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## **PHYSICAL AND CHEMICAL PARAMETERS –COMPARATIVE CRITERIA OF COW’S AND GOAT’S MILK QUALITY ASSESSMENT**

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**Key words:** physical and chemical parameters, cow’s milk, goat's milk

### **SUMMARY**

Milk physical and chemical properties are closely related to its composition for certain animal species. In this paper, researches were conducted to compare the physical and chemical parameters of milk samples taken from cows and goats. Milk samples were collected from different areas near Bucharest and analyzed for various physical and chemical parameters such as: fat, non-fat dry substances, density, protein, titratable acidity, temperature, lactose, pH and ash. Cow milk recorded 4,33% fat, 1,029 density, 3,48% protein, 4,56% lactose, 6,66 pH, titratable acidity 0,17%, 12,78% total solids substances and 0,64% ash. Goat milk recorded 3,90% fat, 1,029 density, 2,85% protein, 4,32% lactose, 6,51 pH, titratable acidity 0,16%, 12,98% total solids substances and 0,82% ash.

Milk is a specific secretion of the mammary glands of all female mammals. Due to its function - food for Youth - is a complete and complex product with high nutritional value. The only food in the first period of life, it contains all substances necessary for life and development. It covers the utmost human needs in food of animal origin. Its composition provides the majority of substances needed to build living tissues and maintenance of metabolic processes that occur in the body. Milk also contains antibodies that protect young infants against infections.

Cow's milk has been and still is considered a highly nutritious and valuable food that is consumed daily in a wide variety of products. Goats play a special role in the lives of small farmers. Goat was referred to as cow replacement for poor men who couldn't afford to maintain a cow.

Goat milk is different from cow's by its superior structural and functional features, including better digestibility.

The purpose of this study was to assess the quality of milk by comparing the physical and chemical parameters of milk samples collected from cattle and goats.

## 1. MATERIAL AND METHODS

**Sampling:** there were collected 30 fresh milk samples from both cows and goats, in sterile plastic containers. After identification, samples collected were sent refrigerated to Bucharest Faculty of Veterinary Medicine, Department of Food and Animal Products Quality Control for laboratory tests.

**Physical and chemical analysis:** the establishing of physical and chemical parameters was performed using the Ekomilk Total device, an automated multi-parameter milk analyzer providing rapid test results for: Fat, Protein, Solids Not Fat, Lactose, Density, pH, Temperature and Conductivity in fresh milk (cow, sheep and/or buffalo, goat) for 40 seconds, based on ultrasonic technology. RS-232 interface, thermal micro printer and automatic data capture are integrated.

Measurements with Ekomilk device were performed after its calibration, in parallel with standard methods described by AOAC (2000), confirming the accuracy of information provided by the manufacturer of the device.

Titration acidity was assessed by titration method and ash content was assessed by gravimetric method using a muffle furnace at 550°C, both methods described by AOAC (2000).

## 2. RESULTS AND DISCUSSIONS

**Fat** in milk samples collected from cows and goats is presented in Table 1. The results illustrated that the fat content was between 3,45 and 5,21% for cow milk and between 3,18 and 4,62% for goat milk. The average value of the fat content in cow's milk was higher than that in goat milk.

*Table 1*

Fat content in milk samples collected from cow and goat

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	3,45	5,21	4,33
Goat	3,18	4,62	3,90

**Density:** Density values in samples collected and analyzed from cows and goats are shown in Table 2. Density ranged between 1,026 and

1,033 for cow milk and between 1,027 and 1,032 for goat milk. Density values for cow milk and goat milk were extremely close.

*Table 2*

Density of milk samples collected from cow and goat

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	1,026	1,033	1,029
Goat	1,027	1,032	1,029

**Protein:** Protein content of milk samples collected from cows and goats is presented in Table 3. According to these results, the protein content ranged between 3,23 and 3,74% in cows milk and between 2,31 and 3,40% in goats milk. The amount of protein content of cows' milk was higher than that of goats' milk.

*Table 3*

The protein content of milk samples collected from cow and goat

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	3,23	3,74	3,48
Goat	2,31	3,40	2,85

**Lactose:** Lactose content of milk samples collected from cows and goats is presented in Table 4. The results show that the lactose content between 4,00 to 5,12% in cows' milk and from 3,72 to 4,75% in goats' milk. The amount of lactose content of cows' milk was higher than in goats' milk samples.

*Table 4*

Lactose content of milk samples collected from cow and goat

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	4,00	5,12	4,56
Goat	3,72	4,75	4,32

**pH:** pH values of milk samples for the two species are presented in Table 5. The results showed that pH values range within 6,60 - 6,73 for

cows and within 6,42 - 6,61 for goat. pH values in cows' milk were significantly higher than those in goats'.

Table 5

pH values of milk samples collected from cows and goats

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	6,60	6,73	6,66
Goat	6,42	6,61	6,51

**Titratable acidity:** Titratable acidity values in milk samples collected from cows and goats are presented in Table 6. As can be seen, titratable acidity values were within 0,15 - 0,19% for cows and within 0,14 - 0,18% for goats. Titratable acidity values were close for both cows and goats milk samples. Still, higher values were recorded in cows.

Table 6

Titratable acidity in milk samples collected from cows and goats

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	0,15	0,19	0,17
Goat	0,14	0,18	0,16

**Total solids:** TSS concentration in milk samples collected from cows and goats is presented in Table 7. These results have shown that the total solids concentration was from 11,26 to 14,31% for cow and from 12,11 to 13,85% for goat. Goats milk samples recorded slowly higher values for this parameter.

Table 7

TSS concentration in milk samples collected from cow and goat

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	11,26	14,31	12,78
Goat	12,11	13,85	12,98

**Ash** samples values collected from cow milk and goat milk are presented in Table 8. The results of this study revealed that the ash content ranged within 0,43 - 0,85% for cows and within 0,66 - 0,98% for goats milk.

The average value of ash content in cows' milk was lower than that in goats' milk.

Table 8

Ash samples collected from cow milk and goat

<b>Milk</b>	<b>Min. value</b>	<b>Max. value</b>	<b>Average</b>
Cow	0,43	0,85	0,64
Goat	0,66	0,98	0,82

### 3. CONCLUSIONS

All parameters tested were within the limits set by law. Cow's milk showed higher values than goat milk for the following parameters: fat, protein, lactose, pH, titratable acidity. Goat milk showed higher values for the parameters: total dry matter and ash. For density, the average values were similar in samples collected from both species.

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## **STUDY CONCERNING SOMATIC CELLS ASSESSMENT IN GOAT MILK**

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**Key words:** physical and chemical parameters, goat milk

### **SUMMARY**

Milk, the mammary gland secretion, is a complete food, ideal for infants during the first months of life and recommended for children and adults regardless of age, because it contains almost all the substances necessary to both human and animal body in a easily assimilating way.

The number of somatic cells in milk is currently used in all countries with advanced animal husbandry, not only to measure the pollution of milk with germs, but also to get quick information about breast health degree from a goats farm, in order to take urgent measures for prevention and treatment of subclinical mastitis (which can not be observed).

To determine the quality of milk depending on the number of somatic cells is necessary to identify the factors determining the variation of this parameter over a year.

Goat milk is considered superior due to nutritional effects, tonic and anti-rickety, antianaemic and anti-infectives. It taste and smells pleasant, if maintenance and milking hygiene and proper feeding, especially in terms of feed varieties.

### **1. MATERIALS AND METHODS**

The study was conducted in a private goats farm located in Southern Romani. This study was conducted over a period of 10 days in 2011. 10 goats were selected for analysis from which there were collected 10 samples from each udder half, a total of 200 samples.

Goat milk somatic cell count was assessed using BactoCount IBCm device.

## 2. RESULTS AND DISCUSSIONS

The results of measurements of goat milk SCC is shown in Table 1.

Table 1

SCC measurements values of goat milk samples

Goat no.	SCC min. $\times 10^3$	SCC max. $\times 10^3$	Average SCC det. $\times 10^3$
1	876	914	895
2	1244	1286	1265
3	1806	1842	1824
4	1589	1781	1685
5	744	778	761
6	1031	1105	1068
7	879	953	916
8	855	871	863
9	1067	1229	1148
10	1244	1470	1357

The analysis of average values show that milk from the goats did not range within the limits allowed in our country (1000000 cells / ml) for 60% of specimens studied. Of the 200 samples collected and analyzed, 40% are fit for human consumption due to the corresponding number of somatic cells, the rest being inadequate in terms of hygiene.

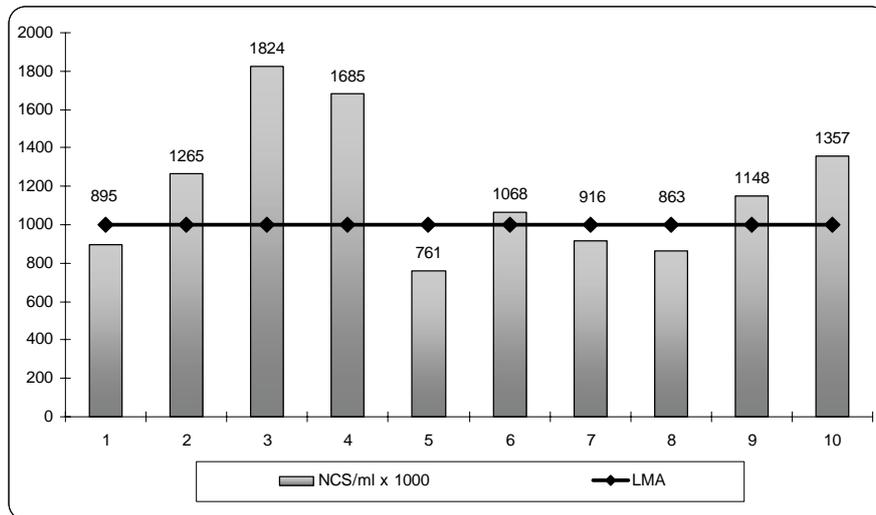


Fig. 1 - The variation of CSS values to maximum

The large number of cells collected from milk has an atypical dynamic, due to the animal rearing technology which has many deficiencies, especially because the milking hygiene is not respected or animal health is not enough tested. This increase in somatic cells is due to the poor health of the mammary gland, and to the poor milking of old animals.

### **3. CONCLUSIONS**

3.1. Among the factors identified in this study that could influence elevated somatic cell count in goat milk, milking incomplete can be remembered; animal's agedness and breast rest, knowing that the somatic cell count values increase before and after breast rest period.

3.2. Milk somatic cell counts showed variations depending on the physiological state of the mammary gland and from one harvest to another. In young animals, the somatic cell count was lower, which increases progressively with age and number of lactations. Increasing the number of somatic cells in milk over the admitted values is abnormal and indicates a progressive inflammatory process in the mammary gland or a poor milking hygiene. This increase in somatic cell count level reaches maximum values at the beginning of the inflammatory process and is gradually decreasing, the number of cells is increased after healing over several days and even weeks.

3.3. In the milk from the goats clinically healthy, at the smear examination, was observed an increased number of neutrophils (50-60% of SCC), which causes infectious mastitis to evolve rarely in goats.

3.4. The increased number of somatic cells in goat milk may be due to the poor hygiene and poor milking technology (hand-incomplete) rather than to a pathological process with the election area in goat mammary gland.

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## **ANIMAL RIGHTS AND THEIR WELFARE IN CONCEPT, LAWS, ACTIONS**

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Key words: animal rights, welfare, laws.

### **SUMMARY**

Pets are comfortable in the situation of homeostasis; the physiological and metabolic parameters are within normal limits. When they feel well-being. Well is a psychological phenomenon difficult to measure and appreciated. The term wellbeing is wellbeing (in English welfare, in other European languages: bien-être (French), bienestar (Spanish), benessere (Italian), etc., which can be appreciated by physical and metabolic parameters of the body.

Approach "modern" science of animal welfare began in the mid 1960s. The first reaction against intensive farming systems was generated by the book published in 1964 by Ruth Harrison "Animal Machines". Between 1960 and 1970 established that the term "welfare" would be closely related to physiological stress response in animals. Later, between 1980 and 1990, greater emphasis was placed on animal feelings, as a welfare measure.

People's interest and welfare rights can be in: humanitarian considerations (animal protection organizations), health reasons (veterinary medical structure), economic reasons (political bodies - administratively).

Pets are comfortable in the situation of homeostasis; the physiological and metabolic parameters are within normal limits. When they feel well-being. Well is a psychological phenomenon difficult to measure and appreciated. The term wellbeing is wellbeing (in English welfare, in other European languages: bien-être (French), bienestar (Spanish), benessere (Italian), etc., which can be appreciated by physical and metabolic parameters of the body (Simona Stan).

People's interest and welfare rights can be in: humanitarian considerations (animal protection organization-target animal protection organizations are that animals feel good), health reasons (veterinary medical structures - the animals do not feel well if sick. Health Assessment is

through physiological and metabolic parameters bodies. These are indicators for animal welfare organizations), economic reasons (political bodies administratively - to changes in living environment, to maintain good physical and mental bodies are efforts to accommodate demand, energy conversion efficiency that reduce energy intake in the product).

Approach "modern" science of animal welfare began in the mid 1960s. The first reaction against intensive farming systems was generated by the book published in 1964 by Ruth Harrison "Animal Machines" (Simona Stan). Between 1960 and 1970 established that the term "welfare" would be closely related to the animal's response to physiological stress. Subsequently, between 1980 and 1990, greater emphasis was placed on animal feelings, as a welfare measure.

Animal welfare expression was chosen to describe animal welfare. The concept of "welfare" still lacks a precise definition, but all the experts who have studied animal welfare agree that this notion includes health, comfort and productivity, the extent:

- Hunger and thirst. Without food or water or animals become agitated, some even aggressive. Most have call tones. It is desirable that the animals free and permanent access to water and food. Water should be fresh and diet quality and quantity to be satisfactory.

- Fear. All animals were afraid. Fear creates stress, mental anxiety.

- Pain caused by wounds or bruises.

- Anxiety caused by disability, a new environment.

Animal welfare is the health of defense reaction of organisms that exteriorizează by diseases (infectious and parasitic diseases in common reactions are fever, tachycardia, polipnee, numerical changes of figurative elements of the blood and symptoms, the nutritional diseases bloating, diarrhea, polyurie, mucosal color change, if tehnopaty noticeable symptoms are lameness, changes aplomb, Behind separation, vaginal or uterine prolapse, mastitis). Avoid sick animals are through: prevention sanitary - veterinary animals in loose housing technology, preserving fodder in good condition.

Lately, the focus is on how the animal is treated in its environment, as well as environmental protection, the impacts of animal welfare rules on the production of food of animal origin. The most important reasons for buying organic products are quality and health safety.

In the European Union since 1980, the Eurogroup for Animal Welfare was established as a civic organization, international observer to the Council of Europe. Eurogroup is open to all organizations, national and regional animal protection. The role of the Eurogroup is receiving, processing and transmitting public pressure animal welfare organizations, local to the European Commission, European Parliament, the Council of Ministers and the Council of Europe, in order to improve legislation and compliance monitoring. Eurogroup mission is supported by the Intergroup Animal Welfare and Conservation (Intergroup on the Welfare and Conservation of Animals), which was established in the European Parliament (1983) and brings together people with different political orientations, but are interested states to support legislative initiatives welfare and animal protection.

In addition to EU bodies, the issue of animal welfare is concerned and the Council of Europe, which already passed six international conventions on animal welfare: protection of animals during international transport, protection of farm animals, protection of animals for slaughter, protection of vertebrate animals used for experimental pet protection and recreation, conservation of wildlife and natural habitats.

Between EU bodies directly involved in animal welfare highlight the European Food Safety Authority (EFSA).E.F.S.A. provide independent scientific advice on all matters which have direct or indirect impact on food safety - including animal health and welfare and plant protection. E.F.S.A. is also consulted on nutrition issues in relation to Community legislation. Communicate with the public authority in an open and transparent on all matters within its competence.

Risk assessments carried out by E.F.S.A. provide institutions managing risks (EU institutions with political accountability, i.e. European Commission, European Parliament and Council) a sound scientific basis for defining legislative or regulatory measures based on policy guidelines, to ensure a high level of consumer protection on food safety. Collection and analysis of scientific data, identify emerging risks and scientific support for the Commission, especially in food crises, are all of the powers EFSA as provided in Regulation of setting (EC) no.178 of 28 January 2002.

In the EU, livestock is known for its high standards in animal welfare. Community legislation on animal welfare in the EU has a long tradition, and the first Community legislation was adopted in 1974. Current Community legislation on animal welfare contains minimum standards to be met by all manufacturers. When applying higher standards in animal welfare, manufacturers must find ways to obtain a price for their product corresponding to the added value it gives to compensate for products and investments. Optional communication on product features animal welfare is another complementary way to stimulate consumer interest.

European legislation can be considered as a useful tool to ensure minimum standards of animal welfare.

European Parliament and Council Regulation (EC) nr.882/2004 on official controls performed to ensure verification of compliance with feed and food law, animal health and welfare and lays down general rules for the conduct of official controls to verify compliance with standards aimed at both preventing, eliminating or reducing to acceptable levels of risk that may arise for people and animals, either directly or through the environment and ensure fair practices in trade with feed and food and protect the interests consumers, including labeling of feed and food products and other forms of consumer information.

Debates on European consumers with information on animal welfare in animal production is very lively in the EU in recent years, at least since the adoption in 2002 of the Community report entitled "Legislation on farmed animals Animal welfare in Third Countries and the Implications for the EU" (farm animal welfare legislation applicable in third countries and its implications for the EU).

Conference on "Animal Welfare - Improving by Labeling?" (Brussels, 03/28/2007), organized by the Economic and Social Committee, European Commission and German Presidency of the Council, allowed the development of an initial discussions with representatives of all stakeholder groups. Following this conference, the Council of Ministers adopted in May 2007 conclusions on animal welfare labeling, inviting the Commission to submit a report to enable a thorough debate on this topic.

The first Community Action Plan for the Welfare of Animals 2006-2010 defined the meaning of Community policies and related activities planned for the coming years in order to continue to promote high standards

of animal welfare in the EU and internationally, taking into account the commercial opportunities offered by manufacturers of animal welfare, while respecting the ethical and cultural dimension of the issue.

Establishing „platform specific information on animal welfare", in the framework of the EU Seventh Framework Programme, designed to promote dialogue and exchange of experiences between stakeholders is a key element of implementing the action plan.

Cattle production occupies an important place in Community agriculture, and satisfactory results depend to a large extent on the use of pure-bred breeding animals.

An example is given of recent changes to European Union Council Directive 2008/119/CE for protection of calves which sets minimum standards for the protection of calves confined for rearing and fattening, given that most Member States have ratified the Convention European animal welfare on farms and also approved this Convention by the Community Council Decision 78/923/EEC of the European Union.

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## **TRACEABILITY IMPORTANCE ON THE TECHNOLOGICAL FLOW FOR OBTAINING SOME MEAT PRODUCTS**

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**Keywords:** traceability, meat products, alimentary chain, technological flow, batching.

### **SUMMARY:**

**Purpose of the work** —The work brings to the fore the checking methods for the traceability of meat products on the manufacturing technological flow.

**Working methods** —The taken-over raw materials and auxiliary materials are stored on entering lots, each lot being identified with a label specifying the identification data of the product. During fabrication, the product's identification and traceability are provided by allotting a number to each product batch and by recording it in all records drafted during fabrication, and finally, parceling is done by labeling with labels where the fabrication batch is to be specified.

**Results** —Demonstratively, I have taken a labeled product to be checked, I have checked the correctness of the batch, I have looked for its launch in the documentation and subsequently, I have checked on flow each stage in the technological process, specific for the product, and the identification data in the traceability charts, the specific documents of the product and the taking-over charts and I have recorded them in the Form "Traceability check".

**Conclusions** —As traceability represents a requirement for products' quality certification, the Company makes much of the products' traceability and permanently checks the correct and in real time drafting of the forms providing traceability. The implemented traceability system keeps all records and shows the way covered by a product or by an ingredient from the supplier, along the technological up to the end consumer;

### **INTRODUCTION**

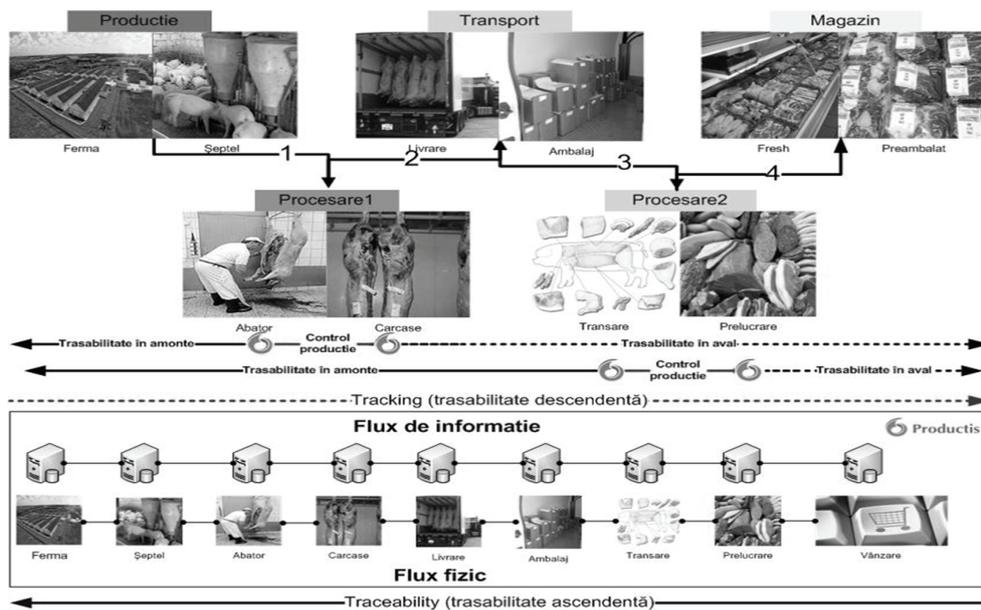
Meat and meat products' traceability is achieved by interlinking the information about the raw material (meat) and about the secondary ones during the whole production process. Traceability may be obtained during the production flow in the meat industry depending on the recipe and technology, by following some well-defined steps. The documentation of the steps and the processing of information in meat products' traceability are

to be done downstream: from the client up to the raw material and auxiliary materials lots it has been manufactured from.

By meat products traceability there are managed and monitored data having informative value with regard to the materials' origin, processing history and delivery to the selling points. The meat processing enterprises are involved in a chain network (livestock, slaughtering, meat transport, trading), with common interconnecting points. In order to obtain an upstream and downstream traceability on the principle "one step forward and one step downward", it is necessary the meat line permanently communicate with the line of the animals that were sacrificed to obtain the meat, and the enterprises for cold meats processing and manufacturing to record the destinations the products' batches have been delivered to.

An information flow results from the recording of the information adherent to the physical flow carried out in the production process within the meat industry.

### MEAT AND MEAT PRODUCTS TRACEABILITY



### *Definitions regarding traceability*

Traceability is defined in Standard ISO 9000:2006 as being the capacity of locating and following up a food, fodder, an animal producing food or a substance intended or wanted to be incorporated in food or fodder, through all production, processing and distribution stages.

According to Law no. 150/2004 regarding aliments' safety and of food for animals safety, updated in the year 2010, denominated "the general law of food" traceability represents the possibility of identifying and following-up, during the all producing, processing and distribution stages of a food, of food for animals, of an animal intended for food production or for a substance that is to be incorporated or that may be incorporated in food or in the food for animals.

According to CAC 60-2006 (CAC = codex alimentarius commission, Principles for traceability product tracing as a tool within a food inspection and certification) by traceability it is understood the capacity of following-up the movement of an alimentary product in different production specific stages, processing and distribution. (Banu C., 2007).

### *Purpose and importance of the work*

The work is within the thematic concerns of current food safety, bringing to the fore the methods for checking the meat products' traceability from the end product up to the used raw and auxiliary materials.

The research objectives consisted in the following:

- Presenting an own traceability system in a meat processing unit;
- Drafting a traceability;
- Demonstrating the fact that the implemented traceability system in the processing unit the survey took place, functions.

### *Traceability requirements*

In article no. 18 of EC Regulation 178/2002 of the European Parliament and of the Council, known as "The general law of food" partially transposed by Law no. 150/2004 regarding foods safety and of food for animals, revised and with the subsequent alterations, it is clearly mentioned traceability's requirements, as follows:

- The traceability of an alimentary product, of food for animals, of animals from which alimentary products are obtained and any other substance that is intended or expected to be incorporated in an alimentary product or in the food for animals it is set up for all the stages of production, processing and distribution;
- The operators in the alimentary sector and the operators with activity in the field of food for animals must identify any person from whom the procurement with aliments, fodders, food producing animals and any substance to be incorporated in food or fodders has been done and, must also have systems/procedures that will allow the information be provided to the authorities requiring them;
- Business operators of food and fodder must have systems/procedures for the identification of other operators to whom the said products have been delivered. The information will be offered to the competent authorities, by request;
- Foods/fodders that are placed on the market or that are to be placed on the market within the European Union will be adequately identified in order to facilitate traceability by labeling, documentation or adequate information.

## 1. MATERIAL AND METHODS

### *Identification and traceability of alimentary products of animal origin*

Each manufacturer must provide the identification and traceability of products during their achievement through specific means for each type of product. In order to provide products' traceability, the manufacturer allots and keeps under control a sole identification for each batch of product. Products' identification is done by labeling and/or recordings.

The quality records through which there are provided the identification and traceability of the achieved products are found in the general, operational procedures and in the working instructions. The procedure founds the practice regarding the identification and traceability of raw materials, spices, auxiliary materials and semi-products, through which there are provided the identification and traceability of end products by specific means, during their processing and until their delivery to the beneficiary, thus, for each taken-over product batch it is allotted and kept

under control a sole identification, and for the end products it is allotted a sole identification (batching) during the whole fabrication process. Products' identification is done by labeling and records. Quality records through which identification and traceability of the achieved products are provided must be identified in the operational procedures and in the work instructions.

Thus, *raw materials and auxiliary materials* are accompanied during storage and up to their introduction in manufacturing by quality records from the producer (for ex.: Certificates of Public Veterinary Health, CMR, Quality Certificates, Conformity Declarations, etc. depending on each product) and by quality records drafted by the department in the unit that is responsible for the quality technical control (for ex.: NIR, taking-over files for spices or raw materials, taking-over charts for raw materials, auxiliary raw materials and spices, other taking-over documents). Raw materials and auxiliary materials are stored on entry lots, each lot being identified with a control label on which there are specified the identification data of the product: intern number, the denomination, taking-over date, manufacturing date, expiry date, but, also, the number of the lot given by the supplier (to provide traceability up to the lot given by the initial manufacturer).

Also, during manufacturing, the *product's* identification and traceability are provided by allotting a number to each batch of products and by entering it in all records drafted during manufacturing and in the end (examples of traceability forms: launching charts, charts for compositions preparation, chart for preparing spices batches or semi-product, production-manufacturing reports, labeling report, batching labels attached on the frames with semi-product).

In the end, the products are identified by labeling with labels where the manufacturing batch is specified. For the achieved products traceability given the order/contract is achieved by records (advice of delivery or the Declaration of conformity where there are specified the delivered end products batches). In addition, when analyzing the end products, these are recorded on batches in the evidence file of samplings and counter-samples, the sampling Minute and in the analysis bulletins, they are identified based on batches.

### *Program for traceability check*

For demonstrating and checking the way the records are done during the manufacturing process, traceability is checked as follows: a labeled product for being checked is taken from the end products storehouse or from the site (from the beneficiaries). After checking the correctitude of the batch, its launch is looked for in the documentation and, subsequently, it is checked by flow each stage in the technological process specific for the said product, and the data identified in the traceability charts, the documents specific for the product and the taking-over charts are recorded in a "Traceability check" form.

This is drafted with columns for recording the documents where there are recorded the data for each stage in the technological flow, and columns for the registration numbers, the dates of the documents and batches' correspondence. It concisely contains the data from the following coded documents:

- The Declaration of Conformity where the chosen product for traceability is found;
- Records of storing the end products packed in a controlled atmosphere (where necessary);
- Packing in controlled atmosphere report;
- Report of boiling-smoking production;
- Chart of prepared filling (if applicable);
- Charts for compositions' preparation;
- Injecting chart (if applicable, depending on the product's type);
- Chart for preparing the spices and additives batches;
- Launching;

Traceability is deemed to be finished when, starting from the end product label, and going on the technological flow, from stage to stage, we arrive to identify the raw material batches, both the ones internally allotted at the respective raw materials taking-over, and to the raw materials' batches from the supplier, to the identification of the spices batches and auxiliary materials used to manufacture the product chosen for traceability check and, implicitly, the check of the taking-over documents.

## 2. RESULTS AND DISCUSSIONS

In order to exemplify the issues mentioned in chapter Material and method, there has been drafted the traceability of a product.

*Table no. 1*

### TRACEABILITY CHECK FOR RUSTIC BACON BATCH 12/14.09.2011

Batch	Document	No./Date	Product	Batch
12	Declaration of Conformity, Code S-01-F-08-02	217/13.09.2011 218/14.09.2011	Rustic bacon	12
	Boiling-smoking report S-01-F-06-01	5225/12.09.2011	Rustic bacon	12
	Injecting chart S-01-F-05-04	12.09.2011	Rustic bacon	12
	Reception chart for refrigerated/frozen raw materials S-01-F-01-03b	08.08.2011	Pork belly with bone	MPC 221
	Register for raw materials of animal origin that are object of the inter-communitary exchanges taking-over	126/08.08.2011	Pork belly with bone	MPC 221
	Notification regarding the arriving of transports coming from the member states	534/08.08.2011	Pork belly with bone	MPC 221
	Accompanying documents: CMR, Declaration of Conformity/delivery bill, invoice	369764/05.08.2011 1211/1/05.08.2011 2000510/05.08.2011	CMR, Certificate of Conformity, Fiscal invoice	MPC 221
	Chart for spices and additives batches preparation S-01-F-01-12, S-01-F-01-13	12.09.2011	Extra fine salt Dextrose Savory Black pepper Fortis garlic Vilma ham Polyphosphate Consal 6	CA 1990 CA 1914 CA 1383 CA 1885 CA 2066 CA 1934 CA 2007 CA 1987

	<p>Taking-over chart for auxiliary materials-spices/additives, labels and packaging materials S-01-F-01-03a</p>	<p>31.08.2011 23.03.2011 29.06.2011 19.08.2011 06.09.2011 25.08.2011 31.08.2011 30.08.2011</p>	<p>Extra fine salt Dextrose Savory Black pepper Fortis garlic Vilma ham Polyphosphate Consal 6</p>	<p>CA 1990 CA 1914 CA 1383 CA 1885 CA 2066 CA 1934 CA 2007 CA 1987</p>
	<p>File for auxiliary materials/spices/additives entry S-01-F-01-07</p>	<p>1022/31.08.2011 982/23.03.2011 717/29.06.2011 962/19.08.2011 1055/06.09.2011 993/25.08.2011 1031/31.08.2011 1017/30.08.2011</p>	<p>Extra fine salt Dextrose Savory Black pepper Fortis garlic Vilma ham Polyphosphate Consal 6</p>	<p>CA 1990 CA 1914 CA 1383 CA 1885 CA 2066 CA 1934 CA 2007 CA 1987</p>
	<p>Accompanying documents: Declaration of Conformity</p>	<p>293/31.08.2011 23.08.2011 28.06.2011 18.08.2011 06.09.2011 0626/25.08.2011 31.08.2011 07655/30.08.2011</p>	<p>Extra fine salt Dextrose Savory Black pepper Fortis garlic Vilma ham Polyphosphate Consal 6</p>	<p>CA 1990 CA 1914 CA 1383 CA 1885 CA 2066 CA</p>

				1934 CA 2007 CA 1987
	Launchings	10-12.09.2011	Rustic bacon	12

Legend:

No./date: registration number and date of the raw or auxiliary material taking-over;

Date: date of effective use of the product;

Batch: internal coding of the end product (12), or coding of the purchased raw materials or auxiliary materials;

CA: auxiliary material;

MPC: frozen raw material.

### 3. CONCLUSIONS AND RECOMMENDATIONS

- 3.1. The Company has drafted an own traceability system through which it intends to provide data regarding the processing origin and history;
- 3.2. The forms set up according to the procedure are filled in from the raw material taking-over up to the end product during the whole technological flow;
- 3.3. By filling in the traceability check form, it has been demonstrated the fact that the set-up traceability system is functional;
- 3.4. As traceability represents a requirement for the products' quality certification, the Company focuses on the traceability of products and permanently checks the correct and in due time drafting of the forms providing traceability;
- 3.5. Although the drafting of a traceability in the Company requires the getting over of many stages due to the production extensive flow, this is observed and the involved personnel is responsible for the tasks it has; this is responsible for the filling in of the forms providing traceability, is conscientious and well trained;
- 3.6. The implemented traceability system keeps all records and shows the way covered by a product or ingredient from the supplier, along the technological flow up to the final consumer;

- 3.7. Due to the functional traceability system that can document the products' history, in case of any raw material or auxiliary material non-conformity, there may be obtained information with regard to the origin of the raw material or the auxiliary material generating the non-conformity;
- 3.8. The enterprises in the alimentary sector that want to keep and strengthen their position on the market must take severe measures in order to provide quality, traceability, logistics' efficiency and information exchange.

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## **ICHTIOLOGIC STUDY ON THE DANUBIAN SECTOR KM 135 (CONFLUENCE WITH PRUT) —KM 197 (GROPENI)**

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**Key words:** fishing, Danube river, ichtiofauna

### **SUMMARY**

The fishing stock captured in 2008-2011 on the Danube river sector km 135 —km 197, made the object of this ichtiologic study. 41 species of fish were identified, which indicates that there are 58.57% out of the entire species in the Danube river, living in this sector. Although the proportion predatory/peaceful species is maintained to a certain equilibrium, there is a noticeable decrease in Danube's ichtiofauna. In addition to the small amount of migratory martime sturgeons, the autochthonous ichtiofauna records a gradual decrease in the amount of species considered to be economically valuable (carp, Danubian wels, pike-perch, pike), as well as a decrease close to extinction in some other species (burbot, orfe, white-eye bream).

The continuous industrialization process as well as the embankments along the Danube's floodplains have determined significant changes to the river's characteristics. The Danube's Basin is one of the most industrialized in Europe. The improvement and exploitation of Danube's floodplains has led to one of the most severe ecological aggressions on the country's territory. Genuine landscape was destroyed alongside a disaster in aquaculture and the pollution of Danubian waters (Bud et al., 2010).

The Danube's ichtiofauna along the romanian sector comprises over 70 species out of which approximately 70% were the object of commercial and recreational fishing (Bănărescu, 1967, 1969).

The aim of the present study is to investigate the ichtiofauna on the fluvial sector km 135 —km 197.

## 1. MATERIAL AND METHODS

The Danubian sector taken into consideration for this study is located between km 135 —Cotu Pisicii, the dischargement of Prut into the Danube and km 197 —Gropeni locality, upstream Brăila municipality. In order to determine the sampling section the simplified and adapted version of the „Microhabitat method” was used in accordance with Romania’s hydroclimatic conditions. For capturing the fishing stock which made the object of our study, the next sections were established: section 1 (km 135 — km 170) and section 2 (km 170 —km 197).

For capturing the fishing stock the classical fishing method was used (Bacalbaşa, 1995). Between years 2008-2011, in the sampling stations, utensils like trammel nets (with a mesh size of  $a = 30 - 80$  mm) as well as gillnets ( $a = 12 - 14$  mm) and some trap-like instruments (fyke and stake nets) were used. Both active (floating gillnets) and passive (fixed gillnets and fyke nets) fishing methods were used in the Danubian floodplain.

Professional observers present in the fishery throughout the study analyzed the collected fishing stock. The identification of the collected species was based on the morphological characteristics and the species’ description in speciality literature (Buşniţă and Alexandrescu, 1971). For 2008, 2009 and 2010 a whole year’s acquired data was processed, compared to 2011 where the data collected between January and August was processed. The species balance was established after their frequency in the following manner: XXXX (>15%); XXX (6 —15%); XX (2 —6%); and X (0 —2%) out of the entire capture.

## 2. RESULTS AND DISCUSSIONS

Beginning with 1990 the ecological concerns regarding the Danubian ichthiofauna gained increasingly importance by introducing and reassessing concepts like population, persistent exploitation of resources and the management and marketing of piscatorial activities. The balance of captured species between 2008-2011 on the considered river sector is described in table no. 1 and 2.

From the Acipenseridae family, specimens belonging to *Huso* (beluga sturgeon) and *Acipenser* (Danube sturgeon, stellate sturgeon,

sterlet) genera have been captured. Among sturgeons, the sterlet was the best represented in 2009-2010 (++) on section 1 compared to other years when it was present alongside sturgeons but in a smaller figure. The study demonstrates the presence of sturgeons in Danubian waters, but in small quantities (to very small quantities).

From the Clupeidae family a relatively small number of specimens was captured. The Black Sea herring was captured in the migration periods (April, May and June) being better represented than the Azov shad and common kilka. Although the specimens of this family are better represented than the sturgeons, small captures indicate that their numbers are gradually declining.

Table 1

The balance of species during 2008 –2011, section 1

No.	Systematic group/species	Year / frequency			
		2008	2009	2010	2011
	<b>Acipenseridae family</b>				
1.	<i>Huso huso</i> –beluga sturgeon	x	x	-	x
2.	<i>Acipenser gueldenstaedti</i> –Danube sturgeon	x	x	x	-
3.	<i>Acipenser stellatus</i> –stellate sturgeon	x	x	x	x
4.	<i>Acipenser ruthenus</i> - sterlet	x	xx	xx	x
	<b>Clupeidae family</b>				
5.	<i>Alosa immaculata</i> –Black Sea herring	xx	xx	xx	xx
6.	<i>Alosa tanaica</i> –Azov shad	x	x	x	x
7.	<i>Clupeonella cultiventris</i> –common kilka	x	x	x	x
	<b>Salmonidae family</b>				
8.	<i>Salmo labrax</i> –Black Sea salmon	x	-	-	-
	<b>Esocidae family</b>				
9.	<i>Esox lucius</i> - pike	x	xx	xx	x
	<b>Cyprinidae family</b>				
10.	<i>Cyprinus carpio</i> –common carp	xx	xx	xx	x
11.	<i>Carassius gibelio</i> –gold fish	xxx	xxx	xxx	xx
12.	<i>Rutilus rutilus</i> - roach	x	x	x	x
13.	<i>Tinca tinca</i> - tench	-	x	-	-
14.	<i>Scardinius erythrophthalmus</i> -rudd	x	x	x	x
15.	<i>Aspius aspius</i> - asp	x	x	x	xx
16.	<i>Chalchaburnus chalcoides</i> –Danube bleak	x	x	x	x
17.	<i>Alburnus alburnus</i> - bleak	xx	xx	xx	xx
18.	<i>Blicca björkna</i> –silver bream	xx	xx	x	x

19.	<i>Abramis brama</i> —common bream	xx	xx	xx	xx
20.	<i>Abramis sapa</i> - white-eye bream	xx	xx	xx	x
21.	<i>Abramis ballerus</i> —blue bream	xx	xx	xx	x
22.	<i>Vimba vimba</i> —vimba bream	x	x	x	xx
23.	<i>Pelecus cultratus</i> —sabre carp	x	x	x	x
24.	<i>Chondrostoma nassus</i> —common nase	x	x	x	-
25.	<i>Barbus barbus</i> - barbel	xxx	xx	xx	xxx
26.	<i>Ctenopharyngodon idella</i> —grass carp	x	x	x	x
27.	<i>Hypophthalmichthys molitrix</i> —silver carp	xx	xx	xx	xx
28.	<i>Aristichthys nobilis</i> —bighead carp	x	x	x	xx
	<b><i>Siluridae family</i></b>				
29.	<i>Silurus glanis</i> —Danubian wels	xx	xx	xx	xx
	<b><i>Gadidae family</i></b>				
30.	<i>Lota lota</i> - burbot	x	-	-	-
	<b><i>Percidae family</i></b>				
31.	<i>Perca fluviatilis</i> —river perch	x	x	x	x
32.	<i>Gymnocephalus cernuus</i> - ruff	x	x	x	x
33.	<i>Gymnocephalus schraetser</i> —striped ruffe	x	x	-	-
34.	<i>Zinger streber</i> —Danube streber	xx	x	x	-
35.	<i>Zinger zingel</i> - zingel	x	x	x	-
36.	<i>Sander lucioperca</i> —pike-perch	x	x	x	x
37.	<i>Sander volgense</i> —Volga perch	x	-	-	-
	<b><i>Gobiidae family</i></b>				
38.	<i>Neogobius kessleri</i> —bighead goby	x	x	x	x
39.	<i>Neogobius fluviatilis</i> —sand goby	x	-	-	x
	<b><i>Cobitidae family</i></b>				
40.	<i>Misgurnus fossilis</i> - weatherfish	x	-	-	x
	<b><i>Centrarchidae family</i></b>				
41.	<i>Lepomis gibbosus</i> —yellow sunfish	x	x	x	x

Legend: X 0—2% ; XX 2—6 %; XXX 6—15 %; XXXX >15 % out of the captures.

Only in 2008 and on section 1, representatives of the Salmonidae family such as the Black Sea salmon were captured in very small quantities. The small numbers and the sporadic presence show that the salmon is not a well represented species in the river's waters.

Throughout the four years taken into consideration small captures of pikes belonging to the Esocidae family were documented. In terms of numbers we can state that the pike has a modest representation in the Danube river, fact leading us to the conclusion that it does not have adequate growth and reproduction conditions thus being endangered.

From the Cyprinidae family specimens belonging to the next genera were captured: *Cyprinus* (common carp), *Carassius* (gold fish), *Rutilus* (roach), *Tinca* (tench), *Scardinius* (rudd), *Aspius* (asp), *Chalcalburnus* (Danube bleak), *Alburnus* (bleak), *Blicca* (silver bream), *Abramis* (common bream, white-eye bream, blue bream), *Vimba* (vimba bream), *Pelecus* (sabre carp), *Chondrostoma* (common nase), *Barbus* (barbel), *Ctenopharingodon* (grass carp), *Hypophthalmichthys* (silver carp) and *Aristichthys* (bighead carp). The barbel, gold fish, silver carp, carp, common bream, white-eye bream, silver bream and the bleak were best represented. The vimba bream, common nase, asp, rudd, roach and the grass carp had medium representation. The bighead carp, sabre carp and the tench were found in significantly smaller amounts, whereas the chub and the orfe were not present at all in the captures over the studied period of time. This family is the best represented in the Danubian sector taken into study, both in genera and species as well as certain species' balance .

Table 2

The balance of species during 2008 –2011, section 2

No.	Systematic group/species	Year / frequency			
		2008	2009	2010	2011
	<b>Acipenseridae family</b>				
01.	<i>Acipenser gueldenstaedti</i> –Danube sturgeon	-	x	-	-
02.	<i>Acipenser stellatus</i> –stellate sturgeon	x	x	x	x
03.	<i>Acipenser ruthenus</i> - sterlet	x	x	x	x
	<b>Clupeidae family</b>				
04.	<i>Alosa immaculata</i> - Black Sea herring	xx	xx	xx	xx
05.	<i>Alosa tanaica</i> - Azov shad	x	x	x	x
06.	<i>Clupeonella cultiventris</i> - common kilka	x	x	x	x
	<b>Esocidae family</b>				
07.	<i>Esox lucius</i> - pike	xx	x	xx	x
	<b>Familia Cyprinidae</b>				
08.	<i>Cyprinus carpio</i> - common carp	xx	xxx	xxx	xx
08.	<i>Carassius gibelio</i> - gold fish	xxx	xxxx	xxxx	xxxx
10.	<i>Rutilus rutilus</i> - roach	x	xx	x	-
11.	<i>Tinca tinca</i> - tench	-	x	-	-
12.	<i>Scardinius erythrophthalmus</i> - rudd	x	x	x	x
13.	<i>Aspius aspius</i> - asp	x	x	x	xx
14.	<i>Chalcalburnus chalcoides</i> –Danube bleak	x	x	x	x

15.	<i>Alburnus alburnus</i> - bleak	XX	XX	XX	X
16.	<i>Blicca björkna</i> - silver bream	XX	XX	XX	X
17.	<i>Abramis brama</i> - common bream	XX	XX	XX	XXX
18.	<i>Abramis sapa</i> —white-eye bream	XX	XX	XX	X
19.	<i>Abramis ballerus</i> —blue bream	XX	XX	XX	X
20.	<i>Vimba vimba</i> - vimba bream	X	X	X	XX
21.	<i>Pelecus cultratus</i> - sabre carp	X	X	X	X
22.	<i>Chondrostoma nassus</i> - common nase	X	X	X	X
23.	<i>Barbus barbus</i> - barbel	XXX	XXX	XXX	XXXX
24.	<i>Ctenopharyngodon idella</i> - grass carp	X	X	X	X
25.	<i>Hypophthalmichthys molitrix</i> - silver carp	XXX	XXX	XXX	XX
26.	<i>Aristichthys nobilis</i> - bighead carp	X	X	X	X
	<b><i>Siluridae family</i></b>				
27.	<i>Silurus glanis</i> - Danubian wels	XX	XXX	XX	XXX
	<b><i>Percidae family</i></b>				
27.	<i>Perca fluviatilis</i> - river perch	X	X	X	X
29.	<i>Gymnocephalus cernuus</i> - ruff	X	X	X	X
30.	<i>Gymnocephalus schraetser</i> - striped ruffe	X	X	X	-
31.	<i>Zinger streber</i> —Danube streber	X	X	X	X
32.	<i>Zinger zingel</i> - zingel	X	X	X	X
33.	<i>Sander lucioperca</i> - pike-perch	X	X	X	X
34.	<i>Sander volgense</i> —Volga perch	X	X	X	-
	<b><i>Gobiidae family</i></b>				
35.	<i>Neogobius kessleri</i> —bighead goby	X	X	X	X
36.	<i>Neogobius fluviatilis</i> —sand goby	X	X	X	X
	<b><i>Cobitidae family</i></b>				
37.	<i>Misgurnus fossilis</i> - weatherfish	X	X	X	X
	<b><i>Centrarchidae family</i></b>				
38.	<i>Lepomis gibbosus</i> —yellow sunfish	X	X	X	X

Legend: X 0—2% ; XX 2—6 %; XXX 6—15 %; XXXX >15 % out of the captures.

From the Siluridae family specimens of Danubian wels were captured, with a medium and relatively constant representation throughout the study, which enables us to infer that the Danube waters offer auspicious conditions for the Danubian wels' growth and reproduction.

From the Gadidae family the burbot was captured in small quantities only in 2008 and on section 1. We can state that the burbot does not have a significant representation among the Danube's ichtiofauna.

From the Percidae family, specimens belonging to the next genera were captured: *Perca* (river perch), *Acerina* (ruff, striped ruffe), *Stizostedion* (pike-perch, Volga perch) and *Aspro* (Danube streber, zingel). Specimens

belonging to this family had a constant presence but with a relatively modest representation indicating that Danube's waters can't assure the best conditions for growth and reproduction. The following families: Gobiidae (bighead goby, sand goby), Cobitidae (weatherfish) and Centrarchidae (yellow sunfish) are modestly represented, but have a constant representation in captures. Based on the data acquired between 2008-2011 on the analyzed river sector, assessments and observations with regards to the ichthiofauna were made. 41 species of fish were identified on section 1 and 38 species on section 2. The investigated fish population comprised peaceful species (60.0% on section 1 and 65.8% on section 2) and predatory species (36.1% on section 1 and 28.9% on section 2). A normal distribution of predatory fish ranges between 15% and 35% (Pora and Oros, 1974). Economically valuable species (peaceful and predatory) were 65.9% and 55.3%. We can state that there is a decrease in the specific ichthiofauna of this area. Various fish population (peaceful and predatory), inclusively migratory species (maritime sturgeons) that come to the Danube for reproduction are being affected (Ciolac et al., 2003). The following factors have determined a dramatic decrease in fish population: excessive fishing as well as the alteration of their habitat, together with hydrologic and morphologic changes secondary to dams' construction (Bacalbaşa, 1989).

### 3. CONCLUSIONS

3.1. The analysis of the captures between 2008-2011 shows the presence of 41 fish species on the studied river sector .

3.2. There is a noticeable decrease in Danube's ichthiofauna, relating in different manners to peaceful, predatory and migratory species, regardless of the fact that the proportion predatory/peaceful species is maintained to a certain equilibrium.

3.3. The migratory maritime sturgeons have a delicate status. The intensity of migration decreased constantly in the past decades and accentuated in the past years, fact that imposed the suspension of fishing on a 10 year period.

3.4. The autochthonous ichthiofauna is in a similar situation. A gradual decrease in the amount of species considered to be valuable (carp, Danubian wels, pike-perch, pike) is noticeable as well as a decrease in some species density close to extinction (burbot, orfe, white-eye bream).

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## FARM TO FORK TRACEABILITY THROUGH DNA TECHNOLOGY

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**Keys Words:** bioeconomy, eoeconomy, food security,

### SUMMARY

In terms of respect international standards of food safety and security; the known european principles “from the farm to the fork” and “from the farm to the plate” which have restricted rules established by European Food Safety Authority, must be respected in agrifood products in perspective also. In this framework, the food safety and security must be correlated with the respect of known principles of Hazard Analysis and Critical Control Points, based on actual international standards from ISO 9001-9002 series (Quality Management System), ISO 14001: 2004 (Environment Management), ISO 22000 (Food Safety). The present study predicts the ecoeconomic and bioeconomic impact of food safety and security in perspective the implemetation the european norms for food trasability in Romania.

The main objective of EU food safety policy is to achieve the highest possible degree of protection of human health and consumer interests in relation to food. In this regard, the EU strives to ensure food safety and proper labeling, given the diversity of products, including traditional ones by specific certification bodies (EFSA). ISO 8402 defines traceability as ‘...The capacity for establishing a product’s origin, process history, use and provenance by reference to written records.....’ The sustainable economy of the future has to become a bioeconomy, adapted to the rural area and based on a large biodiversity that will create first of all an opportunity for more producers of primary organic synthesis and further on for a longer line of consumers up to the final state of dead organic matter that must be mineralized. In this context, Nicolas Georgescu-Roeger’s world-wide-known Bioeconomics paradigm of improving the agricultural efficiency becomes most topical, particularly as mankind’s limited natural resources

are being depleted. Globalization of the food chain causes constant new challenges and risks to health and consumer interests. The EU has developed a comprehensive body of legislation on food safety, which is continually monitored and adapted as new developments. Traceability is managed by European legislation and the regulations nr.178/2002 1642/2003 on food safety and the local law no. 150/2004 on food safety and feed quality and standards, such as: 22005:2007, ISO 22000:2005 and ISO / TS 22004:2006 for traceability in the food chain. The EU actively promotes high standards of consumer safety and consumer support organizations to strengthen their role in decision making. Biotechnology researches and development related to food (including genetically modified organisms) is a way to eradicate hunger, which takes into account the basic principle of EU food safety policy by applying an integrated approach, such as "farm to fork" covering all sectors of the food chain, including feed production. The EU has a comprehensive strategy on food safety. It covers not only food safety but also health and welfare of animals and plants. Although the specifications of particular systems vary from region to region, traceability within the beef sector is typically achieved through animal eartags, meat labels and bar-codes, which identify a meat product and enable it to be tracked back to a production batch or group of animals of origin. The strategy provides the ability to track food from farm to consumer even if it is needed to move within the EU borders. EU food strategy is based on three main elements: legislation on food and feed safety, basic scientific advice necessary decisions in the field and implementing a policy and control. The law covers many areas, from food and feed, up to food hygiene, applying the same high standards throughout the Union. Community legal framework on food safety is common to all Member States, but adapted diversity. EU efforts significant because traditional foods are not removed from the market due to food safety standards and that innovation should not be discouraged and do not have the quality of the Romanian scientific. The context of our paper approach: eco-and bio-economy (the socio-economic priorities and humanities), biodiversity as a resource of sustainable development, biotechnology, food safety including food chemistry, health (a consequence of ecosanogenesis) environmental and implicitly (through the environmental impact on human health aspects and animals;) Each EU country is obliged to ensure that product safety was not compromised in its

food chain, and this can be achieved through the implementation and certification of a Food Safety Management System. HACCP is a system of internationally recognized food safety, based on a systematic analysis and preventive production process, which shows that food safety risks are identified, assessed and controlled. HACCP involves risk identification, control and monitoring of critical points where the process could be compromised food quality. The system is based on the Food Code (Codex Alimentations) developed by the UN Food and Agriculture Organization and World Health Organization. There is a big interest apart of breeders and veterinarians for a certain identity of animals and for their paternity. Current animal identification practice in the EU is based on administrative tracking of the animal ID by using visual animal ID devices (e.g. visual ear tags) and procedures (e.g. animal passport, abattoir batch no.). A major drawback of this approach is its susceptibility to fraud as reliable control instruments are missing. Today, molecular genetic technologies are available to provide such control instruments. These technologies (“DNA fingerprinting”) not only provide the means to 100 % reliable traceability of livestock and livestock products, but also represent a powerful instrument to improve animal health and animal welfare.

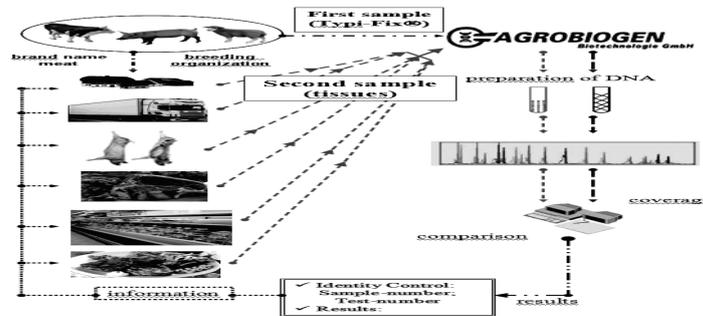
## **2.MATERIAL AND METHODS**

. This scientifically paper presents results of research concerning recognition of genotypes by microsatellites genetic markers collecting and preserving the tissue samples by TypiFix method.

The research team used modern tools to identify the traceability the original materials (meet or milk) of different species from traditional products - molecular tests based on identification, amplification and characterization of nucleic acids for food traceability (PCR techniques).

Many ways and methods were tested and applied. The best of them seems to be the DNA analysis as “Genetic Fingerprint”, which is found in every cell of the body and the more recent method of microsatellites genetic markers. Using PCR techniques to multiply DNA segments it is possible to dispose of enough genetic material to compare DNA from different cells, let say from under skin tissue and from muscle fibers, and know if they have or don't have the same genotype originConcerning traceability of animal

products there are hopes as well. The way to apply genetic identity in monitoring the traceability of products is given in figure:



The leading lab correlation with traceability of meat products through the new method of prof. G. Brem (By A.T. Bogdam at all, published 2009 in Bulletin UASVM, nr. 66 (1-2)/2009 - Veterinary Medicine, Print ISSN 1843-5270; Electronic ISSN 1843-5386, pg. 427)

The method based on microsatellite markers gives concrete results and is a valuable tool for the specific meat of breed. The applicability of the methods is very important because give the transparency needs of the market in very short time.

The analytical methods used for species identification and authenticity of foods rely mainly on protein and DNA analysis. The protein-based methods include immunological assays electrophoretical and chromatographic techniques.

More recently, DNA molecules have been the target compounds for species identification due to the high stability compared with the proteins, and also to their presence in most biological tissues, making them the molecules of choice for differentiation and identification of components in foods, and a good alternative to protein analysis. Most DNA-based methods for species identification in foods consist on the highly specific amplification of one or more DNA fragments by means of polymerase chain reaction (PCR). DNA microsatellite markers are proposed for meat traceability. 10 microsatellites were amplified in multiplex reactions and analyzed on ABI310 genetic analyzer. The probes it was works in Agrobiogen Laboratory at the Vienna.

*Tissue collection with TypiFix™ –System* The TypiFix™ ear tag system is a combination of a conventional ear tag with a simultaneous tissue sampling technology. By ear tagging the farm animals, the tissue samples are automatically collected and sealed in the TypiFix™ sample containers, where the tissue samples are preserved at ambient temperature and can be used for protein or DNA based assays. The easy handling of the TypiFix™ ear tag system allows economic sampling of whole populations and is therefore an effective tool for analysis of genetic markers for paternity control, traceability and breeding traits. The Typi-Fix-System is a procedure for the collection of DNA containing tissue samples avoiding all these hurdles and problems. With the Typi-Fix-ear tags the animal is marked - in the usual convention - with a plastic ear tag. At the same time, however, a tissue sample is taken by the spike of the ear tag which immediately after the collection is packaged in a special plastic container (sample receiving container) labeled with the (bar coded) animals ear tag number.

After collection the preservation and preparation of the DNA is initiated automatically by substances which are hold in stock in the sample receiving container. The identification number of the samples can be registered by a reading device (scanner). The sample container is connected to the ear tag by a plug and socket and is easily removed after the ear tag has been affixed and the tissue sample simultaneously collected. If desired, the sample container can also be used without the ear tag. After pigs tissue collection with ear tagging, we collected meat probes in abattoir. The porcine agreed microsatellite markers use for: Set I is: S0005 for chromosome 5 and range 205-248, S0090 for chromosome 12 and range 244-251, S0155 for chromosome 1 and range 150- 166, SW857 for chromosome 14 and range 144-160, SW240 for chromosome 2 and range 96-115; Set II is: SW24 for chromosome 17 and range 96-121, SW951 for chromosome 10 and range 125-133.

*DNA purification with DNA FIX* columns an extremely simplified and shortened one-step high-throughput separation procedure of genomic DNA from TypiFix samples. The sorbents retain protein and other contaminants, while the DNA passes the column in the exclusion volume. DNA isolation and purification can be automated through the use of a piping robot and a special one-step procedure (Nexttec technology). PCR reactions with these samples can also be prepared automatically. The results

of the multiplex PCR 565 analyses are linked with the scanned identification number and saved in the animal data bank. *Gel electrophoresis of NCC purified DNA from 88 TypiFix eartag samples* : 5 µl (total elution volume: 240 µL) of each sample were loaded on a 1% agarose/ EtBr gel. The DNA concentration is about 10 ng/µl or greater = negative control

The tools of molecular genetics are likely to have considerable impact in the future. For example, DNA-based tests for genes or markers affecting traits that are difficult to measure currently, such as meat quality and disease resistance, will be particularly useful (Leakey et al. 2009). Another example is transgenic livestock for food production; these are technically feasible, although the technologies associated with livestock are at an earlier stage of development than the equivalent technologies in plants. In combination with new dissemination methods such as cloning, such techniques could dramatically change livestock production. Complete genome maps for poultry and cattle now exist, and these open up the way to possible advances in evolutionary biology, animal breeding and animal models for human diseases (Lewin 2009). Genomic selection should be able to at least double the rate of genetic gain in the dairy industry, as it enables selection decisions to be based on genomic breeding values, which can ultimately be calculated from genetic marker information alone, rather than from pedigree and phenotypic information. Genomic selection is not without its challenges, but it is likely to revolutionize animal breeding.

New tools of molecular genetics may have far-reaching impacts on livestock and livestock production in the coming decades. But ultimately, whether the tools used are novel or traditional, all depend on preserving access to animal genetic resources. In developing countries, if livestock are to continue to contribute to improving livelihoods and meeting market demands, the preservation of farm animal genetic resources will be critical in helping livestock adapt to climate change and the changes that may occur in these systems, such as shifts in disease prevalence and severity as the tools and techniques of breeding are changing.

### 3.CONCLUSION

3.1. The main objective of EU food safety policy is to achieve the highest possible degree of protection of human health and consumer interests in relation to food.

3.2. Many ways and methods were tested and applied for identification traceability of animal products; the best of them seems to be the DNA analysis as “Genetic Fingerprint”, which is found in every cell of the body and the more recent method of microsatellites genetic markers. In order to apply DNA analysis using microsatellite test there are much hopes but it is necessary to know precisely how this trait, microsatellite presence in different chromosomes is inherited in the progeny.

3.3. The DNA-based methods, namely the PCR, proved to be reliable, fast, sensitive and extremely specific techniques for the detection of frauds

3.4. The method based on microsatellite markers gives concrete results and is a valuable tool for the specific meat of breed . The applicability of the methods is very important because give the transparency needs of the market in very short time.

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## ANTIMICROBIAL SUSCEPTIBILITY OF *LISTERIA* SPP. STRAINS ISOLATED FROM ANIMAL PRODUCTS

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**Key words:** antibiosusceptibility, *Listeria* spp., food

### SUMMARY

*Listeria* species are ubiquitous bacteria widely distributed in the natural environment. The ubiquitous character of these germs result in contamination of numerous food products. Epidemiological studies have demonstrated that *Listeria monocytogenes* is an important pathogen transmitted by food.

Different commercial food products (raw milk and dairy, vegetables, raw meat, poultry and fish), as well as fast food preparations are frequently contaminated by *Listeria* germs and prove to be the source of *Listeria* infection manifested by different clinical aspects of listeriosis (septicemia, meningitis, encephalitis, abortive disease), as result of the digestive transmission of these germs to humans. The following individuals are at great risk for listeriosis: immunocompromised, elderly, infants, and pregnant women (and their unborn children).

The objective of this study was to evaluate the susceptibility of 25 *Listeria* spp. strains isolated from food-processing samples to 21 antibiotics currently used in veterinary and human therapy. There were studied strains of *L. innocua* and *L. welshimeri* comparatively with *L. monocytogenes*, because they were more genetically related to *L. monocytogenes* than other *Listeria* species.

Antibiosusceptibility tests were performed according to Clinical and Laboratory Standards Institute guidelines. The majority of strains were resistant to oxacillin, cephalosporins, nalidixic ac., colistin. This study shows that *L. monocytogenes* strains from food-processing samples are susceptible to the antibiotics commonly used in veterinary and human listeriosis treatment.

Genus *Listeria* consists of six species: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*, but only *L. monocytogenes*, *L. ivanovii* are considered virulent. *L. monocytogenes* is an important foodborne pathogen and has been isolated from a variety of food

products: meat, poultry, dairy and vegetable products, which have been implicated as vehicles of listeriosis (Benenson, 1995; Caplan, 2001). Little information is available on the antimicrobial susceptibility of *L. monocytogenes*, particularly on strains isolated from food and food environment, indicating the necessity of monitoring the antimicrobial susceptibility of this pathogen.

The present study aimed to evaluate the susceptibility of 25 *Listeria* spp. strains isolated from food-processing samples to 21 antibiotics currently used in veterinary and human therapy.

## 1. MATERIALS AND METHODS

There were investigated for their antibiotic susceptibility spectrum 25 suspicious *Listeria* spp. strains isolated from food-processing samples. Identification and confirmation of strains were performed according to the ISO 11290-1 method. *L. monocytogenes* strains were serotyped by the agglutination test with rabbit adsorbed antisera: antiserum *Listeria monocytogenes* serovar 1a and antiserum *Listeria monocytogenes* serovar 4b.

Susceptibility tests were performed by standard method on Mueller Hinton Agar (MHA), using Oxoid disks, following the procedures recommended by the Clinical and Laboratory Standards Institute (CLSI) 2011. *Staphylococcus aureus* ATCC 29213 was used as control strain. Twenty-one antibiotics were chosen: Penicillin 10U, Ampicillin 10µg, Amoxicillin + Clavulanic Ac. 20µg/10µg, Oxacillin 1µg, Piperacillin 100µg, Imipenem 10µg, Ceftriaxone 30µg, Cefuroxime 30µg, Amikacin 30µg, Gentamycin 10µg, Neomycin 30µg, Nalidixic Ac. 30µg, Ofloxacin 5µg, Ciprofloxacin 5µg, Tetracycline 30µg, Erythromycin 15µg, Chloramphenicol 30µg, Rifampicin 30µg, Colistin 10µg, Sulphamethoxazole / Trimethoprim 1,25µg/23,75µg.

## 2. RESULTS AND DISCUSSIONS

All the 25 suspicious strains isolated from food-processing samples had been belonging to *Listeria* species. Table 1 presents the types of sources for samples analyzed in this study. Among the 25 strains of *Listeria* spp., 7

strains *L. monocytogenes*, 16 strains *L. innocua* and 2 strains *L. welshimeri* were identified and confirmed by Gram coloration, motility, catalase, hemolysis test, CAMP test and biochemically (Table 2). *L. monocytogenes* was represented by two serogroups: 6 strains were *L. monocytogenes* serotype 1a and 1 strain was *L. monocytogenes* serotype 4b, the most circulating serovars (Swaminathan, 1995).

Concerning the antibiotic susceptibility (Table 3), for gentamicin, all isolates were susceptible to this antibiotic; our data are similar to those of Ennaji *et al.*, 2008. All 6 *L. monocytogenes* isolates were resistant to oxacillin, nalidixic acid and colistin. All tested *L. monocytogenes* strains proved to be resistant to the second and third generation of cephalosporins, as mentioned by the literature data (Ennaji *et al.*, 2008); for this reason, these antibiotics should not be used clinically for treating listeriosis. One *L. monocytogenes* isolate was intermediary to ciprofloxacin. *L. monocytogenes* isolates were susceptible to penicillin, ampicillin, amoxicillin+clavulanic acid, imipenem, aminoglycosides, ofloxacin, tetracycline, erythromycin, chloramphenicol, rifampicin, sulphamethoxazole/trimethoprim. The current antimicrobial treatment for listeriosis is ampicillin with gentamicin or penicillin alone, where sulphamethoxazole/trimethoprim is used as a secondary agent (Tuazon, 1982). In the current study, all *L. monocytogenes* isolates were susceptible to these drugs.

All 16 *L. innocua* isolates exhibited resistance to penicillin, oxacillin, nalidixic acid and colistin. The majority of isolates were resistant to cephalosporins, tetracycline and intermediary to chloramphenicol. All *L. innocua* strains were susceptible to ampicillin, amoxicillin+clavulanic acid, imipenem, aminoglycosides, ofloxacin, ciprofloxacin, rifampicin and sulphamethoxazole / trimethoprim.

The two *L. welshimeri* strains were resistant to penicillin, oxacillin, nalidixic acid, ciprofloxacin, chloramphenicol, rifampicin and colistin. One *L. welshimeri* isolate was intermediary to sulphamethoxazole/trimethoprim and both of them were intermediary to ofloxacin. *L. welshimeri* isolates were susceptible to ampicillin, amoxicillin+clavulanic acid, imipenem, ceftriaxone, aminoglycosides, tetracycline and erythromycin.

The current study demonstrated the variability of antimicrobial resistance among the three closely related *Listeria* species. However, little variation was observed among *L. monocytogenes* isolates from various

sources. Ampicillin, rifampin, or penicillin plus gentamicin remain the treatment of choice for most manifestation of listeriosis (Conter *et al.*, 2007). Sulphamethoxazole/trimethoprim is considered to be a second-choice therapy. In general, most *L. monocytogenes* were susceptible to the antibiotics commonly used in veterinary and human listeriosis treatment.

Considering that *L. monocytogenes* is slowly becoming antibiotic resistant by acquisition of known antibiotic resistance genes from gram-positive bacteria, a continued surveillance of emerging antimicrobial resistance of this pathogen is important to ensure effective treatment in listeriosis.

Table 1

Incidence of *Listeria* species from food products (No.)

Products		<i>Listeria</i> spp. strains		<i>L.innocua</i>	<i>L.welshimeri</i>
		<i>L.monocytogenes</i>			
		<i>serotype 1a</i>	<i>serotype 4b</i>		
Raw minced meat (pork and beef)		1	-	7	-
Sausages	Paste of Romanian sausages	1	-	-	-
	Fresh sausages	3	-	3	-
Muscular tissue of	bovine	-	-	1	-
	pork	-	-	2	-
Pork meat	Bacon in processing	-	-	1	-
	Smoked chop	1	-	-	-
	Pork shoulder blade	-	-	1	-
Poultry carcass		-	1	-	-
Dairy products	Romanian pressed cheese	-	-	1	-
	Powder milk	-	-	-	2
TOTAL STRAINS		6	1	16	2

These data can be used to improve general data on antibiotic resistance of *Listeria* strains isolated from food products and for epidemiological and public health studies of *L. monocytogenes*.

Table 2

Identification of *Listeria* spp. strains (No.)

Products		<i>Listeria</i> spp. strains		<i>L.innocua</i>	<i>L.welshimeri</i>
		<i>L.monocytogenes</i>			
		<i>serotype 1a</i>	<i>serotype 4b</i>		
Beta-haemolysis		6	-	-	-
CAMP Test	<i>Staphylococcus aureus</i>	6	1	-	-
	<i>Rhodococcus equi</i>	-	-	-	-
Mobility		6	1	-	-
Catalase Test		6	1	16	-
Acid from: D-Glucose		6	1	16	2
D-Manose		6	1	16	2
L-Rhamnose		6	1	1	1
D-Xylose		-	-	2	-
Serological identification	<i>L. monocytogenes</i> 1a	6	-	-	-
	<i>L. monocytogenes</i> 4b	-	1	-	-

Table 3

## Antibioresistance / susceptibility spectrum of the strains

Antibiotic			<i>Listeria</i> spp. strains		<i>L.monocytogenes</i>						<i>L.innocua</i>			<i>L.welshimeri</i>			
						<i>serotype 1a</i>			<i>serotype 4b</i>								
						S	I	R	S	I	R						
<i>Betalactams</i>	<i>Penicillins</i>	Penicillin	6					1			16			2			
		Ampicillin	6					1	16			2					
		Ampicillin+ClavulanicAc	6			1			16			2					
		Oxacillin			6			1			16			2			
		Piperacillin	6			1			16			2					
		Imipenem	6			1			16			2					
	<i>l o s p</i>	Cefuroxime			6			1			16			2			

		Ceftriaxone			6			1	4		12	2		
<i>Amino-glycosides</i>		Amikacin	6			1						2		
		Gentamycin	6			1			16			2		
		Neomycin	6			1						2		
<i>Quinolones</i>		Nalidixic Ac.			6			1			16			2
		Ofloxacin	6			1			16				2	
		Ciprofloxacin	5	1			1		16					2
<i>Tetracyclines</i>		Tetracycline	6			1			2		12	2		
<i>Macrolides</i>		Erythromycin	6			1			16			2		
<i>Other antibiotics</i>		Chloramphenicol	6			1			2	10	4			2
		Rifampicin	6			1			16					2
		Colistin			6			1			16			2
		Sulphamethoxazole / Trimethoprim	6			1			16				1	1

S = susceptible; I = intermediary; R = resistant

### 3. CONCLUSIONS

- 3.1. The majority of strains were resistant to oxacillin, cephalosporins, nalidixic ac., colistin.
- 3.2. Ampicillin, rifampin, or penicillin plus gentamicin remain the treatment of choice for most manifestation of listeriosis.
- 3.3. Sulphamethoxazole / trimethoprim is considered to be a second-choice therapy.
- 3.4. In general, most *L. monocytogenes* were susceptible to the antibiotics commonly used in veterinary and human listeriosis treatment.

### ACKNOWLEDGEMENTS

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## OPTIMUM TRAINING METHODS FOR THE HAZARDS ASSESSMENT AND CONTROL IN THE MEAT PROCESSING PLANTS

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**Keywords:** training, hazards, contamination, multiplication, survival.

### SUMMARY

The aim of this study is to show how can be trained the HACCP teams from the meat industry to be able to handle more easy the hazards in their plants, using a "Food Safety Pyramid". It was taking consideration all potential hazards (biological, physical, chemical) and also contamination, multiplication and survival risks and it was made a clear separation of the hazards, which kind of risks are addressed to the Prerequisite Programs and which kind of risks are addressed to the HACCP plan.

In this way, the hazards analysis became simple because it was eliminated the risks which cannot be monitored in the HACCP plan.

The HACCP teams from the meat processing plant where I made this kind of training, following the "Food Safety Pyramid", proved a better understanding regarding this system. Much confusion were eliminated, they understood very well that:

- HACCP is not a stand alone system;
- Prerequisite Programs must be in place prior HACCP; and
- Prerequisite Programs are not part of the formal HACCP system.

The safe production of food is a fundamental legal obligation of the food business operator:

*"Food business operators at all stages of production, processing, and distribution within the businesses under their control shall ensure that foods ... satisfy the requirements of food law which are relevant to their activities and shall verify that such requirements are met." Reg.178/2002 EC Article 17.*

*"Food business operators shall put in place, implement and maintain a permanent procedure or procedures based on the HACCP principles." Reg.852/2004 EC Article 5 point 1.*

## 1. MATERIAL AND METHODS

The study was carried out since 2004 to 2011. During this time, I worked with many plants from meat industry (slaughterhouses, deboning plants, meat processing plants). At the beginning, I started to make foods safety assessments to see what is happening in these plants regarding the understanding of HACCP system and how they put in place the HACCP principles.

Unfortunately, I found a lot of misunderstandings regarding the applying of HACCP principles. The main misunderstanding found was the confusion regarding which kind of hazards need to be addressed to the HACCP plan and which kind of hazards are controlled through Prerequisite Programs.

*A hazard is 'a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect'. (Codex Alimentarius)*

It was taking consideration all potential hazards:

*Biological hazards* — bacterial agents (Salmonella, E.coli, Listeria monocytogenes, Clostridium botulinum etc.), viral agents, parasites, prions (BSE agents).

*Physical hazards* —foreign objects, which are normally not found in food (metal fragments, clips, jewelry, buttons, wood splinters, glass splinters etc.

*Chemical hazards* —antibiotics, hormones, growth promoters, pesticides, cleaning and disinfecting agents, lubricants, pest baits, additives, preservatives, coloring agents etc. Another chemical hazards are the allergens. These are usually proteins that trigger a specific type of immune response by the human body.

*Biological contamination* —a product is considered contaminated when this product take a microbiological load.

*Chemical contamination* —a product is considered contaminated when the product catches a foreign chemical substance or a kind of ingredients (ex. sodium nitrite) is put in a big amount (over legal limits).

*Physical contamination* —a product is considered contaminated when the product take a foreign body.

*Multiplication* —it is referring to biological hazard.

*Survival* —it is referring to biological hazard.

Both, multiplication and survival are depending on physical-chemical parameters (time, temperature, acidity, water availability).

Physical and Chemical hazards are characterized by imparting danger only by contamination and not by multiplication and survival.

Taking account of these, it was built a “*food safety pyramid*”. This pyramid has four levels.

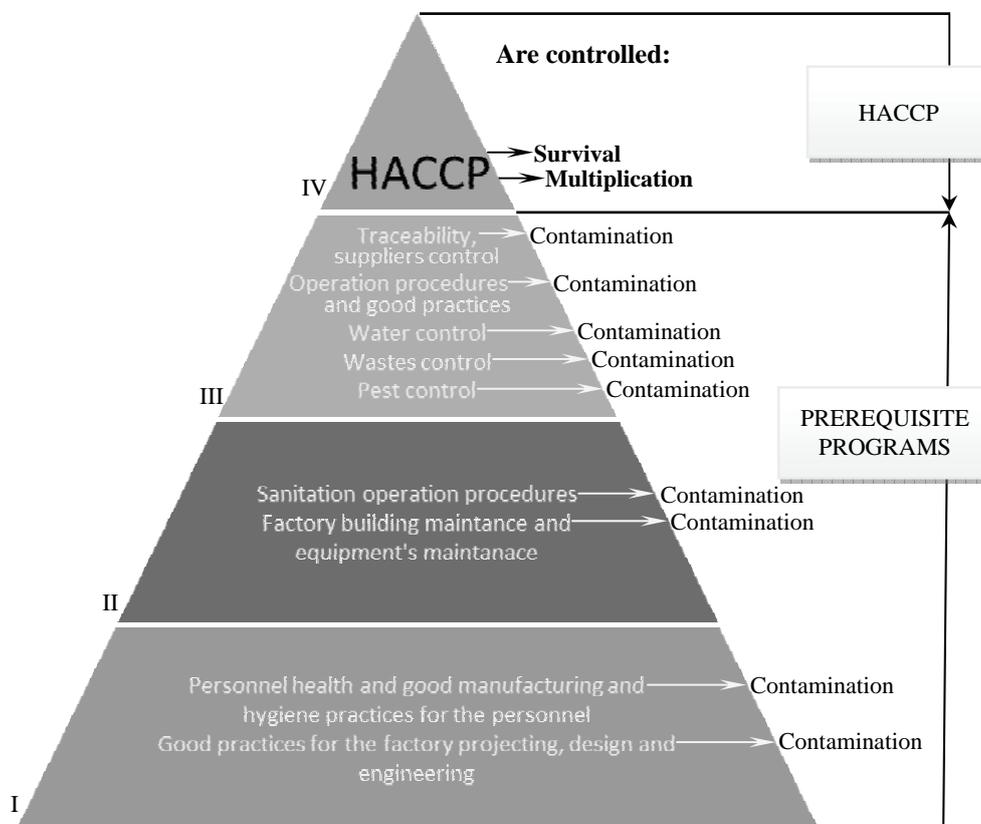


Fig.1 Food safety system

**I.** On the first level we found “*Good practices for the factory projecting, design and engineering*”. The location, design, layout and construction of the factory and the choice of equipment, fittings and facilities are crucial to ensure that food businesses can operate under

hygienic conditions and produce safe food. Poorly designed and constructed buildings and equipment are potential source of physical, chemical and microbiological hazards. The design and the construction of the premises should permit good manufacturing and hygiene practices. Badly designed buildings and equipment could create “dirt traps” and make cleaning and maintenance difficult and thus become a source of microbiological contamination. “*Personnel health and good manufacturing and hygiene practices for the personnel*” —People employed in, or visiting, food plants are an important potential source of microbiological and physical hazards. Personnel health is very important to prevent contamination, for example monitoring of staff for potential Salmonella carriers and monitoring of staff for lesions caused by Staphylococcus. The factory should maintain relevant health records of the personnel. For example, regarding the hygiene practices, we can talk about hands. The hands, frequently in direct contact with foodstuffs, need to be considered as the first operational tool. If are not subjected to strict hygiene rules, the hands constitute the first vector of contamination of food, by germs passed on from the operator. Lack of adequate hygiene facilities, such as toilets and hand-washing basins, would prevent staff from following personal hygiene procedures and could lead to product contamination. Each factory must have written hygiene rules and personnel must be trained and supervised to follow these rules.

**II. “Maintenance”** —establishments, facilities and equipment should be kept in a good state of repair and condition to facilitate all sanitation procedures and to prevent contamination of food, for example physical contamination (metal fragments from rails, clips, tags, machinery, bolts, screws, paint flakes, rust, plastic, rubber bands, glass splinters) and chemical contamination (no food- grade lubricants). Building, fittings, equipment and surfaces must be maintained in a good state of repair to minimize the opportunity for build up of dirt, food and other debris. Poor maintenance may also allow the entry of other sources of physical, microbiological and chemical contamination such as pests, dust, waste water etc. “*Sanitation*” — Sanitation is one of the essential prerequisite programs for the successful implementation and maintenance of a HACCP program. A good sanitation program will control many potential biological, chemical and physical hazards associated with food operations. Poor sanitation allows food to be

contaminated by dirt from the working environment and it increases the chances of cross contamination of the products.

**III.** “*Pest control*” —pests (insects, rodents, birds, dogs, cats) entering or infesting food plants are a significant potential source of microbiological, physical and chemical hazards (use of pesticides). Pests are carries for many microorganisms, pests are source of foreign bodies (insects themselves, hair, larvae etc.) and insects can transfer contamination from dirty areas to clean areas. “*Wastes control*” —food waste, non-edible by-products and other waste materials can be a significant source of microbiological and physical contaminationand will attract pests that can contaminate the production environment. “*Water control*” —water is an important potential source of microbiological and chemical hazards. Microorganisms can survive for many days or even months in water. Water can also be a source of chemical contamination (heavy metals, pesticides, nitrates). “*Traceability*” —“*Traceability*” means the ability to trace and follow a food, food producing animal etc. through all stages of production, processing and distribution —reg.178/2002 EC.Information about suppliers and customers means that if a food safety emergency occurs (microbiological, chemical and physical contamination), the food can be traced back or forwards through the food chain. “*Suppliers and raw material control*” —all raw materials supplied (meat and other ingredients), should be free from microbiological, chemical and physical hazards. A food business operator should not accept raw materials, ingredients and any other material used in processing products, if they are known to be or might reasonably be expected to be contaminated.They should have specifications for all raw and auxiliary materials that enter the plant. These specifications will be communicated to the suppliers and agreed by them, becoming part of the purchasing contract. This will assure the raw materials standardisation and their constant quality, with positive effects on the safety and quality of the final product.“*Good practices and operation procedures*”—poor work practices or failures to follow instructions may give rise to microbiological, physical and chemical hazards. Insufficient training and supervision can lead to unhygienic work practices. Staff must be trained and supervised to know and to understand the consequences of their actions.

**IV.** HACCP —Hazard Analysis and Critical Control Point. To produce safe food, all the important safety hazards that are associated with

the production of food need to be prevented, eliminated or reduced to an acceptable level. Critical Control Point: a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. Multiplication and Survival of microorganisms from the product, depends on measurable parameters (time, temperature, acidity, water activity). When these parameters can be monitored, compared to critical values in the course of the process and be exploited to take actions when there is a loss of control, then a CCP can be defined at this process step.

## **2. RESULTS AND DISCUSSIONS**

The production of safe food products requires that the HACCP system must be built upon a solid foundation of Prerequisite Programs. This has traditionally been accomplished through the application of GMPs. These conditions and practices are now considered to be prerequisite for the development and implementation of effective HACCP plans. Prerequisite Programs provide the basic environmental and operating conditions that are necessary for the production of safe food. Many of the conditions and practices are specified in regulations and guidelines. Prerequisite Programs are established and managed separately from the HACCP plan. Following the explanations of the pyramid levels, the conclusion is that the control of Contamination depends on Prerequisite Programs, while that of the Multiplication and Survival is based on CCP establishment.

## **3. CONCLUSIONS**

3.1. In this way, much confusion was eliminated. The hazards analysis became simple because it was eliminated the risks which cannot be monitored in the HACCP plan.

3.2. The HACCP teams from the meat processing plant where I made this kind of training, following the “Food Safety Pyramid”, proved a better understanding regarding this system.

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## **MONITORING HEAVY METAL CONTAMINATION OF CARP (*CYPRINUS CARPIO*) FROM FISH FARMS IN BRAILA**

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**Key words:** carp, heavy metal, contamination

### **SUMMARY**

The main purpose of this research is to quantify the accumulation of heavy metals (Pb, Cr, Cd, Ni) in various organs (gills, liver, kidney, muscle dorsal) in *Cyprinus carpio* (common carp), depending on age and exposure period to different contaminants (heavy metals).

In addition to other aquatic organisms, fish can be widely used to assess the health of aquatic ecosystems. In this context, because fish are an important food resource and a major component of aquatic ecosystems, assessment of contamination is very important.

In addition, the bioaccumulation of metals in fish tissues may be an indicator of environmental pollution, altering and health fish, even the appearance of degenerative changes observing and oxidative stress, a real biomarker.

Industrial development, that characterizes the current age, and brutal intervention of irrational exploitation of nature by man, has led to fresh water contamination with a wide range of pollutants, become in recent decades, a serious concern [3,5,7].

Natural aquatic systems which is the place of origin, growth, respectively, required environment for all life aquatic organisms, may be contaminated with various pollutants including heavy metals, incredible discharge of industrial and household waste, fertilizers and pesticides, which can not escape the harmful effects of these pollutants.

Contamination with heavy metals can have devastating effects on the ecological balance, with serious repercussions on the health of the fish [1,4,6].

## 1. MATERIAL AND METHODS

The control groups were noted by the following:

- LMP - juvenile control group (n=12), composed of fish from *Cyprinus carpio* species, with the average length of 12-15 cm and average weight of  $38.9 \pm 2.5$  g;

- AML - adult control group (n=10), composed of fish from *Cyprinus carpio* species, with average length of 30-35 cm and average weight of  $1.550 \text{ g} \pm 0.150 \text{ g}$ .

Experimental groups of juvenile and adult fish species coming from *Cyprinus carpio* (common carp) were harvested from the Brăila's Lake, in which were detected the presence of heavy metals.

Experimental groups of common carp (*Cyprinus carpio*), were composed of fish who had approximately the same length (about 12-15 cm) and weight (mean  $38.9 \pm 2.5$  g).

Depending on the period of exposure to the toxic (heavy metals), after harvesting juveniles, were made following batches, denoted as follows:

- LP1 (n=10) harvesting performed at 1 day after exposure to toxic;
- LP2 (n=11)- harvesting performed at 7 days after exposure to toxic;
- LP3 (n=12)- harvesting performed at 14 days of exposure to toxic;
- LP4 (n=10) - harvesting performed at 7 days after exposure to toxic.

Adult experimental plots (*Cyprinus carpio*) were formed by individuals who had approximately the same length (about 30-35 cm) and weight ( $1550 \text{ g} \pm 0,150 \text{ g}$ ).

Depending on the time of exposure to toxic, harvesting was done on different days, forming, experimental groups, noted:

- LP1 (n=5) harvesting performed at 1 day after exposure to toxic;
- LP2 (n=5) harvesting performed at 7 days after exposure to toxic;
- LP3 (n=5) harvesting performed at 14 days of exposure to toxic;
- LP4 (n=5) harvesting performed at 7 days after exposure to toxic.

After harvesting, the fish in each group (control and experimental) were dissected, separated, some organs (gills, liver, kidney and dorsal muscle) according to methods specified by FAO.

Confirmation of the presence of heavy metals in some lakes in the county of Braila, was made by laboratory tests of water samples collected from them.

## 2. RESULTS AND DISCUSSION

In tables 1-2 and charts 1-4 are presented the results of heavy metal dosage, studied in various organs of fish (*Cyprinus carpio*) from control and experimental groups.

Table 1  
The average values of heavy metals level in different organs of *Cyprinus carpio* juveniles from experimental groups

Heavy metals ( $\mu\text{g/g s.p.}$ )		ORGANS EXAMINED				
		GILLS	LIVER	KIDNEY	MUSCLES	
Cr	Control group	0,78±0,02	0,84±0,01	0,92±0,02	1,47±0,02	
	Experimental group	1 day	2,28±0,06**	2,68±0,03**	2,72±0,03**	1,51±0,01*
		7 days	2,64±0,04**	3,02±0,02**	2,93±0,03**	1,74±0,01*
		14 days	3,15±0,04**	3,94±0,02***	3,15±0,03**	1,96±0,02*
		30 days	3,42±0,01**	4,22±0,01***	3,22±0,02**	2,12±0,04**
Ni	Control group	1,03±0,02	0,92±0,02	0,64±0,01	0,60±0,01	
	Experimental group	1 day	2,20±0,07**	2,77±0,02**	1,86±0,02**	0,13±0,01*
		7 days	3,42±0,05**	3,95±0,03**	1,99±0,02**	0,87±0,02*
		14 days	3,64±0,03***	4,42±0,01***	2,21±0,03**	1,23±0,01**
		30 days	3,87±0,02***	4,75±0,02***	2,40±0,02**	1,59±0,028**
Cd	Control group	1,87±0,01	1,42±0,01	1,11±0,01	0,10±0,02	
	Experimental group	1 day	2,80±0,05**	3,76±0,01**	3,29±0,02**	1,12±0,01**
		7 days	3,90±0,04***	4,98±0,01***	4,72±0,03***	1,36±0,01**
		14 days	4,70±0,02***	5,25±0,02***	5,05±0,03***	1,48±0,01**
		30 days	5,10±0,02***	5,58±0,01***	5,31±0,02***	1,62±0,19**
Pb	Control group	1,36±0,02	1,86±0,01	1,78±0,02	0,31±0,03	
	Experimental group	1 day	3,52±0,03**	3,31±0,03**	3,40±0,01**	2,08±0,02*
		7 days	4,12±0,03***	6,74±0,04***	5,43±0,01***	2,31±0,02**
		14 days	4,64±0,01***	8,27±0,04***	6,71±0,02***	2,54±0,01**
		30 days	5,92±0,02***	8,70±0,02***	6,89±0,01***	2,69±0,01**

\*  $p > 0,05$  — insignificant differences  
 \*\*  $p < 0,05$  - significantly differences  
 \*\*\*  $p < 0,01$  — distinct significantly differences

Table 2

The average values of heavy metals level in different organs of *Cyprinus carpio* adults from experimental groups

Heavy metals ( $\mu\text{g/g s.p.}$ )		ORGANS EXAMINED				
		GILLS	LIVER	KIDNEY	MUSCLES	
Cr	Control group	0,64±0,01	0,67±0,01	0,76±0,02	1,10±0,02	
	Experimental group	1,93±0,04*	2,24±0,02*	1,64±0,03*	1,23±0,01*	1,51±0,01*
		2,15±0,03**	2,64±0,02**	2,41±0,03**	1,43±0,01*	1,74±0,01*
		2,48±0,02**	3,13±0,01**	2,62±0,02**	1,65±0,01*	1,96±0,02*
		2,64±0,01**	3,63±0,01***	2,88±0,02**	1,88±0,01**	2,12±0,04**
Ni	Control group	0,98±0,02	0,81±0,01	0,48±0,01	0,45±0,01	
	Experimental group	1,86±0,06*	1,72±0,02*	1,26±0,02*	0,09±0,01*	0,13±0,01*
		2,64±0,04**	2,58±0,02**	1,65±0,02**	0,35±0,01*	0,87±0,02*
		3,36±0,03***	3,95±0,02***	1,91±0,01**	0,68±0,02*	1,23±0,01**
		3,44±0,02***	4,11±0,02***	2,12±0,01**	1,23±0,02**	1,59±0,028**
Cd	Control group	1,74±0,01	1,23±0,01	0,94±0,01	0,10±0,01	
	Experimental group	2,32±0,04*	2,07±0,02*	1,81±0,03*	0,87±0,01*	1,12±0,01**
		3,67±0,04**	3,44±0,02**	2,03±0,03**	1,12±0,01**	1,36±0,01**
		4,50±0,03**	4,20±0,01**	3,36±0,02**	1,34±0,01**	1,48±0,01**
		5,06±0,02***	4,89±0,01***	4,63±0,02***	1,52±0,01**	1,62±0,19**
Pb	Control group	1,10±0,02	1,65±0,01	1,53±0,02	0,25±0,03	
	Experimental group	2,57±0,03*	2,24±0,03*	2,89±0,02*	1,52±0,02**	2,08±0,02*
		3,23±0,03**	4,82±0,03***	4,36±0,02***	1,86±0,01**	2,31±0,02**
		4,06±0,02***	5,43±0,02***	5,83±0,01***	2,20±0,02***	2,54±0,01**
		5,24±0,01***	6,75±0,02***	6,02±0,01***	2,46±0,02***	2,69±0,01**

\*  $p > 0,05$  — insignificant differences  
 \*\*  $p < 0,05$  - significantly differences  
 \*\*\*  $p < 0,01$  — distinct significantly differences

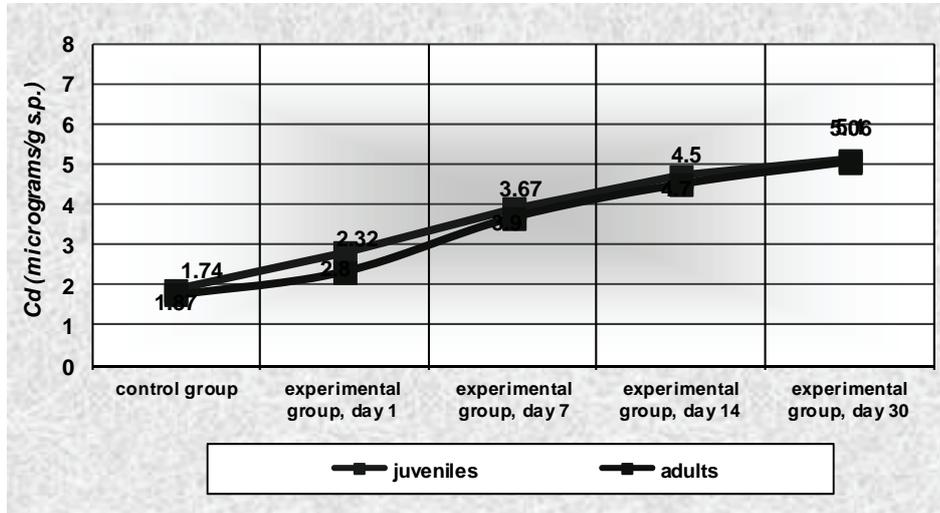


Chart no. 1. The dynamic of average values of cadmium level in gills of *Cyprinus carpio*, juveniles and adults from control and experimental groups

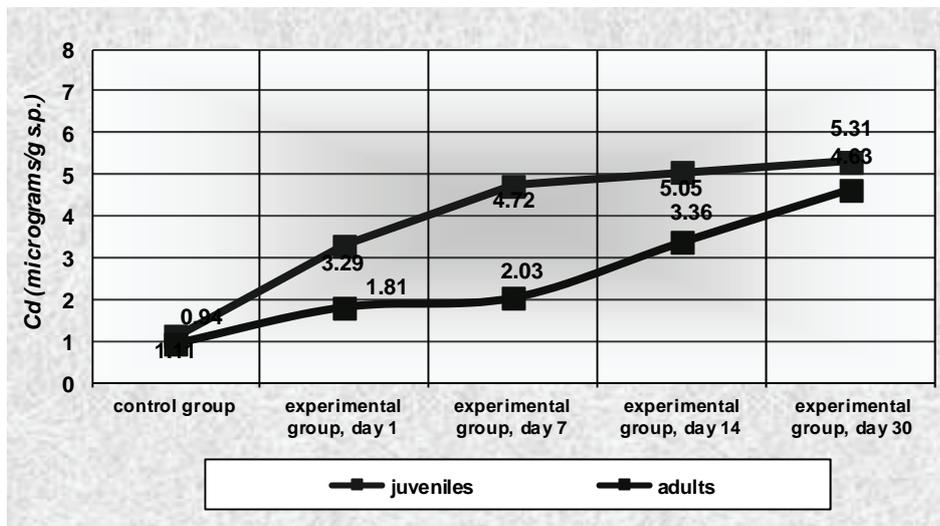


Chart no.2. The dynamic of average values of cadmium level in kidney of *Cyprinus carpio*, juveniles and adults from control and experimental groups

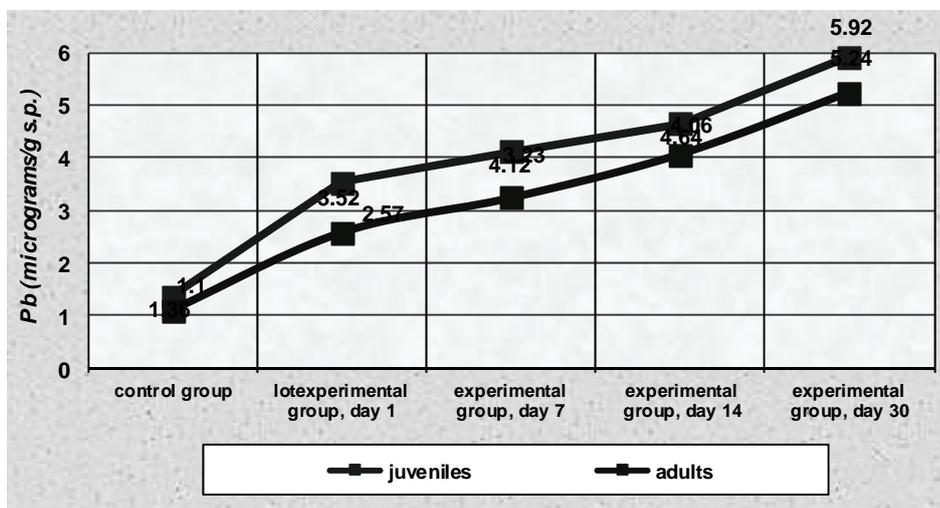


Chart no. 3. The dynamic of average values of lead level in gills of *Cyprinus carpio*, juveniles and adults from control and experimental groups

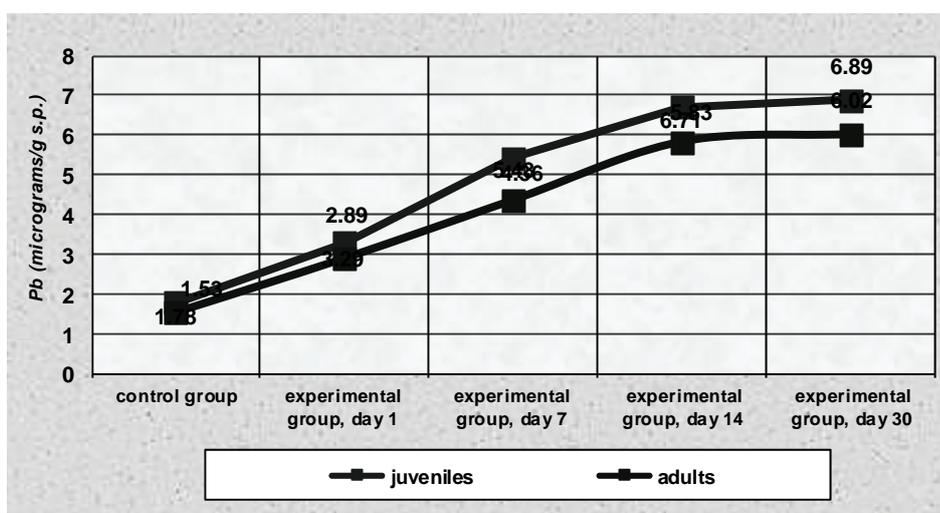


Chart no. The dynamic of average values of lead level in kidney of *Cyprinus carpio*, juveniles and adults from control and experimental groups

After the first day of exposure to toxic juveniles from experimental plots growth is found statistically significant (to the maximum permissible value - according to EC regulation no.1881/2006), for all heavy metals, both in the gills and in the liver and kidneys (table 1).

In contrast, in muscle tissue, which is not considered an active tissue in accumulating heavy metals, as indicated and other authors [2,8], from control group consisting of juveniles, were slightly higher in some cases to maximum limits, without statistical significance ( $p>0.05$ ) in some cases (tabel 1 and charts 1-2).

With increasing time of exposure of fish to heavy metals (7, 14 and 30 days) in adults *Cyprinus carpio*, there is a significant increase ( $p<0.05$ ) and then a separately significant ( $p<0.01$ ) of the mean values of heavy metals (tabel 2 and charts 3-4), in the organs studied (gills, liver, kidney and muscle dorsal).

When the time of exposure to toxic was high (14 and 30 days), there was a proportional increase in the percentage of mortality of adults fish (table 2).

### 3. CONCLUSIONS

3.1. In muscle tissue, which is not considered an active tissue in accumulation of metals, the mean of heavy metals values, that were dosed in the dorsal muscle, were lower than in other organs in both experimental groups (juveniles and adults).

3.2. In all cases studied, there was a greater accumulation of heavy metals in juvenile *Cyprinus carpio*, compared with adults because the juveniles were more efficient in heavy metals assimilation from plankton and detritus formed food, but also through higher capacity of adults to participate in detoxification processes.

3.3. The order in which the concentration of heavy metals accumulated in the organs of juvenile *Cyprinus carpio*, was, in gills and liver:  $Pb > Cd > Ni > Cr$ , in the kidney:  $Pb > Cd > Cr > Ni$ , and in the dorsal muscle:  $Pb > Cr > Cd > Ni$ .

3.4. In adults of *Cyprinus carpio*, order, accumulation of heavy metals in organs was: the gills:  $Cd > Pb > Ni > Cr$ ; liver:  $Pb > Cd > Ni > Cr$ , in the kidney:  $Pb > Cd > Cr > Ni$  and the dorsi muscle:  $Pb > Cr > Cd > Ni$ .

3.5. Bioaccumulation of lead and cadmium in the organs of common carp (*Cyprinus carpio*) from both groups (juveniles and adults) was significantly higher ( $p<0.05$ ) than other studied heavy metals (chromium and nickel).

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## **THE IMPACT OF TOXIC HEAVY METALS ON THE HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN COMMON CARP (*CYPRINUS CARPIO*)**

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**Key words:** carp, heavy metal, contamination

### **SUMMARY**

Hematological and biochemical profile of fish, proved to be a sensitive indicator for the assessment of metabolism under metal stress in these aquatic organisms.

The main purpose of this study was to investigate the hematological and biochemical changes in common carp (*Cyprinus carpio*), according to the period of exposure to different contaminants (heavy metals).

Studies in various species of fish, but in particular on common carp (*Cyprinus carpio*) showed that heavy metals can cause changes in physiological, hematological and biochemical parameters and health alterations.

Industrial waste is the major source of pollution with heavy metals in flowing or standing water. Aquatic systems are exposed to a range of pollutants (including heavy metals) that are mainly coming from discharges from metallurgical industry, steel mills [5,8].

These pollutants are highly toxic for all aquatic organisms, irreversibly altering their health, depending on dose and exposure time.

Cadmium is a heavy metal, considered one of the most toxic contaminants of water and can cause changes in blood cell populations at any level of toxicity [2,7].

Lead causes also changes in blood picture, by inhibiting the activity of many enzymes involved in heme biosynthesis.

Nickel can cause some morphological changes and chromosomal aberrations of cellular systems [1].

Chronic exposure to creatures from Cr (VI) can cause damage to the DNA [3].

## 1. MATERIAL AND METHODS

Investigations in this chapter were made on juvenile carp (*Cyprinus carpio*), with average length of 12-15 cm and average weight  $38.9 \pm 2.5$  g, harvested from Braila county ponds.

Thus, fish from the control group showed, after laboratory analysis, physico-chemical parameters between the normal values provided by law nr.202/2002.

The control groups were noted - LM - juvenile control group (n=12), composed of fish from *Cyprinus carpio* species with the average length of 12-15 cm and average weight of  $38.9 \pm 2.5$  g;

Experimental groups of common carp, were composed of fish who had approximately the same length and weigh. Depending on the period of exposure to the toxic (heavy metals), after harvesting juveniles, were made following groups, denoted as follows:

- LP1 (n=10) - harvesting performed at 1 day after exposure to toxic;
- LP2 (n=10) - harvesting performed at 7 days after exposure to toxic;
- LP3 (n=10) - harvesting performed at 14 days of exposure to toxic;
- LP4 (n=10) - harvesting performed at 7 days after exposure to toxic.

Blood samples were collected by cardiac puncture method, using special tubes. Determination of blood parameters was performed by special methods such as, for hemoglobin using Drabkin and Austin method, for total cholesterol - method of Allain (1974) and Young (1997), red blood cells by the Ochei Kolhatkar method (2005) and of packed cell volume (PCV) by Wintrobe tubes method.

## 2. RESULTS AND DISCUSSION

The results of this study demonstrated the toxic effects of heavy metals in fish from experimental group, causing changes in hematological and biochemical parameters.

Our research showed significant increases in heavy metals in different organs of fish from experimental group, increases that are directly proportional to exposure period.

After blood and biochemical tests which made from common carp (*Cyprinus carpio*), collected from Braila county ponds, there were some differences which are outlined below.

*Table 1*

The average values of hematological and biochemical parameters in fish (*Cyprinus carpio*) from control and experimental groups

Hematological & biochemical parameters	Control group	Experimental groups			
		L1 1 day after exposure to toxic	L2 7 days after exposure to toxic	L3 14 days after exposure to toxic	L4 30 day after exposure to toxic
<b>Hb</b> (g/dl)	7,38±1,3	7,10±1,3*	6,40±1,2*	5,92±1,2**	5,25±1,2**
<b>PCV</b> (%)	30,84±0,4	29,42±0,4*	28,65±0,3*	27,12±0,2**	26,14±0,26**
<b>Red cells</b> (mil./mmc)	4,11±0,1	3,94±0,1*	3,53±0,1**	3,35±0,1**	3,12±0,1**
<b>Glucose</b> (mmol/l)	3,86±0,10	4,82±0,10*	6,25±0,11**	8,67±0,12***	9,20±0,15***
<b>Cholesterol</b> (mg/dl)	2,66±0,06	3,10±0,05*	2,50±0,05*	3,20±0,06*	2,95±0,06*
<b>Vitamin C</b> (mg/dl)	15,9±0,03	12,5±0,02*	9,6±0,01**	7,4±0,02***	5,5±0,03***
<b>Fe</b> (µg/dl)	75,6±0,02	81,1±0,02*	87,9±0,02**	90,5±0,02**	92,4±0,02**
<b>Cu</b> (µg/dl)	79,92±0,03	84,7±0,03*	96,2±0,02*	104,1±0,02**	118,3 ±0,02**

\*  $p > 0,05$  —insignificant differences

\*\*  $p < 0,05$  - significantly differences

\*\*\*  $p < 0,01$  —distinct significantly differences

During the study, hemoglobin concentration decreased significantly ( $p < 0,05$ ) in the blood of fish, exposed to heavy metals (table 1 and chart 1).

Thus, we can see that some heavy metals such as cadmium, chromium, nickel and lead, change the properties of hemoglobin and decrease their affinity to oxygen.

Therefore, erythrocytes are more fragile and more permeable, making them to deform and then to destroy, while determining and reducing the number of red blood cells, a fact noted by other authors [7].

Chart no.1. The dynamics of average values of direct erythrocyte constants in fish (*Cyprinus carpio*) from control and experimental groups

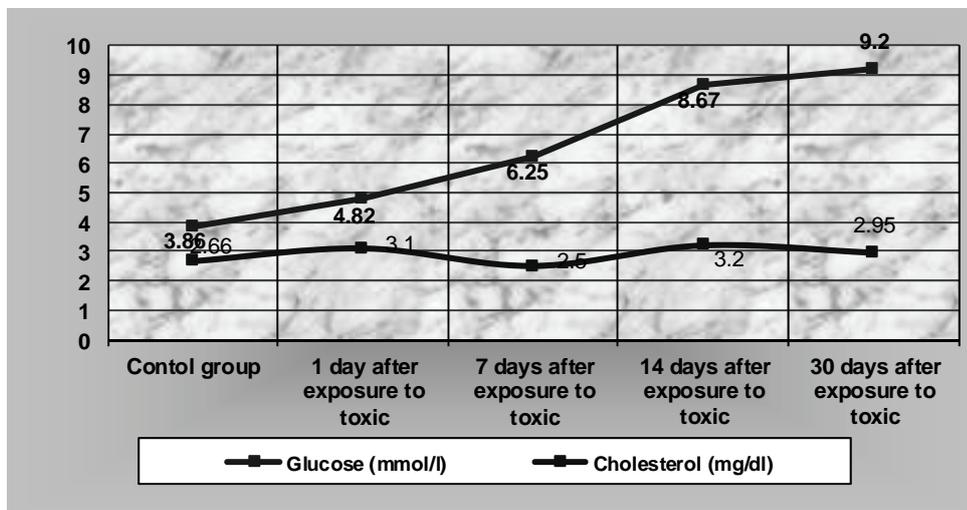
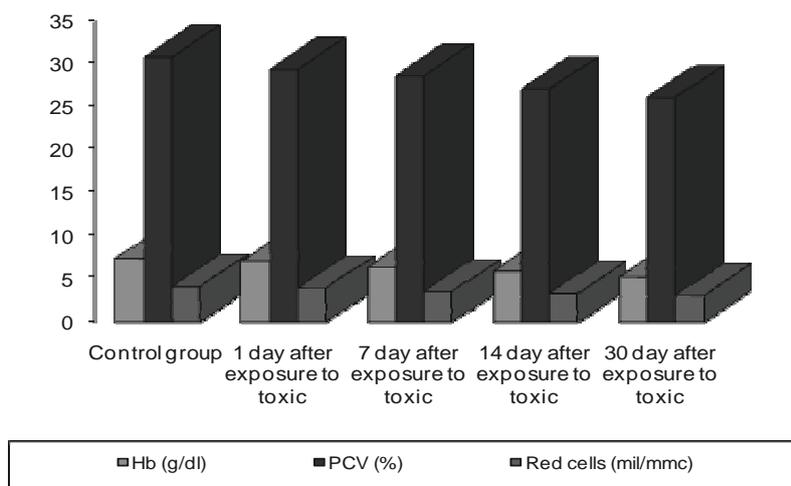


Chart no.2. The dynamics of average values of glucose and cholesterol levels in fish (*Cyprinus carpio*) from control and experimental groups



It was also observed that heavy metals induced a significant decrease ( $p < 0.05$ ) of packed cell volume (PCV) levels in fish from experimental groups (table 1 and chart 2), and our results are in agreement with work of other authors, which also reported a significant decrease in hemoglobin, in average number of erythrocytes and packed cell volume (PCV) in common carp, exposed to heavy metals at different times [4,6].

As a result of biochemical tests in fish from experimental groups (exposed to different heavy metals), we can also notice the existence of changes with statistical significance ( $p < 0.05$ ) of biochemical parameters taken into account (table 1 and chart 2).

### 3. CONCLUSIONS

In this research, we investigated the hematological and biochemical changes in common carp (*Cyprinus carpio*), according to the exposure period to the toxic (heavy metals).

3.1. Hematological and biochemical profile of fish proved to be a sensitive indicator for the assessment of metabolism, under metal stress.

3.2. Studies in various species of fish, but in particular on common carp (*Cyprinus carpio*), showed that heavy metals can cause changes in physiological, biochemical and hematological parameters and alterations of health.

3.3. The results revealed a hemato-toxic effect in fish from experimental groups, because there was a low level of hemoglobin, packed cell volume (PCV) and red blood cell number.

3.4. The result of biochemical tests in fish from experimental groups (exposed to different heavy metals), showed an existence of changes, with statistical significance ( $p < 0.05$ ), of biochemical parameters taken into account.

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## ASSESSMENT OF LEAD LEVELS IN COTTAGE SALTY CHEESE FROM COW'S MILK IN BAI A MARE INDUSTRIAL AREA

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**Key words:** lead, pollution, cheese, milk products

### SUMMARY

Milk products are very important components of human diet. The presence of toxic element in milk products can cause serious health problems. The aim of the study was the evaluation of cottage salty cheese from cow's milk contamination with lead in nine surrounding localities of the industrial area Baia Mare (0-60 km). Lead determination was carried out during 2007-2010 periods on 180 cottage salty cheese samples collected from free market or private households and originated from different localities situated at increasing distances from polluting source, Baia Mare industry (S.C. Romplumb S.A. and S.C. Cuprom Bucuresti/Baia Mare-branch). Lead was determined by mass spectrometer with inductive coupled plasma (ICP-MS) Perkin Elmer. The mean concentration of lead in cottage salty cheese was progressively lower, but statistically insignificant ( $p > 0.05$ ) in all localities studied, in the period 2007-2009, and slightly higher in 2010 in most localities (except Săsar locality, where it was reported the same amount of concentration in 2009 and 2010, and Tăuții Măgheraș locality, where in 2010 there was an insignificant decrease). Comparing the lead levels from the industrial area with the levels obtained from the control area (Sighetul Marmației), the values were significantly higher ( $p < 0.01$ ) during the entire period. There were insignificant differences in the polluted area samples in 2010 comparative to 2007. The mean annual lead level surpassed the maximum levels according to Commission of Regulation (E.C.) 1881/2006 (0.1 mg/kg), excepting those in unpolluted control area (Sighetul Marmației) and Tăuții Măgheraș locality, where in 2010 the concentration value reported was at the limit of maximum permissible concentration.

The danger represented by pollution increases continuously enforcing upon urgent measures against it (Teușdea and Lungeanu, 1996).

The residues from chemical industry, nonferrous metals, are the most important sources of chemical pollution, because they are spread and act on extended areas, even over the state borders (Brown, 2000; Falandysz, 1991; Hura, 1997). In this context, heavy metals represent a problem along trophic chain and represent a permanent risk for environment health.

Lead is considered number one pollutant because of frequent poisoning in animals and an important public health problem (Younes *et al.*, 1995). Lead poisoning is considered to be one of the most difficult environmental health problems to control since it does not show any unique manifestation during its early stage.

Lead is a pervasive environmental pollutant with potential public health hazard as a food contaminant of animal origin (Swarup *et al.*, 2004). The presence of lead in animal products is a consequence of environmental pollution in all its segments, accidental introduction of this element in animal products, the addition of spices and adjuncts contaminated with lead, the storage in various containers with high levels of lead and under the influence of certain factors as a result being transferred to the food products (Boltea *et al.*, 2008). Lead, among other heavy metals, is one of the major pollutants in Baia Mare region where there is a specific industry, because of its cumulative effect with well known toxic effects.

Milk and milk products are foods with major implications in man's health. In the last few years, the contamination of milk and milk products is considered as one of the main dangerous aspects (Abou Ayana *et al.*, 2011). Typically, a food product can be correlated with its geographical localization, the quality of raw material and the production techniques (Brescia *et al.*, 2005; Scott *et al.*, 1998). Research concerning concentration levels of metals in milk and milk products are important for risk assessment for the consumer.

Taking into account the previous affirmation and due to the high pollution level in Baia Mare area, the present research was developed. Hence, the aim of the present study was to evaluate cottage salty cheese from cow's milk contamination with lead in industrial area Baia Mare. The objectives were: determination of lead level in cottage salty cheese collected from free market and private households and originated from different localities at increasing distances from the polluting source, Baia Mare industry (S.C. Romplumb S.A. and S.C. Cuprom București/Baia Mare branch); lead level dynamics related to the distance from the polluting source (annual, and during the whole studied period 2007-2010).

## 1. MATERIAL AND METHODS

The evaluation of lead level in cottage salty cheese from cow's milk in the Baia Mare area extended during 2007-2010 period. Cottage salty cheese samples (n=180) were collected from free market and private households originating from different locations at increasing distances off the polluting source (SC Romplumb S.A. and S.C. Cuprom București/ Baia Mare branch): Ferneziu (Baia Mare suburb.), Satu Nou de Sus (5 km), Groși (7 km), Recea (9 km) Săsar (9 km), Mocira (10 km), Baia Sprie (12 km), Tăuții Măgherauș (15 km) and Sighetul Marmației (60 km-control area).

Lead concentration was determined by mass spectrometer with inductive coupled plasma (ICP-MS) Perkin Elmer model Elan DRC-e equipped with reaction cell, to remove polyatomic interferences created by plasma, after a preliminary mineralization in a mineralization oven, Multiwave 3000 type.

The data were statistically analyzed by Anova Methods and Student *t*-test.

## 2. RESULTS AND DISCUSSION

In this study, lead level was inversely correlated, with different degrees of significance, to the distance from the polluting source, during the entire period.

The annual average concentration of lead in cottage salty cheese samples decreased progressively, but insignificantly ( $p > 0.05$ ) in all analyzed localities, in the period 2007-2009 and was slightly higher in 2010 in most localities, but insignificant ( $p > 0.05$ ). Some exceptions have been reported in Săsar locality, where it was reported the same amount of concentration in 2009 and 2010, and Tăuții Măgherauș locality, where in 2010 there was an insignificant decrease ( $p > 0.05$ ).

Compared to 2007, in 2010, the annual average concentration of lead in cottage salty cheese from cow's milk was lower in the samples from all localities, but the difference between the concentrations recorded in these two years being insignificant ( $p > 0.05$ ).

The results concerning lead level dynamics in cottage salty cheese from cow's milk are summarized in Table 1 and Fig.1.

Table 1

The average concentrations of lead (mg/kg) in cottage salty cheese from cow's milk during 2007-2010 period

Area/Locality	X±Sx			
	2007	2008	2009	2010
<b>Ferneziu</b>	0,179±0,023	0,171±0,016 ns	0,145±0,016 ns	0,150±0,023 ns/ns
<b>Satu Nou de Sus</b>	0,156±0,019	0,135±0,025 ns	0,127±0,021 ns	0,130±0,016 ns/ns
<b>Groși</b>	0,132±0,017	0,129±0,020 ns	0,121±0,014 ns	0,123±0,022 ns/ns
<b>Recea</b>	0,122±0,020	0,116±0,017 ns	0,102±0,013 ns	0,108±0,015 ns/ns
<b>Săsar</b>	0,123±0,009	0,115±0,013 ns	0,108±0,014 ns	0,108±0,008 ns/ns
<b>Mocira</b>	0,120±0,022	0,117±0,019 ns	0,108±0,017 ns	0,111±0,018 ns/ns
<b>Baia Sprie</b>	0,120±0,010	0,114±0,013 ns	0,107±0,009 ns	0,111±0,012 ns/ns
<b>Tăuții Măgherauș</b>	0,114±0,012	0,107±0,013 ns	0,102±0,009 ns	0,100±0,011 ns/ns
<b>Sighetul Marmației</b>	0,021±0,005	0,0187±0,005 ns	0,019±0,004 ns	0,020±0,009 ns/ns
<b>ML</b> - according to Commission Regulation (E.C.) nr.1881/2006				0,1 mg/kg

M.L. – Maximum Level;

ns (p>0.05) -insignificant;

• - the significance of the difference between 2008/2007, 2009/2008;

•/ - the significance of the difference between 2010 and 2009;

/• - the significance of the difference between 2010 and 2007;

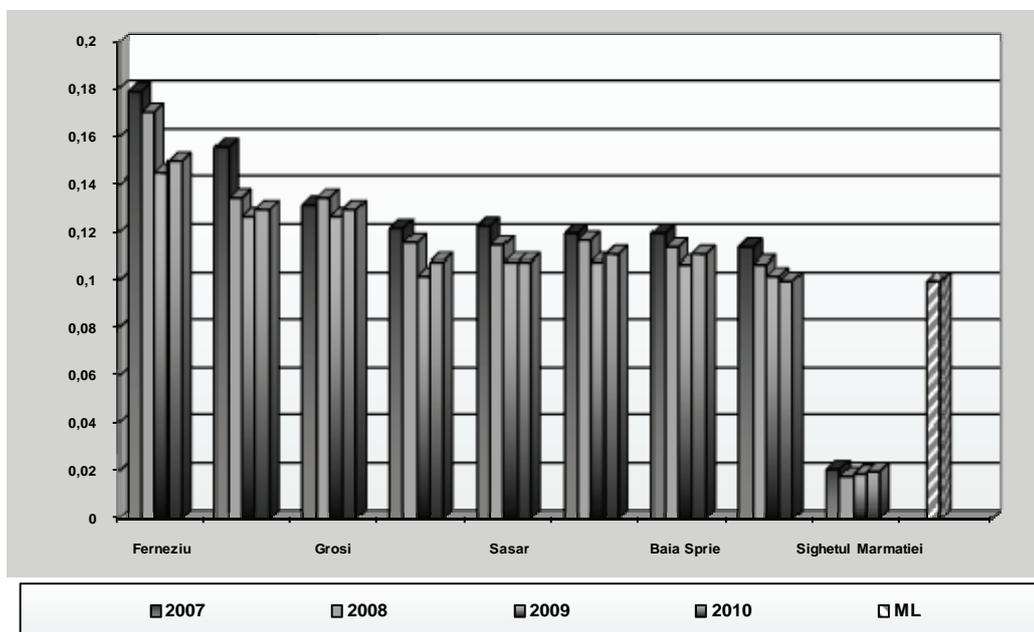


Fig.1 Annual dynamics of lead concentration in cottage salty cheese from cow's milk (mg/kg) during 2007- 2010 period

The percentage increase of annual average concentration of lead in cottage salty cheese from cow's milk differed depending on the locality and the years compared:

- Ferneziu: 2008/2007: -4,46%; 2009/2008: -15,20%; 2010/2009: +3,44%; 2010/2007: -16,2%;
- Satu Nou de Sus: 2008/2007: -13,46%; 2009/2008: -5,92%; 2010/2009: +2,36%; 2010/2007: -16,6%;
- Groși: 2008/2007: -2,27%; 2009/2008: -6,2%; 2010/2009: +1,65%; 2010/2007: -6,81%;
- Recea: 2008/2007: -4,91%; 2009/2008: -12,06%; 2010/2009: +5,88%; 2010/2007: -11,47%;
- Săsar: 2008/2007: -6,5%; 2009/2008: -6,08%; 2010/2009: equal concentrations; 2010/2007: -12,19%;
- Mocira: 2008/2007: -2,5%; 2009/2008: -7,69%; 2010/2009: +2,77%; 2010/2007: -7,5%;

- Baia Sprie: 2008/2007: -5%; 2009/2008: -6,14%; 2010/2009: +3,73%; 2010/2007: -7,5%;
- Tăuții Măgherauș: 2008/2007: -6,14%; 2009/2008: -4,67%; 2010/2009: -1,96%; 2010/2007: -12,28%.

Throughout the period under study was reported over passed M.L. according to Commission Regulation (E.C.) nr.1881/ 2006 in samples from each locality placed in polluted area, except the samples taken from the Sighetul Marmației area (unexposed to pollution risk) and Tăuții Măgherauș locality, in 2010, when the concentration value reported was at the limit of the Maximum Level.

In this study, in polluted area, the obtained lead levels were lower (0.114-0.137 mg/kg) than those of Mendil (2006) in different sort of cheese in Turkey (0.18-0.34 mg/kg). Similar differences were obtained comparing to the results of Gogoasă *et al.*, (2006) (0.193-0.314 mg/kg), in three sorts of sheep cheese in Banat-Romania.

Lead levels in the samples from unpolluted area ranged in limits of other authors as Florea *et al.*, (2006) (0-0.08mg/kg) in Dalia cheese in Harghita county (Romania), as Tahvonon and Kumpulainen (1995), 0.017 mg/kg in Finnish cheese and 0.017-0.06 mg/kg in imported cheese, and as Anastasio *et al.*, (2006), 0.47 µg/g in fresh cheese, 0.391 µg/g in Riceetto cheese and 0.58 µg/g in other types of cheese, in southern Italy. In Baia Mare area, Bușcan (2011) registered in all fresh cheese samples not detectable values and in cottage salty cheese values ranging from 0 to 0.1 mg/kg.

### 3. CONCLUSIONS

Studying the dynamics and the lead level in cottage salty cheese from cow's milk samples taken from localities within a radius of 0-60 km from Baia Mare area polluting industry during 2007- 2010 period revealed the following aspects:

- 3.1.exceeded the maximum levels by the European legislation (Reg. CE 1881/2006) of the lead concentration in the whole period under study, except for the samples taken from the Sighetul Marmației area (unexposed to pollution risk) and Tăuții Măgherauș, in 2010, when the concentration value reported was at the limit of the Maximum Levels;

- 3.2. progressively lower, but statistically insignificant as regarding the annual concentrations of lead in the cottage salty cheese, in the period 2007-2009, slightly higher in 2010 (except Săsar locality, where it was reported the same concentration in 2009 and 2010, and Tăuții Măgherauș locality, where in 2010 there was an insignificant decrease) of the annual average concentrations and the registering in 2010 of some slightly lower concentrations compared to 2007 samples from all localities studied;
- 3.3. annual average concentrations of lead strongly significantly higher in samples taken from locations at risk of pollution as compared to those of the unpolluted area, Sighetul Marmației, and, with different degrees of significance, lower than those from the highly polluted locality, Ferneziu-Baia Mare;
- 3.4. insignificant differences between the villages located within a radius of 0-15 km from the main sources of pollution;
- 3.5. fluctuations, statistically insignificant regarding the annual average concentrations, insignificant differences between the levels recorded in 2010 than in 2007 and framing within the Maximum Levels of annual average concentrations of the samples with minimal risk of pollution (control), located at 60 km from the sources of pollution.

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## CADMIUM CONTAMINATION LEVEL OF MILK IN BAI A MARE INDUSTRIAL AREA

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**Key words:** cadmium, milk, pollution, industry

### SUMMARY

Since cadmium is an element that exists naturally in the environment, all foods contain a certain level of cadmium, so that the entire population is exposed to the risk of contamination of this pollutant. The aim of this study was to assess the degree of milk contamination by cadmium taken from nine surrounding localities around the industrial area Baia Mare located over a distance of 0-60 km from the source of pollution. The study was conducted over a period of four years (2007-2010), being analyzed 660 milk samples. The determination of the cadmium levels in milk samples was performed by the method of Inductively coupled plasma mass spectrometry (ICP-MS), using the Perkin Elmer mass spectrometer with inductively coupled plasma (ICP- MS), Elan DRC model, which is equipped with a reaction cell in order to remove the polyatomic interferences created in the plasma and the mineralization furnace from Perkin Elmer, type Multiwave 3000, with temperatures and time control. The concentrations of cadmium that were recorded both in the milk samples taken from the localities exposed to pollution and from the unpolluted area were lower than maximum levels according to the Order No. 975/1998, throughout the period under review. The annual average concentration of cadmium in milk fell insignificantly ( $p>0.05$ ) in most localities taken in the study (except the locality situated at 7 Km, in 2008, and the locality situated at 9 km, in 2010), the difference between the concentration recorded in the year 2010, as compared with 2007 being insignificant ( $p>0.05$ ). The annual average concentrations of cadmium in the samples collected from the area at risk of pollution were heavily significantly ( $p<0.01$ ) higher than those in the unpolluted area, Sighetul Marmației.

In the industrialized environment, cadmium, together with arsenic, lead, mercury and uranium plays an important role in the environmental pollution (Andersen *et al.*, 1992, Oehme, 1978).

Because it comes from numerous sources, cadmium can enter the food chain air-water-soil, and then directly through the air and water, in animal bodies, or indirectly through fodder plants that can accumulate on average 0,6 ppm cadmium (greater amounts in beet). In animal feed cadmium concentration is much higher (Ansay *et al.*, 1990, Savu and Georgescu, 2004).

The ingestion of cadmium in food is the main source through which it gets into the human and animal body. The average of daily ingested cadmium by humans along with food, was estimated to be around 10-50  $\mu\text{g}/\text{zi}$ , this average value may be growing dozens of times in the polluted areas (Houpert *et al.*, 1995, Hura, 2005).

The most important food intake of cadmium and, at the same time, the biggest risk factor for man is represented by the consumption of milk and meat derived from domestic ruminants from the polluted areas, the more so as their milk and meat are basic foods in human nutrition, but also raw materials for producing other food supply of animal origin. This is proven by numerous studies and research carried out both in our country (Decun *et al.*, 1995, Dumitrache *et al.*, 1993), and in other countries (Jonson *et al.*, 1981, Kluge—Berge *et al.*, 1992).

In milk, normally, 2 to 10 mg Cd/l are found, but the concentration may increase by up to 10 times in the event of contamination of animal body, the more the milk is milked in glazed pots or comes into contact with the devices of cadmium alloys during mechanical milking and/or industrial processing (in which case the cadmium concentration can increase by up to 50 times) (Decun *et al.*, 1995). With this in mind, the purpose of this study was to evaluate the degree of cadmium contamination of milk collected on the free market and private households from the industrial area of Baia Mare.

## 1. MATERIAL AND METHODS

The evaluation of cadmium level in milk from Baia Mare area extended during 2007-2010 period. Milk samples (n=660) were collected from free market and private households originating from different locations at increasing distances from the polluting source (SC Romplumb S.A. and S.C. Cuprom București/ Baia Mare branch): Ferneziu (Baia Mare suburb.), Satu Nou de Sus (5 km), Groși (7 km), Recea (9 km) Săsar (9 km), Mocira (10 km), Baia Sprie (12 km), Tăuții Măgherăuș (15 km) and Sighetul Marmației (60 km-control area).

Cadmium concentration was determined by mass spectrometer with inductive coupled plasma (ICP-MS) Perkin Elmer model Elan DRC-e equipped with reaction cell, to remove polyatomic interferences created by plasma, after a preliminary mineralization in a mineralization oven, Multiwave 3000 type.

The data were statistically analyzed by Anova Methods and Student *t*-test.

## 2. RESULTS AND DISCUSSION

The annual average concentration of cadmium in milk decreased progressively, but insignificantly ( $p>0.05$ ) in most localities in the survey, in the period 2007-2010. Some exceptions have been reported in Groși locality, where in 2008 the concentration of cadmium in milk increased slightly, but insignificantly ( $p>0.05$ ) and in Recea locality, where it has been reported the same value of concentration, both in 2009 and 2010.

Compared to 2007, in 2010, the annual average concentration of cadmium in milk was lower in the samples taken from all localities, but the difference between the concentrations recorded in these two years being insignificant ( $p>0.05$ ).

The annual growth rate of cadmium concentration in milk is summarized in Table1 and Fig.1.

Table1

The average concentration of cadmium (mg/kg) in milk during 2007-2010 period

Area/ Locality	X±Sx			
	2007	2008	2009	2010
<b>Ferneziu</b>	0,00108±0,00029	0,00105±0,00026 ns	0,00088±0,00024 ns	0,00082±0,00023 ns/ns
<b>Satu Nou de Sus</b>	0,00087±0,00023	0,00083±0,00020 ns	0,00081±0,00021 ns	0,00078±0,00022 ns/ns
<b>Groși</b>	0,00072±0,00018	0,00073±0,00022 ns	0,00069±0,00014 ns	0,00067±0,00019 ns/ns
<b>Recea</b>	0,00047±0,00014	0,00042±0,00013 ns	0,00038±0,00014 ns	0,00038±0,00013 ns/ns
<b>Săsar</b>	0,00051±0,00013	0,00048±0,00014 ns	0,00045±0,00014 ns	0,00044±0,00014 ns/ns
<b>Mocira</b>	0,00045±0,00014	0,00043±0,00013 ns	0,00042±0,00012 ns	0,00039±0,00013 ns/ns
<b>Baia Sprie</b>	0,00048±0,00014	0,00045±0,00016 ns	0,00043±0,00014 ns	0,00041±0,00014 ns/ns
<b>Tăuții Măgherăuș</b>	0,00039±0,00013	0,00036±0,00013 ns	0,00032±0,00011 ns	0,00029±0,00012 ns/ns
<b>Sighetul Marmației control area</b>	0,00003±0,00001	0,00001±0,00001 ns	0,00002±0,00001 ns	0,00003±0,00001 ns/ns
ML —according to the Order no. 975/1998			0,01 mg/kg	

M.L.—Maximum Level; ns ( $p>0.05$ ) -insignificant;

- - the significance of the difference between 2008/2007, 2009/2008;
- / - the significance of the difference between 2010 and 2009;
- /• - the significance of the difference between 2010 and 2007;

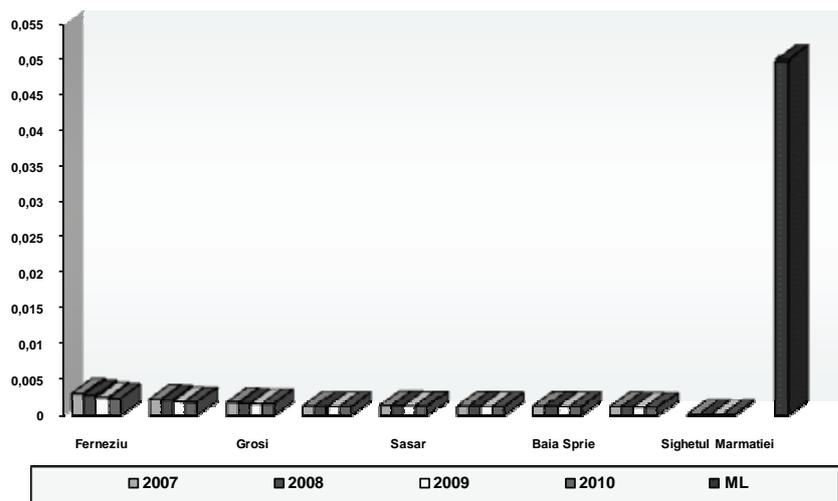


Fig.1 Annual cadmium concentration dynamics in milk (mg/kg) during 2007- 2010 period

The percentage dynamics of annual average concentration of cadmium in milk differed depending on the locality and the years compared:

- Ferneziu: 2008/2007: -2,77%; 2009/2008: -16,19%; 2010/2009: -6,81%; 2010/2007: -24%;
- Satu Nou de Sus: 2008/2007: -4,59%; 2009/2008: -2,4%; 2010/2009: -3,7%; 2010/2007: -10,34%;
- Groși: 2008/2007: +1,38%; 2009/2008: -5,47%; 2010/2009: 2,89%; 2010/2007: -6,94%;
- Recea: 2008/2007: -10,63%; 2009/2008: -9,52%; 2010/2009: concentrations equal; 2010/2007: -19,14%;
- Săsar: 2008/2007: -5,88%; 2009/2008: -6,25%; 2010/2009: -2,22%; 2010/2007: -13,72%;

- Mocira: 2008/2007: -4,44%; 2009/2008: -2,32%; 2010/2009: -7,14%; 2010/2007: -13,33%;
- Baia Sprie: 2008/2007: -6,25%; 2009/2008: -4,44%; 2010/2009: -4,65 %; 2010/2007: -14,58%;
- Tăuții Măgherauș: 2008/2007: -7,59%; 2009/2008: -11,11%; 2010/2009: -9,37%; 2010/2007: -25,64%;

During the survey period, the average concentration of cadmium in milk was lower, compared with the maximum levels according to Order No. 975/1998, both in the unpolluted area and in the regions at risk of pollution.

As a result of the study on milk samples collected from the area exposed to pollution (Baia Mare), it have been reported lower levels of cadmium (0,00052-0,000062 mg/Kg) compared to those found by Elham *et al.* (2011), 0,007 mg/kg, in Egypt, Zugravu *et al.* (2008), 0,008 mg/kg, in Brașov and 0,015 mg/kg in Dâmbovița (Romania). Also, Florea *et al.* (2006) has recorded higher cadmium levels in milk samples, ranging from 0-0,007 mg/kg, in the area of Harghita, Romania and Ogabiela *et al.* (2011), 0,1631 mg/l (range 0,361-0,03 mg/l), in Kano, Nigeria and 0,0986 mg/l (with values between 0,193-0,031 mg/l), in Zaria, Nigeria.

The level of cadmium in milk, recorded in the samples collected, during the survey period, in the area exposed to the risk of pollution was comparable with the one reported by other authors: Patra *et al.* (2008), 0,23 μg/l, in the samples collected from the vicinity of some industrial objectives in India, [Mehennaoui](#) *et al.* (1999) 0,4 μg/l in the sheep's milk and less than 0,2 μg/l in the cow's milk, and Anastasio *et al.* (2006), 0,07 μg/g (with values ranging from 0,05-0,1 μg/kg), in southern Italy.

### 3. CONCLUSIONS

As a result of the study on the assessment of the degree of milk contamination by cadmium from the industrial area of Baia Mare, the following conclusions were drawn:

- 3.1. lower concentrations, as compared to maximum level according to the Order no. 975/1998, both in samples from localities situated in the area exposed to pollution (Baia Mare area), and from the unpolluted area (Sighetul Marmăției area), throughout the period under review;

- 3.2.annual average concentrations of cadmium in samples from localities at risk of pollution significantly strong, higher than those of the unpolluted area, which is, Sighetul Marmației and, with varying degrees of significance, lower than the most intense locality polluted Ferneziu-Baia Mare;
- 3.3.the progressive decrease, but insignificant, of the annual average concentration of cadmium in milk in the period 2007-2010, in most localities studied (except Groși locality, where in 2008, there was a slight increase and Recea locality, where was reported the same amount of concentration both in 2009 and 2010) the difference between the concentration recorded in 2010 compared to 2007 was statistically irrelevant in all localities studied;
- 3.4.irrelevant differences between villages located within a radius of 0-15 km from the main sources of pollution;
- 3.5.fluctuations, statistically insignificant, of the average annual concentrations in samples taken from the unpolluted area and irrelevant differences between the levels recorded in 2010 than in 2007;

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## **THE RELATION BETWEEN AN OPTIMAL ENERGY-PROTEIN RATIO IN MIXED FODDER AND THE MAIN PARAMETERS OF THE GROWTH IN YOUNG RABBITS**

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**Keywords:** energy, protein, weight gain, specific consumption, rabbit

### **SUMMARY**

Research over the last decade on youth rabbit nutrition analyzed unilateral energy requirements and issues of optimal mixed fodder protein in rabbits administered. We considered it necessary to approach a new concept of energy-protein interrelationships, energy-amino acids, energy -cellulose, all with important implications on growth performance, health maintenance and obtaining high-quality housing.

Prolificacy and high speed of growth of farmed rabbits are features that were required for the adoption of intensive farming systems and provide the market with high biological value protein. Rabbits have a specific phenomenon-caecotrophy, which allows recycling of part of the caecal contents, rich in bacterial proteins, a phenomenon that requires the particular requirements of nutritional parameters such as the level and quality of fiber, energy sources etc. nature. Caecal ecosystem plays an important role in animal health, directly and indirectly interfering with the immune status.

Research undertaken in the last 15 years (Garcia J et al., 1996, Falcao E et al., 1996) have shown that daily intake caecotrophy global protein is 10-20% and that rabbits, like other monogastric, must find the 10 essential amino acids in food from the 21 that enter into the composition of proteins normally. Most nutrition researchers accept the idea that rabbits voluntarily adjust their food intake according to energy levels of mixed fodder (Partidge GC, 1989, Drogoul C et al 2004). It was found and was accepted in principle that this regulation is permitted between the concentration limits of

9.2 and 13.4 ED Mj / kg mixed fodder, but in many cases can not achieve the levels of other nutrients, namely protein, essential amino acids or the fiber, the major role in the functioning of the digestive tract.

In this paper we propose to establish an optimum between energy and protein, according to the insurance requirements and highlighting the relationship between cellulose ratio increased energy / protein mixed fodder and the main parameters of the growth of young rabbits hybrids belonging to specialized production meat

### 1. MATERIALS AND METHODS

The biological material used in the experiments was young rabbits specialized in meat production. Rabbits were divided into 3 groups of 20 heads: one control group and two experimental (Table 1)

During the experiment, rabbits were kept in cages arranged horizontally. Administration of compound feed was done ad libitum throughout the experience. Distribution of mixed fodder was performed manually.

Table 1

**Organization of experiment**

LOT	n	Duration of experiment (Age weeks)	Mixed fodder parameters used in experimentation	The objectives
M Control	20	5-13	2160 kcal EM/ kg mix fodder 16.42% PB, 0.68% liz, 0.57% Met + cyst; 0.22% trp; report EM / PB 131.54	-Evolution of body weight; - Daily consumption of mixed fodder, energy and nutrients;
E1 Experimental 1	20	5-13	2280 kcal EM / kg mixed fodder, 16.47% PB, 0.67% liz, 0.58% Met + cyst; 0.23% trp; report EM / PB 138.43	- Specific consumption - The actual output
E1 Experiment 2	20	5-13	2380 kcal EM / kg mixed fodder 16.35% PB, 0.67% liz, 0.59% Met + cyst; 0.23% trp; report EM / PB 146.18	- Slaughter yield and carcass quality

Mixed fodder for growing rabbits were different, depending on the lot, the difference between them being represented by the ratio energy / protein, respectively in control group M 131.54, 138.43 in E1 group and 146.18 in

E2 group. Structure and parameters of mixed fodder given experimental groups are presented in Table 2.

For determining the development of body weight and growth of rabbits made up from the 3 groups were conducted weekly individual weighings recorded data were processed statistically.

Tracking consumption of mixed fodder was made by weighing administered daily and weekly fodder debris (mainly represented by dust from grain handling mixed fodder).

Table 2

**Structure and parameters of mixed fodder**

	Specification	UM	Control group	Lot E1	Lot E2
<b>Structure</b>					
1	Corn	%	36.50	36.50	37.00
2	Wheat bran	%	5.50	3.00	-
3	Wheat straw	%	20.00	20.00	20.00
4	Alfalfa hay	%	5.00	5.00	5.00
5	Full fat soy	%	10.00	10.00	10.00
6	Sunflower meal	%	20.00	21.00	22.00
7	Oil	%	-	1.50	3.00
8	Calcium carbonate	%	1.00	1.00	1.00
9	Dicalcium	%	0.80	0.80	0.80
10	Salt	%	0.20	0.20	0.20
11	MVP	%	1.00	1.00	1.00
12	Total	%	100.00	100.00	100.00
<b>Parameters</b>					
1	Dry matter	%	87.96	88.16	88.36
2	Metabolisable energy	Kcal / kg	2160	2280	2390
		MJ / kg	9.03	9.53	10.00
3	Crude protein	%	16.42	16.47	16.35
4	Lysine	%	0.68	0.67	0.67
5	Methionine + cystine	%	0.57	0.58	0.59
6	Threonine	%	0.72	0.73	0.74

7	Tryptophan	%	0.22	0.23	0.23
8	Arginine	%	1.16	1.17	1.18
9	Crude fiber	%	15.10	15.20	15.25
10	Crude fat	%	2.48	3.89	5.30
11	Calcium	%	0.71	0.71	0.71
12	Total phosphorus	%	0.58	0.57	0.55
13	Forsfor available	%	0.25	0.24	0.22
14	Report EM / PB	-	131.54	138.43	146.17
15	Report EM / liz	-	3176	3403	3567
16	Report EM / Met + cystic	-	3789	3931	4051
17	Report EM / tryptophan	-	9818	9913	10,391

Situation of actual output was monitored daily mortality of each lot during the experimental period 5-13 weeks. The data obtained were processed statistically, comparing the groups E1 and E2 with the control group M. The results were reviewed and were partial conclusions.

## 2. RESULTS AND DISCUSSION

### 2.1 The evolution of body weight of rabbits in experimental groups

Rabbits were weaned at the age of 4 weeks, according to technology intensive growth. The average weight was 522 g was at control group M, 517g in E1 and 523 g in group E2, the differences being statistically insignificant (Table 3)

Growing rabbits in the control group M were fed *ad libitum* during the experiment with a recipe with 2160 kcal ME / kg mixed fodder 16.42% PB and recorded the following average weight: 870 g at the age of 7 weeks, 1385 g 10 weeks and 2315 g at 13 weeks.

Lot E1 recorded values of 852g average weight at 7 weeks, 1411g at 10 weeks and 2663g at 13 weeks. Compared with the control group's weight differences were not significant throughout the experimental period,

recording at the end with an average weight of 38 g more than the control group.

Evolution of body weight in rabbits E2 group was in early growth (until the age of 10 weeks) similar to the control group subsequently recorded significant differences by the end of the growing season. E2 group showed the following average body weight: 885g to 7 weeks, 1425g and 2398g at 10 weeks to 13 weeks.

**Table 3**

**Evolution of body weight in experimental groups of rabbits  
between 4-13 weeks**

Age (week)	Control group		Lot E1		Lot E2	
	$X \pm s_x$	v%	$X \pm s_x$	v%	$X \pm s_x$	v%
7	870 ± 8.02	8.8	852 ± 8.24 <sup>NS</sup>	9.5	885 ± 8.72 <sup>NS</sup>	9.7
10	1385 ± 13.02	8.9	1411 ± 14.72 <sup>NS</sup>	9.9	1425 ± 13.97 <sup>*</sup>	9.4
13	2315 ± 22.65	9.3	2363 ± 24.15 <sup>NS</sup>	9.7	2398 ± 24.45 <sup>*</sup>	9.8

NS not significant differences (P <0.05) \* Significant differences (P > 0.05)

Ratio increased energy / protein of mixed fodder to the recipe used for E2 to the control group had a slight positive influence on the evolution of body weight in the second period of growth.

Statistical analysis of the evolution of body weight in E1 compared to E2 group showed that significant differences are not recorded.

The data presented show that use of mixed fodder recipes with differences in the ratio EM / PB of 6.89 to 7.75, while the other nutrients are maintained at constant values were recorded weights lots of different backgrounds, but the differences are statistically insignificant. By using the relationship between different recipes EM / PB of 14.64 and 220 kcal ME were obtained

larger differences between body weights in the second growth, which are statistically significant.

Between 5-7 weeks rabbits in the control group had an increase of 348g, an average daily gain of 16.57 g/day in E1 group achieved an increase of 335g respectively 15.95 g / day, while the rabbits from group E2 showed an increase of 362g and 17.2 g / day (Table 4).

The next observation period of 21 days (8-10 weeks) increases the weight of experimental groups increased to the following values: the control group had an increase of 515g per period, resulting in an average daily increase of 24.52 g / day, rabbits had a lot E1 average daily increase of 559g per period and 26.62 g / day group and E2 gain was 540g and 25.7 g / day.

At 11-13 weeks there were the highest increases in all groups: the control group 930g per period (44.28 g / day), E1 952g (45.33 g / day) and at 937g E2 (46.33 g / day).

Throughout the experimental period of growing rabbits in the control group had a total increase of 1793g and 28.46 g average daily gain, rabbits in group E1 an increase total of 1846g and 29.30 g / day and rabbits at E2 a lot Total increase 1875g and 29.76 g / day.

**Table 4**

**Weight gain in young cunicul of experimental groups between 5-13 weeks**

Period (week)	Control		E1		E2	
	g / week	g / day	g / week	g / day	g / week	g / day
I 5-7 per week	348	16.57	335	15.95	362	17.2
II 8-10 per week	515	24.52	559	26.62	540	25.7
III 11-13 per week	930	44.28	952	45.33	973	46.33
Total (5-13 weeks)	1793	28.46	1846	29.30	1875	29.76

Following growth ratio energy / protein of mixed fodder for rabbits up from 131.54 to 138.48 and 146.18 respectively to produce an improvement in average daily gain on the total period of increase from 2.95 to 4.56 % (respectively from 0.84 to 1.3 g / day)

## **2.2. Mixed feed intake, energy and nutrients in growing rabbits**

Rabbits were fed *ad libitum* throughout the period of observations. Mixed fodder was weighed daily before use. Weekly combined feed remains more of a presence as grist were weighed and removed from the feeders, calculating weekly consumption, average daily consumption corresponding to the week and mixed fodder consumption throughout the period of observations. To avoid the high quantity remains, mixed fodder was administered in conjunction with voluntary consumption made in rabbits of experimental groups.

Data on feed consumption of growing rabbits in the three experimental groups are presented in Table 5.

According to the data recorded and processed, average daily consumption showed the following trend:

- Growing rabbits in the control group had an average daily consumption of 54g in the 5th week, 75g in week 7, 117g in 10<sup>th</sup> week and 286g in the 13th week;
- Group E1 rabbits had a daily average consumption of 46g in 5th week, 66g in week 7, 143g in the 10<sup>th</sup> week and 271g in the 13th week.
- Group E2 rabbits have had an average daily consumption of 50g in 5th week, 66g in week 7, 121g in 10<sup>th</sup> week and 215g in 13th week.
- Regulation of voluntary food consumption according to energy levels of mixed fodder becomes evident from the age of 7 weeks.

**Table 5****Evolution of average daily consumption of mixed fodder in growing rabbits between 5-13 weeks (g mixed fodder / rabbit / day)**

<b>Week</b>	<b>Control</b>	<b>E1</b>	<b>E1 / M (%)</b>	<b>E2</b>	<b>E2 / M (%)</b>
5	54	46	85.2	50	92.6
7	75	66	88.0	66	88.0
10	117	143	122.2	121	103.4
13	286	271	94.8	215	75.2
Average period (5-13 weeks)	132.8	128.3	96.6	113.6	85.5

Average daily consumption for the entire experimental period (5-13 weeks) was 132.8 g in rabbits of the control group, 128.3 g of the rabbits in group E1 and 113.6 g in rabbits from group E2.

Total average consumption of mixed fodder individual was 8372g in rabbits control group, 8078g to 7161g in group E1 and E2. This consumption was clearly influenced by the energy of the recipes, by the ratio EM / PB of mixed forrage used for feeding rabbits in experimental groups.

**2.3. The specific consumption of rabbits in experimental groups**

In the experimental period (9 weeks) we determined body weight evolution rabbits by weekly weightings and weekly weight gain was calculated. In parallel with this action was recorded daily and weekly consumption of mixed fodder. These data allowed the calculation of specific consumption weekly and the entire period of observations (Table 8)

Table 8

**The evolution of specific consumption of mixed fodder made of rabbits  
in experimental groups**

Age (Weeks)	Control group			E1			E2		
	Increase (g / week)	Consumption (g / week)	Specific consumption (Kg / kg gain)	Increase (g / week)	Consumption (g / week)	Specific consumption (Kg / kg gain)	Increase (g / week)	Consumption (g / week)	specific Consumption (Kg / kg gain)
Average (5-7 weeks)	348	1323	3.80	335	1148	3.43	362	1183	3.27
Average (8-10 weeks)	515	2331	4.53	559	2352	4.21	540	2044	3.78
Average (11-13 weeks)	930	4718	5.07	952	4578	4.81	973	3934	4.04
Average (5-13 weeks)	1793	8372	4.67	1846	8078	4.37	1875	7161	3.82

Analysis of data from Table 8 on the specific consumption of rabbits conducted in experimental groups reveals a relationship between age and inversely proportional to the specific consumption as follows:

- The rabbits in the control group recorded a specific consumption of 4.67 kg mixed fodder per period.
- The rabbits of the E1 group recorded a specific consumption of 4.37 kg mixed fodder per period.
- The rabbits of the E2 group recorded a specific consumption of 3.82 kg mixed fodder per period.

The data analyzed and presented the report shows that by increasing energy / protein fodder from 6.89 to 14.64 is combined with an improvement in feed conversion.

### 3.CONCLUSIONS

3.1. The use of mixed fodder recipes for rabbits with different energy / protein ratio of 6.9 to 7.75, given the differences in energy level of 120-220 kcal ME / kg mixed fodder, while the protein and other nutrients are maintained at constant values, determinate a different average weight increase of rabbits in experimental groups, 2351 kg in the control group, 2363 to 2398 in E1 and E2. But the differences are statistically insignificant.

3.2. Increasing the ratio EM / PB from 131.54 to 146.18 in mixed fodder for young rabbit influenced statistically significant body dynamics of rabbits in the second growth period (E2 compared to the control).

3.3. Weight gain of rabbits made increasing from 2.95 to 4.56% has been improved to the rabbits in groups E1 and E2 by increasing the ratio EM / PB of mixed fodder used to control group from 6.89 to 14.64.

3.4. Regulating voluntary consumption of food depending on the energy level of mixed fodder becomes evident from the age of 7 weeks in young rabbits. Average daily consumption growth over the period was lower by 3.4% in rabbits lot E1 and E2 group 14.5% compared to controls, due to higher energy levels of compound feed used.

3.5. Feed conversion was improved with 6.42 to 18.20% by increasing ratio of energy / protein in mixed fodder with 6.89 - 7.75 in the E1 and E2 groups compared with control group.

3.6. Performances of rabbits in experimental groups recommended to use a mixed fodder recipe having an 146.18 energy / protein ratio.

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## **EFFECT OF GROWTH ENERGY / PROTEIN RATIO IN MIXED FODDER ON CARCASS QUALITY IN RABBITS**

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**Keywords:** energy, protein, slaughter yield, quality housing, rabbit

### **SUMMARY**

This experiment represent a continuation of an complex experiment that aimed to report the relationship between increasing energy / protein ratio mixed fodder and the main parameters of the growth of youth belonging to rabbits hybrids specialized for meat production. Rabbits came from three experimental groups (control, E1 and E2). For 9 weeks, rabbits were fed with mixed fodder by different energy-protein ratio and at he age of 91 days they were slaughtered to determine carcass quality and performance to slaughter.

### **1. MATERIALS AND METHODS**

The biological material used in the experiments was the rabbit belonging to specialized hybrids for meat production, divided into 3 groups of 20 heads: one control group (M) and two experimental (E1 and E2).

Rabbits were fed with different mixed fodder, administrated *ad libitum* during the experiment (9 weeks). Rabbit control group received a mixed fodder with the following parameters: 2160 kcal EM/ kg mixed fodder 16.42% PB, 0.68% liz, 0.57% Met + cyst; 0.22% trp; ratio EM / PB 131 , 54. Group E1 received a mixed fodder with 2280 kcal ME / kg mixed fodder, 16.47% PB, 0.67% liz, 0.58% Met + cyst; 0.23% trp; ratio EM / PB 138.43, and the group E2 a mixed fodder with 2380 kcal ME / kg mixed fodder 16.35% PB, 0.67% liz, 0.59% Met + cyst; 0.23% trp; ratio EM / PB 146.18.

In order to assess the growth ratio energy / protein mixed fodder on performance to slaughter rabbits were selected by 5 rabbits aged 91 days

and the same weight average weight at the end of experimental period (13 weeks).

Comments on slaughter yield and carcass quality indices were performed as recommended harmonization of criteria and technology study of rabbit meat.

Prior to sacrifice the animals were subjected to 24 hours starve. Slaughter was the dislocation of the cervical vertebrae and severing the jugular vein bleeding achieved by (blood was collected to determine its weight). Later they resorted to cutting skinning piecewise anatomy and determining their weight. Averages were calculated for each group of five rabbits slaughtered and measurements have revealed differences between groups were analyzed statistically.

## 2. RESULTS AND DISCUSSION

The use of mixed fodder recipes with EM / PB different ratio of 6.89 to 7.75, while the other nutrients are maintained at constant values determined different weight of groups, but differences are statistically insignificant. Rabbits in the control group showed the following average weight : 870 g at the age of 7 weeks, 1385 g and 2315 g at 10 weeks to 13 weeks. Group E1 recorded values of 852g average weight at 7 weeks, 1411g and 2663g at 10 weeks to 13 weeks. E2 group showed the following average body weight: 885g to 7 weeks, 1425g and 2398g at 10 weeks to 13 weeks (Table 1).

Table 1

### Evolution of body weight in experimental groups of rabbits between 4-13 weeks

Age (week)	Control group		E1		E2	
	X ± s <sub>x</sub>	v%	X ± s <sub>x</sub>	v%	X ± s <sub>x</sub>	v%
4	522 ± 4.75	9.5	517 ± 3.88 <sup>NS</sup>	7.4	523 ± 4.45 <sup>NS</sup>	8.5
7	870 ± 8.02	8.8	852 ± 8.24 <sup>NS</sup>	9.5	885 ± 8.72 <sup>NS</sup>	9.7
10	1385 ± 13.02	8.9	1411 ± 14.72 <sup>NS</sup>	9.9	1425 ± 13.97 <sup>*</sup>	9.4
13	2315 ± 22.65	9.3	2363 ± 24.15 <sup>NS</sup>	9.7	2398 ± 24.45 <sup>*</sup>	9.8

NS not significant differences (P <0.05) \* Significant differences (P > 0.05)

Data obtained at slaughter are presented in Tables 2 and 3. As measured by fasting for 24 hours they were subjected rabbits were recorded following weight loss: rabbits in the control group lost 137 g, rabbits in group E1 have lost 112g and rabbits in group E2 lost 140g.

Live weight at slaughter (code recognition LW) was 2177 g in rabbits in the control group 2241g to 2245 g E1 and E2.

Because blood collection at slaughter was possible, we determined the average weight of blood (code BW) in every group of rabbits: control group 92g, 85g to 78g rabbits in group E1 and E2.

Following the action of skinning, skin weights were determined (code SW) to rabbits: in the control group the average weight was 337 g, the rabbits in group E1 365g and 357g in E2.

**Table 1**

**Slaughter performance of rabbits in experimental group**

No.	Specification	UM	Code recognition	M	E1	E2
<b>CRT</b>						
1	Live weight (before fasting)	g	-	2314	2353	2385
2	Loss of weight during fasting	g	-	137	112	140
3	Live weight at slaughter	g	LW	2177	2241	2245
4	Coat weight	g	SW	337	365	357
5	Head weight	g	HW	106	124	117
6	Distal portion of limbs	g	DLW	79	92	87
7	Gastrointestinal content weight	g	GW	252	364	346
8	Weight of blood	g	BW	92	85	78
9	Cold carcass weight (without head and offal)	g g	HCW	1094	1088.2	1127.7
10	Liver weight	g g	KHLW	50.4	54.3	53.0
11	Weight of kidney, heart, lungs	g g	PFW	36.0	34.4	35.8
12	Fat weight prirenale	g g	CHW	8.9	10.4	15.2
13	Carcass weight + head	g g	CEPW	1200	1212	1245
14	carcass weight + edible internal organs	g g	CHEPW	1189	1187.3	1231.7
15	Carcass weight + head + comestible organs	g g	- -	1295	1311.3	1349
16	The weight of the hindquarters	g g	- -	382	374	407

**Table 2****Qualitative assessments to slaughter**

No CRT	Specification	UM	M	E1	E2
1	Share housing (without head and edible portion)	%	50.25	49.55	50.23
2	Carcass weight + head (without giblets)	%	55.12	54.08	55.46
3	Total yield at slaughter (carcass + head + comestible organs)	%	59.49	58.51	60.09
4	The share of housing hind	%	34.92	34.38	36.09
5	Coat weight of live weight	%	15.48	16.29	15.90
6	Share gastrointestinal contents of live weight	%	11.58	16.24	15.41
7	Edible internal organs weight of live weight	%	4.12	4.42	4.63

The average weight of the head (Code HW) was 106 g in the control group, 124 g in group E1 and 117g in E2.

The average weight of gastrointestinal contents (Code GW) was 252g in the control group, 364g in E1 and 346g in E2 .

Distal portion of limbs (DLW code had the following weights) was 79g in the control group, 92g in E1 group and 87g in E2.

Rabbit housing (code HCW) was weighed after evisceration and the removal of the head and after chilling it for 24 hours at 3-5 ° C. Average weight was 1094 g carcasses in rabbits in the control group of rabbits in group 1088.2 g 1127.7 g E1 and E2 in rabbits in group.

Edible internal organs were very similar average weight between rabbits of experimental groups: the average weight of 50.4 g of the liver in rabbits in the control group, 54.3 g of the rabbits in group E1 and 53g in E2, average weight kidney, heart, lungs (code KHLW) was 36.0 g in rabbits in the control group, 34.4 g in rabbits in group E1 and 35.8 g in rabbits in group E2.

Kidney fat (PFW code) presented differences between rabbits in relatively large experimental groups: 8.9 g to rabbits in the control group, 10.4 g in rabbits in group E1 and 15.2 g in rabbits in group E2. Statistical analysis of data showed that in terms of kidney fat weight differences between rabbits significantly distinct from E2 group compared to rabbits in the control group.

The average weight of hind limbs in rabbits in the control group was 382 g, those in group E1 of 374g and 407 g in rabbits in group E2.

Slaughter performance of rabbits made from experimental groups were analyzed after assessment, of reports (reports generally are made to live weight at slaughter, after fasting for 24 hours) to calculate indices analysis.

For rabbits is determined by dividing the carcass weight + head + offal, live weight at slaughter. This index was 59.49% value for rabbits in the control group, by 58.51% for rabbits in group E1 and 60.09% in rabbits in group E2.

### 3.CONCLUSIONS

3.1. The use of recipes of mixed fodder different from the ratio of energy / protein of 6.9 to 7.75, given by the differences in energy level of 120-220 kcal ME / kg feed, determined a growth of average weight in rabbits of different experimental groups, but differences were not statistically significant.

Rabbits control group achieved an average weight of 2315kg to 13 weeks, group E1 showed an average weight of 2363kg and 2398kg rabbits group E2.

3.2. Slaughter performance of rabbits in the experimental groups were not significantly influenced by the ratio energy / protein of mixed fodder used. An exception is reported kidney fat in large quantities for rabbits fed with

mixed fodder with higher energy level, that report energy / higher protein, but without affecting the overall quality of the carcasses.

3.3 Record of major growth performance for the experimental group E2, maintaining low rates of morbidity and mortality, and obtaining a yield of 60.09% in rabbits slaughter group E2, recommends the use of mixed fodder to growing rabbits with a 146.18 ratio protein -energy.

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## MILK PRODUCTION, MILK COMPOSITION, MILK FATTY ACID PROFILE IN DAIRY COWS FED RAPESEED AND FLAXSEED OIL

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**Keywords:** rapeseed oil, flaxseed oil, milk production, milk composition

### SUMMARY

A total of 18 lactating dairy cows, Holstein-Friesian breed, were assigned to three groups: control group C (no dietary oil), group RO (with dietary rapeseed oil, Canola variety) and group FO (with dietary flaxseed oil). All three groups received corn silage (*ad libitum*), alfalfa hay (limited to 4 kg/day) and compound feed (limited to 6.5 kg/day).

The inclusion rate of two dietary oil was 10.4% in the compound feed or 3.8% on a dry matter basis, so that cows consumed 600 g daily. The monitored experimental parameters were: diet formulation and composition, dietary supply of energy and protein, feed intake, milk yield and composition, milk fat fatty acid profile.

The total intake of dry matter, milk yield, the production of milk protein and milk protein content didn't differ significantly among the experimental groups, or among them and the control group. On the other hand, milk fat content and the production of milk fat were very different ( $P < 0.05$ ). The following trends were noticed concerning the milk fatty acid profile: decrease of C 16:0 ( $P < 0.01$ ) and C 16:1 ( $P < 0.05$ ), increase of C:18:0, C18:1 *n-9 trans* ( $P < 0.05$ ), C18:1 *n-9 cis* ( $P < 0.01$ ), C 18:3 *n-3* ( $P < 0.001$ ), and the stable level of C 18:2 *n-6 trans*, C 18:2 *n-6 cis* and C 18:3 *n-6*.

Compared to the rapeseed oil, the flaxseed oil diet resulted in a lower level of 18:1 *n-9 cis* ( $P < 0.01$ ) in the milk fat and in a higher level of C 18:3 *n-3* ( $P < 0.001$ ). The highest level of milk fat polyunsaturated fatty acid (PUFA) resulted from the use of the dietary flaxseed oil, both compared to the diets with rapeseed oil and compared to the control diet ( $P < 0.01$ ).

Many papers were published during past 10-15 years which describe milk composition, fatty acid (FA) profile of the milk from cows fed on different types of diets using different sources of fats and FA. However, despite the large amount of data published in a number of reviews, no clear conclusion have yet been drawn on the way in which the factors depending on feeding or animals influence milk fat and milk FA.

Milk fat contains over 400 individual fatty acids and their isomers. Cow milk contains large amounts of saturated fatty acid (SFA), particularly C 14:0 and C 16:0, which determine physiological dysfunctions including higher plasma cholesterol, and small amounts of monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and *omega-3* fatty acids with beneficial effects on human health.

The problem is how to modify milk FA profile to make it as good as possible. One solution is to feed whole oleaginous seed or oils of them to the cow. Rapeseed oil and

flaxseed oil may influence milk FA level as follows: (1) decrease SFA, (2) increase MUFA and PUFA, (3) increase the proportion of the conjugated linoleic acid and the *alpha*-linolenic acid, (4) decrease the FA *omega* 6 to FA *omega* 3 ratio. The possible changes relay on the very high level of the rapeseed Canola oil in C 18:1 (50-55%), particularly in C 18:1 *n*-9 and of the flaxseed oil in C 18:3 (40-45%), particularly in C 18:3 *n*-3.

The objective of this study was to assess the effect of including the two oils in dairy cows' diets commonly used in Romania, on milk yield, protein and fat content and on milk fat FA.

## 1. MATERIALS AND METHODS

### *Experimental protocol*

The experiment used 18 lactating cows, Holstein-Friesian breed, homogenous in terms of parity (2.7 ± 0.44 lactations), milk yield (21.6 ± 3.2 kg/day), stage of lactation (135 ± 42 days), milk fat (3.9 ± 0.21%) and milk protein (3.3 ± 0.15%).

The cows were assigned to groups of 6 animals each and fed three dietary regimes: a control diet with no oils (C) and two experimental diets with rapeseed oil, Canola variety (RO) and flaxseed oil (FO). All three groups received corn silage *ad libitum*, alfalfa hay (limited to 4 kg/day or 3.42 kg DM/day) and compound feeds (limited to 6.5 kg/day or 5.79 kg DM/day, with different formulations), which covered cow requirements for the expected milk yields.

The experiment was designed as a Latin square, each rotation lasting for 28 days, of which 21 days of accommodation and 7 days for measurements.

### *Measurements*

Milk yield was measured individually, during the two daily milking sessions, during the 7 final days of each experimental period. Milk samples were collected, stored at 4° C and analyzed for milk fat and protein. Part of the samples was frozen for a subsequent determination of the fatty acids by chromatography.

Feed intake was measured daily and individually. Under the conditions of the limited administration of alfalfa hay and compound feeds, they were consumed entirely and were no leftovers. The administered silage quantities were periodically corrected so the leftovers are around 10%.

During each of the 3 experimental periods we collected two hay samples, two corn silage samples and two compound feeds samples which were assayed for their composition and nutritive value.

The statistical analysis was done using linear model ANOVA.

## 2. RESULTS AND DISCUSSION

### *Diet structure and composition*

Tables 1 and 2 show data on diet structure, on compound feeds formulation and on the dietary protein, fat and fatty acids. The diets were dominated by the two bulk forages, so that the forage to concentrate ratio was about 63/37, normal for the obtained milk yields.

The two dietary oil included in amounts of 10.4% of the compound feed or 3.8% of the dietary DM, otherwise said, the cows consumed every day about 600 g oils.

Table 1

**Diet and compound feeds formulation**

<b>Regimen</b>	<b>C</b>	<b>RO</b>	<b>FO</b>
Diet formulation (% of DM)			
Corn silage	41.0	39.6	40.0
Alfalfa hay	22.0	22.5	22.4
Compound feed	37.0	37.9	37.6
Compound feed formulation (%)			
Corn	58.7	43.2	43.2
Soybean meal	20.7	25.8	25.8
Wheat bran	17.3	17.3	17.3
Rapeseed oil	-	10.4	-
Flaxseed oil	-	-	10.4
Monocalcium phosphate	0.4	0.4	0.4
Calcium carbonate	0.5	0.5	0.5
Vitamin-mineral premix	1.0	1.0	1.0
Salt	1.4	1.4	1.4

Table 2

**Dietary protein, fat and fatty acids**

<b>Regimen</b>	<b>C</b>	<b>RO</b>	<b>FO</b>
Dietary protein and fat (% of DM)			
Protein	14.1	14.5	14.3
Fat	3.1	5.7	5.7
Dietary fatty acids (% of the total detected FA)			
C 12:0	1.48	0.86	0.77
C 14:0	1.76	0.55	0.41
C 16:0	11.49	8.93	9.84
C 16:1	0.17	0.41	0.20
C 18:0	2.48	2.59	2.99
C 18:1	18.55	35.14	18.46
C 18:2	52.51	38.09	34.82
C 18:3	4.96	6.03	26.31
SFA	17.21	12.93	14.01
MUFA	18.72	35.55	18.66
PUFA	57.17	44.12	61.13

The three diets had similar protein levels, while the dietary fat was higher in the experimental diets (5.7% on DM basis) compared to the control diet (3.2% on DM basis).

The rapeseed oil diet had a high level of C 18:1 and C 18:2 fatty acids, the flaxseed oil diet had a high level of C 18:2 and C 18:3 fatty acids; C 4:0, C 6:0, C 8:0 and C 10:0 fatty acids were not detected.

*Dietary energy and protein supply*

Table 3 shows the supply of dietary energy and protein and the ratio of the supply and requirement (for the obtained milk yields), which are around 100% showing the adequacy of the diet formulations.

Table 3

**Dietary energy and protein supply and how much of the requirement was met**

Regimen	C	RO	FO
Supply of MFU <sup>1</sup>	14.42	14.89	14.98
Supply of IDPN <sup>2</sup> (g)	1488	1496	1501
Supply of IDPE <sup>3</sup> (g)	1471	1410	1417
MFU supply/MFU requirement	97	100	101
IDPN supply/IDPN requirement	101	101	102
IDPE supply/IDPE requirement	100	96	96

1) MFU = Milk feed unit; 1 MFU = 1720 kcal NE

2) IDPN = Intestinally digestible protein, nitrogen

3) IDPE = Intestinally digestible protein, energy

*Feed intake, milk yield and milk composition*

Table 4 shows the total DM intake (of which silage), milk yield, protein and fat production, milk protein and fat.

Table 4

**Feed intake, milk yield and milk composition**

Regimen	C	RO	FO	Significance
Total DM intake (kg/day)	15.62	15.25	15.34	ns
- of which corn silage	6.41 <sup>a</sup>	6.04 <sup>b</sup>	6.13 <sup>b</sup>	*
Milk yield (kg/day)	22.05	22.51	21.79	ns
Fat production (g/day)	864 <sup>a</sup>	774 <sup>b</sup>	769 <sup>b</sup>	*
Protein production (g/day)	754	752	738	ns
Milk fat (%)	3.92 <sup>a</sup>	3.44 <sup>b</sup>	3.53 <sup>b</sup>	*
Milk protein (%)	3.42	3.34	3.39	ns

Different superscripts within a row show significant differences; \*:  $P < 0.05$ ; ns: not significant

DM intake was quite similar in the three groups, the differences not being statistically significant. The small differences were noticed in the intake of corn silage ( $P < 0.05$ ) to which the animals had free access. Because of the multiple factors of influence it is difficult to make comparison with the data published by other authors. However, no author among the ones we cited mentioned higher intakes when rapeseed or flaxseed oils, or oils in general, were used in the diets. The authors report either

constant intake (Bayourthe *et al.*, 2000, Ward *et al.*, 2002, Chelikani *et al.*, 2004) or a lower intake (Chouinard *et al.*, 1998, Dhiman *et al.*, 2000, Ueda *et al.*, 2003).

Milk yield was slightly higher in the RO group and slightly lower in the FO group, compared to the control group. Although the difference between the highest and lowest milk yield was of 0.7 kg/day, the differences were not significant. There are diverging opinions on the influence of the rapeseed and flaxseed oils on the milk production. Some authors reported a higher production (Chouinard *et al.*, 1998, Dhiman *et al.*, 2000, Petit *et al.*, 2004, Loor *et al.*, 2005, Zhang *et al.*, 2006), others report an unchanged production (Kennelly, 1996, Mir *et al.*, 1999, Bayourthe *et al.*, 2000, Ward *et al.*, 2002, Loor *et al.*, 2002, Komprda *et al.*, 2005) while others reported a decrease of the production (Chilliard and Ferlay, 2004). Besides the types of oils, there are too many variables taken into account to allow comparisons with the data of this paper.

The production of fat and protein depend both on the influence of the oils and on the production of milk.

In our experiment, the production of fat and milk fat were similar in the two experimental groups, but lower than in the control group ( $P < 0.05$ ), in agreement with the reports of Focant *et al.*, 1998, DePeters *et al.*, 2001, Chilliard *et al.*, 2001, Chilliard and Ferlay, 2004, Bell *et al.*, 2006. Other authors, however, reported an unchanged production of fat subsequent to the administration of dietary rapeseed and flaxseed oil (Bayourthe *et al.*, 2000, Komprda *et al.*, 2005, Zhang *et al.*, 2006. Milk fat might decrease while the production of fat remains unchanged or it may increase if the milk production increases.

The production of protein and milk protein, according to the literature, remain constant or may decrease slightly (Loor *et al.*, 2002, Loor and Herbein, 2003, Zhang *et al.*, 2006). Only Flowers *et al.* (2008) noticed a quadratic increase. In our experiment, these two parameters didn't differ significantly, both between the experimental groups and between them and control group.

#### *Milk fatty acids profile*

Table 5 shows the milk fatty acids profile. We analyzed a wider range of fatty acids but we selected only those with a higher proportion and importance.

The literature data, obtained in various feeding conditions, show a decrease of milk SFA and an increase of milk UFA subsequent to the use of whole oleaginous or their oil. (Kelly *et al.*, 1998, Chouinard *et al.*, 2001, Chilliard *et al.*, 2000, 2002, Gonthier *et al.*, 2005, Petit *et al.*, 2002, 2003, 2007). In many reports, PUFA and sometimes MUFA increase; however the extent of these changes is quite variable.

Table 5

**Diet effects on the milk fatty acids profile (% of the total analyzed FA)**

Regimen	C	RO	FO	Significance
C 4:0	1.33	1.42	1.43	ns
C 6:0	2.02	2.08	2.24	ns
C 8:0	1.78	1.71	1.73	ns
C 10:0	3.03	2.58	2.64	ns
C 12:0	4.33	3.74	3.88	ns
C 14:0	12.70	12.21	12.09	ns
C 16:0	32.05 <sup>a</sup>	25.41 <sup>b</sup>	24.75 <sup>b</sup>	**
C 16:1	2.17 <sup>a</sup>	1.66 <sup>b</sup>	1.70 <sup>b</sup>	*
C 18:0	11.04 <sup>b</sup>	13.48 <sup>a</sup>	14.21 <sup>a</sup>	*
C 18:1 <i>n-9 trans</i>	2.29 <sup>b</sup>	2.56 <sup>a</sup>	2.58 <sup>a</sup>	*
C 18:1 <i>n-9 cis</i>	19.21 <sup>c</sup>	25.17 <sup>a</sup>	23.89 <sup>b</sup>	**
C 18:2 <i>n-6 trans</i>	0.24	0.25	0.24	ns
C 18:2 <i>n-6 cis</i>	2.38	2.34	2.42	ns
C 18:3 <i>n-6</i>	0.30	0.33	0.31	ns
C 18:3 <i>n-3</i>	0.58 <sup>b</sup>	0.68 <sup>b</sup>	1.12 <sup>a</sup>	***
SFA	68.28 <sup>a</sup>	62.63 <sup>b</sup>	62.97 <sup>b</sup>	**
MUFA	23.67 <sup>b</sup>	29.39 <sup>a</sup>	28.17 <sup>a</sup>	*
PUFA	3.50 <sup>b</sup>	3.60 <sup>b</sup>	4.09 <sup>a</sup>	**

Different superscripts within a row show significant differences; \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; ns: not significant

In our experiment we noticed in both experimental groups, compared to the control group, the decrease of C 16:0 ( $P < 0.01$ ) and C 16:1 ( $P < 0.05$ ), the increase of C 18:0 and C 18:1 *n-9 trans* ( $P < 0.05$ ), the increase of C 18:1 *n-9 cis* ( $P < 0.01$ ), the increase of C 18:3 *n-3* ( $P < 0.001$ ), and the unchanged status of C 18:2 *n-6 trans*, C 18:2 *n-6 cis* and C 18:3 *n-6*.

The flaxseed oil diet resulted in a lower milk fat C 18:1 *n-9 cis* ( $P < 0.01$ ) and in a higher milk fat C 18:3 *n-3* ( $P < 0.001$ ) compared to the rapeseed oil diet.

C 18:3 *n-3*, one of the FA most studied during the recent period, represented 1.12% of the total milk fatty acids when flaxseed oil was used, compared to 0.68% and 0.58%, when rapeseed oil was used or compared to the control diet. Moate *et al.* (2007) report in their review on 28 publications, that the average level of C 18:3 *n-3* in the milk was 5.9% of the total FA, with a range from 0.2 to 19%

Milk PUFA was the highest when flaxseed oil diet was used compared to the rapeseed oil diet and to the control diet ( $P < 0.01$ ).

### 3.CONCLUSIONS

3.1. The use of 3.8% on DM basis of rapeseed and flaxseed oils in dairy cows diets with 63/37 ratio of forages to concentrates changed significantly the milk fatty acids profile. C 16:0 and C 16:1 decreases and C 18:0, C 18:1 *n-9 trans*, C 18:1 *n-9 cis*, C 18:3 *n-3* increased.

3.2. C 18:3 *n-3* was 1.12% of the total milk FA when flaxseed oil was used, compared to 0.68% and 0.58% when rapeseed oil was used or compared to the control diet.

3.3. The production of milk, the production of protein and milk protein were similar between the experimental groups and the control group. The production of fat and milk fat were, however, lower in the experimental groups and were similar among them.

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## **DYNAMICS OF ORGANOLEPTIC, PHYSICOCHEMICAL AND MICROBIOLOGICAL MODIFICATIONS DURING VALIDITY PERIOD FOR MEAT PRODUCTS IN MEMBRANE —FRESH PRODUCTS (FOOD AGING)**

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### **SUMMARY**

Before evaluating its nutritional potential, if the product is nourishing or not, consumers decide primarily according to the psychosensorial effect of the product, which, in fact, determined the initiation of this study.

The food products shall be determined mainly according to their nutritional value which is based on the total number of substances from the product composition, namely glucides, lipids, proteins, vitamins, mineral substances, organic acids, vitamins and enzymes. When food quality is to be determined, apart from organoleptic examination it is also required the determination of main physicochemical and microbiological properties with the view of objectivizing the total quality control of that product. Therefore, several fresh meat products in membrane were distinctly observed throughout the entire validity period, the results obtained differing according to each product, thus being registered modifications which might shorten or increase the validity period for some products.

**Key words:** sensorial analysis, microbiological analysis, physicochemical analysis, meat products, validity period.

## INTRODUCTION

The sensory analysis of food products is, basically, as old as mankind, but progress was made no sooner than the latest three decades, by the intensification of the scientific research aiming at objectivizing it. This special interest for the sensory analysis of food raises from the progress made in physics, chemistry, microbiology, biochemistry, histology, technology, merceology, as well as in other domains of the technique and science.

The systematic research made during the last decades in the field of sensorics resulted in a rich resource regarding the way of applying the sensorial analysis in the control and quality assessment of the food products. The interest in the issues raised by the sensorics is currently very high, which is not at all by chance, as the sensorial properties of food products always draw the attention of the consumers, who react sensitively and also promptly to all the basic changes which affect certain products, and especially those changes related to sensory characteristics.

If, for most of the other industrial products, quality is characterised by a well-defined property or group of physical or chemical properties, in the case of food products quality is determined by three different essential criteria: innocuity, nutritional value and sensory quality. Sometimes, food quality is assessed by means of elements such as packaging and labeling, which are important for the product's protection and aspect.

### 1. MATERIALS AND METHODS

We isolated three batches of products from a company which produces sausages in the county of Brasov, a company which occupies an average market segment; the isolation was made as follows:

BATCH I—fresh salami: pork “parizer” (thick rosy sausage made of boiled minced meat), cremwurst, Polish sausages made of chicken breast

These products were subject to examinations and sensory assessment based on scores, and we also made a statistic study regarding the consumers' opinion about sausages in different stages of the validity period, that is, at the beginning, middle, and at the end of the validity period.

The sensory properties will be examined in the following order: the condition of the transport package, outer aspect and shape of the product, the aspect in cross-section, smell, taste and consistency.

Dimensions are checked immediately after checking the outer aspect and product shape.

In order to check the cross-section aspect, the pieces of meat products are cut perpendicular to the longitudinal axis, either in slices or in pieces, using either a thin-bladed and very well sharpened knife or a meat cutting machine (the machine is not used for aspic, black pudding, smoked bacon and sausages).

The order of the examination is the following: we will start with the less spiced sorts (cremwurst, Italian salami, etc.) and we will continue gradually with the more spiced ones (“Agnita” sausages, sausages with cumin, etc.).

Members of the commission are not allowed to exchange opinions, as this might influence the result of the sensory evaluation.

At the sensory evaluation we could notice:

Outer aspect: whole pieces showing a clean surface, without any impurities or spots of mould —except for the raw salami or sausages with special noble mould on the surface). The membrane has to be neat and without any wrinkles for fresh semi-smoked meat products, it must have wrinkles in the case of semi-smoked air-dried salami (classic summer salami covered in natural membrane), it must adhere to the composition, and under the membrane there should not be any air holes, melted fat, juice, larvae or insect galleries.

Cross-section aspect: compact, well-bound composition, with pieces of bacon of equal size and equally spread throughout the whole composition (for meat products with a mosaic aspect). The composition must not have air holes, melted fat blobs, pockets with juice or albumin precipitate.

For fresh salami, the composition is juicy, but without letting out liquids when pressed moderately.

For semi-smoked salami, the composition is compact, well-bound, firm and elastic.

For products which last longer, the composition is relatively hard, firm and homogeneous.

Colour: on the exterior, the colour is specific according to the product and to the technological process used, as well as to the type of membrane (natural, synthetic or semi-synthetic —the last two being able to be coloured and imprinted). On the cross-section, the colour must be homogeneous, specific to the sort, without areas showing a modified colour. The pieces of bacon are either white or pink, without having a grey, green or yellow hue. The semi-smoked products and products which last longer have a reddish, homogeneous colour on the cross-section, without any darker hue on the margins or any other changes in the colour towards the middle.

Smell and taste: characteristic to the sort, pleasant, moderately salted or spiced, without no changed or improper smell or taste.

The sensory quality of meat products is generally assessed on a 1 —10 scale, the first five steps of the scale representing products showing positive qualities. ( **Banu et al., 2007**).

The products labelled “exceptional” and “very good” (grades 9 and 8) are included in the “superior” category; the products labelled “good”, “better than average” or “average” (grades 7, 6 and 5) are included in the 1<sup>st</sup> category. ( **Banu et al., 2007**).

The area referring to the “unsatisfactory” grade represents less than 20% of the whole.

The sensory analysis is completed with the physico-chemical analysis for the integrity assessment: water, fat, proteins, sodium chloride, nitrates/nitrites, polyphosphates, including starch, vegetal proteins or collagen proteins.

These evaluations are necessary in order to check if the product observes the standards imposed by the company or branch regarding the recipes, technological process or final product parameters.

## 2. RESULTS AND DISCUSSION

Sensory characteristics of the PORK “PARIZER”

Validity period: 20 days (stored at 0-4°C and relative humidity of air between 75 —80%) given by the producer; it obtained the following score:

Table no. 1

Table no. 1

<b>Organoleptic properties</b>	<b>12.08.2010</b>	<b>22.08.2010</b>	<b>01.09.2010</b>	<b>11.09.2010</b>
Outer aspect	8	7	7	7
Colour through the section	6	6	6	5
Flavour	7	6	6	5
Taste	6	6	5	4
Consistency	5	7	6	6
Juiciness	7	7	7	7
Overall assessment of quality	5	5	5	4
Total	44	44	42	38
Score	6.28	6.28	6	5.42

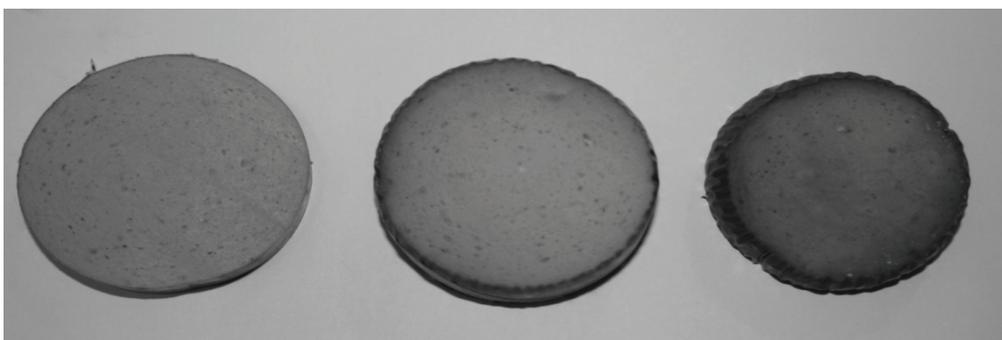
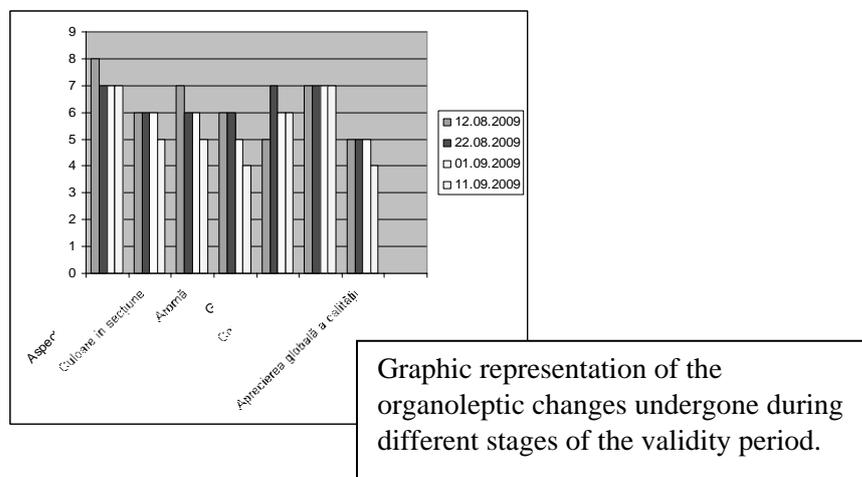


Fig. 1. Pork “parizer” —cross-section during different stages of the validity period

Table 2

Physicochemical parameters monitored for pork „parizer”

Characteristics	Admissibility conditions	12.08.2010	22.08.2010	01.09.2010	11.09.2010
Water,%	Max. 72	67.68	67.02	65.98	65.87
NaCl,%	Max. 2.8	2.69	2.69	2.8	2.57
Nitrites, mg/100g product	Max. 7	3.271	2.615	2.014	2.034
NH <sub>3</sub> , mg/100g product	Max. 30	5.00	5.9	5.9	5.1

Table 3

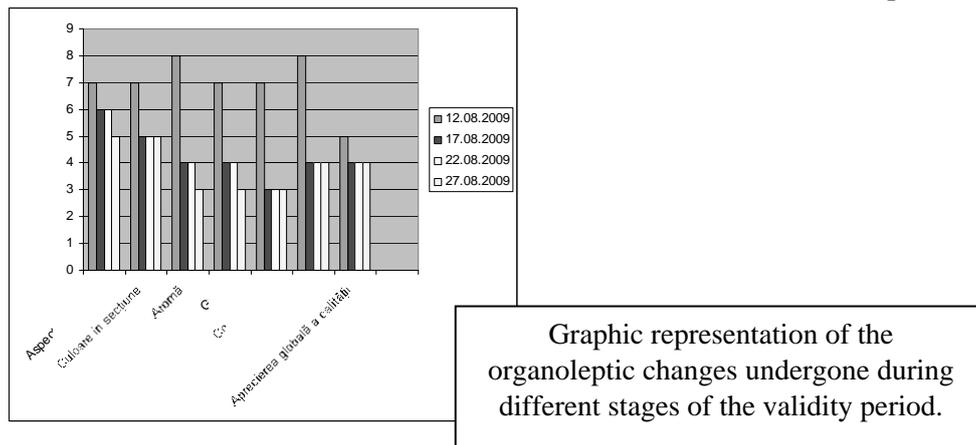
Microbiological characteristics determined for pork „parizer”

Characteristics	Admissibility conditions	12.08.2010	22.08.2010	01.09.2010	11.09.2010
<i>Salmonella</i> /25g	Absent	Absent	Absent	Positive	Absent
Coliform bacteria max./1g	10	< 10	< 10	<10	< 10
<i>Escherichia coli</i> max./1g	Absent	Absent	Absent	Absent	Absent
Coagulase-positive staphylococcus max./1g	10	< 10	<10	<10	< 10
Sulfate reducing bacteria /1g	10	< 10	<10	<10	< 10
<i>Bacillus cereus</i> /1g	10	< 10	<10	<10	< 10

Sensory characteristics of the POLISH SAUSAGES MADE OF CHICKEN BREAST

Validity period: 10 days (stored at 0-4°C and relative humidity of air between 75 —80%) given by the producer. As a result of the score it obtained, we noticed the changes presented in Graph no. 2.

Graph no. 2



Graphic representation of the organoleptic changes undergone during different stages of the validity period.



Fig. 2.

Polish sausages —outer aspect.

Table 4

Physicochemical parameters monitored for chicken breast Polish sausages

Characteristics	Admissibility conditions	12.08.2010	22.08.2010	01.09.2010	11.09.2010
Water,%	Max. 70	65.67	65.24	64.46	64.77
NaCl,%	Max. 3	2.34	2.34	2.69	2.8
Nitrites, mg/100g product	Max. 7	1.439	1.252	0.975	0.475
NH <sub>3</sub> , mg/100g product	Max. 30	8.26	10.03	21.2	21.8

Table 5

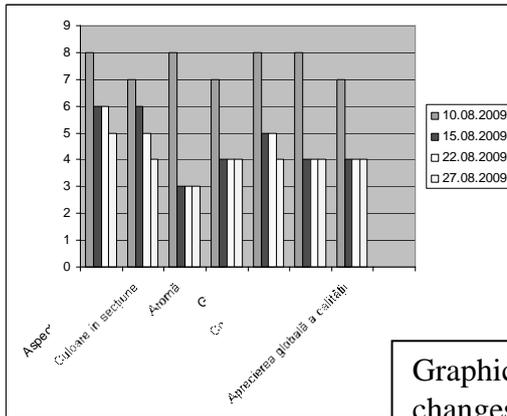
Microbiological characteristics determined for chicken breast Polish sausages

Characteristics	Admissibility conditions	12.08.2010	22.08.2010	01.09.2010	11.09.2010
<i>Salmonella</i> /25g	Absent	Absent	Absent	Absent	Absent
Coliform bacteria max./1g	10	< 10	< 10	<10	< 10
<i>Escherichia coli</i> max./1g	Absent	Absent	Absent	Absent	Absent
Coagulase-positive staphylococcus max./1g	10	< 10	<10	<10	< 10
Sulfate reducing bacteria/1g	10	< 10	<10	<10	< 10
<i>Bacillus cereus</i> /1g	10	< 10	<10	<10	< 10

Sensory characteristics of CREMWURST

Validity period: 10 days (stored at 0-4°C and relative humidity of air between 75 —80%) given by the producer; it obtained the following score:  
Graph no. 3

Graph no. 3



Graphic representation of the organoleptic changes undergone during different stages of the validity period.

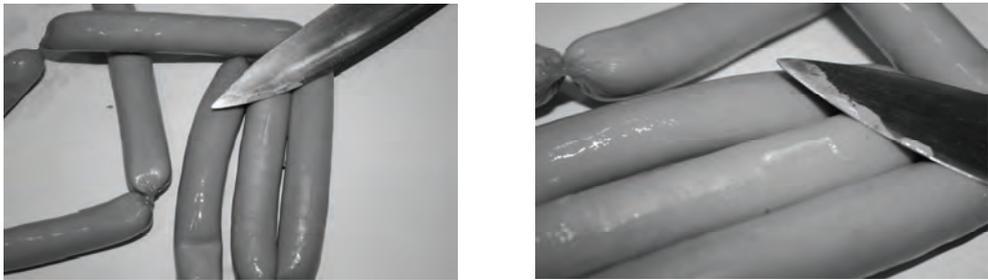


Fig. 4.  
Cremwurst —outer aspect.

Table 6

Physicochemical parameters monitored for cremwursts

Characteristics	Admissibility conditions	10.08.2010	15.08.2010	22.08.2010	27.08.2010
Water,%	Max. 70	66.57	65.42	64.12	64.10
NaCl,%	Max. 3	2.38	2.38	2.29	2.8
Nitrites, mg/100g product	Max. 7	1.439	1.252	0.975	0.475
NH <sub>3</sub> , mg/100g product	Max. 30	8.26	10.03	21.2	21.8

Table 7

Microbiological characteristics determined for cremwursts

Characteristics	Admissibility conditions	12.08.2010	22.08.2010	01.09.2010	11.09.2010
<i>Salmonella</i> /25g	Absent	Absent	Absent	Absent	Absent
Coliform bacteria max./1g	10	< 10	< 10	<10	< 10
<i>Escherichia coli</i> max./1g	Absent	Absent	Absent	Absent	Absent
Coagulase-positive staphylococcus max./1g	10	< 10	<10	<10	< 10
Sulfate reducing bacteria/1g	10	< 10	<10	<10	< 10
<i>Bacillus cereus</i> /1g	10	< 10	<10	<10	< 10

### 3.CONCLUSIONS

3.1. The best score at the category of “fresh” meat was obtained by the “parizer”, in whose case the validity period can be extended at least 5 days.

3.2. The cremwursts and Polish sausages showed visible modifications and, therefore, had a lower score before the end of the validity period, which results in a 4-day decrease of the validity period (40%).

3.3. The sole positive sample was determined by its presence in the pork “parizer” on 01.09.2010 (expired product) of salmonellas. Because it was the only positive sample, it shows the the contamination moment took place after or during the packaging time, the other bars of „parizer” being negative.

3.4. The evolution of physicochemical characteristics of the „parizer” showed that after 5 days after the end of the validity period, the product presented a state of normal freshness.

3.5. The cremwursts and the Polish saussages showed visible modifications and hence a low scoring before the end of the validity period (10 days) which recommends the shortage of the validity period with 4 days ( 40%) to 6 days, although the physicochemical characteristics range within the admitted limits.

3.6. The results of microbiological analyses must be correlated with the results of sensorial and physicochemical analyses, in order to objectivise the total quality control. Therefore:

- a. The evolution of physicochemical characteristics of the „parizer” showed that after 5 days from the end of the validity period, the product presents a normal state of freshness, obtaining the best scoring both for the organoleptic determination which might lead to the extension of the validity period with 5 days.
- b. The cremwursts and the Polish saussages showed visible modifications and hence a low scoring before the end of the validity period (10 days), which recommends the shortage of the validity period with 4 days ( 40%) to 6 days, although the physicochemical characteristics range within the admitted limits.

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**UTILIZAREA BIOTEHNOLOGIEI ÎNSĂMÂNȚĂRILOR  
ARTIFICIALE, LA OVINELE DIN RASA ȚIGAIE CAPNEGRU DE  
TELEORMAN (KARABAȘĂ), UTILIZÂND MATERIAL SEMINAL  
CONGELAT DIN RASA AWASSI DIN SIRIA, ÎN SCOPUL  
DEZVOLTĂRII ȘI CONSERVAREA BIODIVERSITĂȚII OVINELOR**  
**USE OF BIOTECHNOLOGY ARTIFICIAL INSEMINATION, OF  
OVINE BREED TZIGAI BLACK HEAD OF TELEORMAN  
(KARABAȘĂ) USING FROZEN SEMEN AWASSI BREED IN SYRIA IN  
ORDER TO DEVELOP AND BIODIVERSITY CONSERVATION  
SHEEP**

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Key words: biotechnology, ovine, artificial insemination, biodiversity.

### **SUMMARY**

Advantages of using biotechnology to breeding the can be divided into four categories namely: economic, animal husbandry, veterinary science.

The goal, this research was to obtain from the use of biotechnology products matzos artificial insemination, the import of 55 doses of frozen semen of Awassi breed of sheep in

Syria and Tzigai race with black head Teleorman (Karabașă) in Romania.

After 48 hours of pesary extraction and administration of 400 IU of PMSG (Folligon) were identified with clinical signs of heat a number of 13 females of race Karabașă, which have been artificially inseminated with semen frozen and thawed, Awassi breed, according to the technique described above.

After I.A. a total of 29 sheep breed Black Head Tzigai Teleorman , gave birth after a normal pregnancy, a number of sheep, 12 resulting in 12 products (41.37%), 6 females and 6 males.

With artificial insemination (AI) is manipulation male reproduction function.

Advantages of using biotechnology to breeding the can be divided into four categories:

1 - Economic: if a ram by natural service can perform on average 30-40 monte / year from a single ejaculate of a ram can sow an average of 150-200

females using frozen sperm is limited transportation, sperm can be kept and stored in liquid nitrogen at - 196°C and decreases the cost price of gestation;

2 - Breeding: breeding programs are used most valuable male of breed, via frozen semen (m.s.c.) have access to genetic world, as, creates the possibility of long distance transport of sperm in different regions, countries or continents, providing fast and efficient testing by descent, by obtaining the required number of sons and daughters after testing, to obtain offspring of close age, which creates the premise for a correct assessment of skills for milk or meat.

3 - Sanitary-veterinary: using AI are largely preventable infectious and parasitic infectious diseases specific to each species can be transmitted through breeding and thus reduce the risk of introducing diseases with imported livestock, may apply to units that are in quarantine, it is prohibited the movement of animals, leading to early detection of breeding with poor sperm quantity and quality, and various genetic diseases.

4 - Science: man can intervene, manage and control the function of reproduction, it is possible to study the biology of the main processes related to reproduction (fertilization, sperm biochemistry, sperm behavior in different environments outside the animal body, the ability of sperm fertilized, etc.).

## **1. MATHERIAL AND METHOD**

The goal, this research was to obtain from the use of biotechnology products matzos artificial insemination, the import of 55 doses of frozen semen of Awassi breed of sheep in Syria and the breed Black Head Tzigai Teleorman (Karabaşă) in Romania.

The research was conducted in Greek Wine Research Station belonging to the Romanian Academy, on a total of 50 sheep breed breed Black Head Tzigai Teleorman (Karabaşă), property of the Imad Ahmad D, which was imported and made an m.sc. contract with specialists from the Center for Biodiversity Studies and agro "Acad David Davidescu".

Working protocol was to conduct the following activities:

- Group of 50 female and 2 male Karabaşă race were examined clinically and settled ration supplemented with food purchased from MVP IBNA Baloteşti and supervision care and how animals throughout the period of interest, including calving;- 19.09.2008 with a torch in the species were

introduced pesarii vaginal Chrono-gesture to the 50 women in the race and remained Karabaşă 13 days;

- On 02/10/2008 pesary were extracted from vaginal only 46 sheep (four sheep lost pesariile) at a number of 25 sheep received i.m 400 u.i. PMSG (Folligon);

- On 10/04/2008 after 48 hours of extraction pesarr, a young male of the race was prepared Karabaşă apron over the abdomen to detect females in heat, in that day were carried out artificial insemination (AI) to a number of 13 sheep breed Karabaşă;

- On 10/05/2010 after 72 hours of artificial insemination was performed pesariilor extraction (IA) in a total of 19 sheep. Physiological manifestation of oestrus mention that these females was fairly removed synchronized (only 10 females showed IA mucus when heat only other vulva congestion of various degrees, and some not at all).

Technical I.A. used consisted of the following steps: A female was restrained on the ground with the help of two workers, placing female abdomen on a support board, high hindquarters upside down.

This method allowed the media to exert pressure on the abdominal genital cervix so that the identification was made easier and also the actual insemination psitoletul top-down tilting at an angle of 30 °.

B. the actual technique of I.A.:

1. Extraction of container of liquid nitrogen and thawing them straw temperature of 35°C for 1 minute.

2. Straw out of the water, remove and shake them;

3. Laboratory cutting stopper;

4. Cassou straw entering the torch type of A I;

5. Toilet vulva region;

6. Disinfection and vaginal lubrication speculum;

7. Speculum introduction bivalve vaginal genital and locking device with a closure nut.

9. Introduction device - tube valves including lighting and viewing speculum cervical canal;

10. Introduction into the vagina through the tube torch lighting and deposit the semen into the cervix by pressing the push cap mandren plant as a piston.

## 2. RESULTS AND DISCUSSIONS

After 48 hours of extraction pesariilor and administration of 400 IU of PMSG (Folligon) were identified with clinical signs of heat a number of 13 females of race Karabașă, which have been artificially inseminated with semen frozen and thawed, Awassi breed, according to the technique described above.

After 72 hours of extraction pesariilor and administration of 400 IU of PMSG (Folligon) were identified with clinical signs of heat a total of 19 women in the race Karabașă, which have been artificially inseminated with semen frozen and thawed, Awassi breed.

Listed in Table 1 are pooled data and the results recorded breed of sheep Karabașă of Giurgiu County Greek resort were artificially inseminated (AI) by msc Awassi breed after oestrus induction and synchronization with ajitorul pesariilor Crono and 400 IU PMSG Gest (Folligon) and their calving date.

Tabel nr. 1

Nr. curent	Matricol number	Extraction date of pessary +PMSG (400 U.I.) 2.10.2008	Date I.A./ nr of doze	Date of birth	Sex of lamb
1	RO 105 7 031 052	Da + 400	5.10.08	03.01.2009	F
2	RO 104 6 336 062	Da + 400	5.10.08	12.01.2009	M
3	RO 105 7 030 076	Da + 400	4/5.10.08 / 2	17.01.2009	M -mort
4	RO 107 2 949 122	Da	5.10.08	13.01.2009	F
5	RO 105 7 035 123	Da + 400	5.10.08	-	-
6	RO 407 1 711 244	Da	4.10.08	-	-
7	RO 140 0 645 302	Da + 400	5.10.08	13.01.2009	F
8	RO 105 7 031 337	Da + 400	4.10.08.	-	-
9	RO 104 0 645 344	Da + 400	5.10.08	13.01.2009	F
10	RO 105 7 035 379	Da + 400	4.10.08	15.01.2009	M
11	RO 104 0 645 409	Da + 400	4.10.08	-	-
12	RO 105 7 035 485	Da	5.10.08	-	-
13	RO 104 0 644 473	Da	4.10.08 / 2	-	-
14	RO 105 7 031 526	Da + 400	4.10.08	-	-
15	RO 105 7 031 534	Lost pessary	5.10.08	-	-
16	RO 107 2 948 655	Da	4.10.08	-	-
17	RO 107 2 948 657	Da + 400	4/5.10.08 /2	28.12.2008	M
18	RO 107 2 948 676	Da	5.10.08	30.12.2008	F
19	RO 107 2 948 683	Da	4.10.08	28.12.2008	M
20	RO 107 2 948 697	Da + 400	5.10.08	-	-
21	RO 105 2 948 732	Da + 400	4.10.08	-	-

22	RO104 0 421 768	Da + 400	4.10.08	28.12.2008	F
23	RO 104 0 621 769(0)	Da	4.10.08	-	-
24	RO 104 0 621 777	Da+ 400	5.10.08	-	-
25	RO 104 6 328 934	Da+ 400	5.10.08	-	-
26	RO 104 6 328 947	Da	4.10.08	08.01.2009	M
27	RO 104 6 328 948	Da	5.10.08	-	-
28	RO 106 1 633 965	Da	5.10.08	-	-
29	F.M. – U. S. Crestata	Da + 400	5.10.08	-	-
	Total lamb obtained form I.A.	-	6 (46,15%) G.04.10.08 6 (31,57%) G.05.10.08	-	12 = 6 M+ 6 F (41,37%)

From this table that were artificially inseminated a total of 29 sheep breed Karabaşă (including 3 females were double-dose AI) with frozen semen from male Awassi breed, using a total of 32 doses. Stock in the liquid nitrogen container CSCBA There are 21 doses (9 and 12 yellow beads white doses). Mention that of the 55 doses of m.s.c. imported Awassi breed in Syria, two doses were examined by the A.N.A.R.Z. dr. ing. Maria Vasile, who found that its value is of average quality.

### 3. Conclusion

3.1. The method of induction and synchronization of oestrus in sheep with the help of pessary CronoGest and administration of 400 IU PMSG without PGF2  $\alpha$  administration, led into the heat of over 60% of treated sheep and 58% were IA

3.2. After 48 hours of extraction and management pessariior CronoGest 400 IU PMSG were IA with m.s.c. importet Awassi breed in Syria, 13 sheep breed of Black Head Tzigai Teleorman (Karabaşă and six females calved (46.15%).

3.3. After 72 hours of extraction and management pessary CronoGest 400 IU PMSG were IA with m.s.c. 19 sheep breed of Black Head Tzigai Teleorman (Karabaşă) and gave birth after a normal pregnancy 6 women (31.57%).

3.4. After I.A. a total of 29 sheep breed of Black Head Tzigai Teleorman (Karabaşă) have given birth after a normal pregnancy, a number of sheep, 12 resulting in 12 products (41.37%), 6 males and 6 femefe.

3.5. It can be concluded that using ram semen frozen in biotechnology IA products can be obtained in an interest rate beneficial to farmers.

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## ANIMAL WELFARE IN SERVICE OF ECOSANGENESIS, OF ANIMAL HEALTH AND FOOD SAFETY

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based on eco-economy and biochemistry necessary for eco-san-genesis

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### SUMMARY

**Key words:** animal welfare, ecosangenesis, animal health, food safety

Lately, we have been witnessing a background mutation in the concept of animal disease control. If in the past this was mainly preventive, using biological products, treating the animals with drugs and even cutting the workforce, today we also take into consideration additional protection and animal welfare for animal food safety, as a basic element of public health and protection of the environment. The term welfare was chosen to refer to the quality of animal life in a safety and ecological environment, in one word: ecosanogenesis. The introduction of ecosanogenesis is not easy especially in times of crisis when everybody is interested in the quantity and efficiency which is needed in making of a product. One of the most important objectives of ecosanogenesis is to require proper management and having a preventive role, to avoid spasms and irreversible evolutions regarding animal welfare, human and environment health in the next century. In this context, the role of veterinary medicine is growing especially for human and environmental protection, without lowering the responsibility for animal welfare as a biological way of production, but also about their welfare as emotional beings with certain capacities.

The welfare concept still lacks a precise definition, but all experts who have studied animal welfare agree that this notion includes *health, productive comfort and protection of animals*. The Universal Declaration on Animal Welfare, developed by the World Society for Animal Protection, defines animal welfare as: *the degree in which the physical demands, behavioral and psychological characteristics of the animal are met*. Another definition, increasingly accepted, is that of professor *Broom*, this being acquired by the Eurogroup for Animal Welfare (1980) of the European Council.

“The animal welfare” is defined as the mode in which an animal can cope with the conditions in it lives. An animal lives in welfare conditions if it is healthy (this being proved scientifically), lives in a comfortable environment, is well fed, safe, capable of showing a innate behaviour, and doesn't suffer of negative effect states as pain, fear and sufferance. The animal welfare makes references at the animal state of being; the treatment that an animal receives corresponds to other terms which are: animal care, breeding and having part of so called “human like” treatment.

(OIE 2008 Source)

The welfare is not a state that can be conferred to an animal, but it is a natural individual state, as long as the animal makes an effort to accommodate, in relation with its needs, environmental characteristics and the way in which the information are perceived.

The welfare is equally influenced by the feeding conditions, adaptation, microclimate, and also by the informational circuit between animals and their environment. The information can influence in a decisive way the homeostazia and especially the way of using the substances and energy, animal necessities being in correlation with numerous functional systems on which the survival system depends on. (*Decun, 2010*).

Last but not least the animal welfare also refers to animal's life quality. The most widely accepted definition of animal welfare, is that issued by *Broom*, that states that „the animal welfare represents the animals state in relation to their attempts of accommodating to the environment in which they live”.

The modern vision regarding animals is influenced by the idea, confirmed by modern science, that the animals are beings which “have feelings, feel pleasure, but also suffer and feel pain.

So, in the 2005 Report of the Norwegian Council on Scientific Reserch, *Broom's* definition extends to the animal's psychic: „the animal welfare represents the subjective experience of the individual regarding its mental and phisical state in realtion to their attempts of accomodating to the environment in which they live.”

The animals had and still have a „startegic role” in the food supply for humans and are required to maintain any rational process of a nation's food reserves. Nowadays, farming is no longer considered by the European consumers as a simple mean of realising alimentary products. The animals are very usefull for other social fundamental researches such as: certainty

and the quality of alimentary products, the environment protection and sustainable development. Internationally, the link between animal welfare, animal health and food safety, was recognized by the relevant legislation (OIE - Terrestrial Animal Health Code (2005) Appendix 3.7.1., Guidelines for animal welfare).

Food safety is a parameter which involves the consumer, and all the parts that participate at its production, processing and transportation are also involved in ensuring it. On the conservation of food safety in the European Union are the professional training, the civic education, the awareness and the control of state Institutions and NGOs made to the highest standards. The European Union food strategy is based on three main elements:

1. the legislation on food and fodder safety
2. a fundamental scientific advice necessary to taking a decision in this domain.
3. a policy and control implementation

The legislation covers many domains, from food and fodder to alimentary hygiene, applying the same high standards all over the European Union. In terms of potential benefits from animal welfare, the new measures under the rural development policy present a higher interest (The European Commission working document containing a community action plan for the protection and welfare of animals during the period 2006-2010 Action 1, pct.13) According to the „World Declaration on Nutrition” (FAO/OMS, Roma, 1992) and to the „Declaration on World Food Security (FAO/OMS, 1996), „food security exists when all people at all times, have physical and economical access to safe and nutritious food to meet the food needs of the body, to lead a healthy and active life”.

Food security is a state level policy as well as a global policy. Earth's population nutrition is a concern of the international expansion WHO (World Health Organization), FAO (Food and Agriculture Organization), the Codex Alimentarius Commission, etc. Each country's food security can be achieved primarily from internal resources through policies applied by each country.

Animal welfare should be seen as an integrative science, providing a superior understanding of complex interactions between animals and their environment. Recent studies have shown that the use of modern methods of assessing animal welfare, although profitable in the short term, can provide

long-term profit through continuous improvement of farm animal welfare (*Broom theory*, 1995).

The problem of efficiency in today's society means becoming more complex to solve problems contained in the phrase „human-product-nature” context in which the notion of efficiency is rather effective for the ecosystem.

Application of this phrase is not possible in this case, as the existence of a healthy man is not possible in an unhealthy environment. By merging the terms „ecological” and „sangenesis”, *B. Cotigan* used for the first time the term eco-san-genesys.

Animal welfare issues are generally negative consequences of animal-environment interaction, resulting from the management of the accommodation factors, so-called „design criteria”(Anonymous, 2001).

Getting organic products, unpolluted and non-polluting, primarily agricultural products, represents the plunge in obtaining sanogene products for the food, cosmetic or pharmaceutical industry. The most important are, of course, bioproducts and biofodders, with complementary nutritional and therapeutical preventive effect (*Gruia, R.*, 1995), as well as the herbal and forest area products, which became very popular lately.

Obviously the enforcement of ecosangenesis term is not an easy thing, especially in times of economic crisis when the quantity and efficacy are the key factors when a product is made. To avoid such a situation, in addition to new technologies able to support the growing needs of manufacturers and to protect the environment, we believe that successful element is the way of persuasion, at a conceptual level.

The application of HACCP (Hazard Analysis, Critical Control Points), the analysis of risks and critical points for their control, aims, in the concerned field, the protection of animal health care and food safety but also the insurance of animal welfare in close relationship with a healthy environment. The evaluation of animal welfare based on the HACCP system was proposed by *Grandin* (1998) and it was widely adopted in the United States of America. At present, many European countries are making great efforts to introduce the systems for evaluating the animal welfare which stand on HACCP principles. (*Teuşdea*, 2005). The HACCP is considering the establishment of methods and means of identifying the risk ( zoonotici or nezoonotici pathogenic agents) and of critical points ( vectors, places, etc.) through which the pathogenic agents come into the system, effectively and

of removing/controlling them on the basis of monitoring and continuous adaptation to any situation. HACCP is a coherent means of analysis and decision, which applies at all stages of technological process, namely growth, exploitation and protection of animals.

Welfare programs based on HACCP system should consider protecting livestock from holdings of foreign aggression animated hazards, but also each stage of production cycle of the infectious intense pressure, through concerted actions of maintaining this at a low level.

These should be an easy to follow detailed guidance for the implementation and evaluation of the results, based on seven principles that relate to:

- the risk analysis;
- determination of critical control point;
- establish critical limits;
- establish monitoring system of the system;
- establish corrective measures;
- recording data and setting up a data bank;
- establish measures/tests for own or neutral verification of the proper functioning of the system

The risk analysis has as purpose:

1. the identification, for the entire period of growth and operating dangers (risks) for the health workforce as a mean of biological production and food safety derived from them;
2. assessing the likelihood of hazards, identify preventive methods and means for controlling risks

The HACCP system is in constant expansion, moving from food industry to the livestock feed industry and other fields.

## **CONCLUSIONS**

1. Faced with the increased consumer demand around the world regarding the amelioration of health security of food, you need a law and scientific deed on "health security of food of animal origin from alive animals," thus the animal should be investigated and monitored continuously to determine whether the rules and principles of animal welfare were followed;

2. The transmission of information about animal welfare and ecosangenesys from professionals to the general public, accurate and sustained information leading to public education in the spirit of the requirement of quality goods, which are not to be produced at the expense of the environment and human health;

3. Awareness raising ability of state institutions in developing environmental policies, economic and health surveillance and related areas, thus eco-san-genesys can fulfill one of its most important objectives, namely to impose a management appropriate and to have a preventive role, to prevent spasms and irreversible developments on human health and the environment in the next century

In this context the role of veterinary medicine increases, especially in terms of human and environmental protection, but without decreasing its responsibility to animal protection as a mean of biological production, but also of their welfare, as beings with certain emotional capacities, poorly investigated and known so far.

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## **RESEARCHES REGARDING THE MICROBIOLOGICAL PARAMETERS OF WATER IN MURES RIVER**

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**Key words:** water, pollution, microbiological parameters, sampling points

### **SUMMARY**

Water, the environment in which it is supposed that life appeared, is always subject to pollution, this fact becoming an issue of great interest.

This study aimed to assess the fecaloid pollution of Mures River by sampling water from 7 points. From water samples there were assessed the following parameters: total coliforms, fecal coliforms, fecal enterococci and sulphito-reducing bacteria.

The microbiological parameters determination was done according to the provisions of STAS 3001/1991.

Following the researches it can be concluded that the lowest number of bacteria was recorded at 10 km upstream of Târgu Mureş and the highest number occurred at the entrance of the city due to both recreational practices in this area and wastewater discharge from mink farms. There were noticed seasonal fluctuations of germs number, in all sampling points the values being minimal in winter and maximum in summer.

The origin of fecaloid pollution of water in Mures River is represented by human activities in summer time, at the entrance and exit point of the city, and mixed source with animal mainly contribution during the winter in the others sampling points.

Water, the environment in which is admitted that life appeared, has also a major significance upon the perpetuation of life on Earth.

Along with the global economic development, the water pollution takes a large growing, becoming today a highly topical issue (5).

Thus, purification of polluted water is a vital necessity for preserving the ecological balance.

Water pollution is also manifested by unusual loading with microorganisms.

Microorganisms are found in different proportions in natural surface and underground waters.

In the surface waters, the density and nature of microorganisms depend on several factors including: nearby presence of pollution centers, distance from the point of discharge, season etc. (1,6)

As a potential carrier of pathogenic microorganisms, water can endanger consumers' health and life (2,3,4).

Drinking water in most cities is obtained from surface sources, which are often subject to pollution (5).

### 1.MATERIAL AND METHODS

The present paper aimed to assess the Mures River fecaloid pollution extent.

There were taken water samples from 7 points: P1 - at 10 km upstream of Târgu Mureş; P2 - at dam point, where the River enters the city; P3 - in Târgu Mureş city area; P4 and P5 - upstream and downstream of city's water treatment plant; P6 and P7 - at 8 km, respectively 10 km downstream of Târgu Mureş.

From the water samples there were established the following microbiological parameters: total coliforms, fecal coliforms, fecal enterococci and sulphito-reducing bacteria.

The microbiological parameters determination was done according to the provisions of STAS 3001/1991 (7).

In order to differentiate the Enterobacteriaceae species there were performed four tests: indol production by tryptophan metabolism; methyl red test; Voges-Proskauer Test and citrate use as single carbon source.

### 2.RESULTS AND DISCUSSIONS

The results obtained following the total coliforms number establishing are presented in Table no. 1.

*Table 1*

The number of total coliforms in Mures River' water during the four seasons of 2010 (germs/l)

Sampling point	Season			
	Winter	Spring	Summer	Autumn
P1	180	550	8300	3800
P2	400	740	110000	43000
P3	360	740	92000	21000
P4	600	930	43000	54000
P5	740	740	36000	43000
P6	560	830	21000	6200
P7	740	820	28000	4900

Analyzing the data in the table, it can be noticed that the lowest value was determined in water sampled from the point located at 10 km upstream of Targu Mures in winter and the higher value was determined at the entrance of river in the city, during summer.

The explanation could be that the water from Mures River before enters the city is clean in terms of fecaloid pollution and the effect of urban agglomeration is felt up to 10 km downstream of Targu Mures (P7).

The very high values recorded in P2 during summer and autumn are explained by the fact that in warm season, in this area, the residents practice recreational and entertainment activities, also in this sampling point Mures River is linked to the Recreational Complex “Muresul” and in the same spot there were discharged the wastewaters from a mink farm situated on an island at 200 m upstream of dam.

A seasonal evolution is evident in all sampling points: the registered values being minimal in winter, slightly high during spring, high in summer and very high in winter.

The numbers of fecal coliform bacteria established in all the four seasons of year 2010 in the water of Mures River are shown in table no. 2.

*Table 2*

The number of fecal coliforms in Mures River' water during the four seasons of 2010 (germs/l)

Sampling point	Season			
	Winter	Spring	Summer	Autumn
P1	18	95	580	230
P2	54	210	14000	380
P3	180	250	11000	2300
P4	130	330	9200	4300
P5	74	170	6400	2200
P6	78	200	2300	840
P7	61	140	2800	640

The results of determinations from table no. 2 show that, in the case of fecal coliforms number, the highest value was recorded during summer in the second sampling point (P2: 14000 germs/l) and the lowest value in the winter in the first sampling point (P1: 18 germs/l).

For the fecal coliforms was noticed also a seasonal evolution in all sampling points, following the same tendency with minimal values in winter, slightly raised in spring, maximal in summer and close to the summer values in autumn.

The ratio between the values recorded in summer and those recorded in the other seasons varies in closer limits than for total coliforms, the maximum value of the ratio being registered in P2 sampling point.

The numbers of fecal enterococci are shown in table no. 3.

*Table 3*

The number of fecal enterococci in Mures River' water during the four seasons of 2010 (germs/l)

Sampling point	Season			
	Winter	Spring	Summer	Autumn
P1	40	140	430	210
P2	36	83	3100	180
P3	83	94	4300	720
P4	81	98	2100	1700
P5	36	94	2700	910
P6	36	110	1400	620
P7	54	94	1700	620

As can be noticed from the table, the presence of fecal enterococci was detected in all the analysed samples. Their number was higher in summer and autumn in comparison with winter and spring seasons. Unlike the result obtained for the other two above mentioned bacterial groups, the highest values are recorded in P3 sampling point (Targu Mures city area) in summer (4300 bacteria/l).

The nature of fecaloid pollution of surface waters was established by using the proportion between the fecal coliform bacteria (FC) and fecal enterococci (FE): table no. 4.

Table 4

The proportion between fecal coliforms and fecal enterococci, indicator of pollution source - with animal mainly contribution (FC/FE <2) or human origin (FC/FE>2)

Sampling point	Season			
	Winter	Spring	Summer	Autumn
P1	0.45	0.68	1.35	1.10
P2	1.50	2.53	4.52	2.11
P3	2.17	2.66	2.56	3.19
P4	1.60	3.37	4.38	2.53
P5	2.06	1.81	2.37	2.42
P6	2.17	1.82	1.64	1.35
P7	1.13	1.49	1.65	1.03

A proportion FC/FE higher than 4 indicates a certain human origin polluting source. When the proportion has values between 2 and 4, the pollution is mixed with human mainly contribution; when is between 0.7 and 1 is mixed but with animal mainly contribution and when is lower than 0.7 the pollution has strictly animal origin.

According to this indicator, the human source of fecaloid pollution is proved only in summer in P2 and P4 sampling points (at the entrance of River in Targu Mures and at the exit) and the animal source of pollution in P1 sampling point in spring and winter. In the other sampling points, the pollution is mixed, with animal preponderance.

The results of analysis regarding the sulphito-reducing bacteria in water samples are shown in table no. 5.

Table 5

The number of sulphito-reducing bacteria in Mures River' water during the four seasons of 2010 (germs/l)

Sampling point	Season			
	Winter	Spring	Summer	Autumn
P1	37	56	71	19
P2	56	86	152	80
P3	18	52	103	92
P4	53	55	99	64
P5	57	78	74	58
P6	39	83	168	35
P7	75	53	99	52

As can be noticed, the sulphito-reducing bacteria are also present in all analysed samples, their number being significantly lower than the bacteria in other groups. The values exceed 100 only in summer in P2, P3 and P6; the maximum being recorded in P6 (168 germs/l).

The seasonal fluctuation does not follow the same tendency as the other groups of bacteria. Thus, in summer were recorded the highest values, in winter the lowest, but in spring the established values are higher than in autumn, except the samples from the points located upstream of Water Treatment plant Cristesti.

### 3.CONCLUSIONS

3.1. In all seven sampling points, located between 10 km upstream and 10 km downstream from the Targu Mures city was detected the presence of total coliforms, fecal coliforms, faecal enterococci and sulphito-reducing bacteria, regardless of season.

3.2. The lowest number of bacteria is generally recorded in P1 sampling point, located at 10 km upstream of the city and the highest number (up to 110'000 total coliforms/l) in the P2 sampling point, located at the river entrance to city. The differences from the other sampling points are quite high: more than 13 times in summer and more than 11 times in fall — for total coliforms, in P1 sampling point.

3.3. High values recorded in P2, especially in summer and autumn is explained by the effect of intense practice of recreation and entertainment activities perform by the city residents in this area, and the discharge of wastewater from the mink farm located nearby. It can be said that before entering the city, Mures River is quite clean in terms of fecaloid pollution and urban agglomeration effect is felt up to 10 km downstream (P7), where the number of total coliforms/l remains fairly high, even if the values are below those for the city.

3.4. Is recorded a clear seasonal trend in the number of bacteria belonging to the four groups studied. Evolution is parallel in all sampling points, with minimum values in winter, slightly higher in spring, high in summer and autumn, without relevant differences between groups. In fecal coliforms germs, the ratio between the values recorded in summer and the

values from the other seasons varies in smaller limits than for total coliforms.

3.5. Fecal enterococci were present in all samples analyzed. Their number is with one or two orders of magnitude higher in summer and autumn compared with winter and spring seasons. Only in P1 sampling point were not recorded values exceeding hundreds, in any of the seasons.

3.6. The nature of surface waters fecaloid pollution, assessed by an indicator which represents the ratio of fecal coliforms/fecal enterococci, shows a clear human origin in summer in the P1 and P2 points, at the entrance and respectively at the exit of the River from Targu Mures. The animal source of pollution is proved (in the basis of the same ratio) in winter and for the P1 sampling point in winter and spring. For all the other sampling points, the fecaloid pollution is mixed, with animal mainly contribution.

3.7. The presence of sulphite-reducing bacteria is recorded in all samples analyzed. Their number is significantly lower than the other bacterial groups.

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## GENETIC EFFICIENCY ESTIMATION OF MOET AND CLASSICAL BREEDING PROGRAMMES IN CATTLE

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**Key words:** artificial insemination, multiple ovulation and embryo transfer (MOET), genetic progress

### SUMMARY

The aim of this study was to predict the achieved genetic progress by applying MOET in bull dams, comparatively the classical variant based only by artificial insemination using, keeping the bulls testing on their descendants and the analyze the opportunity of bull dams selection on descendants with collateral selection.

Comparative the Artificial Insemination classical variant, MOET applying in bull dams determined the improvement of donor cows' prolificacy, the increasing of selection intensity and also the increasing of the genetic progress per generation, on bull dams' path. This increasing, associated with generation gap decreasing, consecutively MOET (5.5 years, beside 7.5 years in the classical A.I.variant) established the increasing of the annual genetic response ( $\Delta G = +15\% -30\%$  Elite : 70%Testing;  $\Delta G = +13\% -70\%$ Elite : 30%Testing). MOET variant with bull selection per collaterals (full sisters and half sisters) was superior the classical variant A.I., no matter the active population structure ( $\Delta G = +19\% -30\%$  elite: 70%Testing).

Initial, applied in the aim of scientific and later, in the commercial way, the embryo transfer, preceded by the poliovulation (MOET), represents a biotechnology of reproduction used more and more frequent (in the developed countries) in cattle. The multiple scientific, sanitary-veterinary, economical and especially zootechnical advantages (decreasing of generation interval, the substantial growing of the progenies number obtained from a donator with a remarkable zootechnical value, the increasing of the females contribution to the genetic progress, the acceleration of the rhythm in the genetic breeding of cattle etc.) [1, 6, 7, 12], justify higher interest of the geneticists for the large application of MOET in cattle. The unanimous opinion of the specialists is that the main zoo technical advantage of embryo transfer is given by the decreasing of generation interval for the obtaining of a higher number of progenies during the reproductive female life, the creation of some numerous families of full

sibs, maternal and parental half sibs, offering, in this way, the possibility to replace the progenies selection by the collateral one. This substitution raises a few questions: in point of accuracy, is the collateral selection same as the progenies testing?; does the collateral selection offer a better response than the progenies testing?; is it really, opportune to replace the progenies testing by the collateral selection?

## 1. MATERIAL AND METHODS

To answer to the questions mentioned above, in the present study there were effectuated two steps. In the first step, we estimated the possible genetic progress which could be obtained through a classical breeding program, applying the artificial insemination (AI) and progenies testing, and in the second step, the possibility of implementation MOET into a breeding program of dairy in Romania was analysed. In the MOET variant, the genetic progress possible through the keeping the progenies testing and through the replacing it by the collateral selection (full sibs; full and half sibs) was predicted. This problem couldn't be solved without simulation, and the conditions of simulation were the following:

- active population size = 100000 cows; structure of active population, in the variant by the progenies testing = 30% Elite:70% Testing; 70% Elite:30% Testing (there were taking in count only these two structures, due to in a precedent study [13], the minimum genetic progress was obtained in the first variant, and the maximum was recorded in the second variant); average duration of exploitation of reproduction cows = 4 years old; average duration of exploitation in bulls = 3 years old (in the elite group); in the active population, 12 bulls will be used to maintain the inbreeding at a low level; birth rate in the active population = 0.80; surviving to the first calving = 0.85; age at the first calving = 2.5 years old; calving interval = 1 year; one daughter or a male could be obtained either from the three cows in a year, either from a cow during three years (in the variant without MOET); annual losses of 20% till the second calving and of 10% from the second to the third calving, when the bull dams will be classified (number of lactation/cow = 3); average performance of the population before the selection = 4000 kg milk; phenotypic variability coefficient = 25%; phenotypic standard deviation  $\sigma_p = 0.25 \times 4000 = 1000$

kg; genetic standard deviation  $\sigma_A = h \times \sigma_p = 0.5 \times 1000 = 500$  kg; heritability for the milk quantity:  $h^2 = 0.25$ ; repeatability of the milk quantity:  $R = 0.40$ .

In the MOET variant, the cows called as bull dams, also depending the average performances recorded in the first three lactations will be taken out for one year from the reproduction, in order to be used as donators of embryos. In this period, the donators are poliovulated and harvested for four times, at the intervals of three months. The number of viable and transferable embryos (first and second quality), was of 4 embryos/meeting of harvesting/donator, thus obtaining 16 viable embryos/donator/year. Considering a gestation rate of 50% in the receptor cows, a percentage of surviving of calves of 85% and the sex ratio of 1:1, results that annually, there could be obtained about 6.8 viable calves from a donator (3 males and 3 females) thus, 3 young bulls could be introduced in testing.

There were established: the retaining ratio, the intensity of selection, the accuracy of selection and the interval of generation, on the way of dam dams, bull dams, bull bulls, and dam bulls. There were considered the following generation intervals:  $L_{DD} = 5.5$  years,  $L_{BD} = 7.5$  years,  $L_{BB} = 7.92$  years,  $L_{DB} = 4.45$  years, values established by Drăgănescu C. I. (1998) [10] for the classical variant AI, respectively  $L_{DD} = 5.5$  years,  $L_{BD} = 5.5$  years,  $L_{BB} = 7.92$  years,  $L_{DB} = 4.45$  years, for the MOET variant by the progenies testing. In the MOET variant, by the selection of collateral,  $L_{DD} = 5.5$  years,  $L_{BD} = 5.5$  years,  $L_{BB} = 4.3$  years, and  $L_{DB} = 4.3$  years. Finally, it was estimated the genetic progress per generation (R) and the annual genetic progress ( $\Delta G$ , expressed into genetic standard deviations  $-\sigma_A$  -, in absolute values -kg-, and in relative values -%-) [9, 11]. Also, the weight of participation of these four ways to the genetic progress was estimated.

## 2. RESULTS AND DISCUSSIONS

The results presented into the Table 1 shown that, both the classical variant AI, and the MOET variant by the progenies testing, the changing of the percentage structure in the active population through the increasing of the weight in the elite group from 30% to 70% and the decreasing of the weight in the testing group from 70% to 30%, is favourable for the increasing the response to selection. The majoring the selection effect by the increasing the weight in the elite group, is the consequence of the changing the retained ratio and the intensity of selection, through the bull dams and

bull bulls. The increasing of retained ratio and reducing the intensity of selection through the bull bulls, which contributes, of course, to the decreasing of the genetic progress per generation ( $R_{BB}$ ), it was “compensated” by the antagonistic evolution of these two parameters on the way of bull dams, which contributed to the increasing of genetic progress ( $R_{BD}$ ) on this way. The applying of MOET in the bull dams, allowed the substantial decreasing of the number of cows called as bull dams and the increasing of intensity of selection by about 20% in the variant by 30% Elite:70% Testing, and by about 14% in the variant by 70%Elite:30%Testing. This increasing generated the increasing of genetic progress ( $R_{BD}$ ) by the same weight and, together with the decreasing of the interval of generation, consecutively to MOET (5.5 years comparison to 7.5 years in the classical variant AI); finally, it determined the increasing of the annual response of selection. The increasing of the annual genetic progress using MOET as comparison to the classical variant AI was 15.1% for the structure 30%Elite: 70%Testing, respectively 13.3%, in the variant by 70%Elite: 30%Testing.

As regards the weight of participating of these four ways to the genetic progress, the results presented into the table 1, suggest that, through the applying of embryo transfer at the bull dams, it increase the contribution of females to the genetic progress by about 4%. The contribution of females to the response of selection could be supplemented through the MOET also, on the way of dam dams (an alternative non-taken in count in this study). Christensen (1991), shown that into the conventional breeding programs in dairy cows, the contribution of these four ways at the genetic progress depends upon the accuracy, intensity of selection, and interval of generation. These three parameters are influenced by a few factors and they have tendency to be unfavourable linked. For example, the selection on the repeated performances offers a better precision, but increases the interval of generation, and decreases the intensity of selection. The contribution to the genetic progress through the way of dam dams is enough low (about 5%), due to, mainly, to the low reproduction rate, fact which makes the breeders to retain the highest ratio from the female’s descendants. Thus, the intensity of selection, on this way, is very low, but it could increase significant using MOET. Doubling the number of progenies per cow, the total genetic

progress could increase by about 8%, and an increasing of about 5 times of the reproductive rate, could majoring the genetic progress by 15%.

Using MOET on the way of dam dams for the increasing of reproductive rate by 20 times could sensibly increase the total genetic gain (Cunningham, 1986). Numerous researchers (Mc Daniel, 1981; Van Vleck, 1981 etc.) shown that the applying MOET on the way of dam dams, could become economic feasible only if the costs could be substantial decreased. However, it seems that the economical benefits are neglect able as comparison the using of MOET in bull dams (Mc Daniel and Cassel, 1991).

As regards *the selection of bulls by collateral (sibs)*, analysing the results presented into the table 1, it could be seen that on the way of dam dams, the ratio of retaining, the intensity and accuracy of selection, and the genetic progress, presented the same values as in the classical variant AI, and MOET by the progenies testing. Applying MOET at the bull dams determined the changing of selection intensity in this category (+55% as comparison to the classical variant AI, +20.5% as comparison to MOET by the progenies testing in the case by 30%Elite: 70%Testing, respectively +17.1% as comparison to the classical variant AI, and +2, 6% as comparison to MOET by the progenies testing, in the variant by 70%Elite: 30%Testing. Consecutively to this evolution of selection intensity in the bull dams, the genetic progress ( $R_{BD}$ ) was superior to that obtained in the classical variant AI and comparison to the MOET by progenies testing (table 1).

The replacing of progenies testing by the collateral testing (sibs), determined the substantial decreasing of selection accuracy ( $r_{BB} = 0.3873$ , as comparison to  $r_{BB} = 0.9298$  - 30%Elite: 70%Testing, respectively  $r_{BB} = 0.8898$  - 70%Elite: 30%Testing, in the classical variant AI and MOET by progenies testing). This drastic decreases of accuracy by about 56-58%, couldn't be compensated by the decreasing of generation interval on the way of bull bulls and dam bulls (4.3 years), which finally, decreases the annual genetic progress comparison that estimated through the MOET by progenies testing, regardless of population structure ( $\Delta G = -7.9\%$  in case 30%Elite: 70%Testing;  $\Delta G = -21, 1\%$  in case 70%Elite: 30%Testing). MOET, by the bull selection on sibs is superior to the classical variant AI, only for the population structure of 30%Elite: 70%Testing ( $\Delta G_{MOETsibs} = +6.0\%$ ), and for the case by 70%Elite: 30%Testing  $\Delta G_{MOETsibs} = -10.6\%$ . This result demonstrates that the bulls testing only on their sib's

performances, doesn't constitute a viable alternative for replacing the progenies testing.

Analysing the percentage participation of these four ways to the genetic progress (Table 1), it could be seen the increasing of the females contribution (58%), mainly due to bull dams (52%). Through the application of MOET, each candidate young bull will have, besides full sibs, maternal and paternal half sibs, in a higher or lower number, depending on the number of viable embryos harvested from each donator cow. For that, it looks to be rational that *in the selection of each young bull, it has to be taken in count, both its full sibs, and half sibs*. On the way of dam dams, the ratio of retain, the accuracy and intensity of selection, like the genetic progress ( $R_{DD}$ ), presented the same values like in the other analysed variants. On the way of bull dams, the results were similar to that estimated in the MOET, by the sibs selection, and comparison to the classical variant AI and MOET by the progenies testing, the evolution of the factors which conditioning the response in selection, was also, similar to MOET, by the sibs testing. The only difference comparison to the  $MOET_{sibs}$  is given by the increasing of accuracy in bulls selection ( $r_{BB} = 0.5011$ , comparison to  $r_{BB} = 0.3873$ ), which was followed by the increasing of genetic progress on the way of bull bulls and dam bulls ( $R_{BB; DB} = 0.9949\Sigma_A$ , comparison to  $R_{BB; DB} = 0,7689\Sigma_A$ ), finally determining the increasing of annual selection response by 12.8%.

Table 1

Predicted genetic progress values in AI classical and MOET modern variant (Progeny testing; collateral testing)

Active population structure (%)	The way	Ratio retaining (p)	Intensity selection (i)	Accuracy selection (r)	Genetic progress/generation (R) ( $\Sigma_A$ )	Annual genetic progress ( $\Delta G$ ) ( $\Sigma_A$ )	Annual genetic progress ( $\Delta G$ ) (kg)	Annual genetic progress ( $\Delta G$ ) (%)
AI classical variant– progeny testing								
30 Elite 70 Testing	DD	0,7353	0,4452	0,5000	0,2226	0,1774	88,7242	2,2181
	BD	0,0504	2,0597	0,6455	1,3295			
	BB	0,0180	2,4590	0,9228	2,2690			
	DB				0,6807			
70 Elite 30 Testing	DD	0,7353	0,4452	0,5000	0,2226	0,2105	105,2480	2,6312
	BD	0,0139	2,5503	0,6455	1,6462			
	BB	0,0280	2,2950	0,8898	2,0420			
	DB				1,4294			

MOET variant – progeny testing								
30 Elite 70 Testing	DD	0,7353	0,4452	0,5000	0,2226	0,2042	102,0988	2,5525
	BD	0,0171	2,4779	0,6455	1,5995			
	BB	0,0180	2,4590	0,9228	2,2690			
	DB				0,6807			
70 Elite 30 Testing	DD	0,7353	0,4452	0,5000	0,2226	0,2384	119,1967	2,9799
	BD	0,0047	2,9091	0,6455	1,8778			
	BB	0,0280	2,2950	0,8898	2,0420			
	DB				1,4294			
MOET variant – sibs testing								
-	DD	0,7353	0,4452	0,5000	0,2226	0,1881	94,0719	2,3518
	BD	0,0037	2,9855	0,6455	1,9271			
	BB	0,0600	1,9854	0,3873	0,7689			
	DB				0,7689			
MOET variant – sibs and half sibs testing								
-	DD	0,7353	0,4452	0,5000	0,2226	0,2122	105,5997	2,6400
	BD	0,0037	2,9855	0,6455	1,9271			
	BB	0,0600	1,9854	0,5011	0,9949			
	DB				0,9949			

Even the accuracy and intensity of selection, like the genetic progress per generation on the way of bull bulls, presented lower values comparison to the classical variant AI and MOET, by the progenies testing, the plus of about 29% in the accuracy of selection (comparison to the selection on sibs), obtained by taking in count of full and half sibs of each young bull candidate in selection, associated by the decreasing of interval of generation, on the way of males (4.3 years), finally, led to the increasing of annual genetic progress. The MOET, by the bulls selection on full and half sibs, was superior to the classical variant AI, regardless of population structure ( $\Delta G = +19,0\%$  - 30% Elite:70% Testing;  $\Delta G = +0.8\%$  -70% Elite:30% Testing), and comparison to MOET, by the progenies testing, the superiority was maintained only for the active population structure of 30% Elite:70% Testing ( $\Delta G = +3.9\%$ ), while for the structure of 70% Elite:30% Testing, the genetic progress was a little lower ( $\Delta G = -11.0\%$ ).

Also, in the MOET by the bulls selection on full and half sibs, it was seen the superior contribution of females on the annual genetic progress (52%).

The evaluation of bulls could be improved by using of collateral selection, as supplement in the progenies testing. By a selection ratio of 20% at the bulls selected by their progenies, for the commercial aim, and having the records of 3-7 full sibs/bull, the relative genetic superiority of

seminal provided by groups where it was applied the collateral testing combined by progenies, could be 1.10-1.20 times higher than the value of sperm provided by a group where it was applied only the progenies testing (Smith and Ruane, 1987).

### 3. CONCLUSIONS

3.1. Comparison to the classical variant AI, the application of MOET in the bull dams, by the progenies testing, determined the improving of prolificacy in donators, the increasing of their selection intensity, and the majoring the genetic progress per generation on the way of bull dams by about 20% (30%Elite: 70%Testing), respectively by about 14% (70%Elite: 30%Testing). This increasing associated by the reducing, consecutively, of the generation interval consecutively in MOET (5.5 years, comparison to 7.5 years in the classical variant AI), determined the increasing of annual response ( $\Delta G = +15.1\%$  -30% Elite:70%Testing;  $\Delta G = +13.3\%$  -70% Elite:30%Testing).

3.2. The application of bull's selection on sibs was followed by the substantial decreasing of selection accuracy, which led to the decreasing of annual genetic progress, comparison to that predicted through the MOET, by the progenies testing, regardless of active population structure. MOET, by selection on sibs is superior to the classical variant AI, only for the population structure of 30%Elite: 70%Testing ( $\Delta G = +6.0\%$ ), and for the case 70%Elite: 30%Testing,  $\Delta G_{\text{MOET}_{\text{sibs}}} = -10.6\%$ .

3.3. MOET by the selection on collateral (full and half sibs), was superior to the AI classical variant, regardless of population structure ( $\Delta G = +19, 0\%$  - 30% Elite: 70% Testing;  $\Delta G = +0.8\%$  -70% Elite: 30% Testing), and comparison to the MOET by the progenies testing, the superiority was maintained only for the active population structure of 30%Elite: 70%Testing ( $\Delta G = +3.9\%$ ).

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## **RESEARCHES REGARDING THE GENETIC PARAMETERS OF SOME CHARACTERS IN A MEAT HYBRID HEN LINE**

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Key words: additive variance, heritability, repeatability, heterosis.

### **SUMMARY**

Heritability is considered as one of the most important features of a quantitative character, it allow the estimation of the gene contribution in achieving of a quantitative character [1, 2, 4]. Heritability ( $h^2$ ) expresses the proportion of the total phenotypic variance due to the genes effect which made the genotype of the character. Repeatability (R), as a genetic character, refers to the phenotypic expression of the same character in different moments of the individual life, expressing the constant appearance of the character from a performance to another.

The importance and use of the heritability coefficient ( $h^2$ ) in the animal breeding process, mainly consists in the fact that it represents the regression of an individual breeding value beside its phenotypic value. The knowledge of some character heritability is useful for:

- According the priority to the genetic breeding or the unit improving having as aim the increasing of the animal production.
- Choosing the selection method and the establishing of the selection objective.

The correct management of the breeding work has to estimate the heritability coefficient in each generation of the working population. The repeatability (R) represents the superior limit of the heritability because it includes all types of the influences, genetic ones, and general environment ones, which contribute to the real differences among individuals (1, 2, 3, 4, and 5).

Repeatability, as a genetic parameter, will present the same features as heritability, it is a particularity of each character, of each population and also of the environmental conditions the population evolve in.

## 1. MATERIAL AND METHODS

The present researches were made on a maternal meat line within “SC Avicola București”. There were used the performances recorded in 2010 by the control and selection results from half of the entire line. The families were chosen randomly, but furthermore, part of them were too limited regarding their descendents, so, finally, the research was made on 27 roosters families (a mix of sisters and half sisters) and 209 hen families. The average number of hens per one male, and also good families in the roosters family was 7,74, with a variation of minimum 4 and maximum 11. These families were made on 1681 chicks, proven from four successive hatchings. The average number of chicks per hen, and also the average size of full sisters families was 8,04, with a variation between minimum 5 and maximum 15. For a rooster there were 62,25 chicks, with a variation between minimum 34 and maximum 104. There were collected data regarding the following four characters used within the main selection objective:

- Growth speed, by body weight at eight weeks;
- Number of eggs in the control period by trap-nest (almost 26-40 weeks);
- Egg weight, at a significant age in the control period (30-31 weeks);
- Hatching percentage, in the same significant moment (maximum hatching intensity).

The estimation of the genetic parameters starts from the causal compounds of the variance. The knowledge of these compounds is possible after the observational compounds analyse is firstly done.

In the present research there was set an experimental plan which lead to a variance analyse with three sources (mother, father and descendants), in a non-balanced hierarchic model (different number of descendants per mother and different number of mothers per the same father). The statistic model for the observational compounds was the following:

$$Y_{ijk} = \mu + a_i + b_{ij} + e_{ijk}$$

Where:

$\mu$ =general average of the performances;

$a_i$ =exception due to i rooster;

$b_{ij}$ =exception due to j hen mated with i rooster;

$e_{ijk}$ =error that could affect the performances of chick of hen j, mated with rooster i.

To establish the heritability, it was applied the variance causal compounds method, knowing that heritability represent the ratio between the additive variance of the breeding value and the total phenotypic variance ( $h^2=V_A/V_F$ ). Knowing that  $V_A=4 S_{fathers}^2$ ), by dividing  $V_A$  to  $V_F$  the value of the heritability coefficient is obtained. By establishing the additive variance ( $V_A$ ), the only one which is transmitted from parents to descendants, it may be possible to establish the heritability (after the noticing of the observational compounds by variance analyze):

$$h^2 = V_A / V_{TOTAL} = 4S_{Fathers} / S_{TOTAL}^2$$

The precision of the establishing heritability was calculated using the simplified method of Robertson, quoted by Draganescu C. (1, 2):

$$S_{h^2}^2 = (h^2 + 4/ni) \sqrt{\frac{2}{S}}$$

Repeatability (R) expresses the percentage of the variance stabile compounds (they do not change during animals live), so they have not fluctuations from a performance to another one: genotypic variance with all the sources, and the variance of the general environment. Repeatability could be expressed by the following ratio:

$$R = \frac{V_G + V_{Mg}}{V_F}$$

$V_G$  = genotypic variance

$V_{MG}$  = variance of the general medium deviations

$V_F$  = phenotypic variance.

## 2. RESULTS AND DISCUSSION

The calculations started with the carrying out of the variance analyze for the all four characters. The results are shown in table 1.

*Table 1*

Observational compounds of the studied characters

Variance compounds	Body weight at 8 weeks	Number of eggs	Egg weight	Hatching percent
Among fathers $S_F^2$	378,053	588,6112	1545,4508	3,9069
Among mothers $S_M^2$ at the same father	473,1373	783,2722	1981,4703	11,5423
Among descendants $S_D^2$ at the same mother	6130,6539	7910,3020	7079,6805	305,1274
Total $S_T$	6981,8415	9282,1854	10607,0016	320,5766

Based upon these observational compounds, in the next stage there were established the causal compounds of the total variance (table 2).

Upon this study it may conclude that the dominance interactions less contribute to achieve the differences between individuals. Some exception is achieved by the hatching percent (1, 9770%), so at this character may have a heterozis effect that could be taken into consideration. The epistatic interactions, as a source of variability has not been separated in this analyze, being involved in the general environment and especially in the

special environment. In the same comparative study of the data in the table 2 results that the special environment took part less. This low participation of the general environment is caused by the inexistence of a common environment of each family, different from another, that will lead to a low correlation of the common environment ( $c^2$ ) within the phenotypic correlation (t) among members of a family.

Table 2

Causal compounds of the studied characters

Variance compounds	Expressing way	Body weight at 8 weeks	Number of eggs	Egg weight	Hatching percent
Additive variance	Absolute values	1512,2014	2354,4446	6181,8032	15,6278
	Relative values	21,6590	25,6590	58,2804	4,8749
Dominance variance	Absolute values	49,5152	75,4287	79,7116	6,3378
	Relative values	0,7092	0,8132	0,7515	1,9770
General environment variance	Absolute values	82,7080	175,7854	416,4916	6,0510
	Relative values	1,1846	1,8938	3,9265	1,8875
Special Environment variance	Absolute values	5337,4167	6676,4727	3928,9948	292,5600
	Relative values	76,4472	71,9279	37,0416	91,2606
Total ( $V_F$ )	Absolute values	6981,8415	9282,1854	10607,0016	320,5766
	Relative values	100,0000	100,0000	100,0000	100,0000

In table 3 there are presented the genetic parameters of the characters:

Table 3

Heritability and repeatability values of the studied characters

Character	Heritability and its error ( $h^2 \pm S^2_h$ )	Repeatability (R)
Weight at 8 weeks	0,2165±0,0783	0,2212
Number of eggs	0,2536±0,0886	0,2699
Egg weight	0,5828±0,1803	0,6298
Hatching percentage	0,0487±0,0315	0,0874

By its value, repeatability shows the percentage of the constant part of the individual life, the one which could do that the performance be the same at a future control. By difference to the value 1, this parameter shows the percentage of the special environment in the total variance. Regarding the egg weight and the hatching percentage, the repeatability is framed in the part of the expected values. Low values were obtained for the body weight at 8 weeks (0, 2212). The repeatability of the egg number takes values in normal limits (0, 2699).

Heritability shows how the additive interactions participate to population variability, the measure how the individuals differ by their breeding values. So, in a population where the variance due to non additive interactions and general environment variations is low, the values of repeatability and heritability are close, permanently the heritability is lower, that is noticed in table three.

The values of the heritability coefficients in the studied characters are framed in the limits mentioned in the special literature. It may remarked that the optimising of the experimental plan assure the heritability estimation with very low errors.

### 3. CONCLUSIONS

3.1. The non additive interactions less contribute to achieve the differences between individuals, practically in all the studied features.

3.2. The general environment variance has a low percentage in the total variance. It could say that the environment influences are random spread and each family has no special environment, different from another.

3.3. The values of heritability and repeatability coefficients have normal values in the studied characters, excepting the body weight in eight weeks, where the values are under the normal ones. The most probable explanation

is represented by the artificial increasing of the variance due to the special environment by errors in the performances control.

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## **RESEARCHES CONCERNING SOME BREAD SORTS QUALITY PROCESSED IN A MODERN UNIT**

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**Key-words:** bread, chemical analyzes, porosity, acidity, humidity.

### **SUMMARY**

Food stuffs quality and safety are nowadays a consumers right with direct effects upon life quality and the problem of these two desiderates is centred upon world and national organisms for consumers interests protection. Because bread and cereal stuffs are one of the most important fields in the food industry, these products are situated at the base of the nutritional pyramid, a study in the bread and other foodstuffs top processing unit represents an idea regarding the achieving of food safety. Bread and cereal stuffs are products which could affect consumer's health in the case of some physical, biological or chemical contamination, after ingestion or during the time by their noxious accumulation into the organisms. Only strict preserving of some processing rules during their processing could assure the quality and safety of these products.

That is why; the present paper proposes itself to carry out some physical and chemical indicators and sensorial assessment which reveal the bread quality of two bread types obtained in S.C. Vel Pitar S.A. București (Berceni).

### **1. MATERIAL AND METHODS**

The present paper studied two of the bread types obtained in S.C. Vel Pitar S.A. București (Berceni), *White Bread 300g* and *Black Bread 300g*. The analyzes were carried out in the Laboratory of Analyzes in S.C. Vel Pitar S.A. București (Berceni).

There were sampled 4 batches and there were established sensorial analyzes and physical and chemical ones. The physical chemical analyzes consisted in: acidity, pH and humidity and the obtained values were compared with the values stipulated by the standards.

The working methods in our researches are the classical ones for physical and chemical parameters used in the production unit S.C. Vel Pitar S.A. and in other control laboratories in our country.

## 2. RESULTS AND DISCUSSIONS

The first chemical analyzed parameter is the acidity. There are noticed low differences regarding the acidity analyze in the three batches of White bread, being recorded a mean value of 1,7 acidity degrees/ 100 g product. The chart 1 shows a balance of the mean values variation.

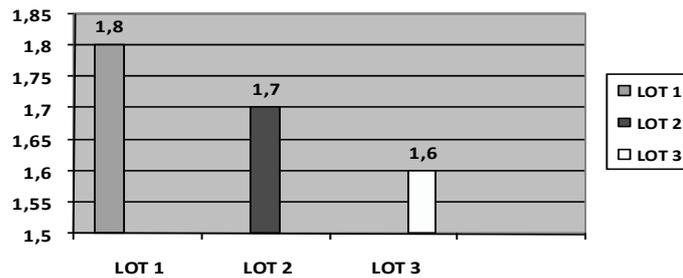


Chart 1. Acidity variation in White bread 300 g

This parameter was also established in Black bread 300g. Chart number two emphasize the mean values of acidity in Black bread 300 g and reveals the mean of 2,03 acidity degrees/ 100 g product and acceptable limits with very low differences within the four batches.

The chart number 3 shows the mean values of humidity recorded in the three batches of White bread, analyzed by the specific methods. The mean recorded value was 42, 56%. The chart shows the mean values for each batch and emphasizes the specific values.

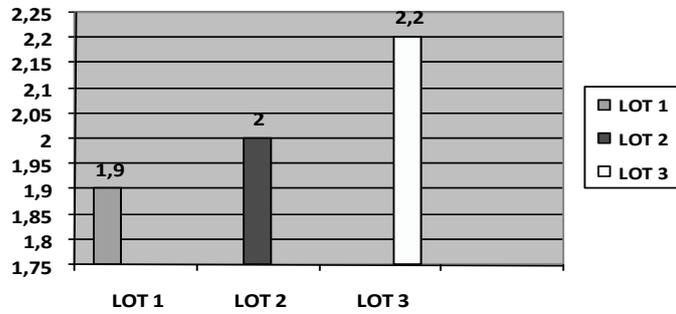


Chart 2. Acidity variation in Black bread 300 g

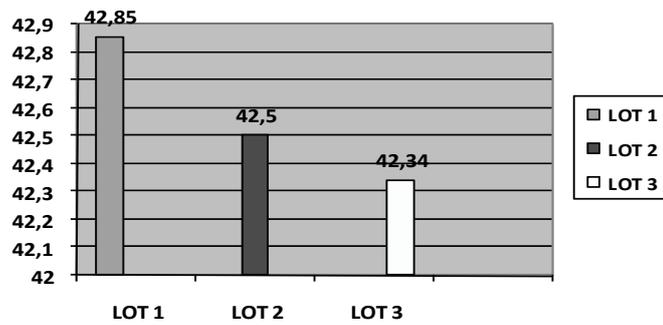


Chart 3. Humidity variation in White bread 300g

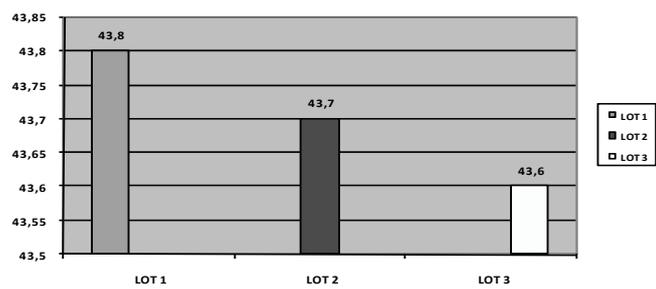


Chart 4. Humidity variation in Black bread 300g

Chart number 4 presented the same parameter recorded in the three batches of Black bread 300g. It may notice that the humidity in the four analyzed batches is framed in the acceptable limits with very low variations and presents a mean value of 43, 7%.

Chart number 5 shows the values recorded in the three batches of White bread 300g. It may notice that the pH values in the three analyzed batches is framed in the acceptable limits with very low variations and presents a mean value of 5, 34.

Chart number 6 shows the values recorded in the three batches of Black bread 300g. It may notice that the pH values in the three analyzed batches is framed in the acceptable limits with very low variations and presents a mean value of 5, 05.

The sensorial examination consisted in evaluation of all the sensorial features of bread with the aid of sense organs. It was made by specialists in the field and has in view the external features, by visual examination of the whole loaves, the aspect of the bread core, by cutting the loaves and core examination and also the taste, aroma, the microbial alteration signs.

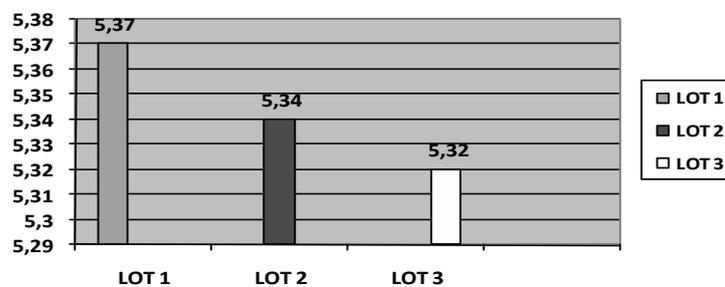


Chart 5. Values of pH variation in White bread 300g

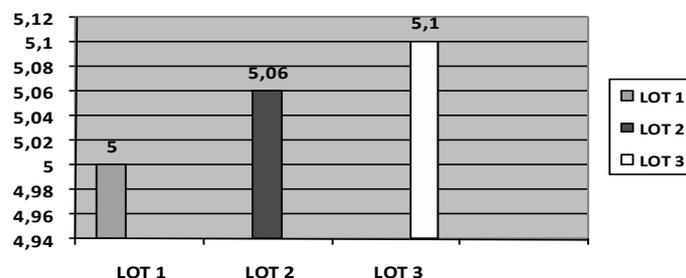


Chart 6.Values of pH variation in Black bread 300g

### 3. CONCLUSIONS

3.1. The sensorial, examination carried out upon the three batches of White bread 300 g and Black bread 300g demonstrates that in the analyzed batches bread does not present alterative disorders having the whole normal psycho sensorial quality for the control period.

3.2. From the physical and chemical points of view it is noticed a low variation of the analyzed but with no exceeding of the stipulated limits.

3.3. The analyzed unit proves to be again a top unit in the field that recommends it to all the consumers.

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## **RESEARCHES CONCERNING THE QUALITATIVE AND QUANTITATIVE PARAMETERS OF LACTATION IN ROMANIAN SPOTTED COWS**

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Key-words: lactation, total lactation, normal lactation, fat, protein.

### **SUMMARY**

The Romanian Spotted cow is a breed which represents 36% of the Romanian breed structure, and in Transylvania it represents 81, 5%, with variations between 65% and 100%. The Romanian Spotted cow generally represents the main milk and meat supplier in the area. Meat production is favourable, because there was kept the dual purpose type, and milk production was improved by the used breeding and exploiting technologies, being predicted in the future the improvement under Red Holstein impact.

### **1. MATERIAL AND METHODS**

The length of the normal lactation period represents the interval starting with the second day after calving to the day 305. Regarding the mean length of the normal lactation, this presents relative constant values, with a low decreasing trend, as in the total lactation, but with a medium increasing beside that, with values from 82-85% to almost 90%, having a great influence upon the calving interval.

The length of lactation is influenced appreciably by the genetic structure (to 10-15%), by the total length of lactation and this rapport beside the normal lactation, the technical and economical support of the exploiting unit (to 18-20% days, after C.Velea), the age at first calving, the parturition season, being shorter in the winter-autumn (depending on the number of lactation, with differences between 15-20%).

The present researches were made upon the whole population of Romanian Spotted cow, raised in the central and western part of the country. There were collected data regarding milk productions, from the quantitative and also qualitative point of view. They were statistically interpreted and there were concluded upon the importance in this breed exploiting within the area.

## 2. RESULTS AND DISCUSSIONS

The evolution of the total and normal lactation, their differences in days and the percentage of normal lactation length period (DLN) from total lactation length period (DLT) in Braşov active cows' population are presented in table 1.

*Table 1*

The evolution of the total and normal lactation in Braşov cows population

Lactation	n	X DLT	X DLN	Difference	DLN/DLT (%)
I	3036	337.32	286.54	50.78	84.96
II	2658	334.24	286.23	48.01	85.63
III	2104	327.64	276.13	51.51	84.27
IV	1651	344.04	298.72	45.32	86.83
V	1153	328.06	283.11	44.95	86.30
VI	789	346.38	299.15	47.23	86.36
VII	422	338.81	288.95	49.86	85.28
VIII	207	306.33	268.35	37.98	87.60
IX and more	28	313.72	280.24	33.48	89.32
TOTAL	15084	331.386	285.714	45.672	86.22

Analyzing the sampled data, it is emphasized that the total lactation length period is framed within 313-346 days, the highest period and its ratio beside the normal lactation was achieved at the sixth lactation when it reached the maximum value (an average value of 346.38 days DLT and 299.15 days DLN), after this being recorded a decreasing trend. The shortest lactation was the eighth one.

Beside the other breeds in Simmental group, raised in different European countries, as the special literature said, the total lactation varied between 273-385 days, with an average of 332 days in the Romanian active population, that situated it close to Slovenian breed (347days), and beside the original country, Switzerland (318 days) the differences being almost 7,5% in the Romanian population.

Following the analyze of the official control upon the active population of Romanian Spotted cow in Transylvania, it may notice differences regarding milk yield per normal and total lactation as it about fat and protein amount per total and normal lactation (table 2 and 3).

*Table 2*

Average values of milk quantitative parameters per normal lactation

District	n	Normal lactation		
		Average milk amount LN	Average fat amount LN	Average protein amount LN
Alba	2262	4028.04	162.97	128.96
Bistrita N.	627	<b>3519.94</b>	147.69	119.04
Brasov	633	3860.05	158.88	132.50
Cluj	1217	4128.94	163.13	129.16
Covasna	357	4078.42	166.33	126.06
Harghita	235	<b>4393.73</b>	160.47	135.39
Hunedoara	566	3606.96	<b>135.25</b>	<b>116.25</b>
Mures	1532	4250.91	<b>172.13</b>	<b>135.63</b>
Salaj	511	3878.63	162.59	134.59
Sibiu	298	3766.49	150.84	118.77
<b>TOTAL</b>	<b>6238</b>	<b>3988.06</b>	<b>160.27</b>	<b>128.90</b>

Table 3

## Average values of milk quantitative parameters per total lactation

District	n	Total lactation		
		Average milk amount LT	Average fat amount LT	Average protein amount. LT
Alba	2262	4600.09	187.38	148.72
Bistrita N.	627	<b>3828.17</b>	163.34	132.34
Brasov	633	4167.25	172.82	143.93
Cluj	1217	4639.98	184.12	146.07
Covasna	357	4432.98	180.94	137.67
Harghita	235	<b>4741.34</b>	174.09	146.97
Hunedoara	566	3852.93	<b>145.13</b>	130.88
Mures	1532	4726.29	<b>192.08</b>	<b>151.36</b>
Salaj	511	4327.89	182.86	151.22
Sibiu	298	4122.32	165.27	<b>130.46</b>
<b>TOTAL</b>	<b>6238</b>	<b>4400.15</b>	<b>177.87</b>	<b>145.02</b>

Conformingly the official control of milk yield, as it looks in the data processing, the Romanian Spotted cows active population achieved at Transylvania level an average amount of milk yield per normal lactation of 3988 Kg and an average amount of milk yield per total lactation of 4400 Kg, the maximum milk amount for a normal lactation (4393kg), and for the total lactation (4741kg) being obtained in Harghita district, and the smallest amount in Bistrita –Nasaud district (3519 kg /LN and 3828 kg/LT).

The fat amount per normal (172.13 kg) and total (192 kg) lactation was maximum in Mures district, with an average of 160.27 kg /LN and 177.87kg/LT, and the minimum value for the fat amount was recorded in Hunedoara district (135.25kg/LN and 145.13 kg/LT).

Regarding the protein amount, Mures district has the maximum value per both lactations, respective 135.63 kg/LN and 151.36 kg/LT . For the whole population, the average protein amount was 128.90 kg/LN and 145 kg/LT, with minimum values in Hunedoara district (135.25 kg/LN) and Sibiu district (130.46 Kg/LT).

### 3. CONCLUSIONS

3.1. The length of total lactation is surrounded between 313-346 days, the highest length of the total lactation and its ratio beside the normal lactation being achieved at the sixth lactation when it reaches the maximum value (an average of 346.38 days DLT and 299.15 DLN), after this recorded a low character. The differences are significant in districts, so the lowest lactation was recorded in Covasna and Harghita, almost 330 days and the highest in Alba (354 days). The length of lactation could be diminished in the future to increase the milk yield per cow's productive life.

3.2. The milk yield per total lactation in the active population of Romanian Spotted cow was almost 4400 kg. The best performances were obtained in Harghita: 4741 kg and Mures: 4726 kg and the lowest in: Bistrita-Nasaud: 3828 kg and Hunedoara: 3853 kg. Regarding the mean production per normal lactation, it was 3988 kg, being with 400 l lower than the one per total lactation.

3.3. The fat amount per total lactation in the active population of Romanian Spotted cow was almost 178 kg. The highest amount per total lactation was achieved by cows in Mures, 129 kg and the lowest in. Bistrita-Nasaud: 163 kg. The amount per normal lactation was 10% lower than the one per total lactation.

3.4. The protein amount was 145 kg per total lactation and 129 kg per normal lactation. The best performances were recorded by cows in Mures (151, 4 kg per total lactation and 136 kg per normal lactation) and the lowest in Sibiu (130 kg per total lactation and 119 kg per normal lactation).

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## **STUDIES CONCERNING LONGEVITY IN MILK PRODUCTION OF ROMANIAN SPOTTED COWS**

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Key-words: milk yield, productive longevity, dual purpose cows.

### **SUMMARY**

The productive longevity represents a physiologic and also economic feature (cows production from the first lactation to the moment of their last dry off period), being expressed by the exploiting period length \*years or lactations) and also by the milk yield achieved during its life and exploiting period (daily yield, total yield).

The exploiting period length has a great breeding importance, productive and economic one, because the life length of parents is a very important part of animal exploiting efficiency, from the economic point of view and also due to the fact that it affects the breeding possibilities.

Under breeding aspect, the length of exploiting period influences directly the interval among generations and indirectly the intensity and precision of the selection.

Thus, the increasing of the length of exploiting period influences the generation interval, leading to the increasing of the descendants' number from the valuable cows, but decreases the selection effect, diminishing the genetic progress. The same time, it increases the intensity and precision of selection. The inverse situation, when the length of exploiting period is short, it increases the selection effect due to the decreasing of the generation interval, leading to low intensity and precision of selection. It also influences the economic efficiency.

### **1. MATERIAL AND METHODS**

The present paper studied the productive longevity in one of the most important Romanian breed, the Romanian Spotted Cows. There were collected data from the most important exploiting unit in the centre and western part of the country, where this breed is most located. The length of the exploiting period represents the time during the first calving and the end of the lactation consecutive to the last calving, corresponding with the leaving from the livestock and it is expressed by number of exploiting years or the number of calving, or both of the expressing ways.

The length of the exploiting period could be established as an event spent in the past or as an expected event. The establishing as a past event is calculated based upon the individual average values of all the cows out of the livestock, using the relation

$$d = V_2 - V_1, \text{ where,}$$

**d** —Length of the exploiting period;

**V<sub>1</sub>** —age at first calving; **V<sub>2</sub>**—outing date.

The establishing as an expected event could be determined based upon the surviving rate at a lactation or age, ( $e_x = T_x / I_x - 1$ ) or upon the percentage of the primiparous cows ( $d = 1/p_1$ ), where:

**e<sub>x</sub>** = average exploiting period length expected at cows of x age;

**T<sub>x</sub>** = total of alive cows and cows after x age;

**I<sub>x</sub>** = number of cows at x age;

**I** = coefficient for cows frequency in the herd;

**p<sub>1</sub>** = primiparous cows frequency.

The estimated length of the exploiting period results from adding the multiplying between the outing cows after lactation with the lactation number of the outing and its dividing to the sum of the total outing cows.

Most of the researchers sustain the favourable effects of the increasing of the length of the exploiting period by using more time of the productive cows and elimination of the low production cows. The milk yield increases proportionally with the added lactations number and the decreasing of the expenses for every day in animals life and the cost per milk litre is based by the fact that the expenses recorded in the non productive period is shared on large productions, going to economic efficiency at the farm level.

## 2. RESULTS AND DISCUSSIONS

The researches regarding the length of the exploiting period established for Romanian Spotted Cow an average length of the exploiting period of six lactations, after the primiparous cows were eliminated.

Cows are eliminated from the livestock due to some involuntary causes (mortalities or culling) and voluntary causes. There were made different studies to elucidate the ratio among these sources. Analyzing these causes, there were concluded lot of afflictions with different percentages, as it follows:

- Reproductive disorders 41, 73%;
- Mammary afflictions 13,91% ;
- accidents– culling 11,98%;
- senility 11,61%;
- traumatic pericarditis 5,71%;
- tuberculosis and leucosis 3.71%;
- mortalities 2,89%;
- feet afflictions 1,46 %';
- Other causes 7%.

For Romanian Spotted cows, the causes are different depending on the farm features, where the cows are, but on the first place are situated the reproductive afflictions (20 %}, followed by senility{16,6%), mammary and digestive afflictions (14% for each); infectious diseases (13,9%) feet afflictions (7,3%).

The researches regarding the causes of livestock eliminations show that in every country which raise cattle in intensive system, the selective reform has a percentage of 18,37%, but in our country it has very low limits 1- 11%. Within Romania, the reproductive disorders represent the most important cause 13 - 42% Regarding the milk yield dynamics during the productive life in Romanian Spotted Cows, the most of the authors who developed researches on Romanian Spotted Cows established its maximum production at the fifth or sixth lactation (table 1)

Table 1

Milk yield dynamics during the productive life in Romanian Spotted Cows

LACTATION	Euro region I		Euro region II	
	Milk (kg)	%	Milk ( kg)	%
I	2.930	76,80	2.527	66,20
II	3.575	93,70	3.258	85,30
III	3.662	96,00	3.544	90,08
IV	3.773	98,90	3.688	96,60
V	3.813	100,00	3.779	99,00
VI	3.801	99,70	3.818	100,00
VII	3.553	93,20	3.572	93,60
VIII	3.453	90,50	3.428	89,80
IX	3.314	86,14	3.577	88,40
X	3.387	88,80	3.336	87,40

The results of different researchers carried out in our country, emphasised the necessity of this breed increasing precocity, but also the ways this process could be assured, by reaching the maximum production in the fifth lactation or maybe later and by the other hand by increasing the milk yield percentage in the first lactation of 78-80% of the maximum one, beside 65-66% in the present.

The specific character of the milk yield dynamics consists in the fact that it starts by a large increasing from the first to the third lactation (when it reaches 90% from the maximum one), it keeps the following three lactations at over 90% from the maximum lactation and it is placed at almost the same values after the maximum one. This evolution of the lactation curve, especially the specific biologic resistance, offers a strong longevity of this breed and a long possibility to use it cows.

Gh. Georgescu (1988) showed that the productive longevity is strict linked to raising conditions and exploiting intensity, varying in the Romanian Spotted Cows between 4,4 and 4,6 lactations, with a milk yield of 12.000-27.365 kg, and a number of 30 cows recorded in the Romanian Herd Book

achieved more than 45000 kg milk in 9,4 lactations.

The character of the evaluative level of milk yield in dynamics permits an easily achieving of an average economic exploiting life of 6-7 years, at high and uniform production levels. The productive performances per exploiting life recorded in some Romanian exploitations of 35-50 tone of milk and 1.000-1.800 kg pure fat, emphasised this breed possibilities, which answer correctly the nowadays and future breeders requiring.

### 3. CONCLUSIONS

3.1. Romanian Spotted Cows raising area is concentrated especially in Transylvania, where it found favourable natural and artificial environmental conditions for developing.

3.2. Romanian Spotted Cows breed origin started with almost 125 years ago, being declared and recognized as breed in 1959. There was used as main method the absorption crossing, practiced between Sura de Stepa and Simmental with different origin, as Switzerland, Austria and Germany. So, there were made large imports with high zoo technical value animals that explain the actual good performances. Lately, the biologic material was structured in lines and families, using the efficient selection based upon own performances and guided mating.

3.3. The fundamental objective of this breed rearing was the reconsidering of the breeding direction, linked to the nowadays requiring of the world and national economy.

3.4. Bull dams in Romanian Spotted Cows are characterized by a hypermetric trend body development and a relative correct conformation. The main height was 136, 6 cm. and the body weight 663 kg. The females present a dual purpose type body shape (119 %), a strong massive body (149%), o a relative correct superior line (different height index is 1, 3%) and a medium developed thorax (thorax width index: 52, 5%).

3.5. Cows in the active population are characterized by a low reproductive precocity in milk yield. The age at first calving is 36, 7 months, with a large variability (29, 9%). The best precocity is recorded by cows Harghita (30, 3 months), relative homogenous character (10%) and the lowest in Cluj (39, 2 months).

3.6. Reproduction cycling could be appreciated as good in Romanian Spotted cows. This is revealed by the value of calving interval, which is almost 114 days. The variable element of reproduction cycling is service period, which recorded a mean value of 132 days. The value of calving interval is influenced by the milk yield performances and the exploiting conditions. The longest calving interval was recorded in Salad (458 days) and the shortest in Hunedoara (399 days).

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## IMPORTANCE OF OFFICIAL CONTROL

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**Key words:** official control, competent authority, national control plan

### SUMMARY

The official control represents any kind of control made by the competent authority or the European Commission and EU member states, in order to verify compliance with food law. These controls are carried out in any stages of production, processing and distribution of animal food or feed and animal products.

The competent authority of Romania as a country of destination can be control throughout its subordinate authorities the compliance with food legislation in the field of foodstuffs by means of non-discriminatory checks. For reasons strictly necessary for the organization of official checks, the competent authority may require operators who trade goods that were supplied from the European Union member states to report their arrival at destination.

Official controls should be carried out without a prior warning, except audit activities, where the prior notice of the food or feed operator is necessary. Also, official checks may be made ad hoc.

Competent authorities who perform official controls should fulfil a number of operational criteria in order to ensure their impartiality and effectiveness. They should have enough staff, with adequate qualifying and experience and to possess adequate facilities and equipment to properly perform their duties.

Since 2007 Romania, the European Union Member State is obliged full application of EU legislation concerning veterinary certification and official controls on products of animal origin are subject to intra-Community trade. Official controls on products of animal origin should cover all aspects that are important for protecting public health and, where appropriate, animal health.

Official beneficiaries of products coming from Member States are required 24 hours before arrival of the consignment at the destination to pre-notify the official veterinarian to check the transport to arrive at the destination. To ensure traceability, operators engaged in intra-community

trade transactions of products of animal origin, must enrol in a special register complete data on consignments of animal products.

At shipment arrival to unit destination, the official representative of the unit will notify the Sanitary Veterinary and Food Safety Directorate and the official veterinarian about this arrival.

Based on operator notification, the official veterinary does documents inspection, identity checks and physical checks as appropriate.

Physical control or any other check will be performed to verify compliance with food law when necessary, especially:

- in case of any doubt or other uncertainty
- based on notifications from Rapid Alert System for Feed and Food;
- under Official Program, prevention and control of animal diseases, those transmitted from animals to humans, animal welfare and environmental protection.

Official controls are carried out randomly, without any purpose for blocking distribution of the products.

The purpose of physical control of animal products is to ensure that products meet the requirements from the veterinary certificate or document: there should be checked the origin guarantees, certified by the third country that should guarantee the subsequent product transportation has not modified the original conditions guaranteed upon departure.

This is achieved by:

- physical and chemical simple tests: cutting, thawing, cooking;
- organoleptic examinations: smell, colour, texture, taste;
- laboratory tests for residues detection, pathogens, obvious modifications as well as alteration presence.
- Despite of product type, it has to be done the following aspects:
- real weight of transport should be compared with what is noticed in the veterinary certificate or document which accompany the transport, by weighing the whole transport, if necessary;
- control of transportation conditions for identification of deficiency and interruption, especially, in refrigerating chain;
- check packing materials and all markings (stamps, labels) to ensure their compliance with Community law;
- compliance of temperature control of products shipment according with European Union legislation;

- in case of the bulk products a whole batch of packaging or samples have to be examined before the sensorial examination, physical and chemical laboratory tests.

These tests must be performed on a series of samples taken from transport that should be partially discharged, if necessary, to allow access to the entire content of the shipment.

The person responsible for the food transport is mandatory to submit a duly completed and the border inspection post of entry into the European Union, Part I of the Common Veterinary Entry Document (CVED) before the physical arrival of the intra-European Union (notification).

Decoiling import operations are performed according to the epizootic situation of the countries of origin, with mandatory compliance of legal provisions.

Animal products can be imported from third countries in Romania only if:

- come from a country approved to export to the EU member states for that type / category of products;
- are accompanied by health certificate in the format / model established for export to the EU, the type / category of products / varieties;
- come from an establishment approved for export to the EU, published in the Official Journal of the European Union.

In order to find out the full name and authorisation export code of manufacturing units in third countries agreed for export into EU, TRACES application may be accessed.

Import operations (from third countries) in the European Union can be made by border inspection points located in Romania, as well as all inspection points on the border of the European Union, located in other Member States.

In order to protect Romania territory against major transmitted diseases to animals, veterinary authority may forbid the development of import of animals / animal products, according to the epizootic situation of the origin country.

Food business operators must comply with the provisions of the national and EU on intra-Community trade regarding import operations.

Official controls on products of animal origin should include all matters that are important to the protection of public health and, where appropriate, animal health. These controls should be based on the most recent and relevant information and therefore should be possible to adapt them to the relevant available information. Official controls on meat production are necessary in order to verify that food business operators comply with hygiene rules, as well as the criteria and objectives lay down by Community legislation.

These should include checks on business activity in the feed and food business, regarding the use of feed and animal food, food and feed storage or any process, substance, activity or operation including transport feed or food and live animals.

The competent authorities must ensure that all their staff performing official controls:

a) receive, for their competence, the appropriate training allowing them to consequently carry out official duties.

b) informs to date in the field of competence and if necessary, carry out additional training on a regular basis;

Member states shall ensure that food business operators provide all necessary assistance to ensure effective implementation of official controls by the competent authority.

Competent authorities should ensure that, in cases in which the official controls require the participation of different control units, they apply and effectively implement appropriate coordination procedures.

Multiannual national control plan should contain general information on the structure and organization of food control systems, in particular on:

- strategic objectives of the plan and how to control priorities and allocating resources reflect these objectives;
- risk-classification of those activities;
- designation of competent authorities and their functions at national, regional and local levels and the resources available to these authorities;
- management and general organization of official controls at national, regional and local level, including official controls in individual companies;
- control systems applying to different sectors and coordination between various control units of the competent authorities responsible for official controls in these sectors;

- where appropriate, delegated tasks to other control bodies;
- methods to ensure compliance with operational criteria;
- staff training for performing official controls;
- documented procedures;
- organization and operation of contingency Plans for emergency animal disease or in case of food poisoning due to contamination of food and other situations of public health risk;
  - organization cooperation and mutual assistance.

Competent authorities should also ensure that, in cases in which the competence of performing official controls has been delegated from a central level to a regional level, between the central and the regional or local level there is an efficient and effective coordination.

If during an official control at the destination or during storage or transport in Romania, the competent authority finds non-compliance, it may proceed to the returning of the incriminated goods back to European Union member state.

## **CONCLUSIONS**

1. Official control means any form of control that made him competent authority or the European Commission and EU Member States, to verify compliance with food law.
2. Responsible authorities for official controls should meet a number of operational criteria to ensure their impartiality and effectiveness.
3. Official controls must be carried out without prior warning, except audit work that is required prior notice to the operator for animal feed business activity or the food. Also, official checks may be made ad hoc.
4. Official controls must be made in any of the stages of production, processing and distribution of feed or food and animal products.

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## THE INSPECTION OF FOOD INDUSTRY UNITS

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**Key words:** inspection, examination, audit, official controls

### SUMMARY

Inspection is the examination of any aspect of feed, food, health and welfare, to verify that such matters are in accordance with the requirements of specific legislation.

Since 2010, official controls on veterinary hygiene in catering units will take place according to the classification of risk groups of units. Classification of these units is done at least once every six months based on the evaluation sheet.

Units that produce, process, store, transport and / or distribute products of animal origin may carry out activities under control only after obtaining veterinary authorization by the competent authority. After obtaining its full responsibility for the manufacturing, processing, storage, transport and / or distribution of products is the food business operator.

Completion of inspection to be recorded deficiencies and lack of conformity to the legal provisions in force, proposals are measures to remedy deficiencies and when appropriate, apply sanctions.

National Implementation Framework for Inspection and Control ensure the existence of an integrated system of official controls "from farm to fork" that help protect public health, animal

The official checks shall be performed using appropriate control methods and techniques, such as: monitoring, supervision, checking, auditing, inspection, sampling and analysis.

Monitoring is achieving planned sequence of observations or measures in order to obtain a general assessment of the level of compliance with food law;

Supervision is a careful examination of one or more businesses with food business operators to work food or their activities. Monitoring means observing the detail of various aspects of products and units which fall within the competence of ANSVSA - DSVSA, following the incidence of potentially having impact on food safety.

Verification is control, by examination and consideration of evidence, in terms of meeting the requirements specified. Verification is a

method of testing by examining objective evidence and analysis on legislative requirements.

The audit is the systematic and independent examination to determine whether the activities and results comply with planned programs and these programs are implemented effectively and are suitable for meeting targets. The audit is a complex evaluation of an operator in the food industry. The audit plan covers the audited areas / activities, date of audit scheduling, system function to audit, applicable reference documents (audit criteria). The findings within the audit will be communicated to the audited throughout the audit so that later misunderstandings to be avoided. The findings must be in writing, actual and in compliance with requirements. Dawn raids are not announced, audit is. Audit aims at continuous improvement.

Audit activities on hygiene best practices must check whether operators within the food area apply, continuously and appropriately, procedures regarding at least:

- controls on information referring to the alimentary chain;
- design and maintenance of facilities and equipment;
- pre- and post-operational hygiene;
- personal hygiene;
- training on labour and hygiene procedures;
- pest control;
- water quality;
- temperature control;
- controls on food getting in / out of the enterprise, as well as any accompanying papers.

Specifics and intensity of audit tasks on every unit are based on the estimated risk. Thus, the competent authority periodically checks:

- risks on public health and, as the case may be, on animal health;
- in case of slaughter houses, aspects concerning animal welfare;
- type of processing performed and the outcome hereof;
- previous registrations of the operator in the good sector on observing the food legislation.

Inspection is the examination of any aspect of feed, food, health and welfare, to verify that such matters are in accordance with the requirements of legislation on feed and food law and health and welfare rules animals.

The inspection is based on the element of surprise. If it is considered that, by explaining why the completion of the inspection can distort the result, will not communicate a particular view.

The inspection will be carried out according to the content and topic requirements. A departure from such matter may occur when additional items to clarify incongruities are needed. Veterinary inspectors will not perform financial controls. They will have access to all documents of the objective.

Document control:

1. Document control is performed in a logical sequence;
2. Annotations will not be made on the documents made available;
3. Documents will be carefully studied and only in the presence of a representative of the objective;
4. No original documents will be taken away, but copies hereof;
5. Before starting the inspection, procedures in place in the unit will be carefully studied;

Inspection of the objective:

- inspection will be performed without disrupting the technological flow or activity carried out inside the objective;
- all throughout the inspection, the veterinary physician shall wear full protection equipment;
- the inspector will observe the principle “from the clean to the dirty”;
- the inspector will require to be accompanied by a person in charge during the inspection;
- the inspector will write down their own notebook the deficiencies found that they will highlight under the control or inspection sheet;
- statements from individuals involved in the case may be taken;
- in cases of denial, the help of other institutions may be asked for;

Drafting the control / inspection sheet:

- the control / inspection sheet will be carefully filled in under all boxes;
- the control / inspection sheet will be as legible as possible;
- the control / inspection sheet must be logic, and recommendations pertinent;
- for every measure taken, an achievement deadline will be provided, and a person in charge will be assigned;
- in case major deficiencies are found, a re-control term will be set;

- the control / inspection sheet will be drafted in two counterparts, one staying with the unit management with a registration number, being signed by the management of the objective.

Syntheses:

Upon concluding the inspection action, the deficiencies and non-conformities against legal provisions in force will be jotted down, and proposals for measure to remedy the deficiencies found will be made, and when the case may be, sanctions will be enforced or the director with the Sanitary Veterinary and Food Safety Directorate will be proposed to issue an ordinance concerning the activity suspension / interdiction.

In all instances, the control sheet drafted at sight and the sanctioning report are submitted to the unit administrator / representative.

The veterinary physician records and assesses the outcome of activities during the inspection. In case inspections highlight the presence of some disease or pathological condition that might affect the public or animal health, or might endanger animal welfare, the veterinary physician must inform the operator within the food sector hereabout.

In case the problem identified occurred during primary production, the official veterinary physician must inform hereabout the veterinary physician dealing with the animals from the source exploitation, operator within the food sector in charge of that exploitation (providing such information will not compromise a subsequent legal procedure) and, as the case may be, competent authority the source herd or hunting territory in case belongs to.

In case the animals in question come from a stock farm of another member state or a third country, the official veterinary physician must inform the competent authority of the member state where that unit is located. Every competent authority must take appropriate measure in accordance with the applicable communitarian legislation.

Veterinary inspectors check the existence, implementation and operation of HACCP programmes so that it should ensure, through specific leverage, the functioning of a compliant production flow and getting a healthful and qualitative end product.

The veterinary inspectors will use the following methods of control:

- gathering information by studying documents; direct questions and observation;

- assessing information;
- establishing requirements;
- assessing requirement observation.

During the inspection, the following will be assessed:

- HACCP programme;
- Monitoring;
- Checking;
- Registration;
- AMC calibration;
- Corrective actions;
- Final assessment.

The frequency of official controls should be periodic and in proportion to the risk, considering the outcome of checks performed by operators in the food sector within control programmes based on the HACCP technique or quality assurance programmes as long as such programmes are designed to meet the requirements of the legislation on food products.

Control bodies are responsible with classifying units within the food sector using raw materials of animal / non-animal origin based on the production activity and actual risk associated to the existent activity. Unit classification is the basis to programming the control activity in terms of quality and quantity, thus achieving a unitary controlling activity.

Controls in food manufacturing units must be performed based on the risk category involved in the activities carried out within the unit in question.

Assessment of relevant risks considers:

- probability of their occurrence;
- severity degree;
- their reoccurrence with every stage of the technological process;

There are two stages to elaborate the risk profiles:

- a. Type of production and control frequency

Identifying the highest number of units having a certain type of production in order to establish intervention priorities from the very beginning; thus, the risk profile that defines the average frequency to perform control is achieved independently from the management and history of manufacturing units. Later on, specific characteristics of every piece of equipment must be

considered, making a difference between control frequencies according to the appropriate specific data;

b. Classification of equipment based on risks

By classifying equipment based on risks there are established objective criteria in order to make a difference between control frequencies and all operation performed by official human and veterinary physician, including sampling activities. The main objective of risk classification is getting a classification of manufacturing units in the food area based on risk, in order to:

- determine the control frequency based on scoring and pre-defined objectives;
- examine units of similar risks, using homogeneous evaluation parameters.

Sampling for analysis is sampling of feed or food or other substances representative of the production, processing and distribution of feed or food or animal health (including environmental) in order to verified by analysis of compliance with legislation in the feed or food law or animal health rules; Sampling methods and analysis used in official controls must comply with EU rules into national law, or:

a) if such rules do not exist, with internationally agreed rules and protocols, as those that the European Committee for Standardization (CEN) has accepted or those agreed by national legislation or

b) where those referred to above, other methods for their purpose or developed in accordance with scientific protocols.

Official controls on food should include, inter alia, the following activities:

1. examining control systems that feed business operators and those with pet food business have implemented and results achieved;

2. inspection:

- primary-production facilities, business activity in feed and food business enterprises, including their surroundings, facilities, offices, equipment, facilities and equipment, vehicles, and feed and food;

- raw materials, ingredients, processing auxiliary agents and other products used in the preparation and production of feed and food;

semi-products;

- materials and articles intended to come into contact with food;

- products and processes for cleaning and maintenance and pesticides;

- labelling, presentation and advertising;

3. controls on hygiene in business activity in feed and food business enterprises;

4. evaluating Procedures for Good Manufacturing Practice (GMP), good hygiene practices (GHP), good agricultural practice and HACCP (Hazard Analysis and Critical Control Point), taking into account the use of guides established in accordance with national legislation transposing EU legislation specifies;

5. examining written materials or other records that may be relevant to assess compliance with legislation in the feed and food law;

6. interviews with animal feed business operators, with the activity in food and their staff;

7. reading values recorded by measuring instruments business activity in the feed or food business enterprises;

8. controls' own instruments made by the competent authority to verify the measurements made by operators of animal feed business and those with food business.

## **CONCLUSIONS**

1. Units that produce, process, store, transport and / or distribute products of animal origin may carry out activities under control only after obtaining veterinary authorization by the competent authority;

2. Official controls on veterinary hygiene in catering units will take place according to the classification of risk groups of units;

3. Inspection is the examination of any aspect of feed, food, health and welfare, to verify that such matters is in accordance with the requirements of specific legislation;

4. The inspection is based on the element of surprise. If it is considered that, by explaining why the completion of the inspection can distort the result, will not communicate a particular view.

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## **THE HEALTH CHECK EVALUATION OF PARAMETERS BEFORE BOARDING THE POULTRY**

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**Key words: indicators, management, hematological, biochemical, parameters welfare, poultry.**

### **SUMMARY**

SEB-122 MI method evaluation health parameters of poultry used to transport, especially in poultry farms. Own method of evaluation the health parameters of poultry for transport, by which to determine and verify the conditions of acceptability within the parameters of health status before and after transport of poultry in order to permit the application of corrections for values of poor conditions requirements transport. Through this method to establish the stages be followed to an correct evaluation of health parameters of poultry, which are subject to proper evaluation scores obtained for these parameters values, while in secondary, according to these values to be established and can provide the degree of mortality poultry during transport to the destination.

The testing stages, before and after shipment, consisting from several stages of verifying the conditions of housing growth and the transport, shelter microclimate interactions destination, biochemical and hematological parameters of poultry, when checking dependency state of health, SS, changes in biochemical parameters, PBIO, hematology, PHEM, microclimate, PMIC, the parameters density per m<sup>2</sup>, D, Time, Pt, and distances, Pd, which considers that the health per bird, is given by an expression of the form:

$$SS = f(PBIO, PHEM, PMIC, PD, Pt, Pd) \quad (1)$$

Or transport is given by an expression form:

$$SS = f(\Sigma PBIO, \Sigma PHEM, \Sigma PMIC, \Sigma PD, \Sigma Pt, \Sigma Pd), \quad (2)$$

Evaluation of parameters based Health Check systems is a numerical method of evaluating integrative spread species of mammals and poultry farm. Their integrative character derives from the fact that they have factors related to housing conditions during transport and management systems and practices (Engineering-based parameters) behavioral factors (animal-based parameters).

Method for measuring health parameters of the poultry for transport in the context of management practice, adequate social interactions and behavioral factors [2] [11], is that poultry from the age of 4-80 weeks to be transported for slaughter Are subjected to two stages of evaluation, a first test step being 3-4 hours before boarding, during which the poultry are fasted 1.5 hours, and 30 minutes before boarding private water and a second test phase immediately after landing from a transport vehicle, test phases consisting of several phases of verification: the housing conditions of to increase, exploitation, and the transport, shelter microclimate interactions arrival, the most important welfare indicators on hematological and biochemical parameters of the poultry [5] [1], check dependence health, SS, changes in biochemical parameters, hematology, microclimate, with the density parameters of 175 m<sup>2</sup> ... 225 kg/m<sup>2</sup>, time and distance course, being played by an expression of the form:

$$SS = f(PBIO, PHEM, PMIC, PD, Pt, Pd) \quad (1) \text{ or transport}$$

$$SS = f(\Sigma PBIO, \Sigma PHEM, \Sigma PMIC, \Sigma PD, \Sigma Pt, \Sigma Pd), \quad (2)$$

Health status and dependence of each parameter is given by equations

$$\begin{aligned} (1)... (8): \quad (3) \quad SS = f(PBIO) & \quad (4) \quad SS = f(PHEM), \\ (5) \quad SS = F(PMIC), & \quad (6) \quad SS = f(PD), \\ (7) \quad SS = f(Pt), & \quad (8) \quad SS = f(Pd), \end{aligned}$$

Where:

- PBIO: are biochemical parameters - PHEM: hematological parameters;
- PMIC: microclimate parameters - PD: the density parameters m<sup>2</sup>;
- Pt: parameters for transport,
- Pd: transport distance parameters;

That link health with a score of between 61 ... 122 points, corresponding to five possible situations as follows:

- a) Situation in which their score is 108 ... 122 points defining a very good health with a level mortality rate up 0.2%;

- b) Situation in which their score is 92 ... 107 points, defining a healthy condition with a grade of mortality of 0.3... 0.7%;
- c) Situation in which their score is 77 ... 91 points, defining a state of poor health with a level mortality rate of 0.8 ... 1.2%
- d) Situation in which their score is 62 ... 76 points defining a very poor health with high mortality of over 1.3... 1.7%;
- e) The situation is to score 61 points, defining a precarious state of health unacceptable with a grade of mortality of over 1.7%.

Health status based on biochemical parameters, SSBIO, is given by the relation:

$$SS = f(\Sigma PBIO, \Sigma PHEM, \Sigma PMIC, \Sigma PD, \Sigma Pt, \Sigma Pd)$$

SSBIO = f (PBIO) (9) or by:

SSBIO = f (CK, AST, LDH, ALP, and GLU) (10) and is considered optimal if the health status values [1]:

- Glucose, GLU = 130 ... 157 mg / dl;
- Creatine kinas, CK = 150 ... 250 U / l;
- Aspartate aminotransferase, AST = 150.6 ... 174.6 U / l
- Lactic dehydrogenises, LDH = 615.5 ... 636 U / l
- alkaline phosphates, ALP = 620 ... 1060 U / l.

Health status point of view hematological SSHEM be described as a dependency of hematological parameters: SSHEM = f (PHEM) (11) or

Reports heterofile / lymphocytes, H / L and basophiles / B lymphocytes / L played by [1]:

$$SSHEM = f(H / L, B / L) \quad (12) \text{ the optimal state of health}$$

SSHEM OPT = f (H / L, B / L) is within the limits Reports H / L, B / L, where reports

$$H / L = \text{between } 0.2109589041 \text{ to } 0.84905660377 \text{ (mii/mm}^2\text{)}$$

$$B / L = 0 \text{ to, } 0050228310\text{-}0, 0540308747 \text{ (mii/mm}^2\text{)}$$

Health status point of view of microclimate, SS MIC is dependent on the parameters of the microclimate by the following expression:

SS MIC = f (T, HR, NH<sub>3</sub>, and CO<sub>2</sub>) (13) where:

- T is temperature, ° C - T = 17 ... 28 ° C.
- HR is the relative humidity, % - HR = 50 ... 80%.
- Is the concentration of ammonia NH<sub>3</sub>, % - NH<sub>3</sub> = 10 ... 25 ppm.
- CO<sub>2</sub> is carbon dioxide concentration%. - CO<sub>2</sub> = 1000 ... 3000 ppm.

The optimum expression is established between the limit values specified below:

Health status according to the parameters D, the density per m<sup>2</sup>, is given by a relationship of dependency type [21]:

$SS PD = f (PD)$  (14) where D is the density of transport/m<sup>2</sup>, 175 ... 225 kg/m<sup>2</sup> for proper health.

The health status during transport, SST is given by an expression of type -  $SST = f (t)$  (15) where t is the transport, which is considered optimal to 4 hours.

Health status of transport distance, SSD, is represented by an expression of form

$DSS = f (d)$  (16) which is considered optimal for a distance up to 50 Km;

For the first test phase, 1-2 hours before loading for transport, check some essential elements in the sequence of phases consisting of:

a1. Verification of the conditions the in the housing consists of verification of growth and increase in exploitation system practiced in the poultry to increase, feeding and housing system, the density of housing per m<sup>2</sup>, care and maintenance conditions reported at 5000 birds, the general condition container/transport cages before their height and poultry category between 1 and 4 kg and the storage density of poultry during the journey [2];

b1. Verification of social interaction and management factors, consisting of verification the level preparedness of the transportation tasks and responsibilities of personnel and reported 5000 poultry transported to Sanitary preparation: vehicle, containers/cages and birds, the area of transport, number of poultry caught and how charge cage, preparation of personnel and verification the type of ventilation system of the vehicle [5];

c1. Verification of housing microclimate boarding, consisting of verification of temperature and humidity levels of ammonia and carbon dioxide before loading poultry [4];

d1. Biochemical verification of poultry in purpose of transport consisting of verification of: glucose, creatine kinase, aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase and haematological parameters consisting of verification of of reports heterofile / basophils and lymphocytes / lymphocytes [1].

For the second test phase, immediately after landing from the transport vehicle, check some essential elements in the sequence of phases consisting of:

a2. Verification of the conditions the arrival of the house consists of verification transport density per m<sup>2</sup>, care and maintenance conditions of poultry reported in 5000, the general condition of the containers / cage after transport to the category of poultry between 1 and 4 kg and the density of storage of poultry during the journey.

b2. Verification of social interaction and management factors in the slaughterhouse, consists of verification the level of preparedness of the transportation tasks and responsibilities of personnel and reported 5000 poultry transported the vehicle Sanitary preparation of, containers/cage and poultry, the area of transport, the establishment number of poultry caught and how of unloading and type of cage ventilation system of the vehicle

c2. Verification of housing microclimate boarding consists of control temperature and humidity, and ammonia levels dioxide carbon discharge poultry [4].

d2. Biochemical parameters verification of poultry consists of verification transport following: glucose, creatine kinase, aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase and hematological parameters consists of verification of reports heterofile / basophils and lymphocytes/lymphocytes [1] .

Before presenting in the detail method is necessary to specify that each stage and phase of the method is correlated with some rules for granting scores established in the invention according to which, ultimately, is appreciates health status and level of mortality of poultry during transport.

## **RULES ESTABLISHING THE SCORES**

- Each indicator, irrespective the stage is noted with a numerical value (score);
- The score is based on animal welfare standard for each indicator monitor in the part;
- Scores which can take granted positive values, or equal to 0;
- Final grade is establishes by adding all the marks awarded, after deducting coefficient method;

- The method is based on two scores granting principles, namely:
- Scoring is done depending on each indicator of the importance of the indicator in the determining the welfare of poultry;
- If the indicators for of evaluation methodology presents a easily higher subjective, has adapted a special measure of recovery, as such, an indicator that allows for subjective interpretations of the home will receive one third less than the one that does not allow subjective interpretations. Thus:
- Maximum of 2 points for each indicator assessed in the first two groups of indicators of the method, irrespective of stage (boarding or disembarking during transport), namely: the evaluation management practices, evaluation the social interactions and behavioral factors. The total of these indicators is: 28 (12 indicators related to management practices + 16 indicators related to social interactions and behavioral factors) and maximum rating awarded for all of them is: 28; finally  $28 \times 2$  points = 56 points
- Maximum of 3 points for each indicator evaluated in the other two groups, namely: evaluation of microclimate conditions, evaluation the most significant biochemical and hematological parameters of paraclinical welfare. Their total number is (8 microclimate indicators 14 indicators on paraclinical parameters), and all of them allow for the maximum score is:  $22 \times 3$  points = 66 points Thus, the maximum final score method is  $56 + 66 = 122$  points.

Determination paraclinical indicators of welfare respectively, biochemical and hematological presented previous, was taken by a set in 10 blood samples before loading in the the vehicle for 10 poultry, and the second set in 10 blood samples in the vehicle landing on the same poultry, three points is given for each indicator when <50% of the performance is falls in the reference of data, birds have been introduced in a container marked with a specific band.

- Findings and explanations of final grade will be passed on the last page of the method (page final conclusions) will be included where the competent authorities in animal welfare in Romania, with a maximum period of remediation

## **DESCRIPTION OF THE METHOD**

The method is structured in two main stages include several phases and are determined in which many welfare indicators, proven and accepted by all international specialty centers, and at the same time adapt the current situation of Romanian ensuring their applicability when present respectively:

I-Evaluation of poultry welfare before boarding;

II-Evaluation of welfare of poultry landing; for each stage was chosen four key evaluation criteria respectively:

– Evaluation of management practices;

– Evaluation of social interactions and behavioral factors;

– Evaluation of microclimate conditions;

– Evaluation of the most significant paraclinical biochemical and hematological parameters of welfare, namely: CK, AST, LDH, ALP, GLU, H / L, B / L.

## **CONCLUSIONS**

1. The value of the scores obtained in the various possible situations can determine the health of poultry and their level of mortality rate during the transport.

2. These data can be used to made recommendations for correcting the facts to ensure optimal conditions of transport, health maintenance and decrease the mortality rate of poultry during the transport.

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## INTEGRATION OF AN E-LEARNING PLATFORM IN PRACTICAL TRAINING OF VETERINARY STUDENTS IN ROMANIA

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**Key words:** e-learning platform, veterinary students, practical training

### SUMMARY

Paper presents the progress of the project Labour Market Integration of Veterinary Medicine Students —Practical Training, co-financed by European Social Fund through the Sectoral Operational Programme Human Resources 2007-2013, implemented by University of Agronomical Sciences and Veterinary Medicine Bucharest, University of Agricultural Sciences and Veterinary Medicine “Ion Ionescu de la Brad” Iasi and S.C. Softwin S.R.L. . Project aims to facilitate the transition to the work place of 2800 students through the use of innovative programs with practical training support, including information technology and communication resources. At present, there was developed an e-learning platform with five modules that cover the practical needs and allow the application of the acquired theoretical knowledge. Platform is visible online at the address [www.stagiupraktica.fmvb.ro](http://www.stagiupraktica.fmvb.ro). The e-learning platform may be used to update professional skills of the personnel involved in the practical training of students and also provide an on-line evaluation methodology of students and tutors. It was already used for training of 19 tutors from institutions where students did practical training, and it was accessed by 901 students.

Today, computers play an important role in all aspects of life. Their use in improving the education quality can no longer be ignored. Computer-aided learning represents an innovative and existing alternative to the traditional teaching methods, even in such a field like veterinary medicine (Bell *et al.*, 2010, McConnell *et al.*, 1997, Pinto *et al.*, 2008, Tugui *et al.*, 2008).

Faculties of Veterinary Medicine of the University of Agronomical Sciences and Veterinary Medicine Bucharest, University of Agricultural Sciences and Veterinary Medicine “Ion Ionescu de la Brad” Iasi and S.C. Softwin S.R.L set up a partnership and proposed the project Labour Market Integration of Veterinary Medicine Students —Practical Training. The

project is co-financed by European Social Fund through the Sectoral Operational Programme Human Resources 2007-2013.

Project implementation will facilitate the transition to the work place of 2800 students through the use of innovative programs with practical training support, including information technology and communication resources. Another objective is developing and strengthening of the existing partnerships in order to facilitate students' labor market integration by involving at least 25 institutions and companies in trying to diversify the workplace experience (farms, clinics, laboratories etc). The increase of the relevance of the learning process and its outcomes by using innovative and interactive approaches for conducting 5 internship programs that cover the practical needs and allow the application of knowledge acquired in the classroom is also one of the project's aims. Update professional skills of the personnel involved in the practical training of students is a desired outcome of the project implementation.

## 1. MATERIAL AND METHODS

Project aims to improve practical competences of the veterinary students and graduates. As a result of the project implementation, students' access of the e-learning platform before participating at practical training will upgrade their practical skills. In order to evaluate students' views about both e-learning and practical training, a questionnaire was developed. Staff from professional and beneficiary institutions was questioned about the opportunity and acceptance of an e-learning platform in veterinary medicine and about specific practical training demanded by the potential workplaces for veterinary graduates too.

A joint of experts from both veterinary faculties delivered written material on five domains, and then, in collaboration with the third partner, platform modules were developed. Modules' subjects were: *Farm Animals Breeding Units, Clinics and Hospitals, Laboratories (research, diagnosis, food control), Slaughtering Houses, Rural Veterinary Clinics.*

Both future tutors from potential beneficiary institutions and students (members of the target group) accessed the platform, which is visible online 24/24 hours at the address [www.stagiupracticala.fmvb.ro](http://www.stagiupracticala.fmvb.ro). Each participant received own password, which has been supposed to be used as long as everyone needs, during and after the project's end.

## 2. RESULTS AND DISCUSSION

In total 357 questionnaires distributed among students were evaluated. Students participating in this investigation were from all years of study. The most important result of this assessment was the highlighter of the domains for which platform modules would be developed: *Farm Animals Breeding Units, Clinics and Hospitals, Laboratories (research, diagnosis, food control), Slaughtering Houses, Rural veterinary Clinics*. Another revealed aspect was that even though classical textbooks have remained the most important instrument for learning, students gave consideration to e-learning as a useful educational tool.

A number of 25 experts from potential beneficiary institutions (animal farms, clinics, laboratories, food processing factories) were questioned about the opportunity and acceptance of an e-learning platform in veterinary medicine and about specific practical training demanded by the potential workplaces for veterinary graduates too. It resulted that although e-learning was considered helpful for some educational purposes, online learning should not completely substitute traditional teaching methods in veterinary medicine. All experts shared the opinion that competences of veterinary students could be improved by the platform access previous to practical activity.

668 students of the last year of faculty accessed the e-learning platform, distributed on all developed modules. 210 students of the first year accessed modules *Laboratories* and *Farm Animals Breeding Units*. The e-learning platform was accepted as a complementary tool of learning. These first sessions of platform utilization revealed some technical difficulties that should be mended in the future.

## 3. CONCLUSIONS

3.1. Platform utilization by graduates and other professionals will result in regularly update knowledge. It will increase the number of veterinary students introduced in real life workplace.

3.2. Innovative computer system will contain background material, increasing the level of preparedness, adaptability and rapid graduates integration in labour market.

3.3. The e-learning support, practical training and tutors trained by the project are key elements for improvement of quality of veterinary higher education and of veterinary medical services.

**ACKNOWLEDGEMENTS: Project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resource Development, 2007-2013.**

**Priority Axis 2 —“Linking life long learning and labour market”.**

**Key Area of Intervention 2.1. “Transition from school to active life”.**

**Project ID: 63915.**

**Project Code: POSDRU/90/2.1/S/63915.**

The content of this paper does not necessarily represent the official position of European Union or the Romanian Government.

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## ETIOMORPHOPATHOLOGY OF THE AIRWAYS IN SWINE IN THE INTENSIVE SYSTEM

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**Key words:** pigs, intensive, rhinitis, bronchitis, bronchiolitis

### SUMMARY

Results of macro- and microscopical investigations of large and medium airways in 53 carcasses of swine from the intensive system are presented in this paper.

Catarrhal rhinitis and bronchitis, caused by different irritant factors that reach the bronchia through the inhaled air, have as starting point, a serous exsudation and a glycosaminoglycans hypersecretion from bronchic glandae and from goblet cells from the epithelium of the bronchic mucosa.

Infiltrative (edematous) serous rhinitis and bronchitis are caused by pathogens that reach the affected anatomic structures through blood and lymph. The constant lesion in this case is the separation of all parietal structures through large accumulations of serous exsudate.

Hyperplasic rhinitis and bronchitis are characterised by a polimorphic cell proliferation with a circular disposition around normal anatomic structures, vessels, glands and nerves. It is easily observable in high resolution microscopical examination that the main cell types in inflammatory foci are the lymphocyte, the hystiocyte and the plasmocyte.

Fibrous inflammation is the final phase of exsudative and proliferative inflammations, both in the nasal cavities as well as in intrapulmonary airways.

The main disease localised in the respiratory system are generally viral, but there are also pathological antities of bacterial or parasitic origin.

### *Influenza*

The etiologic agent is the „type A influenzavirus”, an RNA- *Influenza virus*, of 80-120 nm, with a lipoproteic coating that has hemagglutinin (H) and neuramydase (N) spiculi.

Histopathological examination reveals degeneration and necrosis of the bronchic and bronchiolar epithelium, exsudate containing desquamate cells and neutrophiles in the air spaces, dilatation of the capillaries and the infiltration of the alveolar septum with lymphocytes and hystiocytes, peribronchiolar and perivascular cellular infiltration.

### *Adenovirosis*

Basic characteristics of the adenoviruses are identical for all *Adenoviridae*. A 75 nm diameter sphere containing a double layer of DNA genome, surrounded by an icosahedral capsid formed of 252 capsomeres. Swine adenoviruses are stable at pH 4 or treated with chloroform or ether.

Following the experimental infection of piglets, macroscopical lesions were only identified in the lungs. Virusologic diagnosis can be accomplished only by isolating the virus from the infected tissue. (Perianu T., 2005) (12)

#### ***Coronaviriosis***

The etiologic agent is a nonenteropathogen virus antigenically related to the virus of the transmissible gastroenteritis, isolated on cell cultures (Pensaert *et al.*, 1986). This is considered to be a mutant of the transmissible gastroenteritis virus, adapted to the respiratory epithelium, that multiplies in epithelial respiratory cells and in alveolar macrophages (Pensaert *et al.*, 1989). In contrast with the virus of the transmissible gastroenteritis (VGET), respiratory coronavirus is frequently isolated from the respiratory tract, but with a weak or absent intestinal replication.. (Perianu T., 2005) (12)

#### ***Citomegalic inclusions rhinitis***

Most characteristic and constant lesions are represented by a catarrhal inflammation of the nose cones, with degeneration of the epithelium of mixed glands and mucosa keratinization. On the 10th and 27th day of infection, characteristic citomegalic inclusions are obvious in the nuclei of degenerated inclusions. The intervention of the associated bacterial flora (*Streptococcus spp.*, *Pasteurella spp.*, *Actinomyces pyogenes*, *Escherichia coli*, *Actinobacillus spp.*) further complicates the lesional aspect, leading to either purulent or lymphohistiocytic aspect. (Coțofan Otilia *et al.*, 1997) (2)

#### ***Enzootic pneumonia***

Restriction analysis of the endonucleases and the gel electrophoresis revealed antigenic differences between the different isolated strains of *Mycoplasma hyopneumoniae*. This species is antigenically related to *Mycoplasma flocculare* and *Mycoplasma hyorhinis*, that can be isolated from the lungs of pigs diagnosed with enzootic pneumonia. (Paul I. *et al.*, 1990) (10)

In the case of pigs with enzootic pneumonia lesions, several bacterial germs are frequently isolated (*Pasteurella spp.*, *Escherichia coli*, *Salmonella spp.*, *Streptococcus spp.*), chlamydia and even viruses (adenoviruses, picornaviruses) that in most cases lead to a complication and aggravation of the

primary pulmonary process (Bondoc I. *et al.*, 2004; Perianu T., 2011) (1, 13)

Histologically, enzootic pneumonia is first manifested as a slight catarrhal-purulent bronchopneumonia. This lesion is quickly replaced by a circumscribed lymphohistiocytic inflammation expressed through perivascular and peribronchiolar proliferations, lymphoid pseudonodules and diffuse infiltrations of interalveolar septae (Oprean O.Z., 2002; Paul I., 1996; Paul I. *et al.*, 1990; The Merck Veterinary Manual, 1986) (8, 9, 11, 15)

#### ***Pasteurellosis***

The Etiologic agent is *Pasteurella multocida*, A and D capsular types. Most frequently isolated biotypes in pulmonary lesions are :1A, 3A, 1D, 2D and 10D. Special focus is given to serotype D toxins, that cause necrosis of the derm. Schimmel (1988), states that it is possible to cause both atrophic rhinitis as well as pneumonia by using *Pasteurella multocida* strains in experimental infections. (Moga Mânzat R., 2001) (7)

The acute (edematous) evolution consists of an intense serous or serofibrinous infiltration of the connective tissue in the submaxillary, cervical, substernal regions, and sometimes even in the thoracic cavity or in the interlobular pulmonary tissue. (Enache T. *et al.*, 1994) (4)

The subacute (pectoral) evolution consists of haemorrhagic effusions as well as fibrinonecrotic bronchopneumonia foci and fibrinous pericarditis. Fibrinohaemorrhagic polyarthritis, gastric edema similar to those in colibacillosis have also been noticed. (Secasiu, 1977). (13)

The chronic evolution involves anemia and cachexia, old fibrinous and necrotic bronchopneumonia lesions, pulmonary and pericardic sequelae and fibrinous, often adherent pleuresia.

#### ***Atrophic rhinitis***

The adherence of the *Bordetella bronchiseptica* and *Pasteurella multocida* bacteria to the epithelium of the respiratory tract is a complex interaction between the bacteria and the target cells, which determines epithelial invasion and pathogen and immune effects of the germs.

Histologic examination reveals the stages of the inflammatory process, which is more obvious in soft tissues: epithelium, lamina propria and submucosa. Lamina propria suffers from a serohaemorrhagic and, late on, leukocytic infiltration.

#### ***Contagious pleuropneumonia***

Etiologic agent is *Actinobacillus pleuropneumoniae*. The name of *Haemophilus pleuropneumoniae* was chosen by *Shope et al., 1964* and by *White et al., 1964*, and then conformed by *Kilian et al., 1978*. The name *H. parahaemolyticus* given to the californian strain (*Olander 1963; Biberstein et al., 1963*) and isolated in Switzerland as well (*Nicolet and Konig 1966; Nicolet 1968*) was considered a synonym. (Taylor J.D., 1989) (14)

The pneumonia is bilateral, localised in cardiac and apical lobes, where pulmonary lesions are often focalised and well delimited. In the supraacute evolution, the trachea and bronchiae are full of a foamy reddish exsudate. Only in few cases the pulmonary area appears dense, with a fragile section area.

Fibrinous pleuresia is evidentiated in animals who die during the acute stage of the disease, in at least 24 hours from the infection. According to the evolution of the lesions, fibrinous pleuresia can become fibrous, affecting the whole pulmonary surface. (Lemann D.H., 1992) (5)

#### ***Tuberculosis***

Pulmonary localisations usually coexist with digestive localisations and is expressed by a similar simptomaptology to the one in pulmonary tuberculosis of bovine, but with a faster evolution (a few weeks ) and with a lethal end (Drăghici D. *et al.*, 1982) (3)

#### ***Hidatidosis***

Lesions are mainly localised in the lungs and liver. The punction of the cyst expresses the hydatidic liquid. When sectioned, the lung has caverns, with vesicular debris in the cavities. The parasitated lung shows hidropneumothorax and emphisema, in areas limitrophic to the cysts. The diagnosis is difficult in living animals. Post mortem, the diagnostic is easy when vesicles are not altered. (Mitrea L.I., 2002) (6)

#### ***Metastrongilosis***

The histological examination shows catar and proliferative reactions in the bronchiae and bronchioles. The aerophore spaces are filled with abundant mucus and numerous parasites in all developmental stages (eggs, larvae, adults), the rich exsudate can cause bronchioles obstruction. (Mitrea L.I., 2002) (6)

## **1. MATERIAL AND METHOD**

### ***1.1. Necropsic examination***

### *The exam of the nasal cavities*

After the incision of soft tissues, a transverse section through the maxilar is performed with a saw, in front of the first molar. The inspection starts with the nasal septum, whose aspect is very important in swine; then, nasal cones, mucosa and deposits are examined.

*The exam of the trachea and main bronchiae* is performed by longitudinal sections with the scissors; changes of the lumen, intraluminal deposits and the surface of the mucosa aspect must be recorded.

*The exam of intrapulmonary airwaves* consists of the exteriorisation of the inner context, through light pressure applied on section surfaces.

## **1.2. Histological examination**

Prelevated tissular fragments were paraffine embedded and processed in order to obtain the permanent histological slides according to the specific working protocole: prelevation, fixation, paraffine embedding, sectioning, staining and mounting.

Histological staining used were : *Haematoxiline - Eosine – Methyl Blue (HEA)* for general orientation purposes, *Periodic Acid Schiff (PAS)* for glycosaminoglycans and micetes and *May Grunwald-Giemsa (MGG)* for cellular details .

## **2. RESULTS AND DISCUSSIONS**

### **2.1 Lesions of nasal cavities**

#### **2.1.1 Infiltrative serous rhinitis**

In superficial areas of the skin, the epiderm is slightly displaced off adiacent structures due to serous exsudate, noticeable in histological examination as free disjunctive deposits; the same dissociation is also observable in the superficial derm. (*Fig. 1*)

The intermediate derm and the submucosa are affected by the same serous infiltrate; lacunary spaces dissociate glandular formations and local nerves. (*Fig. 2*)

In the areas where cutaneous and vestibular nasal mucosa conserves hypodermic debris, the severe congestion is followed by serous exsudation that

damages the local adipose tissue; fragments of the hyaline cartilage are visible sometimes. (*Fig. 3, Fig. 4*)

### **2.1.2 Lymphohistiocytic rhinitis**

In the subacute-chronical stage of the inflammatory pathological process, nespecific cellular proliferation takes a restaurative part.

Lymphocytic, hystiocytic and rarely plasmocytic hyperplasia is activated by calcium ions, choline and antigenes. Prolifiration was noticed with a perivascular and periglandular disposition, and seldom as a pseudonodular disposition. (*Fig. 5, Fig. 6*).

### **2.1.3 Fibrous rhinitis**

Fibrous or sclerotic rhinitis is described in 35 of the 53 cases taken in study, which stands for a 66% of the cases.

In the initial area of the nasal cavity, where hair follicles are still present, they appear trapped in fibrous tissue. In the distal area, hair follicles and vascular and glandular structures are replaced by connective tissue, the derm and the submucosa showing a homogenous fibrous aspect. (*Fig. 7*)

Intermediate and deep areas of the submucosa show the same fibrous hyperplasia that dissociates glandular structures and nervous terminations. (*Fig. 8*)

The fasciculs of striated muscle tissue coming from the subcutaneous muscles are also trapped in an excessive stroma; some of the fascicles show the homogene aspect of tissular devitalisation. (*Fig. 9*)

The characteristic of the fibrous prolifiration that constantly appears in all sclerosis areas is the circumscribed aspect of normal structural formations (glands, nerves, blood vessels). (*Fig. 10*)

## ***2.2 Lesions of intrapulmonary airways***

### **2.2.1 Catarrhal bronchitis**

Macroscopically, the catarrhal exsudate has different aspect and composition, being the base of an accentuated stadialization of the catarrhal inflammation.

Histological examination reveals the fact that catarrhal exsudation is caused by changes with two possible localisations: intraparietal and superficial (of the respiratory epithelium).

Both in the submucosa and in the lamina propria, bronchic glands are in hyperactivity, with a cell overload of glicosaminoglycans; histological examination of PAS staining slides show distended cells, with the inner space practically blocked by intense PAS-positive mucopoliosides. (*Fig. 11*)

The surface epithelium of the bronchic mucosa is marked by hyperplasia and hyperfunction and goblet cells. (*Fig. 12*)

The hyperactivity of the bronchic glands and mucinous distrophy of the superficial epithelium lead to intraluminal mucus accumulations that resist to repeated washing during histological processing and that stain intensely PAS-positive. (*Fig. 13*)

The subacute-chronical phase of the pathological process adds to congestive-exsudative changes slightly hyperplasic phenomena; the mucus on the surface of the mucosa is less abundant, and the vascular ectasy in the lamina propria is accompanied by a discrete lymphohistiocytic proliferation. (*Fig. 14*)

### **2.2.2 Hyperplasic bronchitis**

Hyperplasic bronchitis is actually a **lymphohistioplasmocytic bronchitis**, this being the most simple and wide spread proliferative inflammation in comparative pathology.

This lesions appears both as primary lesion as well as a final form of exsudative inflammations.

Macrostructurally, the wall of the bronchia appears homogenous due to nespecific cell compactisation. The submucosa is the place of polimorphic proliferation as very thick polymorphic cell bands. (*Fig. 15*)

Hyperplasia is present arround blood vessels and bronchic glands; vessels are slightly compressed and glandular acins suffer necrosis. (*Fig. 16, Fig. 17*)

High resolution microscopical examination clearly shows morphological characters of mail cellular type from the inflammation foci. Medium sized *lymphocytes*, have a nucleo-citoplasmatic ratio of 8-9/1; the hyperchromatic nucleus is surrounded by a cytoplasmic narrow basophile band. *Hystiocytes* have big vesiculous nucleus and a poorly delimited and ramified cytoplasm. *Plasmociytes* have the shape of a raindrop, with a hyperchromatic nucleus at the rounded pole of the cell. (*Fig. 18*)

In predominantly plasmocytic foci, rare giant cells syncytium are formed, with a relatively well delimited cytoplasm and basophile and with 5-10 nuclei with a horseshoe disposition under the plasmalema. (*Fig. 19*)

In one case the lungs were probably the place of a parasitic infection, since May Grünwald-Giemsa staining evidentiates several eosinocytes; the cells have bilobated hyperchromatic nuclei and big oxyphile grain cytoplasm. (*Fig. 20*)

### **2.2.3 Fibrous brinckitis**

Fibrous (sclerotic) bronckitis is the final stage of other inflammations or other initial lesions of the bronckial wall.

Collagenization of the bronckial wall sometimes leads to its retraction, making it look like a thin band of connective tissue in which fragments of the Reissessen muscle are still visible and which show an alternance of disepithelized areas and areas of hyperplasic epithelium. (*Fig. 21*)

*Obliternat bronckitis* is a variant of the fibrous inflammation, in which the hyperplasia of the connective tissue is mainly centripete. Excessive connective tissue in the lamina propria accentuates the folded aspect of the bronckial mucosa. (*Fig. 22, Fig. 23*)

In interlobular bronckiae, parietal changes justify the name of this lesion, papillar hyperplasia of the chorion leads to a permanent obliteration of the respiratory tract. (*Fig. 24*)

## **3. CONCLUSIONS**

Necropsic examination of the respiratory tract in 68 pigs was followed by histopathological investigations of the respiratory tract in 53 cases.

3.1. Nasal mucosa is relatively hard to approach during the necropsic examination, but histological examination is rather easy in cutaneous and vestibular areas, but in the respiratory area it is hard to perform due to the bone support of the wall of the nasal cavities and nasal cones.

3.2. Microscopical examination were performed in nasal cavities, lobal bronckiae and interlobular bronckiae; distal segments of intrapulmonary airways are considered part of the pulmonary parenchyma and will be further investigated.

3.3. The lesions in the nasal mucosa and in intrapulmonary airways are exsudative (acute) and hyperplasic (chronical) inflammations.

3.4. Serous infiltrative rhinitis and bronchitis are caused by pathogens that attain the affected structures through sanguine and lymphatic circulatory way. The constant change is the dissociation of all parietal structures by large lacunes of serous exsudate (free spaces after histological processing).

3.5. Catarrhal bronchitis, caused by irritant factors that reach the bronchiae with every breath, consists of serous exsudates and glycosaminoglycans hypersecretion in bronchic glands and in goblet cells of the bronchic mucosa.

3.6. Chronical inflammation is initially lymphohistioplasmocytic and then fibrous.

3.7. In hyperplasic rhinitis and bronchitis, polimorphic cell proliferation is circumscribed to normal structures, vessels, nerves and glands. Main cell types in inflammatory foci are easily distinguishable: lymphocytes, hystiocytes and plasmocytes.

In some cases, a cell fusion process begins, and in one of the cases, an induced eosinocytosis is noticed, probably caused by a pulmonary parasitic invasion.

3.8. Fibrous inflammation is the final stage of exsudative and lymphohistiocytic inflammations both in the nasal cavities and in the intrapulmonary airways.

The etiology of rhinitis and bronchitis described is relatively nespecific and is caused by different irritative factors.

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**Board I**

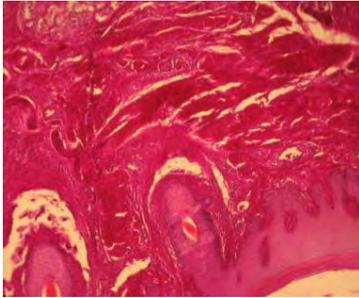


Fig. 1. Infiltrative (edematous) serous infiltration. Subepidermic serous infiltration. PAS, x 400

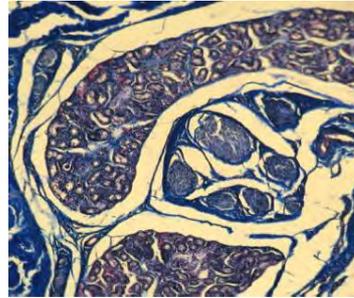


Fig. 2. Edematous rhinitis. Dislocation of glandular formations and nervous endings. HEA, x 100

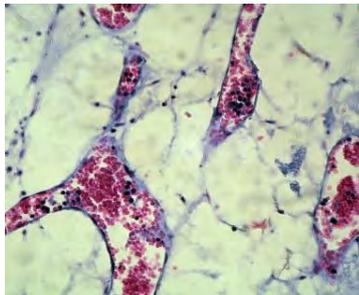


Fig. 3. Edematous rhinitis. Congestion and serous infiltration in the hipodermic area HEA, x 400

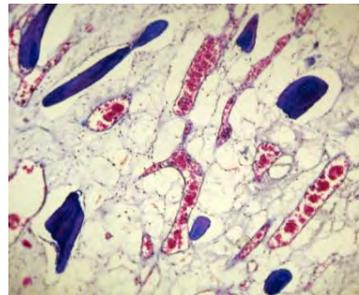


Fig. 4. Edematous rhinitis. Congestion and serous infiltration in hipodermic areas. Dislocation of the hyaline cartilage HEA, x 100

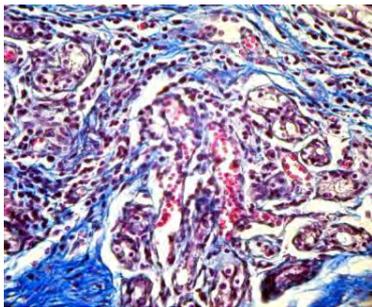


Fig. 5. Lymphohistiocytic rhinitis. Perivascular intradermic proliferations HEA, x 400

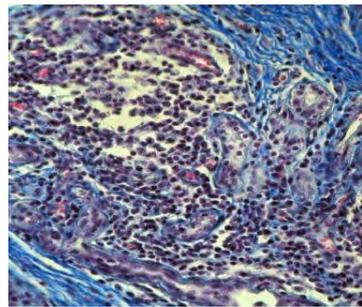


Fig. 6. Lymphohistiocytic rhinitis. Periglandular intradermic hyperplasia. HEA, x 400

**Board II**

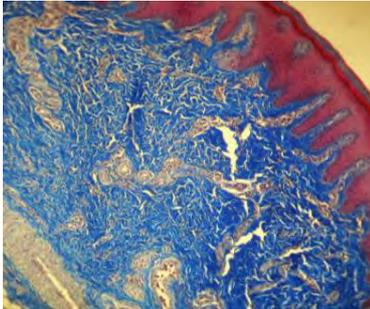


Fig. 7. Sclerotic rhinitis. Subepidermic fibrous proliferation HEA, x 100

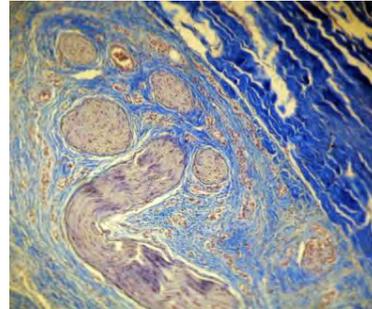


Fig. 8. Sclerotic rhinitis. Perinervous fibrous proliferation. HEA, x 100

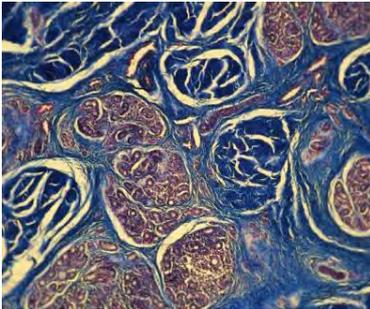


Fig. 9. Sclerotic rhinitis. Profound fibrous proliferation. Muscular necrosis HEA, x 100

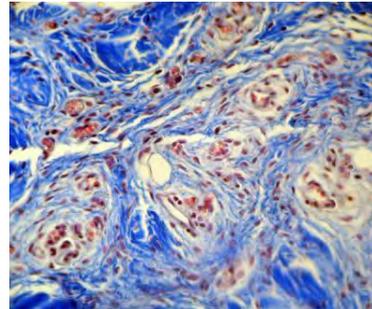


Fig. 10. Sclerotic rhinitis. Connective tissue in perivascular vortex . HEA, x 400

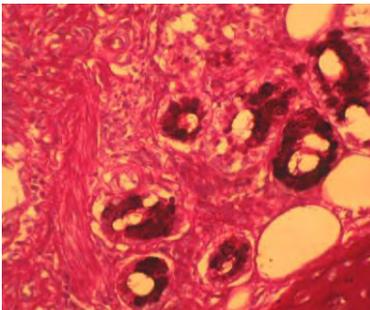


Fig. 11. Catarrhal bronchitis. Hypersecretion of glands in the submucosa PAS, x 400

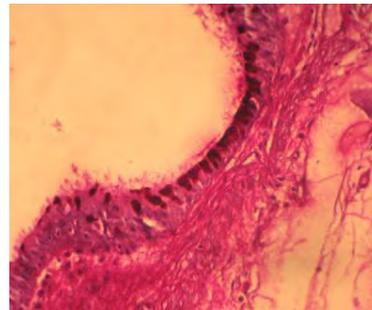


Fig. 12. Catarrhal bronchitis. Hyperplasia and hypersecretion of goblet epithelial cells PAS, x 400

**Board III**

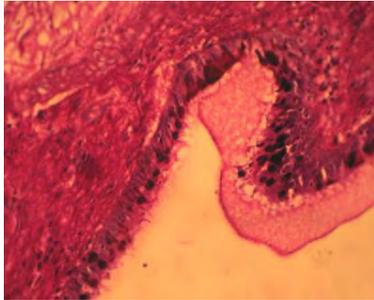


Fig. 13. Catarrhal bronchitis. Goblet cells hypersecretion. Mucus on the respiratory epithelium. PAS, x 100

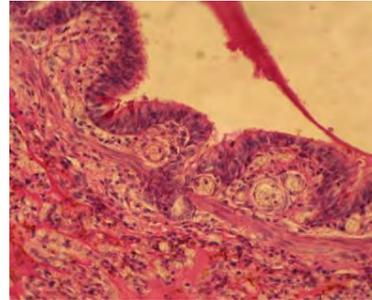


Fig. 14. Chronic catarrhal bronchitis. Mucus debris. Chorionic hyperplasia PAS, x 400

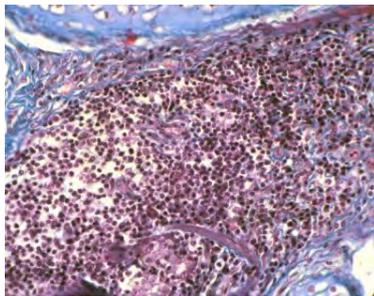


Fig. 15. Hyperplastic bronchitis. Polimorphic cell proliferation in the submucosa. HEA, x 400

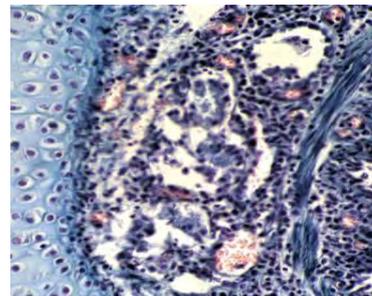


Fig. 16. Hyperplastic bronchitis. Hyperplasia in the submucosa. Sequestration and necrosis of the glands. HEA, x 400

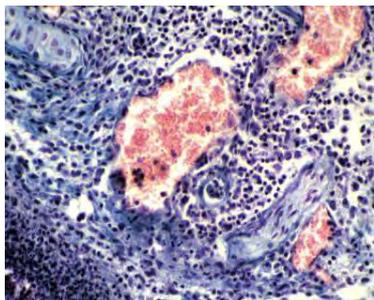


Fig. 17. Hyperplastic bronchitis. Profound polymorphic proliferation. Fragmentation of the hyaline cartilage. Sequestration of blood vessels and nervous endings. HEA, x 400

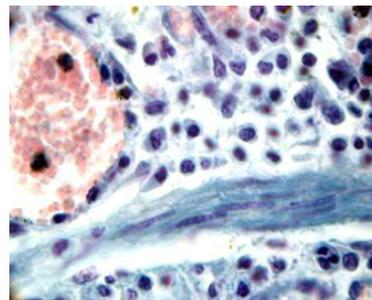


Fig. 18. Hyperplastic bronchitis. Polimorphic proliferation in the submucosa. Lymphocytes, hystiocytes, plasmocytes . HEA, x 1000

**Board IV**

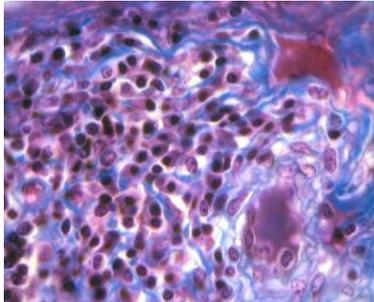


Fig. 19. Hyperplastic bronchitis. Proliferation of the chorion. Plasmocytic proliferation. Gigantocellular proliferation HEA, x 1000

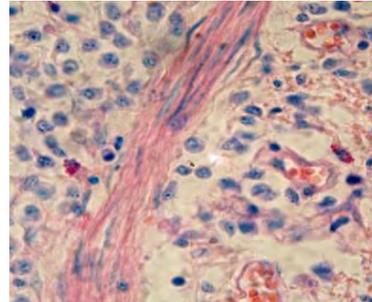


Fig. 20. Eosinocytic bronchitis. Congestion. Eosinocytes. Col. MGG, x 1000

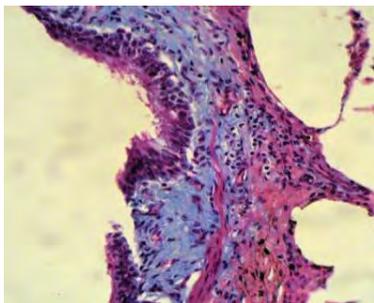


Fig. 21. Sclerotic bronchitis. Retraction of the bronchic wall. HEA, x 100

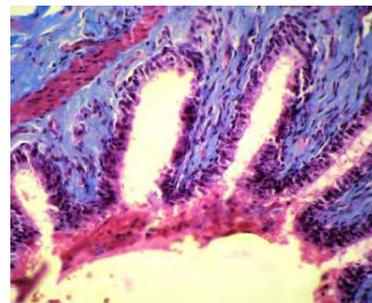


Fig. 22. Centripete (obliterant) sclerotic bronchitis. Chorionic connective tissue hyperplasia. HEA, x 400

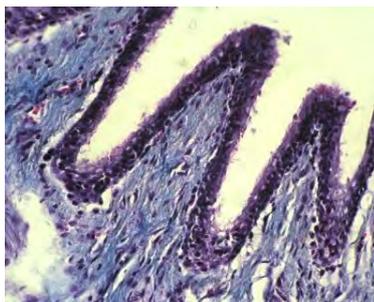


Fig. 23. Obliterant bronchitis. Chorionic fibrous hyperplasia. Crenulation of the mucosa HEA, x 400

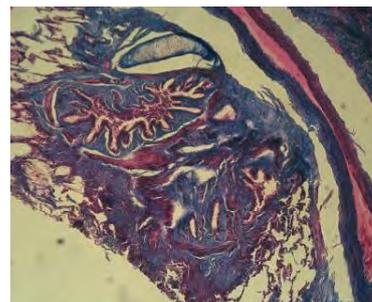


Fig. 24. Obliterant bronchitis. Disappearance of the respiratory lumina. HEA, x 100

## **NONADAPTATIVE BEHAVIOR AS WELFARE INDICATOR IN A DOG TRAINING UNIT**

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**Key words:** welfare, stress, nonadaptative behavior, dog training unit

### **SUMMARY**

Abnormal or nonadaptative animal behavior reflects stress and defective coping mechanism and is a sign of poor welfare. The present study aimed to assess dogs' welfare from a training unit in the center of the country, by using behavioral indicators of stress.

There were used 20 animals, divided into two subgroups: subgroup A, consisting of 10 females from breeding stock and subgroup B, consisting of 10 males used in various missions. Dogs were of different breeds: German Shepherd, Belgian Shepherd and Bloodhound. In order to establish the stress level in the studied dogs, in the first phase it was conducted an objective analysis, using an ethogram and recording time spent by expressing different patterns in a 10 minutes observation period. In the second phase there was conducted a subjective assessment of behavioral stress indicators prevalence using sheets filled by stockmen for each dog.

Following the researches, it can be concluded that the dogs from the training unit are affected by stress and have subsequently welfare issues. Among behavioral indicators of stress, high prevalence was recorded for excessive vocalization and destructive or stereotypic locomotor behavior.

Defined by Broom as "animal state as regards its attempts to cope with its environment", animal welfare is a problem of major importance, entering in the domain of activity of many non-governmental and governmental organizations [7].

The animal welfare could be assessed on the basis of housing conditions, livestock management and practices, laboratory analysis and behavioral indicators which reveal a high level of stress.

Among experts there are many controversies related to notions of natural or normal behavior and abnormal behavior or nonadaptative or inappropriate behavior [2,3,4].

Normal behavior refers either to how animals manifest in natural environment or how animals perform highly motivated patterns or which give them a functional feedback.

Abnormal behaviors include redirected behaviors, stereotypes and heightened aggression. Representing coping mechanism or behavioral pathologies, all these patterns are signs of poor welfare [3,6].

## MATERIAL AND METHODS

Researches in the present paperwork focused on the stress assessment as welfare indicator for 20 dogs, 10 females and 10 males, from a training unit in the center of the country. The animals were housed in cages with exterior individual pens forming a continuous row, with metal grid walls. Breeds distribution was: 14 dogs from German Shepherd breed (eight females and six males), 5 dogs from Belgian Shepherd breed (two females and three males) and 1 dog from Bloodhound breed (male).

The studied group was divided in two subgroups.

Subgroup A consists of 10 females belonging to the breeding stock, chosen either from puppies obtained and selected by mother's reproductive performance, or bought from kennels and selected by a special committee.

Subgroup B consists of 10 males – dogs belonging to the unit personnel, dogs used in tracking missions, patrolling, intervention, explosives detection, narcotics detection, mountain rescue etc.

The estimation of stress behavioral indicators prevalence was done objectively in the same day, by observing each dog for 10 minutes. Dogs were restrained in the inner area of the pens the entire period of observation, and the person who performed the test was not in sight of dogs. Data were analyzed using an ethogram (table 1) which includes only activities and postures that have been proved to reflect the stress in dogs. It was recorded the period spent by each dog to perform a certain activity or posture.

*Table 1*

The ethogram used to assessed the dogs' behavior

<b>Behavior</b>	<b>Symbol</b>	<b>Description</b>
Excessive vocalization	<b>V</b>	Dog barks, growl or whimper
Repetitive jumps	<b>J</b>	Dog constantly jumps on fence or pen walls
Pacing	<b>Pc</b>	Dog walks repetitively on linear trajectory
Circling	<b>C</b>	Dog walks repetitively in circle
Panting	<b>Pn</b>	Dog breathes fast, with mouth open
Trembling	<b>T</b>	Dog is shivering or trembling, could be noticed skin or superficial muscle movements
Digging	<b>D</b>	Dog performs digging movements with forelimbs
Urine and feces eliminating	<b>E</b>	Dog urinates or defecates
Destructive behavior	<b>De</b>	Dog bites or paws walls or objects in the pen or crate
Submissive postures	<b>S</b>	Keeping the ears and tail in low position
Lifting forelimb	<b>LF</b>	Dog lifting a forelimb while sitting
Lips licking	<b>L</b>	Dog is licking its lips or nose
Excessive self-grooming	<b>G</b>	Dog licking or biting its fur, paws and tail

There was also conducted a subjective assessment of behavioral stress indicators prevalence using sheets filled by stockmen for each dog (table 2).

Table 2

Sheet used for assessing the behavioral stress indicators prevalence

<b>Dog name:</b>	<b>Sex: male/female</b>
<b>Age:</b>	<b>Breed:</b>
1. Dog performs an abnormal (nonadaptative behavior) A. Yes B. No	
2. Observed activities or postures	
a. Circling b. Pacing c. Repetitive jumps d. Tail-chasing e. Excessive grooming f. Passivity g. Destroying the bed	h. Destroying the litter i. Turning the water pots j. Turning the food pots k. Licking walls l. Biting pens' grid m. Excessive barks n. Urinating in litter
3. Other noticed nonadaptative behaviors	

## RESULTS AND DISCUSSIONS

The results obtained by objective analyze of the behavior are shown in table 3. The total time spent by dogs with stereotypic behavior (circling, pacing and repetitive jumps) was presented in the column headed „Stereotypes”.

The dogs in the studied group showed the following patterns: vocalization (V), lips licking (L), circling (C), repetitive jumps (J), lifting a forelimb (LF), panting (Pn), digging (D) and destructive behavior (De). Self-grooming, trembling or feces and urine elimination were not observed.

The patterns which appeared only one time (as destructive behavior, lifting a forelimb and digging movements recorded in a dog from subgroup B and adopting a submissive posture in a dog from subgroup A) were not included in statistical analyze.

From the statistical interpretation it results that dogs in subgroup A and those in subgroup B spent slightly similar time for vocalization or performing stereotypic behaviours. It was noticed a large variability of stress responses, especially in animals from subgroup B.

*Table 3*

The results regarding the behavior performed by dogs in housing space (seconds)

Nr.crt.	Dog name	V	L	C	J	Pc	Stereotypes
1	Nija	27	0	0	0	20	20
2	Noba	0	0	37	0	0	37
3	Nipa	0	0	0	0	0	0
4	Naia	30	0	0	10	0	10
5	Noca	68	0	10	5	0	15
6	Naka	0	0	54	0	0	54
7	Noxa	55	8	0	0	0	0
8	Neza	15	0	0	0	0	0
9	Naca	7	2	0	3	0	3
10	Naba	48	4	7	0	0	7
11	Far	10	3	0	7	0	7
12	Nabaru	0	0	0	27	0	27
13	Nacazu	5	0	0	30	25	55
14	Gid	20	0	0	0	0	0
15	Nay	175	0	0	43	0	43
16	Geb	25	4	0	0	0	0
17	Gif	55	0	18	34	0	52
18	Man	200	0	4	20	0	24
19	Cibag	153	0	3	15	0	18
20	Dogu	63	0	15	10	0	25

The results of subjective assessment of stress by using behavioral indicators are shown in tables 4 and 5.

*Table 4*

The prevalence of stress behavioral indicators in dogs from subgroup A

Behavior	No. of dogs showing the pattern	Percent
Excessive barking	4	40%
Destructive behavior	0	0%
Repetitive jumps	3	30%
Urinating in litter	0	0%
Passivity	0	0%
Tail-chasing	1	10%
Walls licking	0	0%
Circling	4	40%
Turning water pots	3	30%
Excessive grooming	0	0%
Grid biting	1	0%
Pacing	1	10%

Table 5

The prevalence of stress behavioral indicators in dogs from subgroup B

Behavior	No. of dogs showing the pattern	Percent
Excessive barking	7	70%
Destructive behaviour	1	10%
Repetitive jumps	6	60%
Urinating in litter	1	10%
Passivity	0	0%
Tail-chasing	1	10%
Walls licking	1	10%
Circling	1	10%
Turning water pots	1	10%
Excessive grooming	0	0%
Grid biting	3	30%
Pacing	0	0%

On the basis of assessment sheets filled by stockmen, there could be concluded that nonadaptative behaviors appeared in both dogs subgroups, without significant differences.

Dogs manifested the following compulsive patterns: excessive vocalization, locomotors and oral manifestations. The higher prevalence was recorded for excessive barking (55%). Different authors [1,5] have reported that, in kennels, the dogs housed in individual pens vocalize more often than those housed with other dogs, fact which is interpreted as a manner to satisfy their need for communication. In the studied group, the visual and olfactive communication were possible due to the fact that the individual pens were placed in continuity on one row, but the tactile communication was absent on the period when dogs were within cages. Therefore, half of the dogs compensated the effects of sensory deprivation with excessive acoustical communication.

Because the dogs are very active animals, their constraint in a limited space is a major stressor. The specific literature cites many nonadaptative behaviors in dogs housed individually, in small cages: excessive grooming, coprophagy, walls biting and stereotypic locomotor activities as pacing or circling, repetitive jumps on pen's walls or tail-chasing.

In the dogs from this study the coprophagy was not observed, but it dominates another stereotypic compulsive oral behavior: the destructive behavior, with a prevalence of 5% (figure 1).

The prevalence of locomotor compulsive behavior – repetitive air jumps or jumps on pen's walls, circling and tail chasing - was also high

(45%). Different studies demonstrate that in dogs housed individually could be observed more locomotor compulsive behaviors than in dogs housed in groups. There were incriminated: low-stimulus environment, lack of exercise or insufficient exercise, space deprivation and lack of social contact, anxiety with different etiologies.



Fig. 1. Destructive behavior – grid biting (Man: male, 3 years age)

It was observed that in some dog breeds tend to occur mainly certain types of compulsive behaviors (e.g. in German shepherd tail-chasing and in English Bulldog passivity [6]).

Similar to other authors, the results we obtained show no significant differences between dogs with possibility to move in large open areas and dogs kept in pens (subgroups A and B). However, could be noticed a slightly higher nonadaptive patterns in the second category

#### CONCLUSIONS

1. The present study demonstrates that the dogs from the training unit are affected by stress and has subsequently welfare issues. The cause is more likely the space limitation and the lack of social interactions.

2. Among behavioral indicators of stress, high prevalence was recorded mainly for vocalization and secondly for destructive and stereotypic locomotor behavior. This could be explained by the fact that dogs from German and Belgian shepherd breeds are characterized by hyperactivity and high exploration trend.

3. Although the behavioral differences between dogs kept in cages and dogs with limited possibility to move in large open areas (during the missions) are not significant, there could be noticed a slightly higher nonadaptive patterns in the second category.

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## OVERVIEW OF THE CUTANEOUS BENIGN EPITHELIAL TUMORS IN DOG - EPIDEMIOLOGICAL AND MORPHOLOGICAL ASPECTS

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**Key words:** benign epithelial cutaneous tumors, dog, epidemiology, morphology

### SUMMARY

The aim of the present study was to evaluate both epidemiologically and morphologically the benign cutaneous epithelial tumors diagnosed in dog between 2007 and 2010. During these four years, a total of 2742 dogs have been specifically examined and 903 (33%) of them were diagnosed with cutaneous lesions. 228 out of 903 dogs (25.2%) had benign epithelial tumors. No breed predilection was observed, but the majority of the dogs were medium and large breeds (German Shepherd, Boxer, Rottweiler, Amstaff, Cocker etc.). There was no sex predilection, either (53% females, 47% males), and the average age was 9-year-old. The neoplasms were located on the trunk (33%), limbs (29%), head (23%) and neck (15%). The attempt to correlate the incidence of the tumors with the season concluded that the majority of the diagnosed lesions were established during spring (35%) and the fewest, during summer (15%).

The specimens were obtained by fine needle aspiration (45%) and surgical removals (55%). Cytological examination was the only method of investigation for 30% of the surgical samples. On the other hand, histological examination was used for only 25% of the surgical removals and 45% of the cases were diagnosed by both methods of investigation. Cytology was dominantly used in this study, 86% of cases been diagnosed by this method. Histological examination enabled the diagnosis of the following types of tumors: trichoblastoma (28.50%), epidermal cyst (20.61%), trichoepithelioma (9.30%), follicular cyst (8.33%), pilomatricoma (3.07%), sebaceous gland hyperplasia/adenoma (3.94%), keratoacanthoma (1.75%), and "other benign epithelial tumors" (10.52%).

Cutaneous neoplasms, especially benign epithelial tumors hold a central position in the skin pathology of the dog (Moulton, 1990; Meuten 2002). The literature describes more than 30 benign epithelial tumors, which originate in various structures of the skin (epidermis, hair sheath, glands) (Goldschimdt *et al*, 1998; Gross *et al*, 2005). The structural complexity of the skin explains the large variety of these neoplasms (Kee and Hyun, 2005). The gross examination must be completed with cytological and histological examination, further stating the importance of the two methods of investigation for veterinary practice (Baba, 2002; Goldschimdt, 2002; Manolescu and Balint, 2009; Raskin 2010).

## MATERIAL AND METHODS

This study was carried between January 2007-December 2010 in Pathological Anatomy Department of the Faculty of Veterinary Medicine Bucharest. During these four years, a total of 2742 dogs have been specifically examined and 903 of them were diagnosed with cutaneous lesions. 228 out of 903 dogs had benign epithelial tumors. We have evaluated the epidemiology (breed, age, sex, location) and the morphology of these tumors. The importance of cytological and histological examination was also demonstrated.

The cytological examination of fine needle aspirates (FNA), imprints and/or cut surface scrapings was performed on May-Grunwald Giemsa.

For histological examination the tissue specimens were fixed in 10% neutral buffered formalin or Bouin solution, embedded in paraffin, sectioned at 4-6 µm and stained by trichrome Masson or H&E.

### 1. RESULTS AND DISCUSSION

Between 2007-2010 was evaluated 2742 dogs and 903 (33%) had cutaneous lesions. This percent makes cutaneous lesions the most frequent diagnosed type of lesion in our department. A number of 228 dogs out of 903, had benign epithelial tumors, which means 25.2%. The distribution over years and seasons is included in the tables 1-4.

*Table 1*

Presentation of the cases of benign epithelial tumors in 2007

Month	No Case	Sex F/M	Age	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	1	1 M	5 Y	0	0	1	0	1	0	0	0	1
Jan	9	3F/6M	8 Y	3	1	5	0	5	4	5	0	4
Feb	3	2F/1M	9 Y	1	1	1	1	2	1	1	0	2
<b>Winter</b>	<b>37%</b>											
March	3	1F/2M	6 Y	0	0	1	3	1	2	1	2	0
April	2	1F/1M	4 Y	1	0	1	0	0	2	0	0	2
May	3	1F/2M	5 Y	1	0	1	1	2	1	0	0	3
<b>Spring</b>	<b>23%</b>											
June	3	3M	7 Y	1	1	1	1	3	0	0	0	3
July	2	1F/1M	5 Y	1	0	1	1	2	0	1	0	1
Aug	1	1F	7 Y	1	0	0	0	1	0	0	0	1
<b>Summer</b>	<b>17%</b>											

Sept	2	1F/1M	8 Y	0	0	2	0	1	1	1	0	1
Oct	4	3F/1M	7 Y	1	0	1	2	4	0	3	0	1
Nov	2	1F/1M	7 Y	1	0	0	1	2	0	2	0	0
<b>Autumn</b>	<b>23%</b>											
<b>Total</b>	<b>35/ 100%</b>	<b>15F/ 20M</b>	<b>7 Y</b>	<b>11</b>	<b>3</b>	<b>15</b>	<b>10</b>	<b>24</b>	<b>11</b>	<b>14</b>	<b>2</b>	<b>19</b>

F = females, M = males, Y=years H=head, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

During the year 2007, 500 dogs were examined and 172 of them (34.4%) had cutaneous lesions. A number of 35 (20.3%) cases out of 172, had benign epithelial tumors.

*Table 2*

Presentation of the cases of benign epithelial tumors in 2008

Month	No case	Sex F/M	Age	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	3	2F/1M	8 Y	0	0	1	2	2	1	2	0	0
Jan	8	2F/6M	8 Y	2	1	2	3	5	3	4	0	3
Feb	10	5F/5M	8 Y	2	2	5	3	7	3	4	1	1
<b>Winter</b>	<b>25%</b>											
March	8	3F/5M	10 Y	1	0	5	2	7	1	5	0	1
April	2	1F/1M	8 Y	0	1	1	1	2	0	1	0	0
May	8	2F/6M	8 Y	2	3	1	2	6	2	2	3	3
<b>Spring</b>	<b>22%</b>											
June	12	8F/4M	8 Y	5	0	3	4	6	6	2	4	2
July	5	2F/3M	8 Y	0	0	2	3	4	1	1	0	0
Aug	2	2F	12 Y	0	0	1	1	1	1	0	1	0
<b>Summer</b>	<b>23%</b>											
Sept	3	2F/1M	8 Y	2	0	1	0	2	1	3	0	0
Oct	12	10F/2M	9 Y	2	2	6	2	9	3	2	3	3
Nov	10	4F/6M	10 Y	3	0	5	3	7	3	8	0	1
<b>Autumn</b>	<b>30%</b>											
<b>Total</b>	<b>83/ 100%</b>	<b>43F/ 40M</b>	<b>8 Y</b>	<b>19</b>	<b>9</b>	<b>33</b>	<b>26</b>	<b>58</b>	<b>25</b>	<b>34</b>	<b>12</b>	<b>14</b>

F = females, M = males, H=head, Y=years, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

During the year 2008 the number of cases increased with 47%, compared to 2007. A number of 735 dogs were evaluated and 277 (37.7%) had cutaneous lesions, 83 out of 277 (30%) being benign epithelial tumors.

Table 3

Presentation of the cases of benign epithelial tumors in 2009

Month	No case	Sex F/M	Age	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	5	4F/1M	9 Y	2	0	3	1	5	0	4	0	1
Jan	4	3F/1M	8 Y	2	0	2	0	2	2	2	1	1
Feb	5	1F/4M	11 Y	1	2	1	2	5	0	3	0	2
<b>Winter</b>	<b>24%</b>											
March	9	4F/5M	7 Y	3	1	3	2	8	1	5	2	2
April	4	2F/2M	10 Y	0	1	1	2	3	1	4	0	0
May	8	7F/1M	10 Y	1	1	5	1	7	1	7	0	1
<b>Spring</b>	<b>36%</b>											
June	7	3F/4M	9 Y	1	1	4	1	7	0	5	2	0
July	3	1F/2M	8 Y	0	0	3	0	2	1	2	0	1
Aug	1	1F	10 Y	0	0	0	1	1	0	1	0	0
<b>Summer</b>	<b>19%</b>											
Sept	5	1F/4M	9 Y	2	1	2	2	5	0	4	0	1
Oct	1	1M	7 Y	0	0	1	0	1	0	0	0	1
Nov	6	1F/5M	9 Y	4	0	1	1	5	1	5	0	1
<b>Autumn</b>	<b>21%</b>											
<b>Total</b>	<b>58/ 100%</b>	<b>28F/ 30M</b>	<b>9 Y</b>	<b>16</b>	<b>7</b>	<b>26</b>	<b>13</b>	<b>51</b>	<b>7</b>	<b>42</b>	<b>5</b>	<b>11</b>

F = females, M = males, Y=years, H=head, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

In 2009 a total of 700 dogs were examined. 288 (41%) of them had cutaneous lesions, 58 (20.6%) being benign epithelial tumor.

The largest number of examined dogs (807) was during 2010. 236 (29.2%) of those had cutaneous lesions and 22% of these lesions were benign epithelial tumors (52 cases). The synthetic presentation of the information permits the complete and comparative evaluation of the cases, which results in incidence of cutaneous lesions, in particular the incidence of benign epithelial tumors.

Thus, 2008 had the most numerous cases of cutaneous lesions (277 cases), one third of them being benign epithelial tumors. Taking into account of all data, including anamnesis, diagnosis and also the fact that the summer of 2008 was extremely hot, with temperatures exceeding 35<sup>0</sup>C, for long periods of time, we can hypothesize that the temperature favored the

debut or recurrence of cutaneous lesions, especially benign epithelial tumors.

*Table 4*

Presentation of the cases of benign epithelial tumors in 2010

Month	No case	Sex F/M	Age (years)	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	7	6F/1M	10 Y	3	0	4	0	3	4	4	2	1
Jan	4	2F/2M	6 Y	1	0	1	2	0	4	0	3	1
Feb	3	2F/1M	11 Y	2	0	1	0	1	2	1	0	2
<b>Winter</b>	<b>27%</b>											
March	9	5F/4M	9 Y	4	1	2	3	4	5	6	0	3
April	6	4F/2M	10 Y	1	0	3	2	1	5	2	1	3
May	4	3F/1M	9 Y	1	0	3	0	2	2	0	3	1
<b>Spring</b>	<b>37%</b>											
June	2	2M	5 Y	1	0	0	1	1	1	2	0	0
July	3	3F	10 Y	3	0	0	0	2	1	3	0	0
Aug	1	1M	11 Y	1	0	0	0	0	1	1	0	0
<b>Summer</b>	<b>12%</b>											
Sept	3	1F/2M	6 Y	2	0	0	1	0	3	0	2	1
Oct	4	1F/3M	10 Y	1	0	3	0	2	2	2	0	2
Nov	6	5F/1M	5 Y	2	0	2	2	2	4	2	0	4
<b>Autumn</b>	<b>25%</b>											
<b>Total</b>	<b>52/ 100%</b>	<b>32F/ 20M</b>	<b>9 Y</b>	<b>22</b>	<b>1</b>	<b>19</b>	<b>11</b>	<b>18</b>	<b>34</b>	<b>23</b>	<b>11</b>	<b>18</b>

F = females, M = males, Y=years, H=head, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

There was no breed predilection, but there was a slight increase in the incidence of benign epithelial tumors affecting Rottweiler, German shepherds, Cockers, their cross-breeds and mongrels.

The males were mostly affected in 2007 (57%) and 2009 (52%), in comparison with 2008 and 2010, when the females were most affected (52%, 62%, respectively). Although the literature mentions males being the most affected by benign epithelial tumor, our study concluded that the differences are small, insignificant (Goldschimdt and Hendrick, 2002; Scott and Anderson, 1991). Following data presented above, the mean age of dogs increased from 7 to 9 years, from 2007 to 2009. In 2010, the median age remained the same as the previous year.

Related to the sites of neoplasm development, there were aspects which remained constant, but also some changes were noted. Thus, the skin

on the head and trunk were the main body site for all the years, but the limbs were the most affected in 2008. The skin of the neck was the least affected.

The main method of sampling was by fine needle aspiration, with a maximum of 88% of cases sampled by this method in 2009 and surgical removals. FNA represents an easy, fast and inexpensive, and permits obtaining a diagnosis in a few hours. This explains why this method is extensively recommended by clinicians and preferred by dog owners.

The large percentage of samples taken fine needle aspiration in 2009 could find an explanation in the financial crisis, which made this low cost method of investigation a suitable one for the owners. Compared to histological examination, which offers more information on the lesion, the limitations of cytological examination are well known.

The samples obtained by fine needle aspiration were submitted only to cytological examination. The surgical samples were submitted only to cytological examination, only to histological examination or to both methods of examinations. According to the number of cases diagnosed by cytological examination increased significantly in 2009, when 72% were diagnosed through this method. The range of lesions diagnosed in our department is shown in Table 5.

*Table 5*

The results of histological examination

Year	No. cases	TE	TB	CE	P	C	CF	PM	SGH	Other BET
2007	35	4	9	8	7	3	0	0	0	4
2008	83	12	25	14	11	1	9	0	4	7
2009	58	3	16	13	6	0	5	5	4	6
2010	52	2	15	12	8	0	5	2	1	7

TE = trichoepithelioma, TB = trichoblastoma, CE = epidermal inclusion cyst, P = papilloma, C = keratoacanthoma, CF = follicular cyst, PM = pilomatricoma, SGH = sebaceous gland hiperplasia BET = benign epithelial tumors.

The most frequently diagnosed lesion has been trichoblastoma (Fig. 4, 5, 7), its incidence ranging from 26 to 31%. Trichoepithelioma (Fig. 8) has been also frequently diagnosed, with an incidence of 45% in 2008 and 11% in 2007, followed by a dramatic decrease in the number of cases in 2009 and 2010. Keratoacanthoma (Fig. 9) was frequent in 2007, when 9% of the dogs had this tumor. Conversely, in 2008 only 1% of the dogs has developed this tumor. Epidermal inclusion cyst was diagnosed constantly in 2007, its incidence ranging between 17 and 23%, with little variations from

one year to the other. Papilloma (Fig. 10) has also been frequently encountered, its highest incidence being in 2007 (20%) and its lowest incidence in 2009 (10%). Except for 2007, when there was no registered case of follicular cyst (Fig. 11), in the other years its incidence was of 9-11%. A particular case was noted for pilomatricoma (Fig. 12), with no registered case in 2007 and 2008, but with a high incidence in 2009 (9%) and a lower one in 2010 (4%). Sebaceous gland hyperplasia/adenoma (Fig. 6), reached its highest frequency in 2009, when 7% of the dogs with benign epithelial tumor had this lesion, but in the other years, the cases were fewer. It is worth mentioning that between 2007 and 2010 there was a diversification in the range of lesions diagnosed. The majority of the benign epithelial tumors included in this study, have originated in the skin adnexa, the lesions originating in the epidermis were fewer.

The category of "other benign epithelial tumor" includes rare tumors, infrequently encountered from one year to another. Examples of this kind of tumors are: epithelioma or basal cell tumors, infundibular tricholemmoma, apocrine gland adenoma, and eccrine gland adenoma.

## CONCLUSIONS

1.1. Cutaneous benign epithelial tumors represented 25.2% a total of cutaneous lesions diagnosed in dog.

1.2. The lowest incidence of the benign epithelial tumor was registered in 2007 (20.4%) and the highest incidence was in 2008 (30%). The most affected breed were Rottweiler, German shepherd, Cocker, there cross-breeds and mongrels.

1.3. The mean age of the affected dogs ranged between 7 and 9 years, but in a progressive increase on the time interval taken into study.

1.4. The females (51.75%) were more affected than males (48.24%) and the neoplasm occurred mostly on the trunk, head and limbs.

1.5. The most used method of sampling was by fine needle aspiration (65-88%) and the majority of the cases were diagnosed by cytological examination (72%).

1.6. The histological examination permitted the diagnosis of the following types of tumors: trichoblastoma (28.5%), epidermal cysts (20.6%), trichoepithelioma (9.3%), follicular cysts (8.33%), pilomatricoma (3.07%), sebaceous gland hyperplasia/adenoma (3.94%), keratoacanthoma (1.75%), and "other benign epithelial tumors" (10.5%).

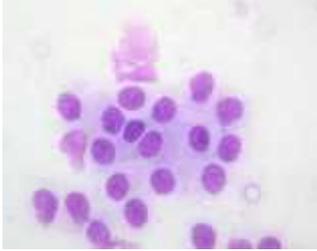


Fig. 1. Basal cell tumor. Cluster of uniform small epithelial cells with high N:C ratio (MGGx100)

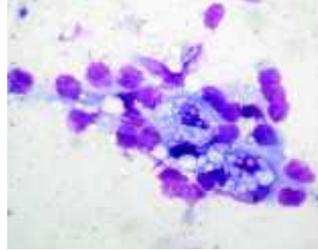


Fig. 2. Basal cell tumor with sebaceous differentiation. Mixed of epithelial and sebaceous cells (MGGx100).

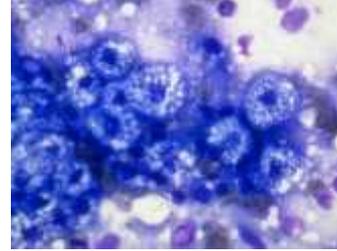


Fig. 3. Sebaceous gland hyperplasia/ adenoma. Pure population of uniform sebaceous cells (MGGx100).

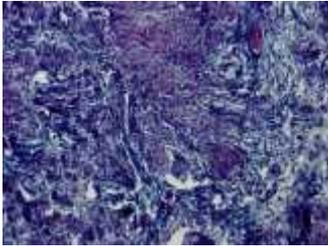


Fig. 4. Trichoblastoma, medusoid type. Stain trichromic Masson x40 x20

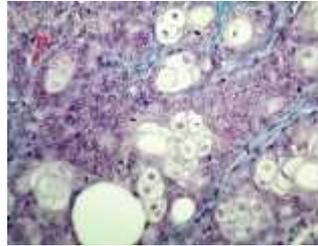


Fig. 5. Trichoblastoma with sebaceous differentiation. Stain trichromic Masson x40

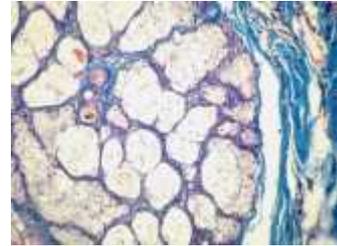


Fig. 6. Sebaceous gland adenoma. Stain trichromic Masson x40

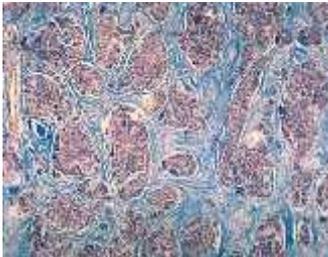


Fig. 7. Trichoblastoma, adenoid type. Stain trichromic Masson x40 x20



Fig. 8. Trichoepitelioma. Stain trichromic Masson x10

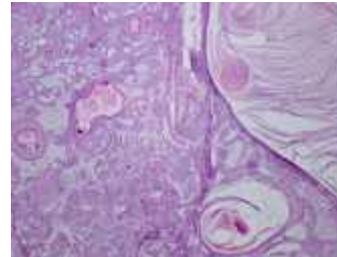


Fig. 9. Infundibular keratinizing acanthoma. Stain HE x20

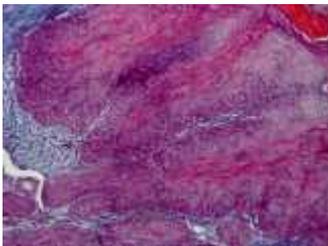


Fig. 10. Exophytic papiloma. Masson x40



Fig. 11. Follicular cyst – infundibular type. Stain trichromic Masson x40



Fig. 12. Pilomatricoma. Stain trichromic Masson x40

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# RETROSPECTIV STUDIES OF THE CUTANEOUS MESENCHYMAL TUMORS IN DOG – EPIDEMIOLOGICAL AND MORPHOLOGICAL ASPECTS

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**Key words:** skin, benign mesenchymal tumors, dog, epidemiology, morphology

## SUMMARY

This paper is a retrospective study that purpose to present comparative assessment of the epidemiology and morphology of benign cutaneous mesenchymal tumors during four years (2007-2010) in dog. This lesions take an important place in canine dermatology. During these four years, a total of 2742 dogs have been specifically examined and 903 (33%) of them was diagnosed with cutaneous lesions. 197 to 903 dogs were diagnosed with benign mesenchymal tumors (21.8%). No breed predisposition could be determined, and the sex distribution fluctuated: in 2007 and 2008 60% of all those affected were females, while in 2009 and 2010, 55% of those affected were males. The average age of the affected animals was 6 years and 8 months of age, with limits between 1 month and 16 years of age. The localization of most of the mesenchymal benign tumors was on the trunk (48%) and limbs (43%).

The majority of the examined samples (80%) were obtained by fine needle aspiration and examined exclusively cytologically. 74% of the surgical specimes were examined exclusively by cytology, 10% were examined exclusively by histological method, while 16% was examined both by cytological and histological investigations.

Lipomas (42%), canine cutaneous histiocytomas (25%), collagenous nevus (14%), hemangiomas (15%) were the most frequently encountered tumors in this study.

The benign mesenchymal tumors represent important lesions in canine dermatology (Gross *et al*, 2005; Meuten, 2002). Speciality data mention over 20 types of tumors in this category, tumors with origin in different cellular or tissular components of the skin (Hendrick *et al*, 1998). The macroscopic aspect does not permit a diagnosis, thus the microscopic, cytological and histological exam is necessary in veterinary practice (Baba, 2002; Scott *et al*, 1995).

## 1. MATERIAL AND METHODS

This retrospectiv study was carried between January 2007 and December 2010 within the Pathological Anatomy Department of the Faculty of Veterinary Medicine of Bucharest. 903 dogs with cutaneous lesions were assessed, out of which 197 were diagnosed with benign mesenchymal tumors.

The incidence of benign mesenchymal tumors was assessed, for the purpose of identifying morphological and epidemiological elements (breed, age, gender, localization), as well as for demonstrating the importance of the anatomopathological, cytological and histological exam in diagnosing cutaneous lesions.

The cytological examination of fine needle aspirates (FNA), imprints and/or cut surface scrapings was performed on May-Grunwald Giemsa stain. For histological examination the tissue specimens were fixed in 10% neutral buffered formalin or Bouin solution, embedded in paraffin, sectioned at 4-6 microns and stained by trichrome Masson (HEA) or H&E.

## 2. RESULTS AND DISCUSSIONS

Between 2007-2010 was evaluated 2742 dogs and 903 (33%) had cutaneous lesions. Out of these, 197 were diagnosed with benign mesenchymal tumors, representing 21.8% out of all the diagnosed cutaneous lesions. The year distribution of the cases is presented in the following tables.

*Table 1*

Presentation of the cases of benign mesenchymal tumors in 2007

Month	No case	Sex F/M	Age	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	1	0/1	9Y	0	0	1	0	1	0	1	0	0
Jan	5	4/1	12Y	0	0	1	4	2	3	4	0	1
Feb	3	2/1	6Y	0	0	1	2	2	1	2	1	0
<b>Winter</b>	<b>20%</b>											
March	2	1/1	8Y	0	1	0	1	2	0	1	1	0
April	3	2/1	8Y	0	0	3	1	3	0	3	0	0
May	7	2/5	7Y	1	0	4	2	6	1	5	1	1
<b>Spring</b>	<b>27%</b>											
June	2	0/2	11Y	0	1	1	1	2	0	0	0	2
July	4	3/1	5Y	0	0	2	2	3	1	2	1	1
Aug	1	1/0	7Y	1	0	0	0	1	0	1	0	0
<b>Summer</b>	<b>16%</b>											
Sept	6	5/1	7Y	2	0	4	1	6	0	4	0	2
Oct	8	4/4	8Y	3	1	1	3	8	0	7	0	1
Nov	3	3/0	10Y	0	0	2	1	3	0	3	0	0
<b>Autumn</b>	<b>38%</b>											
<b>Total</b>	<b>45/ 100%</b>	<b>27/18</b>	<b>8Y</b>	<b>7</b>	<b>3</b>	<b>20</b>	<b>18</b>	<b>39</b>	<b>6</b>	<b>33</b>	<b>4</b>	<b>8</b>

F = females, M = males, Y=years H=head, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

As seen above, 500 dogs were examined in 2007, out of which 172 (34.4%) had cutaneous lesions. Out of these, 45 (26.16%) were diagnosed with benign mesenchymal tumors.

*Table 2*

Presentation of the cases of benign mesenchymal tumors in 2008

Month	No case	Sex F/M	Age	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	4	3/1	9Y	0	0	0	4	4	0	0	1	3
Jan	6	5/1	8Y	1	0	3	2	5	1	3	1	1
Feb	3	2/1	10Y	1	0	3	0	2	1	4	0	0
<b>Winter</b>	<b>23%</b>											
March	4	2/2	9Y	0	0	2	2	4	0	3	1	0
April	4	2/2	5Y	2	0	2	1	2	2	3	0	1
May	6	4/2	9Y	0	0	3	3	6	0	6	0	0
<b>Spring</b>	<b>29%</b>											
June	3	0/3	5Y	0	0	2	1	2	1	2	1	0
July	4	4/0	7Y	0	0	2	2	2	2	4	0	0
Aug	1	0/1	10Y	0	0	1	1	1	0	1	0	0
<b>Summer</b>	<b>14%</b>											
Sept	3	2/1	10Y	1	0	1	1	3	0	1	2	0
Oct	12	7/5	6Y	1	1	3	7	11	1	9	0	3
Nov	6	3/3	7Y	0	0	3	3	4	2	5	1	0
<b>Autumn</b>	<b>38%</b>											
<b>Total</b>	<b>56/ 100%</b>	<b>34/22</b>	<b>8Y</b>	<b>6</b>	<b>1</b>	<b>24</b>	<b>27</b>	<b>46</b>	<b>10</b>	<b>41</b>	<b>7</b>	<b>8</b>

F = females, M = males, H=head, Y=years, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

In 2008, out of 735 dogs examined, 277 (37.7%) had cutaneous lesions, of which 56 (20.2%) were diagnosed with benign mesenchymal tumors. It can be considered that this significant growth was determined by the very high temperature in that summer.

In 2009, 700 dogs were examined and 218 (31%) had cutaneous lesions, out of which, 50 (22.4%) had benign mesenchymal tumors. Many of the diagnosed lesions in this year has started in summer of 2008, so on a very warm climate.

Table 3

Presentation of the cases of benign mesenchymal tumors in 2009

Month	No case	Sex F/M	Age	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	4	1/3	11Y	1	0	3	0	4	0	4	0	1
Jan	7	4/3	6Y	1	0	5	1	6	1	7	0	1
Feb	6	5/1	9Y	1	1	4	1	4	2	6	0	1
<b>Winter</b>	34%											
March	7	4/3	7Y	0	0	4	4	6	1	7	0	1
April	3	2/1	6Y	1	0	2	0	3	0	3	0	1
May	8	3/5	6Y	2	0	4	2	5	3	8	0	0
<b>Spring</b>	36%											
June	2	0/2	6Y	1	0	1	0	2	0	2	0	0
July	1	0/1	2Y	0	0	0	1	1	0	0	0	1
Aug	2	0/2	10Y	1	0	1	2	2	0	2	0	1
<b>Summer</b>	10%											
Sept	4	2/2	7Y	0	0	4	1	3	1	4	0	1
Oct	1	0/1	7Y	0	0	1	0	1	0	1	0	0
Nov	5	3/2	5Y	2	0	2	1	5	0	4	1	0
<b>Autumn</b>	20%											
<b>Total</b>	<b>50/ 100%</b>	<b>23/27</b>	<b>6Y</b>	<b>10</b>	<b>1</b>	<b>31</b>	<b>13</b>	<b>42</b>	<b>8</b>	<b>48</b>	<b>1</b>	<b>8</b>

F = females, M = males, Y=years, H=head, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

In 2010, 807 dogs were examined, the largest number from all four years. Out of these, 236 (29.5%) were diagnosed with cutaneous lesions, out of which 44 (18.6%) were benign mesenchymal tumors. Following the information in the table, we notice that the number of dogs examined increased considerably from 500 in 2007 to 807 in 2010, thus a 61.5%.

Table 4

Presentation of the cases of benign mesenchymal tumors in 2010

Month	No case	Sex F/M	Age (years)	Location				Method of sampling		Method of investigation (SS)		
				H	N	T	L	FNA	SS	Cyto	Histo	C+H
Dec	5	2/3	7Y	1	1	1	2	2	3	3	1	1
Jan	3	2/1	7Y	0	1	1	1	1	2	2	1	2
Feb	2	0/2	3Y	0	0	2	0	2	0	2	0	0

<b>Winter</b>	<b>23%</b>											
March	5	3/2	5Y	2	0	2	1	1	4	3	1	3
April	2	2/0	10Y	0	0	2	1	1	1	2	0	0
May	7	4/3	6Y	2	0	3	2	4	3	5	1	4
<b>Spring</b>	<b>32%</b>											
June	3	2/1	8Y	0	0	2	1	2	1	3	0	0
July	2	1/1	10Y	0	1	1	0	1	1	1	0	2
Aug	3	0/3	9Y	0	0	3	0	1	2	2	0	1
<b>Summer</b>	<b>19%</b>											
Sept	3	1/2	9Y	1	0	2	0	1	2	1	2	0
Oct	6	2/4	5Y	0	2	1	3	2	4	2	1	3
Nov	3	1/2	3Y	1	1	1	0	1	2	1	2	1
<b>Autumn</b>	<b>27%</b>											
<b>Total</b>	<b>44/ 100%</b>	<b>20/22</b>	<b>5Y</b>	<b>7</b>	<b>6</b>	<b>21</b>	<b>11</b>	<b>19</b>	<b>25</b>	<b>27</b>	<b>9</b>	<b>17</b>

F = females, M = males, Y=years, H=head, N=neck, T=trunk, L=limbs, FNA=fine needle aspiration, SS=surgical sample, C+H=cytological and histological examination

The comparative analysis among the four years of the study, revealed that in 2008 we had the largest number of cases diagnosed with cutaneous lesions, that is 277, out of which a third represented benign mesenchymal tumors. If we relate the anamnesis data with the diagnosis data and considering the fact that the summer of 2008 was very hot, with temperatures of more than 35°C over long periods of time, we can suspect that the increased incidence of lesions is due to this fact. If we relate the cases with the time of the year, we notice that during the winters between 2007 and 2010, a constant number of cases was recorded. In the autumn of 2008 we have the most cases of benign mesenchymal tumors.

Analysing the data we have and correlating with the anamnesis data, we notice the apparition or activation of the majority of cutaneous lesions in the summer of 2008, even though it was examined and diagnosed in the autumn of 2009, the winter of 2008-2009 and the spring of 2009. Between the autumn of 2008 and summer of 2009, we notice a significant decrease of cases diagnosed with cutaneous lesions generally and benign mesenchymal tumors particularly. As for the gender distribution, the most affected in 2007 and 2008 were females, 60% and 61% of all cases, while in 2009 and 2010 males were first, in different, but similar proportions: 54% in 2009 and 55% in 2010. Although specialty literature mentions a higher incidence of cutaneous tumors in males, the data we obtained is different without being able to correlate this fact with the year, season or age of the affected

animals. According to the data presented in table 4, the average age of the animals dropped from 8 years in 2007 to 5 years in 2010.

In regard to the localization, benign mesenchymal tumors occur in higher numbers on the trunk and limbs. We notice that in years 2008 and 2010, the degree of limb-localization is high, while in 2009, the degree of localization on the trunk is the highest. We do not exclude the possibility that the high incidence of trunk localization of diagnosed lesions in 2009 is due to the prolonged exposure to sun rays, to which we add the fact that most of the affected animals did not have it trimmed.

In general, the fine needle aspiration has been the most used method, regarding the cases with cutaneous mesenchymal tumors, recording a peak in 2007, when 86% of the cases required this method, and following with 80% in 2008 and 2009. The examined samples had been prelevated by fine needle aspiration (43%-86%) and surgical biopsy(14%-57%). The lowest percent was given by the histological exam, in 2009, used in just 2% of the cases.

The data regarding the lesional field met in the studied case work is exposed in the next tabels, in all 4 years of observation.

*Table 5*

The results of histological examination

<b>Year</b>	<b>No. cases</b>	<b>Collagenous nevus</b>	<b>Lipoma</b>	<b>CCH</b>	<b>Hemangioma</b>	<b>Other BMT</b>
<b>2007</b>	45	8/18%	23/50%	5/11%	7/16%	2/5%
<b>2008</b>	56	6/11%	24/42%	12/22%	11/19%	3/6%
<b>2009</b>	50	4/8%	20/40%	19/38%	5/10%	2/4%
<b>2010</b>	44	7/16%	16/36%	14/32%	5/11%	2/5%

CCH- canine cutaneous histiocytoma, BMT-benign mesenchymal tumors

The most frequent benign mesenchymal tumors in the study are the collagenic nevus or collagenic hemartoma, lipoma or the infiltrative lipoma, canine cutaneous histiocytoma and the hemangioma. Of all cases registered in the year 2007 the most often diagnosed lesions are the lipoma (50%), followed by the collagenic nevus (18%), hemangioma (16%), canine cutaneous histiocytoma (11%). In 2008 we notice a change regarding the incidence of different types of benign mesenchymal tumors, with most frequency being diagnosed the lipoma (42%), followed by the canine cutaneous histiocytoma (22%) and the hemangioma (19%) while the collagenic nevus was the least diagnosed (11%). Except these lesions it was mentioned a number of other benign mesenchymal tumors with a total

percentage of 6%, of which it is to be mentioned the cutaneous fibroma and the nodular fasciitis. Considering 2007 it can be noticed a significant rise of the cases of canine cutaneous histiocytoma, the percentage changing from 11 to 22, while the cases of lipoma reduced from 50 to 42%.

The lesion diagnosed as lipoma has the supremacy regarding the incidence, although we noticed changes of percentage in these four years: from 50% in 2007, to 42% in 2008, 40% in 2009 and 30% in 2010.

Interesting to mention is what happened with the canine cutaneous histiocytoma. If in 2007 it stood for an 11% of all cases, in 2008 the percentage almost doubled, being found in 22% of the dogs diagnosed with benign mesenchymal tumors. The following year it almost doubled again reaching in 2009 a total of 38% while in 2010 it was registered in 32%.

The comparative analysis between the lesional incidence and the age of the animals we found out a significant percentage of young aged dogs diagnosed with benign mesenchymal tumors in the period between 2009 and 2010. This is correlated with the high incidence of the canine cutaneous histiocytoma, which is known to appear in young animals. In our registry of cases the inferior limit was the age of one month, so it is perfectly explained the correlation between the lesion and the age.

The other types of lesions registered a less frequent incidence comparing the previous lesions. In the period between 2007 and 2010, the hemangioma registered limits between 10 and 19%, while the collagenic nevus had a constant and low incidence on the whole period.

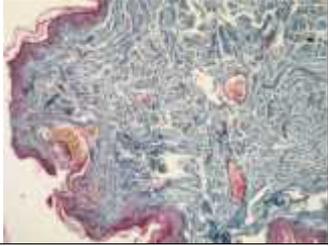
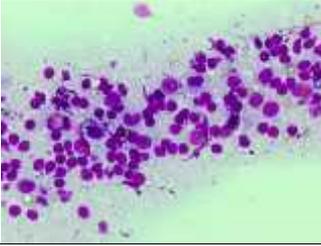
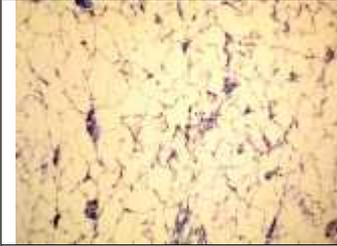
## CONCLUSIONS

3.1 Benign cutaneous mesenchymal tumors represent 21.8% of all diagnosed cutaneous lesions; in the summer of 2008 being recorded the highest amount of cases out of the whole period of time.

3.2 Most frequent localization: trunk and limbs

3.3 Most frequent method for obtaining the specimens was the fine needle aspiration, used in 80% of the cases.

3.4 Lipomas (42%), canine cutaneous histiocytomas (25%), collagenous nevus (14%), hemangiomas (15%) were the most frequently encountered tumors in this study.

		
Fig. 1. Lipoma. Gross morphology	Fig. 2. Collagenous nevus. Gross morphology	Fig. 3. Canine cutaneous histiocytoma. Gross morphology
		
Fig. 4. Lipoma. Cluster of adipocytes with peripheralized nuclei and nonstaining cytoplasm (MGGx100)	Fig. 5. Collagenous nevus. Stain trichromic Masson x40	Fig. 6. Canine cutaneous histiocytoma. Round cells with moderate anisocytosis. (MGGx100)
		
Fig. 7. Lipoma. Stain trichromic Masson x40	Fig. 8. Hemangioma cavernous type. Stain trichromic Masson x40	Fig. 9. Canine cutaneous histiocytoma. Stain trichromic Masson x40

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