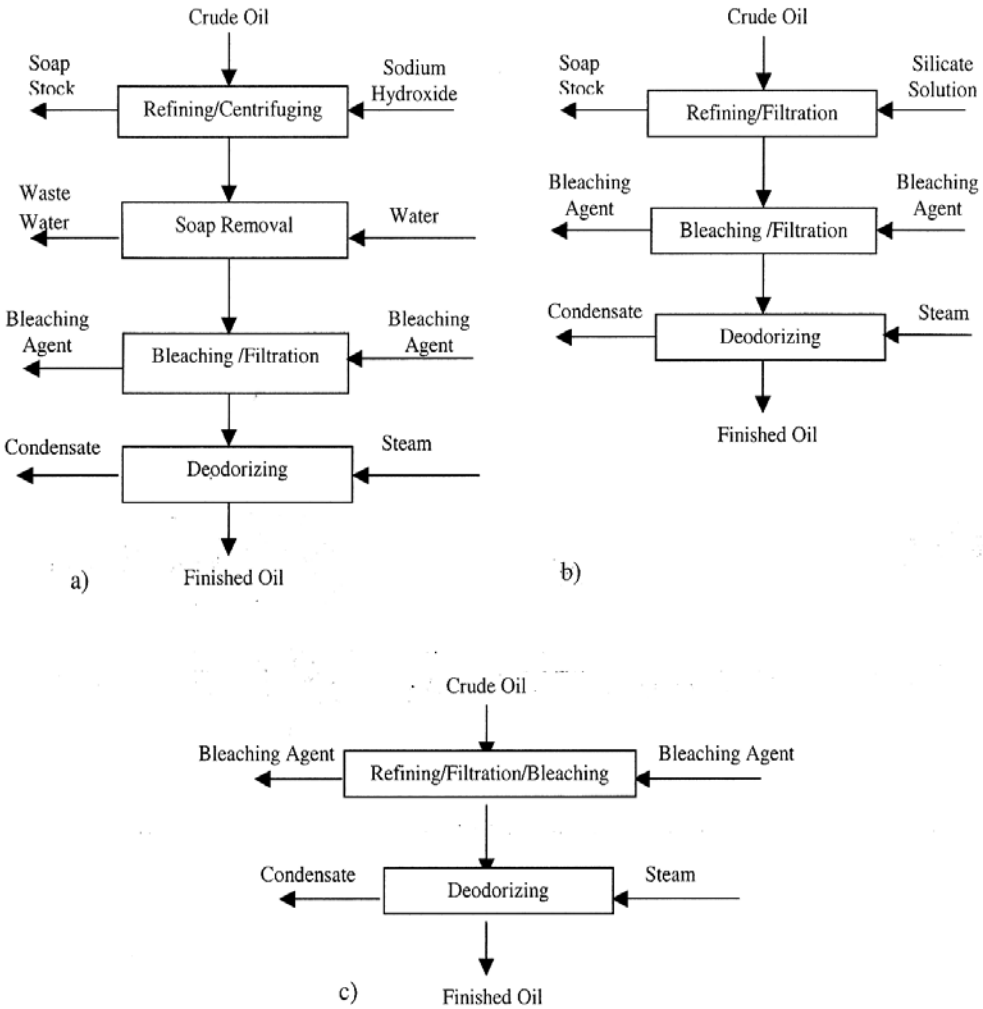

Fig. 1. *Chemical refining using sodium hydroxide followed by water washing (a), sodium silicate refining (b), and physical refining (c).*

The purpose of this paper is to prove that a pharmaceutical grade ostrich oil can be obtained not only using the stripping at 200-270º C in high vacuum and/or molecular distillation, but through a simple procedure based on undesired compound adsorption on clay, in adequate conditions of pH, pressure and temperature.

Materials and Methods

Materials

Crude ostrich oil was provided by Suraki farm and obtained through a process of wet melting. Bentonite (Sigma Aldrich, Germany), Sodium Bentonite Gurasada (Bega Minerale Industriale S.A, Romania), Veguum (Vanderbilt Minerals, USA), Sulfuric acid 95-97% (Sigma Aldrich, Germany) were used.

Methods

Drying of ostrich oil

Drying is achieved maintaining the crude oil at 90-100°C, under vacuum, in the bleaching vessel, with a condenser attached, prior the addition of clay. The operation is continue until there emission of water vapours stops.

Bleaching of ostrich oil

The process is carried out as described in the literature by treating neutralized oil, heated at three different temperatures: 25°C, 90-100°C, 150°C, with an absorbing clay, bentonite, sodium bentonite, acid activated bentonite, under vacuum or protected by nitrogen. The ostrich oil (100 mL) was poured in a 250 mL round bottom reaction flask with 3 necks and heated at chosen temperature, under magnetic stirring. After water vapours stops, bleaching clay is added and the temperature is maintained for different period of time, according to Table 3. Finally, the oil is filtered on a Buchner funnel, using yellow band filter paper. For experiments were used different types and quantities of clay, temperature values, contact time, as well as crude ostrich oil with different characteristics.

Deodorizing the ostrich oil

To eliminate the odor, the ostrich oil was maintained at a low pressure, 2 mmHg, at three different temperatures: 50°C, 100°C, 120°, on different times, in a rotaevaporator or in a special bleaching installation.

Activation of bentonite

The bentonite clay was activated according to the following method: 25% H_2SO_4 solution is prepared from 81 mL H_2SO_4 95,9 % and 425 mL distilled water. 25 g bentonite are added to this solution and the mixture was refluxed for 3 hours and then it was repeatedly washed with distilled water, to obtain a pH value of 6-7. The resulted suspension was filtered, dried at 105-110^{0}C and preserved from

moisture in a desiccator. The bentonite clay activated according to this method was used in the following deodorizing studies.

Peroxide index was determined according to SR EN ISO 3960/2010.

Acidity index was determined according to SR EN ISO 660/2009.

Water and volatile substances were determined according to SR EN ISO 662/2007.

Results and Discussions

Bleaching of ostrich oil

According to the literature data, the ostrich oil bleaching process can be achieved in many ways. Different clays and work conditions are used: low pressure, nitrogen atmosphere, or normal pressure, as well as different work parameters: temperature, clay/oil ratio, time of contact between oil and clay. Depending on the work conditions, different authors are proposing different optimum parameters for an efficient bleaching. During our experimental studies, we developed some variants of this process by modifying the work conditions and parameters, in order to obtain a colourless ostrich oil, with adequate characteristics as peroxide index, acidity index and water content. Several raw materials batches were used (crude ostrich oil) with characteristics displayed in Table 2.

Table 2

Characteristics of the raw materials

Raw material	Characteristics			Observations
	PI* (mE/kg)	AI*(mg KOH/g)	Water (%)	
MP1	19.6	0.50	1.53	old raw material
MP2	8.51	1.07	-	
MP3	6.60	0.65	0.65	
MP4	14.0	0.41	-	improperly processed raw material

*PI - peroxide index
AI - acidity index

In a first series of experiments, the influence of different clays on peroxide index was assayed by using different work parameters and the obtained results are presented in the Table 3.

Table 3

The influence of the clay and of work parameters on discolored oil characteristics

Sample/ Raw material	Purification parameters	Colourless oil characteristics		Observations
		PI (mE/kg)	AI (mg KOH/g)	
P5 / 100 ml MP2 oil	90°C, N_2,1h 5g acid activated bentonite	2.0	1.1	straw yellow
P6 / 100 ml MP2 oil	90°C, N_2, 1h 5g bentonite	5.3	0.5	yellow
P7 / 100 ml MP2 oil	25°C, 12h 5g acid activated bentonite	4.6	1.23	light yellow
P8 / 100 ml MP2 oil	25°C, N_2, 12h 5g bentonite	9.84	0.45	yellow
P 9 / 100 ml MP2 oil	150°C, N_2,1h 5g acid activated bentonite	0	13.28	almost colorless
P10 / 100 ml MP2 oil	150°C, N_2,1h 5g bentonite	2.9	0.65	yellow
P11 /100 ml MP2 oil	150°C, N_2,1h 5g sodium bentonite	6.1	0.6	yellow

According to the data in Table 3, it can be seen that by using bentonite and alkaline bentonite, no decrease of peroxide index can be obtained, and the acidity remains constant or decreases. Acid activated bentonite produced a significant decrease of peroxide index; the higher the temperature was, the bigger the decrease was. In the same time, it can be observed that the acidity index increases parallel with the temperature increase. It was selected as optimum, the temperature of 90-100°C, because at this temperature the peroxide index has a corresponding value (< *2* mE/kg); meanwhile the acidity index increased slightly. Also it has been observed that in the presence of acid activated bentonite it has been produced a strong bleaching of the oil comparatively with using sodium bentonite and bentonite, when the bleaching was insignificant. Note that the higher temperature was used, more intense was the bleaching.

Another experiment was conducted in order to hinder the free acidity increase during the bleaching process, by adding alkaline bentonite in the ostrich oil. The results of these experiments are presented in Table 4.

Data from Table 4 show that using together an acid bentonite and an alkaline one or vegum, the initial oil acidity is kept and it was obtained an advanced bleaching of the oil by using two clays in equal amounts (at least 2% over the amount of oil). It means that if the crude ostrich oil is neutralized and it has an acidity index in accordance with the quality requirements for pharmaceuticals or cosmetics, the bleached oil could also maintain this good quality with respect to

this parameter. It is recommended a bleaching in vacuum, because it is achieved also an additional removal of water. If the raw material used was old, or crude oil was obtained by processing improperly the fat, it can not be obtain a proper oil even though is increased bleaching time or clay quantity.

Deodorizing the ostrich oil

In case that the ostrich oil resulted from the bleaching stage accomplish a pharmaceutical quality with respect to acidity index, acidity index and water content, we assumed that the deodorizing stage could be performed in mild conditions, due to the fact that it is not necessary to remove free fat acids or peroxides. The literature describes a variant of removing the odor in vacuum, during 24 hours, at room temperature. That is why, in a new series of experiments, we tried deodorizing at low pressure, varying temperature and time. The results of these tests are presented in Table 5.

Table 4

The influence of clay and work parameters on colourless oil characteristics

Sample/ Raw material	Purification parameters	Colourless oil characteristics			Observations
		PI (mE/kg)	AI (mg KOH/g)	Water (%)	
P 12 / 100 ml MP3 oil	90-100°C, N_2, 1h, 5g sodium bentonite 5g acid activated bentonite	0.99	0.43	0.44	pale yellow
P13 / 100 ml MP3 oil	90-100°C, N_2,1h 2.5g sodium bentonite 2.5g acid activated bentonite	0.99	0.43	0.3	pale yellow
P14 / 100 ml MP3 oil	90-100°C, N_2,30min 2.5g sodium bentonite 2.5g acid activated bentonite	0.99	0.43	0.24	pale yellow
P15 / 100 ml MP3 oil	90-100°C, N_2 2.5g veegum 2.5g acid activated bentonite	1.57	0.35	0.47	straw yellow
P16 / 100 ml MP3 oil neutralized	90-100°C, vacuum 2.5g sodium bentonite 2.5g acid activated bentonite	1.3	0.10	0.03	straw yellow
P18 / 100 ml MP1 oil	90-100°C, vacuum 2.5g sodium bentonite 2.5g acid activated bentonite	3.66	0.15	0.05	light yellow
P 19 / 100 ml MP4 oil	90-100°C, vacuum 2.5g sodium bentonite 2.5g acid activated bentonite	9.6	0.12	0.05	light yellow

Although the corresponding results were obtained in all three work versions, it was chosen as the optimum solution the deodorizing performed at 50ºC, under vacuum of 2mm Hg, during 5-7 hours, because deodorized samples showed the best stability. After deodorization was added an antioxidant compound, tocoferol, in a ratio of 0,1% and an odorless oil with 6 months stability was obtained.

Table 5

The influence of deodorization parameters on purified oil characteristics

Sam-ple	Purifica-tion parame-ters	Colorless oil characteristics			Odorless oil characteristics			Observa-tions
		PI (mE/ kg)	AI (mg KOH/ g)	Wate r (%)	PI (mE/k g)	AI (mg KOH/ g)	Wat er (%)	
P16.1	50°C, la rotavapor, vid 2mm Hg, 5h	1.3	0.10	0.03	1.28	0.10	0.03	Straw yellow (lighter after deodorizati on), odorless
P16.2	100°C, vid 2mm Hg, 1h	1.3	0.10	0.05	3.43	0.12	0.03	Unchange d color odorless
P16.3	120°C, vid 2mm Hg, 1h	1.3	0.10	0.03	6.5	0.12	0.03	Unchange d color odorless
P20	50°C, la rotavapor, vid 2mm Hg, 5h	0.68	0.05	0.07	1.24	0.07	0.05	Unchange d color odorless

Conclusions

It is possible to obtain an ostrich oil adequate for pharmaceutical or cosmetic purposes, without using purification methods as stripping at 200-270ºC and advanced vacuum or molecular distillation, only if some work parameters are strictly maintained during the oil extraction from the fat and other purification stages:

1. Ostrich fat and oil should be fresh at the beginning of their processing, and in any case rancid raw materials should not be used (ostrich fat or crude oil). The literature indicates that fat should be refrigerated as soon as possible, within a maximum of 2 hours after removal from the carcass. If the fat is to be processed more than 6 hours after removal from the carcass, it should be frozen as soon as possible. Fat to be kept longer than 10 days should be stored at -29°C [*AEA Oil*

Standards, 1998]. In the same time, during the extraction, the fat should not be exposed long time to air or light;

2. After neutralization, the oil acidity has to be below 0,1 g KOH/g;

3. To obtain a quality oil for pharmaceutical industry the bleaching process must proceed in the following conditions:

– Acid activated bentonite must be combined with sodium bentonite in equal amounts (at least 2% over the amount of oil). First is added acid activated bentonite and after 15 min is added the alkaline bentonite;

– Bleaching should be performed at 90-100°C for 30 minutes, under vacuum of 2mm Hg.

4. Two types of bentonite: acid and alkaline have to be used during the bleaching stage, in order to maintain the free acidity in admissible limits. When the colorless oil has admissible values of acidity and peroxide indexes as well as of water content for a pharmaceutical use, the oil deodorizing can be done in mild conditions, meaning 50ºC, 2mm Hg, 5-7 hours. Thus, an odorless oil is obtained, with stable properties during at least six months, if at the end of deodorizing stage 0,05% tocoferol is added, as an antioxidant.

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