CONTENTS

ANATOMICAL AND RADIOLOGICAL STUDY OF SOME CHARACTERISTICS OF THE PONY AND THE HORSE AUTOPODIUM

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Abstract

The investigations were performed on 10 specimens of each species horse and pony. Taken together, the anatomical region of the poney autopodium resembles that of the horse but there are some obvious features. Differences were found especially at the carpal and tarsal bones but also at the synovials of the antebrahio – carpal – metacarpal joint and less than the phalanges.

Key words: *horse, pony, carpal bones, phalanges.*

INTRODUCTION

In terms of the equine autopodial morphology, in the literature are very numerous data, but the data refer on the anatomy of this region in the pony (1,3) are more concise. Also, autopodial bones in this two species are photographed or schematized, but lake of the comparative radiological images (2,4). For this reason we conducted studies to complement existing data from two equine species.

MATERIALS AND METHODS

The studies were performed on 10 specimens of each species - horse and pony. The animals were designed for dissection and research activities in the Anatomy Laboratory of the Faculty of Veterinary Medicine, Bucharest. The autopodies were cleaned of organic debris and subjected of maceration process, after achieving of radiological images. The identification, description and homologation of formation were performed according to Nomina Anatomica Veterinaria -2005.

RESULTS AND DISSCUTION

Generally, the research has shown the significant morphological similarities between the bones of the bazipodial regions of the two species. There are still some specific elements.

So, the joint cavities of the first level of carpal bones for the radius are deeper at the horse than the pony. The joint surface of the pisiform for the radius tends to be flat, also. The ventral edge of the pisiform is placed about 0,5 cm from the level of medio – carpal joint, at pony in 7 of 10 cases. The ditch for the metacarpal bracket of the carpo – ulnar extensor appear to be shallow at pony in 50% of cases, while in horses is always well marked.

The scafo - ulnar interosseus channel has a rectilinear trajectory (Fig. 1) in horse. Because of the little guidance in medial sense of the caudal tubercle of the lunate bone, this channel is slightly medial deviated at the palmar end, in pony. (Fig. 2)

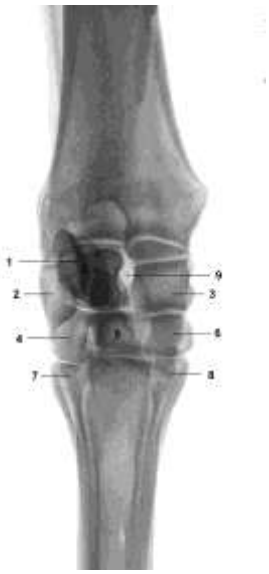

Fig. 1 The X – ray of carpal region, right member, dorsal aspect, horse
1 – the pisiform bone overlapped to lunate bone in X – ray image; 2 – pyramidal bone; 3 – lunate bone; 4 – unciform bone; 5 – capitat bone; 6 – trapezoid bone; 7 – proximal end of the rudimentary metacarpal IV; 8 – proximal end of the rudimentary metacarpal II.

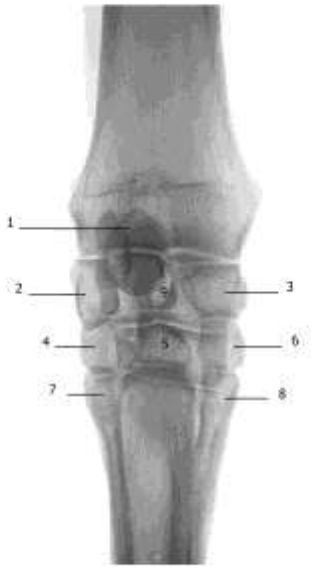

Fig. 2 The X – ray of carpal region, right member, dorsal aspect, pony
1 – the pisiform bone overlapped to lunate bone in X – ray image; 2 – pyramidal bone; 3 – lunate bone; 4 – unciform bone; 5 – capitat bone; 6 – trapezoid bone; 7 – proximal end of the rudimentary metacarpal IV; 8 – proximal end of the rudimentary metacarpal II.

Because of the flattening of the joint surfaces involved in the structure of medio - carpal and carpal – metacarpal joints, the joint capsules corresponding to these joints are less spacious in pony than the horse. This aspect is morphological reflected in the absence of the bottom side bag of the medio – carpal joint in the most cases in pony.

CONCLUSIONS

The bazipodium bones in pony are characterized by a greater degree of flattening than the horse.

The synovial bags of the medio – carpal joints and carpal – metacarpal joints are more spacious in horse than the pony.

The ratio between the extremity width of the main metacarp proximal end and the shaft width at half its is bigger at the horse than the pony.

The non – joint relief accident on palmar side of the carpal bones are out better in horse than the pony.

REFERENCES

Barone, R. – Anatomie compare des mamiferes domestiques, Tome I Ostheologie, Ed. Vigot, Paris, 1966.
Gheţie, V., Hillebrand, A. – Anatomia animalelor domestice, vol. I – Aparatul locomotor. Ed. Academiei R.S.R. Bucureşti, 1971.
Mişcalencu, D., Florica Mailat –Mişcalencu – Anatomia comparată a vertebratelor. Ed. Didactică şi pedagogică, Bucureşti, 1982.
Sisson, S., Grossman, J.D., Getty, R. – The Anatomy of the Domestic Animals, Vol I W.B. Saunders Comp., Philadelphia –London- Toronto, 1975.
***- *Nomina Anatomica Veterinaria* – 2005.

THE ANTEBRACHIAL BONE MORPHOLOGY AND PRONATION AND SUPINATION MOVEMENT POSSIBILITIES IN DOMESTIC MAMMALS AND HUMANS

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Abstract

In according to species, forearm bones have a different topography and development in conjunction with the use of the hand. When mobile they can rotate one around the other to perform pronation and supination movements. Otherwise, they are welded, radius being more developed than ulna. Study was to describe motfologiei articular surfaces in mammals, including humans, linking data obtained with pronation and supination possibilities of autopodium.

Keywords: forearm, radius, ulna, pronation, supination.

INTRODUCTION

Limb morphology in mammals is the result of a long evolution of vertebrates. In a phylogenetic way we can highlight three stages in the progress of terrestrial locomotion: a) transforming the fins in rudimentary, horizontal limbs, perpendicular to the body axis, b) the emergence of zeugo and stilozeugopodial-autopodial angles c) lifting the trunk at ground level, with a tendency to verticality of the limbs (5). because of the lack of works related to limb skeletal development (1,2,3,4,6,7,9) and to aspects of the morphology adaptation of this skeleton, correlated with a certain specialization of limbs (8,10,11), in literature there are few data on the detailed morphology of the anatomical basis of forearm,the begining of the pronation and supination movements of autopodium, which is why I addressed this topic.

MATERIALS AND METHODS

Research has been conducted on parts from Anatomy Laboratory of Domestic Animals Faculty of Veterinary Medicine Bucharest. Actual study was done on 20 horse zeugopodiums, 20 from large ruminants, 30 ovines and swines

zeugopodiums, 30 carnivore zeugopodiums (15 cats and 15 dogs) and 20 from rabbits. The studied bones were from animals of different ages. Also, in the discipline collection were studied also 4 zeugopodiums from human skeletons. Components of articular surface on the proximal and distal end(length, width, depth, etc.) were studied as well as relations between the bodies of the two bones. The most suggestive aspects were photographed.
Identification, description and formations approval was made in accordance with Nomina Anatomica Veterinaria — 2005

RESULTS AND DISSUTION

At horse, the forearm bones are welded, radius encompassing the distal third of ulna,, which is represented at this level, only by a epiphyseal core of ossification. From the antebrachial interosseous space only radioulnar proximal arch remains, and it's elongated in a proximo-distal direction (Fig. 1-9).
Radius presents an articular surface for the ulna, represented by the two diartrodiale relatively planiforme sides, located on the proximo-caudal extremity (Fig. 2-5). Between them and the distal there is a rough surface used for ligament insertion.
In ulna's case, diartrodiale surfaces belonging to radius are supported by lateral and medial coronoid processes. Cranial front of ulna's body is welded to radius, the surface of synostosis resembling a triangle, whose peak, distal oriented, reaches the boundary between the middle and distal third of the diaphysis.
At large ruminants, the forearm bones are shorter and thicker than a horse's. Ulna, welded to the radius, is reduced, but individualized to its distal end (Fig. 3). Antebrachial interosseous space is represented by two radio-ulnar arcades, one proximal, wider, and one distal, which reduced (Fig. 3 to 8.9). Caudal front of radius body presents on its full length a synostosis surface with the ulna, interrupted only at the two radio-ulnar arcade.
Radius has a articular surface for the ulna, represented by two planiforme surface, a lateral one and another medial with dimensions of 1.8 / 1,4 cm, respectively 1,6 / 1,2 cm (wider than at the horse) (Fig. 4 - 3).
Diartrodiale articular surfaces of the ulna are congruent with those of the radius, being slighly widened.

Fig. 1 Horse radius and ulna (lateral surface of the right member)
R-radius; U-ulna; 1 - lateral tuberosity; 2 - bicipitală tuberosity;3- ulnar styloid apophysis;4-olecranon; 5-olecranon beak; 6-semilunar notch; 7 - tendon slide; 8 – ulna body; 9 radio-ulnar proximal arch.

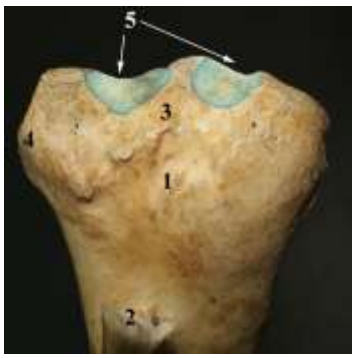

Fig. 2 Caudal face aspect of proximal extremity of horse radius (left member)
1-head of the radius; 2-detachment place of the olecranon;3 -area of ligament insertion; 4-lateral tuberosity; 5 – diartrodial surfaces for ulna.

Fig. 3 Cow radius and ulna (left member side)
R-radius, U-ulna; 1-lateral tuberosity; 2-distal extremity; 3-olecranon; 4-olecranon beak;5-semilunar notch; 6-ulna body; 7-ulnar styloid apophysis; 8-proximal radioulnar arch; 9- distal radioulnar arch.

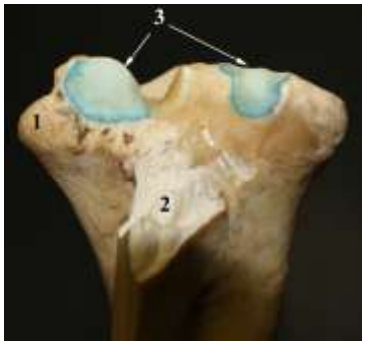

Fig. 4 Caudal face of the proximal extremity of cow radius (the left forelimb)
1-lateral tuberosity; 2-detachment place of the olecranon; 3- diartrodial surfaces for ulna

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Fig. 5 Radius and ulna in pig
(right member, lateral side)
R-radius; U-ulna; 1-radius head 2-distal extremity; 3-olecranon; 4-olecranon beak; 5-semilunar notch; 6-ulnar styloid apophysis; 7-proximal radioulnar arch

Fig. 6 Radius and ulna in rabbit (left member, lateral side) R-radius; U-ulna; 1-semilunar notch; 2-olecranon; 3-ulnar styloid apophysis

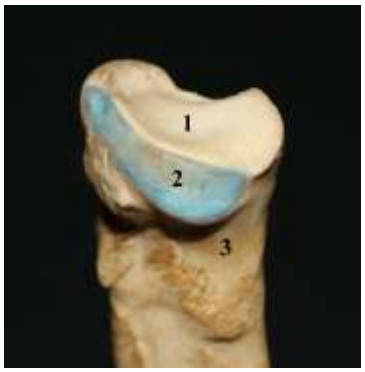

Fig. 7 Proximal extremity in dog radius
(the left member,
caudal view) 1 - proximal articular surface; 2 - joint circumference; 3 –the neck of radius

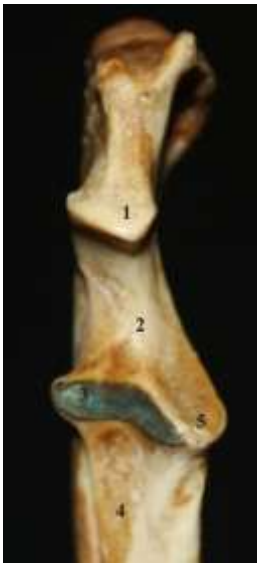

Fig. 8 Proximal end of dog's ulna
(the left member, cranial view)
1 - olecranon beak; 2 - semilunar notch; 3 - radial notch, 4 - interosseous line; 5 - medial coronoid process.

At ovines, bones are thinner and longer and proportionally higher than the large ruminants.
At pig the two bones are rude, short, joined together but seamless(Fig. 5). The link between them is achieved by short fibers, corresponding interosseous membrane from carnivores (fibrous joints). However, there aren't possible any movements between them. Proximal radio-ulnar arch is very narrow, the distal rarely being present in the form of small cracks.
At rabbits, forearm bones, strongly recurved, are fixed between them, throughout their length's contact, which contributes to strengthening the region (Fig. 6). As in pigs, the bond between the two bones's body is realised through a short fibrous interosseous ligament. Planiforme diartrodial articular surfaces, are placed on the perimeter of caudal circumference.
The dog's forearm bones are independent, mobile and crossed with each other. Because of the articulation only between the extremities,at dogs, they delimit an interosseous space (Spatium interosseum antebrachii). The interosseous space is completed by an interosseous membrane, whose proximal portion is very thick and strong. The supination movement is done with an amplitude of about 20 °.
The proximal radius articular surface is represented by a joint circumference (Circumferentia articularis) located on the perimeter of the caudal extremity (Fig. 7). Distal, to the side, concave and elongated proximo-distal direction, for the ulna.
Proximal ulna articular surface for the proximal extremity of the radius is represented by a radial notch (Incisura radialis), supported mostly on medial coronoid process (Fig. 8). Distal articular surface (Circumferentia articularis)for the radius is elongated and slightly convex.
At cat, the forearm interosseous space is wider than the dog's. The ulna joint circumference is larger, located caudomedial. Supination movement is done with an amplitude of about 80 °. At carnivores, the supination movements are favored by the articulation mode of the proximal radius extremity with the humerus. Thus, at these species, the proximal articular surface of the radius only gets to joint humeral condyle, tending to become a spheroidal joint.
At humans, pronation and supination movements are very ample. Unlike other mammals, they do not occur only in the two radio-ulnar joints (Fig. 9). but are accompanied by limb movements in the scapular-humeral articulation.

Judging on the appearance of the articular surfaces, we find features of radio-ulnar joint morphology of the carnivores, animals that can perform supination movement, compared to ungulates and leporidss, at which the forearm bones are attached in the pronation position.

Supination movement a of the thorax autopodium has the center in the forearm region, due to radius rotation around a longitudinal axis and at the same time, oblique to this mobile element, while the ulna remains fixed. The rotation axis passes through the center of the proximal radius extremity and its neck after leaving the bone, so that the distal part of the forearm can pass through the center of the ulna's distal extremity. As in humans, proximal radio-ulnar articulation of this group of animals has the structure of a trochoide joint or swivel joint. Radius joint circumference is maintained in contact with the radial ulna notch, due to annular ligament.

Fig. 9 Radius and ulna (human left member)
R- radius; U-ulna; 1 - joint circumference; 2 - radial tuberosity; 3 – anterior edge;
4 - styloid process; 5 - interosseous edge; 6 - olecranon; 7- ulna trochlear notch; 8 - ulna tuberosity; 9 - ulna interosseous edge; 10 - styloid process.

It surrounds the radius proximal end and inserts ulna with its head in lateral and medial sides of radial notch. Radius proximal extremity, caught in this osteo-ligamentous ring, is able to rotate within the limits permitted by joint surfaces and other configurations of interosseous ligament.

At ungulates and leporids, proximal radio-ulnar joint is an articulation, which judging after joint surface shape can be classified as plane. The articular surfaces, more or less flat are located two by two on the caudal extremity of the radius's circumference, respectively, on the ulna coronoid

process. In these animals, the mobility within the synovial joints is very low, with only tiny sliding movements.
Radius's and ulna's bodies are joined together by the forearm interosseous membrane, which fills the forearm antebrachial interosseous space, forming a syndesmosis. The membrane has a maximum development in carnivores. At horse the fibrous tissue counterpart membrane ossifies earlier and it forms a synostosis, forearm bones being fixed in a pronated position. Also at ruminants occurs interposed fibrous tissue ossification between two bones, but this process is extended for a longer period of time.
Pronation and supination movements are dependent not only of the fusion degree of the two bones, but also of the shape and height of the olecranon's articular head. Compared to species in which the articular head is flattened (horse, ruminant, pig and rabbit) at carnivores (especially cats) it's possible the supination movement. Its magnitude is even greater as the olecranon is shorter. The ratio between the olecranon's and radius's average length for the studied parts is presented in Table 1.

Table 1. The ratio between the radius and olecranon length at some domestic mammals and humans

Species	Olecranon lenght (cm)	Radius lenght (cm)	Ratio Lo/Lr	Observations
Horse	**10**	**37**	**1 : 3,7**	**Ongulate average 1 : 3,38**
Cow	**12**	**33**	**1 : 2,75**	
Sheep	**4,5**	**17**	**1 : 3,7**	
Pig	**7**	**14**	**1 : 2**	
Dog	**4**	**20**	**1 : 5**	**Carnivores 1 : 5,5**
Cat	**1,5**	**9**	**1 : 6**	
Human	**3**	**22**	**1 : 7,3**	**Human 1 : 7,3**

CONCLUSIONS

The forearm represents the center of pronated and supination movements, responsible for autopodium's mobility;
The amplitude of pronation and supination movements is directly proportional to the degree of specialization of the thoracic limb during evolution;
The highest degree of these possibilities is reached by human hands, which is able to transit from pronation to supination by a rotation of 180 °;

From the domestic mammals,the cat can supinate thorax autopodium but the rotation cannot exceed 80 ° . The possibility of executing this movement is closely linked with existing climbing ability in this species;
At dogs, autopodium's mobility is significantly reduced, while forearm bones remain independent;
At other mammals, due to autopodium's unilateral specialization, the thoracic member function is only to support in standing and locomotion, forearm bones being fixed in pronation position . Therefore, on these, the radio-ulnar proximal joint is characterized by lack of annular ligament and consolidation of collateral ligaments. In addition, it is found that pronation and supination movements are dependent not only on the degree of fusion of the two bones but also on the olecranon's articular head shape and olecranon's heigh.

REFERENCES

Antohe, D. Șt., Varlam, H. – Sistemul locomotor, Scheletul, ed. Junimea, Iași 2004.
Christ, B., Brand-Saberi, B. – Limb muscle development, Int. J. Dev. Biol. 46: 905-914, 2002.
Gambarian, P.P. - Evolution of tetrapod locomotion, Zh. Obshch Biol. 2002 sep.-oct; 63 (5): 426-45.
Ganță, C., Pentea, M., Pop, C., Moț Maria, Jivcov, S. – Osteologie veterinară, ed. Mirton, Timișoara, 2008
Gheție, V. – Anatomia animalelor domestice, vol. I, Aparatul locomotor, Editura Academiei Republicii Socialiste România, 1971.
Hall, K.B. – Fins into Limbs: Evolution, Development and Transformation, The University of Chicago Press, 2007.
Predoi, G.- Anatomia topografică a animalelor domestice, ed. Ceres, București, 2012.
Ruff, C.B., Runestad, J.A. – Primate Limb Bone Structural Adaptations, annu. Rev. Anthropol., 21: 407-33, 1999.
Tickle, Ch, Eichele, G. – Vertebrate Limb Development, annual Review of Cell Biology, 10: 121-52, 1994.
Williams, S.B., Wilson, A.M., Payne, R.C. – Functional specialisation of the thoracic limb of the hare (Lepus europeus), J. Anat. 210 (4) 491-505, 2007.
Young, N., Hallgrimsson, B. – Serial Homology and the Evolution of Mammalian Limb Covariation Structure, Evolution, 59(12), pp. 2691-2704, 2005.
*** - Nomina Anatomica Veterinaria, Published by the Editorial Committee Hannover,Columbia, Gent, Sapporo, 2005.

ANATOMOHISTOLOGICAL STUDY REGARDING THE OVARY AND OVIDUCT IN DIFFERENT AGE GROUPS IN THE CHICKEN (*GALLUS DOMESTICUS*)

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Abstract

Introduction: *The importance of the study of the chicken's genital apparatus coincides from an economical point of view with the acquirement of one of the main avian products for which this bird is selected: the egg. In birds only the left ovary and oviduct are developed. The mature ovary has a grape-like shape, suspended form the ceiling of the abdominal cavity, immediately under the cranial extremity of the kidneys. The oviduct is long and flexuous, leading from the ovary to the cloacae.*
Material and Methods: *The research was carried out on the ovaries and oviducts harvested from 15 chickens of various ages (56 to 126 days) that were dissected using common techniques in the Comparative Anatomy Laboratory of the FVM Cluj-Napoca. The samples were also histologically prepared and examined using regular optical microscopy.*
Results and Conclusion: *Following the examinations, the have noticed that in the first group age, the ovary has a smooth surface, at 98 days, its surface becomes uneven, while at 126 days, it completes its development, acquiring its classical grape-like shape. The 56 days and 98 days old oviduct cannot be divided into segments; only the 126 days old oviducts displays macro and microscopic segmentation. In conclusion, results indicate that the ovary and oviduct of chickens reach maturity at the ages of 126 days, when the birds become ready to produce eggs.*

Key words: *chicken, reproduction, ovary, oviduct, age*

INTRODUCTION

Biological features of birds, such as reproductive features, lend a great economical importance, mirrored by the ever growing weight of aviculture as a branch of animal breeding. The importance of the study of the female bird reproductive organs merges, from an economic point of view, with obtaining the most important product from poultry selected in this direction - the EGG. The latter, asides its reproductive importance, has a high nutritional value, being the only complete food besides milk (7). By its

reproductive value, the egg contributes to the procurement of the other main poultry product – MEAT. Poultry meat is obtained from breeds specialized in this direction, turkey, duck, geese, pearl hen and pigeon breeds.
In order to achieve these goals, to increase egg laying populations (which leads to egg and poultry meat production growth), an important emphasis lays on the knowledge of bird reproductive organs' morphology.The study of ovaries development in poultry is a necessary base for researching the increase of egg production and the intensification of their exploitation.
With regards to the existent literature, studies in the last years have paid little attention to the structure of the ovary and oviduct of the *Gallus Domesticus* species.
This study represents a systematic and detailed analysis of these aspects through the usual anatomical and histological investigative methods. **The ovary** has the shape of a cluster of grapes, suspended from the ceiling of the abdominal cavity, on the ventral face of the cranial lobe of the kidney, and it is composed of ovisacs in various stages of development. The main function of the ovary is to produce ovules. The ovary also plays a role in the secretory activity, producing proestogens and estrogen hormones. The ovaries are suspended in the abdominal cavity by means of the peritoneal serosa, forming meso-folds (prolongations of the broad ligament of the uterus). The ovary is sheltered and anchored by the ovarian bursa, which is heavily innervated and vascularized.
The oviduct – only the left one is present and it stretches from the ovary to the cloacae. During the egg laying season it cat reach 60-70 cm in length. It is suspended in the abdominal cavity by a strong dorsal meso-fold. It has five distinct segments, each of them with a specific role in egg production. The cranio-caudal order of succession for these segments is: *the pavilion (Infundibulum)*; *the glandular segment (Magnum)*; *the middle segment (Isthmus); the terminal segment (Uterus)* and *the vagina.* The functions of the oviduct are: sperm depositing and spermatozoid transportation, fecundation, forming of the egg's components, transportation of the egg during the shaping process, oviposition.

MATERIAL AND METHODS

The study was performed on ovaries and oviducts sampled from 15 chickens (*Gallus domesticus*), Leghorn breed, of various ages. We have studied the evolution of the ovary and oviduct, in dynamic until the beginning of

oviposition. The usual euthanasia protocols were used, in the Comparative Anatomy Laboratory of the Faculty of Veterinary Medicine of Cluj-Napoca. The isolation of the reproductive organs requires a rapid opening of the abdominal cavity. We have sampled, as soon as possible after the euthanasia, pieces from the ovary and the oviduct, initially extracted intact to view their macroscopic aspect, then histologically examined. The samples extracted for histological examination were immediately immersed in neutral buffered formalin and Boiun liquid.

RESULTS AND DISCUSSION

The ovary at 56 days

Initially, during the embryonic stage, the ovary develops symmetrically, but soon, the right ovary and oviduct suffer an involution process, so than at the time of the hatching, they present involution processes.

Anatomy: The mass of the ovary weights approximately 0,40g. The ovary presents a solid mass, with a granulated aspect, containing ovary follicles that are not visible to the naked eye (Fig 1). At this age, the ovary is vascularized, as it is noticeable at the tip of the arrows in the image below (Fig 2).

Histology: the surface of the ovary is uneven, with visible prominences separated by grooves, sometimes branched. Secondary hollows begin to appear. A small part of the vascular area remains at the level of the ovarian pedicle. The ration is 2 to 1 (fig. 4).

In the cortex of the ovary there are numerous primary follicles and scarce secondary follicles. The demarcation between the cortex and the medulla is poorly drawn. Visible on the left of the figure 3, there is the ovary with its ovisacs, and on the right, the pavilion of the oviduct.

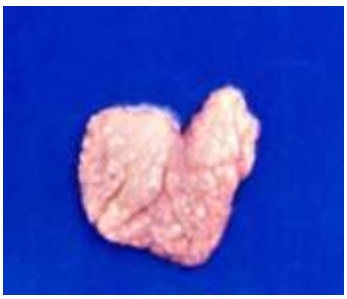


Fig. 1 – The ovary at 56 days

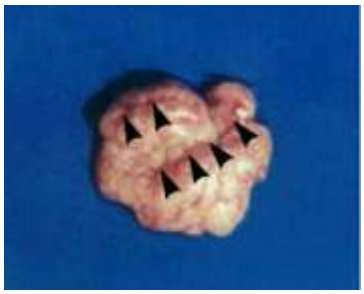


Fig.2 – The ovary at 56 days

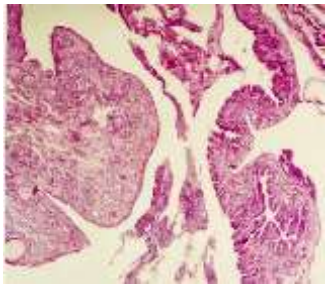


Fig.3. The ovary (left) and the pavilion of the oviduct

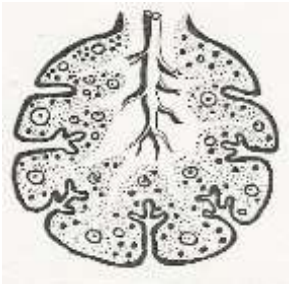


Fig. 4 (right) - 56 days, HE, ob.4x

The ovary at 98 days

Anatomy: The mass of the ovary weights approximately 0,46g. It has an irregular shape, a pale pink color, and is situated on the roof of the abdominal cavity, attached through a serous meso-fold, cranially de anterior lobe of the corresponding kidney. The ovisacs have a whitish color and the size of a grain of pepper. Fig. 6

Histology: The unevenness of the ovarian surface continues to amplify. The prominences gain in height, while the grooves separating them become deeper. Secondary prominences attach themselves to the main ones. They are smaller than the primary ones. At this age, the ovary's structures are defined. The ration between the two areas is approximately 6 to 1(fig.5).

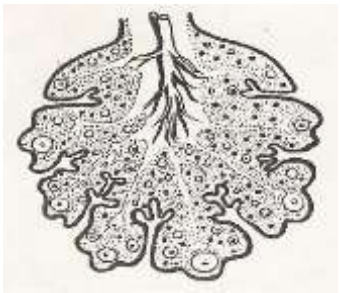


Fig. 5 – Diagram - the ovary 98 days

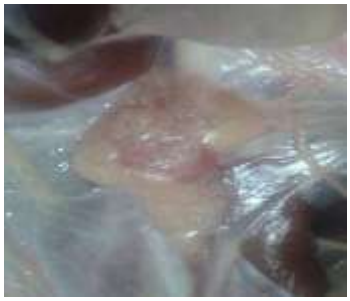


Fig. 6 The ovary anatomy at 98 days

On the histological section, one can notice and ovisacs with primary ovarian follicles and developing ones. The vascularized area presents a lax conjunctive tissue and blood vessels. There is also a nervous ganglion in the ovarian pedicle (fig.7).

The medulla presents vegetative neurons, blood vessels and lax conjunctive tissue, and the cortex is filled with ovarian follicles in different stages of evolution (fig.8)

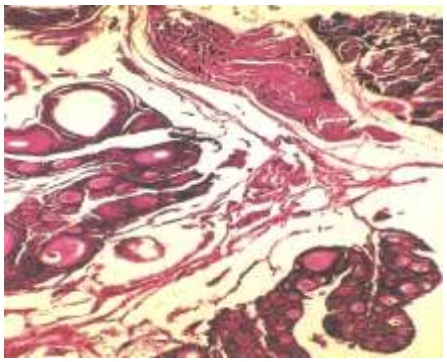


Fig.7. The ovary – 98 days, HE, ob.4x

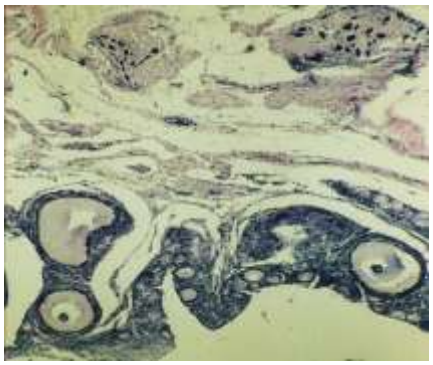


Fig.8. The ovary – 98 days, HEA, ob.4x

The ovary at 126 days

Anatomy: The ovary maintains its topography until this age, before the evolution of the first gestation. It has a round shape of different sizes, and each ovisac contains a future yolk and the oocyte of the future egg, in different development stages. The ovisacs have a yellow to orange color and are attached to the base of the ovary through a serous pedicle. At the age of 126 days it is completely developed (Fig. 9, 10).

Fig. 9 The ovary at 126 days

Fig. 10 The ovary at 126 days

Histology: The prominences continue to grow in height and width, while the indentations on the surface of the ovary become deeper, and the smaller prominences are better separate (Fig. 11).

A follicle is almost ready for ovulation. In birds there is no follicular antrum. Inside an ovisac, the mature follicles occupy the deep area, white the primary and primordial follicles are situated superficially (fig. 11, 12) .

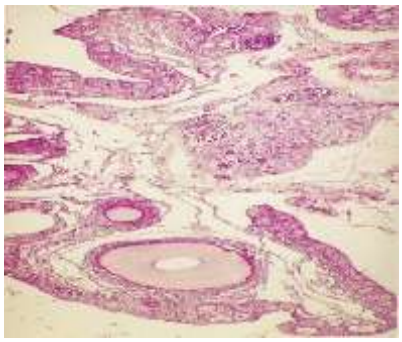


Fig.11. The ovary – 126 days,

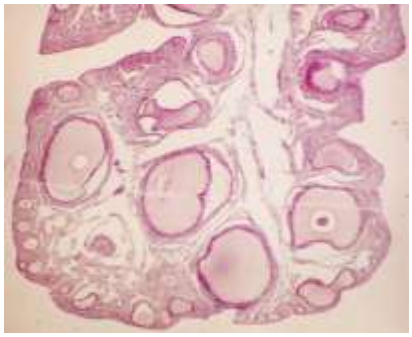


Fig.12. The ovary – 126 days,

The oviduct

The examination of the oviduct has been made at the age of 56, 98 and 126 days after the hatching.

At the age of 56 days

Histology: there are no distinctive segments of the oviduct yet. Thus we have taken 3 samples: from the cranial, the middle and the caudal third of it. Each segment sample has a wall structure represented by: mucosa, musculosa and serosa. In the cranial segment, the mucosa (fig.13) presents large thick folds. The axis of the fold is formed only of conjunctive tissue. The middle segment (fig.14) has shot folds, sometimes branched ones. In the caudal segment, the lymphoid cells of the oviduct's chorion. In the caudal segment, lymphoid cells from the oviduct's mucosa chorion appear much more numerous (fig.15).

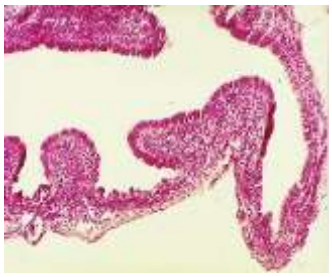


Fig.13 the oviduct- cranial segment

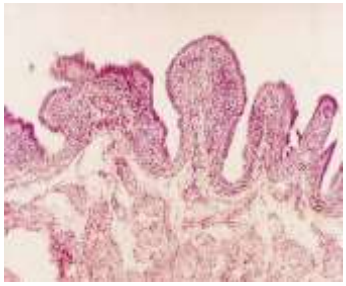


Fig.14. the oviduct - middle segment

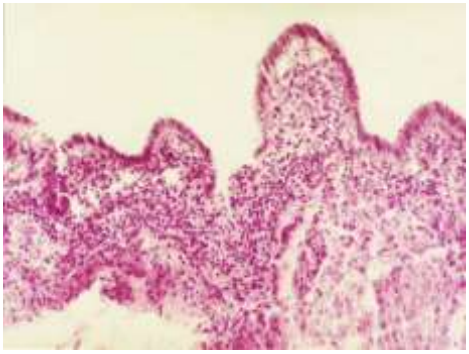



Fig.15 the oviduct – caudal segment

Anatomy: the oviduct at this age is not developed, has the shape of thin and flexuous tubes. It stretches from the ovary until the cloacae, where it opens. It is suspended from the abdominal cavity's wall by two meso-folds. The oviduct-cloacae orifice is closed by a hymen.

The oviduct at the age of 98 days

Anatomy: At this age the segments of the oviduct are still not distinctive macroscopically. The oviduct grows a lot in length and girth. The Segments of the oviduct begin to differentiate (Fig 17).

Histology: In the cranial and middle segments, the folds of the mucosa have a relatively even and unbranched aspect (Fig. 17 and 18). The folds of the mucosa begin to branch. The oviduct musculosa is well defined (Fig.19)

Fig 16- the oviduct at 98 days

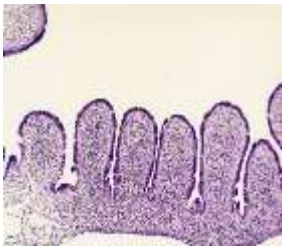


Fig.17. cranial oviduct

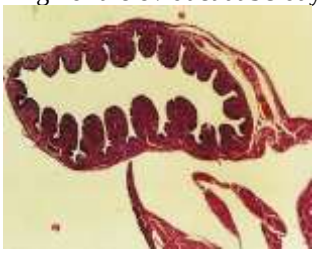


Fig.18. middle oviduct

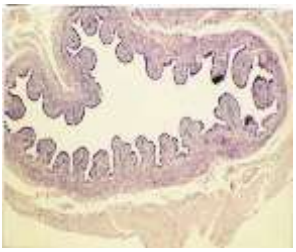


Fig.19. caudal oviduct

The oviduct at the age of 126 days

Anatomy: The five segments of the oviduct are fully developed at this age. The two meso-folds that suspend the oviduct to the abdominal walls are also present and have a length of approximately 60-70 mm.

Fig 20 – the oviduct at 126 days

Fig.21 the oviduct completely formed

Histology: The five segments of the oviduct present the complete development off all of the wall's structures. The mucosa of the pavilion appears extremely creased, presenting richly branched folds. Smooth muscular fibers begin to organize in two layers (fig.22). The magnum or albuminogen chamber occupies a large part of the oviducts length. The walls of the magnum are thicker, due to the growth of the mucosa. The mucosa is folded, presenting primary folds. The musculosa of the magnum is well represented. The folds of the magnum mucosa are thicker and more scarce (fig. 23). In Fig 24 - isthmus, the oviduct's diameter decreases, and through the isthmus' glands the egg shell is produced. Fig.25 - the uterus or the shell chamber presents itself as a dilated segment of the oviduct in which the calcareous shell is produced. Calcium is transported through the blood, which explains the presence of a rich vascular plexus in the wall of the uterus. Fig. 26- In the vagina, the mucosa is strongly folded, well developed.

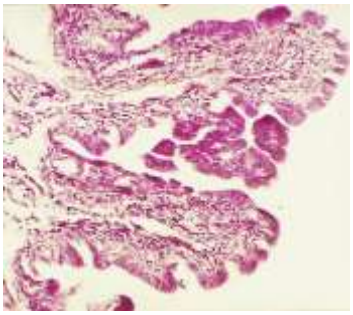


Fig.22 oviduct: the pavilion, 126 days, H

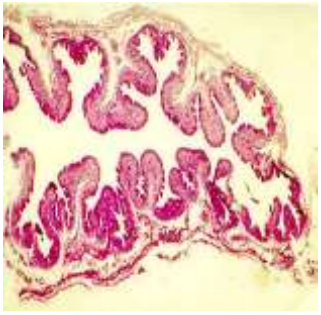


Fig.23 oviduct: the magnum, 126 days, HE,

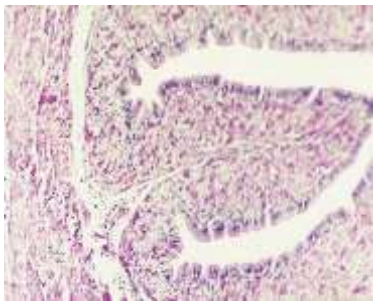


Fig.24 oviduct: isthmus, 126 days, HE, 10x

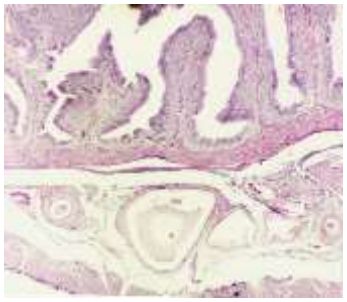
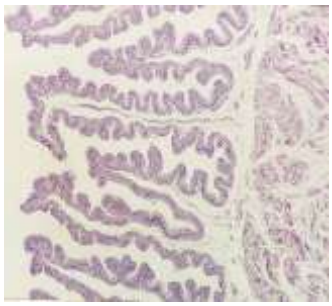


Fig. 25 oviduct: uterus, 126 days, HE, 10x



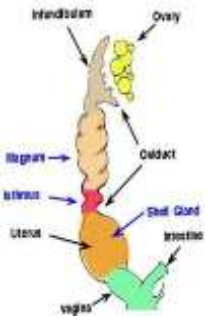


Fig. 26 the oviduct - vagina , 126 days, HE, Ob 10x

CONCLUSIONS

A) histology: Following the histological examination of the ovaries and oviducts sampled from chickens of different age groups, we have assessed that:

In the first days after hatching, the ovary has a smooth surface; the parenchyma and the vascular area are not distinctively delimited.

At 56 days from hatching, the surface of the ovary is uneven, lined by primary and secondary grooves, and the ration between the areas is 3to 1.

At 98 days from hatching on the surface of the ovary there are marked lines, with visible primary and secondary grooves, the ovarian follicles are numerous, the ration is inversed at 5 to 1;

At 126 days from hatching, the ovary is completely formed. It has a grape cluster shape with follicles in different stages of development. The interstitial gland is prominent, and the ration between the areas is 6 to 1 (parenchyma/vascular area).

The oviduct at 56 and 98 days is an even tube, without distinctive functional segments.

The oviduct slowly gains the functional structure of the mucosa, so that only after 100 days from hatching its segments can be differentiated.
The oviduct at 126 days presents the macroscopic and microscopic features of its segments (infundibulum, magnum, isthmus, uterus, and vagina).
B) anatomy Following the examination of the ovaries we have noticed:
At 56 days, the ovary presents a solid mass, with a granulated aspect, with no noticeable blood vessels.
At 98 days, the weight of the ovary slightly increases by comparison to the 56 days stage, and the development of the other elements is insignificant.
The ovary at the age of 126 days reaches sexual maturity, when it is ready for egg laying, its vascularization and innervation become visible, and so do the ovarian follicles.
The oviduct at the age of 56 days, and 98 days respectively, has the shape of a homogenous tube, without functional segments.
Only at the age of 126 days, the segments of the oviduct can be characterized macroscopically as: the infundibulum, the magnum, the white isthmus, the uterus and the vagina.

REFERENCES

Artan E., Daglioglu S. – Relantionship between ovar and oviduct in the domestiv hen. – Istambul Univ. Vet. Fat. Derg. 1984, 10.1.17-28.
Aurel DAMIAN, Ioan POPOVICI, Ioana CHIRILEAN – Tratat de Anatomie Comparată, Editura Academicpres Cluj-Napoca 2003
Bunaciu P. Maria Bunaciu, Dojană N. – Reproducţia păsărilor, Ed. Printech, Bucureşti, 2009
Cornilă N., Manolescu N. Structura şi ultrastructura organelor la animalele domestice, Ed. Creres , Bucureşti, 1995
Federova, N. N. (1987): Growth and development of the reproductive system in White Leghorn hens in different lighting systems. Sbornik Nauchnych Trudov. Moskovaskya Veterinarnya Akademiya;
Gheţie V., Chiţescu St., Coţofan V.,Hillebrabd A. – Atlas de Anatomia de las Aves domesticas, Editorial Acribia, Zaragoza, Espana, 1981, 1985.
http://www.hiroshima-u.ac.jp/en/gsbs/research/kachikuseitai/
Kar, A. B. (1947): Studies on the ligaments of the oviduct in the domestic fowl. Anat. Rec.

HAEMATOLOGICAL RESEARCH ON PIGS AFTER USING SOME NONSPECIFIC IMMUNOMODULATORS

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Abstract

Batches of piglets reared in intensive system were nonspecific immunomodulated During the experiment, three blood samples, necessary for haematological determinations,have been performed.
In group A, it was administrated a bacterial suspension (Corynebacterium parvum). Group B received Levamisol product (for veterinary use) and group C received vitamin E and Selenium, using Romselevit. Group D was used as a witness group, being submissed to vaccination only. Hemoglobin concentration in group A (modulated with Corynebacterium parvum), significantly increased after the second harvest compared to harvest I. The final collection showed a significant decrease in these concentrations, all distinctly significant compared to harvest II. In groups B, C, and D, hemoglobin concentrations showed an increase in statistical terms ,only at an intermediate collection (highly significant), then remained constant.In group A, modulated with Corynebacterium parvum, hematocrit increased significantly distinct from harvest I. The final collection showed a decrease in these levels, manifested statistically significant from the second harvest.In groups B and D, hematocrit increased statistically at the intermediate harvest (significant) The final harvest was similar to the intermediate concentrations. In group C there were changes in the sense that after a distinctly significant increase in hematocrit values ,it decreased at the intermediate harvest without any interest in statistical terms. The number of red blood cells showed a distinctly significant increase in group A,at the second collection compared to the first one ,the final harvest decrease being statistically significant.

Key words: *hemoglobin, immunomodulation, piglets.*

INTRODUCTION

The resistance capacity of the animal body can be increased through the use of imunomodulators (Lee, et al, 2000; Xie and Song, 2000), some of which can act specifically inducing different effects (destruction of pathogen germs or the blocking of their activity), in this category falling the vaccines, immune sera, and even antibiotics (Mikulska-Skupien et al. 2004).

The intensity of the immune response can be increase also nonspecifically (Beuth et al. 2002; Kim and Hyun, 2000) for a certain type of agressor, by the use of a various range of cellular structures, organic or anorganic substances.
The hematomogical reaction of the body after the potentiation with nonspecific substances was observed, over the immune response induced by the the classical swine fever and swine erysipelas vaccination (specific immunomodulation), in piglets reared in intensive system.

MATERIALS AND METHODS

32 piglets were submitted to testing, in 4 groups (A,B,C,D), starting with age of 52 days old, testing being conducted in an intensive piggery.
In group A was administered a bacterial suspension (Corynebacterium parvum) in saline solution (2 mg bacterial body dry residue/ml) through the use of Imunostimulent S.R.E. Corynebacterium parvum, subcutaneous administration.
Group B received Levamisol (for veterinary use), administered subcutaneous, and lot C received vitamin E and selenium, using the product Romselevit, also administered subcutaneous.
Group D was used as a witness group, being submitted only to vaccination.
The vaccination against Classical Swine Fever and Swine Erysipelas was accomplished with a suspension of attenuated Classical Swine Fever virus with the minimal titre of 1000 DICF 50/ml and culture of Erysipelothrix rhusiopathiae (VR_2 strain) with minimal germ concentration of $5x10^7$ UFC/ml, at 60 and 120 days old.
The animals had normal feeding and microclimate the entire experiment.
The experiment was conducted on a period of 85 days, during which there were three blood sampling.

Quantified parameters
- hemoglobinemia;
- hematocrit
- red blood cell count (absolute values)

The hematological determinations were conducted through the electronical method with a Coulter-Counter CBC-5 analyzer.

RESULTS AND DISCUSSION

The Hemoglobin concentration (table 1, graphic 1) in group A (modulated by *Corynebacterium parvum*), increased significantly distinct ($p<0,01$) in the second blood sampling (13,14±1,89 g /dl), opposite to the first blood sampling (10,61±0,81 g/dl), and in the final blood draw decreasing significantly distinct ($p< 0,01$), from 13,14±1,89 g /dl (2^{nd} sampling) to 10,27±2,26 g /dl at 3^{rd} sampling.

In groups B, C and D, Hemoglobin concentrations were increased with statisctical importance only in the 2nd sampling (significant high p<0,001) and after that maintained constant.

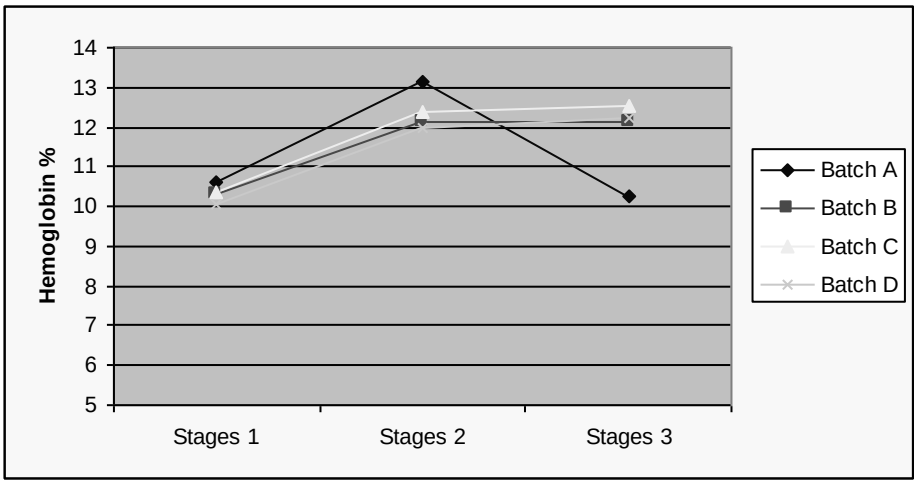
Table 1 - Hemoglobinemia (g /dl)*

Group	Stages		
	I	II	III
A	10,61±0,81	13,14±1,89**	10,27±2,26**
B	10,33±0,7	12,11±0,81***	12,13±1,14
C	10,37±1,06	12,4±0,81***	12,54±0,32
D	10,04±1,11	12,0±0,87***	12,24±1,23

** = significantly distinct difference;
*** = significantly high difference.

Regarding the hematocrit, the statistic result were :
-in group A, modulated by *Corynebacterium parvum*, the increase was significantly distinct (p<0,01) at the second sampling (39,07±5,74%), compared to the first sampling (32,83±2,28%). At the final draw, there was a statistically significant decrease (p< 0,05), from 39,07±5,74 % at the second blood draw to 31,05±6,58 % at the 3rd blood sampling.

Graphic 1 – Representation of the hemoglobin concentration



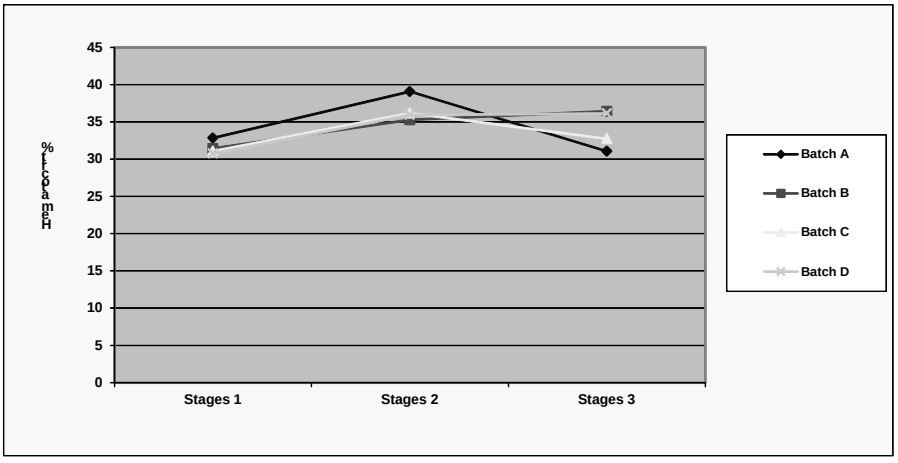
- in groups B and D, the hematocrit increased statistically in the intermediate blood draw (*significantly*–p<0,01), at the final sampling the concentrations were similar to thoseintermediate (table 2, graphic 2);
- in group C, after a significantly distinct increase (P<0,01) in the 2nd sampling (36,17±2,46%), the values dropped without interest from a statistical viewpoint (32,73±12,09 %).

Table 2 - Hematocrit (%)

Group	Stages		
	I	II	III
A	32,83±2,28	39,07±5,74**	31,05±6,58*
B	31,43±2,04	35,27±2,69*	36,43±3,26
C	31,07±2,83	36,17±2,46**	32,73±12,09
D	30,74±3,16	35,93±2,71*	36,13±3,59

* = significant difference;
** = significantly distinct difference.

Graphic 2 – Representation of the hematocrit



The red blood cells count had the following changes :
- in group A there was a significantly distinct increase (p< 0,01), at the second sampling (7,47±1,04%), opposite to the first sampling (36,23±0,4%), and in the final one, the increase was statistically significant (p< 0,05), (table 3, graphic 3);

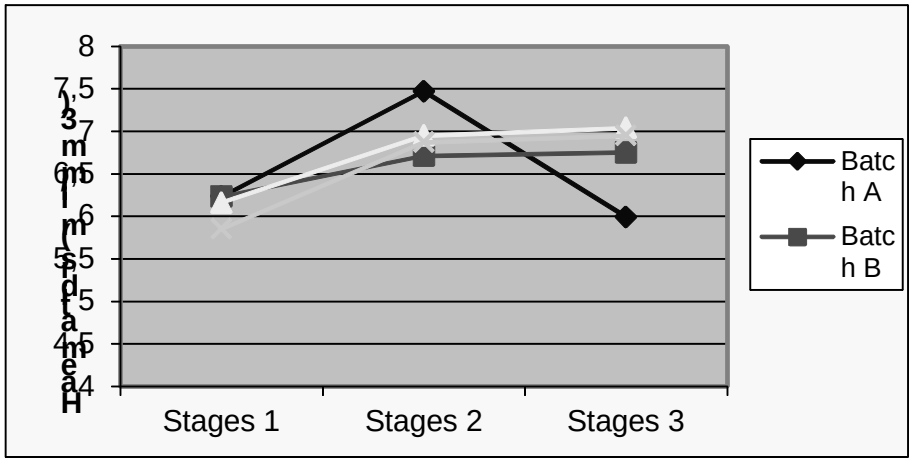
Table 3 - Haematids count in absolute values (mil/mm^3)

Group	Stages		
	I	II	III
A	6,23±0,4	7,47±1,04**	5,99±1,47**
B	6,23±0,41	6,71±0,52	6,75±0,52
C	6,16±0,22	6,95±0,44***	7,04±0,33***
D	5,85±0,73	6,87±0,52	6,95±0,71

** = significantly distinct difference;
*** = significantly high difference.

- in group C, the red blood cells count increased significantly high ($p< 0,001$), in the 2nd (6,95±0,44), and in the 3rd blood sampling also(7,04±0,33), compared to the initial one (6,16±0,22);
- in group B the increase was insignificant.

Graphic 3 – Representation of the Haematids count in absolute values (mil/mm^3)



CONCLUSIONS

Testing was conducted in an intensive system piggery, the animals beeing subjected to normal feeding and microclimate conditions during the experiment.
In group modulated by *Corynebacterium parvum*, the increase was significantly distinct at the second sampling, compared to the first sampling. At the final sampling, there was a statistically significant decrease.
In the *Corynebacterium parvum* modulated group the hemoglobin

concentration, the hematocrit and the red blood cell count had a similar evolution during the experiment, increasing (with statistical value) to the upper physiological limit in the second sampling compared to the first one. In the final sampling , the values decreased to the inferior limit.
In the remaining groups, all the tested hematological parameters presented simmilar manifestations during the experiment, beiing observed a statistically important increase only in the intermediate blood sampling, after which they maintained constant.

REFERENCES

Beuth, J., Ko H.L., G. Pulverer, 2002. Bacterial peptides as immunomodulators, Old Herborn University Seminar Monograph, 15, 151-157.

Kim, J.D., Hyun Y., 2000. Effects of immunostimulators on groth performance and immune response in pig weaned at 21 days of age, Journal of Animal and Feed Sciences, 9 (2) 333-346.

Lee, D.N., Shen T.T., et al, 2000. Effects of chromium supplementation and lipopoly sacharide injection on the immune responses of weanling pigs; Asian-Australian journal of Animal Science, 13(10) 1414-1421.

Mikulska-Skupien, E., Szweda, W., Procajlo, Z.; Platt-Samoraj, A., 2004. Indices of non-specific celular immune response in pigs after intredermal vaccination with deleted Aujeszky's disease vaccine and after experimental infection, Bulletin of the Veterinary Institute in Pulawy 48 (4) 347-354.

Xie Ming Quan, Song Chang Xu., 2000. Effects of five immunostimulants on proliferation of peripherial blood mononuclear cells (PBMC) proliferations pigs, Chinese Journal of Vet. Science, 20(5) 485-486.

THE INFLUENCE OF SOME MICROBIAL IMMUNOSUPRESIVE AGENTS ON THE EFFECTIVNESS OF IMMUNOPROPHYLAXIS PROGRAMS APPLIED IN BREEDING PIGS

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Abstract

This paper presents data obtained from investigations made in pig farms with intensive growing system and from households, the presence and distribution of immunosuppressive pathogens circulating in swine populations in Romania (PRRS, M. hyopneumoniae, Circovirus, etc.) and their immunosuppressive influence on the effectiveness of immunoprophylaxis programs, applied in pig farming.
The benefit of obtaining such data is to obtain and apply some effective immunoprophylaxis methods in order to limit economic losses and increase the food quality and safety.
The influence of the mentioned germs on the anti-erysipelas immune response has taken into account because this vaccination is a currently made action applied in the current technology of growing pigs.
Preliminary results showed that there is some influence of studied immunosuppressive microbial agents including the association germs on the effectiveness of immunoprophylaxis programs applied in breeding pigs.

Key words: *Circovirus, Mycoplasma, PRRS.*

INTRODUCTION

This paper is a study performed in intensive pig farms and households in our country regarding the existence of some pathogens (PRRS, Circovirus, Mycoplasma) and their immunosuppressive influence on the effectiveness of immunoprophylaxis programs, applied in pig farming. The choice of this topic was justified by the fact that there are worldwide encountered pathogens known to have immunosuppressive activity in pig populations.
Some pathogens present in the world, are known to negatively modulate the immune system, significantly interfering with the effectiveness of any vaccination protocol. Such pathogens are Porcine Circovirus type 2 (PCV-2) - major causative agent of Post Weaning Multisystemic Wasting Syndrome

(PMWS) (Gordon et al., 2007; Raymond et al., 1995), PRRS virus - causative agents of Porcine Reproductive and Respiratory Syndrome (Perianu, 2011; Herman et al. 2010), Mycoplasma hyopneumoniae - the causative agent of porcine enzootic pneumonia (PEP) (Perianu, 2011; Silin 2001).
Clinical signs of infection caused by mentioned germs are varied, being dependent on the immune status, conditions of farm hygiene and the presence of other pathogens.
The aim of our research was to obtain data on the influence of immunosuppressive pathogens (PRRS, porcine Circovirus, Mycoplasma) on the effectiveness of immunoprophylaxis programs applied in pig farming. For this reason it was studied the immunological response after anti-erysipelas vaccination in pigs, both *in intensive pig farms and households. The* influence of the mentioned germs on the anti-erysipelas immune response has taken into account because this vaccination is included in vaccination programs performed in pig farms.
Knowing these mechanisms enables us to understand the limits that appear in certain vaccinations in pigs and also enables us to think of the development and implementation of effective immunoprophylaxis methods in order to limit economic losses and to increase the food quality and safety.

MATERIALS AND METHOD

Biological material studied was the clinically healthy pigs of different ages. Animals came from three different locations (A, B, C) of pig growing: 2 intensive farms (Farm A: 172 animals Farm B: 90 animals) and households (50 animals).
These three farms performe different pig growing systems.
In the farm A is used an industrial (intensive) breeding of the young piglets imported from abroad (Holland) and also acquired from the local farmers. The piglets are acquisitioned at 65-75 days old and the growing is carried out up to 150-160 days old, when the pigs are delivered. During this period piglets are vaccinated against erysipelas and PRRS diseases. The serological surveillance is carried out for classical swine fever.
In the farm B is performed a growing system based on the close circuit (breeding, growing and fattening), the breeding material (gilts and young boars) being obtained from the own breeding material. In the past when piglets were acquired from abroad, the farm passed through a disease

occurrence, being declared as PRRS contaminated farm. The sanitary-veterinary surveillance is made for classical swine fever, Aujeszky disease and brucelosis. The self-control is performed by serological tests for PRRS and Circovirus, and also by anatomo-pathological examinations. In this farm is performed the vaccination against erysipelas in pigs of 90-120 days old, sows at 3 days after farrow and at 3 days before weaning.

In the small farm C (of the households), the growth system is extensive, the piglets are acquired from diferent places, animal markets or from the own breeding. In these farms are not performed any type of vaccinations.

The mortality and morbidity parameters recorded in the 3 different locations were in the technological limits; during the period of investigation were not recorded infectious diseases.

By ELISA test, were carried out serological examinations, both for detection of the specific antibodies against *PRRS, M. hyopneumoniae, Circovirus*, and for presence of erysipelas antibodies, before and after vaccination against this disease.

Serological examinations were performed on blood samples (from the jugular confluence) before and after anti-erysipelas vaccination. Pathological and bacteriological examinations from corpses were also performed.

540 blood serum samples obtained from 312 pigs were serologicaly analyzed: 252 young pigs (1.5 - 4 months), 30 fat pigs (6 months - 1 year) and 30 sows. Detection of specific IgG antibodies in blood serum (seroprevalence) was performed using ELISA kits for: PRRS (HerdCheck-PRRS X3 IDEXX Laboratories, USA), Circovirus type 2 (Porcine Circovirus (PCV2) ELISA Test Kit Green Spring, Shenzhen Lvshiyuan Biotechnology Co.., Ltd), Mycoplasma hyopneumoniae (M hyo * CHECK Herd, IDEXX Laboratories, USA) and Erysipelothrix rhusiopathiae (Erysipelothrix rhusiopathiae SE / MR, Cypress Diagnostics). Results were read at wavelengths recommended by manufacturers.

Interpretation of positive results was based on the S / P ratio greater than or equal to 0.4 (PRRS and M. hyo), the value of optical density (OD630) greater than or equal to 0.4 (PCV2) and relative index value x 100 (IRPC) greater than 40 (Erysipelothrix rhusiopathiae).

By anatomopathological examination performed on young pig carcasses were identified different lesions: ecchymosis and petechia in lungs, broncho-pneumonia, enlarged spleen, enlarged and hemorrhagic lymphnodes, catharal and catarrhal-congestive enteritis (Baba, 1996).

In order to isolate and identify the germ of the lesions, bacteriological examinations were made using specific culture media.Isolated strains were characterized biochemically using the API Biomerieux multitest systems. Antibiotic susceptibility testing of the pathogenic bacterial species was performed by antibiogram (Kirby-Bauer - diffusion method).

RESULTS AND DISCUSSIONS

Results of serological examinations:

In the **FARM A** were studied 6 groups of animals; group 1, group 1a, group 2, group 4, group 5a, group 5b, group 6, group 6a.
In the **group 1**, with 50 unvaccinated animals of 65 days old, the ELISA test results were: 49 positive to PRRS virus, 50 negative and 21 suspect to M. hyopneumoniae (Table 1).
The **group 1a**, with 15 animals, represents the group 1 after vaccination against erysipelas and pleuropneumonia. Following to the ELISA test, the results were: 15 positive to PRRS, 8 suspect to Circovirus and none to erysipelas (Table 2).
The **group 2**, with 30 animals of 65 days old, unvaccinated against PRRS, vaccinated against erysipelas with subunitar vaccine and against pleuropneumonia, were recorded 30 positive animals to PRRS, none to M. hyopneumoniae, 6 suspect to Circovirus and none to erysipelas (Table 3).
The **group 3**, with 20 animals of 70 days old, unvaccinated against PRRS and vaccinated against erysipelas, were recorded 20 positive to PRRS, 1 positive to M. hyopneumoniae, 5 suspect to Circovirus and none to erysipelas (Table 4).
The **group 4**, with 20 animals of 70 days old, vaccinated against erysipelas and PRRS, presented 20 animals positive to PRRS, 2 positive to M. hyopneumoniae, 1 suspect to Circovirus and none to erysipelas (Table 5).
The **group 5**, with 20 animals of 65 days old, vaccinated against Circovirosis and M. hyopneumoniae, unvaccinated against erysipelas and PRRS, presented: 1positive animal to Circovirus and none positive to PRRS, Circovirus and erysipelas (Table 6).
The **group 5a**, with 30 animals of 83 days old represents the group 5 after vaccination against erysipelas by subunitar vaccine. Were obtained negative results to PRRS and Circovirus; 4 positive to M. hyopneumoniae and 1 suspect; 2 positive animals to erysipelas.

The **group 5b**, with 30 animals, represents the group 5 at the 91 days old. Were recorded 6 positive results to PRRS; 2 suspect to Circovirus; 1 suspect to M. hyopneumoniae; 5 positive to erysipelas.
The **group 6**, with 32 animals of 70 days old. The animals unvaccinated against erysipelas and PRRS, were grouped in 2 boxes as follows: the 1-st box with 16 animals (1-16) presented 1 animal suspect to M. hyopneumoniae and none to PRRS, Circovirus and erysipelas.
In the second box (17-32) was recorded 1 suspect animal to Circovirus and none to PRRS, M. hyopneumoniae and erysipelas (Table 9).
The **group 6a**, represents the group 6 after vaccination against erysipelas. The vaccination was applied using two vaccines from two different producers. In the 1-st box after vaccination with Eriromvac, 16 animals were positive to PRRS, 1 animal was suspect to Circovirus and the rest of animals were negative to M. hyopneumoniae and erysipelas; in the 2-nd box the animals were vaccinated with Bioveta vaccine and were recorded 16 positive animals to PRRS and negative to M. hyopneumoniae, 1 suspect to Circovirus and 4 positive to erysipelas (Table 10).

Table 1

Group 1. Age 65 days, weight 18 kg					
	Sampling date 06.03.2012	Immunisation: not vaccinated	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			49/50	0/50	0/50	
TOTAL suspect / tested			0/50	0/50	21/50	

Table 2

Group 1a. Sampling II, after erysipelas and pleuropneumonia vaccination on 08.03.2012						
	Sampling date 20.04.2012 (first sampling on 06.03.2012)	Immunisation: vaccinated for erysipelas and pleuropneumonia on 08.03.2012	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			15/15	0/15	0/15	0/15
TOTAL suspect / tested			0/15	0/15	8/15	0/15

Table 3

Group 2. Age 65 days, weight 40-45 kg					
	Sampling date 06.03.2012	Vaccinated for erysipelas and pleuropneumonia (subunit vaccine) on 02.02.2012 Not vaccinated for PRRS	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			30/30	0/30	0/30	0/30
TOTAL suspect / tested			0/30	0/30	6/30	0/30

Table 4

Group 3. Age 60 days					
	Sampling date 20.04.2012	Vaccinated for erysipelas on 15.03.2012. Not vaccinated for PRRS	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			20/20	1/20	0/20	0/20
TOTAL suspect / tested			0/20	0/20	5/20	0/20

Table 5

Group 4. Age 70 days					
	Sampling date 20.04.2012	Vaccinated for erysipelas and PRRS on 05.04.2012	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			20/20	2/20	0/20	0/20
TOTAL suspect / tested			0/20	0/20	1/20	0/20

Table 6

Group 5. Age 65 days						
	Sampling date 20.04.2012	Vaccinated for Circovirus and M. hyopneumoniae on 7 days of age. Not vaccinated for erysipelas and PRRS.	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			0/20	1/20	0/20	0/20

Table 7

Group 5a. Age 83 (65+18) days – sampling II					
Sampling date 07.05.2012	Vaccinated for M. hyopneumoniae and Circovirus on 24.03.2012. Vaccinated or erysipelas on 24.04.2012 (subunit vaccine).	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/30	4/30	0/30	2/30
TOTAL suspect / tested		0/30	1/30	0/30	0/30

Table 8

Group 5b. Age 91 (65+18+8) days – sampling III					
Sampling date 15.05.2012	Vaccinated for M. hyopneumoniae and Circovirus on 24.03.2012. Vaccinated or erysipelas on 24.04.2012 (subunit vaccine)	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		6/30	0/30	0/30	5/30
TOTAL suspect / tested		0/30	1/30	2/30	0/30

Table 9

Group: Lot 6. Age 70 days						
Sampling date 10.05.2012		Not vaccinated for erysipelas and PRRS	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		Box 1 (1-16)	0/16	1/16 (suspect)	0/16	0/16
		Box 2 (17-32)	0/16	0/16	1/16 (suspect)	0/16

Table 10

Group 6a. Age 98 days (70+28) – sampling II						
Sampling date 13.06.2012		Vaccinated for erysipelas on 15.05.2012 (Eryromvac box 1, Bioveta box 2)	Results			
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		Box 1 (1-16)	16/16	0/16	1/16 (suspect)	0/16
		Box 2 (17-32)	16/16	0/16	1/16 (suspect)	4/16

FARM B

The **group 1**, with 28 unvaccinated animals. Following to the serological tests were recorded 28 positive animals to PRRS, 5 positive to M. hyopneumoniae, 1 positive and 5 suspect animals to Circovirus (Table 11).

The **group 1a,** with 28 animals, represents the group 1 after vaccination against erysipelas. Were recorded 25 positive samples to PRRS, 7 suspect to Circovirus and all samples were negative to M. hyopneumoniae and erysipelas (Table 12).

The **group 2** with 30 unvaccinated animals. Were recorded 29 positive animals to PRRS; 6 positive and 4 suspect to M. hyopneumoniae; 1 positive and 6 suspect to Circovirus (Table 13).

The **group 2a,** with 22 animals, represents the group 2 after vaccination against erysipelas. Were recorded 22 animals positive to PRRS; 9 positive and 21 suspect to Circovirus, and all animals were negative to erysipelas (Table 14).

The **group 3**, with 30 vaccinated sows against E. coli and anaerobic germs, unvaccinated against erysipelas. In this group were recorded 27 positive animals to PRRS; 14 positive and 3 suspect to M. hyopneumoniae; 15 positive and 11 suspect to Circovirus (Table 15).

The **group 3a**, with 30 animals represents the group 3 after vaccination against erysipelas. Were detected 30 positive animals to PRRS; 13 positive and 3 suspect to M. hyopneumoniae; 5 positive and 10 suspect to Circovirus and 19 positive to erysipelas (Table 16).

Table 11

Group 1.Grower, age 37 days, F2C2/2					
Sampling date 08.03.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		28/30	5/30	1/30	
TOTAL suspect / tested		0/30	0/30	5/30	

Table 12

Group 1a. Grower, age 79 days (37+42) – sampling II					
Sampling date 19.04.2012	Vaccinated for erysipelas on 17.03.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		25/28	0/28	0/28	0/28
TOTAL suspecte / tested		0/28	0/28	7/28	0/28

Table 13

Group 2. Fattening, age 99 days F2C1/3					
Sampling date 08.03.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		29/30	6/30	1/30	
TOTAL suspect/ tested		0/30	4/30	6/30	

Table 14

Group 2a. Fattening, age 141 days (99+42) – sampling II					
Sampling date 19.04.2012	Vaccinated for erysipelas on 17.03.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		22/22	9/22	1/22	0/22
TOTAL suspect / tested		0/22	9/22	21/22	0/22

Table 15

Group 3. Sows F2C3/4					
Sampling date 08.03.2012	Vaccinated for E. coli and anaerobiosis. Not vaccinated for erysipelas.	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		27/30	14/30	15/30	
TOTAL suspect / tested		0/30	3/30	11/30	

Table 16

Group 3a. Sows - sampling II					
Sampling date 25.05.2012	Vaccinated for E. coli and anaerobiosis. Vaccinated for erysipelas on 23.04.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		30/30	13/30	5/30	19/30
TOTAL suspect / tested		0/30	3/30	10/30	0/30

HOUSEHOLD FARMS

Group 1, with 20 completely unvaccinated animals, of 4 months old. Were recorded 5 suspect animals to Circovirus, the rest of animals being negative to the other diseases including erysipelas (Table 17).
Group 1a, with 20 animals of 5,5 months old, vaccinated against erysipelas with Eryromvac vaccine. All animals have been found negative to PRRS

and M. hyopneumoniae; 3 suspect animals to Circovirus and 12 positive to erysipelas (Table 18).
The **group 2,** with 20 unvaccinated animals of 6 months old. All animals were negative to PRRS and M. hyopneumoniae; 5 animals were suspect to Circovirus and 4 animals were positive to erysipelas.
The **group 2a,** with 20 animals, represents the group 2 of 7.5 months old, after vaccination against erysipelas. All animals were found negative to PRRS and M. hyopneumoniae, 5 animals were suspect to Circovirus and 11 animals were positive to erysipelas (Table 20).
Lotul 3, compus din 10 animale, vârsta de 1 an, total nevaccinate. Au fost depistate toate animalele negative la PRRS și M.hyopneumoniae. 2 dubioase la Circovirus și 5 pozitive la Rujet (Table 21).

Table 17

Group 1. Age 4 months, household farm					
Sampling date 25.04.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/20	0/20	0/20	0/20
TOTAL suspect / tested		0/20	0/20	5/20	0/20

Table 18

Group 1a. Age 5.5 months, household farm – sampling II					
Sampling date 07.06.2012	Vaccinated for erysipelas (Eryromvac) on 11.05.2012	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/20	0/20	0/20	12/20
TOTAL suspect / tested		0/20	0/20	3/20	0/20

Table 19

Group 2. Age 6 months, household farm					
Sampling date 25.04.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/20	0/20	0/20	4/20
TOTAL suspect / tested		0/20	0/20	5/20	0/20

Table 20

Group 2a. Age 7.5 months, household farm– sampling II					
	Sampling date 07.06.2012	Vaccinated for erysipelas (Eryromvac) on 11.05.2012	Results		
			PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested			0/20	0/20	0/20	11/20
TOTAL suspect / tested			0/20	0/20	5/20	0/20

Table 21

Group 3. Age 1 year, household farm					
Sampling date 25.04.2012	Not vaccinated	Results			
		PRRS	M. hyopneumoniae	Circovirus	Rujet
TOTAL positive / tested		0/10	0/10	0/20	5/10
TOTAL suspect / tested		0/10	0/10	2/20	0/10

Percentage representation of the antibody titer induced by the immune-suppressor germs studied (PRRS, M.hyopneumoniae, Circovirus) and the erysipelas antibody level following to the vaccination against erysipelas are presented by the histograms in figures 1, 2 and 3.

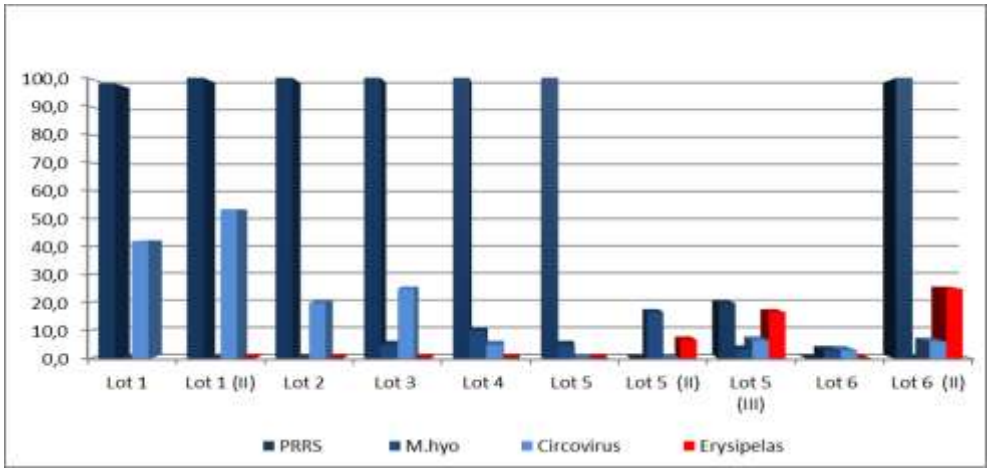

Figure 1. Seroprevalence of immunosupresive germs and postvacination Erysipelas antibody levels in Farm A.

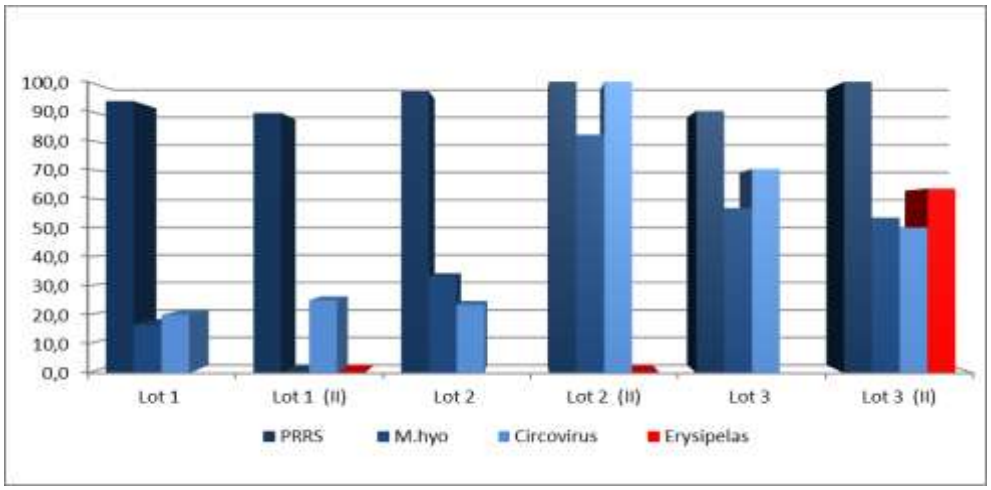

Figure 2. Seroprevalence of immunosupresive germs and postvacination Erysipelas antibody levels in Farm B.

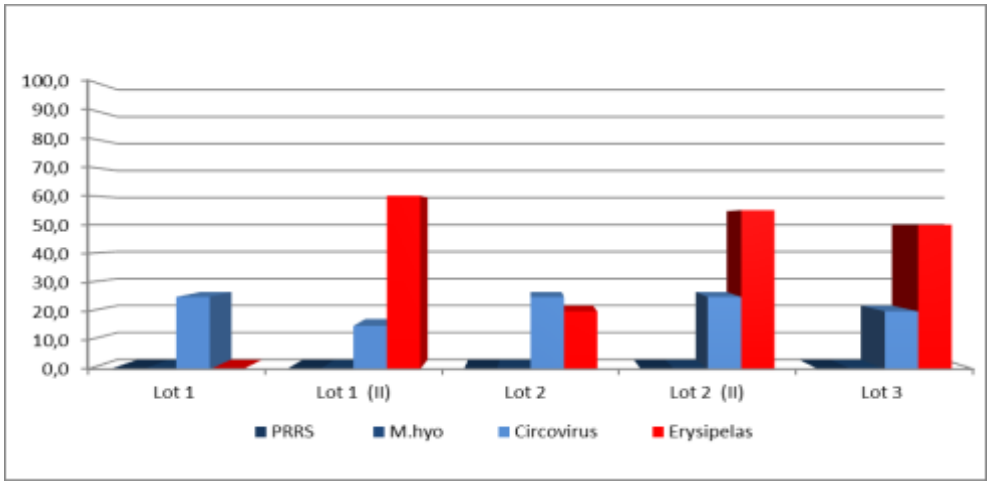

Figure 3. Seroprevalence of immunosupresive germs and postvacination Erysipelas antibody levels in Farm C.

Concomitantly with isolation of the proposed germs (PRRS virus, circovirus or Mycoplasma hyopneumoniae) we had in intention to determine the associated infections, produced by the pathogenic microbial flora or with

pathogenic potential, infections which may have an influence on the immune status of animals.
By bacteriological examinations were isolated bacterial association germs, pathogens or potential pathogens. Have been isolated from pigs, the following bacteria with pathological significance : *Mannheimia (Pasteurella) haemolytica* (1 strain), *Pasteurella multocida* (2 strains), *Pseudomonas aeruginosa* (2 strains), *Streptococcus spp.*(3 strains), *Klebsiella pneumoniae* (1 strain*), Escherichia coli* (12 strains).

CONCLUSIONS

Preliminary results indicated a link between the existence of the studied microbial agents and the immune response after vaccination. Thus, following anti-erysipelas vaccination applied in three pig units, with different immune status and growth conditions, the seroconversion was negative in high proportion, regardless of the type of vaccine used, alive or subunit.
Investigations show the presence of Circovirus and PRRS virus and also M. hyopneumoniae in pigs populations from intensive farming systems which practiced animals import. Clinically healthy animals, suggest a carrier status, clinical signs being dependent on the sanitary status of the farm and the presence of other pathogens.
The study shows that both microbial agents studied (PRRS, Circovirus, Mycoplasma hyopneumoniae) and the germs of association have a negative action on the effectiveness of immunoprophylaxis programs applied in breeding pigs.

REFERENCES

Baba Alexandru Ioan, 1996. Diagnostic necropsic veterinar. Boli bacteriene la suine, 196-213, Ed. Ceres, Bucuresti.
Gordon M. Allan, Francis McNeilly, Michael McMenamy, Irene McNair, Steven G. Krakowka, Sirje Timmusk, Dermot Walls, Maria Donnelly, Donal Minahin, John Ellis, Per allgren, Caroline Fossum, 2007. Temporal distribution of porcine circovirus 2 genogroups recovered from postweaning multisystemic wasting syndrome–affected and –nonaffected farms in Ireland and Northern Ireland. J Vet Diagn Invest 19:668–673.
Herman V., Corina Pascu, Luminita Costinar, Cătană N., Faur B., Ioana Văduva, Anca Surpat, Sorina Irimie, 2010. Investigation of PRRSV Infection in Fattening Farms. Lucrări Ştiintifice Medicină Veterinară Vol. XLIII (1), Timişoara.
Perianu Tudor, 2011. Tratat de Boli Infectioase ale Animalelor. Bacterioze. Pneumonia enzootica suina, vol I, 801-806.

Perianu Tudor, 2011. Tratat de Boli Infectioase ale Animalelor. Viroze si Boli prionice. Sindromul respirator si de reproductie la suine, vol II, 93-99.
Raymond K. Hines, and Phil D. Lukert, 1995. Porcine circovirus: A serological survey of swine in the United States. Journal of Swine Health and Production- Volume 3, Number 2, pages 71-73.
Silin D. S., Lyubomska O. V., Chung Nan Weng, 2001. Mycoplasma Hyopneumoniae Vaccination Influence on Porcine Reproductive And Respiratory Syndrome Virus and Mycoplasma Hyopneumoniae Coinfection. Acta Vet. Brno, 70: 413–420.

STUDY REGARDING THE CORTROSYN-DEPOT EFFECTS IN LACTATING EWES

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Abstract

The literature indicates a positive correlation between maternal behavior in sheep, milk production and administration of the ACTH hormone (Cortrosyn Depot as commercial product) involved in various metabolic processes (specific actions the ACTH hormone on the mammary gland in stimulating the lactopoesis).
Because an intense lactogenesis is associated with a positive maternal behavior, we considered it appropriate to study and quantify the implications of ACTH production and mammary gland development.
Thus, Cortrosyn (ACTH) was series administered in sheep, from the first day after birth until day 15 of lactation, in dose of 1 mg.
It can be observed a positive correlation between maternal behavior in sheep, milk production and administration of the ACTH hormone (in the form of commercial preparation Cortrosyn Depot).

Key words: *Cortrosyn, lactating ewes, maternal behavior.*

MATERIALS AND METHODS

In this experiment we formed two groups as follows:
- LM group - control group - consisting of 10 lactating ewes from Merino breed with their lambs;
- LEC group - experimental group - consisting of five lactating ewes from Merino breed (with their lambs), who were treated with ACTH hormone.

ACTH - based commercial product used in this experiment was Cortrosyn Depot (depot form of ACTH).Through its implications in various metabolic processes and specific actions the ACTH hormone, occurs on the mammary gland, stimulating the lactopoesis (Keller et al., 2008; Dojană, 2010).

Whereas an intense lactogenesis is associated with an intense positive maternal behavior, we studied the role of ACTH in the development of the lactogen potential of the mammary gland (Young et al., 2003; Linares et al., 2008).

Thus, Cortrosyn (ACTH) was series administered in sheep, from the first day after birth until day 15 of lactation, in dose of 1 mg.

Lambs from the control and experimental groups were weighed on days 1, 10 and 21 days after calving, calculating finally batch weaning weight and average daily gain/lamb/group (Acatincăi, 2003).

RESULTS AND DISSCUTION

To lactating ewes from experimental and control groups, we determined indirectly the maternal behavior, by establishing the average weight of lambs at birth, 21 days and at weaning, because a higher lambs weight, reflecting a higher milk production the mother-sheep, which ultimately means better maternal behavior expressed (McKusick, 2001).
An indirect determining of ACTH effect of milk secretion in sheep from this experiment, and while on maternal behavior, was performed by following the dynamics of comparative weight and daily gain of the lambs from the two groups (Codreanu, 2001).
Thus, by weighing lambs derived from experimental and control groups, and after calculating the average weight of lambs groups at birth and at weaning and the daily gain/lamb, there are some changes with statistical significance in some cases that will be mentioned below (Pugh, 2002).
Values of body weight and weight gain in lambs of control and experimental groups are presented in tables 1-2 and illustrated in figure 1.

Table 1. Average weight (kg) in lambs from control and groups treated with Cortrosyn

GROUP	Average weight (kg)			
	3 days after birth	10 days after birth	21 days after birth	weaning (50 days)
CONTROL	4.0±0.180	5.5±0.250	8.3±0.340	16.7±0.680
LEC	4.4±0.190*	6.2±0.310**	9.6±0.400**	18.4±0.860**

** p>0.05 – insignificant difference*
*** p<0.05 - significant difference*

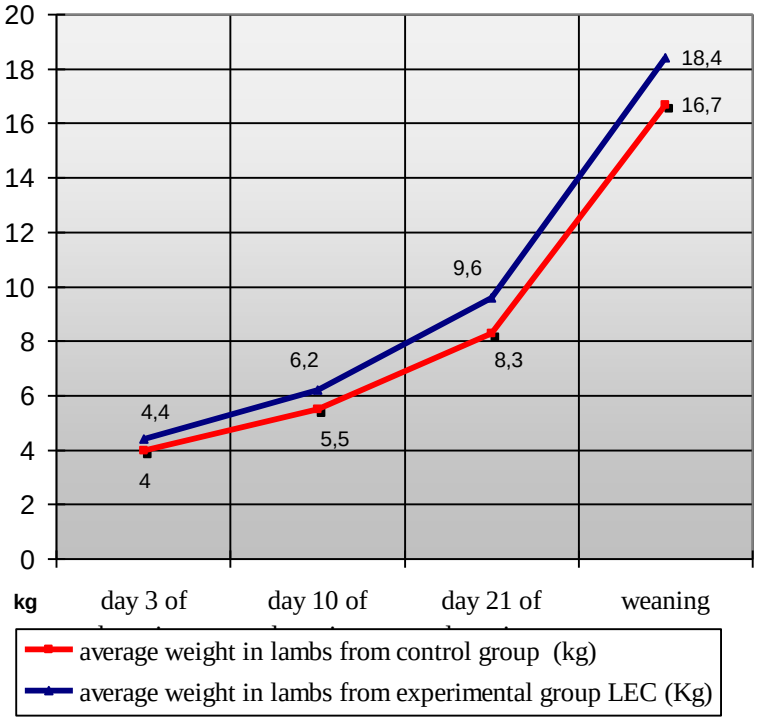
Comparing the average weight per group at weaning and average daily gain on the entire period of lactation, we can see that the lambs from experimental group, these parameters were significantly higher in statistical terms ($p<0.05$) compared with the control group.

Table 2. Comparative results of average daily gain of lambs from control group and experimental group treated with Cotrosyn, during lactation period (g/day)

Group	**Daily gain (g)**		**Average daily gain (g)**
	0-21 zile	*21- 50 zile*	
Control	210±19.2	190±18.8	**200±19.0**
LEC	265±20.2**	225±18.0*	**245±19.4****

** p>0.05 – insignificant difference*
*** p<0.05 - significant difference*

Figure 1. The dynamics of the average weight (kg) in lambs from control group and experimental group treated with Cortrosyn



Cortrosyn Depot administration in sheep from experimental group, is a clear demonstration of the lactogenic role of the ACTH in this species and in the requirement that maternal behavior (Dojană, 2010).

After dosing the biochemical parameters in sheep from the control and experimental groups, revealed the existence of significant increases in average values of albumin, total lipids and triglycerides in sheep from experimental group, increases which probably reflected in milk composition of these sheep (table 3).

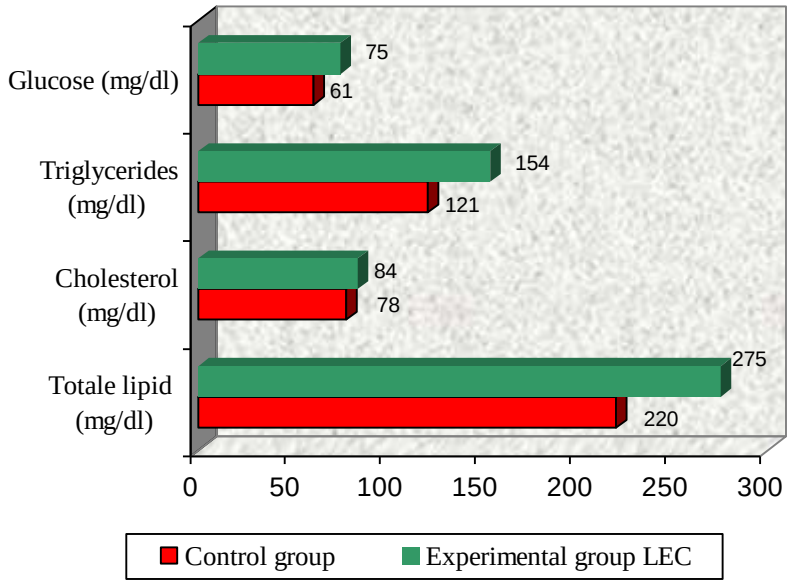
Values of proteic and energetic profiles in ewes, from control and experimental groups, are presented in tables 3 and illustrated in figure 2.

Table 3. Average values of proteic and energetic profiles in ewes from control and experimental group treated with Cortrosyn

PARAMETER		**Group**	
		CONTROL	EXPERIMENTAL
PROTEIC PROFILE	Total protein *(g/dl)*	6.8±0.39	7.2±0.42*
	Albumin *(g/dl)*	3.3±0.19	3.9±0.25**
	Globulin *(g/dl)*	3.5±0.21	3.3±0.19*
	Report Albumin/ Globulin	0.970	1.181*
ENERGETIC PROFILE	Total lipid *(mg/dl)*	220±12.8	275±13.5**
	Cholesterol *(mg/dl)*	78±5.2	84±4.8*
	Triglycerides *(mg/dl)*	121±6.4	154±8.1**
	Glucose *(mg/dl)*	61±4.4	75±4.0*

** p>0.05 – insignificant difference*
*** p<0.05 - significant difference*

Figure 2. The dynamics of average values of proteic and energetic profiles in ewes from control and experimental group treated with Cortrosyn



It can be observed a positive correlation between maternal behavior in sheep, milk production and administration of the ACTH hormone (in the form of commercial preparation Cortrosyn Depot).

CONCLUSIONS

Treatment with Cortrosyn Depot in ewes from experimental group leads indirectly to the improvement of maternal behavior.

Comparative analysis of average weight/group at weaning (18.4 kg) and average daily gain thus the entire period of lactation (245 g/day), we can see that by lambs from the experimental group, these parameters were significantly higher ($p<0.05$) than lambs from the control group (16.7 kg, respectively, 200 g/day).

Although administration of Cortrosyn Depot in ewes has not generated clinical changes of any treated sheep was found, however we observed significant increases of average values of serum biochemical parameters ewes from experimental group (albumin, total lipids and triglycerides).

The other serum parameters (globulin, glucose, cholesterol) in ewes from experimental group were within in normal limits of the species and physiological state, which shows that the ACTH, does not alter the physiological balance animals.
Cortrosyn Depot administration in sheep from experimental group, is a clear demonstration of the lactogenic role of the ACTH in this species and in the requirement that maternal behavior.

REFERENCES

Acatincăi S., 2003. Etologie – Comportamentul animalelor domestice – ed. Eurobit, Timişoara

Codreanu Iuliana, 2001. Elemente de fiziologie a comportamentului matern la animale, Ed. Monitor, Bucureşti

Dojană, N., 2010. Fiziologia animalelor domestice, Ed. Printech, Bucureşti

Keller-Wood M, Wood CE. 2008. Regulation of maternal ACTH in ovine pregnancy: does progesterone play a role? Am J Physiol Endocrinol Metab. Oct;295(4):E913-20.

Linares M., R. Bórnez, H. Vergara, 2008. Cortisol and catecholamine levels in lambs: Effects of slaughter weight and type of stunning, Livestock Science. Volume 115, Issue 1, May 2008, Pages 53-61

McKusick BC, Thomas DL, Berger YM., 2001. Effect of weaning system on commercial milk production and lamb growth of East Friesian dairy sheep. J Dairy Sci.;84 (7):1660-8.

Pugh G., 2002. Sheep & goat medicine. Elsevier Health Sciences

Young S., J. Rose, J. Schwartz, 2003, Ontogeny of Corticotropin-Releasing Hormone Binding in Anterior Pituitaries of Fetal Sheep, Reproductive Sciences, April 1, 2003; 10(3): 130 - 135.

STUDY REGARDING HORMONAL AND BIOCHEMICAL PROFILES IN PREGNANT AND LACTATING EWES TREATED WITH BROMOCRIPTINE

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Abstract

The study of maternal behavior in sheep, can not be conceived without investigating of the anterohypofizary hormones implication in the expression (direct or indirect) of this type of behavior, as, for example, prolactin, FSH and ACTH.
Thus, the literature indicates that prolactin, regardless of species, acting on the central nervous system, inducing and maintaining maternal behavior. It also initiates and maintains the mammary gland secretion, this effect is exercised only in terms of its prior action of estrogen and progesterone.
To approach the role of prolactin in the induction and maintenance of maternal behavior and in the initiation and development of milk secretion in sheep, we used the antiprolactinic drug preparation: Bromocriptine.
Thus, Bromocriptine was series administered in sheep, in doses of 1 mg - 2.2 mg/animal/day, from 4 days before birth up to 3 days after birth, following the effect of bromocriptine on weight gain in lambs, but also on the main biochemical constituents of blood.

Key words: *Bromocriptine, lactating ewes, maternal behavior.*

MATERIAL AND METHODS

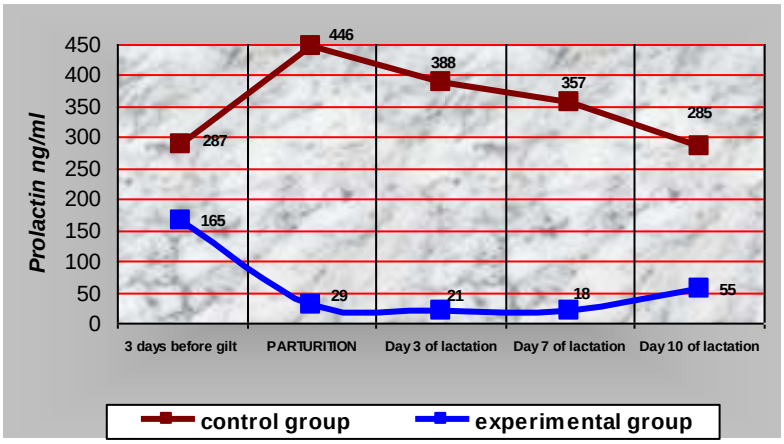
The sheep from this experiment belonged to the Merino breed (artificially inseminated) and they were at the third calving, and all were to girls in the same month (controlled calving).
To assess the antiprolactin effects of Bromocriptine, we proceeded to the composition of the two groups, as follows:
- control group - consisting of seven Merinos sheep;
- experimental group (LE) - consisting of five Merinos sheep, which was administered Bromocriptine series in doses of 1 mg - 2.2 mg/animal/day, from 4 days before birth up to 3 days after birth, following the effect of bromocriptine on weight gain in lambs, but also on the main biochemical constituents of blood. In order to determine hormonal profile and biochemical sheep in the control group we collected blood from the jugular

vein in the morning, between 9.00 to 11.00 in the following days: 70, 100 and 140 of gestation, the day of calving, and days 10,18,35 and 42 of lactation (Brunton, et al., 2008; Negritu et al., 2011). After collection, the blood samples were centrifuged and plasma was frozen at -15^{0}C until they were sent to the laboratory for hormones determination (prolactin, progesterone, FSH and growth hormone) and biochemical parameters.

RESULTS AND DISSCUTION

In addition to hormonal dosages, the research presented in this paper consisted in the development of biochemical tests in sheep, in different physiological states (pregnant and lactating) and consisted of: determination of metabolic profile parameters (activity of aspartate aminotransferase, alanine aminotransferase, dosing mineral elements, but also of total protein). The data were statistically interpreted using T Student test.
The values of prolactin, FSH, progesterone and GH, resulting from hormonal investigations (both for sheep in the control group and those in the experimental group) are suggestively illustrated in figure 1.

Figure 1. The dynamics of average values of prolactine (ng/ml) during pregnancy and lactation in sheep of control and experimental groups



Comparing the results obtained in the study of hormonal profile at birth, in the experimental group, there is observed a distinct significantly decrease ($p<0.01$) of average value of prolactin, a difference which is maintained, also during the 10 days of lactation, when the hormonal dosages were made.

After stopping the antiprolactinic treatment, is observed a significant increase ($p < 0.05$) average value of prolactin in sheep in this group.This highlights the inhibitory and constant action of bromocriptine on pituitary prolactin secretion, secretion which ultimately will change the maternal behavior of sheep in the experimental group (Codreanu, 2001; Dojană, 2011).

Comparing the results after dosing some biochemical parameters (table 1), it is found that the mean values of total protein were slightly higher in the experimental group, but the difference considered statistically insignificant ($p > 0.05$).

Comparing the results after dosing some biochemical parameters (table 1), it is found that the mean values of total protein were slightly higher in the experimental group, but the difference considered statistically insignificant ($p > 0.05$). Bromocriptine treatment of sheep from experimental group did not affect other biochemical parameters (statistically insignificant), observing that they have been close to the average values of the control group (Negritu et al., 2009; Negritu et al., 2010).

Table 1. Comparative analysis of average values of biochemical exams in sheep from the control and experimental groups

Parameter	**GROUPS**	
	Control	**Experimental**
Total Protein *(g/dl)*	7.6	8.0*
Albumin *(g/dl)*	3.75	3.9*
Globulin *(g/dl)*	3.85	4.1*
Ca *(mg/dl)*	9.2	10.1*
P *(mg/dl)*	6.05	6.6*
AST *(U/l)*	47	58*
ALT *(U/l)*	22.6	22.3*

**p>0,05 - insignificant differences*

This highlights the inhibitory and constant action of bromocriptine on pituitary prolactin secretion, secretion which ultimately will change the maternal behavior of sheep in the experimental group (Brunton et al., 2008).

CONCLUSIONS

The studies of indirect role of prolactin in the induction and maintenance of milk secretion (factor that makes the maternal behavior), was performed with a prolactin inhibitor (bromocriptine).
Comparing the results obtained in the study of hormone profile shows a highly significantly decrease ($p<0.01$) of the average value of prolactin (29 ng/ml) in sheep from the experimental group - who was treated with Bromocriptine.
Highly decrease significantly of serum prolactin levels in sheep treated with bromocriptine, shows the constant inhibitory action of Bromocriptine on the LTH - pituitary secretion, which ultimately led to the treated sheep, much weaker expression of maternal behavioral manifestations during lactation.
The comparative analysis of results from other hormones dosage (progesterone and FSH) in sheep from experimental and control groups, were found insignificant differences between the average values of these hormones, either during pregnancy or lactation.
Comparing the average values of total protein, were slightly higher in the experimental group (8.0 g/dl) than the control group (7.6 g/dl), but the difference were considered statistically insignificant ($p>0.05$).
Bromocriptine treatment of sheep in the experimental group did not affect other biochemical parameters, observing that they have been close to the average values of the control group (statistically insignificant).
The whole experiment is a clear demonstration of the lactogen role of prolactin in sheep and maternal behavior in conditioning the default.

REFERENCES

Brunton P., Russell J., Douglas A., 2008. Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. J Neuroendocrinol. 2008 Jun; 20 (6):764-76.
Codreanu Iuliana, 2001. Elemente de fiziologie a comportamentului matern la animale, Ed. Monitor, Bucureşti
Dojană, N., 2011. Fiziologia animalelor domestice, Ed. Printech, Bucureşti
Negritu Gabriela, Iuliana Codreanu, M. Codreanu, N. Dojană, 2011. Investigaţii privind manifestările comportamentale materne la oile primipare şi multipare în perioada postparturientă, al XI-lea Congres National de Medicină Veterinară.

ASPECTS OF LYMPH NODES CYTOMORPHOLOGY ON HORSES - THE BEGINNING CELL IMAGING OF NON-SPECIFIC LIMPH NODES PROLIFERATIONS -

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INTRODUCTION

Pathological processes of lymph nodes are quite frequently found in human and animal pathology, so typing lymph node cell imaging have a significant importance, with anamnesis and clinical examination we obtain the certainty diagnosis.

MATERIALS AND METHODS

We studied the cytomorphological imaging of marrow, peripheral blood and lymph nodes of external and/or internal on 10 cases of slaughtered horses.
The morphocytological investigation of smears was performed using panoptic staining (May-Grunwald Giemsa).

RESULTS AND DISCUSSION

The results allowed the identification of three groups of animals with different cells imaging, as follows:
- the status of these investigated territories was for 3 animals and corresponded to an anatomo-clinical macroscopic examination as quasinormal (fig. 1);
- the status of lymph node irritation, so-called stage 0, of a global proliferation to lymph node cell populations was identified in 3cases;
- the status of massive limfoproliferation (stage 1-2), was found in 4 cases (fig. 2, 3, 4, 5, 6, 7). These cases are characterized by:
- massive lymphocytic blast with a predominance of "B" lymphocytes;

- the presence of a high number of mast cells;
- reticular cells proliferation;
- increased quantitative dendritic cells (spindle and globular).

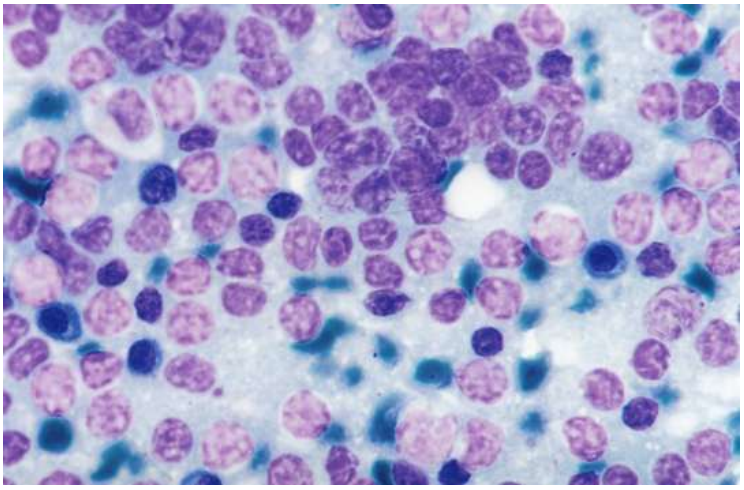

Fig. 1 – Horse limph node -"Quasi normal" aspect of morphological structure. Adult and semiadult lymphocytes are prevalent; rare lymphocytes, MGG, 1000 X

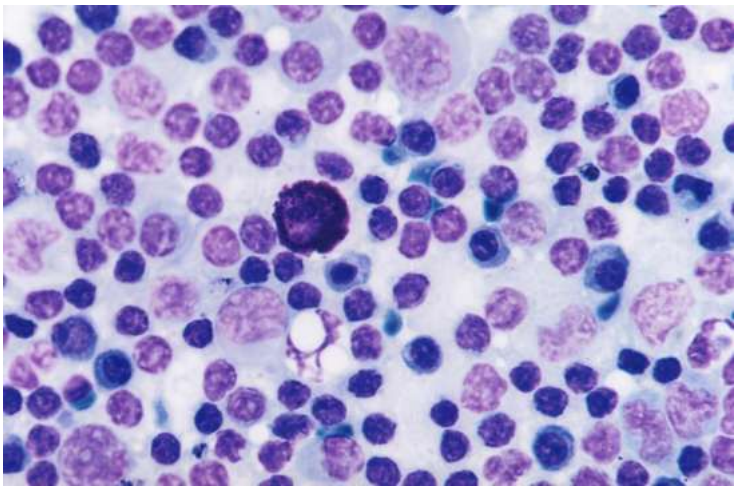

Fig. 2 - Horse limph node - Phase I - "irritation lymph node syndrome" where is a predominant multiplication of lymphoblastic elements with all the "reactive" of lymph (plasma cells, reticular cells, macrophage and mast), MGG, 1000 X

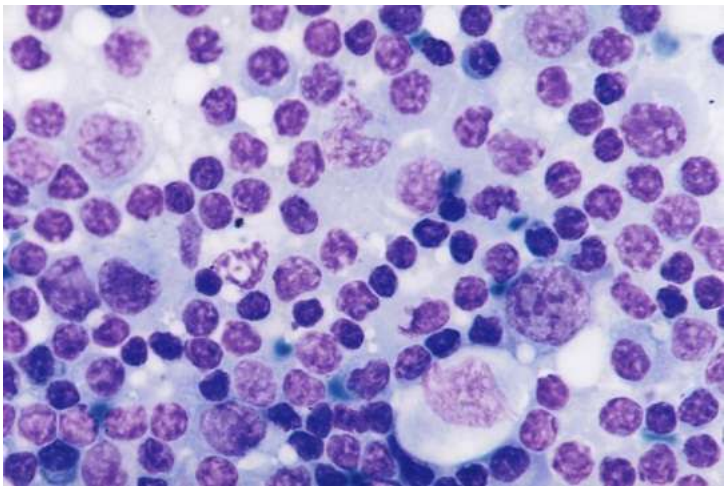

Fig. 3 - Horse limph node - Phase I - "irritation lymph node syndrome" where is a predominant multiplication of lymphoblastic elements with all the "reactive" of lymph (plasma cells, reticular cells, macrophage and mast), MGG, 1000 X

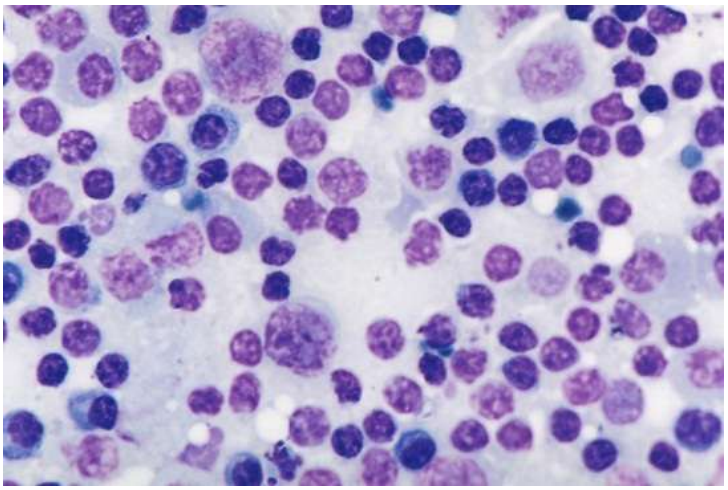

Fig. 4 - Horse limph node - Phase I - "irritation lymph node syndrome" where is a predominant multiplication of lymphoblastic elements with all the "reactive" of lymph (plasma cells, reticular cells, macrophage and mast), MGG, 1000 X

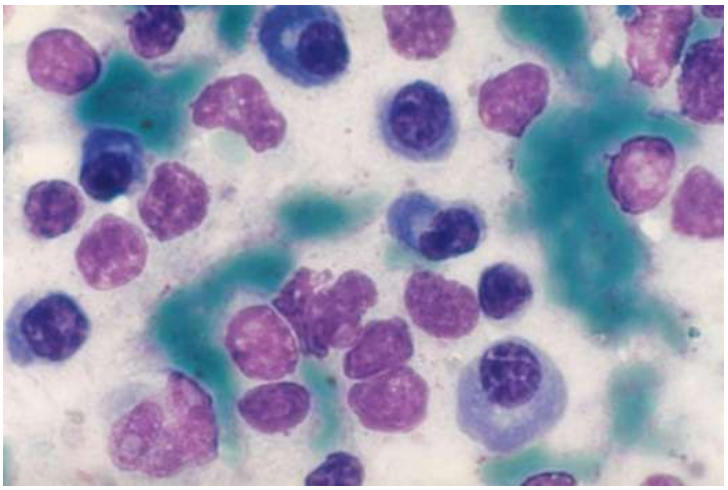

Fig. 5 - Horse limph node - The second phase of "irritation lymph node syndrome" where is a predominant cell with a "reticulosis" with obvious evolutionary trend towards a "malignant lymphoma", MGG, 1000 X

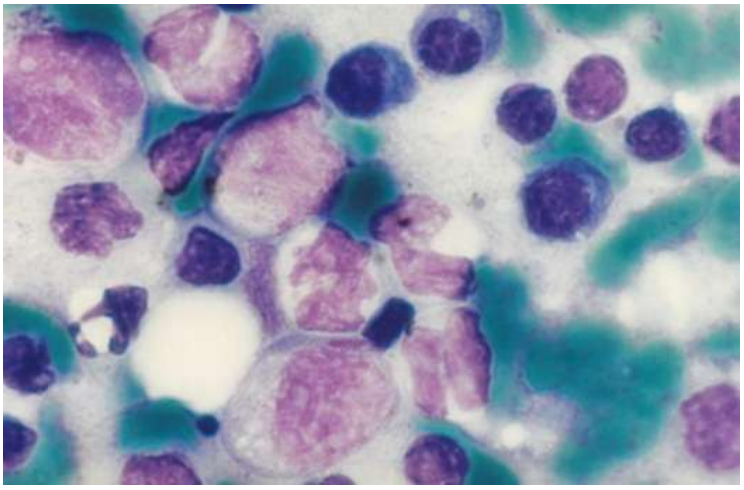

Fig. 6 - Horse limph node - The second phase of "irritation lymph node syndrome" where is a predominant cell with a "reticulosis" with obvious evolutionary trend towards a "malignant lymphoma", MGG, 1000 X

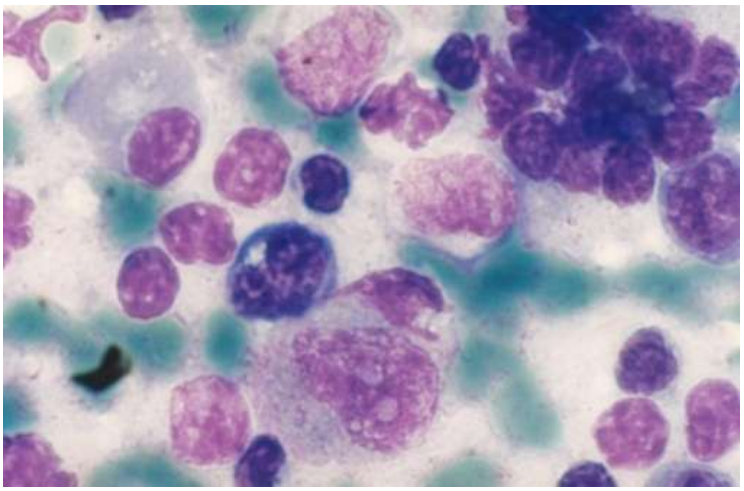

Fig. 7 - Horse limph node - The second phase of "irritation lymph node syndrome" where is a predominant cell with a "reticulosis" with obvious evolutionary trend towards a "malignant lymphoma", MGG, 1000 X

CONCLUSIONS

We highlighted "de facto", present in equine pathology, of "irritation lymph node syndrome";
This “irritation lymph node syndrome” was staged in 4 stages (1-4);
Stages 1-2 are benign totally, with a normal evolution to "restitutio ad integrum" and stages 3-4 is a state of "warning" of their increased risk for their transformation into a malignant hemopathy.

REFERENCES

Balint Emilia, 2010. Veterinary hematology and oncology compared. Old Court Publishing House, Bucharest.
Berceanu S., Manolescu N., Gogiu M., Colita D., Paunescu G., Avram N., 1985. Comparative Hematology. Medical Publishing House, Bucharest.
Faramarz Naeim, P. Nagesh Rao, Wayne W. Grody, 2008. Hematopathology.Academic Press.
Manolescu N. (as editor), 1995 .Aspects of comparative cellular pathology, vol I. Ceres Publishing House, Bucharest.
Manolescu N. (as editor), 1999. Aspects of comparative cellular pathology, Volume II. Teaching and Pedagogical Publishing House, Bucharest.

Manolescu N. (as editor), 2002. Aspects of comparative cellular pathology, Vol III. Ceres Publishing House, Bucharest.
Manolescu N. et al., 1978. Hematology guide to intensive livestock, Ceres Publishing House, Bucharest.
Manolescu N., 1976. Normal and pathological cytology of bone marrow and leukocyte concentrate in domestic mammals. Ceres Publishing House, Bucharest.
Micu D., N. Manolescu, Leukemic cells. Comparative study, 1981. Publishing House Of Romanian Academy, Bucharest.
Zachary James F., M. Donald McGavin, 2007. Pathologic Basis of Veterinary Disease. Mosby-Elsevier Publishing House.

THE STUDY OF THE "LYMPH IRRITATION SYNDROME" COMPARED TO CATTLES AND PIGS

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INTRODUCTION

The lymphatic system is an important component of the immune system. Lymph is filtered through the lymph node sinuses, where the particulates and infectious organisms are detected and removed. Because of the exposure to immune challenges, antibody and cell-mediated immunity is mediated. As a result of such normal processes, the lymph nodes can enlarge by proliferation of normal cells or infiltration by abnormal cells.

MATERIAL AND METHOD

In this study were investigated 17 cases ,7 cattles and 10 pigs, were slaughtered for meat marketing. We examined all major lymph node chains, both external and internal. On this occasion have identified various enlargement of these structures with various aspects of their macroscopic performed on longitudinal section. Were practiced smears of lymph juices and staining them using panoptic technique (May-Grunwald Giemsa). Simultaneously we made peripheral blood and bone marrow smears.

RESULTS

The cytomorphological investigation has revealed a massive proliferation of primordial lymphoid cells with an intense proliferation of support-reticular cells. In some cases, lymph node structure consisted mainly of plasmocitoid and plasma cells.

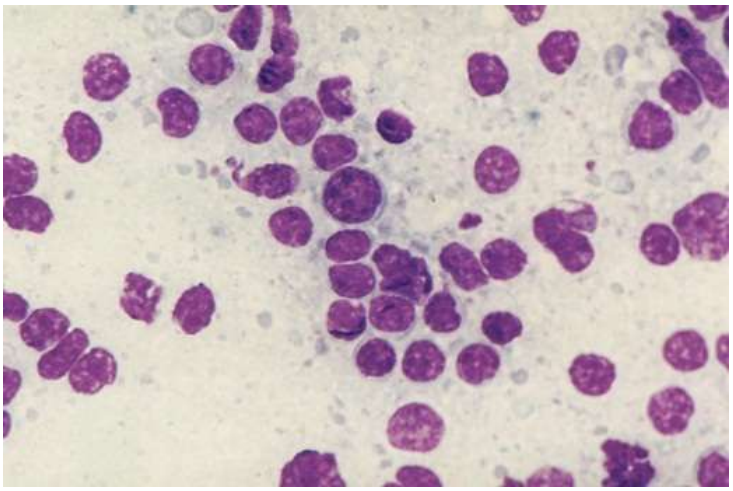

Fig. 1 - Pig lymph node – the "quasi normal" aspect of limphocytomorphology composition of lymph - dominant ratio 20/1 mature lymphocyte / blast cells, MGG, 1000 X

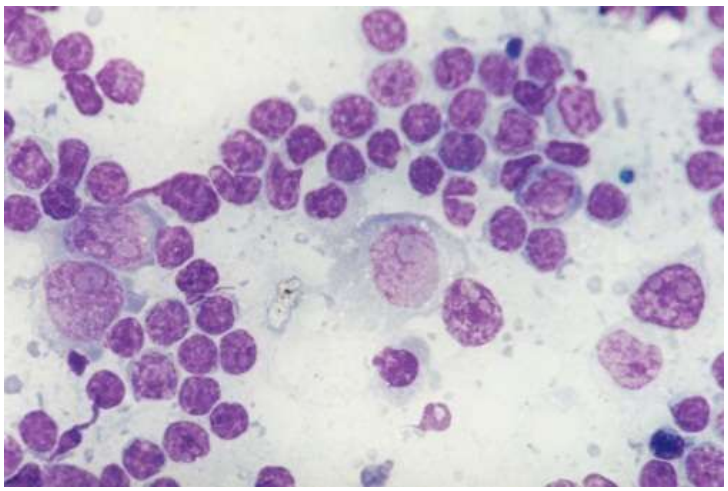

Fig. 2 - Pig lymph node - Intense blast transformation with lymph node-type items exclusively lymphoproliferative, MGG, 1000 X

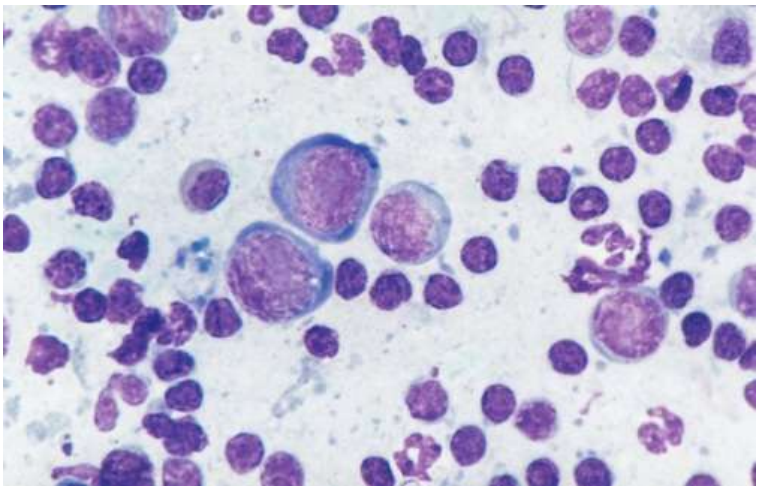

Fig. 3 - Pig lymph node - Intense blast transformation with lymph node-type items exclusively lymphoproliferative, MGG, 1000 X

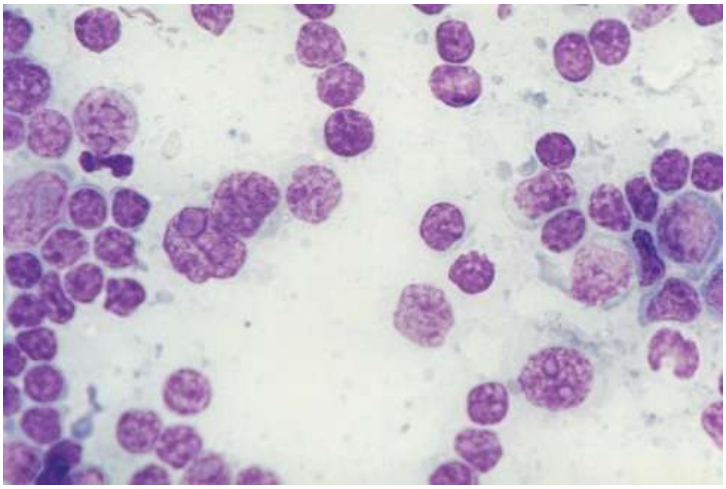

Fig. 4 - Pig lymph node - Intense blast transformation with lymph node-type items exclusively lymphoproliferative, MGG, 1000 X

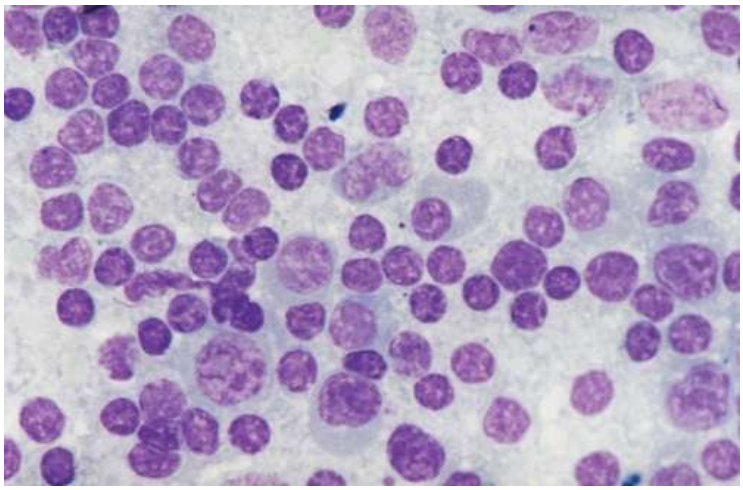

Fig. 5– Cattle lymph node - Same aspect with the appearance of the atypical lymphoproliferative cells, MGG, 1000 X

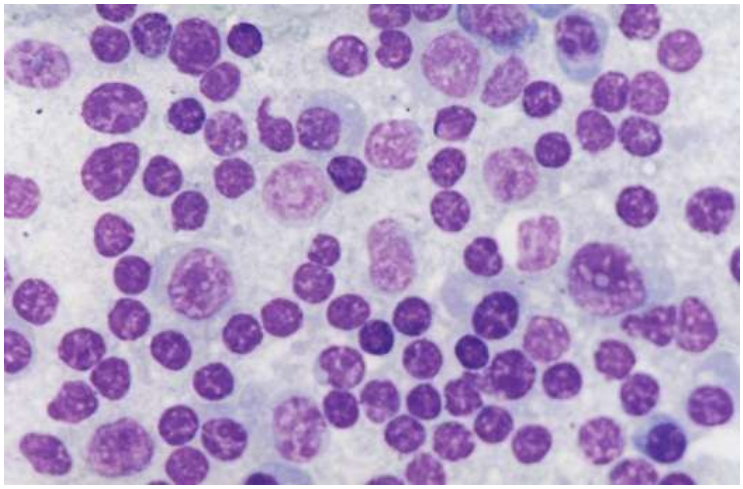

Fig. 6– Cattle lymph node - Same aspect with the appearance of the atypical lymphoproliferative cells, MGG, 1000

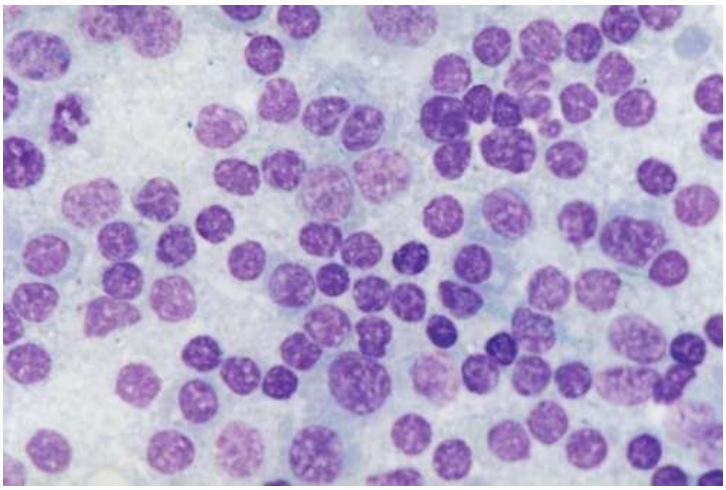

Fig. 7 – Cattle lymph node - Same aspect with the appearance of the atypical lymphoproliferative cells, MGG, 1000 X

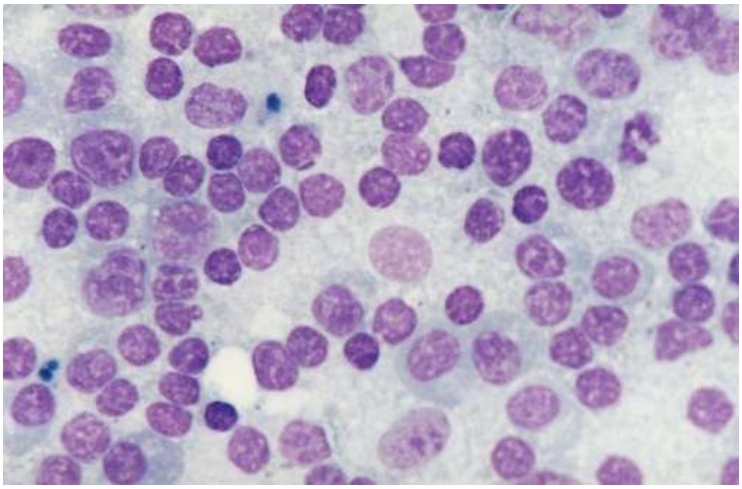

Fig. 8 – Cattle lymph node - Same aspect with the appearance of the atypical lymphoproliferative cells, MGG, 1000 X

CONCLUSIONS

From17 cases, a total of 13animals (9 pigs and 4cattles) wasn’t present visceral lesions that could cause such responses lymph node, so we can discuss this like a"lymph irritation syndrome" present in both species of

animals is due solely to potentially carcinogenic chemicals that are present in specific feeding of these animals.
At these two species could not put in evidence yet (due to the small number of animals investigated) the evolutive stages of this syndrome.

REFERENCES

Balint Emilia, 2010. Veterinary hematology and oncology compared. Old Court Publishing House, Bucharest.

Berceanu S., Manolescu N., Gogiu M., Colita D., Paunescu G., Avram N., 1985. Comparative Hematology. Medical Publishing House, Bucharest.

Faramarz Naeim, P. Nagesh Rao, Wayne W. Grody, 2008. Hematopathology.Academic Press.

Manolescu N. (as editor), 1995 .Aspects of comparative cellular pathology, vol I. Ceres Publishing House, Bucharest.

Manolescu N. (as editor), 1999. Aspects of comparative cellular pathology, Volume II. Teaching and Pedagogical Publishing House, Bucharest.

Manolescu N. (as editor), 2002. Aspects of comparative cellular pathology, Vol III. Ceres Publishing House, Bucharest.

Manolescu N. et al., 1978. Hematology guide to intensive livestock, Ceres Publishing House, Bucharest.

Manolescu N., 1976. Normal and pathological cytology of bone marrow and leukocyte concentrate in domestic mammals. Ceres Publishing House, Bucharest.

Micu D., N. Manolescu, Leukemic cells. Comparative study, 1981. Publishing House Of Romanian Academy, Bucharest.

Zachary James F., M. Donald McGavin, 2007. Pathologic Basis of Veterinary Disease. Mosby-Elsevier Publishing House.

FROM THE HISTORY OF THE ROMANIAN SCIENTIFIC SOCIETIES OF VETERINARY MEDICINE

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Abstract

The union of the young veterinary surgeons who decided to establish the Scientific Medical Society of Romania, at the Veterinary School of Bucharest. The "Statute of the Scientific Medical Society from Romania" drawn in the first meeting of 15 May 1871, were voted in the general assembly of August 14-16, 1871, chaired by Mauriciu Colben. The Scientific Medical Society didn't have its own journal upon establishment. Therefore, in 1879, Alexandru Locusteanu, Mihai Măgureanu, Gheorghe Perşu and Panait Constantinescu, publish the Veterinary Surgeon. Succeeding to dismiss some difficulties, 33 veterinary surgeons met on 10-12 May 1882 in Bucharest for the First Congress of Veterinary Medicine, the first of this kind in Romania. The topics of the congress, set in 1881, included the following subjects: sanitary police, animal husbandry, public hygiene, pathology, miscellanea. Once the Society of Veterinary Medicine was relaunched, the Journal of Veterinary Medicine, Animal Husbandry, Hygiene and Rural Economy also appeared. It has been established in 1888 too, by I. Şt. Furtună, and had an Annex, the Bulletin of the Society of Veterinary Medicine. These meetings debated the important problems of the veterinary pathology confronting that period: the foot and mouth disease, of horses glanders, tuberculosis, the bovine gastro-entero-nephritis (babesiosis) etc. The Society existed until July 21, 1949, when, after this last meeting, it was discontinued abusively by the communist regime. Because the centenary of the Romanian Society of Veterinary Medicine was closing, a group of teaching staff from the Faculty of Veterinary Medicine of Bucharest started in the spring of 1970, the activities necessary to resume the Romanian Society of Veterinary Medicine: it was re-established on May 15, 1971, 22 years after its discontinuation and one hundred years after its foundation.

Key words: history, scientific societies, veterinary medicine, Romanian.

In the Romanian Principalities, because no schools of veterinary medicine existed yet at that time, the authorities employed numerous "masters in surgery and veterinary medicine" who had graduated at Vienna, Pesta, Paris, Lyon etc., and who, after 1831, activated in different army units, in agreement with the "Military rules" (Druţu, 1906; Ioan and Marinescu, 1935; Simionescu and Moroşanu, 1984; Urechia, 1892-1901).

After several failed attempts to establish a school of veterinary medicine, in 1853 the first courses of veterinary medicine started in Bucharest, taught, as

the documents show, by the master in surgery Vasile (Wolfgangus) Lucaci (Figure 1), the future proto-veterinarian of Wallachia. The courses entitled "Lectures of veterinary medicine, particularly about the infectious diseases of the animals" were not taught, however, in an actual veterinary school, but in a school teaching mainly human medicine, including in the school established by Nicolae Creţulescu (Figure 2) which functioned between 1841-1846, within Coltea Hospital.
After the establishment of the "Medical human and veterinary education" in 1853, by Carol Davila (Figure 3), the veterinary medicine education was performed until 1859 at Mihai Vodă Monastery, in whose cells the Army Hospital has been functioning even since 1831. The first School of Small Surgery functioned here too (1853-1859), which subsequently became the School of Medicine and Pharmacy (Curcă, 2000; Gheorghe et al., 1982; Iftimovici, 1994; Pascu et al., 1988), whose professor of "veterinary medicine" was Vasile (Wolfgangus) Lucaci; human medicine was taught by Professor dr. Iacob Felix, Professor dr. Iuliu Teodori and Professor dr. L. Fialla.

Figure 1. State protoveterinarian Vasile Lucaci (1806 – 1890)

Figure 2. Professor Dr. Nicolae Creţulescu (1812-1900)

Figure 3. Professor General Doctor Carol Davila (1828-1884)

During the early days of the veterinary school (1853), after the establishment of the Veterinary School (1856) and until 1887 (Figure 4), the teaching activity was characterized by material difficulties and repeated changes of the regulations of operation. Most of the didactic staff consisted of military doctors, employees of army units, who were departing for military assignments and left aside the teaching activity (Curcă et al., 2002; Iorga, 1910; Iorga, 1928; Iorga, 1938).

Figure 4. 75 years anniversary of founding the Veterinary Medical Education in Bucharest-Romania

Figure 5. Central pavilion of the veterinary campus, on Splaiul Independence 105, build in 1885-1887

Among 1885-1887, the building of the Higher School of Veterinary Medicine was constructed at Splaiul Independenţei nr. 105 (Figure 5), the building plans being developed by architect Nicolae Cerkez. During period of construction the head of the School was dr. Ion Popescu (1885-1890).

At that time, the head veterinarian of the Romanian army was Mauriciu Kolben, from 1871 until his retirement in 1893. In this position, M. Kolben organised the military veterinarian service and struggled to send abroad veterinary doctors for training, particularly in animal husbandry and in the diagnosis and treatment of the contagious diseases in farm animals.

On August 27, 1873, the Ministry of Education announced a competition for the open positions of professor at the School of Veterinary Medicine. Louis Vincent was among the candidates for a position at the department of anatomy, physiology, zoology and surgery. In the same spirit, dr. Mihail Măgureanu the veterinarian of Bucharest volunteered to teach pro bono "meat inspection". After the ministry approved, the courses started in 1880 (see Medicul veterinar, 1880, I, p. 99).

In 1874, General E. Florescu, Ministry of War, established a commission consisting of several military officers among whom Professor Mauriciu Colben, the head veterinarian of the Romanian army, whose task was to purchase Thoroughbred Arabian horses from Arabia. In August 1874 they bought 10 stallions, which were sent to Nucet – Dâmboviţa stud, established at that time for this very purpose (••• 1988; Pascu et al., 1989).

In the first half of the 19th century, in the Romanian Principalities there was a feeling of necessity to establish a scientific forum which to debate the problems of the veterinary medicine profession. This need was particularly felt by the first graduates of the Veterinary School of Bucharest and it was supported by the current created by the human doctors and naturalists from Iasi who had established a Medical Circle of lecture (January 11, 1830), which later became the Society of Doctors and Naturalists from Iasi, headed by doctors Iacob Cihac and Mihai Zotta. A few veterinarians were among the active members of the society (Curcă, 2001-2002; Diaconescu, 2005). The members of the Society of Doctors and Naturalists from Iasi also published a periodic publication, initially called „Foaia Societăţii", which thereafter became „**Buletinul Societăţii de medici şi naturalişti**" din Iaşi (Bulletin of the Society of Doctors and Naturalists from Iasi); later it changed again its title, becoming "Revista medico-chirurgicală" (Medical-surgical review) which is regularly published up to the present time (Fig. 6).

Figure 6. The evolution of journal named "Society of Physicians and Naturalists of Iassy", from 1830 until now

Figure 7. Statutes of Scientific Medical Society of Romania, published in 1857

One of the major events which preceded the union of the Romanian principalities Walachia and Moldova (done by Alexandru Ioan Cuza in January 1859) was the union of the young surgeons who decided to establish the **Scientific Medical Society of Romania**. The "Statute of the Scientific Medical Society from Romania" (Figure 7), published in 1857, is a very interesting linguistic document, because it is typed on two columns: in the left column the text was types in perfect French, while in the right column the text was typed in a Romanian language of

transition, in which not just the words were sometimes approximate, but the text too had both Latin and Cyrillic letters (••• 1931; Bălan and Mihăilescu, 1985; Diaconescu, 2005; Simionescu and Moroşanu, 1984).

The purpose of the **Society of Veterinary Medicine** was to elevate the scientific prestige of the profession of veterinary surgeon. The documents showing this event are found in file 40/1871 in the State Archives, documents of the Ministry of the Interior, Medical Service. These documents show that on **15 May 1871**, 14 veterinarians drew the draft of a project regarding the "**Statute and Regulation of the Society of Veterinary Medicine**" which, together with a "Statement" were forwarded for approval by the Ministry of Cults and Public Education.

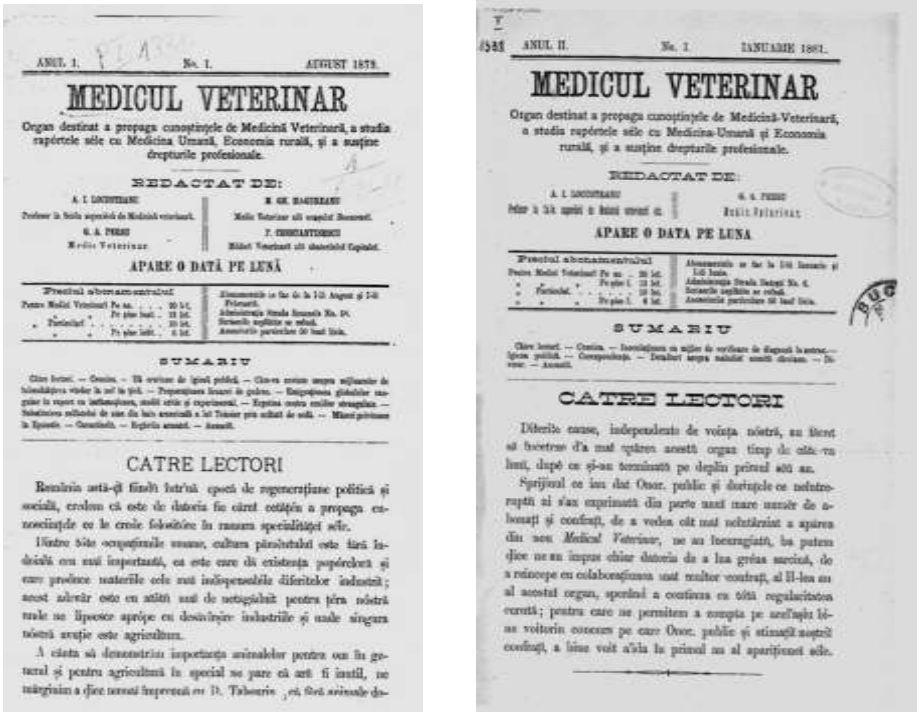
The "Declaration" specifies that the members of the **Society of Veterinary Medicine** aimed to "work jointly to perfect the different branches of the science trying to solve all matters" of interest: animal hygiene, reproduction and education of the domesticated animals, animal diseases, epidemics, contagious diseases and forensic medicine".

At the same time, the veterinary doctors pledged "always to consider the general interests and the dignity of their profession" and to "make disappear the obstacles they were confronted so far" so that the "science of veterinary surgeons develops freely", hoping that the "emulation among the veterinarians will bring useful and profitable developments for all".

This "Statement" was signed by: Professor Mauriciu Kolben, I. Popescu, I. Ioanin, I. Popovici, I. Georgescu, I. Constantinescu, M. Similache, Andronescu, G. Persu, Gh. Putzurianu, Th. Drăgănescu, D. Preotescu and two more signatures that cannot be deciphered (one for sure belonged to Louis Vincent).

The "**Statute of the Society of Veterinary Medicine from Romania**" drawn in the first meeting of **15 May 1871**, were voted in the general assembly of August 14-16, 1871, chaired by Mauriciu Kolben, assisted by Ion Popescu, with secretaries I. Georgescu and a signature that cannot be deciphered, treasurer Dumitru Preotescu, with elected members Panait Constantinescu and Louis Vincent.

The **Society of Veterinary Medicine from Romania** didn't have its own journal upon establishment. Therefore, in 1879, Alexandru Locusteanu, Mihai Măgureanu, Gheorghe Perşu and Panait Constantinescu, publish the „**Medicul veterinar**" -Veterinary Surgeon- (Figure 8) which, unfortunately, cease to appear after exactly 13 issues (Gomoiu V. – History of the Romanian medical press p. 134, Bucharest, 1936).



ANUL I. No. 1. AUGUST 1879.

MEDICUL VETERINAR

Organ destinat a propaga cunoştinţele de Medicină Veterinară, a studia raporturile sale cu Medicina Umană, Economia rurală, şi a susţine drepturile profesionale.

REDACTAT DE:

APARE O DATĂ PE LUNĂ

SUMARIU

CATRE LECTORI

ANUL II. No. 1. IANUARIE 1881.

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REDACTAT DE:

APARE O DATA PE LUNA

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Figure 8. First review of veterinary medical profession entitled "VETERINARIAN" - the first issue appeared in August 1879 and the last number Year II, appears in January 1880

Upon initiative of I. Şt. Furtună, by the time he was first year student of the Veterinary School, the students established, as of **October 19, 1880**, a scientific society, the "**Society of veterinary medicine students**" located on the premises of the "Higher School of Veterinary Medicine". It intended to have a "library" and a "journal" publishing scientific papers. The journal's title was "**Progresul veterinar**" and the board of editors was headed by student I. Şt. Furtună, until October 18, 1883, when he obtained the license of veterinary surgeon. In 1888, the society voted a new statute responding to the requirements of that time.

At the meeting of 15 February 1893 the "**Society of veterinary medicine students**" voted the publishing of a journal, as proposed upon the establishment of the society, but the name was „**Clinica veterinară**" (Figure 9). Much later, in February 1933, started to be published the "Journal of the Society of veterinary medicine students" (Figure 10).

The purpose of the journal was to publish the clinical observations, the results of the experiments conducted by the students in the laboratories of the Higher School of Veterinary Medicine and to publish translations of papers which the students need.

Figure 9. Veterinary Students Magazine named: Veterinary Clinic, published in February 1893

Figure 10. Veterinary Medicine Students Society Magazine, published in February 1933

In ”Clinica Veterinară” journal of the veterinary students, the inaugural paper of professor Paul Riegler was “Bacteriological research in glanders diagnosis, 1893”. His first publication was “A case of tuberculosis in parrots” and the coincidence was that his last paper was also on fowl tuberculosis, “A serious case of epizootic tuberculosis in a pheasant farm”.

The increase of the number of veterinary surgeons who graduated the Veterinary School of Bucharest and the decrease of animal export from the value of 28 million lei in 1877, to just 4 million lei in 1882, prompted the specialists in this field to meet and discuss the problems which caused this alarming decrease and to determine measures for rehabilitation.

Succeeding to dismiss some difficulties, 33 veterinary surgeons met on 10-12 May 1882 in Bucharest for the **First Congress of Veterinary Medicine** (Figure 11), the first of this kind in Romania. The topics of the congress, set in 1881, included the

following subjects: sanitary police, animal husbandry, public hygiene, pathology, miscellanea. Professor Al. Locusteanu presented a report on tuberculosis in humans and animals, proposing to assign this disease to the field of the sanitary-veterinary police. Another report concerned the gastro-entero-nephritis (Babesiosis) and it was presented by the veterinary surgeon of Dolj County, the future Professor Constantin Vasilescu.

Primul Congres National al medicilor veterinari 1882 -randul doi, pe scaune, de la stanga la dreapta: D. Curteanu, Panait Constantinescu, Pandele Constantinescu, M. Colben, G. Persu, A. Locusteanu, M. Magureanu, C. Fometescu

Figure 11. Participants on the first Congress of Veterinary diplomats in Romania, which took place on 10-12th May, 1882, where they discussed issues of veterinary police, public hygiene, veterinary and animal pathology

From 6 to 8 October 1884, the "**First Congress of the human doctors, veterinary surgeons and pharmacists**" took in Bucharest, and Louis Vincent was one of the secretaries. The idea of this meeting of the sanitary specialists was born at Turnu-Măgurele in 1877, being proposed by Iacob Dimitrie Felix, the commander of the army hospitals from that area and by Carol Davila, head of the sanitary service of the Romanian army, whose assistant was the veterinary surgeon Mihail Măgureanu (alias Mihail Ghiuță), who helped him to organize the Romanian troops for the assault on the fort from the right bank of the Danube.

The Romanian Society of Medical Sciences appointed a commission which to prepare and work out the Regulation of the Congress of the Romanian sanitary corps. The provisional secretary of the commission was Louis Vincent (newspaper "Universul", nr. 28, of 21 September 1884, p. 2). The Regulation stipulated three sections: **medical, veterinary** and **pharmaceutical**; each section was to hold

separate meetings, and all sections were to meet in general meetings. Among the participants in the Congress there also were 29 veterinary surgeons.
In the general meeting of October 6, 1884, Professor I. Felix presented the report "About disinfection". After the general meeting from the first day of the Congress, the meeting of the veterinary section took place in the same afternoon one to speak was Louis Vincent, who showed the importance of diagnosing and controlling the disease and proposes a program to study systematically this disease.
On the evening of October 8, 1884, the final day of the Congress, the 130 participants attended the banquet organized at Boulevard Hotel, with sentimental "toasts" of "fraternisation" between the human and veterinary doctors in the field on the compared pathology (newspaper "Universul" of 11 October, 1884, p. 2). This spirit of collaboration didn't die with the end of the Congress.
The collaboration in the field of compared pathology was resumed at a higher intensity in 1964, after the establishment of the **Society of Compared Pathology**, within the Union of the Societies of Medical Sciences; however, the society ceased to function after a period. After 2002, this activity was resumed within the **Institute of Compared Medicine**, approaching various fields of interest.
Given the necessity of discussions to clarify the different scientific matters in the field of veterinary medicine, increasing in number and complexity, during the meeting of November 6, 1888, following the call by Gh. Ionescu Brăila, D. Curteanu and Louis Vincent, addressed to all the veterinary surgeons all across Romania, 38 responded and 21 actually participated in the meeting which revived the activity of the Romanian Society of Veterinary Medicine. The meeting took place on the premises of the Higher School of Veterinary Medicine. This call was prompted by the rather obscure activity of the Romanian Society of Veterinary Medicine at that time.
In the name of the steering committee, Louis Vincent showed that the large number of veterinary surgeons attending the meeting is a sign of approval of the purpose proposed by the steering committee and passed to the establishment of the provisional office. M. Măgureanu is elected as provisional President, while N. Focşa is elected as secretary. The meeting elected a commission consisting of Al. Locusteanu, Șt. I. Furtună, Paul Oceanu and D. Curteanu, who drew up a project of statute of the Society of Veterinary Medicine of Bucharest, written and approved within the same meeting of November 6, 1888 (Bulletin of the Romanian Society of Veterinary Medicine, 1888-1889, p. 3-7). During the same meeting, Louis Vincent is elected secretary general, and subsequently, Vice-president of the Romanian Society of Veterinary Medicine.
Once the Society of Veterinary Medicine was "relaunched", the **Journal of Veterinary Medicine**, Animal Husbandry, Hygiene and Rural Economy, chaired by Professor M. Kolben elected honorary President; Professor Al. Locusteanu, executive President; M. Măgureanu and Louis Vincent, Vice-presidents; Professors Gavrilescu C. and N. Mihăilescu, were appointed secretaries (Georgescu et al.,

2006). During the works of the section, Professor Al. Locusteanu presented the report: „Disinfection of the people and animals in case of cattle plague" (Ioan and Marinescu, 1935; Simionescu and Moroşanu, 1984).
In the meeting of the medical section from October 7, 1884, the president of the section, Professor C. Severeanu, announced that the report of Professor Al. Locusteanu planned "to be delivered during the general meeting", will be delivered within this section attended by veterinary surgeons too. Another report, "Relation between human tuberculosis and animal tuberculosis and hygienic measures to limit it" described information based on findings of experimental compared medicine and of own research. The etiological agent of the disease had been recently isolated and grown by R. Koch, in 1882, followed by a study on the etiology of tuberculosis in humans and animals (1884). On the third day, on October 8, 1884, within the veterinary section, the topic of discussion concerned the gastro-entero-nephritis, because in 1878 this disease killed over 50,000 cattle. The first
also appeared (Figure 12). It has been established in **1888** too, by I. Şt. Furtună, and had an Annex, **the Bulletin of the Society**. The Bulletins of the Society, reporting the regular debates, show that Louis Vincent was one of the most active animators of the debates and there was almost no meeting in which he would not speak, playing a decisive role in drawing the conclusions. These meetings debated the important problems of the veterinary pathology confronting that period: the foot and mouth disease of horses, glanders, tuberculosis, the bovine gastro-entero-nephritis (babeiosis) etc. (Curcă et al., 2001; Curcă, 2002). Although he was quite young, Louis Vincent, who's many sided competence was notorious, was appointed in 1892 in the commission for the development of the third edition of the Romanian pharmacopeia, where he works intensely. This edition was published in 1893.

Figure 12. Journal of Veterinary Medicine, Livestock, Rural hygiene and Economics, under direction of I. Șt. Furtună, head Veterinary service of the General Medical Service. The magazine is published in April of 1888, in city Focșani

The first number of the **Bulletin of the Romanian Society of Veterinary Medicine** appeared in **December 1888** and included the proceedings of the meeting of November 6, 1888, when the Romanian Society of Veterinary Medicine was established and when it statute was drawn up. The meeting of January 4, 1889 discussed and voted the Regulation of the Romanian Society of Veterinary Medicine (Bulletin of the Romanian Society of Veterinary Medicine, 1888-1889, p. 33-55).

The society existed until July 21, 1949, when, after this last meeting, it was discontinued abusively by the communist regime (••• 2001; Simionescu and Moroşanu, 1984). Nevertheless, the spirit of the scientific activity within the Society didn't vanish; the veterinary surgeons working within the Faculties of Veterinary Medicine, of the related research institutes (I.N.Z., I.P.I.A.,"Pasteur" Institute, etc.), continued their activity within the **Scientific Circles from the Faculties** of: **Bucharest** (1949-1971), **Arad** (1949-1957), **Iassy** (1961-1971), **Cluj** (1962-1971) and **Timișoara** (1962-1971). Because the centenary of the Romanian Society of Veterinary Medicine was closing, a group of teaching staff from the Faculty of Veterinary Medicine of Bucharest started in the spring of 1970, the activities necessary to resume the Romanian Society of Veterinary Medicine: it was re-established on May 15, 1971, 22 years after its discontinuation and one hundred years after its foundation.

This is the merit of the veterinary medicine profession from Romania, of the veterinary surgeons acting as teaching staff in the Faculties of Veterinary Medicine and of the specialists from the Research Institutes with medical-veterinary profile who, by their work and support for the steering group, by their perseverance and ability, managed to convince the decision-makers of that time to approve the re-establishment of the Romanian Society of Veterinary Medicine, as well as its statute (Figure 13).

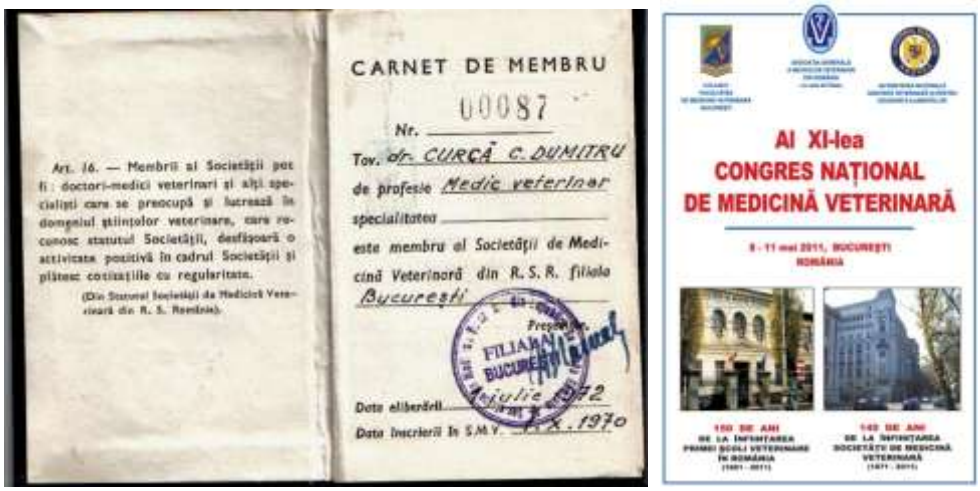

Figure 13. Memberships card with number 00087 - the Society of Veterinary Medicine, with an enrollment date in the Company 1. X. 1970, Prof. Dr. Doc. Şt. Neculai Stamatin signature, the presidents of Bucharest branch (left); 140 years anniversary since its establishment of the Romanian Society of Veterinary Medicine (right).

The Society of Veterinary Medicine, organized festivity omagial of in the 27.11.1970, at Faculty of Veterinary Medicine Bucharest, on the occasion of fulfillmeny of 90 years of life the scholar Academician Professor dr. Alexandru Ciucă, President at the Society of Veterinary Medicine Professor univ. dr. Octavian Vlăduţiu (Figure 14).

Figure 14. Omagial festivity organised of the Society of Veterinary Medicine, in the 27.11.1970, at Faculty of Veterinary Medicine Bucharest, on the occasion of fulfillmeny of 90 years of life the scholar Academician Professor dr. Alexandru Ciucă (left), President at the Society of Veterinary Medicine Prof. dr. Octavian Vlăduțiu (right).

As long as it functioned, judging by the content of the proceedings and of the research published in its Bulletin included in the **Veterinarian Archives** (Figure 15), the journal of the Didactic Corps of the Faculty of Veterinary Medicine, the Society of Veterinary Medicine brought a substantial scientific contribution to the development of the veterinary medicine in Romania, thus accomplishing the goal of its establishment on May 15, 1871.

Professor Paul Riegler published studies and notes on the tuberculosis and para-tuberculous bacilli, on glanders-action of the bacilli; on glanders toxins; on serum therapy; on the experimental transmission in cattle; on the bactericidal coal and on vaccinations; on the symptomatic coal; on experimental transmission and treatment of dourine; on agalactia in the Romanian sheep; as well as other important papers which were published in the Veterinary Archives, journal which he established in 1904 and which he headed for decades (••• 1987; Stancu, 2002). He has been in the board of editors of the Journal of Veterinary Medicine and he published in 1896, 1897, and 1898 the "Bulletin and Memories of the Romanian Society of Veterinary Medicine".

Figure 15. "Veterinary Archives" review appeared on first in March 1904 (left), on cover 4 ads for instruments and equipment for veterinary and human surgery, laboratory microscopes and laboratory instruments, founded by Prof. P. Riegler 1867-1936 (right).

At its meeting on 21 May 1913 of the III National Veterinary Congress, after extensive debates, it has been voted the following "Resolution": This "resolution" was reached because the old Society for Veterinary Health "included only 40 members," as stated in the meeting Prof. Al. Locusteanu and he added that "people should not leave unprotected animal body injustice".

Was first appointed a Commission to draw up draft statutes of the Association then, on **29 June 1914**, it held its constituent meeting of the **General Association of Veterinarians in Romania** (AGMVR), chaired by Prof. Al. Locusteanu. In this meeting, at which 138 veterinarians sent accessions, voted to elect the first committee status and then intervening the First World War until 19 august 1919, by decree of law, the association is recognized as a legal and moral person.

Even before getting the official recognition, it had a "Bulletin" published from January 1, 1919, in the first issue of **Journal of Veterinary Medicine and Zootechnie** (Figure 12), who was none other than former Veterinary Magazine, founded in **1888,** by **Șt. Ion Furtună**, and which, under the new title, is offered to be put on Association property.

Enthusiastically created by an initiative group, the Association, through art. 4 of the Statute, states its goal of its existance, namely: "to cultivate the sense of esteem, fraternities and peer support, defend the right of members and general professional interests, to create an economic background, to set up a bulletin of it and build their own local library, conference room and so on". All the goals set have been performed for over 30 years because in 1949, the association has ceased existence, just as the Society for Veterinary School attached to the Faculty of Veterinary Medicine, founded in 1871.

After 1989 the AGMVR was reestablished and the duties of the SMV were taken over by it. Between 8 to 11 May 2011, in Bucharest, held the prestigious national event "**The XIth Congress of the General Association of Veterinarians Romania**". Congress was held under the title "**2011 - World Year of Veterinary Medicine**" (Figure 13).

The XIth Congress marked two major professional events: **150 years** since the founding of the first veterinary school in Romania and **140 years** since the **establishment of the Society of Veterinary Medicine**.

"**Society for Animal Protecţiunea**" since its founding, was concerned about the protection of animals, giving a great importance to the human and economic problems Figure 16 and Figure 17). Its aim was beginning to convince the owners that it is in their interest to take care of animals, children should not be cruel to them and the teachers and priests to propagate the ideas of animal protection (••• 1904).

Figure 16. The statutes of Society for Animal Protecţiunea published in 1904

Figure 17. Act of approval of the Statute in MONITORUL OFICIAL No. 64, from 24 June-4 July 1905

Among its activities is remarkable setting up different services on behalf of animals, educating people on animal husbandry and care, monitoring enforcement of animal protection (Figure 18 and Figure 19), and a very clear editorial focus between 1907-1913. In the first years of the Society for Animal Protecţiunea Bucharest activity, was present through all those present both in human life but especially in the animals life (••• 1907

Figure 18. Brochure intitled: "Our Birds". Edition II-a

Figure 19. Scientific work "The killing of cattle for food" by E. Perietzianu-Buzeu, published in 1908

In 1929, the **Scientific Society of the Military Veterinary Corps** was established and it had 244 members in 1933, assigned to seven circles: a central one in Bucharest and six branches in Bârlad, Iaşi, Chişinău, Sibiu, Timişoara and Focşani. The meetings of these circles were also often attended by civil veterinarians from those towns.

Once the Scientific Society of the Military Veterinary Corps had been established in 1929, the **Military Veterinary Journal** also appeared (Figure 20 a and b.), scientific publication of the Military Veterinary Corps, under the direction of the general veterinarian Vintilă Rădulescu (1879-1937), head of the army Veterinarian service, an illustrious personality of the entire Romanian Military Veterinary Corps (Figure 21).

A meritorious contribution to the activity of the Romanian Society of Veterinary Medicine came from the military veterinarians, recruited among the graduates of the Veterinary School of Bucharest, some of them being: Dumitru Preotescu, Ion Popescu, Panait Constantinescu, Gheorghe Perşu, Constantin Gavrilescu, Pandele Constantinescu, Nicolae Străulescu, Gheorghe Udriski (Curcă, 2002; Curcă et al., 2002; Picu, 2005). Several military veterinarians also had important publishing activity, either by writing scientific articles, or by issuing books that were very appreciated at that time. Of these, many scientific articles being published in the Military Veterinary Journal, we cite:

- The veterinary captain Nicolae Moga published the brochure: "**The Golubatz fly**", edited in Bucharest, in 1891, which actually is his PhD thesis for the title of

veterinary surgeon, from 1890; another brochure was "Horse and cattle breeding in Romania and sown pastures", published in 1904 la Brăila; "Bee farming", published in 1905, at Brăila;

Figure 20. a, b Military Veterinary journal

Figure 21. General Vintilă Rădulescu, veterinarian, vet army chief

The veterinary general Grigore Hortopan (1880-1957), is the author of a Veterinary Encyclopaedia (Hortopan, 1912) with the subtitle "Rearing and diseases of the domesticated animals", published in Bucharest, with more than 900 pages (Figure 22).

The aim of this encyclopedia was to gather the knowledge on "breeds, breeding, rearing, maintenance and healing the diseases of animals", based on a substantial literature and approaching the whole field, insisting on the anatomy, physiology and pathology of the domesticated animals. It aimed to answer some of the stringent needs of that time concerning the improvement of animal productions by improving the animal breeds (in cattle, pigs, sheep, poultry etc.);

- The veterinary general Petre Stavrescu published two papers, each with more than 600 pages: "Science of horse rearing in modern conception", published in 1930, at Bucharest, and "Hypology" (Stavrescu, 1900), published in 1900 (Figure 23), Bucharest, the latter one receiving the Adamache award of Romania Academy.

The professors from the Faculty of Veterinary Medicine participated actively in the life of other scientific and professional societies too. Thus, Paul Riegler was founding member and several times President of the Society of Biology, Vice-president of the Academy of Medicine, of the Romanian Royal Society of Medicine History, member of Société de Pathologie comparée from Paris, Vice-president of the Romanian General Association of the Veterinary Surgeons, etc.

Figure 22. Growth and animal disease, by veterinary General Gregory Hortopan, Second Edition

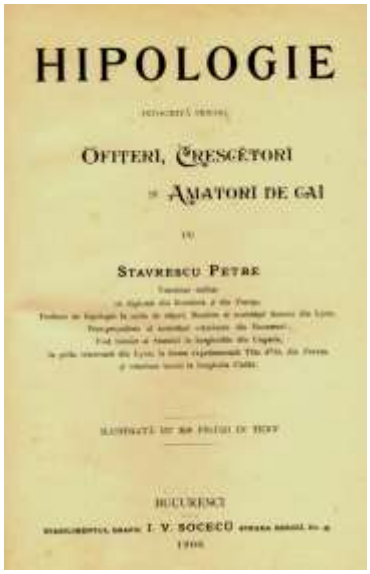

Figure 23. Hippology for officers, breeders and fans of horses, by veterinary General Petre Stavrescu

Of the scientific societies which supported the progress of biology we may mention the "Society of sciences from Bucharest" (1897), from which the "Romanian Society of Naturalists" split in 1899, with its publications edited starting from 1901; the „Society of Biology", established in 1907 by V. Babeş, Paul Riegler, I. Cantacuzino, Gh. Marinescu, I. Athanasiu and D. Voinov, at Bucharest, under the name of „Biological Association" and which published starting with 1911 the „Annals of biology". At the same time, Cantacuzino also was the founder of the "Journal of Medical Sciences", "Annales de Biologie" and "Archives Roumaines de pathologie experimentale et de microbiologie".

REFERENCES

••• 1931. 75 de ani de la întemeierea învăţământului medicinei veterinare în România, 1856-1931, Tipografia Cultura, Bucureşti.
••• 2001. Alma Mater Veterinaria Bucurescensis la a 140-a aniversare, Ed. All, Bucureşti.
••• 1987. Personalităţi din trecutul medicinei veterinare, Vol. I, Societatea de Medicină Veterinară, Bucureşti.
••• Statute – Regulamente - Înştiinţări, 1904, Bucureşti. Atelierele grafice I.V.SOCECU, 16 p (Societatea pentru Protecţiunea Animalelor, fondată în aprilie 1904 sub patronagiul A.S.R. Principesa Maria).

••• Păsările noastre - Apel către şcolari, nr.1, 1907, Bucureşti, Minerva Institut de Arte Grafice şi Editură, 16p (Societatea pentru Protecţiunea Animalelor, recunoscută ca persoană morală şi juridică sub înaltul patronagiu al M.S. Reginei şi Preşedenţia de onoare a A.S.R. Principesa României).
••• Despre necesitatea Protecţiunei Animalelor din punct de vedere social şi economic, 1907, nr.6, Bucureşti. Minerva Institut de Arte Grafice şi Editură, (Societatea pentru Protecţiunea Animalelor, recunoscută ca persoană morală şi juridică sub înaltul patronagiu al M.S. Reginei şi Preşedenţia de onoare a A.S.R. Principesa României).
Bălan Şt., Mihăilescu M., 1985. Istoria ştiinţei şi tehnicii în România, Ed. Academiei Române, Bucureşti.
Curcă D., 2000. First observations on monodactylism (sindactylism) in swine made by Professor C.N. Vasilescu between 1890-1894, 31th International Congress on the History of Veterinary Medicine, 6-10 September, Brno, Czech Republic, Book of abstracts, 64-65.
Curcă D., 2002. The Romanian Scientist - Prof. Victor Babeş, Historia Medicinae Veterinariae, 27, 5-6, 333-347.
Curcă D., 2002. Formarea primelor şcoli de agricultură şi a celor de medicină veterinară din România, Simpozion Facultatea de Medicină Veterinară, Bucureşti, Rezumat 11-13.
Curcă D., Ioana Cristina Andronie, Andronie V., 2001. Romanian priorities in control and eradication of epizootic diseases in veterinary medicine, The 32nd Congress on the History of Veterinary Medicine, 15-19 August, Oslo, Norway, Abstracts, 20-21.
Curcă D., Ioana Cristina Andronie, Andronie V., 2002. The establishment of the first Agricultural Schools and Veterinary Medicine Schools in Romania, Lutherstadt Wittenberg, Germany, 21-24 august, Proceedings: 117-127.
Curcă D., 2001-2002. Romanian priorities in control and eradication of epizootic diseases in veterinary medicine, Lucrări ştiinţifice U.Ş.A.M.V., Seria C, vol. XLIV-XLV, 127-140.
Diaconescu M., 2005. Oameni şi fapte din istoria medicinii militare româneşti, Vol. I and II, Editura Pro Transilvania, Bucureşti.
Druţu Ch. D.,1906. Istoricul învăţământului agricol în România, Institutul de Arte Grafice „Carol Gőbl", Bucureşti.
Georgescu B., Predoi G., Cornilă N., 2006. Profesorul Constantin Gavrilescu (1865-1941), Rev. Rom. Med. Vet., vol. 16, 1, 141-150.
Gheorghe Fl., Popescu M., Rotaru I., 1982. Prezenţe militare în ştiinţa şi cultura românească. Editura Militară, Bucureşti, 121-122.
Hortopan Gr., 1912. Enciclopedie veterinară. Creşterea şi boalele animalelor domestice. Noţiuni de hipologie, Editia a II-a, Tipografia „Revista Geniului", Bucureşti.
Iftimovici R.,1994. Istoria medicinei, Ed. All, Bucureşti.
Ioan D., Marinescu N., 1935. Istoricul învăţământului sanitar militar în România, Tiografia „Ion C. Văcărescu", Bucureşti.
Iorga N., 1910. Viaţa şi domnia lui Barbu Dimitrie Ştirbei domn al Ţerii Româneşti (1848-1856), Vălenii de Munte.
Iorga N., 1928. Istoria învăţământului românesc, Bucureşti.
Iorga N., 1938. Istoria Românilor, vol. VIII, Bucureşti.
Pascu Şt. et al.,1988-1989. Istoria militară a poporului român, vol. V şi VI, Centrul de Studii şi Cercetări de Istorie şi Teorie Militară, Editura Militară, Bucureşti.
Picu Valeria Maria, 2005. Istoria Şcolii de Medicină Veterinară din Bucureşti, Ed. Ceres, Bucureşti.

Simionescu C., Moroşanu N., 1984. Pagini din trecutul medicinii veterinare româneşti, Ed. Ceres, Bucureşti.
Stancu I., 2002. Reprezentanţi de seamă ai medicinei veterinare româneşti (1856-2001), Ed. Coral Sanivet, Bucureşti.
Stavrescu P., 1900. Hipologie întocmită pentru ofiţeri, crescători şi amatori de cai, Stabilimentul grafic I.V. Socecŭ, Bucuresci.
Urechia V.A., 1901. Istoria şcoalelor de la 1800-1864, vol. IV, Impremeria Statului, Bucureşti.

STUDY OF THE AXIAL SKELETON IN THE GIRAFFE (*GIRAFFA CAMELOPARDIS*) COMPARED TO ITS BOVINE COUNTERPART

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Abstract

The giraffe (Giraffa camelopardis) is known as the tallest land mammal of African origins. Its unique evolution has led to the development of singular morphological features. Its rare presence outside its natal continent makes the giraffe a rarely studied species in our country, which makes any new research regarding this species a valuable addition to our anatomic database. ***Materials and Methods:*** *The study was performed in the Comparative Anatomy Laboratory of the Faculty of Veterinary Medicine of Cluj-Napoca. One giraffe body and two cow skeletons were used to determine the anatomical differences between the thoracic, lumbar sacral and coccygeal segments of the vertebral column, the ribs and the sternum in these two species. The body was processed through usual techniques maintaining the thoracic skeleton intact due to the individual's young age. The cow skeletons belong to our ossuary collection. We have chosen to compare the giraffe to the cow, because this is the reference ruminant for veterinary anatomy, well studied both in our country and on an international level.* ***Results and Conclusion:*** *The main differences, underlining biomechanical consequences, were registered in the thoracic, lumbar, sacral segments and in the sternum. The giraffe has an extra thoracic vertebra and an extra pair of ribs, but it has a smaller thoracic length ratio to the total body length. The lumbar and sacral segments each lack a vertebra. The sternum is narrow. The dorsal profile of the trunk has a more pronounced decline in height from the withers to the lumbar segment, and its general shape is dorso-ventrally narrowed to its extremities, making it more compact than in bovines.*

Key words**:** giraffe, cow, trunk, skeleton, anatomy.

INTRODUCTION

The giraffe is rarely studied outside its natal continent, Africa, as it is found only in zoological parks and certain circuses. This research adds to the reduced osteology database currently available on this species, by comparing its trunk skeleton, along with the coccygeal segment to the main reference domestic ruminant species, the cow, and underlines its particular features.

The giraffe (*Giraffa camelopardalis*) is a species of the *Giraffidae* family, along with only one other living species, the okapi. In the prehistoric period, this family contained a great number of *genera.* Stevens (1993), states that the early giraffes resembled the prehistoric antelopes form Europe and Asia, 30-50 million of years ago, and have progressed since then from one meter to three meters in height. The early Miocene presented a stag-like giraffid, the *Climacoceras,* and forward to the middle of the Miocene there two genera, *Palaeotragus* and *Samotherium*, an increased withers height and developed ossicones, similar to today's giraffe, but with still shorter necks. At end of Pilocene, a drastic reduction of the giraffids took place, ending in only two remaining species (Prothero and Schoch, 2003). The remaining genus *Giraffa,* survivor from the Pliocene, contained a list of long-necked species, like the *Jumae giraffa,* some of which did not propagate to the modern era (Savage and Long, 1986). The modern giraffe is the tallest land animal mostly due to its extremely long neck, which can represent half of its total height (Henderson and Naish, 2010), but it also has a great whither height.

MATERIALS AND METHODS

The research was carried out in the Comparative Anatomy Laboratory of the Faculty of Cluj-Napoca, using one giraffe body donated by a circus transiting through Cluj-Napoca and two bovine skeletons belonging to the ossuary of our department. The trunk segment of the giraffe body was obtained using common techniques adapted for the age group of the individual who was under one year old, and had not reached adult development.

Due to the fragility shown by the cervical segment during thermal processing, we have opted to maintain the joints between the thoracic vertebrae, the ribs and the sternum, obtaining a single thoracic piece. After preserving the various body segments at -18ºC, the thorax was de-frozen, and its gross soft tissues were removed of the vertebrae, the ribs and the sternum. The piece was then kept in a 10% formaldehyde solution for 4 days, and underwent a second mechanical cleaning. Finally it was hung to dry in a constant temperature environment.

The lumbar and sacral segments were thermically processed using detergent and whitening solutions, at lower temperatures than usually used for adult skeletons.

After obtaining the giraffe osseous pieces, we have macroscopically examined the distinctive surface features of the thoracic, lumbar, sacral and coccygeal vertebrae, of the ribs and of the sternum, in the two examined species.

RESULTS AND DISCUSSIONS

In cows, there are 13 thoracic vertebrae with short well-developed bodies, with a strong ventral ridge. Their transverse processes are present, smaller than in horses, and the articular processes are reduced and flat (Fig.1A). Except for the last thoracic vertebra, the intervertebral foramina go through the caudal edge of the laminae of the vertebral arch. The spinous processes are thick on their entire length and end with tuberosities. They reach the maximum height in the T2-T5 vertebrae (Fig.1B). Starting from the 5th vertebra, the spinous process decreases up to the 10th vertebra. The spinal processes have a pronounced dorso-caudal orientation (caudoversion) op to T12, which is vertical, and the last vertebra (T13) may have a dorso-cranial orientation (cranioversion) (Barone, R., 1966; Damian, A., *et al*., 2001).

Fig.1. A - T7 in the cow, cranial view; B - Vertebral column of the cow, lateral view.

The giraffe presents an extra vertebra, having a total of 14 thoracic vertebrae (Fig.2B). Due to maintaining the thorax piece intact, this segment was examined only for gross characteristics, without being able to visualize the details. However, we have been able to asses the main differences between species. The vertebrae body is shorter and finer in the giraffe, and the ventral ridge is not prominent (Fig. 2A). The transverse processes, like the cow's are poorly developed (Fig.45). The spinous processes in the giraffe are narrower than those of the cow, but their width is constant along their length. Their height increases suddenly from la T1 to T2, reaches a

maximum at T2 - T4, then it decreases more than in bovines up to the lumbar segment. The first spinal processes are almost vertical, the T4 - T7 segment presents a slight caudoversion, which disappears at the last vertebrae, which are vertical, lacking the cranioversion displayed by cows.

Fig.2. A - T1 in the giraffe, cranial view; B - vertebral column of the giraffe, lateral view.

Bovines have 6 lumbar vertebrae with long and massive bodies, narrow ventral ridges and short but broad spinal processes, decreasing in height form the first to the last (Coțofan, V., *et al*, 1999). The transverse processes are long, narrow and slightly bent forward, having a general aspect of „heron wings" (Fig.3A). Their length increases up to L5, while L6 is equal to L3 (Barone, R., 1966). The mammillary processes are united with the cranial articular surfaces, well developed and form together a particular aspect of „buffalo horns" (Damian, A., *et al*., 2001).

The giraffe specimen has only 5 lumbar vertebrae (Fig.3B), denoting a shorter lumbar region in this species. These have well developed bodies, but they are shorter than in bovines, with reduced ventral ridges. The spinous processes are short but well developed, ending with tuberosities, decreasing slowly from L1 to L5, and bending slightly caudally. The transverse processes are less developed in the giraffe, still decreasing from L1 to L4, with narrow bodies, leaving large spaces between them. Their edges are smoother than in cows and a lot less convex, missing the "heron wings" resemblance. The mammillary processes are united with the cranial articular processes, but the "buffalo horns" aspect is much reduced.

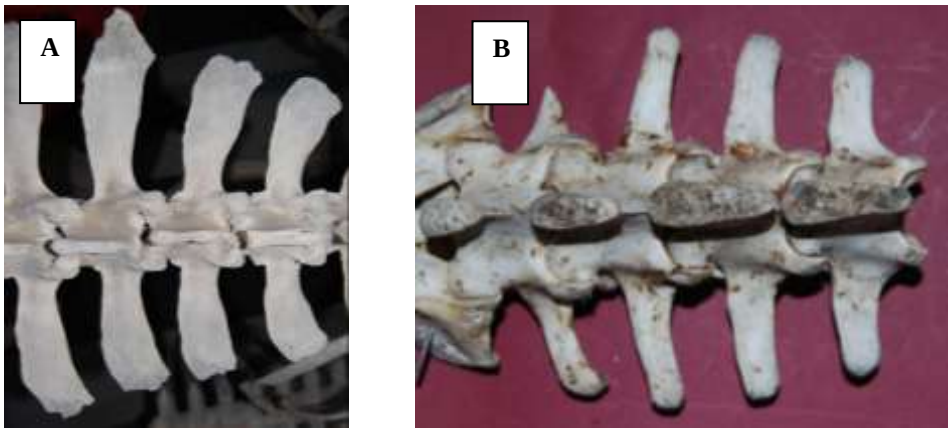

Fig.3. Lumbar segment in the cow (A) and in the giraffe (B), dorsal view.

The sacral vertebrae are united forming the sacrum. The cow presents 5 sacral vertebrae with flattened bodies. The spinous processes are completely fused, forming a median sacral crest (Coţofan, V., *et al*., 1999) with a rough edge. The 4 superior sacral foramina are partially covered by a well developed mammillary ridge, except for the last one which is very large. The articular head is flat, and the first cranial articular processes retain the "buffalo horns" aspect form the lumbar segment. The lateral edges, former transverse processes, are slim, sharp, slightly curved ventrally and rapidly decrease in size form the first element (the sacral wings) to the last. The cranial opening of the sacral canal is shaped like a dorso-ventrally lattened triangle. The ventral side of the sacrum is concave cranio-caudally (Fig.4A)(Barone, R., 1966; Damian, A., *et al*., 2001).

The giraffe has an even smaller sacrum, with only 4 vertebrae (Fig.4B). These have well developed bodies, but, due to the age of the animal they are not fused. The spinous processes are proportionally larger than in the cow, but narrower, ending with well developed tubercles. The mammillary crests are poorly developed, incompletely fused and do not cover the 3 supra sacral foramina. Both supra and sub sacral foramina have a uniform diameter, lacking the obvious variations found in bovines (Fig.4A,B). The transverse processes are smaller than in bovines, with thick rounded edges. The articular head is flat, but the cranial articular processes are simple, lacking the "buffalo horns" aspect. The cranial opening of the sacral canal is shaped like a equilateral triangle. The ventral side has a more pronounced concave profile than in cows.

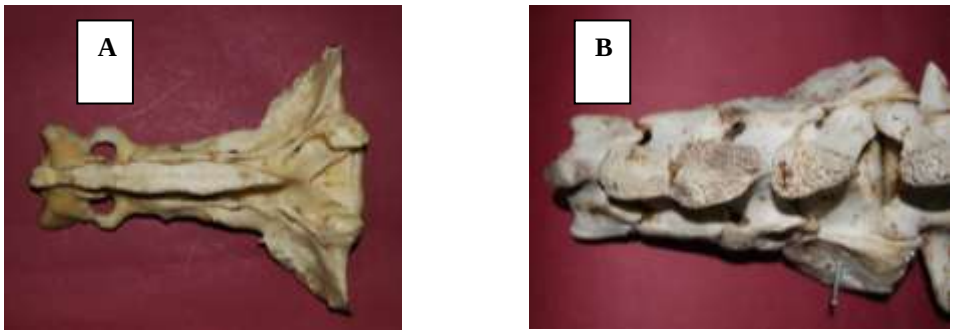

Fig.4. Sacral segment in the cow (A) and in the giraffe (B), dorsal view.

The cow has a well developed tail, containing 18 to 21 vertebrae (Barone, R., 1966; Vasile Coţofan et al., 1999). These coccygeal vertebrae loose their neural arch starting with the 4th one, and the other elements from the 10th one to the last. The first coccygeal segments present a ventral hemal arch.

The giraffe has a proportionally shorter tail, compared to its body size, than the cow. The studied specimen presents 18 coccygeal vertebrae. Their bodies are longer and smoother than in bovines, loosing their surface elements in the same fashion (Fig.5A,B).

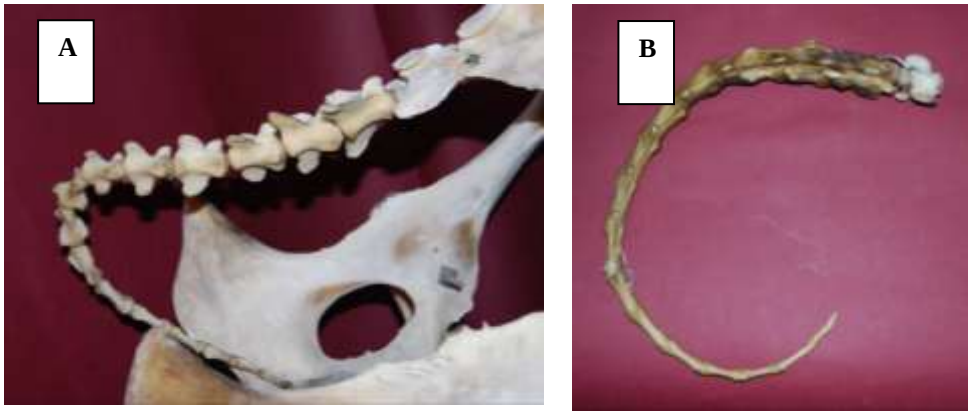

Fig.5. Coccygeal segment in the cow (A) and in the giraffe (B), lateral view.

The cow has 13 pairs of ribs, of which 8 are sternal and 5 floating. They are long, not very arched, with litle mobility (Fig.6A). Their inferior segment is very broad with sharp and thin edges. Their neck is long and their articular

head, well developped (Barone, R., 1966).
The giraffe has 14 pairs of ribs, showing an extra pair of floating ribs. They are not excesively arched, narrower than those of the cow, with rounded edges (Fig.6B) Just like in bovines, the osseous segments of the ribs do not present only a slight ventro-caudal tilt. The costal cartilages are longer and more cylindrical than those of the cow, and the hypocondrum is finer.

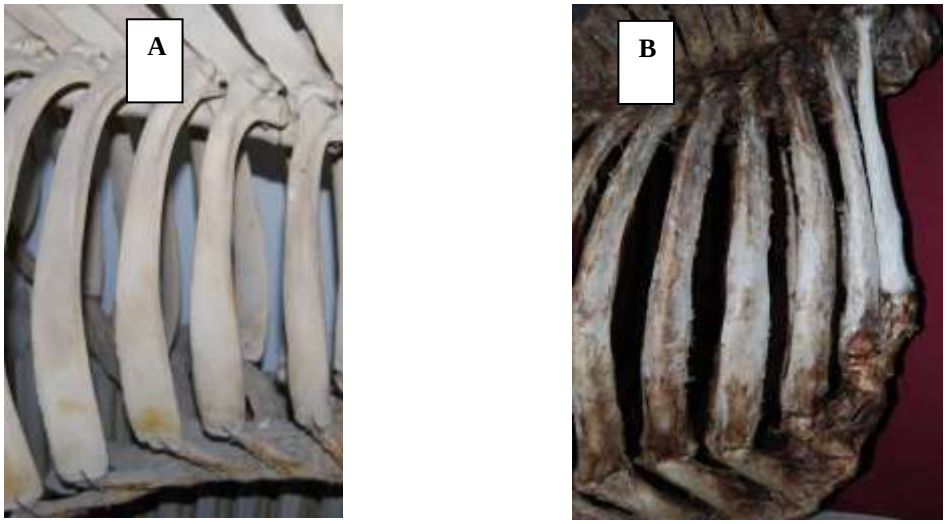

Fig.6. Ribs in the cow (A) and in the giraffe (B), lateral view.

The sternum is an osteo-cartilaginous piece in mammals, placed on the ventro-medial plane, representing the floor of the thorax, composed of sternebrae and articular surfaces for the cartilaginous segments of the sternal ribs (Damian, A, 2001).
The cow's sternum presents 7 sternebrae and has a dorso-ventrally flattened body, narrowed at both extremities and a lot wider cranially (Fig.7A). It offers articular surfaces for eight pairs of ribs. The manubrium is massive and pyramidal, perpendicular on the second sternebrae. There is not marked sternal keel. Consecutive sternebrae are growing larger except the last two. The surfaces of the condro-costal articulations are well defined. The sternum ends in a round small xiphoid process (Barone, R., 1966).
The giraffe's sternum is also composed of 7 sternebrae (Fig.7B), but its general shape shows important differences. The sternebral body is strongly narrowed in the first elements, and the width of the last ones is also much reduced compared to the cow's. The sternal keel is prominent on the ventral face of the sternebrae, with a curved profile, and a marked concavity of the

dorsal face. The manubrium is very narrow and the xiphoid couldn't be examined, as it was detached during the processing of the thoracic piece.

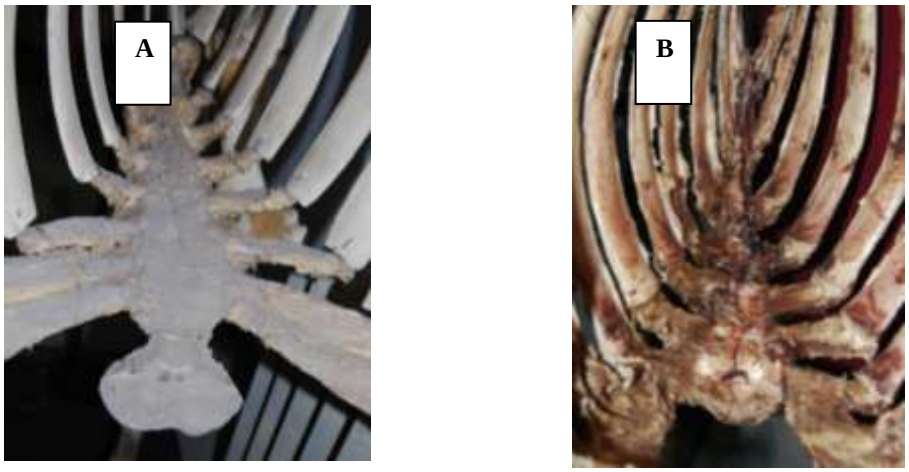

Fig.7. Sternum of the cow (A) and of the giraffe (B), caudal view.

As previously mentioned the ribs together with the thoracic vertebrae and with the sternum create a protective space for the internal organs of the thoracic cavity.

In the cow, due to the displacement of the thoracic legs in an almost exclusively parasagital plane, the thorax is flattened latero-laterally (Fig.8A) (Barone, R. 1966). The line traced by the spinal processes is almost horizontal in bovines, without a slope following the withers. The profile of the thorax skeleton is elongated, with straight angles cranially, which confers it a rectangle aspect. Caudally, the thoracic cavity gains in volume.

In the giraffe, the thoracic cavity, although massive and endowed with an extra pair of ribs, has a shorter longitudinal axis, related to the general body measurements, than in the cow (Fig. 8A). The line of the back is inclined, the spinal processes marking a sudden decrease in height after the peak reached by the withers, which continues to the lumbar segment. The thorax of this species is flattened not only latero-laterally, but also narrowed dorso-ventrally at its extremities.

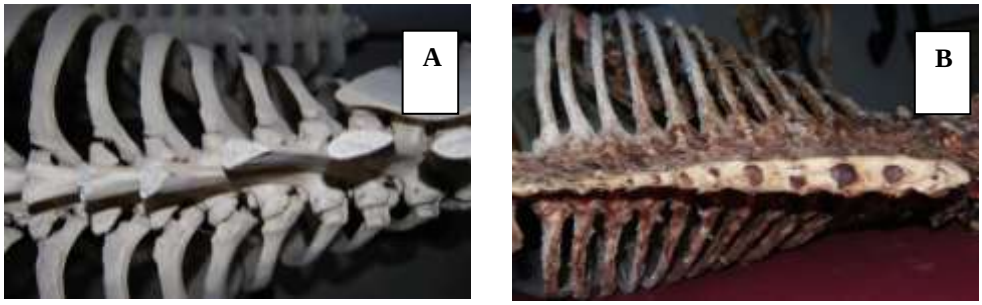

Fig.8. Dorsal view of the thoracic cavity in the cow (A) and in the giraffe (B).

CONCLUSIONS

The thoracic vertebrae are 14, with an even width on the spinal processes, smaller than in the cow; their height rapidly increases from T1 to T2, reaches the maximum in between T2 and T4, after which it decreases to the lumbar segment.

The lumbar vertebrae are 5, with well developed, but short bodies, short spinal processes, slightly inclined caudo-dorsally; less developed transverse processes, linear with rounded ends.

The sacrum contains 4 vertebrae incompletely merged, smaller than in the cow, with higher but narrower spinous processes. There are 3 superior and inferior sacral foramina. The articular surfaces for the pelvis are situated on the latero-dorsal side of the wings of the sacrum.

The coccygeal vertebrae are 18 with long and fine bodies, showing less developed surface elements than the bovines.

The giraffe displays 14 pairs of **ribs**, 8 sternal and 6 floating, with a smaller width than in the cow.

The sternum contains 7 sternebrae, which bodies are strongly narrowed in the first elements, and smaller than in the cow in the last elements. The sternal keel is well marked on the ventral side, and the profile is curved

The thoracic cavity in the giraffe is massive endowed with an extra pair of ribs. It has a shorter longitudinal axis reported to the general body measurements, than in the cow.

Due to the ventro-cranial inclination angle of the thoracic vertebral spine and to the convexity of the sternum, the thorax of the giraffe is narrowed in both extremities.

REFERENCES

Barone, R. (1966). Anatomie comparée des mammiféres domestiques. Laboratoire d'anatomie. Ecole Nationale Veterinaire Lyon.

Coţofan, V., R. Palicica, V. Hriţcu and V. Enciu (1999). Anatomia animalelor domestice, vol.I - Sistemul de susţinere şi mişcare. Ed. Orizonturi Universitare, Timişoara.

Damian, A., N. Popovici and I. Chirilean (2001). Anatomie comparată: sistemul de susţinere si mişcare. Ed. AcademicPress, Cluj-Napoca.

Henderson, D.M. and D. Naish (2010). Predicting the buoyancy, equilibrium and potential swimming ability of giraffes by computational analysis. J. Theor. Biol. 265(2):151-9.

Paştea, E., Gh. Constantinescu, E. Mureşan and V. Coţofan (1978). Anatomia comparată şi topografică a animalelor domestice. Ed. Didactică şi Pedagogică, Bucureşti.

Pratt, D.M., and V.H. Anderson (1985). Giraffe social behaviour. Journal of Natural History. 19:771-782.

Prothero, D.R. and R.M. Schoch (2003). Horns, Tusks and Flippers: The evolution of Hoofed Mammals. The Johns Hopkins University Press.

Savage, R.J.G. and M.R. Long. (1986). Mammal evolution: an illustrated guide. New York, Facts on File. 228-229.

Simmons, R.E. and R. Altwegg (2010). Necks-for-sex or competing browsers? A critique of ideas on the evolution of giraffe. Journal of Zoology. 282:6-12.

Stevens, J. (1993). Familiar Strangers. International Wildlife. 23,6-10.

RESEARCH ON HISTOSTRUCTURE ANTIGENICALLY STIMULATED LUNG IN BIRDS

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ABSTRACT

Birds lung is composed of a network of interconnected intrapulmonary bronchi, gas exchange (hematosis) were achieved at the finest bronchioles.
Left and right primary bronchi resulting from the bifurcation of the trachea to the syrinx cross the lung in caudo-cranial sense, their mucous continue with their lining air sacs. Their skeleton is made of cartilage plates from ring fragmentation.
A tertiary bronchus together with adjacent lung parenchyma forms a structural unit called lobes. At the origin of air capillaries (atria) is observed atrial muscle circumscribing holes communication between para-bronchi and air capillaries. Elastic fibers are well developed surrounding smooth muscle fibers.

Key words: *intrapulmonary bronchi, bronchioles, pulmonary alveoli.*

INTRODUCTION

Respiratory mucosa are continuously exposed to antigenic aggression and play a major role in the immune response that is developed avian body. Respiratory mucosa associated lymphoid formations studys formations belonging to the respiratory mucosal immune system of BALT lymphoma was performed by presenting some details of the normal issues that may serve as a guide in identifying and inventorying respiratory lesions.
Mucous glands disappear and are replaced by goblet cells. In the mucosa, lamina propria contains many blood vessels, which can be infiltrated with lymphoid cells that can organize in lymh nodes. Are present elastic fibers and smooth muscle fibers.

MATERIALS AND METHODS

Research has been lungs from poultry, control or stimulated antigen, normally developed, clinically healthy.For this purpose an experiment was initiated which included 3 groups of birds from breeding industrial

environments.Fragments collected were processed as usual histological techniques and stained with Goldner methods - Szekelly, Mucicarmin Mayer, trichrome Gomorrah, PAS ,Orceina, Alcian Blue.

RESULTS AND DISCUSSION

Gas exchange in birds is carried out at the level of the fine branches of intrapulmonary bronchi that are tertiary bronchi, atria, air clogs and air capillaries both during inspiration and during expiration.
Respiratory mucosal lymphoid tissue occurred during evolution immune defense as a way to inhibit colonization and invasion of the respiratory mucosa by specific and nonspecific immune mechanisms. They act as a local protective immune relatively independent of the systemic.
It is demonstrated that nasal administration of antigens or bronchial aerosol regional causes an immune response in the airways. Application level of antigen influences the type of immune response. In the literature stated that nasal administration limited to be answered purely local, while aerosol administration of antigens stimulating reach the pulmonary alveoli and a systemic response.
Structure of the lungs of birds is completely different from the structure of mammalian lungs. Birds lung is composed of a network of interconnected intrapulmonary bronchi, gas exchange (hematosis) were achieved at the finest bronchioles.

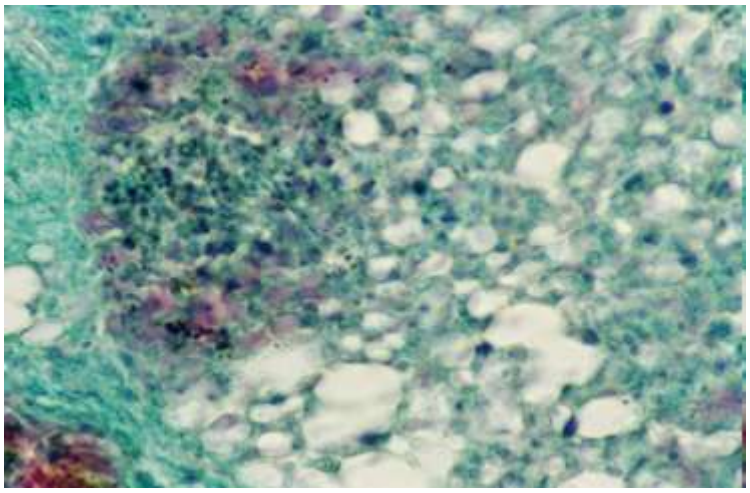

Fig.1 Lung ob. 40 x 4 trichrome stain Gomorrah

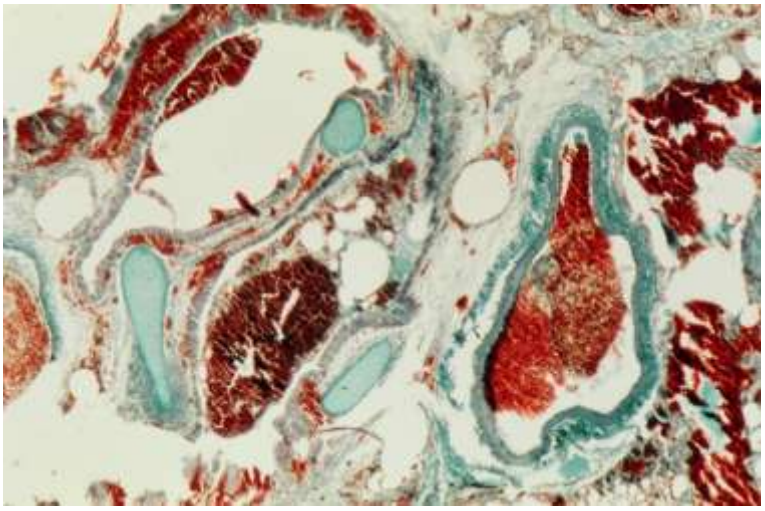

Fig.2 Lung magnifier trchrome stain Goldner – Szekelly witness
primary bronchus cartilage islands located above the vestibule;
lung parenchyma blood drawn during slaughter

Left and right primary bronchi resulting from the bifurcation of the trachea to the syrinx cross the lung in caudo-cranial sense, their mucous continue with their lining air sacs. Their skeleton is made of cartilage plates from ring fragmentation.

Mucous glands disappear and are replaced by goblet cells. In the mucosa, lamina propria contains many blood vessels, which can be infiltrated with lymphoid cells that can organize in lymh nodes. Are present elastic fibers and smooth muscle fibers. From primary bronchi secondary bronchi open arranged into four groups (middorsal, midventral, laterodorsal, lateroventral).

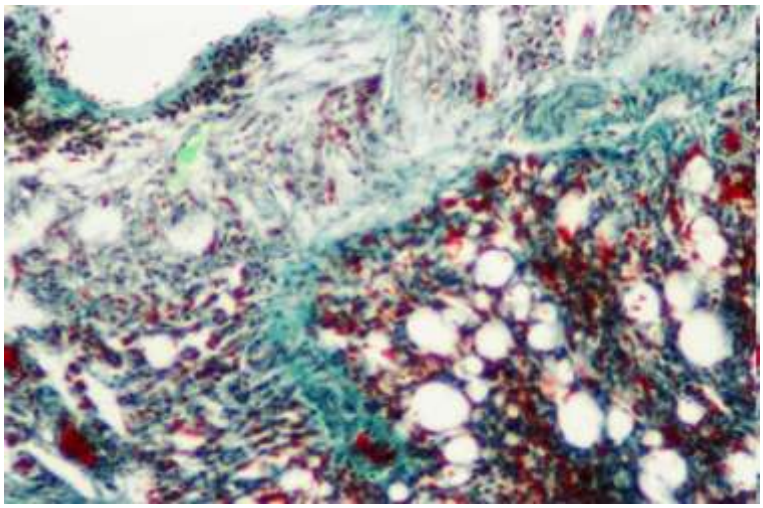

Fig.3 Lung ob. 20 x 4 Goldner- Szekelly stain vaccinated with Avipestisota vaccine lympho-plasma cell concentration near the bronchus

Secondary bronchi ciliated columnar epithelium has a goblet cell. Lamina propria have the same structure as the primary bronchus. Tertiary bronchi called parabronchi drawn from secondary bronchi and form a network of tubes through all lung paranchim. A tertiary bronchus together with adjacent lung parenchyma forms a structural unit called lobes. On the cross section has a hexagonal shape with centrally located parabronhia from which numerous air capillaries radiate to the periphery.

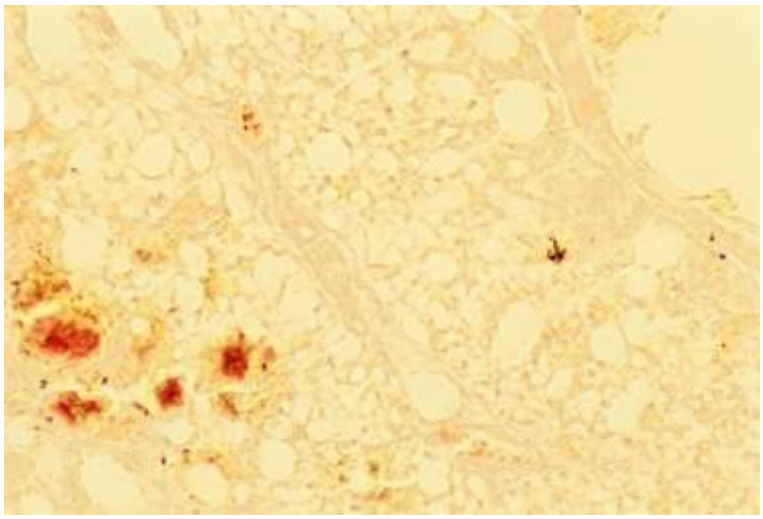

Fig.4 Lung ob. 20 x 4 Mucicarmin Mayer stain vaccinated with Avipestisota Vaccine. It highlights the positive areas. Intense reaction in the densely populated with lymphoid cells.

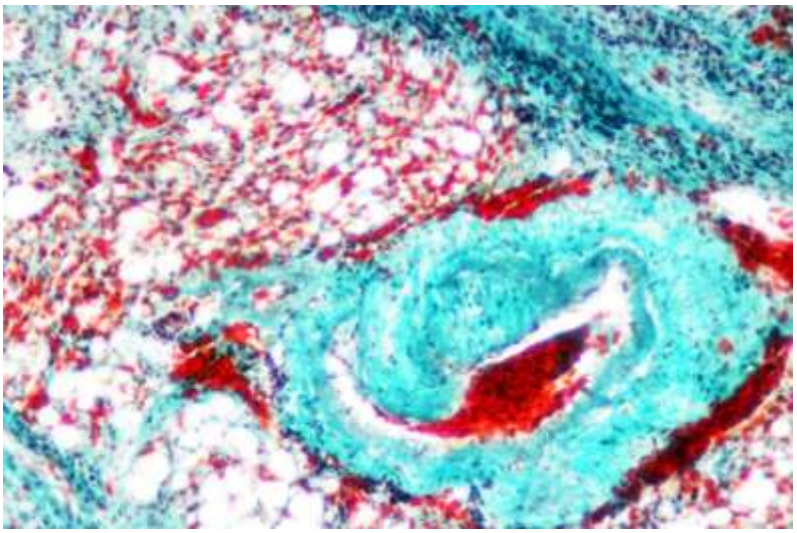

Fig.5 Lung ob. 40 x 4 Alcian Blue staining vaccinated with Avipestisota vaccine .The lymphoid population highlights the interlobular septum.

Parabronchi epithelium is a simple epithelium (flat) squamos separated by connective tissue by a basement membrane.
The origin of air capillaries (atria) is observed atrial muscle circumscribing communication between parabronchi holes and air capillaries.Elastic fibers are well developed surrounding smooth muscle fibers.
Occasionally, elongated dense bundles, characteristic of smooth muscles are placed among miofilaments and also united to the cell membrane. Many pyknotic vesicles follow the contour of cell membrane.

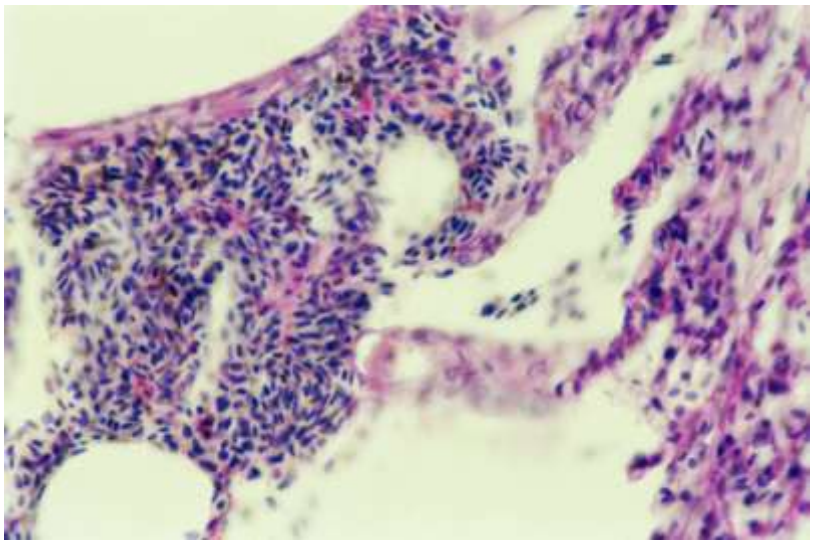

Fig.6 Lung ob. 40 x 4 PAS stain vaccinated with Avipestisota vaccine; diffusely infiltrated area, parechimul lung surface.

Many contemporary authors tend to combine original name of parabronchi with air capillaries or respiratory capillaries term.Air atria are lined by a simple squamous epithelium or cubic.

Atria are separated from each other by narrow connective tissue septa containing elastic and collagen fibers.In connective interstices between air capillaries and blood vessels lymphoid cells are found and alveolar macrophages with vacuolar cytoplasm or not.Pulmonary lobules appear delimited by connective septa rich in elastic fibers, blood vessels, connective and lymphoid cells. At antigenically stimulated birds with Newcastle virus we could see the structure of lymphoid nodules near parabronchi

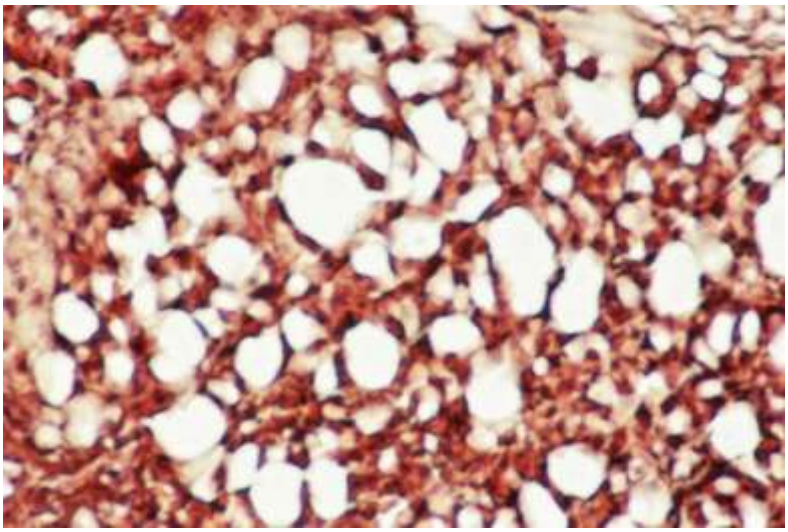

Fig.7 Lung ob. 40 x 4 orceina coloration - witnes.Skin laced given by bronchial capillarie (very weak response to orceine in the lung).

CONCLUSIONS

In primary bronchi, cartilage ring fragment plate cartilage and mucous glands disappear, their role being taken over by goblet cells. Are present in the lamina propria numerous blood vessels and lymphoid cells.

Left and right primary bronchi resulting from the bifurcation of the trachea to the syrinx cross the lung in caudo-cranial sense, their mucous continue with their lining air sacs.

The secondary bronchi simple columnar ciliated epithelium with goblet cells.

Tertiary bronchi or parabronchi occupies the center of lung lobes, in them opening up numerous air capillaries. Parabronchial epithelium seem simple squamous epithelium appears, separated by connective tissue by a basement membrane evident.

Parabronhii air capillary openings are circumscribed by atrial muscles, with characteristics of smooth muscle.Pulmonary lobules appear undelimited by connective septa rich in elastic fibers, blood vessels, connective and lymphoid cells.

Birds antigenically stimulated lymphoid nodules was observed holding in parabronchi adjacent parenchyma.

REFERENCES

Bacha, J.Jr., Wood, L M.(2000) – Color atlas of veterinary histology. Lea and Febiger, Beckembaum, 2nd

Brandtzaeg P., Pabst R., Let's go mucosal:communications on slippery ground, Trends Immunol. 25 (2004) 570–577.

Cornila, N. (2000-2001) - Microscopic morphology of domestic animals. Ed. Bic. ALL, vol. I-II.

Constantin, N., Cotrut M., Sonea A., - Physiology of domestic animals , vol. I, II. Coral Sanivet Publishing,Bucharest,1999.

Crăițoiu Ștefania – Special Histology. University Medical Publishing, 2003.

Maina J.N., A systematic study of the development of the airway (bronchial) system of the avian lung from days 3 to 26 of embryogenesis:a transmission electron microscopic study on the domestic fowl, *Gallus gallus* variant *domesticus*, Tissue Cell 35 (2003) 375–391.

Nganpiep L.N., Maina J.N., Composite cellular defence stratagem in the avian respiratory system: functional morphology of the free(surface) macrophages and specialized pulmonary epithelia, J. Anat. 200 (2002) 499– 516.

Powell F.L., Respiration, in: Whittow G.C. (Ed.), Sturkie's Avian physiology, Academic Press, London, 2000, pp. 233–264.

Phalipon A., Cardona A., Kraehenbuhl J.P.,Edelmann L., Sansonetti P.J., Corthesy B.,Secretory component: A new role in secretory IgA-Mediated exclusion in vivo, Immunity 17 (2002) 107–115.

Radu O. Georgeta – Functional morphology of the respiratory system in birds – Essay II – USAMV – FMV Bucharest, 2000

Toth TE. Nonspecific cellular defense of the avian respiratory system: a review. Dev. Immunol. 2000;24:121–139.

Tschernig T., Pabst R., Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases, Pathobiology 68 (2000) 1–8.

Wieland W.H., Orzaez D., Lammers A.,Parmentier H.K., Verstegen M.W.A., SchotsA., A functional polymeric immunoglobulinreceptor in chicken (*Gallus gallus*) indicatesancient role of secretory IgA in mucosal immunity, Biochem. J. 380 (2004) 669–676.

COMPARATIVE STUDIES ON MICROSCOPIC MORPHOLOGY OF THE SEMINIFEROUS TUBULES IN 120-180 DAYS OLD COCKS

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ABSTRACT

At the age of 120 days in the seminal epithelium are present all types of cells of the seminal line - primary spermatocites, secondary spermatocites, spermatide and spermatozoa, that indicating onset of spermatogenesis process. Sertoli cells were observed in cross sections of seminiferous tubules, they are willing unistratal, with the nuclei located basal, polymorphous nucleolus sometimes triangular course.
There are many cells in semen Sertoli epithelium, in peritubulary are located myofibroblastes.
At cocks 180 days in seminal epithelium are present all cell types of the seminal line. Basement membrane of the seminiferous tubules is evident and intertubular connective tissue is PAS positive.

Key words: *seminiferous tubules, gonocytes, Sertoli cells, interstitial gland.*

INTRODUCTION

At the age of 120 days it observe a quantitative reduction of intertubular.
Interstitial endocrinocytes have different size, some are fusiforme, others have cytoplasm vacuolar, being involved in hormonegenesis.
At the age of 180 days in sections stained by Unna-Tanzer method interstitial gland appears in intertubular tissue, bounded by elastic fibers from basal lamina of the seminiferous tubules. Leydig cells are either isolated, disseminated ,or in groups, with blood vessels and connective tissue.
Sertoli cells are active from mitotic point of view and contain large amounts of rough endoplasmic reticulum and produce antiparamesonephrotic hormone.

As spermatogonia, Sertoli cells are among the most resistant cells and germinal epithelium remain predominant cell type in aging gonads.

MATERIAL AND METHODS

The researches were conducted on testes harvested from cock, race-variety white Leghorn, normally developed, clinically healthy, vaccinated, macroscopically and microscopically examined.
The inspection of histological preparations were made on permanent histology, processed by usual histological techniques and colored by Tanzer-Unna and P.A.S histochemical techniques. Were performed electronmicroscopically studies of seminal epithelium, morphometric measurements of the outside diameter of the seminiferous tubules, the seminiferous tubules lumen diameter and epithelium height of 120 and 180 days cocks.

RESULTS AND DISCUSSION

At the specimens from the age of 120 days in the seminal epithelium are present all types of cells of the seminal line - primary spermatocites, secondary spermatocites, spermatide and spermatozoa, that indicating onset of spermatogenesis process. Sertoli cells were observed in cross sections of seminiferous tubules, they are willing unistratal, with the nuclei located basal, polymorphous nucleolus sometimes triangular course.
There are many cells in semen Sertoli epithelium, in peritubulary are located myofibroblastes.
Seminal epithelium is placed on a thick basement membrane, composed of lamina lucid , electronodense with thickness by 10 nanometers, homogeneous and crossed by fine filaments rare; a lamina thickness of 20-30 nanometers thick, composed by filaments fines abundants, contained in an amorphous matrix, dense and a lamina reticulata which pass to the connective tissue matrix.
Basement membrane has many identary or invagination in the seminiferous epithelium, directed mainly by Sertoli cells and contains numerous electron-light granules.
Is placed on a miofibrilar layer homogeneous devoid of collagen fiber.

Is apparent basement membrane is apparent of the seminiferous tube, quite thick, being placed above the seminal epithelium, composed of cells Sertoli and seminal cells line.
Sertoli cell has a nucleus composed of large coarse chromatin. In his cytoplasm are numerous mitochondria and rough endoplasmic reticulum.

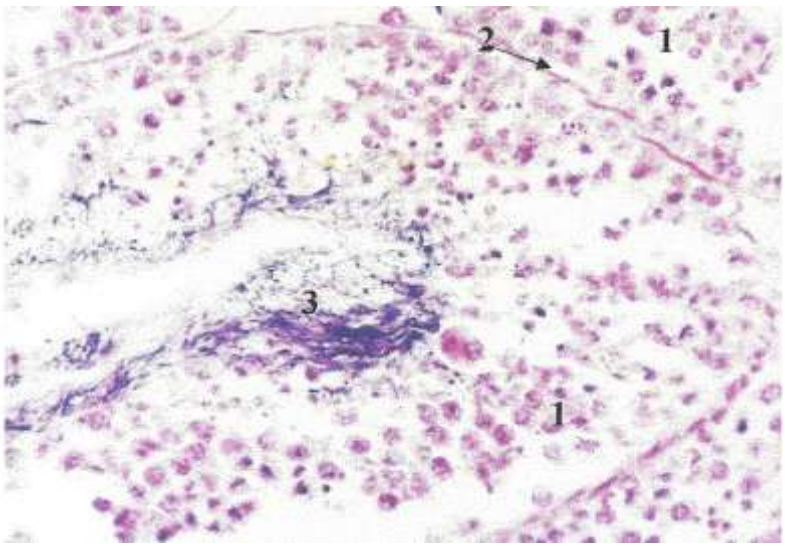

Fig.1- Cock testicle 120 days ;PAS Stain Ob.10X1- lumen of the seminiferous tubules;1- seminal epithelium2- basement membrane; 3-sperm tails

Sertoli cells are active from mitotic point of view and contain large amounts of rough endoplasmic reticulum and produce antiparamesonephrotic hormone. As spermatogonia, Sertoli cells are among the most resistant cells and germinal epithelium remain predominant cell type in aging gonads. Spermatogonia A has an ovoid nucleus with prominent nucleoli and has a large area of contact with the basal lamina.
Type B spermatogonia is a spherical cell with spherical nucleus and nucleolus less prominent. Of mitotic division of spermatogonia B are form the primary spermatocytes.The research on electron microscopy of the testicular parenchyma, from the cocks, I highlighted some outstanding tissues, unreported in the specialty literature.

THE OUTER DIAMETER OF SEMINIFEROUS TUBULES, LUMEN DIAMETER AND EPITHELIUM HEIGHT AT 120 DAYS

Seminiferous tubules	Outer diameter(μm)	Lumen diameter(μm)	Epithelium height (μm)
1	218,182	83,417	67,520
2	2115,342	78,979	68,177
3	168,521	95,750	36,385
4	169,234	71,417	48,572
5	191,917	95,500	47,208
6	187,358	100,458	43,229
7	217,398	145,937	66,396
8	318,687	148,104	84,791
9	158,958	80,062	38,395
10	270,687	146,896	62,448
Average	**217,287**	**104,352**	**56,912**

At the age of 180 days in sections stained by Unna-Tanzer method gland intertubular interstitial tissue occurs, bounded by elastic fibers from the basal lamina of the seminiferous tubules. Leydig cells are either isolated disseminated or in groups, with blood vessels and connective tissue.
At this age in sections stained by the method of Unna-Tanzer elastic fibers appear in the basal membrane of the seminiferous tubules. On the basal membrane is placed stratified epithelium with a settlement seminal feature columns, on top of the columns being placed late spermatids and sperm.
Spermatogonia A have ovoid with a nucleus and nucleolus proieminenţi has a large area of contact with the basal lamina. Type B spermatogonia is a spherical cell with spherical nucleus and nucleolus less proieminent. Mitotic division of spermatogonia to primary spermatocytes B is formed. These are the largest cells of the seminal line located in an intermediate position between spermatogonia and spermatida, are large and round nuclei with conspicuous nucleoli. They gradually lose contact with the basal lamina and moving adluminal compartment through intercellular junctions of Sertoli cells.

Through spermiogenesis the newly formed spermatids differ in sperm. Excess material of spermatids (cytoplasm, water, organelles) unnecessary to sperm resulting from morphological changes during spermiogenesis (after acrosome formation, nuclear chromatin condensation), is phagocytied by Sertoli cells.

At cocks spermatogenesis is four times faster than mammals and are produced four times the number of sperm/g by testicle than in mammals and this difference is associated with a more active transit and reduced survival of sperm in male extratesticular channels at birds than in mammals.

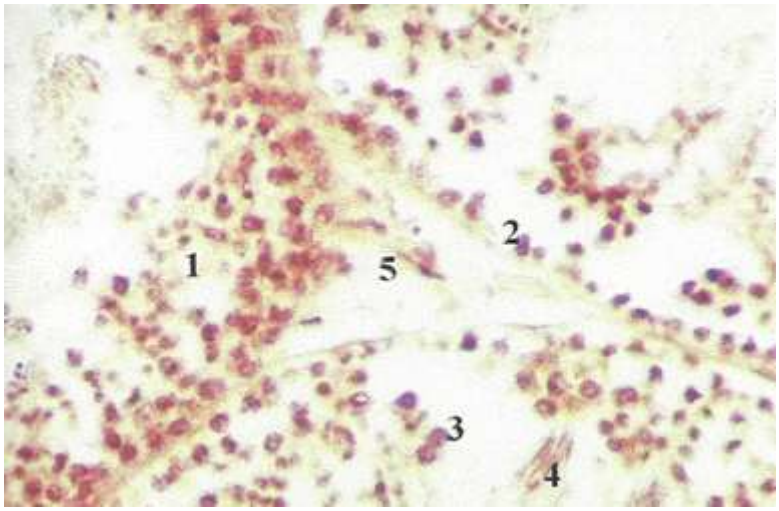

Fig.2- Cock testicle 180 days;Tanzer-Unna Stain Ob.40X
1- seminal epithelium;2- spermatogonia; 3- primary spermatocite;
4- flagelii sperm;5- interstitial gland;

From the age of 180 days in seminal epithelium are present all cell types of the seminal line. Basement membrane of the seminiferous tubules is evident and intertubular connective tissue is PAS positive.

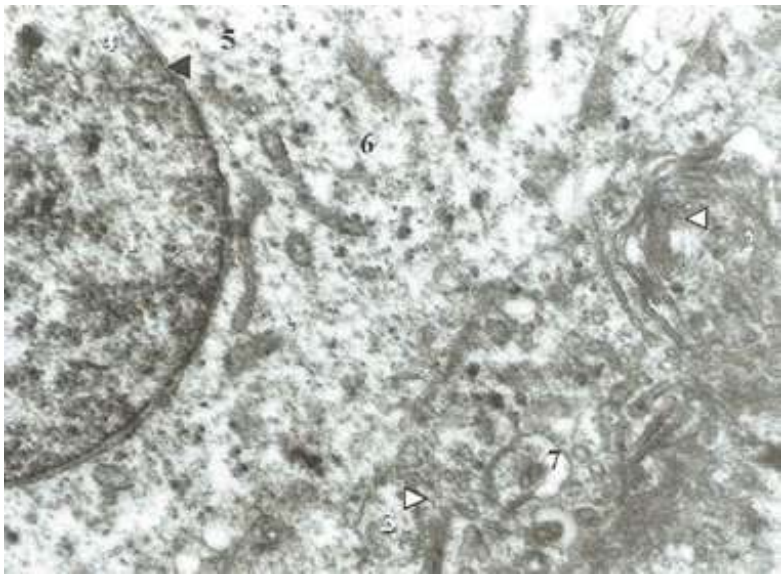

Fig.3 Seminal epithelium – ultrastructure (X 22.000)
1-spermatocit of I;2-Golgi complex;3-ribosomes;4 –nucleolus;5 -nuclear membrane;6-endoplasmic reticulum rough;7-lysosomes

THE OUTER DIAMETER OF SEMINIFEROUS TUBULES, LUMEN DIAMETER AND EPITHELIUM HEIGHT AT 180 DAYS

Seminiferous tubules	Outer diameter(μm)	Lumen diameter(μm)	Epithelim height (μm)
1	377,500	260,000	58,750
2	327,500	200,000	63,750
3	310,000	187,000	61,500
4	382,500	275,500	52,500
5	547,534	160,500	51,250
6	312,432	315,000	76,000
7	400.132	272,500	42,500
8	355,374	195,000	41,250
9	330,000	100,500	67,500
10	297,000	221,300	98,250

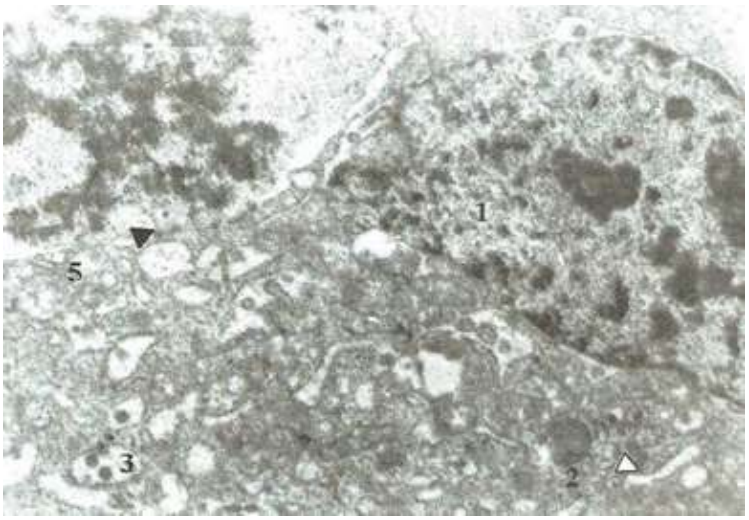

Fig.4 Sertoli cell(x16.500) 1-Sertoli cell nucleus,2-endoplasmic reticulum smooth,3-lysosomes 4-spermatocit,5-desmozomi

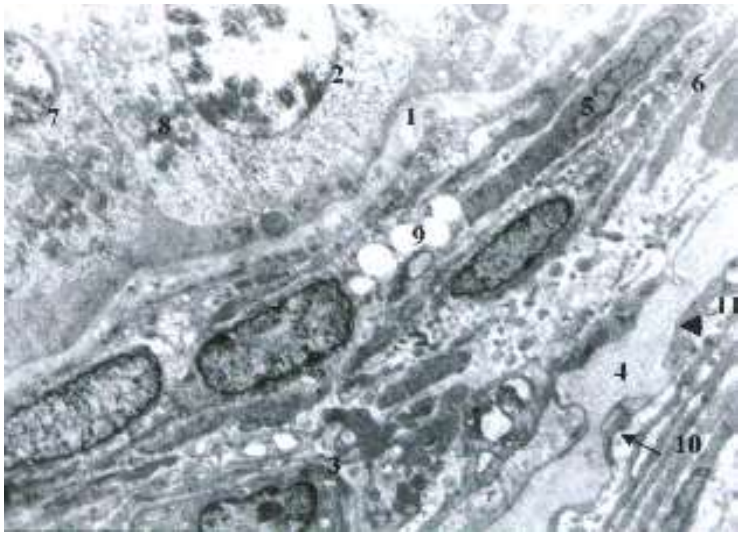

Fig.5 Seminal epithelium – ultrastructure (X 22.000)basement membrane; 2- Sertoli cell; 3-Leydig cell;4- capillary blood; 5- fibroblast; 6- collagen fibres; 7-spermatogonium; 8-mitochondria 9-REN; 10-the capillary basement membrane; 11-edoteliate cells

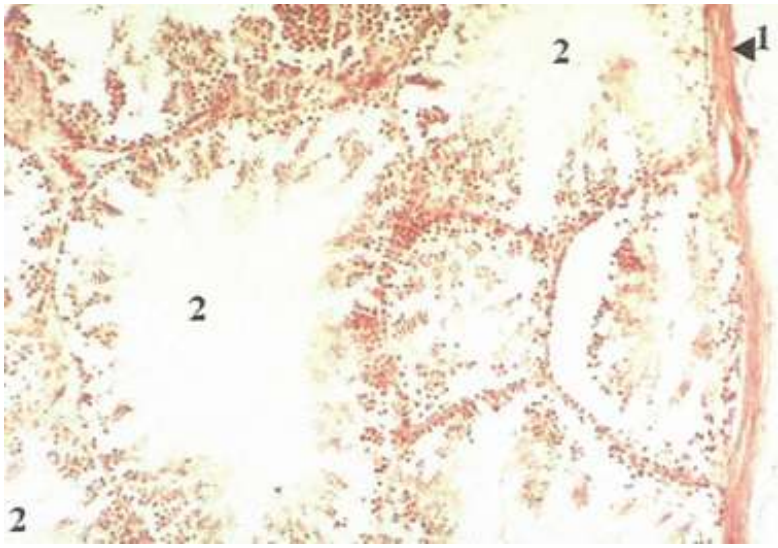

Fig.6- Cock testicle 180 days;Tanzer-Unna Stain Ob.40X
1- Albugineea;2- seminiferous tubules

CONCLUSIONS

At the age of 120 days in the seminal epithelium are present all types of cells of the seminal line - primary spermatocites, secondary spermatocites, spermatide and sperm, that indicating onset of spermatogenesis process. Sertoli cells were observed in cross sections of seminiferous tubules, they are willing unistratal, with the nuclei located basal, polymorphous nucleolus sometimes triangular course.Sertoli cell is located on the basement membrane of seminiferous tube that makes complex jonctionale.

At the age of 120 days the interstitial Leyding gland appears in intertubular tissue, delimited by the elastic fibers from the basal lamina of seminiferous tubules.

It is noted that the basement membrane of seminiferous tube is thick and shows numerous invagination directed to the cytoplasm of the Sertoli cells.

The cytoplasm of the Sertoli cell is dense and has more organites numerous cistern of smooth endoplasmatic reticulum, numerous mitochondria, rough endoplasmic reticulum profiles, lysosomal, Golgi complex, centrioli, etc. Gonocites transformation in spermatogonia is accompanied by a spray of color mass that from granular form switch to powder.

At the age of 180 days spermatogonia located at the periphery of the seminiferous epithelium are basal arranged on a single line and are spaced between them.
Are round or polyhedral cells with dark nucleus, coarse-looking occupying the center of the cell. Sometimes it is polymorphic, being division. Less abundant cytoplasm is basophilic.
These are the largest cells of the seminal line located in an intermediate position between spermatogonia and spermatida, their nucleus are large and round, with obvious nucleolus.
They gradually lose contact with the basal lamina and moving adluminal compartment through intercellular junctions of Sertoli cells.
Size increases progressively seminiferous tubules and intertubular space is reduced proportionally with increasing age, the lowest being observed at 180 days.

REFERENCES

Aire, T. A. – Cyclicaal reproductive changes in the non-ciliated epithelia of the epididymis of birds. Atomonia, Histologia, embryologia, volume 31, bnnIssue 2, Page 113-8, 2002.

Bacha, J.Jr., Wood, L M.(2000) – Color atlas of veterinary histology. Lea and Febiger, Beckembaum, 2nd

Bogdan A.T.., Dorina Bogdan, I.Groza, M.Paraschivescu, G.F.Toba, S. Chelmu, D.L.Diaconescu, Amalia Strateanu, I Surdu, (2009) - More animal production in Agrifood Green Power Development, a New Paradigm of Concept regarding Sustainle Rural Bioeconomics and Eco – Economics. USAMV Cluj vol .66 416-423

Cornila, N. (2000-2001) - Microscopic morphology of domestic animals. Ed. Bic. ALL, vol. I-II.

Hess, R.A., Franca, L.R. (2005)- Strcuture of Sertoli Cell. In: Sertoli Cell Biology. M. Griswold and M. Skinner. New York, Academic Press, 19-40.

Maretta, M., Marettova, E. (2004) – Immunohistochemical demonstration of myoid cells in the testis and its excurrent ducts in the domestic fowl. Vol 45, Number 5, Oct., 585-589.

Miranda, J.R., Rocha, D.C.M., Hess, R.A., Cassali, G.D., Franca, L.R. (2002) – Sertoli cell proliferation in the transition between seminiferous tubules and the rete testis. European Testis Workshop.

Oster, H. (2000) – Sexual differentiation. Semin Reprod Med; 18(1):41-9.

Oatley JM şi Brinster R-(2008) Regulation of spermatogonial stem cell self-renewal in mammals. Annual Review of Cell and Developmental Biology 24 263–286.

Sin, M.K., Cheng, C.Y. (2004) – Extracellular matrix: recent advances on its role in junction dymamics in the seminiferous epithelium during spermatogenesis. Biol Reprod; 71(2):375-91.
Wheater, P.R., Burkitt, H.G., Daniels, V.G. (2001) – Functional histology – A text atlasmedical divisions of Langmann group limited. Churchill Livingstone
Wertz K and Herrmann BG. (2000) Large-scale screen for genes involved in gonad development. Mech Dev; 98:51.

ESTABLISHING SPECIFIC GROWTH RATE OF TWO *LACTOBACILLUS SALIVARIUS* STRAINS ISOLATED FROM DENTAL ROOT CANAL AND SOME *LACTOBACILLUS* PROBIOTIC STRAINS BY INTESTINAL ORIGIN AT PH VALUES 4,5 AND 7,0

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Abstract

In vitro researches were carried out for evaluate specific growth rate and generation time of two Lactobacillus salivarius strains isolated from dental root canal and two Lactobacillus probiotic strains at pH values 4.5 and 7.0. These tests indicated that Lactobacillus salivarius strains isolated from dental root canal showed biger specific growth rate values at both pH values compared with the probiotic Lactobacillus strains.

Key words: *Lactobacillus salivarius, probiotic, specific growth rate*

INTRODUCTION

Lactobacilli have been isolated from specific habitats, including dairy products, plants, meat products, humans and animals (Brizuela M.A. 2001, Cristensen J.E. 2003, Nowel O. 2012 and Teusink B. 2009).
The main goal of our study was to establish the specific growth rate of *Lactobacillus salivarius* strains isolated from dental root canal and two *Lactobacillus* probiotic strains at pH 4.5 and 7.0.

MATERIALS AND METHODS

Two *Lactobacillus salivarius* strains isolated from dental root canal (G1 and G2) and two *Lactobacillus* probiotic strains: *L.salivarius* probiotic and *Lactobacillus rhamnosus* GG were used in this work. These strains were grown in MRS broth in Anglicon fermenters, at 37ºC and CO_2 5% atmosphere, at pH 4.5 and 7.0. The DO_{600} values were determined in the moment of inoculation (T0) and than hourly (moment T1 after one hour, T2 after two hours, T3 after three hours etc).

The DO_{600} values were plotted on logarithmic graphic and the curves growth were obtained. The specific growth rate (μ) and generation time (Δt) were calculated.
The specific growth rate was calculated using the formula:

$$\mu = \frac{\ln OD_{max} - \ln OD_{min}}{T_{max} - T_{min}}$$

Where:
OD max is the value of DO600 in moment Tmax;
OD min is value DO600 in moment Tmin;
The generation time (Δt) (dubling time) was calculated in this main:

$$\Delta t = \frac{\ln 2}{\mu}$$

RESULTS AND DISCUSSIONS

The specific growth rate and generation time of the investigated strains at pH 4.5 are shown in table 1.
Lactobacillus salivarius strains isolated from dental root canal G1 and G2 showed higher values of specific growth rate (0.81 and 0.7) compared with *Lactobacillus* probiotic strains (0.48 for *L.salivarius* probiotic and 0.56 for LGG) at pH 4.5.
Also, the *Lactobacillus* strains by dental origin had smaller generation time values (0.85h for G1 and 0.98h for G2) compared with the probiotic strains (1.43h for *L.salivarius* probiotic and 1.23h for LGG) (table 1).

Tabel 1. The DO600 values, the specific growth rate (μ) and generation time (Δt) at pH 4.5

		DO600 values				pH			
Moment	Time	*L.s.* probiotic	G1	G2	LGG	*L.s.* probiotic	G1	G2	LGG
T0	11:45	0,032	0,038	0,037	0,033	4,57	4,79	4,41	4,62
T1	12:45	0,040	0,057*	0,053*	0,046*	4,35	4,7	4,57	4,58
T3	13:45	0,058	0,121	0,102	0,081	4,61	4,7	4,55	4,49
T4	14:45	0,080*	0,273	0,201	0,140	4,61	4,61	4,51	4,63
T5	15:45	0,121	0,64	0,425	0,243	4,6	6,59	4,55	4,6
T6	16:45	0,206	1,49**	0,89**	0,440**	4,58	4,47	4,57	4,57
T7	17:45	0,342**	1,97	1,59	0,82	4,57	4,55	4,61	4,56
μ (h^{-1})		0,48	0,81	0,7	0,56				
Δt (h)		1,43	0,85	0,98	1,23				

*DOmin, **DO max

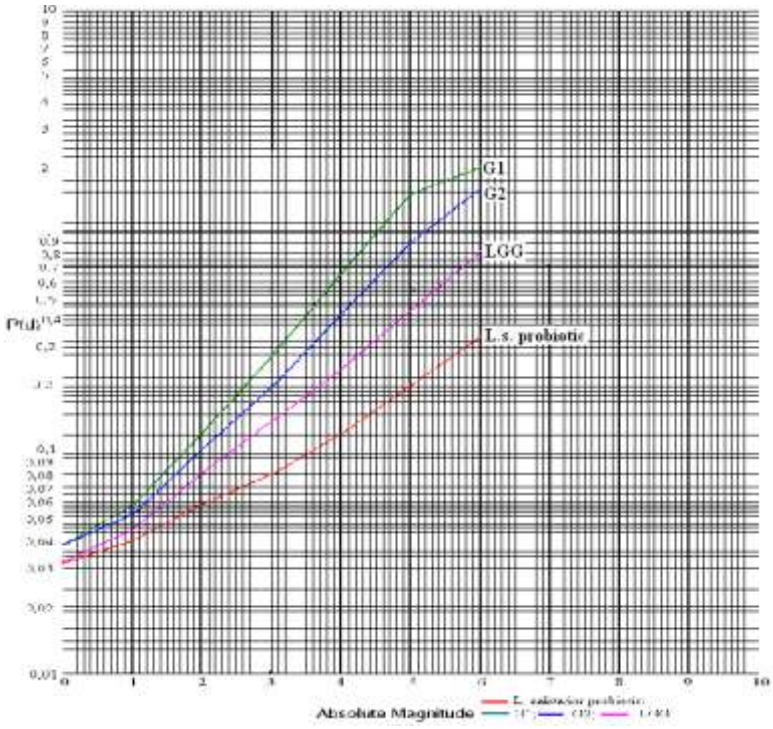

Fig. 1. Graphical representation of DO600 values at pH 4,5

The specific growth rate and generation time of the investigated strains at pH 7.0 are shown in table 2.

Also, at pH 7.0 *Lactobacillus salivarius* strains isolated from dental root canal G1 and G2 showed higher specific growth rate (1.26 and 0.92) compared with *Lactobacillus* probiotic strains (0.77 for *L. salivarius* probiotic and 0.69 for LGG). The *Lactobacillus* strains by dental origin had smaller generation time values (0.54h for G1 and 0.75h for G2) compared with the probiotic strains (0.89h for *L.salivarius* probiotic and 1.0h for LGG) (table 2).

Tabel 2. The DO600 values, the specific growth rate (μ) and generation time (Δt) at pH 7,0

		Valorile DO600				pH			
Moment	Time	*L.s.* probiotic	G1	G2	LGG	*L.s.* probiotic	G1	G2	LGG
T0	13:00	0,033	0,043	0,043	0,033*	7,24	7,14	7,2	7,15
T1	14:00	0,047	0,066*	0,061*	0,048	7,12	6.96	7,01	7,02
T2	15:00	0,072*	0,180	0,135	0,089	7,06	7,03	7,08	7,22
T3	16:00	0,143	0,82**	0,388**	0.151	7,04	6,97	7,03	7,19
T4	17:00	0,338**	2,21	1,36	0,263				
T5	18:00				0,524**				
μ (h^{-1})		0,77	1,26	0,92	0,69				
Δt (h)		0,89	0,54	0,75	1				

*DOmin, **DOmax

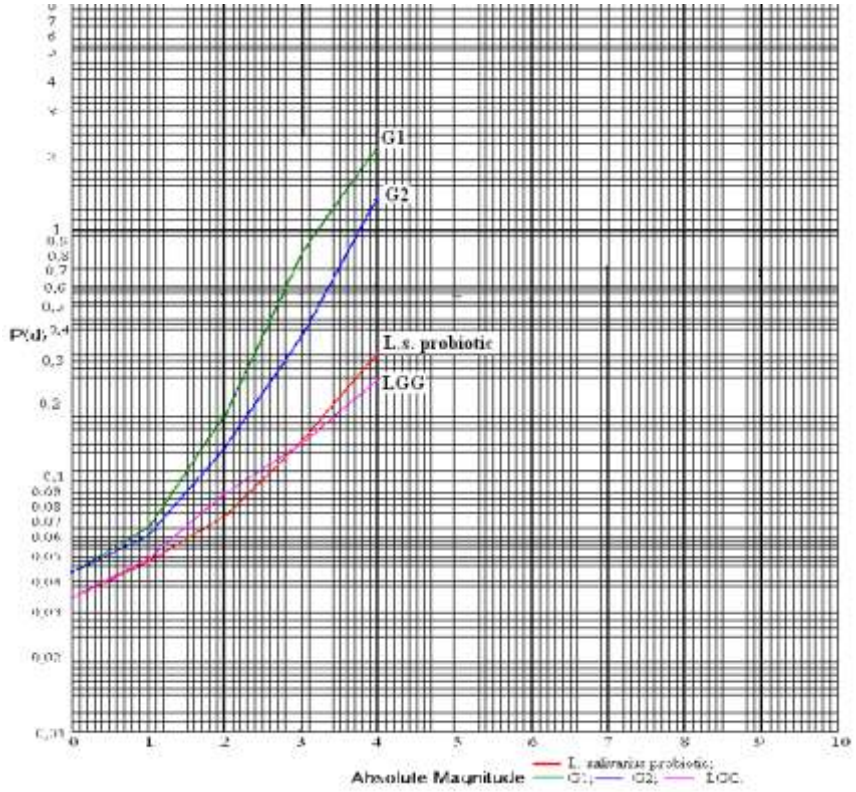

Fig. 2. Graphical representation of DO600 values at pH 7,0

Tabel 3. The values of specific growth rate μ (h^{-1}) and generation time at pH 4,5 and 7,0

	L. salivarius probiotic	G1	G2	LGG
pH 4,5				
Specific growth rate μ (h^{-1})	0,48	0,81	0,7	0.56
Generation time Δt (h)	1,43	0,85	0,98	1,23
pH 7.0				
Specific growth rate μ (h^{-1})	0,77	1,26	0,92	0,69
Generation time (h)	0,89	0,54	0,75	1

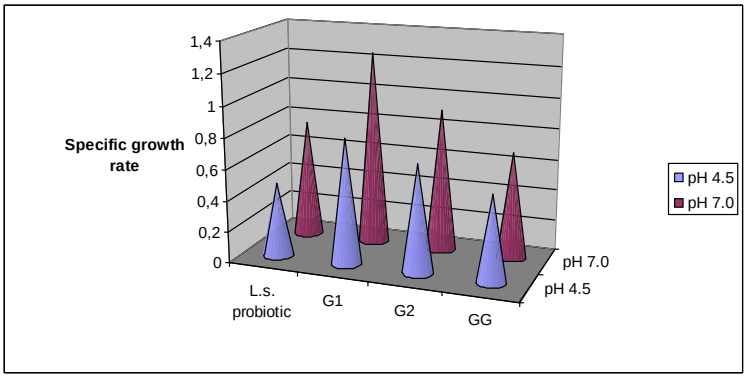

Fig. 3. The specific growth rate μ (h^{-1}) at pH 4.5 and 7.0

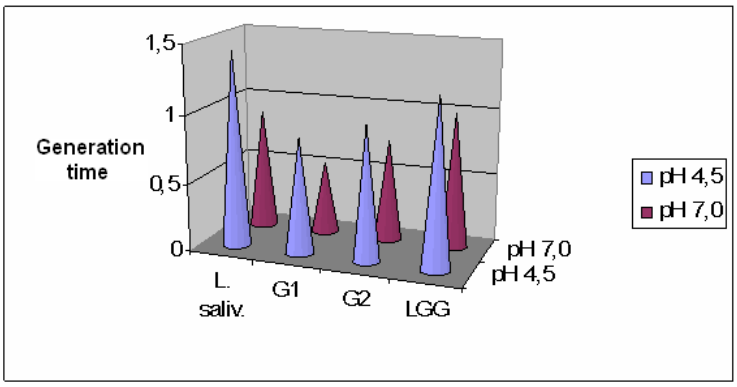

Fig. 4. The generation time values (h) at pH 4.5 and 7.0.

The values of specific growth rate of *Lactobacillus* strains with dental origin were higher than those of probiotic strains at both pH values. The values of generation time were smaller at *Lactobacillus salivarius* strains isolated from dental root canal than those of probiotic strains at both pH values (table 3 and fig.3).

All *Lactobacillus* strains showed smaller values of generation time at pH 7.0 (ranged between 0.54h and 1.0h and the average time was 0,795h) compared with those at pH 4.5 (ranged between 0.85h and 1.43h and the average time was 1,125h) (table 3 and fig.4).

Between the specific growth rate and the generation time is a high negatve correlation. The correlation Pearson factor r= -0,99. These data are correlated with those of Nezhad H.M, 2010 and Wijtzes T, 1995.

CONCLUSIONS

The next conclusions have been formulated:
Higher values of specific growth rate at 7.0 pH were registered for all *Lactobacillus* investigated strains compared with those at 4.5 pH.
The strains with dental origin showed biger specific growth rate values at both pH values compared with the probiotic *Lactobacillus* strains.
The values of generation time for all *Lactobacillus* strains were smaller at pH 7.0 (the average time was 0.795h) than those at pH 4.5 (the averahe time was 1.125h).
A negative correlation between specific growth rate and generation time was observated.

ACKNOWLEDGMENTS

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REFERENCES

Brizuela M.A., P. Serrano, Y. Perez- Studies on probiotic properties of two Lactobacillus strains-2001 Braszilian Archives of Biology and technology Vol 44(1), p.95-99.
Cristensen J.E., J.L. Steele- Impaired growth rates in milk of Lactobacillus helveticus peptidase mutants can be overcome by use of amino acid supplements 2003, Journal of Bacteriology 185 (11), p. 3297-3306.
Nowel O., Ahmed H., Donia zed El. 2012 Effect of the essential oils from Parsley and Fennel seeds on the growth of Lactobacillus casei subsp. rhamnosus- Biotechnology and biomaterials 2 (3)
Nezhad H.M., D.J. Stenzel, M.L. Britz 2010 Effect of growth at low pH on the cell surface properties on a strain of Lactobacillus casei group –Iranian Journal of microbiology, 2 (3), 144-151.
Teusink B., A. Wirsma, Jacobs L., Notebaart R.A., Smid J.E. 2009 Understanding the adaptative growth strategy of Lactobacillus plantarum bz in silico optimization PLOS Computational Biology- www.ploscompbiol.org
Wijtzes T., J.C. de Wit, M.H. Zwietering 1995 Modeling bacterial growth of Lactobacillus curvatus as a Function of acidity and temperature- Applied Environmental Microbiology 61 (7), p. 2533-2539.

THE SENSITIVITY TO ANTIBIOTICS OF SOME *LACTOBACILLUS SALIVARIUS* STRAINS ISOLATED FROM DENTAL ROOT CANAL AND TWO *LACTOBACILLUS* PROBIOTIC STRAINS

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Abstract

In this work the sensitivity of two Lactobacillus salivarius strains isolated from dental root canal (G1 and G2) and two Lactobacillus probiotic strains by intestinal origin: Lactobacillus salivarius probiotic and Lactobacillus rhamnosus GG, against different antibiotics has been examined. All of them were found to be very sensitive (Ø ≥25mm) to rifampicin and penicillin, whereas, all were resistant to acid nalidixic and streptomycin. These data show that cultures should be tested for their antibiotic sensitivity before being used as probiotics or in food industry.

Keywords: *dental root canal, Lactobacillus salivarius, probiotic.*

INTRODUCTION

Some mechanisms regarding antibiotic resistance are known at different bacteria.

-The genes acquired from bacteria which encode enzymes such as lactamases that inactivate the antimicrobial substance before it exerts an effect.

-The efflux pumps of some bacteria can extrude the antimicrobial agent from the cell before it arrives to the target site.

-The final products of some metabolic pathway (genetic acquired by the bacteria) can alter the cell wall and the binding site for antimicrobial agent.

The lactic bacteria can contain several antibiotic resistance genes that can be transferred to pathogenic bacteria.

MATERIALS AND METHODS

Antibiotic sensitivity of two *Lactobacillus salivarius* strains isolated from dental root canal (G1 and G2) and two *Lactobacillus* probiotic strains by intestinal origin: *Lactobacillus salivarius* probiotic and *Lactobacillus rhamnosus* GG was evaluated using the disc diffusion method. Inhibition zone was measured in mm.

RESULTS AND DISSCUTIONS

The obtained data are shown in table 1.

Table 1. The inhibition zone diameter expressed in mm

ANTIBIOTIC	Symbol	L. salivarius probiotic	G 1	G 2	LGG
Ampicilin	AMP	20	15	13	0
Chloranphenicol	C 10	26	20	22	18
Streptomycin	S 10	0	0	0	0
Rifampicin	RD 30	>30	25	26	>30
Nalidixic acid	NA 30	0	0	0	0
Novobiocin	NV	15	0	12	13
Erytromicyn	E 10	18	20	21	22
Penicilin	P 2	30	25	27	25

All investigated *Lactobacillus* strains were resistant to nalidixic acid and streptomycin. These data are according with Hummel et al. (2007) which reported that lactobacilli seem to be intrinsically resistant to quinolones (nalidixic acid). Danielsen and Wind (2003) and Zhou et al. (2005) also reported that some lactobacilli have a high natural resistance to streptomycin. *Lactobacillus rhamnosus* GG was resistant to ampicillin and *Lactobacillus salivarius* G1 strain isolated from dental root canal manifested resistance to novobiocin.

All strains were very sensitive (Ø of inhibition zone ≥25mm) to rifampicin and penicillin. Danielsen and Wind (2003) reported that lactobacilli generally seem to be sensitive to penicillin.

CONCLUSIONS

Major differences regarding antibiotic sensibility between the *Lactobacillus salivarius* strains with dental origin and the probiotic strains were not observed.

All *Lactobacillus* strains were resistant to nalidixic acid and streptomycin.

All strains showed a high sensitivity to penicillin and rifampicin.

ACKNOWLEDGMENTS

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REFERENCES

Danielsen M. and A. Wind, 2003- Susceptibility of Lactobacillus sp. to antimicrobial agents. International Journal of Food Microbiology, 82, 1-11.

Hummel, A. S., C. Hertel, W. H. Holzapfel, and C. M. A. P. Franz. 2007.

Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Applied Environmental. Microbiology 73:730–739.

Zhou J.S., C.J. Pillidge, P.K. Gopal and H.S. Gill 2005. Antibiotic susceptibility profile of new probiotic *Lactobacillus* and *Bifidobacterium* strains. International Journal of Food Microbiology, 98:211-217

PLASMA CORTISOL LEVEL AND MAIN METABOLISM EVOLUTION IN PREGNANT EWE

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Abstract

The purpose of this research was to determine the role of glucocorticoid hormones in enhancing metabolic processes characteristic of gestation in ewe. In this respect a group of pregnant ewes was monitored in terms of evolution of plasma cortisol and metabolism during a period of about 14 weeks from the time of pregnancy diagnosis. Parallel determinations were made in a control group consisting of non pregnant ewes. It was determined the evolution of plasma cortisol, plasma glucose, triglycerides, cholesterol, proteins, urea, uric acid, fibrinogen, creatinin, amylase, calcium, phosphorus and magnesium. Results relived progressively increasing of plasma cortisol concentrations in pregnant ewes compared with control. In parallel it was found different evolution of determined blood parameters by comparing with control, meaning different metabolic evolution of the studied parameters in pregnant vs. control ewes. The metabolic evolution of the blood determined parameters in pregnant ewe correlated to an increased secretion of cortisol.

Key words*: cortisol, ewe, pregnancy, metabolism.*

INTRODUCTION

The pregnancy status of a mammalian in marked by a very intensive and varied metabolic dynamic. This metabolic dynamic is monitored by nervous system as well as endocrine system. In addition to protecting and carrying consecrated pregnancy hormones (e.g. progesterone, estrogen), a special place and role they hold other metabolic hormones, including glucocorticoid hormones (Campbell, 1987; Reisman şi Matheny, 1968; Wood, 1988).

MATERIAL AND METHODS

To reach the objectives of this research the following activities were performed: firstly, it was identified a farm which agreed the collaboration in these experiments. Then, a group of pregnant ewes (experimental) and non-pregnant (control) ewes were diagnosed. The experimental and control groups were constituted by ewes of Spanca, Karakul and Tzigae crossed

breed, two years aged, second gestation. The pregnant ewes were diagnosed using an ultrasound method (LOGIQ 100 TM PRO echograph). This device allows the diagnostic of gestation and the number of fetuses. The groups were clinically monitored for the evolution of gestation and health. Both ewe groups, experimental and control were kept in the herd and fed *ad libitum* using an alpha alpha and grass hay based forage.

Blood was sampled for a period of gestation monitoring: 14 weeks from the moment of pregnancy diagnose.

For blood sampling, only p.v.c. EDTA-type anticoagulant vacuutainers were used, tight locked, that were slowly agitated for homogenization. Sampling was done 14 times, at about one week intervals, from November (from the moment when the diagnostic of pregnancy was technically possible) to February.

The tubes were positioned on 45° angle for a fast velocity sedimentation / separation, stored at laboratory temperature, and transported in time for laboratory determinations, which were done no later than two hours from the moment of sample collection.

Determination of serum hydrocortisone was done by an enzymatic method according to Dima Gesellschaft für Diagnostik. Blood biochemical parameters (glucose, total lipids, cholesterol, total protein, urea, uric acid, fibrinogen, creatinin, calcium phosphorus and magnesium and amylase activity) were determined according to Manta *et al*. (1976).

The obtained data were statistically processed and expressed as mean ± standard error of mean of each analyzed group. The differences between the obtained values from the two groups were statistically analyzed by paired Student't test, according to Tacu (1968). The differences between group were considered significant when $P < 0.05$.

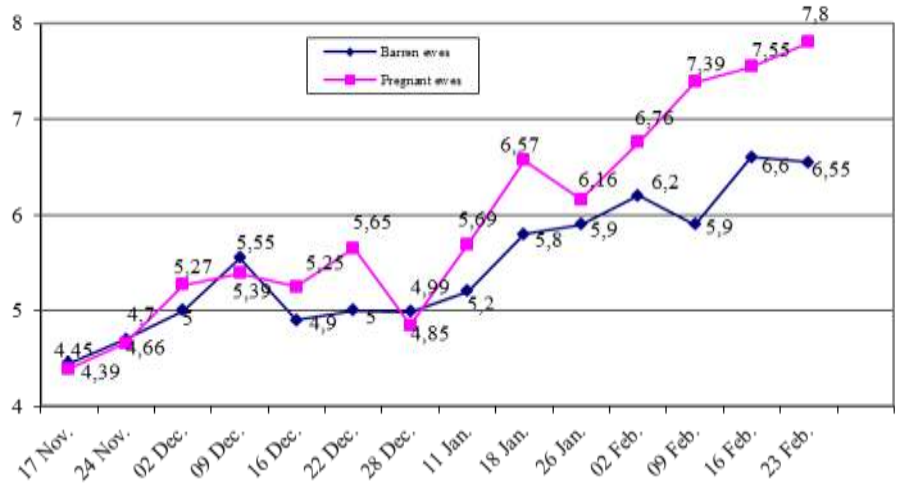
RESULTS AND DISCUSSION

The data presented in Figure 1 show the following: mean plasma cortisol concentrations were not constant over the considered period, ie, November 17, 2009 to February 23, 2010. The values varied between 4.39 and 8.45 ng / mL of plasma in the experimental group (pregnant ewes).

There is a general tendency to increase plasma levels of cortisol, both in the group of pregnant ewes and ewes in the control group (barren, non-pregnant). In this respect it is found that the cortisol levels of November, after installing the latest pregnancy, were located at around 4.45 ng / mL in the control group and 4.39 ng / mL in the experimental group without

statistic significant differences between groups. These values increased to 4.99 ng / mL in the control group and 4.95 ng / mL in the experimental group in mid-December, then to 5.82 ng / ml in the control group and 6.57 ng / mL in the experimental group mid-January.
In mid-February, values of plasma cortisol levels were 6.60 ng / mL in the control group and 7.55 ng / mL in the experimental group. It appears that this growth has characterized both the control group and experimental group. On the other hand it was found a statistic significant difference ($P < 0.05$) between mean plasma cortisol in the experimental group compared with the control, starting from January. This has become a distinct difference statistically significant ($P < 0.01$) in February, after which the mean plasma cortisol of the control group amounted to 6.55 ng / mL, while those in the group of pregnant ewes values were 7.80 ng / mL. Increased plasma cortisol in the group of barren ewes could be explained by the gradual cooling of the weather and switch to maintenance in stalls, which may create some discomfort and stress in a row, that can be reflected in increased cortisol secretion (Damjanovic, 2008).

Figure 1. The evolution of the plasma cortisol concentration in pregnant ewes vs non pregnant ewes along a period of 14 weeks of gestation, from the moment of diagnose (in ng/mL)



For the characterization of metabolic pathways in pregnant ewes, and if this trend is related or not with cortisol level it was monitored the development of the blood plasma concentration of glucose, triglicerides, proteins, urea, uric acid, fibrinogen and amylase. It was also monitored

blood levels of certain minerals: calcium, phosphorus and magnesium. Findings of metabolic pathways of calcium, phosphorus and magnesium were carried out based on monitoring the evolution of these elements in serum. The results are presented in Table 1.

Table 1

The evolution of the main blood biochemical parameters in pregnant ewes vs. non pregnant ewes along a period of about 14 weeks of gestation, from the day of pregnancy diagnostic

Item	Values of reference	Data of blood sampling and the obtained values											
		17 Nov.		09 Dec.		28 Dec.		11 Jan.		26 Jan.		23 Feb.	
		C	P	C	P	C	P	C	P	C	P	C	P
Glucose (mg/dL)	40–80*	54± 21	112± 34a	78± 32	157± 12 a	74± 32	147± 16 a	87± 23	143± 31a	76± 29	132± 43 a	77± 29	132± 33 a
Total lipids (mg/dL)	~300	132± 21	420± 150 a	229± 99	309± 176 a	140± 54	300± 45 a	134± 17	350± 134 a	143± 54	409± 135 a	152± 44	454± 143 a
Cholesterol (mg/dL)	60-150*	98± 32	143± 22	207± 35	232± 43	190± 109	154± 43	132± 31	143± 87	176± 39	219± 86	194± 52	136± 40
Total proteins (g/L)	58-60***	79± 43	165± 71aa	68± 21	113± 15	80± 23	143± 29	87± 28	145± 43	75± 28	140± 54	79± 31	158± 32a
Albumins (g/L)	42% from total***	39± 16	69± 20	30± 6	50± 8	46± 18	60± 21	40± 8	68± 21	36± 19	65± 22	38± 21	77± 21
Globulins (g/L)	58% from total***	40± 12	96± 25	38± 4	63± 14	43± 16	83± 15	47± 16	77± 29	41± 9	85± 32	41± 9	81± 20
Urea (mg/dL)	35	29± 3	44± 10	49± 12	40± 5	24± 20	37± 10	39± 12	46± 12	41± 10	52± 20	34± 11	54± 15

* Normal values in adult ewe, according to Reece, 1996.
** Normal values in adult ewe, according to Kolb, 1974.
*** Normal values in adult ewe, according to Rudas, 1996.
C = Group of control, non-pregnant ewes (n = 8)
P = Experimental, pregnant ewes (n =12)

a P<0,05 (statistic processed vs. control in the same day)
aa P<0,01

The evolution of blood glucose values in the group of pregnant ewes were significantly higher (P <0.05) than non-pregnant ewes throughout the monitoring period, varying between 112 and 145 mg / dL of plasma, maintaining relatively constant higher an showing an increase of the metabolism of this metabolic fuel.

Table 1 (continued)

A similar situation is recorded concerning the plasma total lipids, to whom

Item	Values of refer-ence	Data of blood sampling and the obtained values											
		17 Nov.		09 Dec.		28 Dec.		11 Jan.		26 Jan.		23 Feb.	
		C	P	C	P	C	P	C	P	C	P	C	P
Uric acid (mg/dL)	0,1-2*	0.5± 0.1	4.4± 0.5	2.3± 1.2	3.5± 0.5	1.8± 0,8	1.1± 0.3	1.1± 0.5	3,2± 0,3a	1.5± 0.3	3.3± 1.2	1.5± 0.4	3.9± 0.9a
Fibrinogen (mg/dL)	250-450**	244± 121	232± 65	324± 85	251± 132	386± 174	321± 200	210± 44	238± 39	200± 75	222± 32	236± 213	231± 65
Creatinin (mg/dL)	1-2*	1.0± 0.3	1.2± 0.4	0.4± 0.2	0.9± 0.3	0.8± 0.5	1.5± 0.5	1.8± 0.8	2,0± 1,0	1.1± 0.2	1.8± 0.6	2.0± 0.5	2.5± 1.2
Amylase (mg/dL)	200-500*	230± 76	300± 109	180± 55	180± 76	420± 210	430± 289	350± 210	460± 321	240± 209	360± 210	140± 90	260± 213
Calcium (mg/dL)	4.5 – 6.0*	4.0± 0.3	7.6± 2.1	4.9± 1.5	6.5± 0.5	4.2± 2.2	6.6± 2.6	4.5± 2.2	7.0± 3.0	4.4± 1.6	7.5± 2.3[a]	3.8± 2.7	8.4± 2.2a
Phosphorus (mg/dL)	2 – 7*	2.4± 0.1	3.0± 0.8	2.8± 0.1	3.2± 1.0	23± 0.2	3.1± 0.6	2.5± 1.1	2.5± 0.9	2.9± 1.0	3.1± 1.6	3,0± 1.8	2.9± 2.0
Ca/P	1 – 2	1.6	2.5	1.7	2.0	1.8	2.1	1.8	2.8	1.5	2.4	2.6	2.8
Magne-sium (mg/dL)	1.8 – 2.3*	1.4± 0.7	2.0± 0.4	3.2± 0.7	1.8± 0.2	2.6± 1.7	2.2± 1.0	2.0± 1.1	2.1± 0.6	1.9± 0.7	1.6± 0.5	2.2± 0.6	1.9± 0.5

Legend:

* Normal values in adult ewe, according to Reece, 1996.
** Normal values in adult ewe, according to Kolb, 1974.
*** Normal values in adult ewe, according to Rudas, 1996.
C = Group of control, non pregnant ewes (n = 8)
P = Experimental, pregnant ewes (n =12)
[a] P<0,05 (statistic processed vs. control in the same day)

metabolism was found significantly higher (P <0.05) in pregnant ewes compared with non-pregnant. Lipid levels ranged between 300 ± 45 and 454 ± 143 mg / dL of plasma in pregnant ewes and 124 ± 21 and 152 ± 44 in barren ewes. The high level of lipids illustrates their high tissue depot mobilization, which is due at least in part, to the high levels of plasma cortisol in pregnant ewes, as just it was related.

Pregnant ewe plasma total protein values varied between 113 ± 14 and 165 ± 71 of plasma vs. 68±21 and 100±21 g / L in control (P <0.01). High

concentration of plasma proteins can be partly attributed to high levels of cortisol, but, given the physiological state of advanced pregnancy, probably other factors, maternal or fetal, such as increased protein synthesis capacity of the liver, could modify the metabolism of that large category of substances (Reisman and Matheny, 1998, Leach and Taylor, 2002). On the other hand it should be noted that the albumin / globulin ratio remained relatively constant. A special mention must be done on serum total proteins, which have exceeded in pregnant ewe twice the values in non-pregnant ewes

As a reflection of enhanced protein catabolism under the influence of cortisol it was found elevated serum urea and uric acid in pregnant ewe group, compared with barren ewes. In pregnant ewes, the values of the urea and uric acid concentrations were above the accepted physiological limits: 35 mg / dL urea and 0.1 - 2 mg / dL uric acid.

Concerning the developments of plasma fibrinogen and creatinine in pregnant ewes, they were not found significant differences compared to the group of barren ewes. A similar situation was found in the serum amylase activity whose value did not differ significantly between the group of barren ewes and pregnant ewes, along the entire monitored period.

With regard to calcium metabolism, such as it was presented, in advanced stage of pregnancy, birth and immediately postpartum, metabolism of this chemical element is experiencing a very intense dynamic, calcium (and phosphorus) showing a very high turn-over. According to literature data, glucocorticoid excess (Cushing's disease in the human, for example) causes muscle weakness, atrophy and loss of bone strength and bone matrix (Christiansen *et al*., 2007**;** Johnson *et al*., 1981). With an increased secretion of glucocorticoids, bone formation is reduced and less quantity of calcium is absorbed, but calcium is excreted in higher amount, which is reflected in the evolution of its plasma concentrations, exceeding in some way regulating functional systems for its homeostasis.

For these reasons we started to monitor calcium plasma concentrations together with other minerals (phosphorus and magnesium). Table 1 (continued) shows the evolution of serum calcium, phosphorus and magnesium levels in the group of pregnant ewes compared to non-pregnant ewes on the same monitoring period, from November up to February.

The data analysis presented in Table 1 (continued) shows that serum calcium was significantly higher ($P < 0.05$) for most data we have been done the determinations in the group of pregnant ewes vs. non-pregnant ewes throughout the all monitored period. Also, the Ca / P ratio was significantly

higher in the same batch of pregnant ewes compared with control. Plasma calcium concentration in non-pregnant ewes ranged from 3.8 ± 2.7 and 4.9 ± 1.4 mg / dL of plasma while in the group of pregnant ewes, oscillations ranged from 6.5 ± 0.5 to 8.8 ± 2.0 mg / dL of plasma.

The data analysis presents a constant rise in the concentration of plasma calcium in pregnant ewes, this growing being associated with intensive fetal skeleton growing.

Regarding phosphorus, serum phosphorus concentration is maintained within normal limits, but not so closed limits as those of calcium. In the young lambs, serum phosphorus levels are 3 to 4 times higher than in adult ewes. Also, plasma levels of phosphate shows large diurnal variations with maximal values at night.

In our measurements, the Ca / P ratio was between 1.5 and 2.6 in barren ewes and between 1.8 and 2.8 in pregnant ewes, which indicates increased metabolism of both calcium and phosphorus taken into account, which again can be considered as an effect of high plasma levels of cortisol, which in turn causes a high turn-over of these components.

CONCLUSIONS

During the period of gestation in sheep, progressively increasing plasma cortisol occurs, which indicates involvement of this hormone in the regulation of metabolic processes, exacerbated by the physiological state of pregnancy.

Intensification of metabolic processes such as protein, lipids, carbohydrate, calcium and phosphorus metabolisms during pregnancy may be at least in part attributed to increased secretion of cortisol.

REFFRENCES

Campbell, E.A., E.A. Linton, C.D. Wolfe, P.R. Scraggs, M.T. Jones, P.J. Lowry. 1987 - Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. J. Clin. Endocrinol. Metab. 64:1054–1059.

Challis J.R., A.N. Brooks. 1989 - Maturation and activation of the hypothalamic - pituitary adrenal function in fetal sheep. Endocrine Reviews, Vol 10, 182-204.

Christiansen, J.J., B.D. Christian, H. Claus, J.S. Christiansen, O. Schmitz, J. Weeke, J.O. Lunde Jørgensen, N. Møller. 2007 - Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. The Journal of Clinical Endocrinology & Metabolism Vol. 92, No. 9, pp. 3553-3559, 2007.

Damjanovic, S., R. Stojic, N. Lalic, M. Petakov, Al. Jotic, T. Isailovic, B. Popovic, D. Macut şi I. Bozic. 2008 - Changes in cortisol and insulin during pregnancy in relation to basal metabolic rate. Endocrine Abstracts, 16 P361.

Johnson, J.W., Mitzner W., Beck J.C., London W.T., Sly D.L., Lee P.A. 1981. Long-term effects of betamethasone on fetal development. Am J Obstet Gynecol ;141:1053-64.
Kolb, E. – Physiologie des animaux domestiques, Vigot Frères Èd., Paris, 1965.
Leach, G.L., Taylor, M.H. 2002 - The effects of cortisol treatment on carbohydrate and protein metabolism in Fundulus heteroclitus. General and Comparative Endocrinology. Volume 48, Issue 1, p. 76-83.
Manta, I., M. Cucuianu, G. Benga, A. Hodârnău. 1976 - Metode biochimice în laboratorul clinic. Editura "Dacia", Cluj-Napoca.
Reece, W.O. 1996 – Physiology of domestic animals. 2nd Edition, Williams & Wilkins Ed.
Reisman, L.E., A. Matheny. 1998. Corticosteroids in pregnancy. Lancet; 1:5923.
Rudas, P. 2001 – Veterinary physiology. Department of veterinary physiology, University of Veterinary Science, Budapest, Hungary.
Tacu, A. 1968: t-test in Statistic methods in zootechny and veterinary medicine, pp. 21-53. Editura Agrosilvică, Cluj-Napoca, România.
Wood, C.E. 1998 - Insensitivity of near-term fetal sheep to cortisol: possible relation to the control of parturition. Endocrinology, Vol 122, 1565-1572.

ENZYME OUTPUT CAPACITY OF THE RABBIT EXOCRINE PANCREAS TO ADAPT DIFFERENTLY TO FOOD SUBSTRATE CONCENTRATION CHANGES

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Abstract

Three groups of adult rabbits were fed for 35 days with high starch diet (high starch group, HSG), high protein diet (high protein group, HPG), or high fat diet (high fat group, HFG) compared with a control group (CG) fed with a specific diet. Then pancreatic juice was collected and measured in acute experiments, in two variants of secretion: basal and stimulated by secretin. Pancreatic juice samples were analyzed for protein content and amylase, trypsin and lipase activities. Basal values of juice flow showed no significant differences between any experimental fed group vs. CG (P>0.05). Secretin stimulated juice flows were increased in all the groups, but the increase was significant higher only in HSG vs. CG (P<0.05). Basal protein flows of experimental fed groups did not differ significantly vs. CG (P>0.05). In contrast, the stimulated protein output was significant higher in HSG vs. CG (P<0.05). Amylase activities were significant higher in HSG vs. CG, both in basal (144.3×10^3 and, respectively, 52.0×10^3 amylase units (AU), P<0.001) and in the stimulated pancreatic juice (422.0×10^3 and, respectively, 162.1×10^3 AU, P<0.001). Moreover, the activities of trypsin and lipase in HSG did not differ significantly vs. CG (P>0.05), nor for basal neither for stimulated juice. Trypsin activity (in nmols benzoyl-argynil-ethyl-ester decomposed / 10 min / kg b.w.) increased significantly in HPG vs. CG, both in basal (62.5 vs. 22.2, P<0.01) and in stimulated juices (166.0 vs. 31.5, P<0.001). On the other hand, amylase and lipase activities of HPG group were similar to those of CG. Basal lipase activity (in mequivalents of liberated oleic acid per mg protein per h, 37°C) was higher in HFG vs. CG (122.4 and 86.5, respectively). In the stimulated juice, lipase activity increased to 246.0 in HFG and 184.1 in CG, but no significant differences were found in HFG vs. CG nor for lipase neither for amylase and trypsin (P>0.05).

Key words: *food composition, pancreatic enzymes, rabbit*.

INTRODUCTION

Many works show the influence of different factors on the rabbit enzymatic digestive system, intestinal glands and the pancreas in main. Gilliland and Glazer (1980) found that enzyme secretion by the rabbit pancreas remained proportional (parallel) after acute stimulation despite a 100% rise in protein output. The researches of Gutierez *et al*. (2002) indicated that digestive capability of early-weaned rabbits is limited and should be taken into

account to establish optimal levels and sources of carbohydrates in diet. Debray *et al*. (2002) found a different development of trypsin, chymotrypsin, amylase and lipase activities into the small intestine contents, not related to changes in pancreatic or intestinal enzymatic profiles but more dependent on quality of dietary ingredients. In vitro rabbit pancreas experiments showed that direct bath administration of pancreozymin or acetylcholine produced prompt increases of protein output (Welch and Littman, 1974). The influence of diet on digestive parameters and not only has still many unknowns (Gidenne and Fortun-Lamothe, 2002). The aim of our work is to find the rabbit pancreas ability to change flow ratio of different digestive enzymes in the secreted juice depending to the composition of diet.

MATERIAL AND METHODS

Six months old New Zealand white male rabbits, 3.230± 0.120 kg b.w. were used in this experiment. The rabbits were housed in common metallic cages, two rabbits per cage, in a naturally lighted room at 24±3°C, and 65% humidity. The cages were made of galvanized wire net and equipped with automatic drinkers and manual feeders. The animals were fed *ad libitum* and have free access to water. Four groups of seven rabbits each one were constituted according to the composition of their diet:

- a control group (CG) fed with a specific diet
- a group (HSG) fed with a high starch diet
- a group (HPG) fed with a high protein diet
- a group (HFG) fed with a high fat diet.

Duration of feeding with experimental prescriptions was 35 days. Main ingredients of the diets were: maize, wheat bran, soybean meal, dehydrated lucerne (*Medicago sativa*), and a vitamin-mineral supplement. The starch content was enriched by addition of maize. Protein content was enriched by addition of soybean meal. Fat content was enriched by addition of flaxseeds. The chemical composition of the diets are presented in Table 1.

Table 1

Chemical composition of the experimental diets

Diet	Dry matter (DM) (g/Kg)	Crude fiber (g/Kg DM)	Starch (g/Kg DM)	Crude protein (g/Kg DM)	Fat (g/Kg DM)
Control diet	**877**	**202**	**188**	**123**	**28.3**
High starch diet	**897**	**176**	**261**	**120**	**18.0**
High protein diet	**868**	**196**	**178**	**162**	**27.1**
High fat diet	**870**	**184**	**181**	**120**	**58.3**

At the end of the experimental feeding period, four or five rabbits of each group were anesthetized with chloralose 1% in a 0.9% saline solution injected intravenously (*v. auricularis*), in dose of 100 mg/Kg b.w. The *vena femuralis* was prepared by inserting a cannula to inject secretin for stimulation of pancreatic juice flow. The abdomen cavity was opened and the main pancreatic duct was spotted. A silver cannula (outer diameter of 0.05 mm) was inserted into the main pancreatic duct just before its opening into the intestinal lumen to collect pure pancreatic juice. A calibrated polyetilene tube (0.01 mL/mm) was attached to the free end of the silver cannula for pancreatic juice flow measurement. Then, the abdominal wall was closed with sutures. The rectal temperature of the animals was maintained at 38°C by a heating lamp.

The basal pancreatic juice was collected and measured for a period of 50 minutes from the moment of the attachment of the cannula to the main pancreatic duct. The calibrated tube was detached when the period of 50 minutes ended. Another empty calibrated tube was attached immediately to the free end of the silver cannula. Then, a single dose of synthetic secretin (BioVision, San Francisco), 100 μg/Kg b.w. was injected to collect stimulated pancreatic juice. Measurement of pancreatic juice flow continued for another 50 minutes.

The volume of juice was measured on the calibrated tube every 10 minute from the beginning of the collecting, so five periods of measurements were done: 0-10, 10-20, 20-30, 30-40 and 40-50 minute periods both, in basal and stimulated experiments. At the end of the 50 minute periods, all volume of pancreatic juice was measured. The obtained values from each rabbit were used for the calculation of the mean values of the two periods of secretion (0-50 minutes for basal and 0-50 minutes for stimulated secretion).

The contents of calibrated tubes from each rabbit and from each sample (basal or secretin stimulated) were separately collected and diluted 10 or 20

times with a buffered saline solution and conserved at -20°C up to biochemical determinations. The anesthetized animals were killed at the end of the experiment, by cutting the a. *carotidis communis*.
Protein contents, amylase (EC 3.2.1.1), trypsin (EC 3.4.21.4), and lipase (EC 3.1.1.3) activities were measured in each pancreatic juice sample, basal or secretin stimulated, following previous methods mentioned by Dojana *et al*. (2000). The obtained data were statistically analyzed and presented as mean ± standard error of mean. The significance of differences between control and experimental groups was evaluated using Student's unpaired ***t*** test.

RESULTS AND DISCUSSION

The evolutions of basal and secretin stimulated juice flow for 50 minute periods are presented in Figure 1. Mean basal value (in microL / 10 minutes / kg b.w.) of juice flow of the CG was 27.0 (not shown in Figure 1) and maintained relatively constant, ranging between 20 and 35 along the 50 minutes of acute experimental monitoring. Mean basal values of juice flow of experimental diet groups along the same period of time were (not shown): 40.2 in HSG, 31.2 in HPG and 29.2 in HFG, with no significant differences vs. CG ($P>0.05$).
Secretin stimulated the juice flows. Peaks of the juice flow were reached in 20 minutes following the moment of secretin administering in all the four groups. The peak of the secretin stimulated pancreatic juice flow (in μL / 10 minutes/kg b.w.) was 176 in CG. In HPG and HLG groups, the peaks of secretin stimulated juice flow were 169 and 150, respectively, with no significant differences vs. CG ($P>0.05$). The highest peak of juice secretion was reached by the HSG, with a value of 234 and a significant difference was found vs. CG ($P<0.05$). Mean stimulate values of juice flow (not shown) were: 130 in CG, 180 in HSG, 125 in HPG and 103 in HFG, a significant difference being between HSG and control ($P<0.05$).
It seems that the pancreas of HSG rabbits has undergone some functional changes during or due to feeding with high starch diet since the peak value and mean juice flow of the stimulated secretion in this group were significant above the CG. Some similar results were found in piglets. Jakob *et al*. (2000) reported that potato fiber in the diet in growing pigs evoked in tendency an increase in the volume of secretion of pancreatic juice.

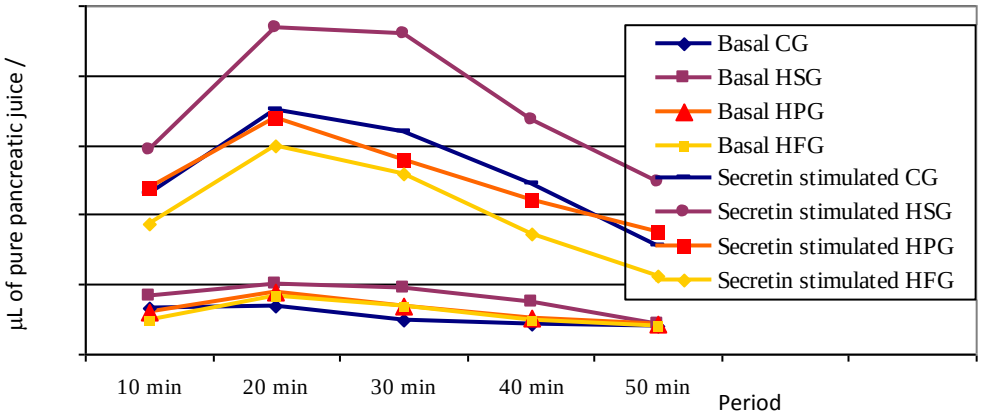

Figure 1. Basal and a single dose of secretin (100 µg/Kg b.w.) stimulated pancreatic juice flow levels over 50 minutes of monitoring periods in adult rabbits fed with high starch, fat or protein diets for 35 days vs. a control rabbit group fed with a specific diet.

Protein contents and enzyme activities of the collected pancreatic juice samples are shown in Table 2.

No statistic differences were found between groups fed with experimental diets and CG concerning the basal *protein* output ($P>0.05$). Secretin administering induced a 4-fold increase of protein output in both HSG and HPG while in CG and HFG the increase was about 3-fold. The stimulated protein output was higher in both HSG and HPG rabbit groups vs. CG even though the differences were not significant from statistic point of view ($P>0.05$). Protein outputs induced by the administration of secretin in our experiments, although smaller, are comparable to those induced by cholecystokinin (Gilliland and Glazer, 1980) or cholecystokinin and methacholine chloride on pancreatic exocrine secretion in rabbits (Adelson *et al*., 1995). Significant differences regarding pancreatic protein output were reported by Jakob *et al*. (2000) in growing pigs fed with high potato fiber diet vs. control.

Amylase activity was found about 3-fold more increased in basal pancreatic juice of HSG vs. CG ($P<0.001$). Secretin stimulated pancreatic juice of HSG registered an amylase activity value about 3-fold higher vs. the secretin stimulated amylase activity of pancreatic juice in CG ($P<0.001$). In the same HSG, the activities of trypsin and lipase did not differ significantly vs. CG ($P>0.05$), nor for basal neither for secretin stimulated pancreatic juice samples.

Table 2

Protein output (μg protein per 10 min per kg b.w.) and enzyme activities of the pancreatic juice collected during 50 minutes periods as a basal and as a single dose of secretin (100 μg/Kg b.w.) stimulated secretions in adult rabbits following 35 days of feeding with high starch, protein or fat diets vs. a control group. B = basal secretion, S = secretin stimulated secretion. The values are expressed as mean ± standard error of mean of four of five animals in acute experiments

Group	Protein output		Amylase activity[1]		Trypsin activity[2]		Lipase activity[3]	
	B	S	B	S	B	S	B	S
CG	32.1 ± 2.3	88.9 ± 17.4	52.0 ± 7.4	162.1 ± 32.2	22.2 ± 4.4	31.5 ± 4.0	86.5 ± 20.0	184.1 ± 42.1
HSG	35.3 ± 5.2	124.3 ± 32.1	144.3 ± 33.3**	422.0 ± 51.4**	26.5 ± 6.0	42.0 ± 10.0	90.0 ± 19.4	201.0 ± 33.2
HPG	28.6 ± 4.1	105.4 ± 19.5	25.3 ± 5.5	144.3 ± 4.3	62.5 ± 12.5*	166.0 ± 21.5**	71.3 ± 18.5	176.5 ± 46.1
HFG	25.2 ± 6.2	85.6 ± 22.2	32.2 ± 8.4	156.7 ± 5.0	18.7 ± 5.0	42.9 ± 5.4	122.4 ± 31.3	246.0 ± 61.3

CG – control group, rabbit group fed with a specific diet
HSG – rabbit group fed with high starch diet
HPG – rabbit group fed with high protein diet
HFG – rabbit group fed with high fat diet
*P<0.01, unpaired Student's *t* test
** P<0.001, unpaired Student's *t* test

[1]Amylase activity is expressed as amylase units, AU×10^3, mg of starch hydrolyzed in 30 minutes at 37°C.
[2]Trypsin activity is expressed as nmols benzoyl-argynil-ethyl-ester decomposed / kg b.w./10 min.
[3]Lipase activity is expressed as mequivalents of liberated oleic acid per mg protein per h, 37°C, using triolein as a substrate.

Trypsin activity was found significantly increased in basal pancreatic juice of HPG vs. control (P<0.01), with a 3/1 ratio. In the same group, the trypsin activity of the secretin stimulate pancreatic juice was significant higher vs. CG (P<0.001). On the other hand, amylase and lipase activities values of HPG group were similar to those of CG. According to the results of this experiment, the pancreas could discharge large quantities of trypsin into the small intestine, although total protease activity of the small intestine content could be lower than that of the caecum (Marounek *et al*., 1995).

Lipase activity was also increased in basal and stimulated pancreatic juice of HLG by comparing to CG. However, lipase activities in HLG were not significantly different vs. CG (P>0.05), nor for basal nether for stimulated pancreatic juice. Our results regarding lipase activity are partially in agreement with the results reported by other authors: Debray *et al*. (2003) found that small intestine activity of lipase was higher in high fat diet fed

rabbits than in low fat diet fed rabbits, but they found that the other pancreatic and intestinal enzyme activities were not influenced by the energetic sources of the diet. Although amylase and trypsin secretion showed parallel increases (3-fold increases each one in our experiment), in the case of lipase, the situation seems to be different. Lipase activity increased much less than the other two studied enzymes, thus seeming to depend on other reasons, not only the presence of the food fat substrate. Adelson *et al*. (2005) consider that the nonparallel secretion of the digestive enzymes occurs routinely, even during constant stimulation, and is due heterogeneous intrapancreatic sources. Instead, parallel increase in the mean values of activities of lipase, trypsin and amylase was found by Jakob *et al*. (2000) in growing pigs fed with high potato fiber diet.

CONCLUSIONS

Higher starch, protein or fat diets do not alter specifically the basal or the stimulated pancreatic juice volume flow, or protein output in rabbits. In contrast, pancreatic enzyme output adapts differently to food substrate concentration changes for starch, protein or fat. Further researches could find the velocity of adaptation of pancreatic exocrine secretion to changes in substrate levels and to what degree the pancreas can respond adequately to increased supply of various substrate levels.

REFERENCES

Adelson J.W., Clarizio R., Coutu J.A. 1995. Pancreatic digestive enzyme secretion in the rabbit: Rapid cyclic variations in enzyme composition. Proc. Natl. Acad. Sci., 92, 2553-2557.

Debray L., Le Huerou-Luron I., Gidenne T., Fortun-Lamothe L. 2003. Digestive tract development in rabbit according to the dietary energetic source: correlation between whole tract digestion, pancreatic and intestinal enzymatic activities. Comp Biochem Physiol A Mol Integr Physiol. 135, 3, 443-55.

Dojana N., Dinischiotu A., Militaru M. 2000. The effect of thyroxin, insulin, hydrocortisone or adrenaline administration on pancreatic exocrine secretion in rabbit. World Rabbit Science. Journal of World Rabbit Association, vol. 8, suppl. No. 1, 175-182.

Gidenne T., Fortun-Lamothe L. 2002. Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. Animal Science, 75, 169-184.

Gilliland E.L., Glazer G. 1980. Paralel secretion of enzymes by the rabbit pancreas. J. Physiology, 303, 33-41.

Gutierrez I., Espinosa A., Garcia J., Carabano R., De Blas J.C. 2002. Effect of levels of starch, fiber, and lactose on digestion and growth performance of early-weaned. J. Anim. Science. 80, 4, 1029-1037.

Jakob S., Mosenthin R., Thaela M.J., Weström B.R., Rehfeld J.F., Olsen O., Karlsson S., Ahrén B., Ohlsson A., Karlsson B.W., Pierzynowski S.G. 2000. The influence of potato fibre on exocrine pancreatic secretions and on plasma levels of insulin, secretin and cholecystokinin in growing pigs. Archives of Animal Nutrition, 53, 3, 273-291.
Marounek, M., Vovk S. J. 1995. Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. British Journal of Nutrition, 73, 463-469.
Welch R., Littman A. 1974. Protein output by the in vitro rabbit pancreas. J. of applied physiology, 37, 2, 235-23.

COMPARATIVE EFFICIENCY OF TIAMULIN AND DIMETRIDAZOLE IN CONTROLLING SWINE DYSENTERY

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Abstract

The purpose of this study was to evaluate the comparative efficiency of Tiamulin and Dimetridazole for treating and controlling swine dysentery. The growing performance was evaluated. Levels of average daily weight gain revealed significant differences ($P < 0.0001$) between Tiamulin and Dimetronidazole. We found strong positive correlation of the average daily gain and treatment with Tiamulin ($P < 0.0001$) and coefficient of determination ($P < 0.001$). For Dimetridazole, the correlation coefficient and the coefficient of determination have low values ($P = 0.006$).

Key words: *comparative efficacy, dimetridazole, piglets, tiamulin.*

INTRODUCTION

Piglets are susceptible to gastrointestinal disorders and digestive disturbances as a result of their immature digestive system. An effect of this is an increase in the prevalence of post-weaning scours, which leads to retarded growth, increased mortality, and additional medical costs. Antibiotic prophylaxis is one of the management methods of gastrointestinal problems. Tiamulin hydrogen fumarates are a semi-synthetic derivative of the diterpene antibiotic pleromutilin. Products based on tiamulin hydrogen fumarates have good activity against Gram-positive bacteria, mycoplasmas, anaerobes, spirochet, and Gram-negative pathogens. Dimetridazole is an antihistomonal drug traditionally used for prevention and treatment of haemorrhagic enteritis in pigs.

The purpose of this study was to evaluate comparative efficiency of tiamulin versus dimetridazole in development of clinical signs of infective enteritis in piglets. Tiamulin and Dimetridazole are not recognized as factors involved in raising piglets, therefore we sought to determine the confidence interval for the mean unknown average daily gain, corresponding to piglets affected by infective enteritis and treated with Tiamulin or Dimetridazole.

MATERIALS AND METHODS

Forty weaned piglets aged 4–5 weeks and weighting from 6–10 kg, healthy or showing clinical signs of gastrointestinal disease (poor growth, diarrhea) were equally divided into four groups: T group which received five consecutive days tiamulin, soluble granules added to the drinking water (60 mg per liter), D group which received five consecutive days dimetridazole insoluble powder feed medication (added 25 mg/kg bw), M group that not treated and S group, healthy piglets. Clinical observations were made over a period of 15 days, registering individual body weight.
A Fisher's Exact test was used to assess coefficients of variation of the individual body weight and differences between treatment groups, while ANOVA was used to analyze productivity data. Significant differences were considered when $p<0.05$. Linear regression was used to analyze connection between initial body weight and average daily gain.

RESULTS AND DISCUSSIONS

After Tiamulin drinking water medication and Dimetridazole feed medication was initiated for the T and S group piglets, within 5 days the clinical signs of gastrointestinal disease has decreased. Piglets were not treated (M group) showing clinical signs (Figure 1).

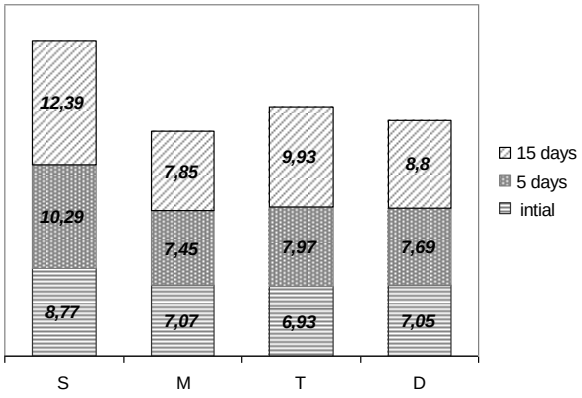

Figure 1. Dynamics of average piglet weight (kg) for the four groups.

Average daily weight gain (ADG) was also significantly greater ($P<0.05$) in the groups with antibiotic treatment compared with the untreated animals: T

group 199.8 ± 11 g and D group 116.6 ± 30 g vs. M group 51.8 ± 12 g (Figure 2).

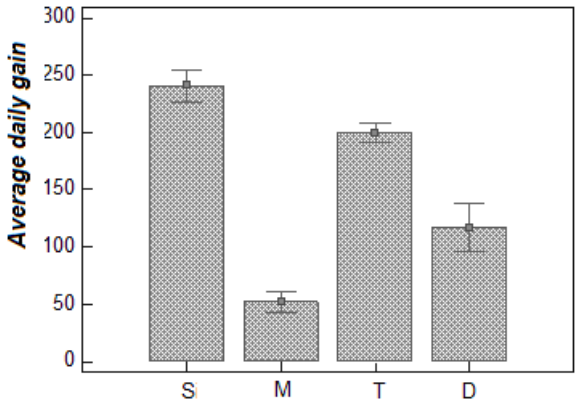

Figure 2. Dynamics of average daily weight gain (ADG, g) for the four groups.

Very good positive correlations of the average daily gain and treatment with Tiamulin and significantly coefficient of determination ($P < 0.001$) were found.

Linear regression analysis (Figure 3) indicated only 44% of ADG is determined by the linear relationship with weight piglets the first day ($P=0.03$).

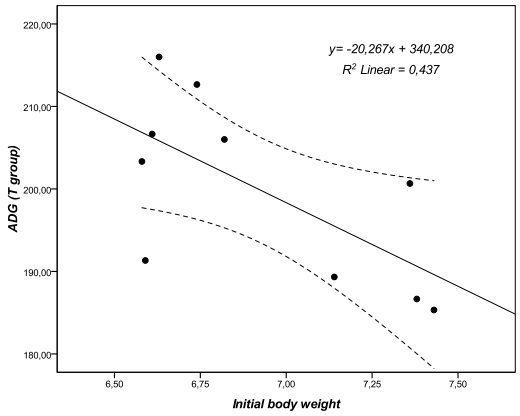

Figure 3. Representation of linear regression for T group.

To determine a 95% confidence interval, ADG values recorded were compared to groups T and D with characteristic value ADG 250 g / day

healthy piglets of the same age and weight. For Tiamulin, 95% confidence interval determined is [191.92 g/day – 207.67 g/day]. For Dimetridazole, 95% confidence interval determined is [95.25 g/day – 137.94 g/day].

CONCLUSIONS

The clinical signs of gastrointestinal disease were decreased after Tiamulin medication and Dimetridazole were initiated, within 5 days.
Average daily weight gain was significantly greater in the group with Tiamulin treatment compared with Dimetridazole treatment and the untreated animals.
Very good positive correlations of the average daily gain and treatment with Tiamulin and significantly coefficient of determination were found.
Approximately half of the ADG is determined by the linear relationship with weight piglets the first day.
The results of our experiment confirm the high effectiveness of Tiamulin in drinking water in treating and controlling the clinical, pathological, and negative productivity, main effects of enteritis in piglets.

REFERENCES

Gebhart T., Asawakarn S., Deen C., 2006. Efficacy and cost benefit study on the use of tiamutin for the treatment of porcine. IPVS, Copenhagen.

McOrist S., Smith S.H., Shearn M. F. H., Carr M. M., Miller D. J. S., 1996. Treatment and prevention of porcine proliferative enteropathy with oral tiamulin. Journal of the British Veterinary Association. 139:615-618.

Nueleanu V.I., et al., 2007. The economic implications of the enteric disease complex and its control by the utilization of pleuromutillins in swine. Vetrerinary drug, 2, 71 – 76.

Schwartz K, Knittel J, Walter D, et al. 1999. Effect of oral tiamulin on the development of porcine proliferative enteropathy in a pure-culture challenge model. *Swine Health Prod.* 7:5-11

Taylor, D.J. 2004. Treatment of Actinobacillus pleuropneumoniae (APP) infection by water medication with tiamulin. Proceedings. 18th IPVS Congress, Hamburg, Germany. Vol. 2, p.509

Walter D., Knittel J., Schwartz K., Kroll J., Roof M., 2001. Treatment and control of porcine proliferative enteropathy using different tiamulin delivery methods. Journal of Swine Health and Production, 9, 109-115.

MULTILINEAR CAPACITY ASSESSMENT OF MOUSE MESENCHYMAL STEM CELLS

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Abstract

Mesenchymal stem cells (MSCs) are defined as bone marrow derived cells which have the capacity to differentiate into the three classical mesodermal lineages (adypocites, osteoblasts and chondrocytes). Different studies revealed the ability of bone marrow derived MSCs to differentiate into ectodermic lineages including neurons. The aim of our study was to evaluate the multipotency of mouse bone marrow derived MSCs by phenotypic characterization during neuronal induction.
Mouse MSCs were isolated from bone marrow by flushing the femurs with α MEM (Gibco) medium supplemented with 1% penicillin-streptomycin (Gibco). Isolated cells were cultured in a propagation medium containing DMEM-F12 medium supplemented with 20% FCS (Gibco), 1% penicillin-streptomycin (Gibco), 5% horse serum (Sigma) and 10µg/5ml MycoZap (Mycoplasma Elimination Reagent, Lonza).
For neural induction, cells were cultured in Neurobasal medium supplemented with 0.1mM β-mercaptoethanol and 1% glutamax for 2 weeks. For phenotypic characterization, were evaluated the expression of S-100 protein and neuron specific enolase (NSE) during differentiation. Our results confirmed the multipotency of isolated cells by neuronal differentiation. At 3 days after neurogenic induction, cells morphology changed, appearing star-shaped cells and at day 4 were present specific neuritic networks. At 2 weeks after induction, the immunostaining showed the presence of S-100+ cells, confirming the glial differentiation, as well as NSE+ cells, an indicator of neuronal differentiation.

Key words: *mouse MSCs, multipotency, neuronal differentiation.*

INTRODUCTION

Mesenchymal stem cells (MSCs) are defined as bone marrow derived cells which have the capacity to differentiate into the three classical mesodermal lineages (adypocites, osteoblasts and chondrocytes) (Pall. et al., 2010; Ippokratis et al., 2005; Tondreau et al., 2004). Based on these properties, MSCs arise interest of many researchers, by generating important promises as a potential source for cellular therapies and tissue engineering (Witte et al., 1997; Baksh et al., 2004,). Currently, hematopoietic stem cells transplantation is used for the treatment of different types of leukemia (Tabbara et al., 2002). The potential use of bone marrow derived MSCs in different types of degenerative disease is related with the ability of MSCs to differentiate into ectodermic lineages including endothelial cells (Groza et

al., 2011), neurons, astrocytes and oligodendrocytes (Shihabuddin et al., 2000; Sekiya et al., 2002). In 2002, Kim et al., indicated the capacity of embryonic stem cells to differentiate in dopamine neurons after transplantation into a rat model of Parkinson's Disease. Also, in a rat model with spinal cord injury the transplantation of differentiated stem cells leeded to a long term functional improvement (Kim et al., 2002). The results of this studies advocate the use of MSCs in neurodegenerative cellular therapy, but the extrapolation to human medicine requires the development of rapid and less complicated protocols for isolation and culture. Also human medicine involve rigorouse protocols for transplantationin in order to prevent the rejection and graft-versus-host-disease.
To minimize this challenges, the aim of our study was to evaluate the multipotency of mouse bone marrow derived MSCs by phenotypic characterization during neuronal differentiation with a simple induction medium.

MATERIALS AND METHODS

Mouse MSCs were isolated from 12-14 weeks old CD1 mice in accordance with the international ethical standards. The bone marrow was harvested after femoral dissection, by removing the epiphyses and flushing the shaft with a 27G needle and 1 ml washing medium (figure 1).

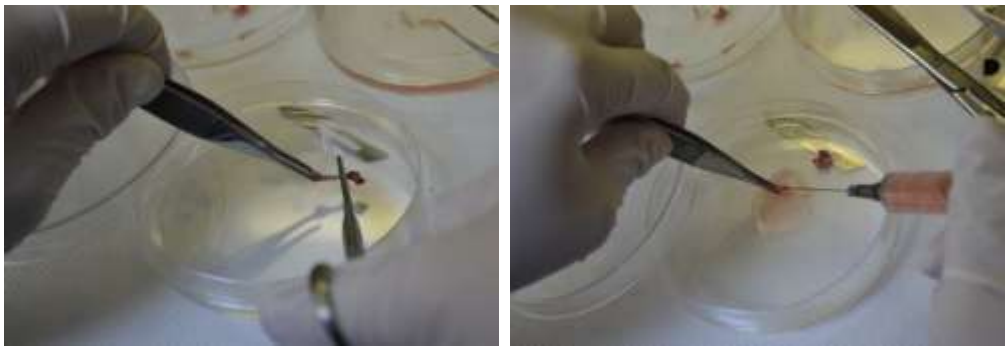

Figure 1. – Bone marrow isolation

Isolated cells were cultured in DMEM-F12 medium. For neural induction, MSCs 4x were cultured in Neurobasal medium for 2 weeks. The phenotypic characterization was done by evaluation of the expression of S-100 protein and neuron specific enolase (NSE) during differentiation. The

immunostained cultures were examined using a fluorescence microscope. In figure 2 the detailed protocol of work is shown.

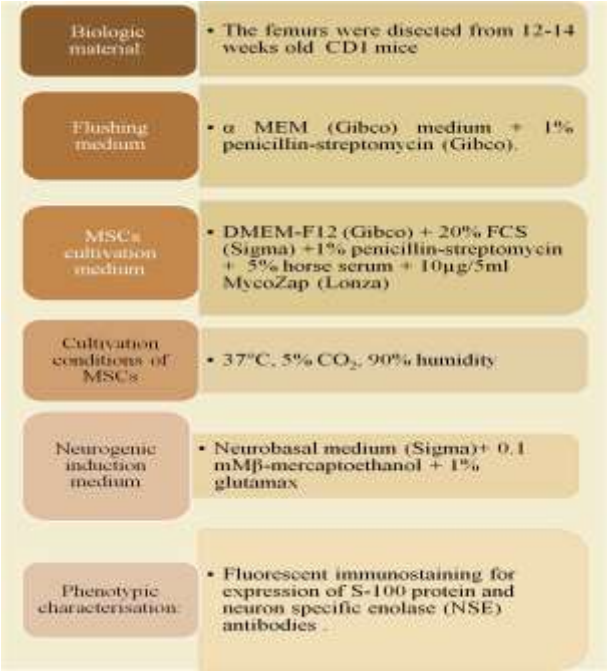

Figure 2. – Detailed protocol of MSCs isolation and characterization

RESULTS AND DISCUSSIONS

The study of MSCs biology and phenotypic characterization can be realized through experimental research on mouse, because it is a very suitable animal model. These cells are promising for regenerative medicine due to their capacity to regenerate injured tissue, to prevent pathologic fibrotic remodeling and to stimulate endogenous progenitors (Meirelles et al., 2009; Rodrigues et al., 2010). MSCs could be use as treatment of coronary artery disease due to their capacity to generate the novo myocardium after local transplantation (Orlic et al., 2001), and as a treatment of myocardial infarct (Stamm et al., 2003). Also, it was demonstrated their potential use in the therapy of muscular dystrophy (Gussoni et al., 1999), and their protection properties in lung injuries, including inflammation and collagen deposition (Ortiz et al., 2003). In orthopedic medicine, MSCs are used for segmental

bone repair (Quarto et al., 2001), craniotomy defects (Krebsbach et al., 1998) and in regeneration of meniscus tissue (Murphy et al., 2003).
In order to evaluate the multipotent capacity of mouse MSCs, the cells after recovery and culture were characterized both morphologically and imunophenotipically.
The primary culture (P1) (figure 3) was maintained for 7 days. In the d 4 of propagation, in culture was present a heterogenous cell population, composed by adherent fibroblast-like cells, rounded and hexagonal shaped cells. Also in suspension were present macrophages, monocytes and erytrocites. Martin et al demostrated in 1997 that the primary cultures are usually maintained for 12–16 days, during which time the nonadherent haematopoietic cell fraction is depleted. Due to the highly incresead doubling number of MSCs, we maintened the culture only until d7, in order to avoid the contact inhibition. First passage was done at a 70% confluence.

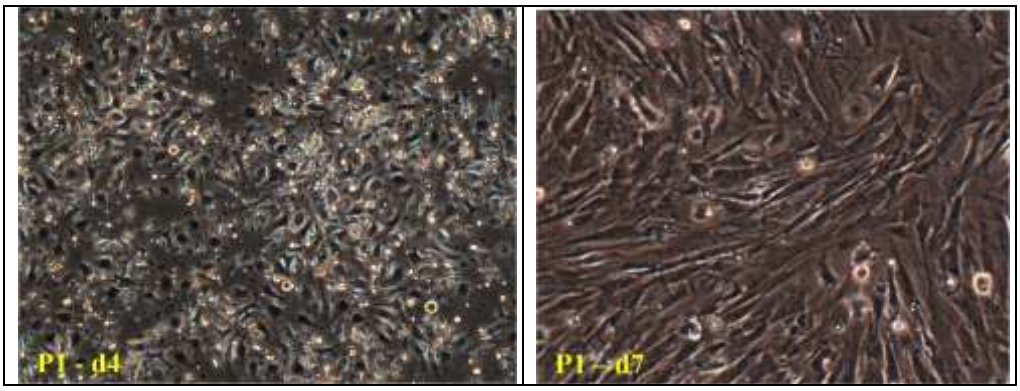

Figure 3. – The morphological aspect of primary culture

At P2, the heterogeneity of the cultured cell population was maintained, but the number of fibroblast-like cells arrived at 60%, a diverse morphology being observed at 40% of the cells. At P3, in culture were present just a percentage of 10% hexagonal shaped cells, the rest of 90% being adherent fibroblast-like cells, special characteristic of MSCs. A homogenous fibroblast-like cells culture was obtained at P4, reason why we started the neuronal induction.
After neurogenic induction with Neurobasal medium, at d3, the cells morphology started to change, appearing neuron-like cells, characterized by rounded somas and long spindly processes, indicating an early stage of neuronal differentiation. At day 4, the number of neuron-like cells increased

significantly (60%) and the neuritic networks started to be apparent (figure 4).

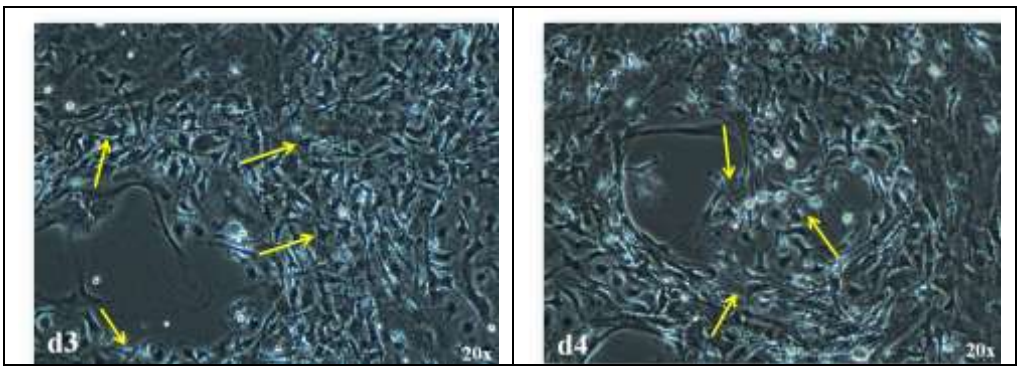

Figure 4. – The morphological aspect during neuronal differentiation

The immunostaining was done at d14 of differentiation. In the differentiated culture, positive cells for S-100+ protein were found, which are specific for astrocytes and glial cells, and also NSE+ cells, an indicator of neuronal differentiation (figure 5).

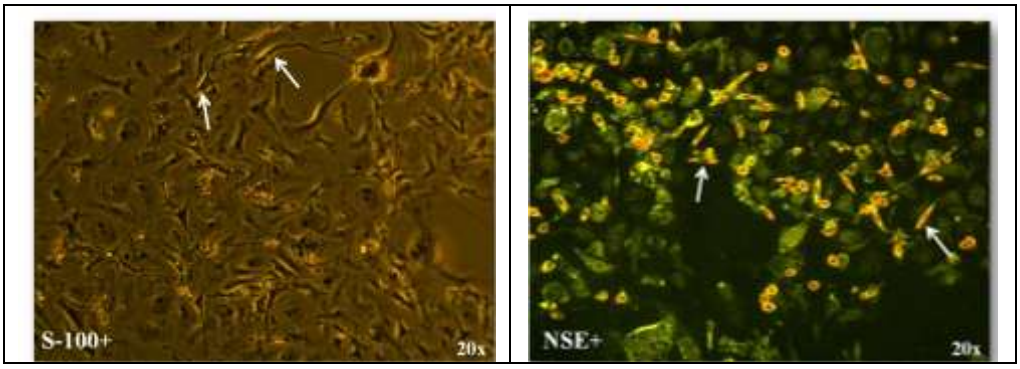

Figure 5. – Immunofluorecent staining

S-100 is a protein found in vertebrates and expressed on the cells derived from the neural crest, including glial, astocytes, and dentritic cells (Krampera et al., 2007). Previous reports showed that bone marrow derived MSCs cannot differentiate in astrocytes, neither *in vivo* and *in vitro* (Wehner et al., 2003). Contrary to these results, in our study we showed the possibility to obtain S-100+ cells after induction with Neurobasal medium.

NSE is a protein that usually is used for the identification of neuronal cells and other cells with neuroendocrine differentiation (Völlner et al., 2009). The expression of NSE on culture-differentiated cells suggest the presence of immature and differentiating neurons (Muńoz-Elías et al., 2003,), accompanied by changes in cell morphology and mitotic activity (neuronal). According to these findings, the morphological and phenotypical changes suggest that during differentiation, the neuron-like cells express diverse specific neuronal proteins, which means that the culture was composed by cells in different stages of differentiation.

CONCLUSIONS

Neuronal differentiation of mouse bone marrow derived MSCs is recommended at P4, when there is a homogenous culture of fibroblast-like cells.

The supplemented Neurobasal medium was optimal to induce neurogenic differentiation after 14 days.

The multipotency of mouse bone marrow derived MSCs was confirmed by the presence of S-100+ cells and NSE+cells, indicating differentiation capacity.

REFERENCES

Baksh D., Song L.,. Tuan R.S., 2004, Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J. Cell. Mol. Med. Vol 8, No 3, 301-316;

Groza I., Pall E., Ciupe S., Ilea C., POP R., ARYAN H., BERCE C., 2011, Immunophenotypical assessment of mouse mesenchymal stemm cells differentiated on endothelial lines. Revista Română de Medicină Veterinară, vol.21;

Gussoni E., Soneoka Y., Strickland C.D., Buzney E.A., Khan M.K., Flint A. F., Kunkel L. M. and Mulligan R.C., 1999, Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature, 401, 390–394;

Ippokratis P., Peter V.G., 2005, Biology of mesenchymal stem cells. Injury, Int. J. Care Injured, 36S, S8—S12;

Kim J.H., Auerbach J.M., Rodriguez-Gomez J.A., Velasco I., Gavin D., Lumelsky N., Lee S.H., Nguyen, J., Sanchez-Pernaute R., Bankiewicz K., and McKay R., 2002, Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature, 418, 50–56;

Krampera M., Marconi S., Pasini A., Galiè M., Rigotti G., Mosna F., Tinelli M., Lovato L., Anghileri E., Andreini A., Pizzolo G., Sbarbati A., Bonetti B., 2007, Induction of neural-like differentiation in human mesenchymal stem cells derived from bone marrow, fat, spleen and thymus, Bone, Volume 40, Issue 2, 382–390;

Krebsbach P.H., Mankani M.H., Satomura K., Kuznetsov S.A. and Robey P. G., 1998, Repair of craniotomy defects using bone marrow stromal cells. Transplantation, 66, 1272–1278;
Martin I., Muraglia A., Campanile G., Cancedda R. and Quarto R., 1997, Fibroblast growth factor-2 supports ex vivo expansion and maintenance of osteogenic precursors from human bone marrow. Endocrinology, 138, 4456–4462;
Meirelles L.S., Fontes A.M., Covas D.T. and Caplan A.I., 2009, Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Review, 20(5-6), 419-427;
Muńoz-Elías G., Woodbury D., Black I.B., 2003, Marrow Stromal Cells, Mitosis, and Neuronal Differentiation: Stem Cell and Precursor Functions. Stem Cells,21, 437-448;
Murphy M., Fink D.J., Hunziker E.B. and Barry F.P., 2003, Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheumatism, 48, 3464–3474;
Orlic D., Kajstura J., Chimenti S., Jakoniuk I., Anderson S. M., Li B., Pickel J., McKay R., Nadal-Ginard B., Bodine D. M., Leri A., and Anversa P., 2001, Bone marrow cells regenerate infarcted myocardium, Nature, 410, 701–705;
Ortiz L.A., Gambelli F., McBride C., Gaupp D., Baddoo M., Kaminski N., and Phinney D.G., 2003, Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proceedings of the National Academy of Sciences of the United States of America, 100, 8407–8411;
Pall E., Groza I., Cenariu M., Ilea C., Soritau O., Ciprian T., Berce C., 2010, Isolation, characterization, phenotypization and differentiation of stem cells from rat placenta. Simpozion Progrese şi Perspective în Medicina Veterinară, Iaşi, Volum program and the abstracts, p.61;
Quarto R., Mastrogiacomo M., Cancedda R., Kutepov S. M., Mukhachev V., Lavroukov A., Kon E., and Marcacci M., 2001, Repair of large bone defects with the use of autologous bone marrow stromal cells. The New England Journal of Medicine, 344, 385–386;
Rodrigues M., Griffith L.G. and Wells A., 2010, Growth factor regulation of proliferation and survival of multipotential stromal cells. Stem Cell Research Therapy, 1(4), 32;
Sekiya I., Larson B.L., Smith J.R., Pochampally R.,. Cui J.G, Prockop D.J., 2002, Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of earlyprogenitors and evaluate their quality, Stem. Cells 20, 530–541;
Shihabuddin L. S., Horner P. J., Ray J. And Gage F. H., 2000, Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. The Journal of Neuroscience, 20, 8727–8735;
Stamm C., Westphal B., Kleine H.D., Petzsch M., Kittner C., Klinge H., Schumichen C., Nienaber C.A., Freund M. and Steinhoff G., 2003, Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet, 361, 45–46;
Tabbara I.A., Zimmerman K., Morgan C., and Nahleh Z., 2002, Allogeneic hematopoietic stem cell transplantation: complications and results. Archives of Internal Medicine, 162, 1558–1566;
Tondreau T., Lagneaux L., Dejeneffe M., Delforge A., Massy M., Mortier C. and Bron D., 2004, Isolation of bone marro mesenchymal stem cells by plastic adhesion or negative selection phenotype, proliferation kinetics and differentiation potential. Cytotherapy, 6:372–379;

Völlner F., Ernst W, Driemel O., Morsczeck C., 2009, A two-step strategy for neuronal differentiation *in vitro* of human dental follicle cells. Differentiation, Volume 77, Issue 5, 433–441;
Wehner T., Bontert M., Eyupoglu I. et al., 2003, Bone marrow–derived cells expressing green fluorescent protein under the control of the glial fibrillary acidic protein promoter do not differentiate into astrocytes in vitro and in vivo. J Neurosci, 23, 5004 –5011;
Witte P.L., Robinson M., Henley A.,. Low M.G, Stiers D.L., Perkins S., Fleischman R.A., Kincade P.W., 1987, Relationships between B-lineage lymphocytes and stromal cells in longtermbone marrow cultures. Eur. J. Immunol. 17, 1473–1484.

RESEARCH ON THE THORACIC LIMB JOINT AT THE DOMESTIC PIG (*SUS SCROFA DOMESTICA*)

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Abstract

The paper work was done on pieces from four adult specimens. They describe the articular surfaces, means of connection and additional items scapular-humeral joint, humeroradial, antebrahio-metacarpal and finger joints . Morphological similarities were confirmed with data from the literature but differential elements that have not been reported were identified and described.

Key words: *articulation, thoracic limb, pig*

INTRODUCTION

This case study aimed to perform a systematic and detailed description of the domestic pig thoracic limb joints, because in the literature there are still a number of insufficiently clarified issues. For example, discrepancies between authors concerning the collateral ligaments if there is an antebrahio-carpal articulation. (1,3,4,5). The main objective of this paper is to present the anatomical reality, demonstrated by images taken directly from the dissected parts. The authors generally noted and described morphologic similarities to the situation seen in ruminants. There are significant differences, the result of the growth and maintenance of the species, generally in areas which restrict the movement of animals. (2,6)

MATERIALS AND METHODS

Research has been conducted on joints from four adult specimens. They described the articular surfaces, means of contact and have photographed the most important elements. Description and homologation formations were performed according NAV – 2005.

RESULTS AND DISSUTION

In the case of the scapula-humeral joint it was observed that the tendon belonging to the brachial biceps muscle is included in the articulation for a distance of 3-4

cm, cranially united to the articular capsule with the help of the lax connective tissue.
Regarding the humero-radio-ulnar joint, the existence of a collateral medial ligament was established. It is formed out of three fascicles and the vertical one is included in the round pronator muscle.
Apart from the dorsal radio-pyramidal ligament and the collateral ligament, the antebrachio-carpal joint also includes a pisi-ulnar ligament and two funicular ligaments that are in contact with the profound side of the common palmar ligament, but incompletely enshrouded in the synovial membrane.
In the case of the medio-carpal joint (Fig.4) the means of connection are: a) scafo-unciform ligament, dorsally located; b) pisi-unciform ligament, laterally located; c) the strong fascicle belonging to the collateral medial ligament, medially located d) two strong ligaments in connection with the profound side of the common ligament, situated in the palmar area.

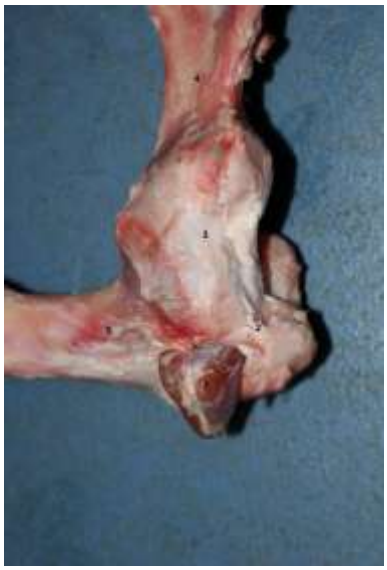

Fig. 1 Scapulo-humeral pig joint – medial side
1-biceps muscle; 2-anterior wall of the articular capsule; 3-capsular ligament; 4-scapula; 5-humerus.

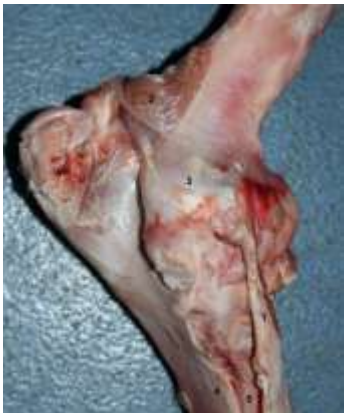

Fig. 2 Articulaţia humero-radio-ulnară la porc –vedere medială
1-vertical fascicle of the collateral medial ligament; 2-anconeus muscle; 3-distal humerus extremity; 4-radius; 5-ulnar body.

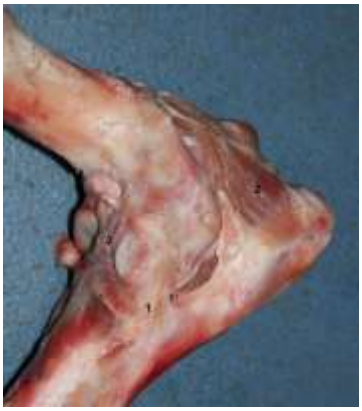

Fig. 3 Humero-radio-ulnar pig joints-lateral side
1-anterior fascicle of the collateral lateral ligament; 1'-posterior fascicle of the colateral lateral ligament; 2- anconeus muscle; 3-articular capsule.

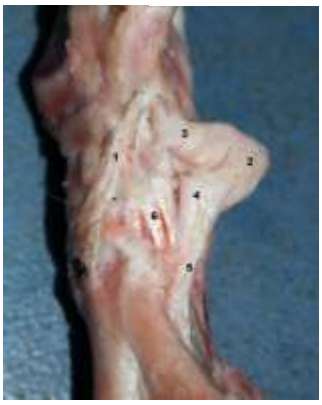

Fig. 4 Antebrachio-arpo-metacarpial pig joint – lateral side1-the long fascicle of the collateral lateral ligament; 2- pisiform; 3 –pisiulnar ligament ; 4- pisiunciform ligament; 5- pisimetacarpian ligament; 6- dorsal carpo-metacarpal ligaments

Beside the commune ligaments, thecarpo-metacarpal joint contains: a-three dorsal carpo-metacarpal ligaments; b-pisi-metacarpal ligament; c-trapezo-metacarpal ligament; d-some fibrous fascicles on the palmar side which can proximally be confused with the palmar ligament; they distally continue with the palmar metacarpial ligaments; e-two strong ligaments, interosseous.
Each metacarpo-sesamo-phalanxial joint contains: 1- a proper intersesamoidian ligament ; on the other hand there is an intersesamoidianinterdigital ligament between the main fingers; 2-two sesamoidian collateral ligament that are attached

on the lateral sides of the proximal termination of the proximal phalanx; 3- four distal sesamo- phalanx ligaments, from which two are cruciate ligaments and two are short ligaments, the abaxial one being more distinct; 4- two collateral ligaments – medial and lateral – each featuring the main fascicle and the accessory fascicle; 5- one dorsal reinforcement of the joint capsule, thick and fribo-cartilaginous in its'central part.

Between the proximal phalanges of the fingers there is the proximal interdigital ligament. Distal interdigital ligaments have a particular topography on unequal fingers. They will form a complex, in which we can recognize two transverse ligaments and four longitudinal ligaments.

CONCLUSIONS

The assembly of elements, now relatively simple represented in the scapular-humeral joint, humero-radio-ulnar joint and in the antebrachio- carpo-metacarpal joint become far more complex in the finger joints.

At the autopodium level, interosseous muscles, well represented and fleshy, form three fibrous cords originating in some ligament formations at the carp level, supporting the main fingers,.

The distal interdigital ligaments provide functional unity to the acropodium components and maximum resistance mechanical requests; this exceeds far beyond the one seen in ruminants.

REFERENCES

Cornilă, N.- Morfologia microscopică a animalelor domestice (cu elemente de embriologie),Vol.II. Ed. ALL, Bucureşti, 2001.

Coţofan, V. Hriţcu Valentina, Cura, P. – Particularităţi anatomice diferenţiare între oasele membrelor pelvine la mistreţ (Sus scrofa) şi porcul domestic (Sus scrofa domesticus), Lucr. Şt. Inst. Agr. Iaşi, vol.25 Seria Zootehnie–Medicină Veterinară, 41–46, 1981;

Kratochvil, Z. – Discriminative characters on the acropodium of the domestic and the wild pigs (Sus scrofa dom. Sus scrofa L.), Acta Veterinaria Czechoslovakia, Brno,1973,42:2, 109-133.

Ninov, L. K.– Morphological differeces between the skeletons of domestic and Wild swine, Sbornik Nauchnykh Trudov Moscovskaya Veterinarnaya Akademyia, 105, 93–95, 1979.

Predoi, G. – Anatomia comparată şi topografică a animalelor domestice, Ed. Casa Cartii de Ştiinţa, Cluj–Napoca, 1999.

Sisson, S., Grossman, J. D. – The Anatomy of the Domestic Animals, Ed. Saunders Comp,Philadelphia, London, Toronto, 1975.

NUTRITIONAL AND METABOLIC PARAMETERS IN LAYING HENS FED WITH DIFFERENT LEVELS OF CALCIUM, PHOSPHORUS AND PHYTASES

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Abstract

The experiment was made on a Lohmann Brown classic hybrid from 32 to 42 weeks of age. There were used feeds containing various levels of Ca, P or phytase: NC1 and NC2 were commercial recipes, NC3 was supplemented with Phyzyme (500 TFU/kg), NC4 was supplemented with monosodium phosphate and NC5 with dicalcium phosphate according to nutrient requirements (NRC 1998). There was tested the possibility to use the phytase included at a level of 0.12%nPP to make available enough phytic phosphorous to satisfy the birds requirements. (0.25% nPP).
When included at a level of 500 FTU/kg feed, Phyzyme hydrolyzed 75% of the phytic phosphorous contributing to a 0.30% aP in the feed.
The biological value of the phosphorous in NC3 was of 91.19% and it did not influence either the eggs production or the eggs' quality.

Keywords*: laying hens, phytase, egg production,*

INTRODUCTION

Food standards for P at laying hens decreased up to 0.25% (NRC 1994) but it was experimentally shown that the level of P can decrease up to 0.11% nPP associated with food supplementation with phytase.
Aim of this study was to determine phosphorus digestibility and its metabolic utilization at laying hens fed with different levels of calcium and phosphorus in food.

MATERIALS AND METHODS

Experiments were made on six laying hens (GOC) which were fed according to five mixed fodder recipes which contained different amount of total phosphorus (tP), nephites phosphorus (nPP) and available phosphorus (aP), with or without phytase (table 1).

Table 1

The quality condition of combined fodder with different levels and supplementary sources of P at GOC

Specification	NC1	NC2	NC3	NC4	NC5
	0,36 nPP	**0,41 nPP**	**0,12 nPP**	**0,25 nPP**	**0,25 nPP**
Calculated nutritional values					
EM (Kcal)	2790	2773	2794	2783	2773
PB (%)	16,85	16,94	16,85	16,85	16,85
Ca (%)	3,50	4,18	3,43	3,43	3,43
tP (%)	0,75	0,76	0,358	0,505	0,505
nPP (%)	0,36***	0,41***	0,126	0,251****	0,251****
Methionine	0,39	0,41	0,37	0,37	0,37
Lysine	0,75	0,81	0,82	0,82	0,82
Threonine	0,64	0,66	0,58	0,58	0,58
Phytase (FTU/kg)	1000*	300**	504***	-	-

* Natuphos 5000TFU/g
** Physine XP 2400 TPT/g
*** P supplemented from monocalcium phosphate
**** P supplemented from monosodium phosphate

NC 1 and NC 2 recipes were commercial ones used in chicken farms, Lohmann Brown-Classic hybrid. Recipes NC 3, NC 4 and NC 5 had the same structure corn/soybeans; At NC 3 there was no supplementation with fodder phosphates, at NC 4 the supplementary monosodium phosphate with P's biological value of 100% was used as a reference for calculating the biological value of P in the other recipes.

Clasic Lohmann Brown hybrid hens were introduced in the experiment at the age of 32 weeks and they were housed individually in battery type BPC4

cages providing them with an area of 0.22 m²/hen. To get accommodated, hens were fed for 10 days with each recipe, then followed by three days of measurements when there were measured the amounts of given fodder and unconsumed fodder debris to determine the quantity of ingested fodder and feces production. The fodder samples, the unconsumed remains and fecal samples were collected for 3 days to determine the content of dry matter (D.M.), calcium and phosphorus. During these three days of measurements, eggs were collected from each hen, they were weighed and the lay percentage was calculated.

On the morning of the third day of measurements, immediately after laying of eggs, blood was collected from axillary vein from each hen to determine seric level of calcium and phosphorus.

The chosen time for blood collection was immediately after the eggs laying because reference can be given by taking into consideration that the formation of egg shell causes fluctuations of calcium and phosphorus levels. In this way, it can be obtained information regarding the metabolic status of calcium and phosphorus in comparison with the level of Ca and P from the diet.

The analytical measurements of substance content of D.M., Ca and P from fodder and feces were effectuated as follows:

- D.M. content was determined by drying in an oven at 105 Celsius degrees;
- Total calcium and phosphorus content was determined after calcination of the samples at 500 °C and colorimetric determination of the spectrophotometer;
- nPP or free P content was determined by *Megazyme* method;
- Alkaline phosphathesis (FAL) was realized from sanguine serum during the third day of measurements with Hospitex Diagnostics kits and reading at 405 nm.
- Phytic phosphorus (PP) was calculated from the difference between total phosphorus and free phosphorus (from which it was subtracted, where appropriate, the intake of

  supplement mineral phosphorus);
- Levels of phosphorus and calcium from sanguine serum was determined colorimetrically (with a-cresolftaleine for calcium and with ammonium molybdate for phosphorus) utilizing the spectrophotometer at 578 nm for calcium and 340 nm for phosphorus using Hospitex Diagnostics kits;

- Statistical calculation of differences between lots was performed by multiple T test and Fischer test for analysis the variation.

RESULTS AND DISCUSSIONS

From the measurements regarding average daily consumption (ADC), it was found that the lowest ADC was recorded at NC 3 recipe (109.83 g/day), consumption which suggests that the absence of fodder phosphate supplements may be the cause of a lower ingestion. The highest ADC was recorded at NC 2 (123,56 g/day).
Statistical calculation shows that between hens fed with the same recipe and also between types of ingested mixed fodder, there are no significant statistical differences. Calcium content, tP, nephites phosphorus and available phosphorus as well as calculated available calcium/phosphorus ratio are presented in table 2.

Table 2

Calcium level and total, nephites and available phosphorus level calculated for experimental fodder recipes

Specification	NC1	NC2	NC3	NC4	NC5
Ca	3,16	4,18	3,43	3,43	3,43
tP	0,62	0,73	0,36	0,50	0,50
nPP	0,36	0,41	0,12	0,25	0,25
aP	0,42	0,46	0,17	0,25	0,23
Ca/P	7,52	9,09	20,18	13,17	14,91

At commercial recipes, the available phosphorus content was calculated by the manufacturers of combined fodder based on an algorithm given by the phytase suppliers.
At NC 4 and NC 5 recipes, the available phosphorus does not contain phosphorus released from phytates. At NC 3, it was estimated that phytase free up 20% of phytic phosphorus.
Based on the mineral chemical composition of ingestion and excretion, there were calculated the amounts of calcium and phosphorus retained in the body and the digestibility coefficients of DM, Ca and P. It was found that between digestibility coefficients of DM there are no significant statistically differences. In fact, weighing hens after each experimental phase (approximately every 14 days), revealed that weight variations were on average approximately (±) 50 g. Concerning digestibility of calcium, the

lowest value was recorded at NC 3 (65.82%), significantly lower than at NC 5 (81.44%), probably due to the lowest level of phosphorus, which is known to promote calcium absorption.
Regarding phosphorus digestibility, the lowest value was recorded at NC1, possibly due to inhibition of phytase through a feed-back mechanism in a high level of free phosphorus. At NC 3 there were no significant statistically differences of phosphorus digestibility comparing to NC 2, NC 4 and NC 5, suggesting that the biological value of Nephites phosphorus and of the phytic one laid off by phytase was high (91.19%) compared to phosphorus from sodium phosphate (100%).
Based on digestibility coefficients of phosphorus and on Nephites phosphorus content, values of real available phosphorus were calculated (table 3).

Table 3

Average digestibility coefficients of dry matter (D.M.), of calcium (Ca) and of total phosphorus (tP) at GOC fed with different levels of phosphorus

Specification	D.M	Ca	tP	nPP	calculated aP	measured aP
NC1	72,17	77,54	62,16	0,36	0,42	0,46
NC2	72,10	73,64	75,55	0,41	0,46	0,68
NC3	71,01	65,82	71,43	0,12	0,17	0,30
NC4	73,96	73,61	78,33	0,25	0,25	0,47
NC5	70,59	81,44	75,00	0,25	0,25	0,42

Compared to values of calculated available phosphorus, it was found that the real available phosphorus was higher in all recipes.
Of interest is the NC 3 recipe, which from the calculated value (0.17 g aP/100g), at an available of 20% from phytic phosphorus under phytase action, it was achieved an availability of 75% ensuring absorption of 0.3 g phosphorus /100g fodder, respectively 0.12 g from nPP and 0.18 g from phytic phosphorus (fig. 1).

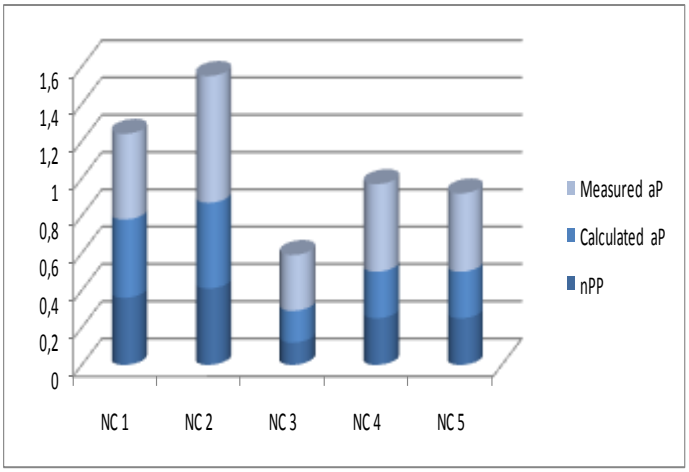

Fig. 1. Nephites phosphorus content (nPP) and real available phosphorus of fodder recipes

Thus, even at a level of 0.12 nPP in diet, by supplementation with 500 FTU phytase Phyzime (2400 FTU / g), it is ensured the need of aP according to NRC regulations (1994).

Values of seric level at calcium and phosphorus are presented in tables 4 and 5.

Table 4

Seric level of calcium (mg%) immediately after laying of eggs at GOC, fed with experimental NC

Specification	NC1	NC2	NC3	NC4	NC5
G 1	26,78	25,25	25,51	26,87	31,87
G 2	25,57	25,55	22,25	26,41	33,72
G 3	23,84	23,13	24,17	23,26	23,95
G 4	21,26	25,01	24,80	27,74	25,44
G 5	23,73	26,79	30,36	28,66	29,90
G 6	23,72	24,71	27,91	27,18	27,69
X	**24,15**	**25,07**	**25,83**	**26,69**	**28,76**

Table 5

Seric level of phosphorus (mg%) immediately after laying of eggs at GOC, fed with experimental NC

Specification	NC1	NC2	NC3	NC4	NC5
G 1	6,14	5,02	4,07	4,04	1,91
G 2	7,40	4,47	2,20	3,86	5,36
G 3	6,05	4,90	3,61	3,85	4,60
G 4	6,40	4,64	4,94	5,23	5,07
G 5	5,70	4,18	4,39	4,17	4,56
G 6	6,03	3,91	3,47	3,88	4,33
X	**6,29**	**4,52**	**3,78**	**4,16**	**3,41**

The obtained results show that in terms of calcium level, in case of all recipes, there are significant differences of calcium level between hens fed with the same level of calcium and phosphorus (F = 4.01). Compared with the NC 4 recipe there are no statistically significant differences. From comparing the calcium level between lots, it was found that at NC 1 and NC 2 lots, the calcium level is significantly lower (P <0.001) than at NC 5. At NC 3 lot, calcium level is significantly lower (P <0.05) compared with NC 5, but it is not significant different statistically from that of NC 4.

In case of phosphorus level, there were significant differences (F = 8.85, P <0.01) between hens at each experimental recipe. Compared with phosphorus level recorded at NC 4, it was found that at NC 1 phosphorus level 1 is significantly higher (P <0.001). Moreover, phosphorus level at hens fed with NC 1 recipe is significantly higher also than of NC 2 (P <0.01) and of NC 3 and NC 5 (P <0.001). Compared with NC 4, NC 3 recipe is of interest, where phosphorus level is not significantly different.

Plasma level of alkaline phosphatase (FAL) involved both in phosphocalcic metabolism in bones but also in eggshell is presented in Tables 6; 7 and 8.

Table 6

Seric level of alkaline phosphatase (ALP) / (U/I) immediately after laying of eggs at GOC

Specification	NC1	NC2	NC3	NC4	NC5
G_1	1047,98	1092,12	1571,35	934,33	762,44
G_2	1751,43	1128,10	1141,96	855,60	1931,32
G_3	1188,37	969,09	1710,16	1573,29	1543,31
G_4	1712,59	2338,32	578,40	820,98	1443,38

G_5	2370,66	1001,68	881,74	970,01	865,68
G_6	3527,57	1172,53	1273,40	1100,22	2066,99
X	1933,10	1283,62	1192,83	1042,40	1435,52

Table 7

Values of correlation coefficients (Sperman) between alkaline phosphatase and different sources of calcium and phosphorus

Variant	Character	Calcium Seric level	Phosphorus Seric level	Calcium from shell	Phosphorus from shell
NC1	Alkaline phosphatase	-0,543	-0,429	0,029	-0,486
NC_2	Alkaline phosphatase	-0,029	-0,314	-0,771	0,657
NC_3	Alkaline phosphatase	-0,200	-0,486	0,486	0,257
NC_4	Alkaline phosphatase	-0,314	-0,600	-0,257	0,714
NC_5	Alkaline phosphatase	-0,143	0,371	0,086	0,243

$r_{5\%} = 0,811$; $r_{1\%} = 0,942$; $r_{0,1\%} = 0,974$

Table 8

Values of correlation coefficients (Pearson) between alkaline phosphatase and different sources of calcium and phosphorus

Variant	Character	Calcium seric level	Phosphorus seric level	Calcium from shell	Phosphorus from shell
NC1	Alkaline phosphatase	-0,301	-0,218	0,090	-0,546
NC_2	Alkaline phosphatase	-0,002	0,075	-0,615	0,062
NC_3	Alkaline phosphatase	-0,231	-0,415	0,257	0,522
NC_4	Alkaline phosphatase	$-0,831^0$	-0,471	-0,573	0,871*
NC_5	Alkaline phosphatase	-0,149	0,619	0,331	-0,218

$r_{5\%} = 0,811$; $r_{1\%} = 0,942$; $r_{0,1\%} = 0,974$

It was found that, among laying hens fed with different levels of Ca and P, there are no significant differences in seric level of alkaline phosphatase when consuming any of the combined fodders. Compared with NC 4, it was observed that at NC 1, the alkaline phosphatase level is significantly higher ($p < 0.05$). Between lots there were found statistically significant differences only at NC 1 compared to NC 3 ($p < 0.05$) and NC 4 ($p < 0.005$).
This indicates a much higher osteoclastic activity at NC 1, suggesting a greater mobilization of calcium from the bones on a less calcium background in food. Mineral analysis of bones would support this finding.
Calculation of correlation coefficients (Pearson and Spearman) between alkaline phosphatase and seric levels and respectively in eggshell, were as follows:
At Pearson test:

- correlation between alkaline phosphatase and seric calcium is negative and insignificant at NC 1, NC 2, NC 3, NC 5, being positive ($p < 0.05$) only in NC 4 lot ($r = -0.831$).
- correlation between alkaline phosphatase and seric phosphorus is negative and insignificant in NC 1 - NC 4 lots and at NC 5, correlation is positive but insignificant.
- correlation between alkaline phosphatase and calcium from eggshell was positive at NC 1 and NC 5 and negative at NC 2, NC 3 and NC 4, statistically insignificant.
- correlation between alkaline phosphatase and phosphorus in egg shell was positive ($r = 0.871$) ($p < 0.05$) at NC 4, NC 2 and NC 3 and at NC 1 and NC 5 was negative, without being statistical significant.

It follows from these correlations that the lowest level of calcium in the diet (3.16% at NC 1) determines significant increase of alkaline phosphatase, reflected in plasma level of calcium which is the lowest (24.15 mg%). This suggests mobilization of calcium from bones, without affecting calcium deposit from eggshell.
Seric level of phosphorus is positively correlated with alkaline phosphatase at NC 2 and NC 5 and negatively at NC 1, NC 3 and NC 4 without statistical significance. Instead correlation of alkaline phosphatase with phosphorus from eggshell is statistically significant ($r = 0.871$) ($p < 0.05$) only at NC 4.

CONCLUSIONS

Ensuring a low level of Nephites phosphorus (0.12%) in recipes without mineral phosphate supplement, but supplemented with 500 FTU reduce statistically insignificant the combined

2400 FTU Phyzyme phytase supplementation at 500 FTU / kg combined fodder without mineral phosphates supplement, lays off 75% of phytic phosphorus, increasing the value of free and available phosphorus from vegetal raw materials (corn and soybean meal, 0.12% nPP) at 0.30 available phosphorus (aP) for absorption.

Compared with NC 4, phosphorus level at NC 3 with minimal level of nPP do not differ significantly statistically. Compared to phosphorus from sodium phosphate (NC 4), the biological value of nPP and of aP phosphorus from phytates at NC 3 was 91.19%.

Seric level of alkaline phosphatase was not significantly different from the level of calcium and phosphorus in hens food.

Between alkaline phosphatase and seric calcium, there is a negative and statistically significant correlation only at NC 4 recipe ($r = -0.831$); there is a positive and statistically significant correlation ($p < 0.05$) ($r = 0.871$) between alkaline phosphatase and the level of phosphorus in the eggshell

REFERENCES

Diarra, S.S., Usman, S.A., , Igwebuike, J.A., A.G. Yisa, A.G., 2010, *Breeding for Efficient Phytate-phosphorus Utilization by Poultry*, International Journal of Poultry Science 9 (10): 923-930, 2010 ISSN 1682-8356 © Asian Network for Scientific Information, 2010

Slominski, B.A., 2010, *Recent Advances in Enzymes for Poultry* Dietshttp://www.thepoultryfederation.com/public/userfiles/files/2-6%20Wed%20-%20Bogdan%20Slominski%20-%20Enzymes%20for%20Poultry.pdf

USDA, National Nutrient Database for Standard Reference, 2011, http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/SR24/sr24_doc.pdf

Wu, G., Liu, Z., Bryant, M.M. Roland, D.A. Sr., 2006, *Comparison of Natuphos and Phyzyme as Phytase Source for Commercial Layers Fed Corn-Soy Diet*, Poultry Science, 2006; 85:64–69

THE SUPPLEMENTATION EFFECT OF FEED WITH SELENIUM, ZINC AND MAGNESIUM ON BIOCHEMICAL SANGUINE PARAMETERS IN LAYING HENS

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Abstract

The experiment was conducted on 144 Isa Brown hens, for the duration of a production cycle. The biological material used in the experiment was divided into 4 experimental groups, each group being constituted of 36 hybrid hens. In the experiment we used a structure of mixed supplemented fodder in three experimental variants with selenium, zinc and magnesium. The blood biochemical parameters were determined in the three experimental stages. In the ascending phase of the egg laying curve, proteinemia ranged below the lower limit reference with the hens in the control group and those in the group supplemented with magnesium. In the steady phase proteinemia was in reference values at all experimental variants. In the downward phase although proteinemia was within reference limits, significant differences were confirmed between the control group and the groups supplemented with minerals. In the upward and downward phase of the egg laying curve, enzymatic activity of SGOT was within reference limits in all groups of hens, and in the steady phase, its activity was greatly reduced in the groups of hens supplemented with minerals. In the upward phase SGPT had lower values for all the hens in the experiment. In the downward phase its activity was higher in hens in the control group compared to the groups supplemented with minerals. In the upward and downward phase the enzyme activity of GGT was within normal limits, only in the steady phase it was more intense. ALP showed higher values for all hens in the experiment.

Key words: *biochemical, hens, parameters, sanguine.*

INTRODUCTION

The health and equilibrium state of the body is determined by the normal functioning of the metabolic processes which take place simultaneously. The occurrence of metabolic disorders in the animal is determined by numerous factors which are independent from the body and/or dependent, such as: nutrition, imbalances between various components of the ration, modification of the composition of fodders, technical – organizational factors and others (Pârvu, 1992; Stoica 1997).

The purpose of our study was to evaluate and interpret the modifications induced by the basic food rations supplemented with selenium, zinc and

magnesium used in egg-laying hens during a biological cycle on certain sanguine biochemical parameters.

MATERIALS AND METHODS

The experiments were carried out on „Isa Brown" hybrid egg-laying hens in a zootechnical farm in the Western part of the country, where the recommended microclimate conditions have been provided. The combined fodder recipes have been observed according to the requirements of the hybrid in the experiment in all the three operational phases (upward, steady and downward). A control lot (CG) with 36 hybrid hens and 3 experimental lots of 36 hens each have been constituted (EG-Se, EG-Zn and EG-Mg). The experimental groups have been fed with combined fodder (CF) supplemented with 20 g Sel-Plex concentration 1000 mg/100 kg CF with 9.5 g zinc oxide conc. 72%/100 kg CF and the 3rd one with 100 g magnesium oxide conc. 75%/100 kg CF. The monitored parameters have been analyzed for the three phases of the egg-laying curve, for 57 weeks. In order to determine the biochemical parameters the kinetic method was used. The statistical interpretation of the data was carried out by means of the Minitab 14 program.

RESULTS AND DISCUSSIONS

Tables 1, 2 and 3 show the significance of the differences between the groups regarding the sanguine biochemical parameters.
In the ascending phase of the egg laying curve, proteinemia ranged below the lower limit reference with the hens in the control group and those in the group supplemented with magnesium.
Carrying out the enzymatic profile in egg-laying hens represents an essential condition in specifying the diagnosis of the health state and can identify the action of certain etiological factors which can influence the egg production.
In the upward phase of the egg-laying curve, aspartate-aminotransferase (SGOT) was within normal limits in all the hens in the experimental lots. Alanine-aminotransferase (SGPT) has had a lower activity in comparison with the data in the specialty literature in all the experimental hen lots.

Table 1

Comparative evolution of the sanguine biochemical parameters with egg-laying hens and the significance of the differences (upward phase)

Parameters	Group	x ± Sx	CV %
Total proteins g/dl	CG	4,11 ± 0,03	2,61
	EG-Se	498,12 ± 21,94	14,62
	EG-Zn	483,29 ± 25,30	17,38
	EG-Mg	4,45 ± 0,09	6,38
SGOT U/I	CG	147,95 ± 2,47	5,54
	EG-Se	162,59 ± 7,00	14,29
	EG-Zn	149,96 ± 8,48	18,77
	EG-Mg	124,07 ± 2,78	7,45
SGPT U/I	CG	5,38 ± 0,25	15,48
	EG-Se	3,73 ± 0,07	5,93
	EG-Zn	2,11 ± 0,08	13,17
	EG-Mg	3,88 ± 0,13	11,14
GGT U/I	CG	13,82 ± 0,10	2,33
	EG-Se	14,15 ± 0,84	32,09
	EG-Zn	13,75 ± 1,09	24,20
	EG-Mg	16,09 ± 0,23	4,66
ALP U/I	CG	797,92 ± 37,50	15,60
	EG-Se	715,58 ± 69,16	32,09
	EG-Zn	716,12 ± 52,20	24,20
	EG-Mg	812,50 ± 33,55	12,68

The activity of gamma-glutamyltransferase (GGT) is within the physiological limits in all the hens in the experiment. Alkaline phosphatase (ALP) has had a high activity in all the hen lots, in comparison with the data in the specialty literature, the increase of the activity of this enzyme being determined by the hepatic or hepato-biliary disorder or deficiencies of calcium metabolism (Ghergariu, 1995; Pârvu, 1992).

In the steady phase proteinemia was in reference values at all experimental variants (table 2). In the downward phase (table 3) although proteinemia was within reference limits, significant differences were confirmed between the control group and the groups supplemented with minerals.

Diaz et al. (1999) have studied the activity of several enzymes AST, ALT, LDH, regarding egg-laying hens. The research result shows that their activity can suggest a hepatic lesion and some of them can be part of the protocol for diagnosing the syndrome of hemorrhagic hepatic steatosis.

In the steady phase of the egg-laying curve the activity of the GOT enzyme was higher in comparison with the data in the specialty literature which can confirm certain hepatic or muscular disorders.

Table 2

Sanguine biochemical parameters with egg-laying hens (steady phase) - Multiple Comparisons Tukey HSD

Parameters	Group		Standard error	Sig.	95% CI	
					Lower limit	Upper Limit
Total proteins g/dl	CG	EG-Se	0,25206	0,994	-0,6129	0,7449
		EG-Zn	0,25206	0,091	-1,2889	0,0689
		EG-Mg	0,25206	0,**000***	-2,1329	-0,7751
	EG-Se	EG-Zn	0,25206	,051	-1,3549	0,0029
		EG-Mg	0,25206	**0,000***	-2,1989	-0,8411
	EG-Zn	EG-Mg	0,25206	**0,010***	-1,5229	-0,1651
SGOT U/I	CG	EG-Se	4,67415	**0,000***	114,7474	139,9246
		EG-Zn	4,67415	**0,000***	53,1974	78,3746
		EG-Mg	4,67415	**0,000***	73,9974	99,1746
	EG-Se	EG-Zn	4,67415	**0,000***	-74,1386	-48,9614
		EG-Mg	4,67415	**0,000***	-53,3386	-28,1614
	EG-Zn	EG-Mg	4,67415	**0,000***	8,2114	33,3886
SGPT U/I	CG	EG-Se	1,69049	0,893	-5,7519	3,3539
		EG-Zn	1,69049	**0,001****	-11,8119	-2,7061
		EG-Mg	1,69049	**0,000***	-19,9339	-10,8281
	EG-Se	EG-Zn	1,69049	**0,005****	-10,6129	-1,5071
		EG-Mg	1,69049	**0,000***	-18,7349	-9,6291
	EG-Zn	EG-Mg	1,69049	**0,000***	-12,6749	-3,5691
GGT U/I	CG	EG-Se	2,67468	1,000	-7,4305	6,9765
		EG-Zn	2,67468	0,742	-4,4905	9,9165
		EG-Mg	2,67468	**0,000***	-45,1845	-30,7775
	EG-Se	EG-Zn	2,67468	0,692	-4,2635	10,1435
		EG-Mg	2,67468	**0,000***	-44,9575	-30,5505
	EG-Zn	EG-Mg	2,67468	**0,000***	-47,8975	-33,4905
ALP U/I	CG	EG-Se	44,21266	0,177	-26,8007	211,3487
		EG-Zn	44,21266	**0,000***	115,8743	354,0237
		EG-Mg	44,21266	0,329	-43,0727	195,0767
	EG-Se	EG-Zn	44,21266	**0,014***	23,6003	261,7497
		EG-Mg	44,21266	0,983	-135,3467	102,8027
	EG-Zn	EG-Mg	44,21266	**0,005****	-278,0217	-39,8723

*p<0,05, **p<0,01, ***p<0,001

GPT was within the reference values in CG and EG-Se, EG-Zn, except for the hens group whose ration was supplemented with magnesium. GGT has had a higher activity in all the hens in the experiment. At this moment it is considered the most sensitive enzyme which proves the secreting function of the liver. ALP has had the most intense activity in the hens in the CG, compared with the supplemented groups with selenium, zinc and magnesium.

Table 3

Sanguine biochemical parameters with egg-laying hens (downward phase) Multiple Comparisons Tukey HSD

Parameters	Group		Standard error	Sig.	95% CI	
					Lower limit	Upper limit
Total proteins g/dl	CG	EG-Se	0,15017	**0,000*** **	-1,7480	-0,9429
		EG-Zn	0,15017	**0,000*** **	-1,4171	-0,6120
		EG-Mg	0,15017	**0,000*** **	-1,9216	-1,1166
	EG-Se	EG-Zn	0,15017	0,140	-0,0716	0,7334
		EG-Mg	0,15017	0,657	-0,5761	0,2289
	EG-Zn	EG-Mg	0,15017	**0,009** **	-0,9071	-0,1020
SGOT U/I	CG	EG-Se	12,65708	0,820	-22,8972	44,9554
		EG-Zn	12,65708	0,156	-6,7335	61,1190
		EG-Mg	12,65708	0,543	-16,9454	50,9072
	EG-Se	EG-Zn	12,65708	0,583	-17,7626	50,0899
		EG-Mg	12,65708	0,965	-27,9745	39,8781
	EG-Zn	EG-Mg	12,65708	0,851	-44,1381	23,7145
SGPT U/I	CG	EG-Se	0,34183	**0,000*** **	1,8838	3,7162
		EG-Zn	0,34183	**0,000*** **	4,6256	6,4581
		EG-Mg	0,34183	**0,000*** **	4,6101	6,4426
	EG-Se	EG-Zn	0,34183	**0,000*** **	1,8256	3,6581
		EG-Mg	0,34183	**0,000*** **	1,8101	3,6426
	EG-Zn	EG-Mg	0,34183	1,000	-0,9317	0,9008
GGT U/I	CG	EG-Se	1,70684	1,000	-4,7532	4,3969
		EG-Zn	1,70684	0,996	-4,2005	4,9496
		EG-Mg	1,70684	0,974	-5,2950	3,8550
	EG-Se	EG-Zn	1,70684	0,988	-4,0223	5,1278
		EG-Mg	1,70684	0,989	-5,1169	4,0332
	EG-Zn	EG-Mg	1,70684	0,918	-5,6696	3,4805
ALP U/I	CG	EG-Se	64,93866	**0,000*** **	141,0299	489,1556
		EG-Zn	64,93866	**0,000*** **	130,3808	478,5065
		EG-Mg	64,93866	**0,001** **	101,9944	450,1201
	EG-Se	EG-Zn	64,93866	0,998	-184,7119	163,4138
		EG-Mg	64,93866	0,931	-213,0983	135,0274
	EG-Zn	EG-Mg	64,93866	0,972	-202,4492	145,6765

*p<0,05, **p<0,01, ***p<0,001

AL-Bustany et al. (1998) found that at the same time with the aging of the egg-laying hens the activity of ALP is considerably reduced. As a result of the determinations carried out one can notice a significant increase in the GOT activity, in all the hens compared with the reference data. GPT has a significantly lower activity in all the hens, the enzymatic activity being under lower limit of the reference values. GGT has had a higher activity in all groups, the average values being comprised between 19-20 U/l, without statistical significant differences between the groups. ALP has had a higher activity in CG compared with supplemented groups. The increased values of serum activity of ALP can be traced back to a more intense osteoblastic activity and even to the existence of hepato-biliary disorders.

CONCLUSIONS

In the ascending phase of the egg laying curve, proteinemia ranged below the lower limit reference with the hens in the control group and those in the group supplemented with magnesium.
In the downward phase significant differences were confirmed between the GC and the groups supplemented with minerals.
In the upward phase of the egg-laying curve, the activity of AST enzyme was within the reference limits in the hens in the experimental groups, while in the steady phase its activity was lower in the hen lots supplemented with mineral substances.
In the upward phase ALT had lower values, justified by the more laborious hepatic activity for egg production.
In the steady phase, its activity was more intense in the lot supplemented with magnesium, and in the downward phase its activity was higher in the hens in GC, compared with experimental groups.
In the upward phase the activity of GGT was within the reference limits, in the steady phase it was above the maximum limit because of a higher egg production, and in the downward phase it came back to normal.
ALP in the upward and steady phase had values above the maximum limit and in the downward phase it had a higher activity in GC compared with experimental groups.

REFERENCES

AL-Bustany, Z., AL-Athari, A.K., Abdul-Hassan I.A. 1998. Plasma alkaline phosphatase and production traits in laying hens as influenced dietary protein, strain and age. Br. Poult. Sci., 39, 568-571.
Diaz, G.J., Squires, E.J., Julian, R.J., 1999. The use of selected plasma enzyme activities for the diagnosis of fatty liver-hemus Lagic syndrome in laying hens, Avian Disease, 43, 4, 768-773.
Ghergariu S., 1995. Patologia nutriţională şi metabolică la animale. Editura Medicală Veterinară Bucureşti.
Pârvu G., 1992. Supravegherea nutriţional metabolică a animalelor, Editura Ceres, Bucureşti.
Stoica, I., 1997. Nutriţia şi alimentaţia animalelor, Ed. Coral Sanivet, Bucureşti.

THE SUPPLEMENTATION EFFECT OF FEED WITH SELENIUM, ZINC AND MAGNESIUM ON EGGS AND MEAT BIOPRODUCTIVE INDICES IN LAYING HENS

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Abstract

The experiment was conducted on 144 hens, for the duration of a production cycle. The biological material used in the experiment was divided into 4 experimental groups. In the experiment we used a structure of mixed supplemented fodder in three experimental variants with selenium, zinc and magnesium. The supplementation of the feed, designed for hybrid hens in the upward phase of the egg laying curve with selenium, zinc and magnesium led to the reduction in the total consumption of feed with additional variants compared with the control group. Feed supplementation with selenium determined the insignificant increase in the egg production, compared to the other variants tested in the upward phase, but a significant increase in the steady and downward phase of the egg laying curve. Zinc supplementation has also led to an insignificant increase in egg production in the upward and downward phases versus the control group. Magnesium supplementation led to the worst results on egg production and their average weight.

Key words: *laying, hens, bioproductive, parameters*

INTRODUCTION

Mineral substances are chemical elements which are indispensable to life, because of the interactions between the ions and the important functions in the development of cells and metabolism (Bârză, 1982; Ghergariu, 1995). They have a plastic role (they make up complex compounds, which are essential for the proper functioning of the body) and a functional role. Microelements are present in small quantities in the tissues of birds, but they perform essential functions for life and growth. In practice a few require supplementation in feeding with fodder: iron, copper, zinc, magnesium, iodide and selenium thus create problems to the birds regarding their productive yield (Crăiniceanu, 2006; Pârvu, 2003; Stoica, 2005). The purpose of this study was to evaluate the modifications induced by the basic food rations supplemented with selenium, zinc and magnesium used in egg-laying hens during a biological cycle on certain bioproductive indicators.

MATERIALS AND METHODS

The experiments were carried out on „Isa Brown" hybrid egg-laying hens in a zootechnical farm in the Western part of the country, where the recommended microclimate conditions have been provided. The combined fodder (CF) recipes have been observed according to the requirements of the hybrid in the experiment in all the three operational phases (upward, steady and downward). A control lot (CG) with 36 hybrid hens and 3 experimental lots of 36 hens each have been constituted (EG-Se, EG-Zn and EG-Mg). The experimental lots have been fed with CF supplemented with 20 g Sel-Plex concentration 1000 mg/100 kg CF with 9.5 g zinc oxide conc. 72%/100 kg CF and the 3rd one with 100 g magnesium oxide conc. 75%/100 kg CF. The statistical interpretation of the data was carried out by means of the Minitab 14 program.

RESULTS AND DISCUSSIONS

During the experimental period of 57 weeks there were no illness cases in the hens in the experimental lots as a consequence of observing the nutritional parameters regarding combined fodder used in the food and of the operating technologies. The average daily consumption (ADC) of fodder recorded at the experimental groups is given in tables 1, 2 and 3 for each phase of the egg-laying curve.

Table 1

Combined fodder consumption (kg) recorded in experimental groups in upward phase

Period (weeks)/ Hens age (weeks)	**CG**		**EG-Se**		**EG-Zn**		**EG-Mg**	
	CF/ hens	ADC / hens	CF/ hens	ADC / hens	CF/ hens	ADC / hens	CF/ hens	ADC / hens
1 (21)	0,770	0,110	0,756	0,108	0,770	0,110	0,742	0,106
2 (22)	0,805	0,115	0,784	0,112	0,791	0,113	0,770	0,110
3 (23)	0,826	0,118	0,805	0,115	0,805	0,115	0,805	0,115
4 (24)	0,840	0,120	0,833	0,119	0,826	0,118	0,805	0,115
5 (25)	0,840	0,120	0,840	0,120	0,854	0,122	0,826	0,118
6 (26)	0,861	0,123	0,854	0,122	0,854	0,122	0,840	0,120
7 (27)	0,875	0,125	0,875	0,125	0,868	0,124	0,840	0,120
8 (28)	0,875	0,125	0,875	0,125	0,875	0,125	0,847	0,121
Total	**6,692**	**0,119**	**6,622**	**0,118**	**6,643**	**0,118**	**6,475**	**0,115**

From the analysis of the data in table 1 (upward phase) it results that during the entire experimental period, the highest CF consumption was recorded at the CG (6.692 kg) followed by the group supplemented with zinc and selenium. The consumption varies from one week to the other, increasing in all the experimental versions, and during the last two weeks analyzed it showed close values.

Table 2

Combined fodder consumption (kg) recorded in experimental groups, in steady phase

Period (weeks)/ Hens age (weeks)	CG		EG-Se		EG-Zn		EG-Mg	
	CF/ hens	ADC / hens	CF/ hens	ADC / hens	CF/ hens	ADC / hens	CF/ hens	ADC / hens
1-4 (32)	3,31	0,118	3,38	0,121	3,32	0,119	3,29	0,118
5-8 (36)	3,32	0,119	3,39	0,121	3,33	0,119	3,3	0,118
9-12 (40)	3,36	0,120	3,42	0,122	3,35	0,120	3,32	0,119
13-16 (44)	3,38	0,121	3,44	0,123	3,35	0,120	3,32	0,119
17-20 (48)	3,40	0,121	3,44	0,123	3,37	0,120	3,35	0,120
21-24 (52)	3,44	0,123	3,48	0,124	3,42	0,122	3,36	0,120
25-28 (56)	3,45	0,123	3,52	0,126	3,44	0,123	3,38	0,121
29-32 (60)	3,47	0,124	3,52	0,126	3,48	0,124	3,4	0,121
33-36 (64)	3,50	0,125	3,54	0,126	3,48	0,124	3,45	0,123
Total	**30,63**	**0,122**	**31,13**	**0,124**	**30,54**	**0,121**	**30,17**	**0,120**

The average daily consumption for the entire experimental period (8 weeks) is found within the limits mentioned in the technological guide of the hybrid. In the steady phase the highest CF consumption was recorded at the EG-Se, followed by the CG (table 2). The ADC also has values which can be found in the technological guide of the hybrid.

The consumption of CF (table 3) in the downward phase in higher in EG-Se (11.30 kg) followed by the CG (11.19 kg) and by the one supplemented with zinc (11.14 kg).

Table 3

Combined fodder consumption (kg) recorded in experimental groups, in downward phase

Period (weeks)/ Hens age (weeks)	CG		EG-Se		EG-Zn		EG-Mg	
	CF/ hens	ADC / hens	CF/ hens	ADC / hens	CF/ hens	ADC / hens	CF/ hens	ADC / hens
1 (65)	0,875	0,125	0,882	0,126	0,861	0,123	0,861	0,123
2 (66)	0,868	0,124	0,875	0,125	0,861	0,123	0,854	0,122
3 (67)	0,875	0,125	0,875	0,125	0,854	0,122	0,854	0,122
4 (68)	0,868	0,124	0,868	0,124	0,861	0,123	0,84	0,12
5 (69)	0,854	0,122	0,875	0,125	0,854	0,122	0,84	0,12
6 (70)	0,861	0,123	0,868	0,124	0,854	0,122	0,84	0,12
7 (71)	0,854	0,122	0,868	0,124	0,854	0,122	0,84	0,12
8 (72)	0,854	0,122	0,868	0,124	0,854	0,122	0,84	0,12
9 (73)	0,854	0,122	0,861	0,123	0,861	0,123	0,84	0,12
10 (74)	0,861	0,123	0,861	0,123	0,854	0,122	0,854	0,122
11 (75)	0,861	0,123	0,868	0,124	0,854	0,122	0,854	0,122
12 (76)	0,854	0,122	0,861	0,123	0,861	0,123	0,854	0,122
13 (77)	0,854	0,122	0,868	0,124	0,861	0,123	0,854	0,122
Total	**11,19**	**0,123**	**11,30**	**0,124**	**11,14**	**0,122**	**11,03**	**0,121**

The egg production was recorded daily, being converted and presented in weekly egg-laying percentages (which were subsequently transformed into angular degrees in order to be able to determine the dispersion index), and for the comparison between groups was used *Anova* test with Tukey test.

From table 4 it is noticeable that the egg production is increasing in all the experimental groups, pointing out the EG-Se which produced a number of 1666 pieces with a weekly average of 208±10.88 pieces, but statistical insignificant compared with the other groups ($p \geq 0.05$).

Table 4

Summarizing table of the evolution of the number of eggs in the upward phase

Period (weeks)	Total /week/group	Mean/day/group		
		$\bar{x} \pm S\bar{x}$	s	CV %
21-28	**CG**			
	1568	196,00±10,33	29,22	14,91
	EG-Se			
	1666	208,00±10,88	30,79	14,79
	EG-Zn			
	1624	203,00±9,89	28,00	13,79
	EG-Mg			
	1484	185,00±8,57	24,24	13,07

Statistical analysis of results in this production phase, revealed that supplementation of CF with minerals did not increase statistical significant the production of eggs.

Table 5

Summarizing table of the evolution of the number of eggs in the steady phase

Period (weeks)	Total /week/group	Mean/day/group		
		$\bar{x} \pm S_{\bar{x}}$	s	CV %
29-64	**CG**			
	7242	804±9,38	28,14	3,5
	EG-Se			
	7442	826±6,61	19,85	2,40
	EG-Zn			
	7298	810±6,97	20,93	2,58
	EG-Mg			
	7003	778±9,13	27,39	3,52

In the steady phase of the egg-laying curve, table 5, egg production is in a slight decrease in all groups and EG-Se stands out, group which produced 7442 pieces with a monthly average of 826±6.61 eggs at a significant difference compared with EG-Mg (7003 eggs). The highest egg-laying percentage during the 36 weeks of experiment is observed in EG-Se. The variability coefficients of the egg-laying percentage for the entire experimental period are low, comprised between 1.78% and 3.49%. In the downward phase of the egg-laying percentage the egg production is decreasing in all groups (table 6), more accentuated decrease being noticed in the EG-Se, which produced a total of 2135 pieces, with an average of 164 eggs, at a significant difference compared to CG.

Table 6

Summarizing table of the evolution of the number of eggs in the downward phase

Period (weeks)	Total /week/group	Mean/day/group		
		$\bar{x} \pm S_{\bar{x}}$	s	CV %
65-77	**CG**			
	2002	154±2,63	9,48	6,15
	EG-Se			
	2135	164 ±2,82	10,15	6,18
	EG-Zn			
	2079	159±3,05	11,01	6,89
	EG-Mg			
	1981	152±2,12	7,64	5,02

For the entire period analyzed in this study we can sustain that the egg production had a low variability coefficient, comprised between 5.02% in EG-Mg and 6.89% in EG-Zn. Analyzing the data cumulated during the 13 weeks of study, it is noticed that EG-Se had the highest lowest egg-laying percentages, being followed by the EG-Zn. As in the case of the number of eggs, evidently the egg-laying percentage registers significant difference during the period of the experiment between EG-Se and CG, but also between EG-Se and EG-Mg.

CONCLUSIONS

Supplementing the CF in the upward phase of the egg-laying curve with selenium, zinc and magnesium led to the reduction of the total CF consumption compared to CG.

Supplementing the CF with selenium determined the significant increase of the egg production compared with the rest of experimental groups.

Supplementing the ration with zinc led to the insignificant increase of the egg production in the upward and downward phase of the egg-laying curve compared to CG and EG-Mg.

In the steady phase of the egg-laying curve, zinc supplementation determined the significant increase of the egg compared with EG-Mg.

Magnesium supplementation of the CF determined the most insignificant results regarding egg production during the entire egg-laying cycle.

REFERENCES

Bârză, H., May, I., Gherghariu, S., Hagiu, N., 1982. Patologie şi clinică medicală veterinară. Ed, Didactică şi Pedagogică Bucureşti.

Crăiniceanu E., Matiuţi M., Crăniceanu, D., 2006. Nutriţia animalelor, Editura Brumar, Timişoara.

Ghergariu S., 1995. Patologia nutriţională şi metabolică la animale. Editura Medicală Veterinară Bucureşti.

Pârvu, Gh., 2003. Tratat de nutriţia animalelor. Ed. Coral Sanivet, Bucureşti.

Stoica Liliana-Mădălina, 2005. Bazele fiziologice şi nutriţionale ale producţiei de ouă. Ed. Coral Sanivet, Bucureşti

MONITORING OF HEAMATOLOGICAL INDICES IN A SAMPLE GROUP OF CATS SUBJECTED TO SERIAL BLOOD COLLECTIONS FOR BIOEQUIVALENCE TESTING

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Abstract

The bioequivalence evaluation of a drug is based on the bioavailability of its active molecule. Serial blood sampling might be limited in cats, due to their reduced blood volume and other morphophysiological characteristics that will be analyzed in this study. The bioequivalence testing of an antihelmintic product was conducted on 37 common breed cats, between 2 to 4 kg, fed with dry and canned food. The testing protocol consisted of two phases, 14 days apart. Eleven blood samples were collected at increasing time periods (0-24h). The total blood volume collected in each stage was evaluated based on the volemia, estimated at 7.5% from the total body weight, representing a maximum of 13 ml/kg and 2.2 ml for each sample. Along with the serial blood sampling, at the start and ending of the two phases, haematological evaluations (on EDTA) and biochemical profiles (on Li-Heparin) were performed. Addtionally, morphological assessments were carried out on panoptic stained smears. No major alterations of the physiological parameters were recorded, except for a small decline in the erythrocitary parameters, associated with oscillations of the total white blood cell count and a tendency to monocytosis. The results of this study reveal the necessity to associate physiological parameters of the tested animals with the requirements of drugs bioequivalence testing protocols, in order to respect ethical and good practice standards while collecting multiple blood samples.

Keywords: *bioequivalence, cats, hematology, volemia.*

INTRODUCTION

The bioequivalence tests require actions that facilitate sample collection, processing analysis and the interpretation of the recorded data. Collecting appropriate samples, both qualitatively and quantitatively, is essential or the success of these tests, with regard to the professional ethics, the existing laws and the good practice and animal welfare requirements. The protocols for these tests include a series of stress factors generated by the serial

collection of blood samples used for determining pharmacokinetic parameters and monitoring the evolution of o the haematological and metabolic profile indices.

Bioequivalence testing is based on the comparative assessment of bioavailability of two or more formulae of the same active ingredient, administered in the same way (Yilmaz and Elmas, 2010; Qayyum, 2012).

The bioequivalence of a drug product is considered to be achieved when the proportion and rate of absorption don't differ significantly from the ones recorded in the reference product, administered in the same molar dose (Altintas and Yarsan, 2009; Posyniak et al., 2001; Sumano et al., 2001, Martinez et al., 2001).

Systemic bioavailability is determined by the absorption time of the active substance, being influenced by the nature of the active substance, dosing form and their interactions with the absorption environment, bioavailability measuring the proportion of active molecules that are available to exert their action (Mycek et al., 1997; Stroescu, 19997, Mungiu, 1995).

The present paper aims to analyze the haematological test used to monitor the health in a group of cats used in bioequivalence investigations and the sampling and processing techniques used in these tests.

MATERIALS AND METHODS

The research has been carried out on a group of healthy adult cats (n=37), European race, originating from rural areas; there were 15 females and 22 males. When creating the group of animals to be included in the study, the following inclusion/exclusion requirements were taken into account (Table 1).

Table 1. Inclusion and exclusion criteria of the animals for the experiment

Inclusion criteria	Exclusion criteria
Male or female	Hypersensitivity to the administered formula or to other drugs containing it
Age 1-5 years	Acute diseases expressed 14 days before given the product
Negative results in the clinical and laboratory tests	Pregnant women and lactating
Weight 1-5 kg	Treatments using barbiturics/phenotyazinics in the last 30 days
	Case history regarding the evolution of major diseases (cancer, hepatic, kidney diseases, etc.)

During the two phases of the experiment, the cats were housed in individual cages, at the same time having permanent access to food and waters; the main food used was granulated feed, supplemented with canned food. As the testing protocol required, a 7 days period preceded the actual tests, needed for accommodation to the experimental conditions. During this period, the animals were subjected to usual clinical, haematological and biochemical tests to confirm their health status. The clinical tests followed mainly the evaluation of vital signs, additional clinical investigations, focused on the different apparatus, haematology and metabolic profile.
In each case, the maximal volume of blood that could be collected was determined using the weight ratio method.
Following the test protocol, the randomizing scheme was set up for the animals used for testing. Blood sampling was carried out from the brachiocephalic vein in most cases, except for few cases when blood was collected from the jugular or saphenous vein.
Blood samples were collected, at the beginning and at the end of the test, using EDTA as anticoagulant, for haematological test, and Li-Heparin, for the biochemical tests; during the tests, 22 samples of blood were collected from each animal, in the two phases, in order to determine the plasma concentration, at the intervals presented in Table 2.

Table 2. Time schedule for the blood sampling of the cats from the experiment

Interval	Schedule of sampling
Day 1	(0.0) and 0.5; 1.0; 1.5; 2.0; 4.0; 8.0; 12.0
Day 2	24.0 and 36.0
Day 3	48.0

Immediately after sample collection, the blood samples were centrifuged at 2000 G to separate the plasma necessary in the bioequivalence study.
The haematological tests were carried out using the Abacus Junior Vet automatic analyzer and consisted of: total and differentiated leukocyte count (WBC), red blood count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC). Morphological tests were also carried out separately, using blood smears coloured using the Dia-Quick Panoptic method.
The metabolic profile tests were carried out using the VetScan VS2 – ABAXIS high-throughput analyzer, using Comprehensive Diagnostic Profile kits for dogs and cats; the main indices determined were: total protein (TP), albumins (ALB), alanine aminotransferase (ALT), amylase (AMY) and globulins (GLOB) concentrations.
The individual data were statistically analyzed using the GraphPad InStat 3.05 software; arithmetic mean and standard deviation were calculated.

RESULTS AND DISCUSSIONS

Following the standards used in bioequivalence testing protocols, the tests were carried out on healthy animals. The experiment started with an initial selection based on the results recorded in the clinical, haematological and biochemical tests; after this selection, a total number of 15 cats were excluded from the test, 7 based on clinical criteria and 8 based on the laboratory criteria. Regarding the clinical tests, two cats were excluded based on their low body weight, two cats being pregnant, two cats being hard to control and one cat suffered from severe coagulopathy. As concerns to the haematological results, 8 cats were eliminated, deviations being recorded in the erythrocyte, leukocyte or biochemical parameters (Table 3).

Table 3. Subjects excluded from the testing and the criteria on which they were eliminated (n=15)

Examination	Nr of cases	Observations
Clinical	2	Low body weight
	2	Pregnancy
	2	Aggressiveness
	1	Coagulopathy
Haematological	5	Deviations from the physiological eritrocitary ranges
	3	Deviations from the physiological leucocitary ranges
Biochemical	3	Deviations from the normal metabolic ranges

Most of the animals that passed the inclusion/exclusion requirements stated in the test protocol, represented the experimental group, composed of 22 cats. Regarding them, it could be noted that the results of the clinical haematological and biochemical tests revealed that their vital functions were in normal physiological limits. The criteria used to select the feline donors for transfusion were chosen In order to estimate the volemia/total collected blood correlation (Kohn and Weingart, 2006). These criteria include the following requirements for the ideal feline donor: friendly temper, body weight of 4.5 kg, a minimum hematocrit of 35%. It is known that 10-15% of the total blood volume can be safely collected, the mean volemia in cat being estimated at 60.1 ± 9 ml/kg. So, the maximum amount of blood that can be collected from a potential donor is estimated at 11-13 ml/kg, once in 3-4 weeks (Donahoe, 2012). It is widely accepted that the acute loss of over 30% of the blood volume can determine hypo-volemic shock, with possible fatal consequences (Donahoe, 2012; Kohn and Weingart, 2006).

By analyzing the main erythrocyte parameters, recorded at the beginning and at the end of the experimental period, it can be observed that they are in normal physiological limits, indicating a good health status, without any signs of anemia (Figure 1). These values were constant throughout the two phases of the experiment, indicating that the serial blood sampling did not affect the main erythrocyte parameters, with regard to the experimental protocol and the good practice requirements. A global view of the erythrocyte mass parameters in the tested animal indicate that, despite the appearances, the cat is a suited animal for this type of blood sampling and can be used without reserve in bioequivalence studies of drugs.

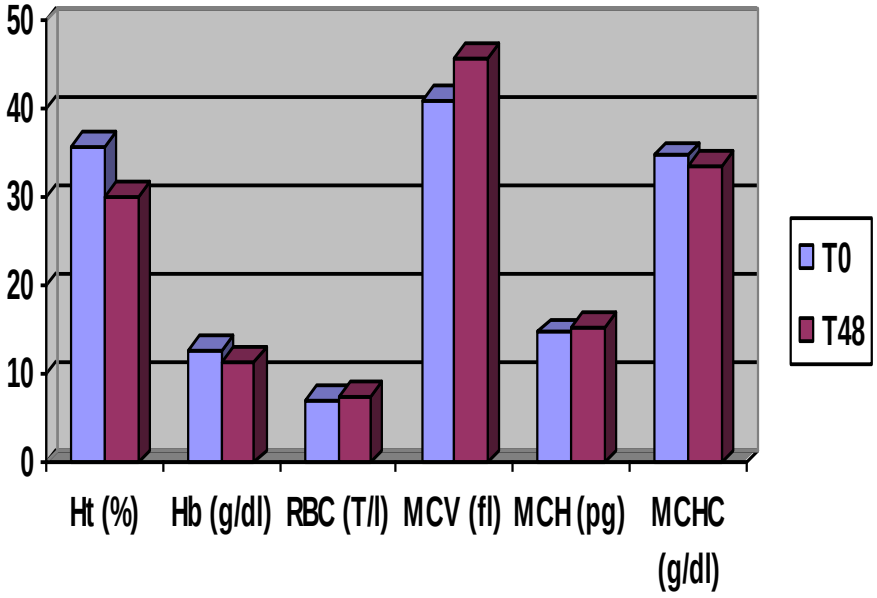

Figure 1. The evoultion of eritrocitary parameters recorded at the beggining and end of the experiment

The evolution of the leukocyte parameters of the tested cats also revealed that serial sampling had no negative influence on the leucopoiesis, leukocyte functions and on the immune system. Special relevance, in this case, has the leukocyte parameters that maintained within the normal physiological range, throughout the experimental period (Figure 2).

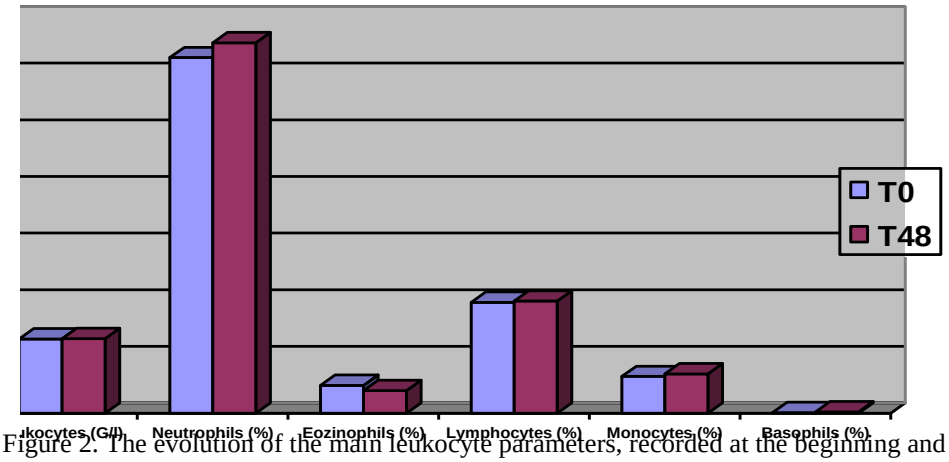

Figure 2. The evolution of the main leukocyte parameters, recorded at the beginning and at the end of the experiment.

The evolution of biochemical indices must also be analyzed from a general point of view. These parameters were determined at the beginning and at the end of the experimental period; as seen in table 4, these parameters were in the normal physiological ranges, cited by the literature (Campbell and Chapman, 2000).

The evolution of the metabolic profile indices also indicated that the serial blood sampling had no negative influence on the health status o the tested cats, also indicating that the animals adapted to the stress conditions generated by the bioequivalence tests (Figure 3).

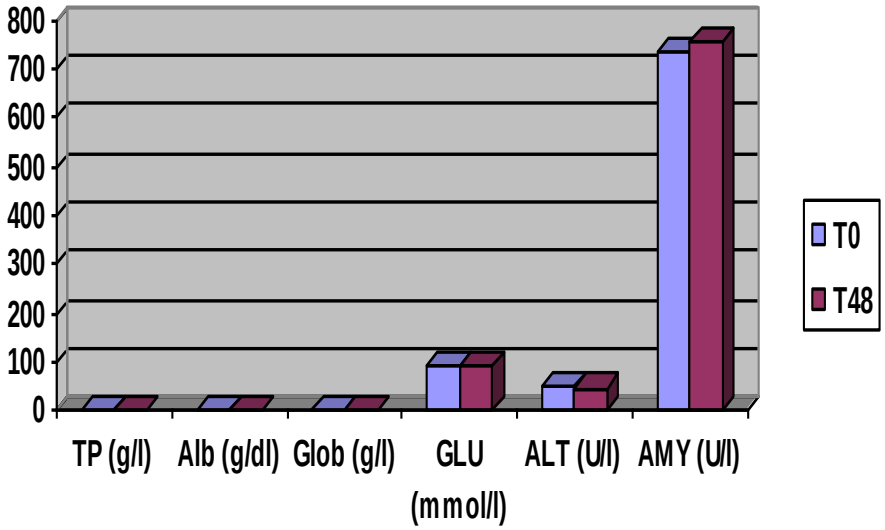

Figure 3. The evolution of the main biochemical blood indices, recorded at the beginning and at the end of the experiment.

The results recorded in this study must be correlated with the requirements of the European testing and approval rules and methods applied in the case of drugs for veterinary use. These imply the use of UE certified laboratory tests for monitoring health status of animals used in the pharmacokinetics and/or bioequivalence studies of various pharmaceutical formulae. Measuring the active substance concentration at the primary site of action, in biophase, is not yet possible, thus limiting the possibility to use these methods to asses the biological reaction or the therapeutic effect. These evaluations are based on determining the drug concentration in plasma or other body fluids; the intensity of the biological reaction is proportional to the plasma concentration of the substance determining that reaction (Posyniak et al., 2001).

The new testing methodology for drugs is focused on assessing and monitoring the risks and adverse effects that occur as a result of using various active molecules in veterinary therapy. All these aspects are reflected in quality and safety assurance of the drug, according to the European Union legal requirements.

CONCLUSIONS

Serial blood sampling allows determining the evolution of certain parameters within a timeframe; nevertheless they can act as stress factors.

All of the cats could be used or blood sampling from the brachiocephalic, saphenous or jugular vein, the last two being less used. The cannulas were well tolerated for 3 days, by the majority of cats; only two cases required changing the cannula during testing.

The total volume of blood collected during the serial blood samplings was within the limits given by the individual values o volemia (13 ml/kg), the initial and final analysis of the blood profile indicating non-significant decreases of the erythrocyte mass indices.

The leukocyte profile revealed small variations of the total leukocyte count, a mild monocitosys being also observed, confirming a good health status and the absence of adverse effects.

Comparative analysis of the biochemical indices revealed non-significant variations, with no consequences whatsoever on using cats in serial blood sampling based tests.

REFERENCES

Altintas L. and Yarsan E., 2009. Bioequivalence of some sulphonamide formulations following oral administration in broilers. *Kafkas Univ. Vet. Fak. Derg.*, 15 (2), 217-223.

Campbell A., Chapman M., 2000. Handbook of poisoning in dogs and cats. Wiley-Blackwell.

Donahoe Charlotte, 2012. Fluid therapy for veterinary technicians and nurses. Wiley-Blackwell.

Kohn B., Weingart C., 2006. Feline blood typing and transfusion: a practical approach. Proc. WSAVA.

Martinez M.N., Pedersoli W.M., Ravis W.R., Jackson J.D. and Cullison R., 2001. Feasibility of interspecies extrapolation in determining the bioequivalence of animal products intended for intramuscular administration. *J. Vet. Pharmacol. Ther*., 24, 125-135.

Mungiu O.C., 1995. Elemente de farmacologie fundamentală. Ed. UMF "Gr. T. Popa", Iasi.

Mycek M.J., Harvey R.A., Champe P.C., Fisher B.C., 1997. Lippincott's Illustrated Reviews, Pharmacology, 2nd edition. Lippincott Williams and Wilkins, Maryland, USA.

Posyniak A., Zmudzki J., Niedzielska J., Biernacki B., 2001. Bioequivalence study of two formulations of enrofloxacin following oral administration in chickens. *Bull Vet Inst Pulawy*, 45, 353-358.

Posyniak A., Zmudzki J., Niedzielska J., Biernacki B., 2001. Bioequivalence study of two formulations of enrofloxacin following oral administration in chickens. *Bull Vet Inst Pulawy*, 45, 353-358.
Qayyum Aisha, 2012. Bioequivalence Studies. In Noreddin A. (Ed.), Bioequivalence Studies, Readings in Advanced Pharmacokinetics - Theory, Methods and Applications. InTech, 3-16.
Stroescu V., 1997. Bazele farmacologice ale practicii medicale. Ed. Medicală, Bucureşti.
Sumano L.H., Ocampo C.L., Gutyerrez O.L., 2001. Non-bioequivalence of various trademarks of enrofloxacin and Baytril® in cows. *Dtsch Tierarztl Wochenschr*, 108, 281-320.
Yilmaz I., Elmas M., 2010. The Bioequivalence Determination of Two Different Formulations of Enrofloxacin in Heifers Following Intramuscular Administration. *Kafkas Univ Vet Fak Derg*, 16 (3), 377-382.

COMPARATIVE PHENOTYPIC ASSESSMENT OF PALATAL SUBEPITHELIAL CONNECTIVE TISSUE ISOLATED FROM DOG AND HUMAN

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Abstract

Stem cells isolated from various tissues can self-renew and produce different cell types. Oral cavity may be a valuable source of mesenchymal stem cells (MSCs) that can be isolated and expanded in vitro, providing a unique reservoir of stem cells from accessible tissue resources. The aim of this study was to isolate and assess the multipotent characteristics of MSCs dog and human in order to cover gingival recessions in future with these cells. Human and dog MSCs were obtained from palatal subepithelial connective tissue, expanded and cultured in DMEM/F12 culture medium supplemented with 10% fetal calf serum (FCS) and antibiotics. The cell surface phenotype of the presumed palatal MSCs was characterized at the 4th passage. All flow cytometry measurements were made using a FACS Canto II flow cytometry system (BD Biosciences, San Jose, CA, USA) and analysed using the DIVA program.
Our data confirmed that the isolated and cultivated dog and human MSCs cells have multipotent character based on specific surface antigen expressing (CD44, CD34/45, CD29).

Key words: connective tissue, mesenchymal stem cells, multipotent

INTRODUCTION

The aim of this study was to isolate and assess the multipotent characteristics of MSCs dog and human in order to cover gingival recessions in future with these cells. Mesenchymal stem cells (MSCs) are adult multipotent progenitor cells having the capacity to differentiate into cells of mesenchymal lineage, including bone, fat, and cartilage (Meierlless & Nardi 2009). Oral cavity may be a valuable source of MSCs. Multitudinous types of dental stem cells have been isolated from the human dental pulp (Grontos et al.2000), and dog dental pulp (Wang et al., 2012), exfoliated deciduous teeth (Suchanek et al.2010), apical papilla (Sonoyama et al.2008), tooth germs (Morsczeck et al.2009), palatal periosteum

(Caballero et al.2010), healthy or inflamed periodontal ligament (Park et al.2011). The multipotent cells isolated from these primary sources can self-renew and produce different cell types. Oral cavity may be a valuable source of mesenchymal stem cells (MSCs) that can be isolated and expanded *in vitro*, providing a unique reservoir of stem cells from accessible tissue resources.

MATERIALS AND METHODS

Human and dog MSCs were obtained from palatal subepithelial connective tissue (approximately 2 to 3 mm of the split-thickness connective tissue graft), expanded and cultured in DMEM/F12 culture medium supplemented with 10% foetal calf serum (FCS) and antibiotics. The cell surface phenotype of the presumed palatal MSCs was characterized at the 4th passage. The cultured cells were trypsinized with trypsin-EDTA, washed and then fixed by adding 4% paraformaldehyde for 15 minutes. 1x105/sample were stained at room temperature with isotype control mAbs and then incubated with 3% FBS albumin and centrifuged for 6 minutes at 1800 rpm. The cells were resuspended in 300 to 600 µl of PBS and 2% FBS. All flow cytometry measurements were made using a FACS Canto II flow cytometer (BD Biosciences, San Jose, CA, USA) and analysed using the DIVA program. More than 105 cells were used to detect nonspecific unions or autofluorescence. Data from 10.000 events were recorded.

RESULTS AND DISCUSSIONS

Human tissue (Fig 1.) approximately 1 weeks of culture, some colonies consisting of fibroblast-like cells were observed. These cells were trypsinized and replated for expansion. In order to obtain single cell-derived hPMC clones, cells were serially diluted in 96-well culture plates (BD Biosciences) at a final density of 60 cells/ plate. Cells with homogeneous bipolar morphology were expanded.

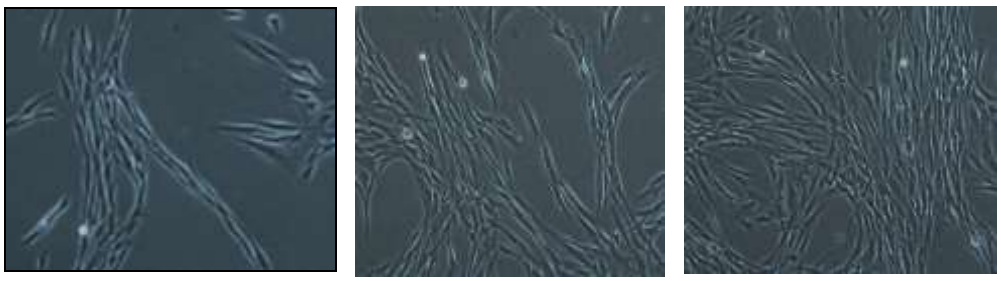

Figure 1 – Human mesenchymal setem cells after isolation and culture

Dog tissue: cells were isolated in explant culture (Fig 2.). After 5 days rapidly proliferative population of cells were isolated.

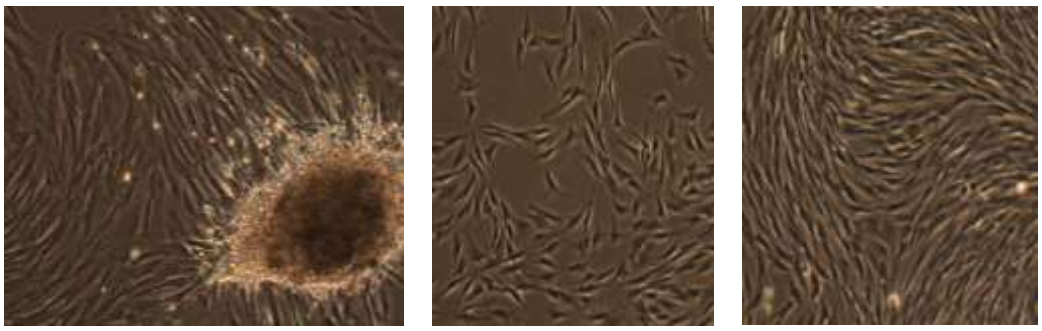

Figure 2 – Dog cells morphology – explant culture

To identify the MSCs, single-cell suspensions were generated from palatal tissue. The ability of palatal-derived cells to form adherent clonogenic cell clusters of fibroblast-like morphology, similar to those recorded for different mesenchymal stem-cell populations, was shown by the formation of about 170-single colonies, generated from 10^4 single cells cultured at low density. These cells firmly attached to the surface of cell-culture plates. The results of the immunophenotypic characterization indicated positivity for CD44, CD29 (Fig 3) and negativity for CD34/45.

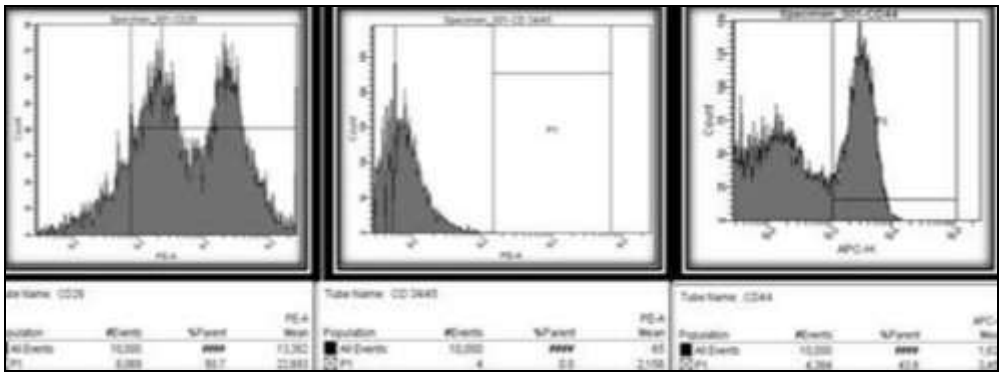

Figure 3 – Phenotypic assessment

CONCLUSIONS

The MSCs obtained (from both sources- human and canine) in this study presented a stable undifferentiated phenotype under normal culture conditions after prolonged cell culture.

MSCs one type of adult stem cell, are easy to isolate, culture, and manipulate in *in vivo* culture. These cells are characterized by high plasticity and can become important cell sources for regenerative therapy.

Our data confirmed that the isolated and cultivated dog and human MSCs cells have multipotent character based on specific surface antigen expressing (CD44, CD34/45, CD29).

REFERENCES

Caballero M., Reed C.R., Madan G., van Aalst J.A., 2010. Osteoinduction in umbilical cord- and palate periosteum-derived mesenchymal stem cells. Ann Pla. Surg. 64(5):605-9.

Gronthos S., Brahim J., Li W., Fisher L.W., Cherman N., Boyde A., DenBesten P., Gehron Robey P., Shi S., 2002,. Stem Cell Properties of Human Dental Pulp Stem Cells, J Dent Res 81(8):531-535.

Meirelles L.S., Nardi N.B., 2009. Methodology, biology and clinical applications of mesenchymal stem cells. Front Biosci., 1;14:4281-98.

Morsczeck C., Götz W., Schierholz J., Zeilhofer F., Kühn U., Möhl C., Sippel C., Hoffmann K.H., 2005. .Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol., 24(2):155-65.

Park J.C., Kim J.M., Jung I.H., Kim J.C., Choi S.H., Cho K.S., Kim C.S., 2011. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: in vitro and in vivo evaluations. J Clin Periodontol., 38(8):721-31.

Sonoyama W., Liu Y., Yamaza T., Tuan R.S., Wang S., Shi S., Huang G.T. 2008, Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study, J Endod., Feb;34(2):166-71.

Suchanek J,. Soukup T., Visek B., Ivancakova R., Kucerova L., Mokry J. ,2009. Dental pulp stem cells and their characterization. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 153(1):31-5.

Wang Y.Y., Zhao Y.M., Ge L.H., 2012. Isolation and identification of Beagle dog dental pulp stem cells, Zhonghua Kou Qiang Yi Xue Za Zhi, ;47(4):241-5.

PSYCHIC STRESS AND ANIMAL WELFARE IN DAIRY CATTLE PRODUCTION

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Abstract:

The animal welfare, the animal protection organizations claim to be sustained, is treated as a psychical state induced by the psychic stress. Understanding of animal psychic stress requires a sensible treatment of the knowledge concerning the brain physiology. The answers to stress differ with the species, the breed, the category, the physiological state and the nervous type of the individuals. In dairy cattle psychical stress may cause alterations of the animal welfare associated with poor health and conducting to financial losses and lower labor productivity. But it is very difficult to appreciate how costly is dairy cows to experience the psychical stress. There are also claims that the organic synthesis taking place under stress condition is resulting in undesirable components for a safe human food. So, some causes of psychic stress and the way to avoid it in dairy cattle are discussed. It is concluded that animal welfare in dairy production is more a veterinary medicine question than a humanitarian one.

Key words: *central nervous system; dairy cattle; farm management; housing system; stress;*

INTRODUCTION

"Animal protection" is a social movement initiated mostly by old ladies fond of their company animals. They thought, and they were right, that having a brain, dogs, cats, cage birds and generally speaking all vertebrate animals suffer like humans of pain, distress, fear or invalidity. Since some bad educated people don't understand these true, they have fought to have good laws obliging for animal protection.

To ask for good laws it is easy but to write such laws is rather difficult. There for veterinarians and other people involved in animal sciences (3, 4, 5) developed the concept of "animal welfare" intending to show and if it is possible to grade or measure the physiological processes causing animals to suffer pain, distress, fear or invalidity in a given moment.

In order to understand the concept of "animal welfare" knowledge about the relation function of superior animals with the environment and the control of central nervous system upon the inner organism functions have to be considered. Among this knowledge special attention must be given to Seyle's theory of stress.

GNOSEOLOGIC FOUNDATION OF "RELATION FUNCTION" IN SUPERIOR ANIMALS

There is three of the objective reality existing outside human mind: Information, Energy and Substance.

Information is *perishable and reproduces*. The *contrary* is its unit of existence and the *bit* is unit of measure.

Energy means *movement* and has *entropy* (lost of movement) when transforms from one type of energy in other one. The *quanta* are its unit of existence and the Joule is unit of measure.

Substance has *mass*. The mass is conserve when kinds of substances are transformed in other kinds of substances (Lavoisier's law). The *molecule* is the unit of substances existence defining their quality and the gram is unit measuring their quantity of mass.

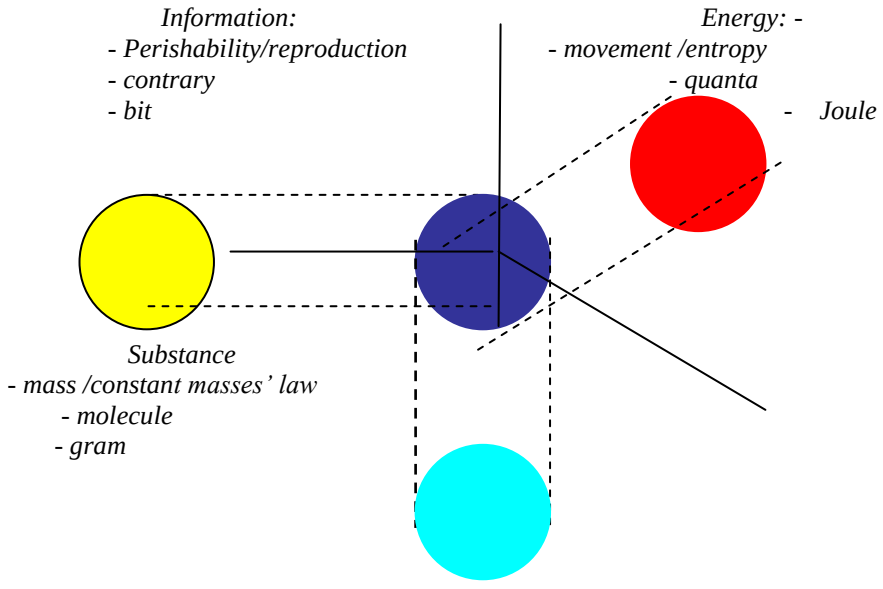
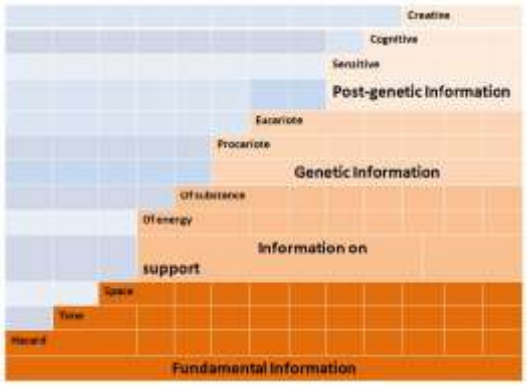


Fig. 1. Unity of existence (objective reality) in triadic thinking mode



Information has no movement and no mass and can exist both without and on energy or substance support. Energy is support of information but has no mass. Substance is support of information and is depositing energy both in their molecules and in the atoms formatting the molecule.

Figure 2. Evolved types of information

Information has evolved from the fundamental type of time and space to the information on support of energy or lifeless substance which perishes and reproduces by hazard together with its support and afterward to genetic information has as support kinds of organized
live substance whose perish and reproduction is done under the supported genetic information control. For a more precise reproduction of their live support cognitive information has been developed by the highly evolved genetic species. In humans information evolved up to the creative stage.

ABOUT THE NERVOUS ACTIVITY IN SUPERIOR ANIMALS

In order to save the live support of animal genetic species and to favor its reproduction, genetic information includes organisms' sensitivity as function of the nervous system. Thus organisms *fell* what is good or bad for its sake. There is sensitive information in cattle unperceived by the cognitive information, and there is also sensitive information recognized by the cognitive information able to *know* what is happening around.
The cognitive information of the cattle is a conscious one. It is made of associated reflexes part of them being innate, hereditary transmitted, as instincts and part of them formatted during the life as conditioned reflexes. The first ones are imprinted in the genetically information at the genom level. The last ones are fixed by the memory and are loaded as the individual knowledge. There are decent hypothesis supposing the memory mechanism at the cerebral neuron level is of the same kind with that of the genetic imprinting phenomenon consisting in methylization and dismethylization of some component bases of nucleotides.

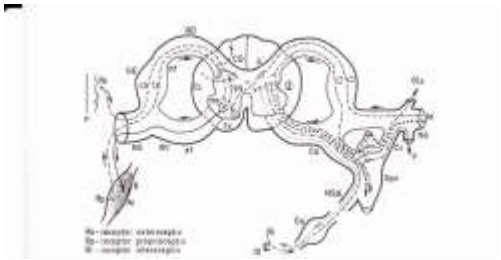


Fig.1. Vegetative and somatic reflex act (CD,CL,CV = dorsal,, lateral and ventral cords. mn – somatic motor neuron, v-vegetative effectors neuron, ps – polysynaptic reflex arc, RD- dorsal rachidian root, af – afferent way, RV – ventral rachidian root, ef – efferent way, NS – rachidian nerve, Ca – white communicant branch, Gpv – paravertebral ganglion, CC – gray communicant branch, Gls – from skin glands, V – to shin vessels, P – skin, M – muscle, Nspl – splanchnic nerve, OI – internal organ, GE – spinal ganglion, Gs – splanchnic ganglion GE – spinal ganglion 1.ventral horn, 2. – lateral horn) (after N. Constantin)

Thus there is a mental activity of fixing by memory new conditioned reflexes and using the innate and after birth obtained reflexes in the current life. There are so called motor reflexes inducing actions and inhibitor reflexes braking action. Reflexes might be expressed quickly or lately after stimulus action, they can be expressed strongly or feebly and with the same intensity both for motor and inhibitor reflexes or unbalanced in favor of one or other of them. These traits differ with individuals and determine the nervous types of animals (see table 1.)

The cognitive information of the cattle is a conscious one. It is made of associated reflexes part of them being innate, hereditary transmitted, as instincts and part of them formatted during the life as conditioned reflexes. The first ones are imprinted in the genetically information at the genom level. The last ones are fixed by the memory and are loaded as the individual knowledge. There are recent hypothesis supposing the memory mechanism at the cerebral neuron level is of the same kind with that of the genetic imprinting phenomenon consisting in methylization and dismethylization of some component bases of nucleotides exercised by the in some cases by the sex of organism generating the gamets.

Nervous activity requires efforts and fatigue is inherent. The rest of the nervous system is received by sleep and by the satisfaction resulting at the end of some reflex acts. But there are also reflex acts finishing with unpleasant sensation like sour or fear. Thus in animal minds states of content or states or discontent install.

Table 1

Table1, Nervous types in superior animals

Nervous type	**Mobility**	**Intensity**	**Balance**	**Ability for life**
Choleric	*good*	*high*	*good*	+
Phlegmatic	*small*	*low*	*good*	±
Lymphatic	*small*	*low*	*inhibition*	-
Sanguine	*good*	*high*	*excitation*	±

Causes of animal mind discontent were disclosed by Seyle in his theory of stress. According to Seyle stress is a shock received by the nervous system. There is sensitive stress received a part of one or other of the sensitive organs. Stress of this type is caused by pain, sour, noise, dazzle, body balance lost. There is also psychic stress, received on the mind level, caused by distress, anxiety, unrest, anger. Effects of stress modify the physiological metabolic and hematological parameters of organisms. This is the scientific approach to evaluating animals' welfare.

CONCEPT OF ANIMAL WELFARE

"Animal welfare" concept is a new one and normally is not fully understood. Three main function are involved in animal physiology: the function sustaining relations of organisms with the environment by means of nervous system, the nutrition function covering the exchange of substances between organisms and the surrounding medium (metabolism) and the reproduction function ensuring the genetic species existence along the time. "Animal welfare" has to serve all of them. A non specific alteration of these functions might be caused by stress. Seyle, which has created the stress theory, has considered *stress as the sum of nonspecific alterations in animal physiology caused by functional disturbances or injures at organism level* (9). Also he said *intensity of stress could be appreciated by the degree of suffering and worn out state of organism (9).* That means stress intensity can be measured and found out. D. M. Broom (1, 2,3,4) has published valuable papers in this respect.

THE DAIRY CATTLE CASE

Dairy cattle are superior animals. They are vertebrates, homeostatic, mammals, herbivorous animal descendants of the wild biological species *Boss Taurus*, now disappeared that was support of a distinct genetic species. Dairy cattle, as well as beef cattle, preserved the molecular mechanism able to close their reproduction as genetic species but have changed the quantitative traits of their biological production as result of artificial selection they undergone. Dairy cattle are specialized to produce much milk as excreted production, beef cattle are specialized to produce meet (muscles) as deposited biological production. There are at present a multitude of dairy cattle breeds. Breeds are artificial biological populations created by humans by artificial selection and reproductively isolation with artificial means (caging, territorial insulation, herd book).

In time breeders of dairy cattle have changed the habitat of dairy cattle as well. In principle there two farming systems to produce cow milk, the extensive system with grazing cattle and the intensive system with cattle fed from manger. For a long time before the intensive system acted with chained animals in 4 walls closed barns. The system is still in use in many parts of the world as combined system with grazing animals in the summer and chained animals in the winter.

Feeding animals from the stock, keeping them chained in closed barns, watering them at fixed time and milking the cows induce great shock on the animal's behavior formatted in nature as a systemic ensemble of innate and memorized conditioned nervous reflexes of cattle. Natural behavior had to be changed. Animals should accommodate to the new habitat but some needs had to be satisfied. Among these the need for rest is the most important. Cows have to lay and to sleep. But cattle are animals of heavy bodies and their leg joints have evident bones that must be protected when cows lay down. If the barn floor is hard the leg joints have to suffer and the hoofs as well. The wet floor will be bad for animals' skin.

The wrong floor produces sensitive stress to animals. Sensitive stress is produced by high temperature in the barn, by thirst or famine, by noise of the barn's machines as well and may be some time by insects. As psychic stress agents, proper to cattle, is the "fear of abyss". That means cattle refuse to pass over ditches. Cattle are afraid of unknown animals. They are able to recognize up to six herd mates and remember one recognized herd

mate no longer then 6 days. Both sensitive and psychic stress agents can produce animals to be sick or hurt and to alter its welfare. Thus organism physiological, metabolic and hematologic parameters are modified and the degree animal welfare degradation might be appreciated.
In dairy cows most of stress agents are of artificial nature, caused by breeders and it is a question of bioethics to avoid their actions.

BAD BIOETHICAL PRACTICES

Most frequent causes of dairy cows welfare deterioration refer to barns. Here chained animals, high density of heads, altered air composition, hard, cold or slippery floor and poor bed for rest should be mentioned. Grill floor on the access alleys is not recommended since cows hesitate to walk on it. Underfeeding or insufficient water are frequently met. Beat or strike cows so as to hurt them is a very bad practice and should be punished by law.
But the mentioned bad practices are not only acts of bad bioethics. Such practices result in alterations of mental state of animals and, par consequence they indulge welfare deterioration and misbalance of homeostatic condition of organisms. Organisms will do efforts to compensate them up to homeostasis using important quantities of energy. The metabolic efforts to remake homeostasis will take energy out of the energy dedicated to milk production. That gives important financial loses that aren't yet properly evaluated. Even the compensate homeostasis is adding energy consummation to the needed one for the basic metabolism.

GOOD BIOETHICAL PRACTICES

The main good bioethical practices referring to dairy cattle address to the housing of cows. The best recommended one is the free stall housing. In the free stall barn must exist three areas: resting area, feeding area and milking area. There is also necessary to have access alleys from one area to the other areas.
The largest and the most important is the resting area. It is build up from rows of individual stalls where cows may lay, ruminate and sleep. One stall must look as a open berth 1½ longer than the cow and narrow, the width measuring half of the stall length. Berth are separated one from the other by bars impeding cows to live the stall other way then retiring back. This way

the bedding of the berth will stay clean and dry. Cows like to have clean, dry, soft and warm bedding. The best bedding is made of 30 cm sick layer of straws. The next one preferred is the sawdust. Rubber bed is not warm enough and the sand bedding is cold.

The feeding area is done out of a low manger along the alley for bringing fodder. The manger is separated from the access alley to the feeding area by a fence permitting cow heads to pass trough and eat. The access alley to manger is the place where their feces and urine are eliminated. The accumulated manure is get of better with a mechanic plough. Hydrolith remove of the manure is most costly, and requires grill floor of the access alley not pleased to cows. Cows might have free access to feed and clean water. They will be never over fed except the case when they are under stress influence. Control of the metabolic energy intake can be done by the energy concentration in the cows' diets, when necessary.

The best air composition inside the barn is given by three walls open buildings limiting the air droughts at the animals' level and permitting free air droughts at the upper levels of barns to have good ventilation;

Keeping on the same herd mates as long as possible in a barn section is good for the psychic of cows. Usually cows are group in the free stall barn in three or four groups according to the time from parturition. There is a group of recent parturient cows, less than 3 month from the calving that need the highest energy concentration of diets and a superior protein / energy rate being also supervised for showing heats. There is group of cows in the high lactation losing in body weight because they could not ingest enough energy, there is a third group of milking cows with decreasing daily milk production and the group of weaned cows which are not milked. The second of the mentioned before group may be absent. This grouping of cows helps the correct feeding of cows and it is good to be respected, but grouping of cows must be done not more frequently than once at four wicks. It is better if the group size is smaller. That will protect against the psychic stress of changing the herd mates.

Milking area is better to be ensured by a milking parlor as a special space dedicated to this purpose. That will help cows to fill the need for the udder discharge and to have better hygiene of milking. One group of cows has to be milked in almost 1 hour including the waiting time to enter the parlor. From the psychical point of view cows will fill better than in case of milking them in the resting area as is done in case of chained animals.

Such practices will ensure animal welfare and good benefit for breeders. The nearby figures present models of modern facilities helping cow protection against psychic stress.

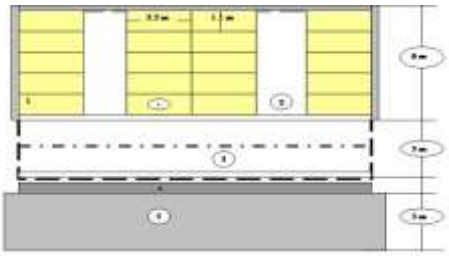


Figure 4. Free stall barn for 20 dairy cows (Legend: 1stall (berth), 2 access alley resting area, 3 manure cleaner, 4 manger 5 – feed transport alley)

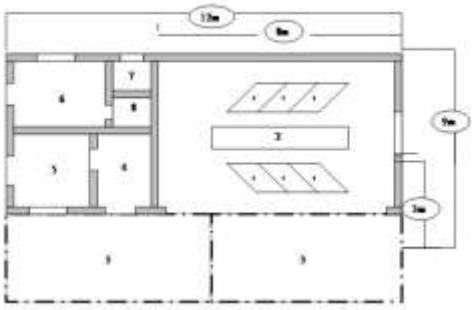


Figure 5. Milking parlor and filter (Legend:1 milking stands, 2 milkier place, 3 waiting pens, 4 washing room, 5 milk room 6milkier room, 7 pumps' room, 8 toilet

FUTURE

Dairy cattle organisms are pressed to support huge efforts apart from the genetic progress induced by artificial selection for more and more milk production.

The body type in dairy cows has be of large stature for much ingesta of feeds, the support offered by the legs must be strong, the vertebral column should be straight indicating good suspension for internal organs and the udder has to be strongly attached to the sacral bones doesn't descend under hocks .

Cows need comfort. They must be left in peace, dispose any time of water and feed, have the possibility of laying down on a convenient clean, dry, soft and warm bedding and be milked regularly.

Research has demonstrated existing liaisons between bioethics standards and animal welfare. Psychic stress alters animal welfare. Since animal welfare influences the necessary metabolic energy for body's maintenance the new technical concepts require to respect the good bioethical practice when dairy cattle farms are planed and managed.

Bioethics principles have to be spread by education in families and in schools.

Dairy cattle breeders have to learn and apply good bioethical practices in order to save time and money.
Animal protection rules against psychic stress are more questions of veterinary medicine than questions of moral.

REFERENCES

Broom D.M. (1983) – Indicators of poor welfare. Br. Vet. J., 142: 524
Broom D.M. (1988) - The scientific assurement of animal welfare. Appl. Anim. Behave. Science, 20: 5
Broom D.M. (1988) - Les concepts de stress et bien etre. Rec. Med. Vet., 164 : 715
Duncan I.J.H. (1987) - The welfare of farm animals. An ethological approach.
Fraser A.F., Broom D.M. (1990) – Farm animal Behavior and welfare. Saunders, New York.
Georgescu Gh. şi colab. (1995) – Tratat de creşterea bovinelor.Vol. 3 Ed. Ceres. – Bucureşti.
Hafez E. (1962) – The behavior of domestic animal. London Baillere – Tindal.
Paraschivescu M. (2008) – Bazele Gnoseologice ale Diversității Biologice, Simposion CSCBAS, AcademiaRomână
Paraschivescu M. (2012) – Diversitatea biologică şi resursele genetice din Zootehnie – Revista de Zootehnie nr 1. 2012.
Paraschivescu M. Th., Bogdan A.T., Paraschivescu M. (2009) - Biodiversity in Farm Animals: Sources, Using, Conservation - Simposion CSCBAS, AcademiaRomână
Popescu A.N., Popescu A.V. (1990) – Stressul animalelor de fremă. Ed. Ceres. – Bucureşti.
Seyle H. (1968) – De la vis la descoperire. Ed. Medicală – Bucureşti.
Solomon M. (19…) – Moduri de Gândire .
* * * H. G. privind aderarea României la Convenţia Europeană privind protecţia animalelor în transportul internaţional. Bucureşti

STUDIES CONCERNING THE DEVELOPMENT OF LIPID NANOSTRUCTURES IN BIOPRODUCTS ENCAPSULATION

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Abstract

In recent years, a considerable effort was dedicated to researching methylxantine derivatives (MX), because of their effect on the hemorheology, increasing deformability, decreasing the aggregation trend of the red blood cells and the fibrinogen concentration. All of these properties turned MX into a drug eligible to be in peripheral and cerebral vascular disorders. At the same time, the pharmacological profile of MX and their short half-life make it a good candidate for encapsulation of drugs.
Lipid nanostructures are a new technology for the encapsulation and delivery of bioactive agents. Because of their biocompatibility and biodegradability, along with their nanosizes, they have potential applications in a vast range of fields. They are able to improve the solubility, bioavailability and stability of bioactive agents, to provide protection of drugs and as well to prevent their unwanted interactions with other molecules, to ensure cell-specific targeting, to minimize adverse effects on healthy cells and tissues.
This study aimed at developing the encapsulation of MX into biodegradable, biocompatible and non-toxic carriers. Lipid vesicles were prepared through a physical dispersion method using different ratios of lipids. We studied the changes that occurred in the entrapment efficiency, the particle size and the drug stability when different formulation parameters were modified. The physicochemical properties of the vesicles were significantly affected by the formulation parameters.

Key words*: liposomes, methylxantine derivatives, physicochemical characterization*

INTRODUCTION

MX derivatives are used both in humans to treat cerebrovascular and peripheral vascular diseases, and in dogs to improve microcirculation and as a consequence, to diminish inflammation and enhance healing of many kinds of skin lesions including: ulcerative dermatosis of Collies and Shelties; dermatomyositis; ear margin seborrhea; atopic disease; and other skin diseases with underlying vasculitis and in horses to treat endotoxemia, laminitis and navicular disease. Because of their pharmacokinetic

properties, they are recommended as good candidates to from the modified release, in order to improve our bioavailability and compliance (Banderas et al., 1997; Hardman et al., 1992; Grigoleit and Leonhardt,1997).
This study was aimed at the development of a method for encapsulating MX derivatives in order to improve their bioavailability and to achieve a controlled drug release profile. Also, the evaluation of the encapsulated forms was studied.
Current research show the preoccupation for the production of vectors ensuring a selective targeting and a controlled release of the drug at the targeted organ or cell (Varshosaz et al., 2010; Lian and Ho, 2001). This approach involves modifying the pharmacokinetic profil of various therapeutic classes of bioactive compounds through their incorporation in colloidal nanoparticulate carriers in the submicron size range such as liposomes or nanoparticles (Lamprecht A., 2009).
Lipid nanostructures are colloidal carriers, usually with a 0.05-5 μm diameter, which form spontaneously when certain lipids are dispersed in aqueous media. They have been reported to improve the solubility, the stability, the bioavailability and the pharmacokinetic properties of the encapsulated bioproducts. Also, they reduce the associated side effects and improve *in vitro* and *in vivo* activity of encapsulated bioproducts (Achim et al., 2009). One of the great benefits of lipid nanostructures is that they can incorporate both hydrophilic and hydrophobic bioproducts. They generally have a large carrying capacity, but usually not large enough to ferry large molecules (such as proteins). Hydrophilic drugs can be readily entrapped within the aqueous interior of the vesicles, while neutral and hydrophobic molecules may be carried within the hydrophobic bilayers of the vesicles (Popovici and Lupuliasa, 2004).

MATERIALS AND METHODS

Materials
Egg phosphatidylcholine (PC) (Sigma– Aldrich)
Dipalmitoil-phosphatidylcholine (DPPC) (Sigma–Aldrich)
Cholesterol (C) (Fluka)
Pentoxifylline (P) and xantinol nicotinate (XN), which were offered as samples from Terapia.
All the other chemicals, reagents and solvents (potassium chloride, potassium dihydrogen phosphate, acetone, chloroform, methanol) used were of analytical or

HPLC purity degree and were purchased from Merck, Germany. All other materials were of analytical grade or equivalent.

Methods

a) Preparation of designed liposomes

From the various lipid nanostructures preparation methods, the hydration of a lipid film was considered as being the most suitable for the encapsulation of MX derivatives. Four formulas for both derivates were prepared in order to select an optimum formula (with an adequate size, yield and stability).

Lipid solutions were prepared by dissolving accurate amounts of PC or DPPC and C in chloroform. The solution was introduced in a 100 ml round-bottomed flask and the solvent was evaporated in a rot evaporator Heidolph Laborota 4200**,** at 35°-40°C, under vacuum. Quantities determined in MX derivatives (P or XN) were dissolved in 10 ml phosphate buffer pH 7,4 (0.25 M) solution and then used for hydrating the dry lipid films formed in bottom glass flask. The lipids were mechanically dispersed in the aqueous medium by stirring for 2 hours. The hydration process was performed 40°C. The liposomes were separated from the unincorporated drug by centrifugation over 30 min. at 12000 rpm and at 5^0C. The supernatant was removed and the liposomes were reconstituted in 5 ml aqueous medium phosphate buffer (pH 7.4). This suspension was allowed to hydrate for 2 h in order to anneal any structural defects.

Four formulations of liposome (Tables 1 and 2) were prepared in order to select an optimum formula (with an adequate size, yield and stability).

b) Size measurement

The determination of the diameter was performed by a Mastersizer 2000 Malvern apparatus.

c) Determination of drug content

The content in drug was analyzed spectrophotometrically.

The percentage of encapsulated MX was calculated using the formula:

$$\% P_e = \frac{MX_s}{MX_t} \times 100$$

in which: MX_e = amount of drug in sediment; MX_t = total amount of drug/ml liposomal suspension (Shivhare et al., 2009).

Also, the influence of the lipid composition and of the temperature during the hydration process on the yield was studied.

RESULTS AND CONCLUSION

a) Preparation of designed liposomes

In the design of the experimental plan for the preparation of the MX-loaded liposomes, we varied the ratio between PC, DPPC, C and P or XN (Tables 1, 2).

Table 1: The composition of the experimentals liposomes loaded with P (mg)

Sample	PC	DPPC	C	P
L1P	3	3	3	2
L2P	3	3	2	2
L3P	4	4	1	2
L4P	9	9	1	2

Table 2: The composition of the experimentals liposomes loaded with XN (mg)

Sample	PC	DPPC	C	XN
L1XN	3	3	3	1
L2XN	3	3	2	1
L3XN	4	4	1	1
L4XN	9	9	1	1

b) Size measurement

The results show that the vesicles are polydisperse, with an average size ranging from 200 to 570 nm, the majority sizing between 300 and 380 nm (Table 3).

Results of particle size analysis showed that with decreasing ratio of phosphatidylcholine and cholesterol occurs and a decrease in the average size of the designed liposomes.

Table 3:Evaluation of the size for experimentals liposomes loaded with MX derivatives (nm)

Type of liposomes	Mean particle size (nm± SD)	Type of liposomes	Mean particle size (nm± SD)
L1P	380 ± 4.81	L1XN	320 ± 5.07
L2P	365 ± 3.84	L2XN	290 ± 4.32
L3P	310 ± 2.72	L3XN	220 ± 2.91
L4P	260 ± 1.71	L4XN	180 ± 1.82

(Mean ± SD, n=3)

c) Determination of drug content

The analysis the UV spectra of MX, both in the presence and in the absence of phosphatidylcholines has shown that this method of assay is adequate, as the lipids and any MX derivatives have peak absorbtions at different wavelengths.

Table 4: The influence of the lipid composition on the encapsulation degree

Type of liposomes	Degree of encapsulation (%)	Type of liposomes	Degree of encapsulation (%)
L1P	31.73 ± 0.87	L1XN	28.13 ± 0.66
L2P	35.28 ± 0.67	L2XN	31.93 ± 0.73
L3P	42.92 ± 0.46	L3XN	36.22 ± 0.86
L4P	46.32 ± 0.32	L4XN	38.27 ± 0.48

(Mean ± SD, n=3)

The data shows that an increase in the cholesterol percentage results in a decrease of the degree of encapsulation from 46 % to 32 % for P, and that the increase of phosphatidylcholine proportion leads to an increase of the degree of encapsulation, as described throughout the literature (Popovici et al., 1998; Gregoriadis G., 2007). The same observations are valid and for XN. It seems that the degree of encapsulation is lower in each case for P comparative with XN due to its higher solubility.

CONCLUSION

Lipid film hydration is an appropriate method for obtaining liposomes loaded with MX derivatives.

Using this method we obtained a liposomal dispersion type MLV with heterogeneous size and entrapment efficiency according to the composition.

An increase in the cholesterol percentage results in a decrease of the degree of encapsulation.

An increase in PC proportion leads to an increase of the entrapment efficiency.

From results of MX derivatives entrapped it was observed that as the amount of cholesterol increased there was subsequent increase in the

stability and rigidity of liposomes but at the same time entrapment efficiency reduced due to reduction in PC and DPPC.
We have determined the optimal parameters for the preparation of MX-loaded vesicles, which ensure the reproductibility of size, content and stability.
In conclusion, these results regarding preparation and characterization of some liposomal formulations could be developed in some sustained or controlled release dosage forms.

ACKNOWLEDGMENTS

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REFERENCES

Achim M., Precup C., Gonganăunițu D., Barbu-Tudoran L., Porfire S.A., Scurtu R., Ciuce C., 2009. Thermosensitive liposomes containing Doxorubicin. Preparation and in vitro evaluation, Farmacia, 57(6), 703-710.
Bandera L., Schorderet M., Tognoni G., 1992. Médicaments "vasoactifs" et "cérébroactifs". Pharmacologie. Des concepts fondamentaux aux applications thérapeutiques, Ed. Frison – Roches, Paris, 213 – 223.
Gregoriadis G., 2007: Liposome technology: Liposome preparation and related techniques, 3rd Edn.,Vol. l, Informa Health care, New York, 21, 112 – 186.
Grigoleit H.G., Leonhardt H., 1997. Rheology of blood and pentoxifylline, Pharmatherapeutica, Vol. 1, 10, 642 – 651.
Hardman J.G., Limbird L.E., Gilman A.G., 1992. The pharmacological basis of therapeutics, 8th ed., Vol. II., New York: Maxwell Macmillan Publishing Corporation: 1699 -1711.
Lamprecht A., 2009. Nanotherapeutics: Drug Delivery Concepts in Nanoscience, Pan Stanford Publishing Pte.Ltd., 105 -232.
Lian T., Ho R.J.Y., 2001. Trends and Developments in Liposome Drug Delivery Systems, J.Pharm.Sci., Vol. 90 (6), 667-680.
Popovici A., Ban I., Tokes B., Nicolaescu I., Suciu G., Mathe I., Nastasă V., Stavri N.,1998. Baze teoretice ale tehnologiei farmaceutice, Editura Mirton, Timişoara, 514 – 533.
Popovici I., Lupuliasa D., 2004, Tehnologie farmaceutică, I, Editura Polirom Iaşi: 25 – 37
Shivhare U. D., Ambulkar D.U.,. Mathur V. B, Bhusari K. P., Godbole M.D., 2009. Formulation and evaluation of Pentoxifylline liposome formulation. Digest Journal of Nanomaterials and Biostructures, 4(4), 857 – 862.
Varshosaz J., Minayian M., Moazen E., 2010. Enhancement of oral bioavailability of pentoxifylline by solid lipid nanoparticles., J. Liposome Research, Vol. 20(2), 115–123.

ATTENUATION OF OXIDATIVE STRESS BY ETHANOLIC EXTRACT OF NETTLE (*URTICA DIOICA*) IN MICE

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Abstract

This study was undertaken to evaluate the antioxidative potential of ethylic fraction of nettle (Urtica dioica) in stressed mice. In this present study mice were divided into the four groups and each group containing five mice. Group I mice were orally administered with 1 ml of 0.9% NaCl with is the positiv control group. Group II mice were administered with ethylic fraction of Urtica dioica. Group III stressed mice were orally administered with 1 ml of 0.9% NaCl with is the negativ control group. Group IV stressed mice were administered with ethylic fraction of Urtica dioica.

The activity of enzymic antioxidants (U/ mg of protein) such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were found to be significantly high in ethylic fraction treated stressed mice when compared to the negative control mice. The levels of nonenzymic antioxidant such as reduced glutathione in the ethylic fraction treated stressed mice was found to be significantly higher than that found in control mice.

These results suggest that nettle (Urtica dioica) has very good antioxidant and hepatic protective effect of oxidative stress in mice. Currently, the importance of oxidative stress in the pathophysiology of many disorders has been highlighted, thus use of this plant as an herbal medicine is highly recommended.

Key words: antioxidative enzymes, glutathione, polyphenols, thiobarbituric acid reactive substances (TBARS).

INTRODUCTION

In this modern era, stress has become an integral part of human life (Ravindran R. *et al.*, 2005). It is vital that stress is kept under control and normal functioning is not hampered due to excessive stress (Verma N. and Khosa R.L., 2009). Stress is considered to be any condition which results in perturbation of the body's homeostasis (Emeny R.T. and Lawrence A. D., 2007). If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. (Lakshmi B.V.S. and Sudhakar M., 2009). Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states; hypertension, peptic ulcer, diabetes, immunosuppression, reproductive dysfunctions and behavioural disorders like anxiety due to involvement of the central nervous

system (CNS), endocrine system, and metabolic system (Rai D. *et al.*, 2003). Drugs having antistress properties induce a state of non-specific resistance against stressful conditions. Herbal formulations have been globally in use for many for human well-being. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body (Lakshmi B.V.S. and Sudhakar M., 2009).

MATERIALS AND METHODS

Preparation of ethanolic extracts

In this study, there were used dried aerial parts of nettle (*Urtica dioica*). The interest parts of plant were powdered and extracted with ethanol 60 % (1:10 ratio, w:v) for 3 hours at 60ºC. The homogenates obtained were filtered using filter paper Watman no. 1 and the filtrates were then centrifuged for 20 min at 5000 rpm and 5ºC. After the ethanol was evaporated, the aqueous residues were utilized.

Animals

The animals used in this study were purchased from Cantacuzino Institute. In this study, twenty adult female albino mice (25-30 g weight, nine weeks old) were used as experimental animals. They were kept in polypropylene cages under standard laboratory conditions of 12 h/12 h light/dark, 22 ± 2 °C temperature, fed with a normal rodent diet one week before the experiment. Starvation was used prior to all assays because polyphenols extracts were always administered orally (by gavage) using distillated water as vehicle. Oxidative stress was induced in experimental albino mice by keeping them in special lighting conditions (six hours of daylight and 18 hours of darkness). The administration of plant extracts to mice began seven days before inducing oxidative stress. All the pharmacological experimental protocols respected European legislation for experimental animals.

Experimental protocol

The mice were randomly divided into 4 groups of 5 animals each, as follows:

- Group 1: Normal control mice treated with 0.9 % NaCl: mice were orally administered 1 mL 0.9 % NaCl, for 21 days.
- Group 2: Normal control mice treated with nettle (*Urtica dioica*) extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.
- Group 3: Control mice group was stressed and treated with 0.9 % NaCl:

mice were orally administered 1 mL 0.9 % NaCl, for 21 days.

- Group 4: Stresed mice were treated with nettle (*Urtica dioica*) extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.

Twenty-four hours following last administration, the animals were sacrificed by cervical dislocation. The abdomen was excised and the liver was removed immediately by dissection, washed in ice-cold isotonic saline and blotted between two filter papers. The liver was transferred into preweighed vials to determine the wet weight. A 10% (w/v) liver homogenates was prepared in ice-cold 0.1 M potassium phosphate buffer, pH 7.5.

Determination of lipid peroxidation

The measurement of liver lipid peroxide by a colorimetric reaction with thiobarbituric acid was done as described by Ohkawa et al. (Ohkawa *et al*., 1979), and the determined lipid peroxide is referred to as malondialdehyde. Briefly, in a test tube, 20% trichloroacetic acid solution and 0.67% thiobarbituric acid solution were added to the homogenate. The color of thiobarbituric acid pigment was developed in a water bath at 100°C for 20 min. After cooling with tap water to room temperature, 2mL *n*-butanol was added and shaken vigorously. After centrifugation, the color of butanol layer was measured at λ_{max} 532 nm. The TBARS concentration of the sample was calculated using the extinction coefficient of MDA (1.56×10^5 $M^{-1}cm^{-1}$) and the values were expressed as nmol/mg protein.

Determination of superoxide dismutase activity (SOD)

The activity of superoxide dismutase in liver was measured using a commercial kit (Fluka analytical). This method uses xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4- iodophenyl)- 3- (4- nitrophenol)- 5- (2, 4- disulfophenyl)-2H-tetrazolium, monosodium salt to form a water soluble formazan dye. The values are expressed as Units/mg of protein in liver tissue.

Determination of catalase activity (CAT)

Catalase activity was measured by the method described by Aebi (Aebi, 1984). Supernatant was added to cuvette containing 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as Units/mg of protein.

Determination of glutathione peroxidase activity (GPx)

The method is based on the oxidation of gluthatione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate, Reduced) (Gupta and Baquer, 1998). Final reaction mixture (3 ml) was 65 mM KH_2PO_4/K_2HPO_4, pH 7.5, 2 mM GSH, 1 U glutathione reductase, 0.12 mM NADPH and 8 mM tert buthyl hydroperoxide. Activity of glutathione peroxidase was expressed as Units/mg of protein.

Determination of liver reduced glutathione (GSH)

Reduced glutathione in liver was determined by the method of Jollow *et al.* (Jollow *et al.*, 1974). An aliquot of liver homogenate (10% in 0.1M phosphate buffer) was precipitated with sulfosalicylic acid (4%). The samples were kept at 4°C for 1h and then subjected to centrifugation at 4000 rpm for 15 min at 4°C. The assay mixture contained 0.1 mL aliquot from the supernatant, 0.1M phosphate buffer (pH 7.4) and dithionitrobenzene (DTNB) in a total volume of 3.0 ml. The optical density of the yellow color developed was read immediately at 412 nm in a spectrophotometer. GSH was expressed as mg / 100 g tissue using a GSH standard curve.

Determination of total proteins

Total proteins were determined according to the method of Lowry, using bovin seric albumin (BSA) as a standard (Lowry *et al.*, 1951).

Statistical data interpretation

Statistical data interpretations were calculated with EXCEL program from Microsoft Office package. All the data are shown as mean value ± standard deviation (SD). Number of mice per group n = 5. Statistical data interpretation considered the corresponding differences for a given significance threshold: $p>0.05$ statistically insignificant; $*p<0.05$ statistically significant; $**p<0.01$ strong statistical significance; $***p<0.001$ very strong statistical significance.

RESULTS AND DISCUSSION

Determination of lipid peroxidation

When the antioxidant capacity is insufficient against ROS, lipid peroxidation occurs and TBARS is formed. In the present study, stress induced oxidative damages in liver characterized by increased MDA concentration. TBARS contents of the liver tissue in group 2 was found to significantly decreased as compared to group 1 ($P < 0.01$). Pretreatment of normal mice with nettle (*Urtica dioica*) alcoholic extracts decreased

TBARS level in the liver; however the polyphenolic treatment in stressed mice (G4) have the same effects on TBARS in this tissue (Fig. 1).

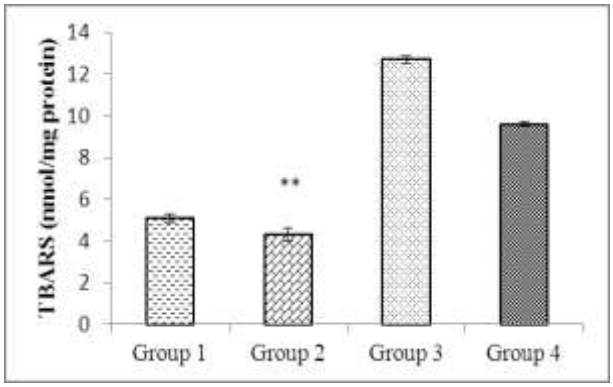

Fig. 1. The influence of oral administration of plant polyphenols (100 mg/kg) on lipid peroxidation in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. ** $p < 0.01$ vs 1st mice group.

Determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities

SOD, GPx, CAT, GST and G6DH activities of liver mice in the experimental groups are given in Figure 2, 3 and 4. When stressed mice were treated with nettle (*Urtica dioica*) extracts, SOD, CAT and GPx activities were found to be increased from those of the group 3 (G3).

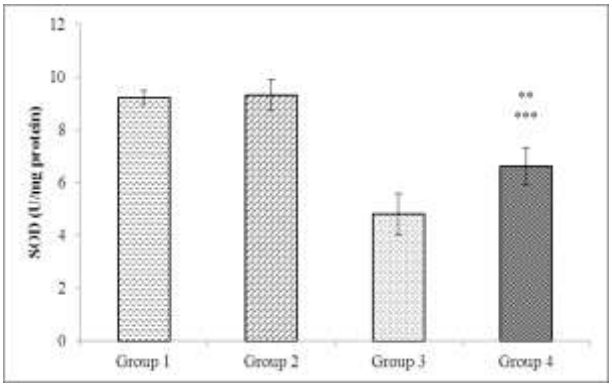

Fig. 2. The influence of oral administration of plant polyphenols on superoxide dismutase (SOD) activities. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 2nd mice group and ** $p < 0.01$ vs 3rd mice group.

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of O_2•– (Crivineanu M., *et al.* 2010). In the present study, nettle (*Urtica dioica*) extract administration caused a very strong significant decreased ($p<0.001$) of SOD activities in liver, when it is compared with G2, and a strong significant increase ($p<0.01$) of liver SOD activity when it is compared with G3.

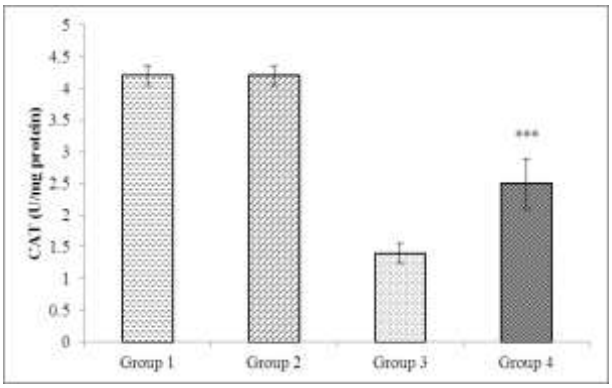

Fig. 3. The influence of oral administration of plant polyphenols on catalase (CAT) activities. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 3rd mice group.

In the present study, the very strong significant increase ($p<0.01$) of CAT activity was observed in liver stressed mice treated with nettle polyphenolic extract. Also, strong significant decrease of liver GPx activity was observed in stressed mice treated with nettle polyphenolic extracts (G4).

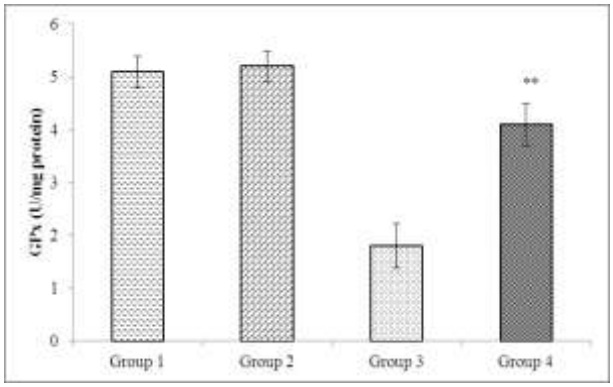

Fig. 4. The influence of oral administration of plant polyphenols on glutathione peroxidase (GPx) activities. Data are expressed as mean ± S.D. Number of mice per group n = 5. ** $p < 0.01$ vs 2nd mice group.

Determination of liver reduced glutathione (GSH)

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (GPx) (Verma N. and Khosa R.L., 2009). Decreased level of GSH is associated with an enhanced lipid peroxidation (Papuc C. et al., 2010). Administration of *Urtica dioica* very strong significantly ($P<0.001$) increased the level of GPx in group 4 as compared with group 3. The significant ($p<0.01$) reduction in the liver non enzymatic antioxidant system (GSH) in stressed mice (G4) as compared to the control unstressed treated group (G2) could be responsible for increased lipid peroxidation levels observed during oxidative stress induced. Olso, GSH concentration for stressed mice group (G3) was significantly ($p < 0.001$) decreased when it is compared with G1 (Fig. 5).

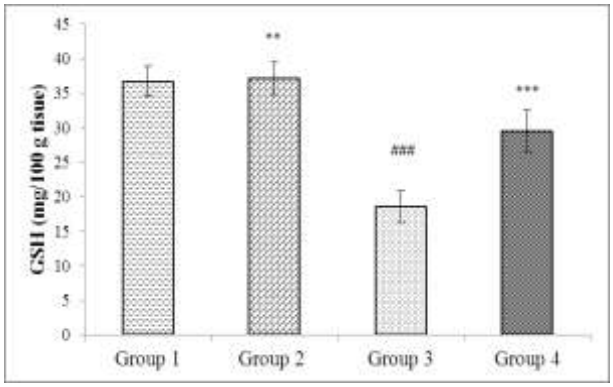

Fig. 5. The influence of oral administration of plant polyphenols on GSH levels in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 3rd mice group; ### $p < 0.001$ vs 1st mice group; ** $p < 0.01$ vs 4th mice group.

CONCLUSIONS

Polyphenols supplementation to stressed mice decreased the level of TBARS, compared to control group.

The treatment of stressed mice with plant polyphenols in quantity of 100 mg/kg significantly improved the levels of SOD, CAT and GPx activities in liver homogenates.

Polyphenols extracted from *Urtica dioica* improved the levels of GSH in

stressed mice liver homogenates when compared with stressed untreated mice.

REFERENCES

Aebi H., 1984, Catalase in vitro, Methods Enzymol, 105, 121-6.

Crivineanu Maria, Camelia Papuc, D. Crînganu, Corina Durdun, V. Nicorescu, 2010, The effect of polyphenolic extracts upon some haematological and biochemical blood parameters in rats with ascitogenous hepatic tumors. *Scientific Works C Series Veterinary Medicine*, LVI (3-4), ISSN 1222-5304, pp. 58-63.

Emeny R.T., Lawrence A. D. 2007, Psychoneuroimmunology, 4th ed., Elsevier.

Gupta BL, M. Azam, N.Z. Baquer, 1998, Changes in erythrocyte glutathione peroxidase and glutathione reductase in alloxan diabetes. Biochem Int; 21(4):725-31.

Jollow, D.J., J.R. Mitchell, N. Zampaglione, J.R. Gillete, 1974, Bromobenzene induced liver necrosis: Protective role of glutathione and evidence of 3, 4 - bromobenzene oxide as the hepatotoxic intermediate. Pharmacol. 11, 151-169.

Lakshmi B.V.S., Sudhakar M., 2009, Screening of Psidium guajava Leaf Extracts for Antistress Activity in Different Experimental Animal Models. Pharmacognosy Research, 1(6), 359-366.

Lowry, OH, N.J. Rosenbrough, A.L. Farr, R.J. Randall, 1951, Protein measurement with Folin phenol reagent. J. Biol. Chem., 193, 265–75.

Ohkawa, H, N. Ohishi, K. Yagi, 1979, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95, 351-358.

Papuc Camelia, Nicoleta Durdun, Valentin Nicorescu, Costin Papuc, Delia Crivineanu, 2010, The effect of sea buckthorn polyphenols upon discoloration and lipid peroxidation of pork and beef ground meat during refrigeration. *Scientific Works C Series Veterinary Medicine*, LVI (2), ISSN 1222-5304, pp. 136-144.

Rai D., Gitika Bhatia G., Sen T., Palit G., 2003, Anti-stress Effects of Ginkgo biloba and Panax ginseng: a comparative study. Journal of Pharmacological Sciences, 93, 458 – 464.

Ravindran R., Sheela Devi R., Samson J., Senthilvelan M., 2005, Noise-Stress-Induced Brain Neurotransmitter Changes and the Effect of Ocimum sanctum (Linn) Treatment in Albino Rats. Journal of Pharmacological Sciences, 98, 354 – 360.

Verma N., Khosa R.L, 2009, Effect of Costus speciosus and Wedelia chinensis on Brain Neurotransmitters and Enzyme Monoamine Oxidase Following Cold Immobilization Stress. Journal of Pharmaceutical Sciences and Research, 1(2), 22-25.

COMPARATIVE RESEARCH ON SPINAL-DORSUM-LUMBAR MORPHOLOGY COMPLEX IN SHEEP AND DOG

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Abstract

Spinal-dorsum-lumbar musculature, located near the thoracic-lumbar spine and ribs, is divided into three longitudinal muscle systems, sideways, intermediate and medial, each of them composed of overlapping muscle bundles. These systems continue in the neck region. The research aimed to demonstrate the special development of this system in carnivores, compared with sheep, rapid movement within the first species being related to the possibility of hyperextension of the rachides.
Key words: *sheep, dog, spinal muscles.*

INTRODUCTION

The muscles in the spinal region form the spinal-dorsum-lumbar muscles, characterized by the extensor role of rachis. It arises by merging metameric muscle material, muscle bundles appearing from multiple segments (1,2,5,6).
Different possibilities for extension and flexion of the rachis correlate with spinal-dorsum-lumbar muscle growth, the reason why we made a comparative study of these at the two species (3,4,7,8).

MATERIALS AND METHODS

Research was conducted on a total of five sheep specimens and five canines, muscles being dissected on successive plans to visibility limit using SMZ 2-T Nikon stereo microscope. Most important elements were photographed. Description and approval formations were performed according N.A.V.-2005.

RESULTS AND DISSUTION

Ilicostal ovine lumbar muscle originates via a tendon on the iliac crest and hip angle, to insert on the last rib, distinct from the lumbar part of the great long dorsum lumbar. Its fleshy portion starts from the fourth lumbar vertebra and is visible under the thorax lumbar fascia, in front of the third lumbar vertebra. Ilicostal thoracic muscle bundles are very tendinous and jump four to six ribs.

The big long lower back muscle is tendinous in its lumbar portion and more muscular in its thoracic portion. Its origin is the median ridge of the sacrum, the iliac crest and ilium angles. The muscle is strengthened with beams that emerge from the lumbar spinous processes and last thoracic vertebrae. It sends medial beams on its ventral face that bundle on articular and mamilare lumbar processes, as the thoracic transverse processes. Other lateral beams catch on the lumbar transverse processes, on the dorsal extremities of the ribs and transverse process of seventh cervical vertebra.

Spinal and semispinal thoracic muscles are connected in the caudal part with own aponeurosisof the great long dorsum lumbar muscle, separating themselves from it in front of the first lumbar vertebras. Spinal fibers result from the spinous processes of the last four thoracic vertebras and first lumbar vertebra, as well as the supraspinatus ligament. Beam paths are nearly horizontal, lateral to the multifida muscle. Cranial, the muscle flattens and receiveshalf-plane bundles, from the transverse processes of the thoracic vertebrae six and seven. Together whit these they enter the cervical territory, to join and insert on the spinous processes of the last four cervical vertebras.

The dog'silicostal lumbar muscle is very well represented. It originates on the endopelvinaface of the ilium wing, on the iliac crest and an intermuscular septum that separates the lumbar part of the great long dorsum lumbar muscle. It inserts himself through some reduced cogging on the last four ribs. The thoracic portion is represented by a long, narrow fleshy mass that attaches to the ribs, except the first and last rib, reaching the transverse process of the last cervical vertebra. Its beams jump over 4-5 ribs. The great long dorsum lumbar muscle is covered with a very strong aponeurosis, separated from the thoracic lumbar fascia by adipose tissue. His beams origin on the iliac crest, on the endopelviana face of the iliac palette and on the lumbar spinous processes. The muscle send medial directed beams, from the ilium and the intermuscular septum, which separates it from the lumbar ilicostal muscle. These beams cover the lumbar transverse processes roots and ends on processes accessories, from the sixth thoracic vertebra to the first lumbar vertebra. The great long dorsum lumbar muscle's thoracic portion shows cogging that catch the tail endsof the ribs through widened and bifurcated tendon slides. Medial tendons are attached to the thirteen to sixth thoracic vertebrae accessories processes and fifth vertebra, which is missing the accessories processes, they catch the transverse processes, in their caudal part. Lateral tendons insert on the thirteen to sixth rib, right next to their tubers. Cranial to the sixth rib the muscle becomes so narrow that the tendons do not appear divided, catching them directly on the ribs and the transverse processes of the vertebrae.

CONCLUSIONS

Compared with sheep, the dog's spinal-dorsum-lumbar muscles are better represented, with a better represented insertion base.

At neither of these two species we cannot speak of a genuine "common ground" because of ilio-costal-lumbar muscle independence.

Development of the spinal-dorsum-lumbar complex is correlated with functional ability of the dog to move quickly by allowing hyperextension on the rachis.

REFERENCES

Cornilă, N. – Morfologia microscopică a animalelor domestice, vol I-II, Ed. ALL, Bucureşti, 2000-2001.

Cornilă, N.; Manolescu, N. – Structura şi ultrastructura organelor la animalele domestice. Ed. Ceres, Bucureşti, 1995.

Coţofan, V. – Anatomia topografică a animalelor domestice, vol. I – II. Lito. AMD – Inst. Agronomic Iaşi, 1975.

De Lahunta, A.; Habel, E.R. – Applied Veterinary Anatomy, W.B. Saunders comp., 1986.

Dornescu, G.T.; Olga Necrasov – Anatomia comparată a vertebratelor, vol. I. Ed. didactică şi pedagogică, Bucureşti, 1968.

Gheţie, V., Hillebrand, A. – Anatomia animalelor domestice, vol. I – Aparatul locomotor. Ed. Academiei R.S.R. Bucureşti, 1971.

Predoi, G.; Belu, C. – Anatomia animalelor domestice, Anatomie clinică, Ed. ALL, Bucureşti, 2001

MORPHOFUNCTIONAL CORRELATIONS OF THE FOREARM MUSCLE AT SHEEP AND DOG

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Abstract

At both ovines and canines, the carpal, metacarpophalangeal and interphalangeal joints are predominantly organized in order for flexion and extension to be possible.cSince these movements are made in the same way for all joints mentioned, extensor muscles are grouped on the back and flexor muscles are on the palmar. At dogs, supinator muscles are framed in terms of topography, in extensor, dorsal muscle group and the pronator muscle in flexor, palmar muscle group.

Key words: muscle, forearm, sheep, dog

INTRODUCTION

Muscles found in the forearm region have large variations in domestic mammals,which corresponds to the specialization and diversification of the fingers numerical differences (1,2,5). At dogs, there are well represented pronator and supinator muscles, responsible for mobilization possibilities of autopodium in this species (3,4,6,7). This paper aims to detail the last two groups, correlating their morphology with specific functions.

MATERIALS AND METHODS

Research was conducted on a total of 5 sheep and 5 canine; muscles were dissected on successive plans untill reaching visibility limit, using Nikon's SMZ2-T Stereomicroscope. They photographed the most important elements. Description and formations approval was made according to N.A.V. - 2005.

RESULTS AND DISSUTION

At canines, on the cranial side of the forearm the brahio-radial muscle is sometimes,very reduced. It is accompanying the dorso-medial edge of extensor carpo-radial, from epicondyle ridge to the distal part of radius's medial edge.

Carpo-radial expander, which is very bulky in sheep, at dogs has a division tendency.

At canines the common digital extensor muscle divides his tendon in four branches who are distributed to distal phalanges of fingers II-V. In sheep, the muscle has two components: one medial, whose tendon ends on finger III and one on the side, with the tendons that reach the distal phalanges of the fingers III and IV.

Lateral digital extensor tendon in sheep ends at the finger four, while the dog is divided, with branches to finger IV and V.

If at sheep, the round pronator muscle is the superficial bundle of the medial collateral ligament and has some fleshy fibers interlaced with fibrous tissue, in dogs,the round pronator muscle is developed. It is thick, spindle-shaped, palpable under the skin and fascia on the medial forearm and elbow joint proximal third of the radius, which ends in a tendon blade. In addition to that,on the dog's cranial side there is the supinator muscle, caught between extensor muscles. It starts with a strong flat tendon that inserts on humerus, together with the lateral collateral ligament of the elbow. Fleshy body, flattened, covered by a thin aponeurosis that covers the proximal quarter of the dorsal side and medial edge of radius.

Also the dog has the square pronator muscle, which extends along the full length of the forearm palm face. Its fibers are oriented obliquely from the interosseous edge of the ulna on the palmar side, near the medial edge radius.

Carpo-ulnar extensor muscle at sheep is moved caudally and serves as autopodium`s flexor.

Superficial and deep digital flexors topography corresponds to the two species, except that tendons are divided into branches which correspond to the number of fingers

Fig. 1 Sheep forearm muscles - lateral aspect
1 carpo-radial extensor; 2-finger extensor digital media; 3-common digital extensor finger III and IV; 4-digital extensor lateral; 5-carpo-ulnar extensor; 6-carpo-ulnar flexor; 7-Superficial digital flexor; 8-digital flexor deep humeral portion; 9-flexor carpo-radial.

Fig. 2 Forearm muscles in sheep - medial aspect
1 carpo-radial extensor, 2 - pronator round, 3 - carpo-radial flexor, 4 - digital extensor side; 5 - carpo-ulnar extensor, 6 - carpo-ulnar flexor; 7 - superficial digital flexor, 8 - digital flexor deep humeral portion 9 - carpo-radial flexor.

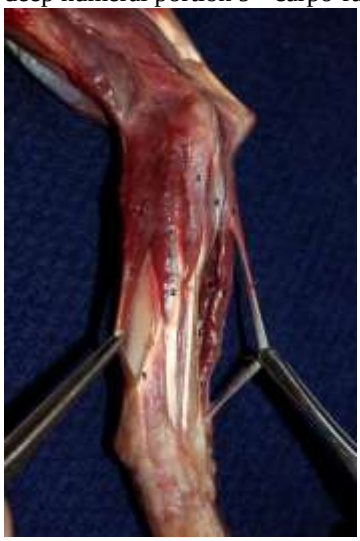

Fig. 3 Forearm muscles in dogs - lateral aspect
1 - carpo-radial extensor, 2 - common digital extensor, 3 - lateral digital extensor, 4 - carpo-ulnar extensor, 5 - policis abductor longus, 6 - humeral portion of carpo-ulnar flexor; 7 - ulnar portion of the flexor carpo-ulnar, 8 - deep digital flexor, humeral portion.

Fig. 4 Forearm muscles in dogs - medial aspect
1 - carpo-radial extensor, 2 - pronator round, 3 - square pronator, 4 - superficial digital flexor; 5 - carpo-radial flexor, 6 - carpo-ulnar flexor, portion of humerus; 7 - digital flexor deep humeral portion, 8 - digital flexor deep radial portion.

CONCLUSIONS

Both at sheeps and in dogs the forearm muscles do not cover the radius`s medial edge ,which can be palpated under the skin.
The largest portions of venters muscles are located in the proximal half of the forearm, the distal third of the forearm, most of the muscles being represented by tendons.
Supinator muscle contractions cause radius rotation around an longitudinal axis, while ulna remains fixed;
Forearm muscles that control the movements of pronation and supination are: supinator muscle, quadrate pronator muscle and round pronator muscle whose morphological topography was described above.

REFERENCES

Cornilă, N. – Morfologia microscopică a animalelor domestice, vol I-II, Ed. ALL, Bucureşti, 2000-2001.

Cornilă, N.; Manolescu, N. – Structura şi ultrastructura organelor la animalele domestice. Ed. Ceres, Bucureşti, 1995.
Coţofan, V. – Anatomia topografică a animalelor domestice, vol. I – II. Lito. AMD – Inst. Agronomic Iaşi, 1975.
De Lahunta, A.; Habel, E.R. – Applied Veterinary Anatomy, W.B. Saunders comp., 1986.
Dornescu, G.T.; Olga Necrasov – Anatomia comparată a vertebratelor, vol. I. Ed. didactică şi pedagogică, Bucureşti, 1968.
Predoi, G.; Belu, C. – Anatomia animalelor domestice, Anatomie clinică, Ed. ALL, Bucureşti, 2001
Sisson, S.; Grossman, J.D.; Getty, R.- The Anatomy of the Domestic Animals, vol. II. W.B. Saunders Comp., Philadelphia – London – Toronto, 1975.

STUDIES ON HISTOLOGICAL STRUCTURES OF THE ABDOMEN ON ADULT WORKER BEES (APIS MELLIFERA CARPATHICA)

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Abstract
In an initial phase, the studies aimed possibilities of including chitinous anatomical segments by using different fixing solutions in order to produce chitin lysis to facilitate cutting them. Various fixing solutions have been used (glacial acetic acid, Carnoy fixative, trichloroacetic acid, picric acid, acetone, absolute alcohol, Bouin's fixative, etc.) with variable terms in determining the degree of chitin lysis, which proved to be inefficient, the parts included being improper to sectioning (cutting friability and lack of integrity in tissue resulting from staining). In a later stage, they proceeded to a careful dissection, under the microscope, of the anatomical organs of the abdominal cavity in order to dissociate the anatomical segments present at this level to prepare it for inclusion. Processing of samples was also performed under the microscope until their inclusion in paraffin.
Detailed histological examination of tissue and cellular structures, captures the structural aspects at a time. The serial sections revealed the histological structure of several organs located in the abdominal cavity. This positioning of organs in sections led to their identification, facilitating their microscopic interpretation.

Key words: worker bees, abdominal cavity, histological structures.

INTRODUCTION

Bees are very little known at the microscopic and molecular level, despite the fact that long have been subject to studies of social behavior (Winston, 1991).
The first data on bee anatomy has a considerable experience, one of the works on this topic being the drawing of the italian microscopist Francesco Stella. It was the bee's anatomy as was revealed under the microscope, sketch published in 1625 (Bazin et all., 1976).
Later, with the development of the microscopic techniques the knowledge about the insects and about the bees were also improved, with the disadvantages related to the chitinous exoskeleton, preventing proper sectioning insects embedded in paraffin (Graham, J. M., 1992).
Lately they started electron microscopy examination of the various structures, with a higher resolution (Dade, 1977). They brought major

improvements such as knowledge of anatomy and physiology of bees (Goodman, 2003).

MATERIALS AND METHODS

The samples collected were fixed in formol saline, were then prepared for inclusion in paraffin and then sectioned in series in order to capture the histological structure of organs in the abdominal cavity.
By histological examination after sampling, fixed in neutral formalin saline and further processed for inclusion in paraffin. Paraffin blocks were sectioned at 6 μm, stained preparations were obtained by HE and the trichromic method of Mallory, examined and microphotographied.

RESULTS AND DISCUSSION

The serial sections revealed the histological structure of several organs located in the abdominal cavity. This positioning of organs in sections led to their identification, facilitating their microscopic interpretation.

Venom glands (*venom sac*) appear obvious, bulky-looking tube. Gland lumen cross-sectional appear anfractuos and filled with secretions.
Microscopic structure of this type of gland cross-sectioned reveals the presence of two histostructural components (fig. 1):

- the epithelial component with secretory role;
- muscle component.

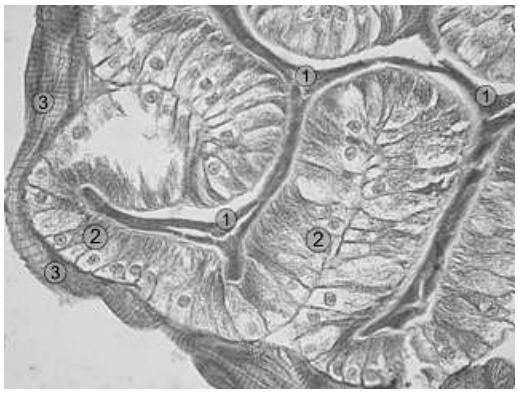

Fig. 1 - Venom gland (cross section);
Col. HE; Ob. 40x
1. lumen of gland; 2. epithelial layer;
3. muscular layer.

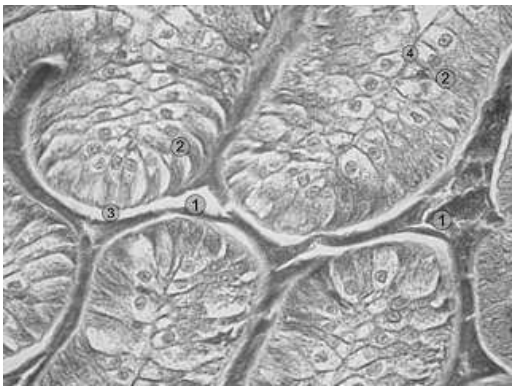

Fig. 2 - Venom gland (cross section);
Col. HE; Ob. 100x
1. lumen of gland; 2. prismatic cells;
3. apical pole; 4. basal pole.

The epithelial component consists of simple columnar epithelium (prismatic) located on an evident basement membrane. The secretory epithelial cells have prismatic appearance, showing a high pole located on the basal membrane and an apical pole filled with secretory vacuoles. The nucleus is spherical and located in the lower third of the cell showing a clear nucleolus. The overall secretory cells form numerous folds in the glandular lumen that gives an anfractuos appearance (fig. 2).

The ovary is presented as a devolved body, consisting of capsule conjunctiva located on the periphery of the organ and internally organized as individual mass of germ cells. Between germ cells and capsule a perigerminative space is defined (fig. 3).

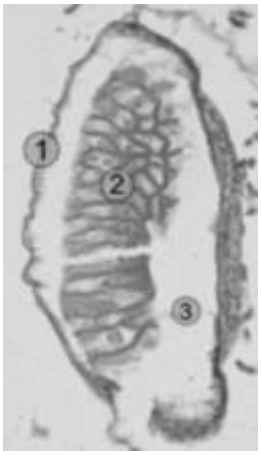

Fig. 3 - Ovary; Col. HE; Ob. 20x
1. cuticle; 2. germ cells; 3. perigerminative space.

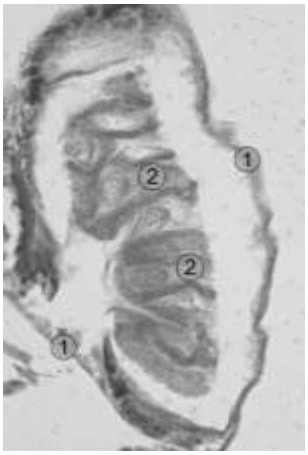

Fig. 4 - Ovary; Col. HE; Ob. 40x
1 - cuticle; 2 - germ cells.

Detailed examination (Ob. 40x) shows an evident capsule consisting of collagen fibers arranged in beams, arranged concentrically around the atrophied ovary (fig. 4).

Cubic form germ cells with a spherical nucleus centrally located in the mass of germ cells appear surrounded by stroma separating ovarioles between them.

The goiter (*honey stomach*) is a big hollow section, which presents particular external peripheral muscle structure composed of muscle fibers arranged in two concentric layers of longitudinal and transverse layout.

To internal lumen epithelial cells are disposed at different heights showing apical plasmalemma changes in appearance of cytoplasmic veils (fig. 5).

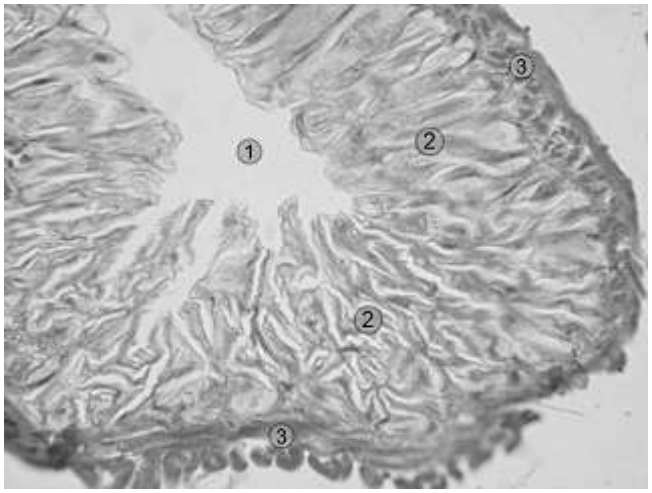

Fig. 5 - Honey stomach; Col. Mallory trichromic; Ob. 40x
1. lumen; 2. epithelial cells, 3. muscular layer.

High waist epithelial cells have a nucleus with an obvious nucleolus, not so intense in activity in terms of protein synthesis, with role in secreting some products needed for honey maturation produced for regurgitation. Sounds like a storage tank and processing honey to be removed later by the concerted action of the muscle layer, highly developed than other hollow organs.

The intestine has a totally different histological architecture than honey stomach. The epithelium is evident with higher cellularity compared to honey stomach, while the peripheral muscular structure is much finer (fig. 6).

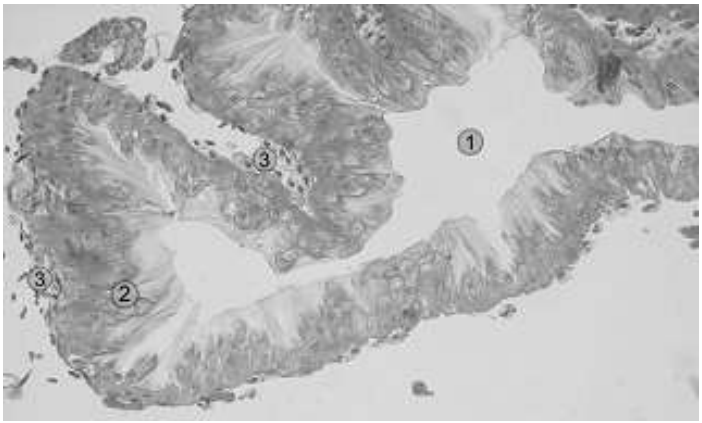

Fig. 6 - Intestine (longitudinal section); Col. Mallory trichromic; Ob. 10x
1. lumen; 2. epithelial cells, 3. muscular layer.

Detailed examination (Ob. 20x; 40x) observed similar characteristics to honey stomach cells in terms of changes in the apical pole plasma;the exquisite muscle layer consists of an orderly succession of muscle fibers arranged longitudinally (fig. 7).

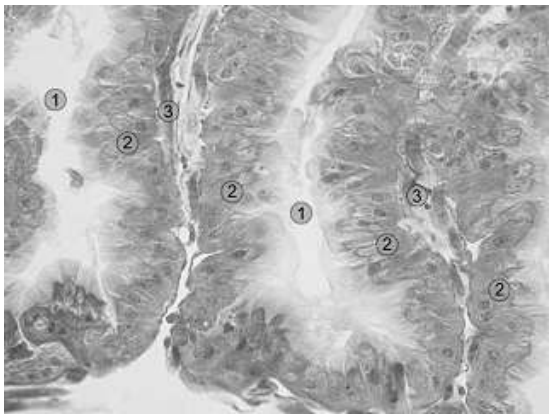

Fig. 7 - Intestine (longitudinal section); Col. Mallory trichromic; Ob. 20x
1. lumen; 2. epithelial cells, 3. muscular layer.

Highlighting arrangement and intercellular relationships in the epithelium is shown by fig. 8 in which intestinal cells have a uniform arrangement on the basal membrane. It can be said that there are basal cells (with regenerative role) and proper intestinal cells. Plasma changes appear obvious on the apical pole and also an intensely colored area which may be similar to ,,corrugated plate" with a role of increasing power reabsorption at this level.

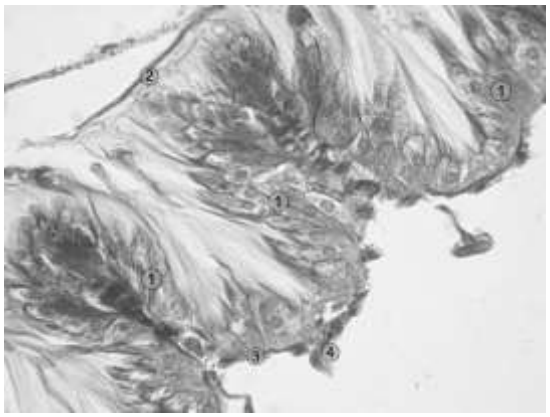

Fig. 8 - Intestine (longitudinal section); Col. Mallory trichromic; Ob. 40x
1 - intestinal cells, 2 - apical pole, 3 - basal pole, 4 - muscle cells.

CONCLUSIONS

Histostructural characterization and early identification of abdominal hollow organs, in conjunction with topographic arrangement have been made.
These organs are the main sections in correlation with hemo-lymphatic compartment, responsible, probably in the process of activation of humoral and cellular immune mechanisms in the haemolymph.
In the future it is intended to increase the accuracy of the characterization and identification of abdominal organs in working bee by enzymatic and histochemical research.
As will be identifying histological bodies will be able to make correlations with various pathological conditions (deficiency, parasitic, bacterial, etc) present in bee populations and relevant in terms of histopathological examination.

REFERENCES

Bazin, B., Kümmel, G., Zerbst, Boroffka, 1976, Studies on the rectal pads of the honey bee Apis mellifera, Naturforsch, 31 (7-8), pp. 489-490.
Dade, H.A., 1977, Anatomy and dissection of the honey bee, International Bee Research Association, London, pp. 158.
Goodman, L.J., 2003, Form and function in the honey bee, International Bee Research Association, Cardiff, pp. 220.
Graham, J. M., 1992, The Hive and the Honey Bee, Revised Ed. Dadant & Sons, Hamilton Illinois, U.S.A.
Winston, Mark, L., 1991, The biology of the honey bee, First Harvard University Press paperback edition, pp. 13-45; pp. 181-198.

THE OPTIMIZATION OF HISTOLOGICAL TEHNIQUES FOR ANATOMICAL PIECES GATHERED FROM BEES

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Abstract.

In order to identify structural modifications produced on the intestinal epithelium of the bee, consecutive with sporozoa Nosema spp parasitism, examination studies have been performed for fixing and histological coloration steps of anatomical pieces gathered from medium and posterior intestine of these species.

Keywords: intestine, histological techniques, medium and posterior bees, Nosema spp.

INTRODUCTION

The purpose of this study consists in optimization of fixing and coloration histological steps of anatomical pieces gathered from medium and posterior intestine of a bee and identification of structural modifications produced at the level of intestinal epithelium parasited by Nosema spp.

The Nosema is a parasitic disease, produced by Nosema spp. a protozoa located in Microsporidium order, which affects digestive tracts of bees.

The disease affects adult bees and is very contagious, having temporal character, mostly the end of the year, winter and spring. The Nosema is conditioned by adjuvant factors represented by: weak families, long winter without cleaning flies, mana honey, adding lot of flours in food and increased humidity in hive. Only the laboratory examinations certifify the presence of disease.

When life conditions are not favorable, when the parasite is eliminated on the external medium once with excrements of bees or when the parasite dies, it sporulates, the form in which he is stronger and resistent. From that moment, by different causes, spores comes at intestinal level of the bee, germinate and produces the active parasite, that enters in the intestinal cell

wall level, feeds, reproduces and produces toxins. The dissemination of disease from one family to another and from one hive to another is realised through the apicultor, bees, and parasites like polish moth.

MATERIALS AND METHODS

The probes have been sampled at the beginning of the active season, the period April – May, before applications with anti-parasitic treatments.
Probes (digestive system) have been sampled from healthy bees, unparasited by Nosema spp, from two Apiary noted with A and B (forming control group I experimental), with the purpose of optimization histological techniques.
For identification of existent modified structures at intestinal tissue level, lot II has been created with live bees, and parasitized with Nosema spp gathered from two Apiary studied.
The technique of intestine sampling consists in catching with mini pliers with a sharp head of the last abdominal tergit and easy shooting of this, through horizontal move.
Sampling of digestive system from live bees, euthanatized with chloroform in closed spaces and fixing anatomical pieces have been made in side the Reference Laboratory for Bees Diseases in the building of IDSA (fig. 1).

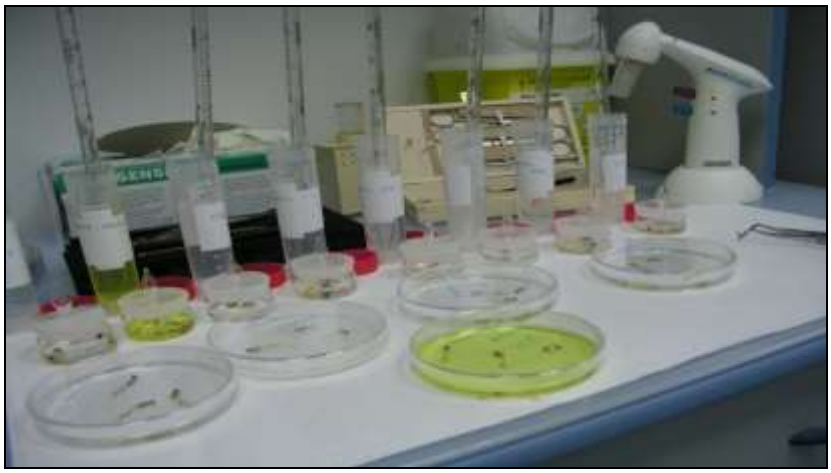

Fig. 1 Digestive system sampling and anatomical pieces fixation in different usual and specific liquids (original)

In obtaining methods for microscopical preparation more successive steps have been implicated: fixing (formalin – 5%, 10%, 20%, neutral formalin, acid formalin, mix Carnoy, mix Dubosq – Brasil, mix Lillie – for each lot studied), including them in paraffin, microtomia and pasting of sections, unparaffining and hydrating, and finally coloration (technique Masson modified, known as coloration tricromical), dehydrating, clarification and sections mounting. The tricromical coloration needs: latinium water, methyl blue (aqua sol. 0,5%), eosin (aqua sol. 1%), Mayer hematoxylin (alcoholic sol.).
The efficiency for every kind of fixating has been appreciated for volumes about ×100; ×200; ×1000 and ×1200.

RESULTS AND DISCUSSIONS

The appreciation of the best fixator has been made by:

a. Conserving capacity of general tissue structure and evidence of topography of digestive system in bees.
b. Cell integration keeping capacity for specifically analyzed tissues.
c. The capacity of cellular components evidence.
d. The capacity of chemical unalteration of the core and basal membrane.
e. The capacity of unalteration tissue reactivity opposite colorant solutions.

For this, a value scale has been made, with absolute natural numbers, from 0 to 5 in which every morphological section examined has been situated. Using large numbers of fixing liquids, *usual* and *specials*, have permitted the evaluation of quality efficiency in report with specifically tissue substrate of the bees, and on the other side selection of the most adequate indicate fixing to be used in terrain conditions for sampling and fixing probes necessary for histopathological diagnostic. In table number 1 histological sections examined have been situated through anterior criteria, and in table number 2 percentage values of chemical efficiency have been showed, specific at 8 usual and special fixating liquids, seeing the scale with absolute values from 0 to 5 (table 1 and 2).

Table nr. 1 Valorical fit of histological sections obtained from anatomical pieces fixated in different usual and specific liquids

Liquid fixating	**Total number of morphological sections**	**Scale 0 – 5 (absolute values)**					
		0	**1**	**2**	**3**	**4**	**5**
Formalin 5%	30	0	23	5	2	0	0
Formalin 10%	30	0	23	4	3	0	0
Formalin 20%	30	0	16	7	7	0	0
Neutaal formalin	30	0	17	8	5	0	0
Acid formalin	30	0	6	11	8	4	1
Carnoy	30	0	0	6	15	6	3
Dubosq - Brasil	30	0	0	0	0	9	21
Lillie	30	0	0	0	14	11	5

Table nr. 2 Percentage values of chemical efficiency specifically of 8 fixator liquids

Liquid fixating	**Total number of morphological sections**	**Fixating efficiency (in procents %)**					
		0	**1**	**2**	**3**	**4**	**5**
Formalin 5%	30	0	76,67%	16,67%	6,66%	0	0
Formalin 10%	30	0	76,67%	13,33%	10%	0	0
Formalin 20%	30	0	53,34%	23,33%	23,33%	0	0
Neutral formalin	30	0	56,66%	26,67%	16,67%	0	0
Acid formalin	30	0	20%	36,67%	26,67%	13,33%	3,34%
Carnoy	30	0	0	20%	50%	20%	10%
Dubosq - Brasil	30	0	0	0	0	30%	70%
Lillie	30	0	0	0	46,66%	36,67%	16,67%

From usual fixating liquids, chemically the best is acid formalin which has an efficiency for tissue fixing of 13,33% and 3,34%, in value scale from 4 and 5. The rest of the usual fixating liquids, formalin 5%, formalin 10%, formalin 20% and neutral formalin, didn't override the value step scale 3, for that it will be considered as inefficient.

The inefficiency of liquids presented, can be associated with a weak stable capacity of unalterative conservation for fixated tissues but also because of very brutal chemical activity resulting in tissue disruptions on the core, cytoplasm and/or basal membrane (fig. 2, 3, 4, 5)

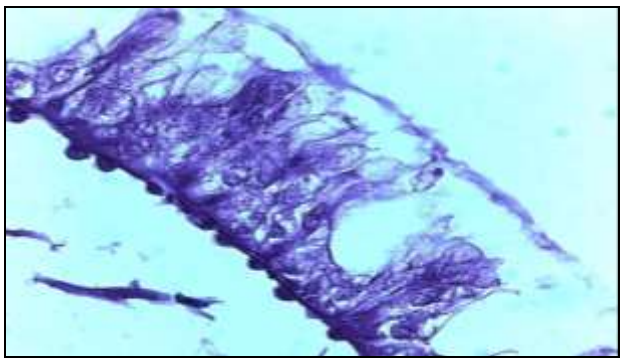

Fig. 2 Vacuolations, with core-cytoplasmic wrecks on epithelial cells of small intestine, adult bee; Method Masson modified, with fixing in neutral formalin x 900 (original)

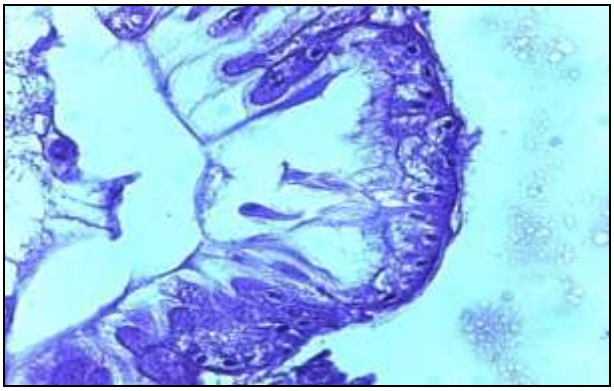

Fig. 3. Subtotal wrecks of epithelial intestine. Small intestine, adult bee. Method Masson modified, with fixing in neutral formalin x 1200 (original)

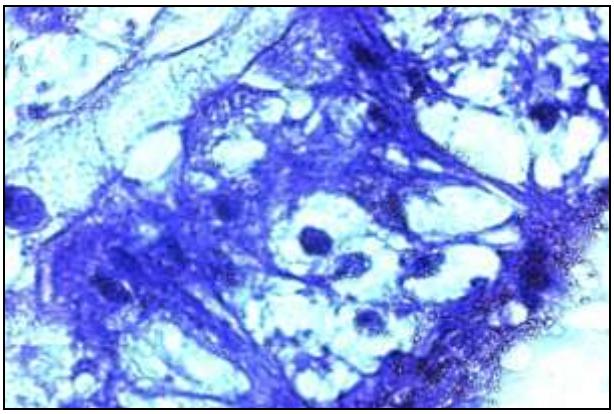

Fig. 4 Vacuolations and cytoplasmic aggregation, karyolysis with dilaceration on basale membrane. Small intestine, adult bee. Method Masson modified, with fixing in formalin 10%, x 1000 (original)

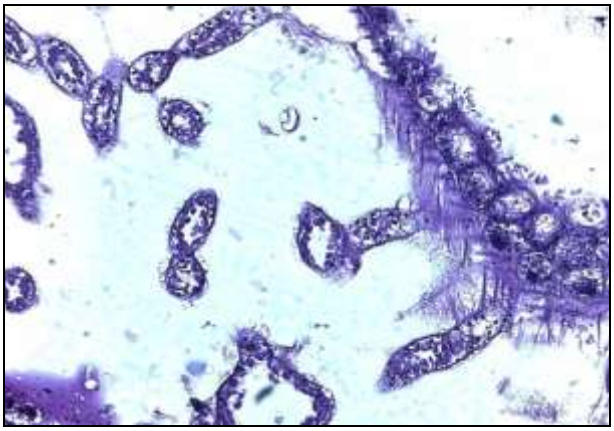

Fig. 5 Vacuolations and cells wrecks of intestine epithelium on Malpighi's tubes. Small intestine, adult bee. Method Masson modified, with fixing in licquid formalin 20% , x 150 (original)

In case of acid formalin the quality of fixing allowed us to observe one result at the limit of accessibility seeing evidence of topography medium intestine tissue at the place of insertion of Malpighi's tubes (fig. 6).

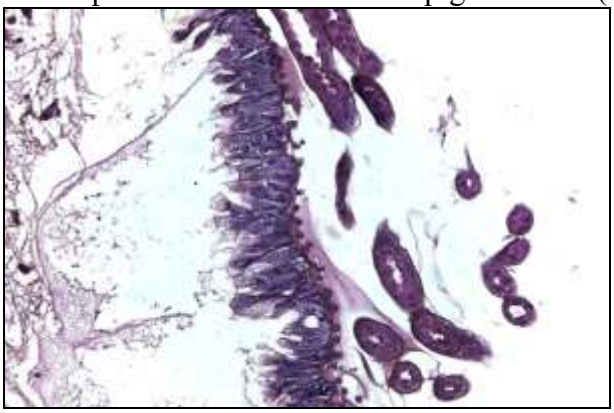

Fig. 6 Intracytoplasic modifications, vacuolation type of epithelial cells. Small intestine, adult bee. Modified Masson method, with fixing in formalin acid, x 400 (original)

From *special* fixating liquids: Carnoy, Dubosq-Brasil and Lillie, *Dubosq-Brasil* (fig. 7) have been more efficient by far: 70% maximum step 5 at values series 0 – 5, beside 16,67 % fixating Lillie (fig. 8) and only 10 % fixating Carnoy (fig. 9).

The fixating Dubosq-Brasil, with picric acid and one small proportion of glacial acethic acid – formaldehyde acid 37% by 1/60 in alcoholic solution has been proved to have a good and rapid permeability in digestive tissue of

adult bees. In the same time this complex fixing, conserved and chemically potentiated the tissues so that treated to realize a reaction "tissue substratum – basics coloring solutions", which allows structural details with an unnoticed grade of alterability.

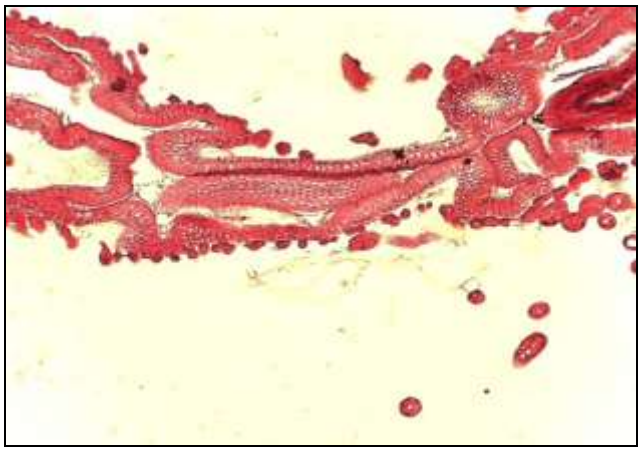

Fig. 7 Medium intestine, adult bee.
Modified Masson method, with fixing in liquid Dubosq-Brasil, x 100 (original)

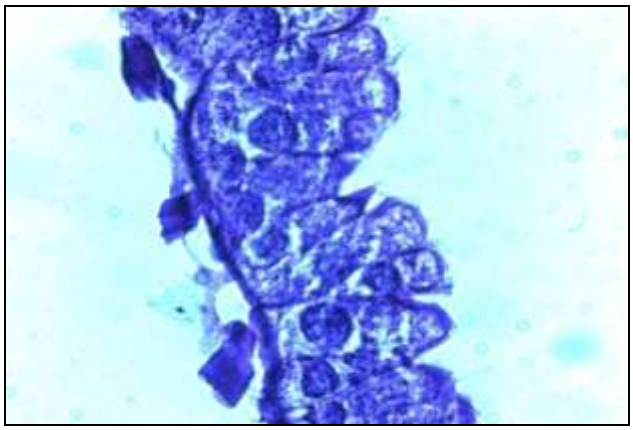

Fig. 8 Granulation modifications on intestine epithelium cytoplasm cells. Small intestine, adult bee. Method Masson modified, with fixing in licquid Lilie x l000 (original)

Fig. 9 Wrecks of parabase and supracores cytoplasm of epithelial cells. Small intestine, adult bee. Method Masson modified, with fixing in licquid Carnoy, x 500 (original)

In conclusion, it can be stated that fixating Dubosq-Brasil fixator is by far the most efficient, which can reveal normal aspects, but also modifications of tissues from the digestive system level of adult bees and others.
In histological samplings obtained from fixed pieces with this special liquid, maximum fidelity was obtained, at all volumes, celullarity of the specific tissue of medium intestine (who delineates through cardiac valve by anterior intestine and through pylorus by posterior intestine) (fig.10).

Fig. 10 Medium intestine (detail), adult bee.
Method Masson modified, with fixing in licquid Dubosq-Brasil, x 200 (original)

The fixating Dubosq-Brasil and general coloration Masson modified clearly revealed the structure of medium intestine and its celullarity (fig. 11).

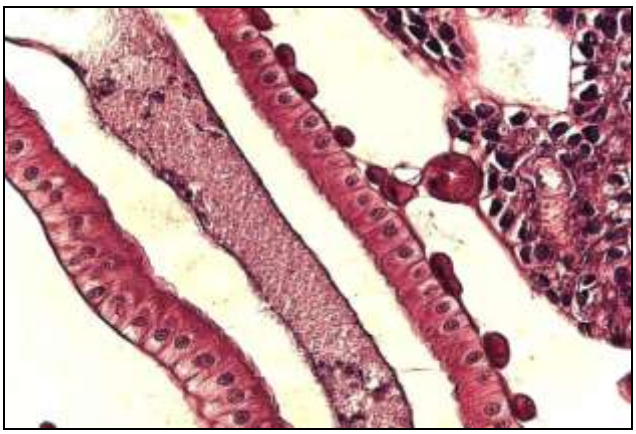

Fig. 11 Medium intestine, adult bee.
Method Masson modified, with fixing in licquid Dubosq-Brasil, x 1000 (original)

Histological preparations obtained from fixated pieces with Dubosq-Brasil, evidenced that the cells form median parts of intestine are cylindrical, have a large base, are strongly unified, and the cores are situated at different pitches of the cells, placed on the inferior half, near the basal membrane. The cores are circle shaped, and karyoplasm presents quick granulation. This structural detail can be observed using NG filter. If the filter is not used, the Masson coloration can't evidence the structural details of the cells.
The same fixator Dubosq-Brasil evidenced normal Malpighi's tubes and characteristics, originally from medium intestine, after passing in pylor at the first higher curbure.
At histological examination preparation, obtained from processed intestine sampled from bees in control group *II*, modifications have been observed in epithelial cell structure of medium intestine (fig. 12).
In one of the histological preparations, fixated in neutral formalin, , microvacuolations have been observed, in apical part of epithelial cells, consecutive with cytoplasm lysis. Acid formalin is one of the fixators with high efficiency grade used by many examiners for permeability of tissue in order to evidence Morrison's corpuscles, in case of using one tricromical colorations which differentiate and evidence cellular elements.

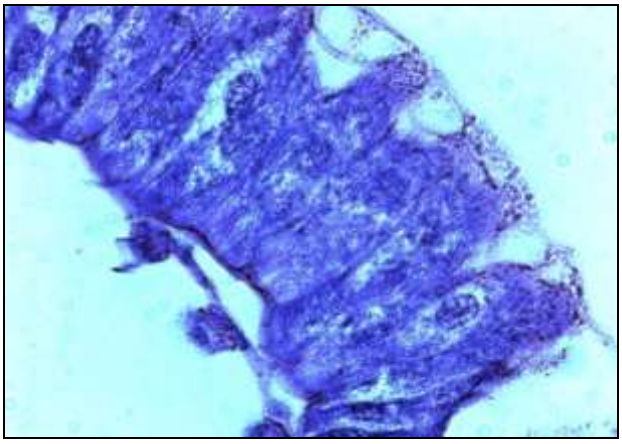

Fig. 12 Microvacuolations, apical lysis cytoplasmic in epithelial cells of small intestine. Method Masson modified, with fixing in acid formalin, x 1000 (original)

Partial wreck on small zones of cytoplasm from basal membrane zone can be easily observed, but also the apical zone from the core. Also on the level of bee's epithelium from the same control group intra-core and intra-cytoplasmic microvacuolizations can be observed, at the same time with apical lysis of epithelial cells.

The fixator with the highest efficiency Dubosq-Brasil and Masson modified coloration evidenced in bees from this lot another kind of histological modification which can be translated through appearance of one proliferative process observed only in medium intestine level of epithelium, where physiologically, the most intense metabolic reactions exist.

In fact, this proliferative process appearss like hyperplasia of intestine epithelium, associated with the appearance of microvacuols in enterocyte level. The cores have an abnormal disposition and karyolysis can be observed. Although epithelium hyperplasia can be observed as a proliferation by conjunctive tissue (fig. 13).

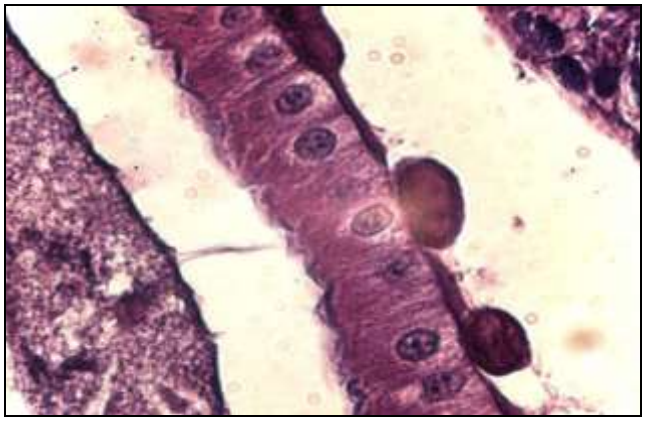

Fig. 13 Medium intestine, cytoplasmic microvacuolations, core base, adult bee (detail). Metoda Masson modificată, cu fixare în lichid Dubosq-Brasil, x 1200, filtru NG (original)

CONCLUSIONS

From usual fixating liquids chemically the best is acid formalin whose fixing efficiency of tissues was 13,33% and 3, 34%, in numerical scale steps 4 and respective 5.

From special fixating liquids, Dubosq-Brasil is the most efficient: 70% at maximum step 5 of valoric series 0 – 5, opposite by 16,67 % fixating Lillie and only 10 % fixating Carnoy.

The fixing Dubosq-Brasil and general modified coloration Masson clearly evidenced the medium intestine structure and cellularity, and also normal structure and characteristics of Malpighi's tubes.

In the process of examination of histological preparations obtained from processed intestine sampled from bees in control group *II* and fixed with usual solutions a lot of modifications have been observed (microvacuolisations, lisys, epithelial cells, etc.) in epithelial cells structure of medium and posterior intestine.

The fixator with the highest efficiency Dubosq-Brasil and modified Masson coloration evidenced in bees from control group another kind of histological modifications which can be translated to existence of a proliferative process observed only in medium intestine epithelium level, where physiologically intense metabolic reactions occurr.

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REFERENCES

Fries I, Feng F, Da Silva A, Slemeda SB, Pieniazek NJ. 32:356–365 (1996). Nosema ceranae n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). Eur J Protistol.

Geoffrey R. Williams, Aaron B.A. Shafer, Richard E.L. Rogers, Dave Shutler, Donald T. Stewart. 97 (2008) 189–192. First detection of Nosema ceranae, a microsporidian parasite of European honey bees (Apis mellifera), in Canada and central USA, Journal of Invertebrate Pathology.

Higes M, Garcia-Palencia P, Martin-Hernandez R, Meana A,. Pathol 94: 211–217. (2007). Experimental infection of Apis mellifera honeybees with Nosema ceranae (Microsporidia). Journal Invertebr).

Jimenez D.R., Gilliam M.,. 261: 431-443.(1990). Ultrastructure of the ventriculus of the honey bee (Apis mellifera L.): cytochemical localization of amid phosphatase, alkaline phosphatase, and nonspecific esterase. Cell Tissue Res.).

Mariano Higes, Raquel Martín-Hernández, Encarna Garrido-Bailón, Amelia V. González-Porto, Pilar García-Palencia, Aranzazu Meana, María J. del Nozal, R. Mayo José L. Bernal. (2009). Honeybee colony collapse due to Nosema ceranae in professional apiaries, Environmental Microbiology Reports.

Scanlon M, Shaw AP, Zhou CJ, Visvesvara GS, Leitch GJ. 47: 525–531. (2000). Infection by microsporidia disrupts the host cell cycle. J Eukaryot Microbiol.

SOME OBSERVATIONS ON EXPERIMENTAL MODEL FOR INDUCING DIABETES IN MICE AND RATS.

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ABSTRACT

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a organism has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). It is known that some dietary behaviors may increase the likelihood in which certain diseases occur both in humans and animals. The unidirectional diet may alter both the metabolism, as well as the level of some blood components (hormones, sugars, lipids etc.), which may be markers for the incidence of some morbid entities. These concepts may be applied to laboratory animals in order to induce metabolic syndromes in experimental conditions similar to the ones seen in humans. Diabetes in animals most commonly affects middle-aged and older animals and is most common in female dogs and male cats. There are two types of diabetes in animals, uncomplicated diabetes and diabetes with ketoacidosis. When diabetes occurs in young animals, it is often genetic and may occur in related animals.
The purpose of the experimental investigations was the possibilities of inducing a hyperglycemic syndrome in mice (C57Bl6 strain) and in rats (Sprague Dawley strain), a syndrome similar to that found in humans after consuming fructose-containing processed foods.Pure substances like casein, maltodextrin, sucrose, fructose, cellulose were used, in two diets: - standard diet according to AIN-93M; - experimental diet consisted in total replacement of the corn starch, of the sucrose and of the maltodextrin with a 60% fructose diet. Compared to the standard diet fed lot, the glucose tolerance was disturbed in the experimental lot after 39 days of feeding with the 60% fructose diet, and glycosuria was detected at female with the two species after 79 days, which may indicate the disturbance of the dietary metabolism, that is characteristic to the hyperglycemic syndrome.

Key words: *diabetes experimental, mice, rats*

INTRODUCTION

In the last decades the demand for sucrose, and more recently for high fructose corn syrup (HFCS) has increased in order for them to be added to juices, jams, jellies, pastry and dairy products. Therefore, if in 1970 the demand for HFCS was an insignificant 0.23 kg per person per year, in 1997

it had reached 28.4 kg (Putnam and Allshouse, 1999). HFCS is cheaper and sweeter than sucrose, it increases the palatability of the products it is included in, thus replacing sucrose in industrial food products, making possible an over-feeding in humans (Yudkin, 1967; Bray et al., 2004). Details of the different types of sweeteners tendencies in the USA are presented in Figure 1 and Figure 2 (Putnam and Allshouse, 1999).

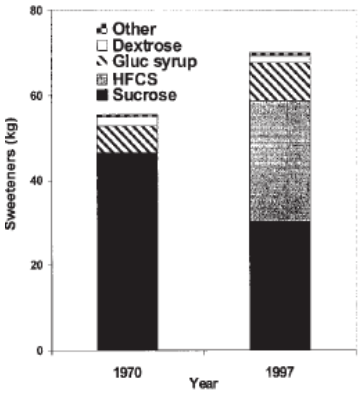

Figure 1. Annual intake per person of sucrose, fructose syrup (HFCS), glucose syrup, dextrose and other sweeteners (bee honey etc.).

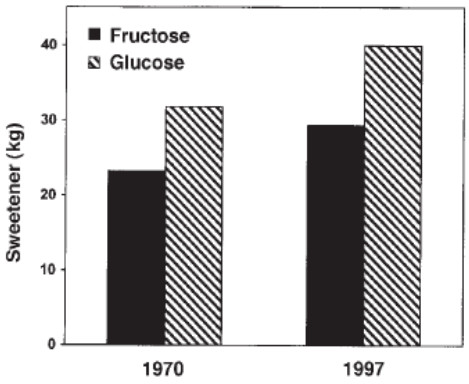

Figure 2. Annual intake per person of fructose and glucose syrup.

In the last decades numerous clinical observation and experiments have gathered that have pointed out that an increased intake of refined sugars, such as HFCS (High Fructose Corn Syrup) and sucrose (a disaccharide formed of fructose and glucose) is associated with arterial hypertension, obesity, diabetes mellitus, kidney and cardio-vascular diseases, both in humans and laboratory rodents.

These harmful effects of fructose excess on health may be attributed to the way fructose is metabolized. The assimilation of monosaccharides, of glucose, galactose and fructose as well of some pentose respectively, implies two mechanisms: *active transport against gradient* and *facilitated transport (passive)*. The greatest absorption capacity is observed in the duodenum and superior jejunum, progressively decreasing in the inferior jejunum and ileum.

Glucose and *galactose* are actively transported thru the microvillous epithelial cell by a transporting protein called SGLT-1. This SGLT-1 protein uses the energy of the Na^+ gradient in the active transport of glucose and galactose. SGLT-1 transports two Na^+ and one molecule of either

glucose or galactose that compete in crossing the brush border of the small intestinal mucosa. The potential difference of Na^+ ions is regulated by the Na^+/K^+/ATP-ase dependent pump that is present in the bazo-lateral membrane of the intestinal epithelium cells.

Glucose and galactose leave the enterocytes by facilitated (passive) transport in the bazo-lateral membrane. The transporting protein responsible with the efflux of glucose and galactose is GLUT-2, this protein being present in the liver, the kidneys and the pancreatic cells as well.

The transport of fructose is facilitated (passive) by a transporter protein named GLUT-5, which is *specific* for fructose and is not inhibated by glucose, galactose or other sugars.

Fructose leaves the enterocytes by crossing the bazo-lateral membrane of the intestinal epithelial cells with the help of the same GLUT-2 transporter used by glucose and galactose.

A first advantage of fructose is the lack of competition in intestinal absorbtion, the transporter protein GLUT-5 being *specific* for fructose and not being inhibited by glucose or galactose. After absorbtion from the gastrointestinal tract, the fructose is transported by the portal circulation to the liver, in which case it will enter the hepatocytes with the help of GLUT-5 transporter – independent of insulin, and is quickly metabolized (Smith Jr et al., 1953; Sato et al., 1996).

In hepatocytes, the fructose is phosphorilated in the presence of ATP, forming fructose-1-phosphate, in a reaction catalyzed by fructosekinase. The resulting fructose-1-phosphate is cleaved by aldolase B in glyceraldehyde and dihydroxyaceton phosphate. Both metabolites may be converted to glyceraldehyde-3-phosphate. Thus, the fructose molecule is metabolized in two phosphate trioses that avoid the main pathway controlling the glycolysis, that being 6-phosphofructokinase, phosphofructokinase is activated by AMP and AMP-cyclic and is inhibited by ATP, representing the direct link between the cell's energetic state and glycolysis (Elliott et al., 2002).

As well, phosphofructokinase, a hepatic enzyme that regulates glycolysis in hepatic cells, acts negatively on the glucose anabolism, while fructose may bypass this control mechanism and be metabolized in glycerol-3-phosphate and acetil-coenzime A. These two intermediary metabolites are then used as substrate for glyceride synthesis, contributing to very low density lipoproteins (VLDL) production (Basciano et al., 2005; Qu et al., 2007).

More, because of the lack of expression of the transporter protein GLUT5 in β-pancreatic cells, fructose, unlike glucose, does not directly stimulate the

insulin secretion (Sato et al., 1996). High quantities of fructose may rapidly stimulate lipogenesis and tryglicerides accumulation in liver, that will consequently reduce the sensibility to insulin and the hepatic resistance to insulin and to glucose intolerance (Basciano et al., 2005).

MATERIAL AND METHOD

The ingredients used to make the purified diets were pure substances, acquired from firms from country and from Germany, that had Quality Certificates and conformity declarations in order to attest both their quality, as well as their purity. Thus: Casein (Kuk Romania SRL); methionine (Nutristar Romania); corn starch, fructose and maltodextrin (Brenntag SRL); sucrose (Lemark SRL); cellulose (J. Rettenmaier & Sohne GMBH); soy oil (S.C. Ultex SA); vitamin-mineral premix (Nutristar Romania); food die Brilliant blue G (Sigma-Aldrich).
The two types of diet were made from the pure substances mentioned above, the first type of diet was a standard one according to AIN-93M (Table 1), and the second experimental diet was imagined for the study, of 60% fructose (Table 2), in which the corn starch, sucrose and maltodextrin have been fully replaced with fructose.

Table 1.

The composition of the diet made of purified ingredients AIN-93M

Index	Ingredients	Quantity (gr.)
1	Casein	140
2	DL- methionine	3
3	Corn starch	465
4	Maltodextrine	155
5	Sucrose	100
6	Cellulose	50
7	Soy oil	40
8	Vitamin-mineral premix	45
9	Choline	2

After the individual dosing of the substances, the samples were mixed for 15 minutes, while distilled water was added (about 1 litre/10 kg mix), in order to ensure the needed consistency to pellet the product. The pelleting was done with the help of a Alvan Blanch granulator from the Institute of Cellular Biology and Pathology “Nicolae Simionescu”. After pelleting, the two diets were dehydrated by depositing them for 48 hours in a

thermostated room at 37^0C. The excess fructose diet is kept in spaces lacked in humidity, because fructose is highly hygroscopic, and the pellets become sticky if kept in humid conditions.

Table 2.

The composition of the diet made with fructose constituted of purified ingredients

Index	Ingredients	Quantity (gr.)
1.	Casein	210
2	DL- methionine	3
3	Fructose	600
4	Cellulose	79.85
5	Palm oil	50
6	Vitamin-mineral premix	55
7	Bitartrate choline	2
8	Food coloring	0,15

The lab animals used came from Animaleria SPF "Stațiunea Băneasa" of Cantacuzino Institute, using: - Mice from strain C57Bl6 6 weeks old, males and females equally, with roughly the same body weight (Table 3).

Table 3.

Groups of mice strain C57Bl6 used in the experiment

Sex Group / ID-animal		Group 1 witness weight	Group 2 fructose weight
♂	N-normal-uncut ears	18.7gr	19.0 gr
	UD-right ear	16.9 gr	18.4 gr
	US-left ear	16.7 gr	19.0 gr
	AU- both ears	17.0 gr	18.2 gr
	2UD – twice the right ear	18.1 gr	16.5 gr
♀	N-normal-uncut ears	15.2 gr	14.4 gr
	UD-right ear	14.1 gr	13.8 gr
	US-left ear	15.2 gr	13.9 gr
	AU- both ears	13.8 gr	14.3 gr
	2UD – twice the right ear	13.8 gr	13.1 gr

Rats from strain Sprague Dawley (SD) 6 weeks old, males and females equally, with roughly the same body weight (Table 4). The identification of animals was possible with the help of some cuts made to the ear pavilion, as seen in Tables 3 and 4. First, the animals were habituated in the accommodation rooms for experimental animals, in regard with the microclimate, hygene and feeding norms needed for these protocols (Curcă, 2004).

Table 4.

Groups of rats strain Sprague Dawley (SD) used in the experiment

Sex Group / ID-animal		Group 1 witness weight	Group 2 fructose weight
♂	N-normal-uncut ears	160.5 gr	152.8 gr
	UD-right ear	116.6 gr	183.2 gr
	US-left ear	152.9 gr	126.2 gr
	AU- both ears	152.5 gr	131.1 gr
	2UD – twice the right ear	166.5 gr	185.8 gr
♀	N-normal-uncut ears	139.0 gr	125.1 gr
	UD-right ear	141.1 gr	137.2 gr
	US-left ear	154.0 gr	145.2 gr
	AU- both ears	142.9 gr	136.2 gr
	2UD – twice the right ear	142.2 gr	165.7 gr

The experimental room has a microclimate controlled by the conditioning system, ensuring the optimum of temperature and humidity (t^0C 18-22; UR 55-65%). The acclimatization period was 7 days, meanwhile the animals were accommodated 5 individuals of the same sex in one per cage. During that period, the transition from the natural diet (of natural ingredients: cereals, proteic shrots of soy and sun-flower) to the standard purified one administered *ad libitum*. The composition of this diet is mentioned above (Table 1).

Blood samples were collected for dosing glycemia, both in the glucose tolerance test (GTT), as in the control dosing, which were made by penetrating the lateral vein of the tail, found at the limit of the anterior third and the middle third of the tail, practicing a small incision with a scalpel blade, collecting the blood drop directly on the reactive strip.

Glycemia was measured with ACCU CHEK ACTIVE Glucometer (Roche diagnostics). Prior to sampling, the animals were dieted, with no hydric diet, for 16-18 hours. The glucose tolerance test or *the orally provoked hyperglycemia* is a functional test that explores the reaction of the organism to the glucidic overload. Thus, after 16-18 hours of alimentary diet the basal glycemia (T0) is determined, after which glucose solution 20% is administered as gavage in dose of 1 gram per 1 kg live weight. Then the glycemia is measured after 15, 30, 60 and 120 minutes after the gavage, revealing information about the capacity of insulin releasing or the peripheric resistance to insulin (http://EMPReSS). If there are altered values for basal glycemia or after feeding fructose, this test can be redone of it may

infirm diabetes mellitus diagnostic. In the glucose tolerance test gavage needles 18G for rats and 22G for mice were used.
The urine analysis was done for the animals that presented increased values of the glycemia, these being tested for glycosuria with the help of CYBOW strips (DFI Co, Ltd). The urine was directly sampled on the reactive area of the strip.Animals were weighted weekly during the experiment with the help of the SARTORIUS-type scale for animals.

RESULTS AND DISCUSSION

The ingredients of the purified diet that ensure the nutrients needed are presented in Table 5, resulting in the quantity of components, as well as the chemical parameters that were estimated and realized.

Table 5.

The purified diet that ensures the upkeep necessary, AIN-93M

No.	**Ingredients:**	**Quantity** (gr)	**Estimated analytical parameters**	
			Estimated	Realized
			Nutrient Quantity%	Nutrient Quantity%
1	Casein	140	Protein 14,02%	Protein 14,0%
2	DL- methionine	3	Sugars 68,0%	Sugars 68,0%
3	Corn starch	465	Lipids 4,1%	Lipids 4,1%
4	Maltodextrin	155		
5	Sucrose	100		
6	Cellulose	50		
7	Soy oil	40		
8	Vitamin-mineral premix	45		
9	Betartrate choline	2		

This diet was administered to the animals of both groups during the habituation period for the first week and then only to the witness group for the whole testing period. The purified diet with fructose was administered to the animals in the second group during the testing period (Table 6). The diet and the water were administered *ad libitum.* At the end of the habituation period, which lasted a week, the animals were evaluated from the glucose tolerance point of view. The glycemia values resulted were considered reference values for the normal animals before the diet alteration. The results of the glucose tolerance test are given in real values in the next tables (Table 7 for mice and Table 8 for rats). From the data analysis in Table 7 and the respective diagrams it can be observed the uniformity of reaction to

the glucose tolerance test in mice C57Bl6 of both sexes, befor administerng the fructose diet and to the rapid decrease of the glycemia after T15.

Table 6.

The purified experimental diet with fructose 60%.

No.	**Ingredients:**	**Quantity** (gr)	**Estimated analytical parameters**	
			Estimated	Realized
1	Casein	210	Nutrient Quantity%	Nutrient Quantity%
2	DL-methionine	3	Protein 18.5%	Protein 18.5%
3	Fructose	600	Sugars 60.0 %	Sugars 60.04 %
4	Cellulose	79.85	Lipids 5.0%	Lipids 5.05%
5	Palm oil	50		
6	Vitamin-mineral premix	55		
7	Bitartrate choline	2		
8	Food coloring	0.15		

It can be noticed that the glycemia value of the entire male group in T0 with 108,8 mg/dl blood average and the standard average 5,59. The results of the glucose tolerance test done on SD rats after the habituation period was over are presented in Table 8. After analysing the data found in table 8, as well as the respective diagrams, it can be observed the uniformity of reactions between sexes to the glucose tolerance test and the continous glycemia between T15-T30 minutes.

Also, there is a slower response to the glucose load in rats as opposed to mice. After determing the glucose tolerance test after 39 days of feeding with the experimental diet in mice, the data were written in table 9.

Table 7

The results of the glucose tolerance test (Gl) in mice strain C57Bl6 that will be fed with experimental diet

Sex Group ID-animal		Live weight gr	ml. sol. Gl 20% gavage	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120 Gl mg/dl
♂	N	17.6	0.088	114	250	231	188	107
	UD	17.1	0.0855	105	211	188	147	107
	US	17.8	0.089	113	171	201	144	123
	AU	18	0.09	111	233	194	162	165
	2UD	16.5	0.0825	101	237	235	186	147
Average		**17.4**	**0.087**	**108.8**	**220.4**	**209.8**	**165.4**	**129.8**
d.s.		0.60	0.00	5.59	30.98	21.72	20.88	25.60
♀	N	14	0.07	107	228	122	170	96
	UD	13.3	0.0665	118	150	209	111	101
	US	14.2	0.071	97	266	158	138	84
	AU	13.7	0.0685	127	252	221	142	116
	2UD	13.2	0.066	139	168	192	155	111
Average		**13.68**	**0.0684**	**117.6**	**212.8**	**180.4**	**143.2**	**101.6**
d.s.		0.43	0.00	16.46	51.35	40.35	21.92	12.62

After 39 days of feeding the mice C57Bl6 with fructose diet the folowing observations can be made, by analising the diagrams above:

- the uniformity of reaction between the individuals from the same group as well as between sexes dissappears;
- the average value of the basal glycemia both in males and females decreases. This decrease of the average basal glycemia in both sexes may be the consequence of the alimentary intake of glucose with decompensation of glycogenolysis and gluconeogenesis;
- the basal glycemia values in male mice have a wider distribution with a standard derivation of 35,27 compared with the moment T0 of the test when the animals ate the standard diet and the basal glycemia values were concentrated around the average, the standard deviation being 5,59;
- a remakable important aspect in both sexes is the tardiv hypoglicemia and the decrease of the glicemia to T120 under the basal glicemic value T0. This situation may be the consequence of exhausting the hepatic glycogen reserves; - the male mouse identified US is remarked by a flat curve that may be the expresion of hyperinsulinemia, characteristic to the incipient stages of hyperglicemic syndromes.
- the male mouse identified 2UD is remaked because of the slow response, with no return to the initial value, not even after 120 minutes after the oral

administration of glucose solution, indicating insulin resistance. In the female group, the female identified UD is noticed due to the peak in T15, when glycemia reached 411 mg/dl, indicating a weak secretion of preformed insulin. After determining the glucose tolerance test after 39 days of feeding rats SD with experimental diet, the data presented in table 10 were obtained. After analyzing the data from the table, it can be observed that the male rat identified "N" died in the first day of the experiment, after his weight dropped from 180.2 gr to 147.3 gr. Following the tendency of weight evolution in the male "N" it was observed a slight increase of the weight after 7 days of fructose diet intake, then a constant decrease. As well, in this rat in the 11th day of basal glycemia control test, his 85 mg/dl glycemia dropped under the average of the group of 100 mg/dl blood.

Table 8

The glucose tolerance test (Gl) done on rats strain Sprague Dawley (SD) that are fed with the experimental diet

Sex Group ID-animal		Live weight gr	ml. sol. Gl 20 % gavage	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120 Gl mg/dl
♂	N	180.2	0.901	110	225	213	159	127
	UD	215.3	1.0765	105	160	200	147	107
	US	150.8	0.754	134	237	192	183	130
	AU	156.5	0.7825	114	167	236	195	113
	2UD	223.3	1.1165	108	185	203	168	122
Average		**185.22**	**0.9261**	**114.2**	**194.8**	**208.8**	**170.4**	**119.8**
d.s.		33.13	0.17	11.54	34.5	16.9	19.0	9.63
♀	N	142.2	0.711	98	199	204	166	112
	UD	158.4	0.792	107	179	197	129	109
	US	159.5	0.7975	92	188	204	126	110
	AU	151.5	0.7575	102	206	181	126	109
	2UD	192	0.96	99	231	241	193	115
Average		**160.72**	**0.8036**	**99.6**	**200.6**	**205.4**	**148**	**111**
d.s.		18.80	0.09	5.50	19.88	22.01	30.32	2.55

By correlating this evolution of the body weight with the basal glycemic value in the 11th day and the aspect of the liver during the necropsy (yellow clay colored with rounded edges), the perturbation of the metabolism due to the lack of food intake of glucose may be deduced.

Table 9

The glucose tolerance test (Gl) done in the 39th day of feeding the mice strain C57Bl6 with experimental diet

Sex Group / ID-animal		Live weight gr	ml. sol. Gl 20 % gavage	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120 Gl mg/dl
♂	N	14.6	0.073	104	179	117	67	63
	UD	15.4	0.077	124	214	129	69	48
	US	13.1	0.0655	45	114	75	62	48
	AU	14.6	0.073	46	166	87	57	37
	2UD	13.8	0.069	90	278	273	221	111
Average		**14.3**	**0.0715**	**81.8**	**190.2**	**136.2**	**95.2**	**61.4**
d.s.		0.88	0.00	35.27	60.81	79.53	70.48	29.23
♀	N	9.8	0.049	78	275	146	78	60
	UD	10.4	0.052	64	411	174	85	59
	US	9.4	0.047	60	183	172	121	60
	AU	12.5	0.0625	67	177	169	155	66
	2UD	10.7	0.0535	56	174	155	108	53
Average		**10.56**	**0.0528**	**65**	**244**	**163.2**	**109.4**	**59.6**
d.s.		1.20	0.01	8.37	102.4	12.15	30.81	4.62

Table 10

The results of the glucose tolerance test (Gl) done in the 39th day of feeding the rats SD with experimental diet

Sex Group ID-animal		Live weight gr	ml. sol. Gl20 % gavat	T0 Gl mg/dl	T15 Gl mg/dl	T30 Gl mg/dl	T60 Gl mg/dl	T120Gl mg/dl
♂	N	147.3	-	-	-	-	-	-
	UD	214.9	1.0745	87	182	197	178	155
	US	138.1	0.6905	66	172	159	127	29
	AU	167.7	0.8385	168	170	189	161	145
	2UD	233.3	1.1665	138	182	184	130	116
Average		**180.26**	**0.9425**	**114.75**	**176.5**	**182.25**	**149**	**111.2**
d.s.		37.51	0.22	46.63	6.40	16.40	24.70	57.27
♀	N	123.3	0.6165	105	143	268	156	91
	UD	133	0.665	97	185	238	230	121
	US	145.5	0.7275	244	272	272	243	180
	AU	148.9	0.7445	119	153	194	157	144
	2UD	212.8	1.064	151	177	230	195	120
Average		**180.26**	**0.9013**	**143.2**	**186**	**240.4**	**196.2**	**131.2**
d.s.		41.94	0.21	60.00	51.03	31.73	40.27	33.13

By analysing the diagrams of the glucose tolerance test done after 39 days of feeding with fructose diet of the rats SD the following observations occur:

- the basal glicemia increases only in some individuals, both males and females. Fron the male group the individuals identified with "AU" and "2UD" are noticed due to the basal glicemia that increased with 47.3% and 21% respectively to the group average. From the female group the female "US" is noticed, having the basal glicemia increase by with 70.3%;
- there is no more uniformity of reaction both between individuals of the same groups as well as between different sexes. The T0-T15 interval represents the oral glucose asimilation phase, meanwhile the preformed insulin is consumed. Thus, in the male rat group the stabilization of the average value of glicemia to 141.2 mg/dl, with the standard deviation 6.40 noticed at T15 is observed, demonstrating the presence of preformed insulin. 15 minutes after oral administering of glucose the newly sinthesised insulin secretion begins, marked by the increase of standard deviation, indicating the individual capacity of synthesis and captation of glucose. The curves made by glicemia in T30, are ascendant both in males as in females,showing the existance of an insufficient secretion of a peripheric resistance to insulin.

It can be noticed that after 39 days of feeding with fructose diet, the answer to provoked hyperglicemia in the glucose tolerance test, is much slower so that the relative plateau constant between T15 and T30 initially, transformed in the case of females in an ascendant curve. The data concerning the dynamics of the average of glicemia in mice C57Bl6 and rats SD, at the beginning of the feeding with experimental diet, then in the 11^{th}, the 39^{th} and the 79^{th} day of experimental diet, ar seen in table 11 and their respective diagrams. By analysing the charts above, it can be noticed that although the rats Sprague Dawley and mice C57Bl6 belong to the same family (*Muridae*), they have different ways of metabolic response to the fructose substitution, of any glucidic source from diet. If rats have a hiperglicemic answer to the diet change, the mice C57Bl6 have a first hipoglicemic answer. Just by analising the glicemic evolution in the first phase in mice, without correlations with other metabolic parameters, the fructose excess may be considered a beneficial nutritional factor in hiperglicemic syndromes. The correlation between these two parameters, the basal glicemia and the glucose tolerance, monitored in this study by insulin, cholesterol, tryglicerid, uric acid dosage and arterial tention determination, may create a complex and complete image of the metabolic perturbances provoked by the excess of fructose in diet.

Table 11

Synoptic table with the average of glicemia values in mice C57Bl6 and rats SD, at the beginning of the feeding with experimental diet, then in the 11th, 39th and 79th day

DATE	Mouse C57/Bl6 ♂	Mouse C57Bl6 ♀	Rat SD ♂	Rat SD ♀
12.05.2011 - **initially**	**108.8**	**117.6**	**114.2**	**99.6**
23.05.2011 – **11th day**	**89.6**	**100.2**	**100**	**97.4**
17.06.2011 – **39th day**	**81.8**	**65**	**114.7**	**143.2**
27.07.2011 – **79th day**	**126.5**	**110**	**152**	**170.6**

In the 79th day of feeding with experimental diet in mice and rats, the glicemia and glucosuria were determined, the data being written in Table 12 and table 13 respectively.

Table 12

Values of glycemia and glycosuria in mice C57Bl6 in the 79th day of experimental diet

Sex	**Group**	**ID. Animal**	**Glycemia mg/dl**	**Glycosuria mg/dl**
Male	**Fructose**	US	133	Negative
		2UD	120	Negative
	Witness	2UD	173	Negative
Female	**Fructose**	N	162	+/- 100
		UD	58	+/- 100
	Witness	AU	137	Negative

Table 13

Values of glycemia and glycosuria in rats SD in the 79th day of experimental diet

Sex	**Group**	**ID. Animal**	**Glycemia mg/dl**	**Glycosuria mg/dl**
Male	**Fructose**	AU	152	Negative
		US	114	Negative
	Witness	N	216	Negative
Female Male	**Fructose** Witness	N	216	+/- 250
		US	169	+/- 100
		AU	127	Negative
	Fructose	AU	141	Negative

After analyzing the data presented in tables 12 and 13, it may be noticed the presence of glycosuria with different intensities in females of both species, while in males of both species it hyperglycemia was installed with no glycosuria.

CONCLUSIONS

The diets made from purified ingredients allowed the selective elimination of all sources of sugars and their replacement with fructose, making possible the evaluation of its effects over the metabolism.

The quantitative alteration, at a given moment, of a nutritional factor from the diet makes the evaluation of the effect very precise. This means that there are practically unlimited possibilities of alterations that can be done in a purified diet, making them a powerful research instrument.

Fructose excess diets administered to mice from strain C57Bl6 and rats from strain Sprague Dawley lead to:

- The increase of basal glycemia and the alteration of glucose tolerance;
- Glyosuria in females of both species while the males in both species were hyperglycemic and not glycosuric.

REFERENCES

ACCU CHEK ACTIVE - Roche diagnostics. ACCU CHEK ACTIVE – Glucometru; Manual de instructiuni; Roche diagnostics.

Basciano H., Federico L. and Adeli K., 2005. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr. Metab. (Lond.), 2, 5.

Bray G. A., Nielsen S. J. and Popkin B. M., 2004. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am. J. Clin. Nutr., 79, 537-543.

Curcă D., 2004. FIZIOPATOLOGIE. Lucrări practice şi protocoale experimentale. Editura Printech, Bucureşti.

Elliott S.S., Keim N.L., Stern J.S., Teff K. and Havel P.J., 2002. Fructose, weight gain and the insulin resistance syndrome. Am. J. Clin. Nutr. 76,911-922

Putnam J.J. and Allshouse J.E., 1999. Food consumption, prices, and expenditures, 1970–97.Washington, DC: Economic Research Service, US Department of Agriculture.

Qu S., Su D., Altomonte J., Kamagate A., He J., Perdomo G., Tse T., Jiang Y. and Dong H.H., 2007. PPAR (alpha) mediates the hypolipidemic action of fibrates by antagonizing FoxO1. Am. J. Physiol. Endocrinol. Metab., 292, 421-434

Sato Y., Ito T., Udaka N., Kanisawa M., Noguchi Y., Cushman S.W. and Satoh S., 1996. Immunohistochemical localization of facilitated-diffusion glucose transporters in rat pancreatic islets. Tissue Cell 28, 637-643.

Smith Jr L.H., Ettinger R.H. and Seligson D., 1953. A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. J. Clin. Invest. 32, 273-282.

Yudkin J., 1967. Evolutionary and historical changes in dietary carbohydrates. Am. J. Clin. Nutr. 20,108-115.

http://EMPReSS/by Phenotype Platform/Hormonal and Metabolic Systems/Oral Glucose Tolerance Test (O.G.T.T).

MONITORING THE RISKS OF SOME PHYSIOLOGIC FACTORS OF LACTATION IN PIC SOWS ON THE HEALTH OF SUCKLING PIGLETS

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Abstract

The influence of the major physiological factors (age, weight at first farrow and some genotypic, phenotypic and environmental characteristics) on prolificacy and sow lactation capacity as well as the growth and health indices in piglets was monitored on a PIC industrial multiplication farm with a breeding effective of 200 sows and a total of 255 farrows, in 2011. Evaluations were based primarily on quantification of biometric parameters of the farrows. Am overview of the data regarding the influence of age (farrow rank) on milk production in sows revealed a maximum production level in the 2nd or 3rd lactation in correlation with increased prolificacy (11.9 or 11.7) and lactation capacity (65 and 64) respectively with reduction of mortality in piglets (3.6% and 3.7%). The evolution of these indices revealed that sows can be effectively exploited in the first 5 lactations, after which advancing age significantly limits milk production. Achieving the first fertile optimal age of first farrowing (233-238 or 348-353 days) also contribute to ensuring milk production. The lactation curve was characterized by an upward phase in the first 7-10 days, a plateau of about 10 days and a downward phase, consisting of slow decrease milk production followed by its marked reduction around weaning. In last year the total recorded farm losses (10.2%) were represented largely by mortality in suckling piglets (45%).

Key words: health and growth indices, lactation risks, PIC sows, piglets.

INTRODUCTION

Health status of the lactate sow and piglets is influenced by several intrinsic or extrinsic factors, which in certain conditions can generate real risks (Cotruţ, 1986; Ghergariu and Baba, 1990). The progress achieved in

monitoring lactation risk factors is well known for cattle and other ruminant species (Peeler, 1987; Sheldrake and Mc Gregor, 1990; Knight and Peaker, 1982). In recent decades these developments have been observed in the case of lactating sows with piglets, leading to significant reduction of losses during the lactation- weaning period (Ognean et al., 2010). In this context it is important to mention that the level of milk production in sows is extremely high, reaching over an average lactation period of 8 weeks, approximately 68-77 kg dry matter. This nutrient rich substrate composed of 23-26 kg of protein, 24-27 kg of fat, 3.5 to 4 kg minerals and 17.5 to 20 kg lactose ensures a piglet weight-gain of up to 5 times in the first month and 10 -12 times in the second month. After the 21st day the piglet becomes dependent on additional feeding. A sow with 10 piglets has to produce around 10-12 kg milk/day and needs 1 kg compound feed per 100 kg body weight and 0.4 to 0.5 kg for each piglet that suckles (Polen, 2007). It is essential for the newborn piglet to maintain normal glycemia levels. The period between the farrow, when transplacental transfer of glucose ceases and suckling is extremely critical, as stabilization of the newborn is dependent upon its own hepatic glycogen reserves. Thus the presences of an adequate hepatic glycogen reserve at birth a factor for increasing the survival rate during this transitional period. Glucose level decline occurs immediately after birth and lasts 1-3 hours, due to the fact that hepatic glycogen reserves are rapidly depleted, being offset by an increased gluconeogenesis, of approximately 10% within hours from birth, (Pabst and Rothktter, 1999).

MATERIALS AND METHODS

This study was conducted in order to identify some of the risk factors acting on prolificacy and suckling ability of lactating sows, after correlating age and weight at first farrow with certain genotypic and phenotypic characters, respectively monitoring some growth and health indices in piglets. To this end observations and investigations were made in a PIC multiplication farm (Coroi Sânmartin, Mureş), with a total number of 2463 of pigs, in 2011. Research has been conducted on samples of breeder sows (n=200) with suckling piglets and relied on quantifying the evolution of some biometric parameters of the piglets, resulted from a total farrowing number of 255. The newly farrowed sows and those with suckling piglets were kept in the farrowing ward in adequate compartments with proper maintenance and

feeding. A farrowing group consisted of the farrowings that occurred in no more than 2 days, managed by the same caregiver.
Investigations were performed in the form of surveys to record data and observations that were followed, according to necessity, by clinical examination and sampling for hematological, biochemical and morpho-pathological investigations. The investigations were focused on the health monitoring of lactating sows and suckling piglets in the feeding and maintenance conditions of the farrowing-nursery ward, namely temperature, humidity and air flow when using fans and humidifiers during heat waves. The influence of some pathological factors on the health of lactating sows and piglets was determined as well based on the development of the data from the "movement reports" respectively from Eurohyb computerized management. Data recorded included aspects regarding the influence weight, age, weaning conditions, namely individual and collective compartments on the unit lactating sow-suckling piglets, in which the sows were kept during insemination and the first month of gestation.
Individual and mean data were subjected to statistical analysis using current statistical processing software (Graph Pad Instant V3.0, V4.0 Graph Pad Prism, Microsoft Excel, Variance and covariance study) and calculating the mean, standard deviation and probability index p.

RESULTS AND DISCUSSION

The data from correlative interpretation of the first fertile breeding age and body development of the sows at first farrowing, stage of lactation, body weight and some genotypic, phenotypic or environmental characteristics (Table 1), was the basis for the following analysis of potential risks resulting from certain physiological factors, which influence lactation in sows and consequently the health of the suckling piglets.
Analysis of the data regarding the correlation of age with body development of the sows at first farrowing showed significant influence on milk production. Regarding this factor it should be mentioned first that the age when the first fertile mount occurred decreased with increasing weight group of the sows. Thus, concerning the influence of age (farrowing rank), we found that the maximum production level was achieve in the 2nd and the 3rd lactation, which were positively correlated with increased prolificacy (11.9 to 11.7), suckling capacity (65-64) and reduced mortality (3.6% - 3.7%).

Also an essential index proved to be the lactation curve. This included an upward phase in the first 7-10 days of lactation, followed by a plateau of about 10 days, in which the milk production remained at the highest level, a downward phase, characterized by a slow decline of milk production and a marked reduction up to weaning. The obtained results showed that, the period of lactation influences milk composition, respectively a slight decrease in protein content, minerals and especially fat during the upward phase and the increase of the same parameters during the downward phase, similar developments being noted by other researchers in the field (Cotruţ, 1984; Cotruţ, 1986; Polen, 2007; Boe, 1993).

Achieving an adequate body weight exerted a major influence on milk production that acted in conjunction with age; an increased body weight resulted in positive effects by augmenting the abdominal volume and hence feed intake capacity. Achieving optimal age at first fertile mounts (233-238 days) respectively at first farrowing (348-353 days) corresponded to breed standards and the provided conditions. The high level of milk production in PIC sows was also ensured by achieving an optimal correlation between the age at first farrowing and body development. Thus, achieving a weight between 120 and 130 kg at the first fertile mount in sows ensured a proper gestation and a large number of piglets at farrowing. Correlating genetic characteristics of breed with the provided maintenance and feeding standards led to the development of highly productive indices. Thus, the L03 line of sows gave an outstanding farrowing rate performance (85%), total prolificacy (12.7 piglets/farrowing), the number of farrows (2.11/year, with 27pigs/year/sow) and mean piglet weight at farrowing (1.7 to 2.1 kg).

Prolificacy analysis revealed that the number of piglets farrowed and sucked can significantly influence milk production, sows with 12-15 piglets producing 25-35% more milk than those with 8-10 piglets, because in smalls farrows the milk produced in the first 3-4 days is not entirely consumed, this factor negatively influencing milk production (Pârvu, 2003).

Statistical analysis of the data regarding genetic factors, phenotypic and environmental factors reveled close correlations between the weight at farrow and weight of the piglets at 21 days (rfxy = 0.494) and between number of live piglets and size of the farrow at 21 days (rfxy = 0.487), summed action of these factors ensured a higher level of lactation capacity.

Behavioral observations of 20 farrows of suckling piglets during the first 72 hours after birth, showed that the newly farrowed piglets with body weight less than the mean weight of the farrow made smaller weight gains, suggesting loss of competition for the mammary glands with higher milk

production and therefore a reduce consumption of colostrum/milk. It was also found that the usage of the pair of mammary glands, according to their anatomical position decreased in the order 1-8, smaller piglets getting those with low milk production, leading to a strong correlation (rxy = 0.686) between body weight and the amount of milk consumed.

Daily monitoring of the main microclimate factors, led to the use of fans equipped with water dispensers during heat waves. Thus, after the controlling the temperature-humidity parameters an improvement, of 10-20%, was obtained in the breeding index.

The consequences of the cumulated action of the risk factors, shown in Table 1, are relevantly expressed by the development of losses in the piglets. Mortality losses occurred predominantly in the first week of life, and were often due to crushing of piglets by the sows, especially in large farrows, followed by mortality due to digestive diseases, correlated in some cases with agalactia in the sows. Cases of diarrhea syndromes in pigs were sporadically reported, beginning with the second week of life, the main pathogen being E.coli. The affected piglets showed mild forms of diarrhea, which were sensitive to antibiotic therapy (Gentocin or Spectam). In the analysis of the cumulative action of the investigated risk factors was also observed their negative influence on individual milk consumption in the first days of life. The consequence of this reduction in energy and structural intake led to morphological and functional changes of the mucosal integrity of the small intestine and consequently in its permeability, with the onset of diarrhea (Lloyd et al, 1998; Bailey et al, 1998; Kok and Ahn, 2007; Rooke et al, 2002).

Agalactia in sows was expressed predominantly mammary edema and reduced milk production and did not exclude developments such as mastitis-metritis-agalactia type syndrome (MMA). One of the severe effects of MMA syndrome is the production of lipopolysaccharides, and their passing from the circulation into the colostrum (Bertschinger et al., 2000). Fewer were the cases reporting strictly mammary lesions (localized in the nipple or even mastitis), sometimes they were associated with hormonal infertility. Changes seen in hypoxemic piglets were correlated with the following negative effects found by other researchers in the field (Sheldrake and Mc Gregor, 1990): reduced vitality due to general malnutrition caused by disruption of the sow-piglet relationship, affecting the energy and material balance of feeding capacity and morpho-functional integrity of the digestive tract and annexed glands.

Monitoring the effect of various risk factors on health of the unit lactating sows- suckling piglets made remarkable progress in recent decades, leading to fewer losses during the lactation-weaning period (Ognean et al., 2010). In this context also fall the results of the final analysis regarding the main health and production indices recorded in the investigated farm where the reduced rate of annual losses through mortality (10.2%) reveals a good control of the risk factors. Regarding this aspect it is relevant to mention that from the total losses only 45% were recorded in the piglet category, 40% in growing youth and 15% in case of testing youth and commercial pigs.

Metabolism in pregnant sows is characterized by intensified anabolic synthesis processes in order for the body to cope with its needs and those of the developing fetuses. Thus a reduction in the concentration of glucose, cholesterol, total lipids and serum calcium can be observed. The physiologically high intake during processes lactopoesis and lactogenesis leads to higher metabolic losses in lactating sows than in other species. The highest losses are observed in energy and mineral metabolic profile. In this context also fall the results of behavioral observations that made on 20 farrows during the first 72 hours after birth. The data showed that piglets with a body weight at birth below the mean weight of the farrow made smaller weight gains, suggesting loss of competition for mammary glands with higher milk production and respectively a reduced consumption of colostrum/milk.

Also noticed was the fact that usage of the pairs of mammary glands according to their anatomical position decreased in the order 1-8, accounting for smaller piglets getting those with lower milk production. Statistical analysis of this data revealed a strong correlation ($r_{xy}=0.686$) between body weight and the amount of milk consumed by the piglets.

Typically, average daily gain of piglets with body weight below the mean weight of the farrow is less than the mean weight gain of the group, suggesting that smaller piglets loose the competition for mammary glands with more milk in favor of bigger ones (Rada, 2010). Also the age, at which the maximum number of new cases of diarrhea appeared, was variable, being dependent on the number of piglets and frequency of parturition, respectively the number of mammary glands with edema, hypo- and agalactia in the first days after farrowing. Hypoxia at birth and hipogalaxia had a convergent effect on piglets, triggering malnutrition and non-infectious diarrhea. Thus, in a farrow during in the first days of life the

piglets showing signs of diarrhea were those with parturition hypoxia and those malnourished especially in the 2-4 days of life.
Metabolic status of piglets with diarrhea is characterized by weight loss, reduce glycemia and morphological changes of the intestinal mucosa, especially the jejunum, which is associated with a negative energy and protein balance, triggering non-infectious or nutritional diarrhea. A small number of piglets may appear a mild osmotic transient diarrhea due to excessive consumption of milk. Diarrhea in the first days of life is generally triggered by malnutrition and is caused by hypoxia and/or hipogalaxia in sows affected by mammitis mastitis-metritis-agalactia (Rada, 2010).
As shown in mortality distribution analysis, the losses caused by crushing of the piglets prevailed. This was seen especially in large farrows, in which the piglets either were not able to avoid the sows or did not have enough room on the rest bed and were crushed by their mothers. This also concludes that reducing this percentage is almost impossible. Diarrhea due to E.coli infection was the second leading cause of suckling piglet mortality. Regarding the efficacy of the used therapies, it is important to mention that all farrows responded well to the usual treatments.

Table 1. Correlations between the characteristics influencing nursing ability in sows

Couple of characters	Correlation			Regression
	Phenotype	Genotype	Environmental	
Total no. of nipples	0.463	0.459	0.469	0.696
Total no. of functional nipples				
Total no of functional nipples	0.767	0.169	0.826	1.046
No piglets/group at 21 days				
Total no. of functional nipples	0.451	0.360	0.443	5.055
Weight/group at 21 days				
Wright/group at farrow	0.494	0.551	0.529	3.158
Weight/group at 21 days				
No of live piglets at farrow	0.487	0.764	0.526	0.889
No piglets / group at 21 days				
No piglets / group at 21 days	-0.336	-0.247	-0.317	-0.613
Individual weight at 21 days				
Daily feed intake	0.630	0.439	0.321	1.412
Weight/group at 21 days				

CONCLUSIONS

Influence of age (farrowing rank) on milk production in PIC sows highlighted maximal levels in the 2nd and 3rd lactation and their correlation with the highest rates of prolificacy (11.9) and lactation capacity (65), respectively with reducing mortality rates (3.6%);

Feed ration of sows during lactation and gestation was characterized initially by an increased energy and reduced protein level, being mineral balanced in the second and third part of gestation, respectively an increased energy and protein level in the final part, when very rapid development must be ensured;

Milk production performances in PIC sows were also insured by realizing optimal age at first fertile mount (233-238 days) respectively at first farrowing (348 to 353 days);

The optimal conditions provided by the farm have enabled high productivity indexes, farrowing rate reached 85%, prolificacy 12.7 piglets/farrowing and the number 2.11 farrows/year, with 27 piglets/year/sow and average weight at farrow between 1.8 and 2.1 kg;

Lactation curve presented an upward phase in the first 7-10 days, a plateau of about 10 days and a descending phase of slow decrease in milk production, followed by a sharp decline around weaning;

Prolificacy analysis revealed that the number of piglets farrowed and sucked can significantly influence milk production, sows with 12-15 piglets producing 25-35% more milk than those with 8-10 piglets, because in smalls farrows the milk produced in the first 3-4 days is not entirely consumed, this factor negatively influencing further milk production;

Statistical analysis of the influence of genetic, phenotypic and environmental factors revealed a strong correlation between weight of the piglets at farrow and weight the piglets at 21 days ($r_{fxy} = 0.494$), between the number of live piglets and size of the farrow at 21 days ($r_{fxy}=0.487$) and a very important influence on the ability of suckling;

Distributed by category the mortalities prevailed in suckling piglets (45%), followed by growing youth (40%), youth in testing (7%) and commercial pigs (8%);

Usage frequency of the anatomical pairs of mammary glands decreased in the order 1-8, smaller piglets getting access to those with lower milk production, resulting in a positive correlation between body weight and the amount of milk consumed by the piglets ($r_{xy} = 0.686$).

Abrupt weaning procedure at the age of 28-35 days, gave very good results PIC farms, consisting in the transfer of piglets in the youth compartment and of the sows in the breeding compartment, prior a 24 hour diet the day before weaning;

Analysis of the causes producing mortality in piglets from farm A showed as predominant causes crushing (55%), diarrhea syndromes, especially E.coli infections (35%), starvation (7%) and less due to other cause (3%).

REFRENCES

Bailey M., Plunkett F., Clarke A., Sturgess D., Haverson K., Stokes.,1998. Activation of T cells from the intestinal lamina propria of the pig. Scandinavian Journal of Immunology, 48, 177–182.

Bertschinger H.U., Nief V., Tschape H., 2000. Active oral immunisation of suckling piglets to prevent colonisation after weaning by enterotoxigenic Escherichia coli with fimbriae F18. Veterinary Microbiology, 71, 255–267.

Boe, K., 1993. Maternal behaviour of lactating sows in a loose housing system. Appl. Anim Behav. Sci, 35: 327.

Burlacu R., Grosu Valentin Doina, Marinescu G. Al., Burlacu G., 2005. Modelarea matematică a proceselor de metabolism energetic şi proteic la suine. Ed. Cartea Universitară, Bucureşti.

Cotruţ Maria, 1984. Valoarea unor parametri de profil metabolic la porcine. Rev. de Creşterea Animalelor, Bucureşti, 11, 17-19.

Cotruţ Maria, 1986. Variaţii ale unor indici ai mediului intern la scroafe în lactaţie şi la purcei sugari. Lucr. Şt. I.A. „Ion Ionescu de la Brad" Iaşi, Seria Zoo-Med.Vet., 30, 55-56.

Ghergariu S., Baba I. Al., 1990. Patologia nutriţională şi metabolică a animalelor, Ed. Academiei Române, Bucureşti.

Knight CH. and Peaker M., 1982. Development of the mammary gland. J. Reprod. Fert., 65, 521 – 536.

Kok Y., Ahn D.U., 2007. Preparation of Immunoglobulin Y from Egg Yolk Using Ammonium Sulfate Precipitation and Ion Exchange Chromatography Poultry Science 86, 400–407.

Billey L., Erickson A., Francis D., 1998. Multiple receptors on porcine intestinal epithelial cells for the three variants of Escherichia coli K88 fimbrial adhesion. Veterinary Microbiology, 59, 203-212.

Ognean L., Bereş M. Gh., Geta Pavel, Vlasiu A., Cristina Cernea, M. Cernea, Meda Moldovan and Trîncă S., 2010. The Evolution of the hemogram and certain biochemical parameters from blood and milk of sows during the first week post-partum. Bull. UASVM Cluj-Napoca, 67 (1), 158-165.

Ognean, L., Vlasiu A., Bereş M. Gh., Meda Moldovan, Oroian R., Carmen Jecan, 2011. Peculiarities Regarding the Testing of Milk Physicochemical and Cytology at a PIC Sows Sample Bull. USAMV Cluj-Napoca, 68 (1), 284-290.

Pabst R. and Rothktter H.J., 1999. Postnatal development of lymphocyte subsets in different compartments of the small intestine of piglets. Veterinary Immunology and Immunopathology, 72, 167-173.
Pârvu Gh., 2003. Nutriţia, răspunsul imun şi sănătatea animalelor, Bucureşti, Ed. Ceres.
Peeler E. J., 1987. Risc factors associated with clinical mastitis in low somatic cell count british dairy herds. J. Dairy Sci., 83, 64 – 72.
Rada Olga Alina, 2010. Statusul metabolic şi endocrin la purcei în perioada neonatală. Teză de doctorat USAMV Timişoara.
Rooke J.A., Baland I.M., 2002. The acquisition of passive immunity in the new-born piglet. Livestock Production Science, 78, 13-23.
Polen T.,2007. Marele Alb - robust, prolific şi performant. *Revista Ferma, 1, 45.*
Sheldrake J. K., Mc Gregor G. D., 1990. Lactation stage, parity and infection affecting somatic cells, electrical conductivity and serum albumin in milk. Dairy Food Protect. 40, 125.

VIRULENCE FEATURES OF *L. MONOCYTOGENES* STRAINS ISOLATED FROM MEAT PRODUCTS

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Abstract

Listeria monocytogenes is an emerging bacterial foodborne pathogen responsible for listeriosis outbreaks. Frequently, listeriosis is transmitted through cured or processed meat, poultry, fish, seafood, dairy products, vegetables. This illness is characterized by septicaemia, meningitis, encephalitis and abortive disease (stillbirth or premature birth of the fetus). Listeria monocytogenes isolates from raw and processed meat were studied for the production of cell associated (adherence to HEp-2 cells) and enzymatic virulence factors, i.e.: pore forming toxins (hemolysine, lecithinase, lipase) and exoenzymes (gelatinase, amylase, caseinase, esculinase, DNase). The majority of the tested strains revealed adherece to HEp-2 cells with a predominant diffuse-aggregative pattern, as well as hemolysine, esculinase, caseinase and lipase. All L. monocytogenes strains harbored the hlyA gene. The presence of different virulence features in L. monocytogenes strains isolated from food products may explain the implication of these strains in the occurrence of severe illness.

Key words: *Listeria monocytogenes, meat products, virulence factors.*

INTRODUCTION

Taxonomically, *Listeria* genus is divided into ten species: *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, L. grayi* (McLauchlin, 2005), and the new described species *L. rocourtiae* (Leclerq et al., 2010), *L. marthii* (Graves et al., 2010), *L. fleischmanii* (Bertsch et al., 2013) and *L. weihenstephanensis* (Lang Halter et al., 2013). From them, only *L. monocytogenes* and *L. ivanovii* are pathogenic (Liu, 2006). *L. monocytogenes*, an important human and animal pathogen, is responsible for major outbreaks associated with food products. Although low-level *L. monocytogenes* contamination of meat products is relatively common, suggesting widespread exposure, foodborne listeriosis occurs in only a small proportion of susceptible individuals. In pathophysiology of

listeriosis, a main role is played by many virulence factors of this microorganism. In the present study, *L. monocytogenes* strains isolated from raw meat and meat products were tested for the presence of soluble virulence factors and ability to adhere to HEp-2 cells, as well as for the presence of *hlyA* gene.

MATERIALS AND METHODS

Bacterial strains

L. monocytogenes 1 (ATCC 19111) reference strain used in the study was obtained from NIRDMI Cantacuzino Bacterial Collection, Bucharest.

The experiment was performed on *L. monocytogenes* strains, collected from NIRDMI Cantacuzino *Zoonosis* Laboratory Collection, isolated from animal meat: 4 strains in raw meat - raw minced meat, pork and beef muscular tissues, poultry (carcass); 5 strains in meat products - sausages and other pork and beef preparations and 1 strain from boiled shell snails (Table 1).

For the detection and confirmation of *Listeria* spp. and *L. monocytogenes* there were used SR ISO 11290/2004 standards (Part 1 and Part 2). The following steps were followed: resuscitation, enrichment in demi-Fraser broth, selective enrichment in Fraser broth, isolation on 7% sheep blood agar and identification on PALCAM agar. Green colour colonies surrounded by a black zone on PALCAM agar plates were collected for further biochemical confirmation (CAMP test, carbohydrates use reactions), along with the reference strain *L. monocytogenes* type 1 ATCC 19111.

All the samples were subjected to serotyping tests performed with hiperimmune rabbit adsorbed sera against *L. monocytogenes* serotype 1a and *L. monocytogenes* serotype 4b (Table 1).

The stock cultures were maintained at -80ºC in Brain Heart Infusion (BHI) broth (Oxoid) with 20% glycerol and next there were streaked on 7% blood agar plates at 37ºC for 24 hrs, prior the experiment.

Table 1. Source of isolation and identification/confirmation of *L. monocytogenes* from the investigated strains (No.)

Samples	*B-hemo-lysis*	*CAMP Test*		*Acid from*				*L. monocytogenes Serological identification*	
		Staphylococcus aureus	*R. equi*	*D-Glucose*	*D-Manose*	*L-Rhamnose*	*D-Xylose*	*serotype 1a*	*serotype 4b*
Raw minced meat (pork, beef)	1	1	-	1	1	1	-	1	-
Pork muscular tissue	1	1	-	1	1	1	-	1	-
Beef muscular tissue	1	1	-	1	1	1	-	1	
Poultry carcass	1	1	-	1	1	1	-	-	1
Paste of Romanian sausages	2	2	-	2	2	2	-	2	-
Fresh sausages	2	2	-	2	2	2	-	2	-
Smocked bacon	1	1	-	1	1	1	-	1	-
Boiled shell snails	1	1	-	1	1	1	-	1	-

Characterization of bacterial adherence to HEp-2 cells

Adherence tests were performed on HEp-2 cells, by using Cravioto's adapted method (Cravioto et al., 1979 and Lazar, 2003). The cells were cultured in Eagle Minimum Essential Medium (EMEM), supplemented with 10% bovine fetal serum, without antibiotics. The HEp-2 cells were grown to 70-80% confluent monolayers in tissue culture plates. The cell monolayers

were washed 3 times with phosphate buffered saline (PBS) and to each well was added 1 mL of fresh medium without antibiotics. Suspension from bacterial mid-logarithmic phase cultures grown in nutrient broth was adjusted to 10^8 CFU/mL and 1 mL was used for inoculating each well. The inoculated plates were incubated for 2 hrs at 37°C. After the incubation period, cells were washed 3 times with PBS, fixed with 70% methanol for 3 min, and stained with 10% Giemsa solution for 20 min. The plates were washed, dried at room temperature overnight, and examined by light microscopy (x2500) to evaluate the adherence patterns.

In vitro characterization of enzymatic virulence factors

Bacterial strains grown for 18 hrs incubation at 37°C, in nutrient broth, were cultivated on available media containing specific substrate for enzymes activity detection (Delcaru et al., 2012).

Lecithinase and **lipase** are enzymes implicated in pore production and bacterial invasion. Their activity was tested by spotting onto 2.5% yolk agar, respectively Tween 80 agar with a substrate at a final concentration of 1%. After incubation at 37ºC for 72 hrs, the reactions were read. A positive reaction was considered as a clearing zone surrounding the growth area for lecithinase activity, respectively an opaque (precipitation) area for lipase activity.

Caseinase and **gelatinase** are enzymes implicated in the tissue damage, evolution of infection process and rapid bacterial multiplication. For detection of these enzymes' production, the tested strains were spotted onto agar plate with 15% soluble casein, and respectively on agar with gelatin at 1% final concentration, after incubation at 37°C for 72 hrs, the reactions were examined. Proteolytic activity is showed by an opaque (precipitation) area for caseinase production, and respectively a transparent zone around the culture for gelatinase.

The **amylase** activity, an enzyme implicated in the polysaccharides hydrolysis, offering a nutritional competitive advantage to producing bacterial strains, was determined using starch as a substrate (1% final concentration in nutritive glucose). After 72 hrs incubation at 37°C the reaction was read. Starch hydrolysis appeared as a precipitation zone surrounding the culture spot.

DNase production, an enzyme that catalyses the hydrolytic cleavage of cellular DNA, with lesions in host cell, was studied by spotting the strains onto DNA agar medium. After incubation for 24 hrs at 37°C, a drop of HCl 1N solution was added to each spotted culture and the reactions were

examined. A clearing area surrounding the spot area was registered as a positive reaction.

PCR analysis

For molecular confirmation of the biochemically and serologically identified *L. monocytogenes* strains PCR was used for the detection of *hlyA* gene, which encodes for the main virulence factor of *L. monocytogenes*, listeriolysin O.

Preparation of bacterial DNA. Bacteria were cultured in 1 ml BHI overnight then centrifuged and resuspended in 1 ml of sterile water (2x) for washing. Genomic DNA was extracted from the isolates by boiling at 100^{o}C for 10 min followed by cooling at -20^{o}C. The primers used in this study are listed in Table 2.

Table 2. Primers used for the PCR assay

Primer	**Sequence**	**Gene**	**Source**
LL7 LL8	TTG CCA GGA ATG ACT AAT CAA G ATT CAC TGT AAG CCA TTT CGT C	*Hly A*	Amagliani, 2004

Amplification was performed in a total reaction volume of 25 µl. The reaction mixture contained 12.5 µl PCR Master Mix 2x, 0.5µl each primer, 1µl of template, and double-distilled H_2O was added to make a total volume of 25 µl. The parameters for the amplification cycles were as follows: denaturation for 15 s at 95^{o}C, annealing of primers for 20 s at 61^{o}C, and primer extension for 30 s at 72^{o}C (50x). Prior to the first cycle, the PCR mixture was incubated for 15 min at 95^{o}C. After the last cycle, the mixture was incubated for 5 min at 72^{o}C for the final elongation. The *L. monocytogenes* type 1 (ATCC 19111) strain was used as known positive strain in PCR analysis. The PCR products were analyzed by electrophoresis in 1.5% agarose gel.

RESULTS AND DISCUSSIONS

During this study we have studied the virulence feature of *L. monocytogenes* strains isolated from different food products. The tested strains formed β - haemolytic colonies on blood agar plate, while on PALCAM agar they have shown green colour colonies surrounded by a black zone, indicating the presence of esculinase, responsible for esculine

hydrolysis, resulting esculetol, which could act as an iron chelating agent, providing iron even in limited conditions. When tested for fermentation reactions, *L. monocytogenes* strains were positive for D-Glucose, D-Manose, L-Rhamnose and negative for D-Xylose (Table 1). CAMP test was positive, with an arrow head shape haemolysis in the presence of *Staphylococcus aureus* (Figure 1).

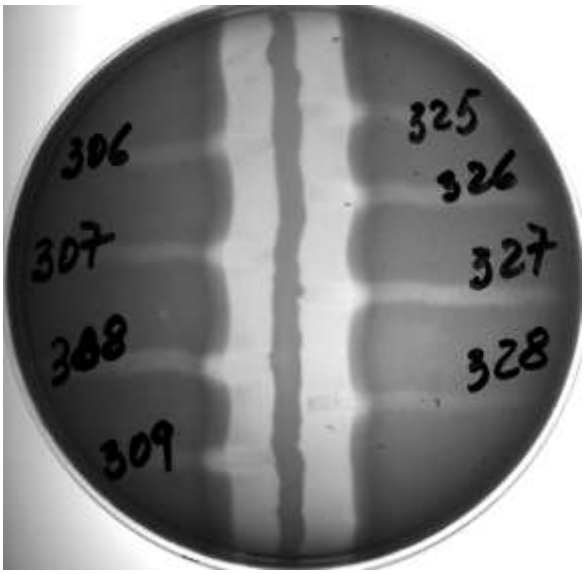

Figure 1. Positive CAMP Test for *L. monocytogenes* strains in the presence of *S. aureus* strains

The microbial adherence to different substrata, specially to epithelial cells, represents a *sine qua non* condition for the colonization of the host organism and the progression of the infectious process.

The *L. monocytogenes* strains exhibited three distinct adherence patterns to HEp-2 cells, i.e.: localized adherence, in which bacteria form characteristic microcolonies adhered on the surface on the host cell (Figure 2a), diffuse adherence, in which isolated *L. monocytogenes* cells adhere uniformly to the whole surface of the eukaryotic cell (Figure 2b), and aggregative adherence, in which large bacterial aggregates adhere both to the host cell surface and between them in a stacked brick appearance (Figure 2c). Some mixed patterns, i.e.: diffuse-localized, aggregative-localized and diffuse-aggregative have been also observed.

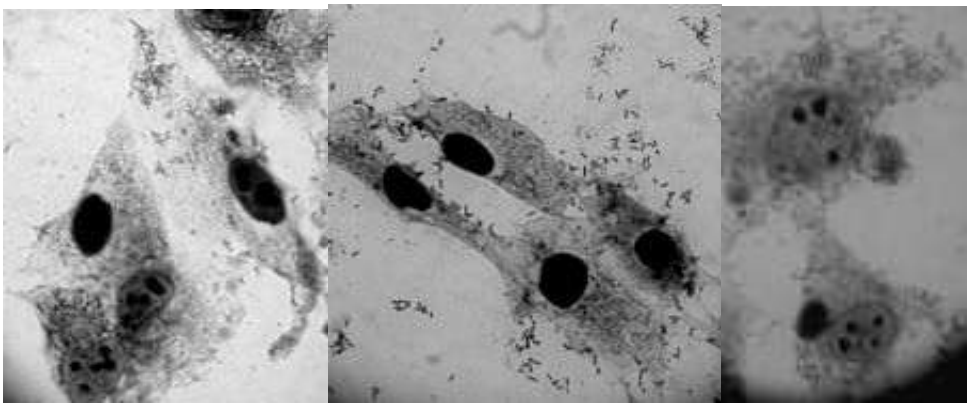

Figure 2. a. Localized adherence - characteristic microcolonies on the surface of HEp-2 cells. b. Diffuse adherence – bacterial cells dispersed over the cell surface. c. *L. monocytogenes* diffuse-aggregative adherence - both to the host-cell surface and between them in a stacked brick appearance (x2500).

In this study, the majority of the tested strains adhered to HEp-2 cells with a predominant diffuse-aggregative pattern (Table 3).

Table 3. Adherence patterns of *L. monocytogenes* strains isolated from meat

Adherence patterns		***L. monocytogenes (No.)***	
		Serotype 1a	***Serotype 4b***
Localized adherence		1	-
Diffuse adherence		2	-
Aggregative adherence		1	-
Mixed pattern	*diffuse-localized*	1	-
	aggregative-localized	1	-
	diffuse-aggregative	3	-
Negative		-	1

The secretion of soluble virulence factors is very important for the evolution of *L. monocytogenes* infectious process. The expression of the tested soluble virulence factors is shown in Table 4.

Lipase, acting as pore-forming toxin, was present in the majority (9 from 10) of *L. monocytogenes* strains isolated from meat products.

Caseinase, which increases by its activity the progression of the infectious process, was revealed at all *L. monocytogenes* tested strains.

Lecithinase, gelatinase, amylase and DNase were absent in this food isolates.

Table 4. Presence of soluble virulence factors in *L. monocytogenes* strains isolated from meat.

Virulence factors	***Tested strains (Nr.)***	***Positive strains (Nr.)***
Lipase	10	9
Lecithinase	10	0
Caseinase	10	10
Gelatinase	10	0
Amylase	10	0
DNase	10	0
Esculinase	10	10
Listeriolysin O	10	10

All the analyzed strains have been confirmed by the PCR analysis of the hlyA gene (Figure 3).

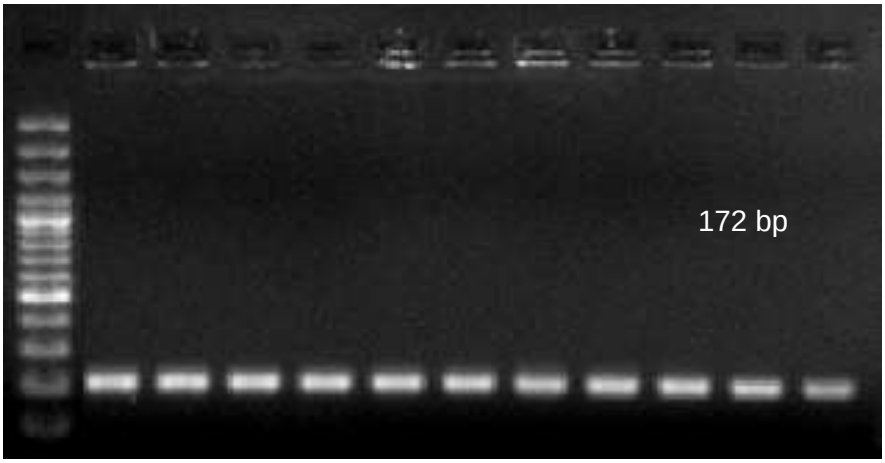

Figure 3. Agarose gel electrophoresis (1.5%) of *hlyA* gene amplification product: 1 = Ladder 100bp; 2–10 = *L. monocytogenes* 1a; 11 = *L. monocytogenes* 4b; 12 = *L. monocytogenes* ATCC 19111.

CONCLUSIONS

All investigated strains were confirmed as *L. monocytogenes* by biochemical activity and detection of *hlyA* gene, encoding listeriolysin O.
The majority of *L. monocytogenes* strains presented soluble virulence factors: lipase, caseinase, esculinase, and listeriolysin.
The adherence assay reveals correlations between the virulent character of tested strains and their source of isolation, the majority of them being with diffuse-aggregative pattern.
These tests can be used like *screening* methods for identification of pathogenic *L. monocytogenes* strains.

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REFERENCES

Bertsch D., Rau J., Eugster M.R., Lawson P.A., Lacroix C., Meile L., 2013. *Listeria fleischmanii* sp. nov., isolated from cheese. Int J Syst Evol Microbiol, 63, 526-532.
Cravioto A., Gross R.J., Scotland S.M., Rowe B., 1979. An adhesive factor found in Strains of *Escherichia coli* belonging to the traditional infantile Enteropathogenic Serotypes. Curr. Microbiol, 3, 95-99.
Delcaru C., Chifiriuc M.C., Dracea O., Iordache C., Limban C., Nitulescu G.M., Missir A.V., Chirita I.C., Badiceanu C., Bleotu C., Sakizlian R., Israil A.M., 2012. *In vitro* evaluation of the influence of compounds with tricyclic structure on the microbial growth and expression of virulence factors. African J Pharmacy and Pharmacology, 6(23), 1631-1638.
Graves L.M., Helsel L.O., Steigerwalt A.G., Morey R.E., Daneshvar M.I., Roof S.E., Orsi R.H., Fortes E.D., Milillo S.R., den Bakker H.C., Wiedmann M., Swaminathan B., Sauders B.D., 2010 – *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. Int J Syst Evol Microbiol, 60, 2280-2288.
Lang Halter E., Neuhaus K. and Scherer S., 2013 - *Listeria weihenstephanensis* sp. nov., isolated from the water plant *Lemna trisulca* of a German fresh water pond. Int J Syst Evol Microbiol, 63, 641-647.
Lazar V., 2003. Microbial Adherence. Romanian Academy Publishing House, Bucharest.
Leclerq A., Clermont D., Biset C., Grimont P.A.D., Le Flèche-Matéos A., Roche S.M., Buchrieser C., Cadet-Daniel V., Le Monnier A., Lecuit M., Allerberger F. 2010 – *Listeria rocourtiae* sp. nov. Int J Syst Evol Microbiol, 60, 2210-2214.

Liu D., 2006. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. J Med Microbiol, 55(6), 645-659.
McLauchlin J., 2005. *Listeria*. Topley&Wilson's Microbiology&Microbial Infections, 10th Ed, Bacteriology, vol. 2, ASM Press, London, chapter 37, 953-969
*** SR EN ISO 11290-1, 2000. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method, 1-25.
*** SR EN ISO 11290-2, 2000. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method, 1-24.
*** SR EN ISO 11290-2/A1, 2005. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method, Amendament 1: Modification of the enumeration medium, 1-4.

EFFECTIVE TEACHING-LEARNING METHODS AND TECHNIQUES APPLICABLE TO VETERINARY MEDICAL PRACTICE

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Abstract

This paper highlights the importance of diversifying teaching methods and learning techniques in practical training in veterinary medical field, aiming a more efficient instructive and educational process.
This study was performed using the methods: investigation and psycho-pedagogical experiment on groups of internships students in livestock units, veterinary laboratories and food processing units.
Within the scientific approach were investigated several teaching and learning methods and techniques: brainstorming, brainwriting, case study , simulation , reciprocal teaching and learning and stellar explosion.
After applying these methods and techniques, it was observed a significant progress in understanding and assimilation of content and better transposition of the theory in practice.
Interactive teaching strategies promote active learning, the students organized in small groups are working together and colaborating, to achieve and realize their objectives.
All teaching-learning investigated methods can be used successfully in the practical training of veterinary medical students.

Key words: *learning, medical, methods, practice, teaching.*

INTRODUCTION

Practice of speciality represents a method, a very efficient process in reaching to a specific result.
Practical training requires great skills not only from students but especially from practical coordinators and all those involved in the training process.
For obtaining the desired results, students need to be trained in different and various activities using effective an attractive methods of learning for them (Neacsu, 1999).
In this paper we intend to highlight some applicable teaching-learning methods wich are effective in veterinary medical practice.

MATERIALS AND METHODS

In veterinary medical fields, practice is conducted in specialized units (farms, laboratories, sausage factories, breeders of birds etc.), where students attend and participate to certain veterinary activities, coordinated by veterinarians, helping the investigation, diagnosis and the applying of appropriate treatments for some diseases. Most indicated teaching strategies in veterinary practice could be:

Methods and techniques based on solving problems (brainstorming, techinique 6/3/5)e.g. performing differential diagnosis, techniques for semen collection, diagnosis of podal affections;

Methods and techniques based on experience (case study method, simulations); e.g. dystocia simulation;

Methods and techniques for development of comunicative competence (reciprocal teaching-learning method) e.g. surgery;

Methods and techniques of interactive-creative learning (stellar explosion) (Dogaru-Ulieru, Draghicesu, 2011).

Methods are carried out in several stages as seen in Figure 1.

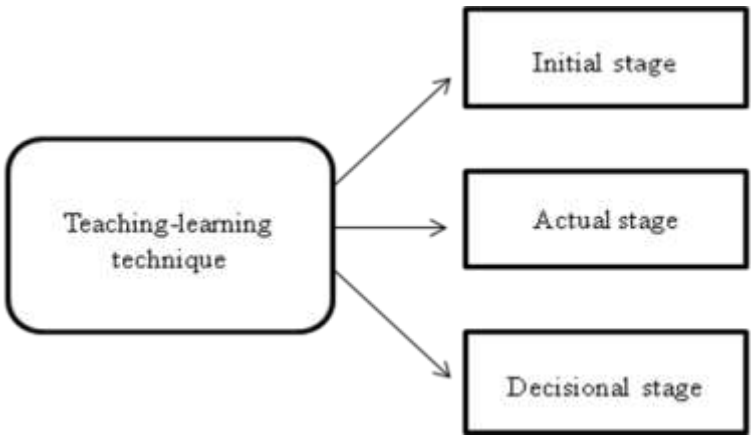

Figure 1. Working stages of teaching-learning techniques

RESULTS AND DISCUSSIONS

Brainstorming (assault of ideas) purpose is solving of a case by issuing a large number of solutions, ideas. The method is conducted within a group of up to 30 students, the theacher/ the coordinator assuming the role of moderator.

The rules of this methods are: do not criticize any suggestion, all ideas has knowledge character; are required the ideas of all group members; the quantity of ideas is less important than the quality.

The method is carried out in several stages: preliminary stage comprising three phases (phase of organizing, creative training and preparation phase of the session, the second stage of issue of creative alternatives by fixing the case, the problems to debate and by solving subproblems through issuance of ideas; the third stage which aimes the issued selection ideas – the list of ideas is analyzed and they opt for the final solution (diagnosis and treatment).

Figure 2. Teaching in a pig farm

Technique 6/3/5 (brainwriting) is realized in writting, it is similar with brainstorming. The ideas are noted on the sheet (six students issue three solutions in five muntes).

Also this method involves several steps: firstly the students are divided into groups of six, in the second stage, each student receives a sheet as shown in table 1; in the next stage, for the problem we discuss, each student

notes on sheet three ideas in five minutes and then the sheets walks fom left to right until the original teacher ; finally the solutions are analyzed and selected the correct ones.

Table 1. Brainwriting worksheet type (by Negreţ-Dobridor, Pâinişoară,2005)

Problem/case:			
Student/ Name and First name	Idea 1	Idea 2	Idea 3

Case study method

This method target is the analysis of one or more cases after which is possible to reach general conclusions. It is used for the creative application of one experience already appropriated, and not for enriching the knowledge with new aquisitions.

The stages of this method are: presentation of the case (clear, accurate, complete); clarify the possible misunderstandings; individual case study (students are documenting, they identify solutions to solve the case and complete the observation worksheet); the possible diagnoses are discussed in group (is made the differential diagnose), it takes a decision regarding the suitable solution (diagnosis and treatment); the solutions are evaluated with the students (Oprea, 2006).

Figure 3. Teaching in a milk processing unit

Simulations

Students are faced with the situation to live an experience of learning simililar to real experience, without negative consequences.

Firstly are determined the objectives of learning, then ensure the necessary materials for simulation (instruments, casts,consumables etc.); the team is divided into minigroups (if necessary) and is fixed the working time. The method is completed by making activity reports in which students describe the simulation activity (Painisoara, 2008).

The method of reciprocal teaching-learning

This method was introduced in 1986 by Palincsar and it refers to dividing the group of the students participating to the practice speciality in groups of reciprocal teaching-learning.

It involves four learning strategies: summarizing (exposure of what is important in the case study), making questions regarding the case presented, discuss the unclear informations and solving the misunderstanding, express students opinions about what will heapen next (evolution).

The ways it can be used:

The student group receives the case study and it is divided in four subgroups: first subgroup consists of „people to summarize'' summarize the esential informations; the second subgroup consists of „questioning people" (adress questions concerning the described case); third subgroup consists of „clarifying people" (clarify the possible unclear information); the fourth subgroup consists of „predictors" which presume the further evolution.

Finally, after discussions, each group exert their role in front of the other groups.

Stellar explosion

This method is similar to brainstorming, which develops the construction of ideas on ideas. Stellar explosion stimulates creation of questions to questions, so it can not be confused with brainstorming.

The problem to debate is written on the panel (to be seen) then are made as many questions are related to this method.

Stages: propose the problem; the group of students is divided into subgroups, everyone noting the problem; every subgroupe elaborates a list with questions about the proposed theme; are communicated the group activity results ; are highlighted the most interesting questions.

Within these activities students are involved in their own training.

Interactive teaching strategies improves the formation of some competences such as restructuring abilities and the practical use of knowledge, in training and development of investigation and exploration capacities, to value students knowledge and abilities in real situations, making the connection between theory and practice.
Interactive teaching methods has evident formative effects , but it is not excluded the possibility of, some manifestation limits as: lot of time to use, encourage passivity of students if the tasks are not carefully distributed by the teacher, the superficial treating of work tasks, difficulties in identifying and evaluating individual progress.
For all that teaching strategies has more advantages than disadvatages , for that we recommend practicing this to all teachers.

CONCLUSIONS

Interactive teaching strategies promote active learning, the students organized in small groups are working together and colaborating, to achieve and realize their objectives;
The teacher becomes a facilitator of learning activities, an organizer of a learning environment tailored on students specific needs and particularities;
All teaching-learning investigated methods can be used successfully in the practical training of veterinary medical students.

REFERENCES

Dogaru-Ulieru V., Drăghicescu Luminiţa – „Educaţie şi dezvoltare profesională”, p.142-173, Editura Scrisul Românesc, Craiova, 2011;
Neacsu I.,- „Instruire si Invatare”, p. 60-61, Editura Didactica si Pedagogica., Bucuresti, 1999;
Negreţ-Dobridor I., Pâinişoară I.O. – „Ştiinţa învăţării. De la teorie la practică”, p.215 Editura Polirom, Iaşi, 2005;
Oprea C.L. – „Strategii didactice interactive”, p. 220-221, Editura Didactică şi Pedagogică, Bucureşti, 2006;
Pâinişoară I.O. – „Comunicarea eficientă” ediţia a III-a, p. 376, Editura Polirom, Iaşi, 2008;

ASSESSMENT OF OWNER'S PERCEPTION CONCERNING ROLE OF NEUTERING AND SPAYING IN WELFARE OF DOGS

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Abstract

The study involved 400 non-sterilized dogs (232 males, 168 females) and 300 sterilized dogs (123 males, 177 females). Both groups have been divided in three subgroups: purebred, crossbreed and adopted community dogs. Four pathological events were questioned and correlated with spaying *and* neutering: obesity, *pyometra, nervous lactation after pseudo-pregnancy and prostatic hyperplasia.*
In our study, reproductive disorders imposed the sterilization in 127 dogs (51.18% males, 48.81% females), of which: 37.00% (47/127) purebred, 41.74% (53/127) crossbreed and 21.26% (27/127) adopted community dogs. Prevalence of obesity was 18.40% (46/250) in purebred, 30.00% (72/250) in crossbred and 18.00% (36/200) in adopted community dogs. A correlation between obesity and sterilization in the population of dogs studied was not observed. Pyometra prevalence was 21.05% (24/114) in purebred, 18.26% (21/115) in crossbred and 16.19% (17/105) in adopted community bitch. Nervous lactation prevalence was 71.05% (81/114) in purebred, 58.26% (67/115) in crossbred and 19.05% (20/105) in adopted community bitch. Prevalence of prostatic hyperplasia was 10.29% (14/136) in purebred, 2.22% (3/135) in crossbred and 7.37% (7/95) in adopted community dogs. The highest prostatic diseases in group of purebred males were correlated with sterilization in old ages (86.95% after 2 years age). The low prevalence of prostatic diseases in crossbred and community males were correlated with high proportion of early age neutered dog (58% before 2 years age). Therefore, early sterilization of non-breeding dogs could be a good decision to reduce the unnecessary distress associated with reproductive pathology.

Key words*: animal health, dog hygiene, pest control, vaccination.*

INTRODUCTION

Analysed often enough in terms of its impact on the quality of the animal life, sterilization continues to suscite polemic when is carried out on request and not out of necessity (Arkow, 1998; Fielding *et al.*, 2002; Fusfeld, 2007). Among the advantages of sterilization, reducing the reproductive apparatus related diseases and breast neoplasia, reducing the associated pregnancy and parturition pathologies, such as metritis, mastitis and dystocia, reducing the hormono-dependent disorders and mammary hypertrophy and reducing the unwanted sexual behaviour are included (Romagnoli, 2008).

Among the disadvantages of sterilization can be retained: anesthesia and surgical complications, increased risk of malignancies of various apparatus and systems of organs, increased incidence of musculoskeletal and endocrine disorders, obesity and urinary incontinence (Angioletti *et al.*, 2004; Brodbelt, 2009; Burrow *et al*, 2005; Knapp *et al*., 2000; Pollari *et al*., 1996).

In this context, evaluation of owners' perception about the sterilization advantages and disadvantages, knowledge the age at which owners prefers to sterilize their dog, the reason of sterilization and the main pathological events correlated with reproductive system can be helpful in assessing animal welfare.

This study was designed to give key information about the owner's knowledge of dog welfare and the role of sterilization in welfare.

MATERIALS AND METHODS

Seven hundred questionnaires completed by dogs' owners from Bucharest have been reviewed in order to assess the effect of the dog sterilization/non-sterilization on welfare.

The descriptive epidemiological study involved 400 non-sterilized dogs (232 males, 168 females) and 300 sterilized dogs (123 males, 177 females). Both groups have been divided in three subgroups: purebred, crossbreed and adopted community dogs. Purebred group included dogs with known origin, declared by owner with both parents from the same breed. Crossbred group included dogs with known origin, declared by owner with parents from different breeds. Community dogs group included adopted dogs, declared by owner without known origin. For the sterilized animals were determined the age of animals at that time and the reason of sterilization.

The survey form contained questions designed to determine the owners' knowledge in dog welfare issues and to obtain information about how owners care for their dogs in correlation with reproductive system. Also, questionnaire contained questions about four pathological events that could be correlated with female spaying or male neutering: obesity, pyometra, nervous lactation after pseudo pregnancy and prostatic hyperplasia.

RESULTS AND DISCUSSIONS

Interviews with 700 owners in Bucharest city indicated that many people have a limited knowledge of the law and animal welfare issues and welfare

perception is heterogeneous and even contradictory from one owner to another.

Out of the total of females that were not sterilized, 48.00% (81/168) were purebred, 39.88% (67/168) were crossbred, and 12.12% (20/168) were adopted community dogs (figures 1 and 2). Considering the high female reproductive value of pure breeds, research pursued and identified the reasons that led to the rather large percentage of purebred females that were sterilized, 28.95% (33/114), respectively, out of the total purebred females that were subject to this survey.

Decision to sterilize the purebred females (44/125) was taken in 77.77% (34/44) of cases after the age of 24 months, whereas in 54.54% (24/44) of cases the decision was taken out of necessity (figure 3). In contrast, adopted community female category was dominated by sterilized specimens, representing 80.95% (85/105). Sterilization in adopted community females was decided for 82.35 % (70/85) before the age of 24 months, 80% (68/85) was decided upon request and only 20% (17/85) out of necessity. It is important to note the polarization of the decision to perform castration depending on the category of breeds: out of 115 females sterilized upon request, 17.39% (20/115) were purebred, 23.48% (27/115) crossbred, and 59.13% (68/115) adopted community females; out of 62 females sterilized out of necessity, 38.71% (24/62) were purebred, 33.87% (21/62) crossbred, and 27.42% (17/62) adopted community females (figure 4).

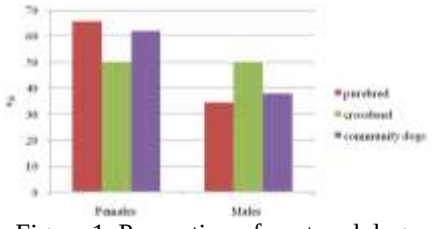

Figure 1. Proportion of neutered dogs

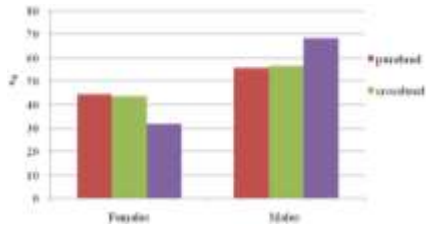

Figure 2. Proportion of non-neutered dogs

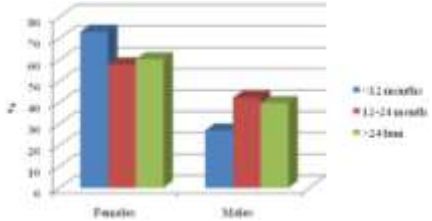

Figure 3. Proportion of the age groups when dogs are sterilized

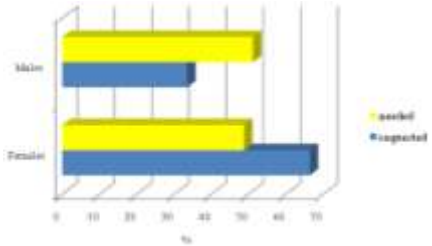

Figure 4. Motivation of dogs' sterilization

Prevalence of obesity, pyometra, nervous lactation after pseudo pregnancy, prostatic hyperplasia and cryptorchidism are listed in table 1.

Table 1. Morbid conditions diagnosed in the population of dogs investigated

Pathological issues	**Dogs**							
	Total		Purebred		Crossbred		Adopted community dogs	
	No.	%	No.	%	No.	%	No.	%
Obesity	154/700	22.00	46/154	29.87	72/154	46.75	36/154	23.38
Pyometer	62/345	17.97	24/62	38.71	21/62	33.87	17/62	27.42
False lactation	168/345	48.70	81/168	48.21	67/168	39.89	20/168	11.90
Prostate hyperplasia	24/355	6.76	14/24	58.33	3/24	12.5	7/24	29.17
Cryptorchidism	12/355	3.38	9/12	75.00	3/12	25.00	0/12	0.00

Out of the 700 dogs evaluated, 22% (154/700) were declared by owners as obese. It is noted that 46.75% (72/154) of cases of obesity belonged to crossbred, and the remainder in roughly equal proportions of other categories, 29.87% (46/154) purebred and 23.38% (36/154) of adopted community dogs. This situation might be due to either overeating, lack of movement, or a combination of these. Analyzing environmental conditions offered, it appears that most dogs stay in apartments (77.71%, 544/700); restriction of movement in house may play a role in apparition of obesity, especially if the food intake is not correlated with the status of sedentary; also, it observed that 55.29% of dogs received rewards between meals.

Obesity seems to be one of the most common issues raised by veterinarians in the United Kingdom (Yeates and Main, 2011). Other retrospective studies have singled out a prevalence of obesity in the canine population of up to 2.80% (Mason, 1970; David and Rajendran, 1980). In the present study, the main contributory factors of obesity are sterilization in association with living in apartments. Other risk factors of obesity have also been reported, such as old age, overweight owners or owners older than 40 years, as well as the breed of the dog (Colliard, 2006). Breeds with highest risk of becoming obese are Beagle, Cairn Terrier, Cavalier King Charles Spaniel, and Labrador Retriever (Mason, 1970; Edney and Smith 1986; Crane, 1991; Colliard, 2006).

Nervous lactation or pseudo-gestation was recorded in surveys by 50.29% (168/334) owners of females out of which 24.25% (81/334) owned purebred females, 20.05% (67/334) crossbred, and 5.99% (20/334) adopted community females. The prevalence of false lactation was of 71.05%

(81/114) in purebred, 58.26% (67/115) crossbred and 19.05% (20/105) adopted community females.

Pyometra seems to be a rather serious problem in the population of females included in the research (18.56% 62/334), however it was smaller than in other studies (Hagman *et al.*, 2011) and was not associated with postsurgery morbidity or mortality (Johnston *et al*., 2001). Pyometra was most frequently detected in purebred females (38.71%, 24/62), but the difference was not large compared to the crossbred females (33.87, 21/62), and adopted community females (27.42, 17/62). Placing purebred females and crossbred on the first two positions is also correlated with increased proportion of sterilized females aged over 10 years in these categories. It is known that the incidence of pyometra in female dogs that were not sterilized after the age of 10 years is of 24-25% (Hagman *et al.*, 2011). In this study it was noticed that the prevalence of pyometra was of 21.05% (24/114) for purebred, 18.26% (21/115) for crossbred, and 16.19% (17/105) for adopted community females.

Correlating these data with the data presented above, pyometra was the only cause of necessary sterilization of females. This is considered the method of restoration in the shortest time of the welfare of the females, with the lowest medical risks. Although the prevalence of nervous lactation was recorded for over half of the females, a correlation between nervous lactation and the decision of sterilization, including dogs already sterilized, could not be made.

The decision-making mechanism of male sterilization is largely the same to that observed in females. In purebred male stands absence of sterilization on demand (0/23), all cases are the result of conditions that imposed sterilization in order to restore those dogs' welfare as soon as possible. However, males that were not sterilized present an increased risk for the development of many diseases in old age, such as testicular cancer and prostate diseases; therefore, the recommended age for sterilization is six months (Dodman, 1999). 65.35% (232/355) out of the males were not sterilized, where 43.97% (102/232) were purebred dogs, 37.5% (87/232) crossbred, and only 18.53% (43/232) adopted community dogs. As for the females, there is the same tendency to decide upon sterilization mainly within the first 24 months of life for adopted community females (80.79%, 42/52), and mainly after 24 months of life (86.95%, 20/23) for purebred females.

In addition to health problems, the males that were not sterilized may create behavioral problems to their owners, due to elevated levels of testosterone.

Dogs can perceive the smell of a female in heat from great distances and will try to escape the leash or the courtyard, running free and thereby exposing themselves to the risk of accidents, fights with other males for dominance, and even abandon (Dodman, 1999). Even more, if they fail to escape, some males that are not sterilized can express aggression or other unwanted behaviors on their owners (Overall, 1997; Murray, 2008). Generally, sterilized dogs integrate better as pets. It is considered that by sterilizing the risk to escape from the courtyard or from the leash drops by 90%, aggression towards other males, dominant aggression and marking and with urine instinct drops by 60% (Landsberg, 1997). However, sterilization should not be used as a substitute for appropriate training. In some cases, sterilization rather reduces the frequency of certain behaviors than to eliminate them.

In this study, out of the male-specific diseases, the owners reported, for the non-sterilized dogs, enlarged prostate, and cryptorchidism. Out of the 36 reported cases, 66.66% involved prostate and 33.34% the testicles. The prevalence of these diseases in all males was 6.76% (24/355) for the prostate diseases and 3.38% (12/355) for cryptorchidism. The most frequent prostate illnesses were found in purebred dogs, 58.33% (12/24) of all cases; and in the group they were reported in 10.29% (14/136) of the males in this group. Crossbred dogs accounted for only 12.5% (3/24) of total cases of prostate disease reported, and in the group of crossbred 2.22% (3/135) of the males in this group. Adopted community dogs represented 29.17% (7/24) of all prostate diseases reported and within this group to 7.37% (7/95) of the community males. Cryptorchidism was reported only by owners of purebred dogs or crossbred dogs, 75% (9/12) in purebred and 25% (3/12) in crossbred. The purebred in this group had a prevalence of 6.62% (9/136) and the crossbred 2.22 (3/135).

In the context where the risk of malignancy of cryptorchid testicles is increased, cryptorchid surgery is often recommended. Analyzing purebred males undergoing sterilization surgery, it is found that any male having a prostate disease and the dogs with cryptorchidism were sterilized. Consistent with the recommendations of the current veterinary medical practice is that benign hyperplasia of the prostate is a very common condition of elderly dogs, with a reported incidence of 50% for the age of 2.5 years and 75-80% for dogs older than 6 years (Zirkin and Strandberg, 1984; Lowseth *et al.*, 1990).

It was observed that the decision of obligatory castration crossbred and adopted community dogs had also other reasons than prostate diseases and

cryptorchidism. A rate of 81.25% (26/32) of crossbred males and 30% (3/10) of adopted community dogs underwent necessary sterilization for reasons other than prostate disease or cryptorchidism.

CONCLUSIONS

Comparatively with the purebred dogs (23.33%, 67/300), a higher proportion of crossbred (32%, 96/300) and adopted community dogs (45.66%, 137/300) in the neutered group has been observed. This can reflect the interest in reducing the community dogs in the population of pets.
Adopted community dogs were usually neutered under 24 months old (81.75%112/137), while purebred dogs over 24 months (80.60%, 54/67). Castration was necessary to 42.33% (127/300) dogs (48.81% females and 51.18% males), of which: 37.00% (47/127) purebred, 41.73% (53/127) crossbred and 21.27% (27/127) adopted community dogs.
The prevalence of false lactation was 71.05% (81/114) in purebred, 58.26% (67/115) in crossbred and 19.05% (20/105) in adopted community females. The prevalence of pyometra was 21.05% (24/114) in purebred, 18.26% (21/115) in crossbred, and 16.19% (17/105) in adopted community females. The prevalence of prostate hyperplasia was 6.76% (24/355). The prevalence of cryptorchidism was 3.38% (12/355). Early sterilization of non-breeding dogs could be a good decision to reduce the unnecessary distress associated with reproductive pathology.

REFERENCES

Angioletti A, DeFrancesco I, Vergottini M, Battocchio ML, - Urinary incontinence after spaying in the bitch: incidence and oestrogen therapy. Vet Res Commun, **28**(Suppl 1), 153–155, 2004.

Arkow, P. - Application of ethics to animal welfare, Applied Animal Behaviour Science; **59**(1–3):193-200, 1998.

Brodbelt, D. - Perioperative mortality in small animal anaesthesia. Vet J **182**:152–161, 2009.

Burrow, B., Batchelor, D., Cripps, P. - Complications observed during and after ovariohysterectomy of 142 bitches at a veterinary teaching hospital. Vet Rec, **157**:829–833, 2005.

Colliard, L., Ancel, J., Benet, J.J., Paragon, B.M., Blanchard, G. - Risk factors for obesity in dogs in France. J Nutr. **136**:1951–1954. 2006:

Crane, S.W. - Occurrence and management of obesity in companion animals. J Sm Anim Prac, 32:275–282, 1991.

David, G., Rajendran, E.I. - The after-effects of spaying in bitches and cats. Cheiron, **9**:193–195, 1980.
Dodman, N. - Dogs Behaving Badly: An A-to-Z Guide to Understanding and Curing Behavioral Problems in Dogs. Bantam Books, pp 186-188, 1999.
Edney, A.T.B., Smith, P.M. - Study of obesity in dogs visiting veterinary practices in the United Kingdom. Vet Rec **118**:391–396, 1986.
Fielding, W.J., Samuels, D., Mather, J. - Attitudes and actions of West Indian dog owners towards neutering their animals: A gender issue?. Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals, **15**(3):206-226, 2002.
Fusfeld, L. - Sterilization in an Animal Rights Paradigm; J. Animal L. & Ethics; **2**:255, 2007.
Hagman, R., Lagerstedt, A.S., Hedhammer, A., Egenvall, A. - A breed-matched case-control study of potential risk-factors for canine pyometra. Theriogenology **75**:1251–1257, 2011.
Johnston, S.D., Root Kustritz, M.V., Olson, P.N. - Disorders of the canine uterus and uterine tubes (oviducts). In Johnston SD, Root Kustritz MV, Olson PN (eds), Canine and Feline Theriogenology. WB Saunders, Philadelphia PA, pp. 206–224, 2001.
Knapp, D.W., Glickman, N.W., DeNicola, D.B., Bonney, P.L., Lin, T.L., Glickman, L.T. - Naturally-occurring canine transitional cell carcinoma of the urinary bladder. A relevant model of human invasive bladder cancer. Urol Oncol **5**:47–59, 2000.
Landsberg, H. A. - The Handbook of Behaviour Problems of the Dog and Cat. Butterworth-Heinemann, p 32, 1997.
Lowseth, L.A., Gerlach, R.F., Gillett, N.A., Muggenburg, B.A. - Age-related changes in the prostate and testes of the beagle dog. Vet Path **27**:347–353, 1990.
Mason, E. - Obesity in pet dogs. Vet Rec, **86**:612–616, 1970.
Murray, L. - Vet Confidential: An Insiders's Guide to Protecting Your Pet's Health. Ballantine Books, p 206, 2008.
Overall, K. - Clinical Behavioral Medicine for Small Animals. Mosby Press, pp 262-263, 1997.
Pollari, F.L., Bonnett, B.N., Bamsey, S.C., Meek, A.H., Allen, D.G.- Postoperative complications of elective surgeries in dogs and cats determined by examining electronic and paper medical records. J Amer Vet Med Assoc **208**:1882–1886, 1996.
Romagnoli, S. - Surgical gonadectomy in the bitch and queen: should it be done and at what age? Proceedings, Southern European Veterinary Conference and Congreso Nacional AVEPA, Barcelona Spain, 2008.
Yeates, J. W., Main, D. C. J. - Veterinary surgeons' opinions on dog welfare issues. Journal of Small Animal Practice, **52**:464–468, 2011.
Zirkin, B.R., Strandberg, J.D. - Quantitative changes in the morphology of the aging canine prostate. Anat Rec **208**:207–214, 1984.

THE POTENTIAL USE OF NEAR-INFRARED SPECTROSCOPY FOR THE QUALITY ASSESSMENT OF EGGS AND EGG PRODUCTS

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Abstract
In a context of high productivity, eggs' quality assessment is necessary for enhanced safety and quality assurance towards the consumers and feedback for producers. The quality assessment of eggs and egg products is performed using destructive and time-consuming methods, therefore the use of rapid tools becomes mandatory, especially in the case of a high production rate. Near Infrared (NIR) spectroscopy is considered a very reliable and rapid technique with large use in food industry. At the farm level, NIR spectroscopy technique would be an interesting tool to determine the chemical and physical properties of eggs, eggshell and internal quality and, moreover, this information may help the layer farm manager when a problem occurs in the flock. Its application in the egg industry is aimed at the quality changes in eggs during storage and quality assessment of the egg products, through the compositional analysis. It is possible and in some cases successful the prediction and/or determination of different parameters such as: protein, total lipid and total solids content (for liquid egg products), polyunsaturated fatty acids (for freeze-dried egg yolk), moisture, fat and protein content (for spray-dried whole eggs). In addition, for the white colored shell eggs, the detection of blood and meat spots is also successful. Further studies with NIR and near infrared hyper spectral imaging system (NIR-HSI) are needed in this direction, as the results obtained until now are very promising for the development of a rapid tool for quality assessment of eggs and egg products.

Key words*: chemometric tools, eggs and egg products, NIR, non-destructive methods, quality assessment.*

INTRODUCTION

Egg quality is a general term which refers to several standards defined by external and internal quality. External quality is focused on shell cleanliness, shell integrity and pigmentation and eggs shape, whereas internal quality refers to egg white (albumen) viscosity, lock of meat and blood spots, size of the air cell, yolk shape, strength and color. Internal egg

quality involves functional, aesthetic and microbiological properties of the egg yolk and albumen (Leeson, 2006).
Nowadays new technologies, fast, fully automated, reliable and nondestructive, such as near infrared spectroscopy, fluorescence spectroscopy, nuclear magnetic resonance, vibration analysis, or analysis of acoustic vibrations offer the possibility to evaluate the quality of a complete batch (control all the eggs from a lot), and not only a sampling (Mertens et al., 2010). Bamelis et al. (2004) mention that the availability of powerful computers and new detection technologies has enabled the development of fast, objective and accurate technologies.
In 2010, Mertens et al. presented several novel methods for eggs quality assessment, as it follows:
– near infrared spectroscopy: the measurement of the shell color as an indicator of stress and the general health of laying hens (the transmission color value linked to dynamic stiffness are related to less healthy hens possibly producing less strong eggs which are more sensitive to breakage);
– fluorescence spectroscopy: shell color and albumen quality;
– nuclear magnetic resonance or vibration analysis: eggshell integrity, eggshell strength;
– analyses of acoustic vibrations: albumen quality, fishy taint, shell integrity and dirt detection.
In this review we will focus on NIR spectroscopy technique because it ensures fast, low cost, reliable and non-destructive measures. The objectives of this paper are to describe the principle of NIR spectroscopy analysis, its advantages and disadvantages, the benefit for egg quality analyses by NIR spectroscopy. Finally, overviews of possible applications in egg industry, for quality assessment are presented.

NIR SPECTROSCOPY – general facts

Principle and theory

NIR spectroscopy is a vibration spectroscopic method very close to visible region, where the organic and inorganic compounds show good reference or transmission proprieties (Aenugu et al., 2011). Different systems such as visible-near infrared spectroscopy (VIS-NIR, wavelengths range: 450-1,100 nm), fourier transform-near infrared spectroscopy (FT-NIR, wavelengths range: 800-2,500 nm) or NIR spectroscopy (wavelengths range: 750–2,500

nm) reflect the absorbance of electromagnetic radiation at different wavelengths (Huang et al., 2008).
Near Infrared (NIR) spectroscopy is a well known technology which allows chemical information acquisition from samples (Rodriguez-Otero, et al., 1997). By NIR spectroscopy, C-H, N-H and O-H bonds are induced to vibrate. This principle is used to identify and quantify components. NIR spectroscopy allows the acquisition of the reflectance spectra of opaque milled or intact materials (Fig. 1).

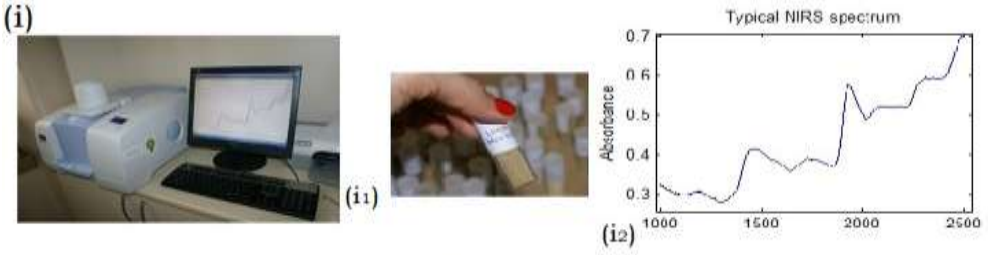

Figure 1. Acquisition of spectrum/spectra by conventional NIR spectroscopy system (source USAMV Cluj, Romania)

Legend: (i) NIR spectroscopy system (Forage Crops Lab, USAMV Cluj, Romania, Prof. PhD Rotar Ioan); (i_1) Sample support for NIR SPECTROSCOPY system; (i_2) Typical spectrum of NIR SPECTROSCOPY system.

NIR spectroscopy is characterized by the acquisition of a typical NIR spectrum which can be considered as the spectral signature or spectral fingerprint of the material. It is an adequate technique for the analysis of major components (chemical composition, detection and quantification of microorganisms) in agro-food products with minimum sample preparation.

Spectral analyses

Near infrared spectrum is recorded with the aim of getting information about the structure of a compound. In order to get efficient qualitative and quantitative information from data coming from NIR spectroscopy, chemometric tools are necessary further on (Roggo et al., 2005). Chemometrics is the science of extracting relevant informations from measurements made in chemical systems, using mathematical and statistical procedures (Massart et al., 1988). Qualitative and quantitative analyses by NIR spectroscopy usually require the application of calibration algorithms based on physicochemical measurements.

The building of calibration models starts with a spectral pre-processing treatment: after collection of spectra, it is necessary to perform a pre-treatment to remove high- or low-frequency interferences. Different types of pre-processing treatments are used: polynomial baseline correction, Savitzky - Golay derivative, Standard Normal Variate (SNV), mean-centering and unit variance normalization among others (Gowen et al., 2007).
After the spectral pre-processing treatment the calibration algorithms can be applied for classification and quantification. In order to analyse the data, many multivariate analytical tools are used, such as: principal component analysis (PCA), principal component regression (PCR), multi-linear regression (MLR), partial least squares regression (PLS), modified partial least squares regression (MPLS), partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (LDA) (Gowen et al., 2007).
The spectral pre-processing treatments and calibration algorithms are extensively reviewed in the literature (Roggo et al., 2005; Wise et al., 2006).
Assessment of calibration performance through NIR calibration model performances can be characterized by several parameters: standard error of calibration (SEC) or standard error of cross validation (SECV). For acceptable calibration model performance, an independent set of samples is used to get the standard error of prediction (SEP) and the squared coefficient of correlation (RSQ), which are used to describe the NIR analytical error when analyzing samples of unknown quantitative composition (Hartmann and Buning-Pfaue, 1998).

Advantages and Disadvantages

Major advantages of spectroscopic techniques are: the ease of use, repeatability and reproducibility, reasonable start-up cost, non-polluting, non-invasive and non-destructive analyses and the possibility of online or directly in the field implementation. By NIR spectroscopy, an average of spectra for one sample can be recorded.
The disadvantages of NIR spectroscopy are: the relatively high price of instruments; the requirement of huge hardware speed; the necessity of calibration models for standardization; the possible presence of spectrum which do not contain chemical information like for example bad spectrum (ElMasry and Sun, 2010), such as spies (Dale et al., 2012).

EGGS AND EGG PRODUCTS QUALITY

Table eggs composition and factors related to quality

The avian egg is characterized through its destination, the one of vehicle of reproduction, but also through its importance as a food product (Karoui et al., 2009). The most important characteristics related to eggs quality are: its nutritional composition, the shell characteristics and the quality of the internal components (the albumen and the yolk).

Eggs' nutritional composition is characterized by a high content of proteins and lipids, being considered one of the most complete food product for the human diet. Moreover, it contains several vitamins and mineral elements that are regarded as essential nutrients. The shell is considered the most important physical barrier for preventing microbial entry, therefore cracking and abrasive damage to this barrier enhances the possibility for microbial penetration and may lead rapidly to spoilage of the egg (Shebuski and Freier, 2009). Its composition includes: approximately 94 % of calcium carbonate, small amounts of magnesium carbonate, phosphate and proteins, as part of the organic matter (Nys and Gautron, 2007).

The albumen nutritional quality resides in its protein content, of approximately 10 % (Powrie and Nakai, 1986). Its quality depends very much on the storage conditions, due to the known loss of CO_2 and the pH increase. The albumen becomes thinner and more transparent due to the aging process, which involves also a change in the protein conformation (Karoui et al., 2009). Jin et al. (2011) observed that during an extended period of storage, from 2 to 21 days, and due to an increase in temperature, from 5 to 21 and further on to 29 ^{o}C, the albumen pH increased (mainly due to temperature value), while the percentage of the albumen significantly ($p < 0.001$) decreased.

The yolk represents 33 % of the liquid weight, and its composition consists of fat (almost all the fat in the egg) and proteins. Also, liposoluble vitamins are present in its composition, with a highlight on vitamin D, egg yolk being one of the few food products naturally containing vitamin D. The pH of the yolk is 6.0 and during storage it remains constant, due to no CO_2 losses occurring (Karoui et al., 2009). Jin et al., (2011) showed that the yolk weight increases significantly during the storage, with increasing storage temperature and time.

Eggs are purchased and consumed as such, but at the same time, they are a very important part of the food industry, as they are extensively used as

ingredients, due to the components' unique functional properties: foaming and gelling. For example, foaming is important in food industry for the production of bread, cakes, crackers, ice cream (Stadelman and Schmieder, 2002). As different changes occur during storage – the thinning of the albumen (Li-Chan and Nakai, 1989), the increase of pH value, weakening and stretching of the vitelline membrane and increase in yolk water content (Karoui et al., 2009) – their monitoring is very important for an immediate quality assessment. These changes depend mostly on storage time (Berardinelli et al., 2005), environmental temperature and relative humidity (Roberts, 2004), but also on hen age and strain (Silversides and Scott, 2001).

Table eggs and egg products and their quality indicators

UNECE standards for the certification and the control of shell eggs have been published in 2010. Concerning the grading of eggs, different parameters have to be observed, mainly regarding the physicochemical proprieties such as egg weight, shell quality (strength, color, cleanliness) and internal quality (freshness indicators).

The freshness is assessed through: air cell height (Sauveur and DeReviers, 1988) and Haugh unit measurements of the white (Silversides and Villeneuve, 1994). Storage temperatures as well as environmental conditions were the most significant factors influencing the quality of fresh eggs (Rossi et al., 2001). During storage, Kato et al. (1981) showed that a loss of CO_2 occurs from the egg through the pores in the shell, causing a thinning of the albumen. Also, Hill and Hall (1980) showed that the pH increases with the age of the egg, depending on the equilibrium between dissolved CO_2 bicarbonate ions, carbonate ions and proteins.

Rossi et al. (1995) proposed a new chemical index for the description of shell egg freshness, the determination of uridine ($C_9H_{12}N_2O_6$) and pyroglutamic acid ($C_5H_7NO_3$) concentrations. The index increase in the hen yolk or albumen is directly dependent on the storage temperature.

Considering the egg products, according to the UNECE standards for the marketing and commercial quality control of egg products (2010), these that are used nowadays in the food industry, are: the whole egg without shell (melange), liquid egg product, the egg yolk, the egg albumen, the frozen egg product, the dried egg product, the concentrated (condensed) egg product, the blended egg product, the fermented egg product, the stabilized egg product, the acidified egg product, the heat-treated egg albumen and the

salted or sugared egg product (UNECE, 2010). Different indicators are used in order to assess the quality of conventional egg products: minimum solids matter content, minimum fat content, minimum protein content, extraneous matter, minimum concentration of hydrogen ions (pH), maximum beta-hydroxybutyric acid, maximum lactic acid, maximum succinic acid (UNECE, 2010).

NIR SPECTROSCOPY APPLICATIONS TO THE QUALITY ASSESSMENT OF EGGS AND EGG PRODUCTS

Nowadays, NIR spectroscopy is used from a large scale to a microscopic level. For this reason, new areas of work based on the NIR technology have been developed combining NIR systems and a microscope to create the NIR microscopy (NIRM) (Baeten et al., 2012). More recently, NIR was combined with imaging technologies creating the Near Infrared Hyperspectral Imaging System (NIR-HSI) (Fernández Pierna et al., 2004). Near-infrared spectroscopy technology offers the possibility to quickly and non-destructively analyze different constituents and properties of a product (Benson, 1995). Frequently hyperspectral imaging technique was used to detect omega-3 fatty acids in designer eggs, egg embryo development, differences between egg‐shell cracks and other egg‐shell features, or detection of hatching and table egg defects.
By NIR spectroscopy, diversified broad area of applications in eggs and egg products quality were carried out, revealing the possibility to use this technique in different ways, with a highlight on: whole egg and albumen quality, blood and meat spots detection and compositional analysis of egg products.

Whole egg quality assessment

Eggs' freshness

Together with shell integrity, the freshness is considered as one of the most important criteria to classify whole eggs.
NIR spectroscopy was used by Schmilovitch et al. (2002) for the estimation of egg freshness, using the transmittance mode (530-1130 nm). By applying PLS regression to the data, NIR can predict the number of days after

hatching, the air chamber size, the weight loss as well as the pH value. The determination coefficient (R^2) varied from 0.90 to 0.92.
The use of NIR spectroscopy by Norris (1996) for the assessment of egg quality during storage revealed changes in the spectral data, occurring immediately after the lay, but without any correlation with the internal egg quality. However, as Kemps et al. (2007) revealed later, there is a great variation between eggs of the same age, possibly due to measurement errors, egg-dependent effects and different times of the analysis. Therefore, Bamelis (2003) used Vis/NIR to monitor the quality changes in eggs that were stored at 18^{o}C for 21 days. The transmission spectra were acquired daily on egg samples, giving a total number of 16 spectra for each egg. The spectra were scanned between 500 and 800 nm, with an integration time of 250 ms. An increase around 674 nm (correlated positively with an increase of the storage time) and a decrease around 663 nm (correlated positively with a decrease in Haugh units) were observed.
Using FT-NIR spectroscopy system (MATRIX™-F, Bruker Optics, MA, USA), Giunchi et al. (2008) acquired diffuse reflectance spectra in the range of 833 to 2500 nm. After each spectral acquisition, the freshness parameters (air cell height, thick albumen heights and Haugh unit) were also destructively measured. The predictive models showed an R^2 value of up to 0.722, 0.789 and 0.676 for air cell height, thick albumen heights and Haugh unit respectively, therefore the diffuse reflectance FT-NIRS appears to be able to discriminate shell eggs during storage.

Albumen quality

The work of Bamelis (2003) continued through Kemps et al. (2006), through a record of the transmission spectra of table eggs stored for 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 days at 18^{o}C, with a relative humidity of 55 %. Through Vis/NIR spectra, intact eggs showed a large variation considering the proportional transmission values between eggs with a comparable albumen quality. Further on, a PLS regression was applied and the results showed that the correlation coefficient between the measured and the predicted Haugh unit values were 0.84 and 0.82 for the calibration and validation set, respectively, with better results for the pH. The most information regarding albumen transmission values was found between 570 and 750 nm, the team attributing these bands to Maillard reaction, with products inducing the formation of melanoidins (Burley and Vadehra, 1989).

Blood and meat spots detection

According to De Ketelaere et al. (2004) blood and meat spots are considered the most common defects found in eggs, these being able to influence the choice of the consumer. For their detection, candling is currently used; however this method is imperfect and costly. Candling accuracy for the blood and meat spots' detection is also varying from 20 to 90 %, therefore the use of spectroscopic techniques such as Vis/NIR is regarded as an improvement in this direction.

The first study of using spectroscopic techniques for detection of the blood presence in the eggs was a trial of Brant et al. (1953). They started by assuming that the calciferous shell of the egg absorbs all transmitted light under 550 nm, therefore only the 577 nm absorption peak could be used in detection of the presence of blood in the eggs. Using this technique, they obtained good results with an accuracy of 99.70%. Later, Gielen et al. (1979) suggested that a wavelength between 585 and 610 nm should be chosen. Karoui et al. (2009) mention that the ratio between the two values has been called "blood value" and the ratio was used as an index.

De Ketelaere et al. (2004) showed that there is a difference between the transmission spectra of eggs containing blood and eggs without blood. These authors divided the transmission at 577 nm by the transmission at a reference wavelength in order to correct the values for eggshell thickness, egg size and other non-haemoglobin-related characteristics. Based on unpublished data, Karoui et al. (2009) mention that a small amount of blood in the albumen could only be detected when part of it is diffused in the albumen, while very small blood spots are often not accompanied by dispersed blood, and, consequently, hard to detect. A factor that has a great influence on the success of the meat and blood spots' detection is the color of the shell. It has been demonstrated that the rate of blood detection in white-colored shell eggs is higher than in brown colored shell eggs. The basis of this difference is the protoporphyrin, the brown pigment of the eggshell that presents optical properties which are very close to those of haemoglobin. This shows a band located around 589 nm, very close to the absorption peak of the haemoglobin (577 nm).

Compositional analysis of egg products

The analytical methods used nowadays for the compositional analysis of the egg products, in order to evaluate their quality, are time-consuming. The use of NIR reflectance may improve this, by a better time management. NIR reflectance was used to determine the moisture, fat and protein in spray-dried whole egg (Wehling et al., 1988), the results showing a SEC of 0.15 %, 0.20 % and 0.28 % respectively, using a calibration based on three wavelengths. The authors concluded that the use of additional wavelengths is mandatory for a successful analysis of this product.

Dalle Zotte et al. (2006) used NIR reflectance to assess its ability for the prediction of physicochemical composition of freeze-dried egg yolk samples from laying hens fed with four different diets enriched with different sources of *n*-3 polyunsaturated fatty acids. The samples were analyzed using NIR spectra between 1100 and 2498 nm. The results showed an unsuccessful prediction of pH and cholesterol. However, the prediction of polyunsaturated fatty acids was accurate, with an R^2 value of 0.98 for the alpha-linoleic acid. Contrary to this, for the pH, R^2 value was 0.21, 0.75 for the crude protein, 0.81 for the dry matter, and 0.06 for the ash.

A new way for the use of NIR spectroscopy in the egg products industry is the prediction of the F_{70}^{10} parameter (Zardetto, 2005). Usually, thermal treatment causes a reduction in the nutritional value of the food, as a result of the Maillard reaction, which makes amino compounds biologically unavailable. The F_{70}^{10} parameter is then calculated with the purpose to compare different combinations of time and temperature for the thermal processing, in order to attain the initial aim (inactivation of foodborne pathogens) but also to maintain the highest nutritional value possible (Bigelow, 1921). Zardetto (2005) evaluated the thermal treatment of fresh egg pasta, using reflectance spectroscopy in the range 1000-2500 nm. The models predicted the F_{70}^{10} values with a standard error of 0.16 and a R^2 of 0.91. Therefore, the use of NIR spectroscopy as a rapid tool for the determination of this parameter could be successful.

Considering liquid egg products, their specifications lead to mandatory assessment of total solid content, therefore a rapid method is needed for current use. Osborne and Barrett (1984) measured the protein, total lipid and total solid contents, using NIR transmission. They obtained a R^2 of 0.96, 0.98 and 0.99 for protein, total lipids and total solid contents, respectively, compared with standard procedures. The researchers suggested that NIR could be successfully applied for the determination of these parameters.

The prediction of macronutrients is nowadays regarded as necessary for egg product manufacturers, therefore the accuracy of NIR in their prediction

should be investigated further on. Buning-Pfaue et al. (2004) used the NIR reflection spectra on liquid shell egg samples and sample mixtures from yolk, egg white and water. Using PLS regression of the NIR spectra and physicochemical parameters, a good correlation was found for dry matter, crude protein, total fat, cholesterol and lecithin phosphate, with a R^2 higher than 0.98 (except for the crude protein, 0.79).

CONCLUSIONS AND FUTURE DEVELOPMENTS

NIR spectroscopy is a very extremely reliable, nondestructive and rapid technique for the prediction of quantitative and qualitative chemical and physical properties of many foods. Nevertheless, these techniques need to use chemometric tools, preprocessing treatment techniques and assessment of calibration models in order to extract the maximum of information available.

NIR spectroscopy and Vis/NIR spectroscopy can be successfully used for the assessment of source indicators of eggs and egg products' quality but further studies are needed. Moreover, the hyperspectral imaging system is an emerging technology for diversified applications in food quality and safety, which is able to determine the internal constituents of food products and is of prime importance in the food industry. It will be interesting to implement this technology with egg's sector.

REFERENCES

Aenugu, H.P.R., Kumar, D.S., Srisudharson, Parthiban, N., Ghosh, S.S., Banji, D. 2011. Near Infra Red Spectroscopy – An Overview. IJCRGG. 3(2): 825-836.

Baeten, V., Fernandez Pierna, J.A., Michotte Renier, A., and Dardenne, P. 2005. Imagerie proche infrarouge: analyse de l'alimentation animale, Techniques de l'Ingénieur. 34(3): 1-8.

Bamelis F. 2003. Non invasive assessment of eggshell conductance and different developmental stages during incubation of eggs. PhD thesis. Catholic University of Leuven, Leuven, Belgium.

Bamelis F., Kemps B., Mertens L., Tona K., De Ketelaere B., Decuypere E., De Baerdemaeker J. 2004. Non destructive measurements on eggs during incubation. Avian Poultry Biol Rev. 15 (3-4):150-159.

Berardinelli A., Giunchi A., Guarnieri A., Pezzi F., Ragni L. 2005. Shell egg albumen height assessment by FT-NIR spectroscopy. Trans ASAE. 48(4):1426-1428.

Benson I.B. 1995. The characteristics and scope of continuous on-line infrared measurement. Spectroscopy Europe. 7(6):18-24.

Bigelow, W.D. 1921. The logarithmic nature of the thermal death time curves. J. Inf. Dis. 29: 528-536.

Brant A.W., Dull G.G., Renfore W.T., Kays S.J. 1953. A Spectrophotometric method for detecting blood in white shelled eggs. Poult. Sci. 32:357-363.
Buning-Pfaue H., Mielke K., Wambold C. 2004. Near infrared spectrometric analysis of egg products. In: Near Infrared Spectroscopy: Proceeding of the 11th International Conference (Davies A.M.C., Garrido-Varo A., Eds.). Chichester: NIR Publications, pp. 627-630.
Burley R.W., Vadehra D.V. 1989. The albumen chemistry. In: The Avian Egg. Chemistry and Biology (Burley R.W., Vadehra D., Eds.). New York: John Wiley and Sons, pp. 65-128.
Dale, L.M., Thewis, A., Boudry, C., Rotar, I., Dardenne, P., Baeten, V., Fernandez Pierna, J.A. 2012. Hyperspectral imaging applications in agriculture and agro-food product quality and safety control: A review, Appl. Spectrosc. Rev. doi: 10.1080/05704928.2012.705800.
Dalle Zotte A., Berzaghi P., Jansson L.M., Andrighetto I. 2006. The use of near-infrared reflectance spectroscopy in the prediction of chemical composition of freeze-dried egg yolk and discrimination between different n-3 PUFA feeding sources. Animal Feed Sci. Tech. 128:108-121.
De Ketelaere B., Bamelis F., Kemps B., Decuypere E., De Baerdemaeker J. 2004. Non-destructive measurements of egg quality. W. Poult. Sci. J. 60:289-302.
ElMasry, G., and Sun, D.-W. 2010. Principles of Hyperspectral Imaging Technology. In: Sun, D. (Eds.): Hyperspectral Imaging for Food Quality Analysis and Control, Academic Press, San Diego. 3-43.
Fernández Pierna, J. A., Michotte Renier, A., Baeten, V., and Dardenne, P. 2004. IR Camera and Chemometrics (SVM): the winner combination for the detection of MBM. Stratfeed Symp., Namur. 39.
Gielen R.M.A.M., De Jong L.P., Kerkvliet H.M.M. 1979. Electro-optical blood-spot detection in intact eggs. IEEE Transactions on Instrumentation and Measurements IM-28. 177-183.
Giunchi A., Berardinlly A., Ragni L., Fabbri A., Silaghi F.A. 2008. Non-destructive freshness assessment of shell eggs using FT-NIR spectroscopy. J. Food Eng. 89:142-148.
Gowen, A. A., O'Donnell, C. P., Cullen, P. J., Downey, G., and Frias, J. M. 2007. Hyperspectral Imaging - an emerging process analytical tool for food quality and safety control. Trends Food Sci. Tech. 18: 590-598.
Hartmann, R. and Buning-Pfaue, H. 1998. NIR determination of potato constituents. Am. J. Potato Res. 41: 327-334.
Hill A.T., Hall J.W. 1980. Effects of various combinations of oil spraying, washing, sanitizing, storage time, strain and age upon albumen quality changes in storage and minimum samples sizes required for their measurement. Poult. Sci. 59:2237-2242.
Huang, H., Yu, H., Xu, H., Ying, Y. 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review, J. Food Eng. 87(3): 303-313.
Jin, Y.H., Lee K.T., Lee W.I., Han Y.K. 2011. Effects of storage temperature and time on the quality of eggs from laying hens at peak production. Asian-Aust. J. Anim. Sci. 24(2):279-284.
Karoui R., De Ketelaere B. Kemps B., Bamelis F. Mertens K., De Baerdemaeker J. 2009. Eggs and egg products. In: Infrared Spectroscopy for Food Quality Analysis and Control. Sun D.-W. (Eds.). MacMillan: U.S.A. pp. 399-414.

Kato A., Ogata S., Matsudomi N., Kobayashi K. 1981. Comparative study of aggregated and disaggregated ovomucin during egg white thinning. J. Agric. Food Chem. 29:821-823.
Kemps B., Bamelis F., De Ketelaere B., Mertens K., Kamers B., Tona K., Decuypere E., De Baerdemaeker J. 2006. Visible transmission spectroscopy for egg freshness. J. Sci. Food Agric. 86:1399-1406.
Kemps B., De Ketelaere B., Bamelis F., Mertens K., Decuypere E., De Baerdemaeker J., Schwagele F. 2007. Albumen freshness assessment by combining visible near-infrared transmission and low-resolution proton nuclear resonance spectroscopy. Poult. Sci. 86: 752-759.
Leeson S. 2006. Defining and predicting changes in nutrient requirements of poultry. World Poultry Sci. J. 62, (Abstracts & Proceedings CD).
Li-Chan E., Nakai S. 1989. Biochemical basis for the properties of egg white. Poult. Biol. 2(1):21-50.
Massarat, D. L., Vandeginste, B. G. M., Buydens, L. M. C., De Jong, S., Lewi, J. P., Smeyers-Verbeke, J. 1988. Chemometrics: A Textbook; Elsevier: Amsterdam, vol. 2.
Mertens K., Perianu C., Kemps B., De Ketelaere B., Decuypere E., De Baerdemaeker J. 2010. Nouvelles techniques non invasives d'evaluation de la qualite de l'oeuf. Jeudis WPSA France, 25.03.2010.
Norris K.H. 1996. History of NIR. J. Near Infrared Spectroscopy 4:31-37.
Nys Y., Gautron J. 2007. Structure and formation of the eggshell. In: Bioactive egg compounds. Huopalathi et al. (Eds.). Springer-Verlag, New York: U.S.A. pp. 99-102.
Osborne B.G., Barrett G.M. 1984. Compositional analysis of liquid egg products using infrared transmission spectroscopy. J. Food Tech. 19:349-353.
Powrie W., Nakai S. 1986. The chemistry of egg and egg products. In: Egg Science and Technology (Williams J.S., Owen J.C. Eds.). Westport: AVI Publishing Co. pp. 97-139.
Roberts J.R. 2004. Factors affecting egg internal quality and egg shell quality in laying hens, J. Poult. Sci. 41:161-177.
Rodriguez-Otero, J.L., Hermida, M., and Centeno, J. 1997. Analysis of dairy products by near-infrared spectroscopy: a review. J. Agric. Food Chem. 45: 2815-2819.
Roggo, Y., Edmond, A., Chalus, P., and Ulmschneider, M. 2005. Infrared Hyperspectral Imaging for qualitative analysis of pharmaceutical solid forms. Anal. Chim. Acta. 535: 79-87.
Rossi M., Pompei C., Hidalgo A. 1995. Freshness criteria based on physical and chemical modifications occurring in eggs during aging. Ital. J. Food Sci. 7:147-156.
Rossi M., Hidalgo A., Pompei C. 2001. Reaction between albumen and 3,3', 5,5'-tetramethylbenzidine as a method to evaluate egg freshness. J. Agric. Food Chem. 49:3522-3526.
Sauveur B., De Reviers M. 1988. Egg quality. In: Reproduction des volailles et production des oeufs (Sauveur B., De Reviers M., Eds.). Paris: INRA. Pp. 377-436.
Schmilovitch Z., Hoffman A., Egozi H., Klein E. 2002. Determination of egg freshness by NNIRS. In: Proceedings of Agricultural Engineering Conference (Paper Number 02-AP-023). Budapest, Hungary.
Shebuski J.R., Freier T.A. 2009. Microbiological spoilage of eggs and egg products. In: Compendium of the Microbiological Spoilage of Foods and Beverages (Sperber W.H., Doyle M.P., Eds.). Springer Science and Business Media. New York. 351 p.

Silversides F.G., Scott T.A. 2001. Effect of storage and layer age on quality of eggs from two lines of hens, Poult. Sci. 80:1240-1245.
Silversides F.G., Villeneuve P. 1994. Is the Haugh unit correction for egg weight valid for eggs stored at room temperature? Poult. Sci. 73, 50-55.
Stadelman W.J., Schmieder H. 2002. Functional uses of eggs – an overview. In: Eggs and Healt Promotion. Ross Watson R. (Eds.). Iowa State Press: U.S.A., pp. 3-9.
UNECE, United Nations Economic Commission for Europe. 2010. Standard EGG-2 concerning marketing and commercial quality control of egg products. United Nations. New York and Geneva. p. 1-20.
Wehling R.L., Pierce M.M., Froning G.W. 1988. Determination of moisture, fat and protein in spray-dried whole egg by near infrared reflectance spectroscopy. J. Food Sci. 53: 1356-1359.
Wise, B. M., Shaver, J. M., Gallagher, N. B., Windig, W., Bro, R., and Koch, R. S. (2006). PLS_Toolbox Version 4.0 for use with Matlab™. Wenatchee, WA, USA: Eigenvector Research Inc. 420p.
Zardetto S. 2005. Potential applications of near infrared spectroscopy for evaluating thermal treatments of fresh egg pasta. Food Control 16: 249-256.

AFLATOXIN AND OCHRATOXIN CONTAMINATION IN POULTRY -A REVIEW-

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Abstract

Aflatoxin and ochratoxin are the most common mycotoxins in poultry feed. Their presence contributes to significant health disorders and decrease in production performances. This leads to considerable economic losses for the poultry industry by increasing mortality, decrease in body weight, number and quality of eggs, greater feed conversion and immunosuppression. The risk associated with mycotoxin residues in poultry meat and eggs represents a concern in human health.

The present article reviews the most important scientific literature on aflatoxin and ochratoxin contamination in poultry and their relationship with food safety.

Recent studies showed that young poultry are more sensitive to aflatoxin and ochratoxin than adults. Ochratoxin has high affinity for liver and kidney, meanwhile aflatoxin has high carcinogenic potential and hepatotoxicity. Lesions in the liver include hepatomegaly, hydropic degeneration, fatty changes, bile-duct hyperplasia and periportal fibrosis.

In order to prevent and reduce the negative implications of these mycotoxins in poultry production, it is necessary to create both global and national strategies to reduce the amount of mycotoxins in grain, to use advance analytical techniques and to establish new limits concerning the maximum amount of mycotoxins allowed in poultry feed and products from poultry for human consumptions .

Keywords: *aflatoxin, food safety, lesions, ochratoxin, poultry feed.*

INTRODUCTION

Mycotoxicoses are intoxications of animals caused by toxin ingestion coming from contaminated grains, feed or litter. In general, fungi produce a large variety of complex chemicals. Some are toxic to animals (mycotoxins), some to bacteria (antibiotics) and some to both. The type and the amount of toxin produced depend on the strain of fungus, temperature, moisture, grain substrate and degree of stress on the host plant.

Nowadays, food safety policy is orientated to possible contamination of poultry feed and consecutively processed food from poultry with fungi and the risk of mycotoxin contamination. High levels of mycotoxins in feed contribute to acute mycotoxicoses and high mortality rate. Lower levels

cause chronic mycotoxicoses with or without clinical symptoms, followed by considerable decrease in production performances, immunosuppressive effects and presence of residues in poultry meat and eggs. Primarily, the toxicity of mycotoxins depends on the type, quantity and duration of ingestion of mycotoxins, species, gender and age of the animal, general health, immune and nutritional status, as well as environmental factors.

Aflatoxicosis and ochratoxicosis are the most common mycotoxicoses in commercial poultry (Pattison et al., 2008).

AFLATOXICOSIS

Aflatoxin is the most prevalent and economically significant mycotoxin that can be ingested by poultry. In the United States the total annual loss due to aflatoxins in corn is about $163 million. The annual market loss through corn rejected for food is about $31 million, while the loss through corn rejected for feed and through livestock losses is estimated at $132 million. The cost of research and monitoring activities are between $500 million and $1,5 billion a year to manage mycotoxin-producing fungi.(Abbas, 2005).

The name ‘‘aflatoxin” derives from the first letter of the word Aspergillus and the first three letters of flavus. Structurally, aflatoxins (AFs) are difurocoumarin derivatives with specific fluorescence under ultraviolet light. Depending on the colour of the fluorescence, AFs are divided into aflatoxin B1 and B2 (AFB1, AFB2) for blue florescence, and G1 and G2 (AFG1, AFG2) for green florescence. Aflatoxin M1 and M2 (AFM1, AFM2), known as milk-AFs, are the metabolites of AFB1 and AFB2. Other metabolites of AFB1 are aflatoxin Q1 (AFQ1) and aflatoxicol. Aflatoxin is the most studied mycotoxin, due to both its toxicity to animals and people and its high carcinogenic potential. Out of AFs group, AFB1 is the most toxic and is classified as human carcinogen (Talebi et al. , 2011).

Etiology

Aflatoxins are secondary metabolites produced by the common moulds of *Aspergillus flavus*, *A. parasiticus* and *A.nominus*. These fungi are ubiquitous in the environment and produce aflatoxin in warm (30–35°C) and high-humidity conditions.

The occurrence of aflatoxins in agricultural commodities depends on region, season and the conditions under which a particular crop is grown, harvested or stored. Crops grown under warm and moist weather in tropical or subtropical countries are more prone to aflatoxin contamination than those in temperate zones. Aflatoxin production is also stimulated by high zinc concentration in feed (Pattison et al., 2008).

Stressed plants by insect damage, drought, poor nutrition or delayed harvest increase aflatoxin production. Aflatoxin is stable once formed in grain and is not degraded during normal milling and storage (http://www.worldpoultry.net).

Host sensibility

Young poultry are more sensitive to aflatoxin than adults. There are also large species differences: ducks being 10 times more sensitive than chickens. Furthermore, certain poultry breeds are more sensitive than others. Chickens and quails are considered relatively resistant, so acute intoxication occurs relatively rare. Chronic intoxication with aflatoxin is the results of aflatoxin ingestion for several weeks (one week minimum). Aflatoxin in concentration of 0.7 ppm reduces the growth rate of turkey but without any effect in quails and chickens (Arafa et al., 1981). A diet containing 400 ppm AFB1 severely affects body and liver weights in turkeys, with no effect in chickens (Leeson et al., 1995). Dalvi (1986) showed that the lethal dose is different between poultry species (Table 1).

Table 1. Oral LD 50 for different poultry species

Species	Oral LD50 (ppm)
Chick embryo	0,025
Duckling	0,3
Turkey poultry	0,5
Chicken, New Hampshire	2
Chicken, Rhode Island	6,3

Pathogenesis

After absorption, the highest concentration of the toxin is found in the liver (target organ). At liver level, aflatoxin B_1 is metabolized by microsomal enzymes into different metabolites.

In one day-old chicks, AFB_1 reduces the activity of liver UDP glucose-glycogen transglucosylase resulting in depletion of hepatic glycogen stores (Shankaran et al., 1970). Also, lipid accumulation occurs in the liver of chickens and ducklings exposed to aflatoxin (Carnaghan et al., 1966; Shank and Wogan, 1966). Aflatoxin is rapidly excreted in the bile (eliminated in faeces) and urine and does not accumulate or persist in body tissues. This perhaps explains the rapid recovery of egg production and hatchability after cessation of toxin ingestion. Aflatoxin reduces resistance of poultry to infection with *Pasteurella multocida*, *Salmonella spp.*, Marek disease virus, infectious bursal virus, *Coccidia*, and *Candida albicans* (Smith et al., 1969; Hamilton and Harris, 1971).

Clinical sings

Aflatoxicosis does not induce mortality directly, although high levels (>10 ppm) may be lethal. Acute aflatoxin poisoning leads to impaired

coordination of movement, vertigo, and paresis, followed by diarrhea with admixtures of blood, haemorrhages, tumescence, jaundice (icterus), coma and death. The most economically significant effects of aflatoxicosis on poultry are decreased growth, weakness, reduced food consumption and poor feed conversion. Poultry exposed to aflatoxins are pallid, as a consequence of poor pigmentation, which is the result of reduced ingestion, resorption, and transport of carotenoid.

Intoxicated adult hens, with concentration above 2 ppm have decreased egg production and the hatchability of their eggs is reduced. In adult male breeder, testicular weights and sperm counts are reduced.

Dietary exposure of broiler hens to AF (10 ppm) resulted in embryonic mortality and reduced the immunity in the progeny chicks. Embryonic exposure with AFs resulted in long-term depression of the immune function in chickens (Resanovic et al., 2009).

The levels of serum proteins are reduced by aflatoxins. The synthesis of albumin and of the most of the globulins takes place in liver and in chronic hepatic diseases, hypoalbuminaemia occurs. The level of globulins decreases, but not as much as the albumin because not all the globulins are formed in the liver, such as gamma-globulins (Fernandez et al., 1995).

Lesions

Lesions depend on the age of the host and the dose of toxin ingested and can include: hepatosteatosis or fatty liver, kidney hypertrophy, splenomegaly, atrophy of the thymus, testes and bursa of Fabricius. Bruising associated with an increase of capillary fragility and haemorrhagic points (petechial haemorrhages) on the surface of muscles of the leg and breast have been described in aflatoxicosis of broiler chickens (Biro et al., 2002).

Acute toxicity of aflatoxins in chickens may be characterized by liver necrosis with icterus, distended gallbladder and bile ducts, white pinhead-sized lesions and paleness in liver. Histopathological, liver damage is manifested as vacuolation of hepatic cells and bile duct proliferation. Metabolic alterations caused by aflatoxins in chickens result in elevated lipid levels, disruptions in hepatic protein synthesis, which result in several blood coagulation disorders, immunosuppression and decreased plasma amino acid concentrations (Sumit et al., 2010).

In aflatoxicosis, a thickened basement membrane in the glomeruli and associated hyaline droplets in the renal tubules are noted. It is not known if the glomerulus is damaged by toxins or by a leakage of unusual protein from a severely damaged liver. Liver lesions in chicken are characterized by

retrogressive and regenerative parenchymal changes.(Herenda and Franco, 1996).

Acute and chronic toxic effects of aflatoxins, particularly AFB1, in turkeys are not very different from those in chickens: diffuse necrosis of liver parenchyma, proliferation of the bile duct epithelial cells and small haemorrhages. The prominent cellular changes in liver included swelling and vacuolation of the parenchyma cells and enlargement of the nucleus. There has also been observed: hypertrophy and congestion of the kidneys.

In ducklings over 3 weeks subcutaneous haemorrhages of legs and feet are characteristic. Lesions of the liver have been reported to be common in acute and chronic cases of aflatoxicosis in ducks. The duckling has been recommended as a convenient species for experiments because of its rapid response to aflatoxin, manifested by marked bile duct hyperplasia 48 to 72 hours after exposure. Prolonged exposure of the duck to low levels of aflatoxins leads to marked nodular hyperplasia of the liver, bile duct proliferation and fibrosis and hepatocellular carcinoma. Chickens and turkeys have not been shown to develop liver neoplasm after ingestion of aflatoxins. Since metabolites of aflatoxins are implicated in carcinogenesis and ducks are known to metabolize aflatoxins rapidly, they appear to be prone to aflatoxin-induced carcinoma (Resanovic et al., 2009).

OCHRATOXICOSIS

Ochratoxicosis occurs less frequently in poultry than aflatoxicosis but is more lethal because of its acute toxicity. Ochratoxins are a family of toxic compounds consisting of three members, A, B and C, which are structurally related and are produced as secondary metabolites of several species of fungus. Ochratoxin A contaminates agricultural products and due to its accumulation in food, represents a serious threat to human and animal health worldwide (Pattison et al., 2008).

Etiology

The name “ochratoxin” derives from *Aspergillus ochraceus*, the first fungus discovered to produce this toxin. Ochratoxins are mostly produced by *Penicillium verrucosum*, but five other species of *Aspergillus* and six other species of *Penicillium* produce it as well. Ochratoxin A (OTA) is the most commonly detected and most toxic member of the family.

OTA is a common contaminant of cereals (corn, wheat, barley, oats, rye, sorghum) and peanuts, as well as soya, coffee and cocoa beans. Environmental conditions for ochratoxin production are similar to those for

aflatoxin and simultaneous contamination with both is common (Pattison et al., 2008).

Host sensibility

Young poultry are more sensitive to ochratoxin ingestion than adults and ducks are seven times more sensitive than chickens. Quail and turkeys are also more sensitive to ochratoxicosis than chickens. Variations in sensitivity towards OTA exists among avian species, as LD 50 ranges from 0.5 to 16.5 ppm body weight for ducks and Japanese quail, respectively, chickens 2-4 ppm (Pattison et al., 2008).

Pathogenesis

After resorption, the highest quantity of ochratoxin can be found inside kidneys and liver, and a considerably smaller extent in muscle. It is characteristic of poultry to have a more efficient and faster excretion of ochratoxins than other animals, approximately 48 hours.

Ninety percent of the ingested OTA is excreted. OTA in poultry diets leads to reduction in growth rate, feed consumption and feed efficiency and increased mortality. One of the profound effects of OTA is the its ability to alter the function of the immune system in avian species, causing severe leucocytopenia, impaired complement activity, reduction in immunoglobulin and several functional properties of macrophages and heterophils and finally it causes atrophy of the lymphoid organs along with depletion of lymphocytes. OTA causes enlargement of the kidney and subsequently impairing its function, therefore, considered as a nephrotoxic mycotoxin in birds (http://ntp.niehs.nih.gov).

Ochratoxin A inhibits protein synthesis, produces acute proximal tubular epithelial necrosis in the kidneys and inhibits normal renal uric acid secretion. A decrease in the concentration of proteins, triglycerides, cholesterol, calcium, phosphorus and potassium is followed by an increase in the level of uric acid and creatinine and a decrease in glomerular filtration (Elaroussi M.A , 2008).

Some authors explain how OTA inhibits respiration in mitochondria, where it acts as a competitive inhibitor of the carrier's proteins, localized on the inner membrane of mitochondria. Furthermore, it is considered that OTA represents a teratogenic agent for chickens, but not for other domestic animals (Bennett and Klich, 2003).

Clinical sings

The unspecific clinical image of chronic ochratoxicosis in poultry is followed by a decrease in egg production of laying hens, whereas, as far as broilers are concerned, their growth is hindered and conversion of food is

weakened. The egg shell often becomes thin and fragile, with different discoloration appearing on the surface.

Growth inhibition is linked with malabsorption syndrome, as confirmed by the presence of hypocarotenoidemia. The minimum amount of ochratoxin also causes reduced bone firmness and poor pigmentation.

Nephropathies are not clinically manifested, although polydipsia accompanied by a substantial amount of moist excrement appears. Acutely intoxicated birds are depressed, dehydrated and often polyuric and die in acute renal failure. Survivors will be poorly feathered, have delayed sexual maturity, increased clotting times, anaemia and immunosuppression.(Resanovic R, 2009)

Lesions

The enlargement of the liver and kidney in OTA intoxications is caused by the involvement of these organs in detoxification and elimination. Affected kidneys are white to tan, swollen, hard and may have white pinpoint urate crystals. If damage is extensive enough to cause renal failure, dehydration, hyperuricaemia and visceral urate deposition appears at kidney level. Pasty white urates are deposited on pericardial, perihepatic, peritoneal and articular surfaces. These deposits may be mistaken with inflammatory exudates but their true nature can be determined by microscopic examination. More commonly, birds survive in compensated renal failure and kidneys appear enlarged, fibrotic and pale (Biró et al., 2002).

In ochratoxicosis, hypertrophy, hyperplasia, mitosis and individual cell necrosis of proximal tubules are noted. Other lesions include a thickened basement membrane in the glomeruli and lymphoid depletion from the lymphoid organs.

In addition to the renal lesions there is mild to moderate glycogen deposition in hepatocytes, mainly at the periphery of the liver lobes at higher levels of dietary OTA (4 and 8 ppm), resulting in yellow enlarged livers. Signs of liver disease were further supported by the significant decrease in total protein, albumin and globulin. There is also some mild decrease in bursal and thymic size consistent with immunosuppression.(Herenda and Franco, 1996).

Since gross lesions observed in ochratoxicosis seem to be neither characteristic nor consistent in poultry, microscopic and ultrastructure changes in the liver, kidney and lymphoid organs can be considered the best diagnostic features for OTA toxicity. Tubular dilatation and hypertrophy, swelling of tubular epithelial cells, localized necrosis, and desquamation of the tubular basement membrane as signs of tubulonephrosis were reported

by Dwivedi and Burns (1984). The changes were usually confined to proximal convoluted tubules, but distal convoluted tubules could also be affected. Thickening of the capillary walls of glomeruli and the presence of granular eosinophilic casts in the tubular lumen were also reported. (http://en.wikipedia.org/wiki/Ochratoxin_A).

Glomerulonephrosis, tubulonephrosis, focal tubular epithelial cell proliferation and the multiplex adenoma-like proliferation of renal parenchyma are considered to be primarily related to the toxin, while focal intertubular infiltration of lymphocytes and histiocytes can also occur either primarily or secondly as reparation of tubulonephrosis or as a consequence of immune stimulation (Elaroussi et al., 2008).

The reduction seen in the number of lymphocytes in the pulpar region of the spleen has also been reported by Dwivedi and Burns, (1984), who detected a marked degree of lymphocytic depletion and obscure distinction between red and white pulp in some areas of the spleen. The lack of visible damage in heart and muscles indicates a low sensitivity of these tissues to OTA toxicity.

Prevention and control of mycotoxin formation

The best way to control aflatoxin and ochratoxin formation is to prevent the growth of fungi on harvested and stored grains and other susceptible commodities. Crops should be harvested at maturity and pre- or post-harvest mechanical damage should be avoided. Moisture contents of harvested crops should be reduced to a safe level. Moisture build-up in the stored grain should be prevented by measures such as regular aeration. Aflatoxin production can be decreased by storing food in a low-oxygen, high-CO2 environment. In areas of the southern United States, where the preferred conditions for aflatoxin production are common (25-30°C, humidity 85%), refrigeration of food is often necessary to prevent aflatoxin production.(Ritchie, 1994)

In the United States, the Food and Drug Administration, has established a tolerance of 20 ppb of aflatoxin for foods other than milk, but European markets are striving for a lower Codex importation standard of 2 ppb. (Abbas, 2005);

AFB_1 contamination is practically unavoidable, chemoprevention strategies aimed to reduce AFB_1 toxicity in poultry and in other animals have been the subject of numerous studies.(Arafa et al., 1981; Leeson et al,1995).

Several chemopreventives have been evaluated in poultry for reducing symptoms of aflatoxicosis. Because of their sensitivity, poultry have been used as models for discovering AFB1 chemopreventives.

Since 1990s, particular studies (Maciorowski et al., 2007; Wyatt, 1991) have shown the value of non-nutritive clays, such as aluminosilicates, zeolites, bentonites and clinoptilolites on aflatoxicosis prevention. They have high binding capacity against aflatoxin, reduce the absorption from the gastrointestinal tract and are generally inert, nontoxic and economical in use. Antifungal agents such as

gentian violet and propionic acid have been evaluated and appear to be most promising substances in the control of aflatoxin-producing fungi. Similarly, benzoic acid has been found to be quite effective against *A. flavus*. Other feed additives including selenium and carotenes have also been reported to have the some value in reducing the toxicity of AFB_1 in chickens and in turkeys. Also, high protein diet has been found to have protective effect against aflatoxins in chickens (Sumit et al., 2010).

The aflatoxin and ochratoxin content in food can be determined by analytical techniques such as: thin layer, gas or liquid chromatography, spectrofluorometry and spectrophotometry (Talebi , 2011). HPLC (high-performance liquid chromatography) still remains the technique of choice for aflatoxin and ochratoxin analysis. HPLC methods include HPLC with fluorescence detection and HPLC with near-ultraviolet, laser- induced fluorescence detection (near- UV LIF) (Abbas, 2005). ELISA test for poultry are available for identification of total aflatoxin and ochratoxin A. Detection of aflatoxin and ochratoxin residues in tissues requires 100 g of fresh or frozen liver or kidney. Samples for analysis should be placed in sealable plastic bags. Although not ideal, tissues from several dead birds can be pooled for analysis if necessary.(Ritchie, 1994);

Chemical detoxification of aflatoxins (acid treatment, alkaline treatment with hydroxide, bisulfites, chlorinating compounds and oxidizing agents) in foods and feeds is important as a short-term postharvest solution to the problem. Although there are many chemical methods, ammoniation is still the most utilized and approved method for decontamination. New methods such as ozonation treatment do show promise, but require further testing for safety and scalability. With any chemical method, studies must be done to determine if new toxins are formed as a result of the treatment. It is also important to determine whether the treatment will alter the functional and nutritional characteristics of the products. (Abbas, 2005).

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REFERENCES

Abbas H., 2005. Aflatoxin and Food Safety, Taylor & Francis Group,pg 10,254,261, 461,474, 549;

Arafa, A., Bloomer S., Wilson R., Simpson H., Harms, R., 1981. Susceptibility of various poultry species to dietary aflatoxin. British Poultry Science. 22: 5, 431-436

Bennett J. W., Klich M., 2003. Mycotoxins. Clin Microbiol Rev. 6(3): 497–516.

Biró K., Solti L., Barna-Vetró I., Bagó G., Glávits R., Szabó E., Fink-Gremmels J., 2002. Tissue distribution of ochratoxin A as determined by HPLC and ELISA and histopathological effects in chickens, Avian Pathology, 31:2, 141-148;
Branson W. Ritchie, Greg J. Harrison, Linda R. Harrison- Avian Medicine: Principles And Application, 1994, Pg 378;
Carnaghan, R. B. A., Lewis, G., Patterson, D. S. P., Allcroft R., 1966. Biochemical and pathological aspects of groundnut poisoning in chickens. Pathol. Vet., 3:601-615;
Dalvi R.R., 1986.An overview of aflatoxicosis of poultry: its characteristics,prevention and reduction;Veterinary Research Communications**,** 10, 429-443
Drago C.Herenda and Don A. Franco- Poultry Diseases and Meat Hygiene, Iowa State University Press, Ames, first edition , 1996;
Dwivedi, P., Burns, R.B., 1984. Pathology of ochratoxicosis in young broiler chicks. Research in Veterinary Science, 36, 92–103;
Elaroussi M.A., Mohamed A, Elgendy M.S, El Barkouky E.M., Abdoul A.M. Hatab M.H, 2008. Ochratoxicosis in Broiler Chickens: Functional and Histological Changes in Target Organs; International Journal of Poultry Science 7 (5): 414-422;
Fernandez A., Verde M.T., Gomez J., Gascon M., Ramos J.J. 1995. Changes in the prothrombin time, haematology and serum proteins during experimental aflatoxicosis in hens and broiler chickens; Research in Veterinary Science, 58, 119-122 ;
Hamilton, P. B., Harris, J. R. 1971. Interaction of aflatoxicosis with *Candida albicans*: infections and other stresses in chickens. Poult. Sci., 50:906-912;
Leeson S., Diaz G.J., Summers J.D., 1995, Trichotecenes in Poultry Metabolic Disorders Mycotoxins, University books, Canada , 199-226
Maciorowski K.G., Herrera P., Jones F.T., Pillai S.D., Ricke S.C. 2007. Effects on poultry and livestock of feed contamination with bacteria and fungi; Animal Feed Science and Technology 133, 109–136;
Pattison M., McMullin P., Bradbury J.,Alexander D., 2008.- Poultry Diseases(Sixth Edition),Chapter 38, pages 435-442;
Resanovic R., Ksenija N., Nesic V., Palic T., Jacevic V, 2009. Mycotoxins in poultry production , Proc. Nat. Sci, Matica Srpska Novi Sad, 116, 7-14
Shank, R. C., Wogan, G. N., 1966. Acute effects of aflatoxin B I on liver composition and metabolism in the rat and duckling.. Toxicol. Appl. Pharmacol., 9:468-476.
Shankaran, R., Raj H. G., Venkatasubramanian T. A., 1970. Effect of aflatoxin on carbohydrate metabolism in chick liver.Enzymlogia, 39:371-378;
Smith, J. W., Prince, W. R., Hamilton, P. B., 1969. Relationship of aflatoxicosis to *Salmonella gallirarem* infections of chickens. Appl. Micobiol., 18:946-947;
Sumit R., Kim E.J., Coulombe R., 2010. Aflatoxin B1 in poultry: Toxicology, metabolism and prevention, Research in Veterinary Science, 89, 325–331
Talebi E., Khademi M., Rastad A, 2011 - An Over Review on Effect of Aflatoxin in Animal Husbandry; Asian J. Exp. Biol. Sci. Vol: 2(3): 754-757
Wyatt R. D., 1991 Poultry. In: Smith J E, Henderson R S (eds) Mycotoxins and animal foods. CRC Press,Boca Raton, p 553–605;
(http://en.wikipedia.org/wiki/Ochratoxin_A)
http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/OchratoxinA.
http://www.worldpoultry.net/Breeders/Nutrition/2010/4/Mycotoxins-Part-2---Current-thoughts-on-global-mycotoxicoses-WP007353W/

IMPORTANCE OF THE GOAT SLAUGHTER AGE ON TECHNOLOGICAL PARAMETERS OF THEIR CARCASS

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Abstract

In this study we analyzed the evolution of technological parameters of goats carcass according to age. For this study we created two groups of samples, namely: group I represented by the goatling and group II represented by adult goats. On these meat samples we analyzed: meat chemical reaction (pH), water holding capacity, cooking loss test and drip loss (losses from refrigeration). For this samples we analyzed a total of 30 samples of goat meat for 15 for each group considered to be studied. Samples were collected from goats slaughtered during 2012 in a slaughterhouse in SE Romania. The values for the chemical reaction of the first group samples (goatling) varied between 6,20 and 6,32, the average being 6,26 and for the group II between 6,31 and 6,39, with an average of 6,35. Water holding capacity showed an average value of 61,23% for group I to 64,17% for group II. Cooking loss are recorded 37,62% to group I to 32,28% in group II, and for drip loss 4,06% for young kids to 4,88% to adult samples. Results lead to the conclusion that slaughtering goats at a younger age represents a disadvantage for manufacturing and processing, generating qualitative and quantitative losses in finished products.

Keywords: *goat meat, goat slaughter, public health risks*

INTRODUCTION

Goats represent a species of domestic animals with high biological, technological and economic flexibility. To support this statement there are several reasons, resulting in the ability of goats to use poor feed resources and rough surfaces, the possibility of integrating them into different rearing systems contributing to the fund of livestock products (meat, milk, hides and skins) and farms development is carried out without too much investment.

For goats rearing, cheap feeding resources are efficiently used, depending on the breeds' characteristics and their morpho-productive features.

Lately, consumer preferences for goat meat and dairy products made from goat milk have increased, due to the food traditions, animal protein deficiency in human diets worldwide, and the special nutritional qualities of these products.

Goat meat is a valuable food in human diet because it contains high quality protein and is rich in essential amino acids that can not be synthesized by the human body. Goat was and still is the main source of meat for the people of

South America, Africa or Asia, significantly exceeding the production and consumption of goat meat, lamb or sheep in Europe. Various studies show that this meat has a much stronger flavor, much higher nutritional and biological values compared to other kinds of meat, due to the specific proportion of the constituent trophins. If until recently the main sources of meat were pork and cattle, lately it was noticed an increased preference for poultry and fish compared to mammals, and of these the preference for goat meat (Tăpăloagă Dana, 2012). Poultry meat contains less fat than pork or beef, chicken liver is rich in vitamin A, and the proportion of unsaturated fatty acids is higher than the saturated ones, suggesting that birds may be an alternative to red meat.

Goat breeders' concerns should be directed towards increasing and improving meat quality and milk production, which can be achieved by encouraging breeds, populations and specialized lines for meat or milk production, by improving rearing technologies (Memisi N, Bauman F, 2007).

Establishing the relation between various production and the limits within which they can increase without prejudice the physiological balance represents an issue of major practical importance for increasing profitability in goats' husbandry.

It is still difficult to ensure a steady supply of goat meat for consumers. Due to the ignorance or other reasons, consumers are often faced with the purchase of meat that is not safe for their consumption due to potentially dangerous effects of low quality meat or meat substitutes (substitutions with reduced trade and nutritional value).

Imposing certain standards of sanitation (cleaning, sanitation) in slaughterhouses, in the processing circuit, handling, transportation and sale of meat is of great importance because meat is an ideal environment for the development and multiplication of microorganisms, especially bacteria, cited as causes in the etiology of zoonoses.

Between meat and milk production there is no physiological antagonism, but rather a low positive phenotypical correlation. However, even under good care and nutrition, meat and milk production can not be limitlessly increased in parallel.

Along with increased productions, the standards of feeding, fodder conservation, superior fodder capitalization must continually be improved, by ensuring a higher digestibility, the preparation of feed ratios based on nutritional requirements and ingestion capacity of different goats categories, in order to increase economic efficiency (Shrestha JNB, 2005).

In order to avoid specific health risks (sources of pathogens and food poisoning) it is necessary to provide optimal conditions for transport, slaughter, rapid refrigeration and proper handling of carcasses. Also it will be avoided long

distance transport of animals, extended maintenance in slaughterhouses, brutally slaughtering, unclean instruments, and contamination during skinning, evisceration, cutting, chilling, storage and prepackaging.

MATERIALS AND METHODS

The pH value was determined by the potentiometric method using the device probe and meat aqueous extract from 10 g of shredded meat sample, adding distilled water up to the 100 ml capacity of the cylinder (AOAC, 2000). Solution was left to rest for 15 minutes, during which for 2-3 times was mixed with a glass rod, then the cylinder content was passed through the filter paper. The pH value was read on the device display.

Water-holding capacity was expressed as milliliters of sodium chloride for 100 grams of meat, following this protocol: from the sample were weighed 8 grams, this quantity being shredded and placed in test tubes. It was added a volume of 12 milliliters sodium chloride solution and then the content was mixed. The tubes were left to rest for 15 minutes at 5^{o}C then were centrifuged at 10000 rpm and 4^{o}C. By decantation it was separated the supernatant and it was measured and used to express the water-holding capacity (AOAC, 2000).

The cooking losses: there were used 100 grams from the analyzed meat sample, which were placed in a capped glass vial and were heated to 72^{o}C (temperature of the meat) on Julabo TW12 water bath. The liquid was removed and the solid portion was cooled and accurately weighed. Finally weight loss of analyzed meat samples was reported.

Drip loss was measured by the following working protocol: 100 grams from the analyzed meat sample were placed in a glass vial with cap and grid and were maintained at a temperature of 4^{o}C for 24 hours in a FTC Velp 90i cooling thermostat. After this, the sample was weighed and the obtained value was related with initial weight of the sample.

RESULTS AND DISCUSSIONS

pH values for examined goat meat samples are shown in Table no. 1 and Figure no. 1. It can be noticed that the pH recorded increasing. Lower values were recorded for samples in group I collected from goatlings and higher values for samples in group II collected from adult goats. The extreme pH figures recorded were 6,20 and 6,39 respectively, mean values being 6,26 for goatling and 6,35 for adult goats.

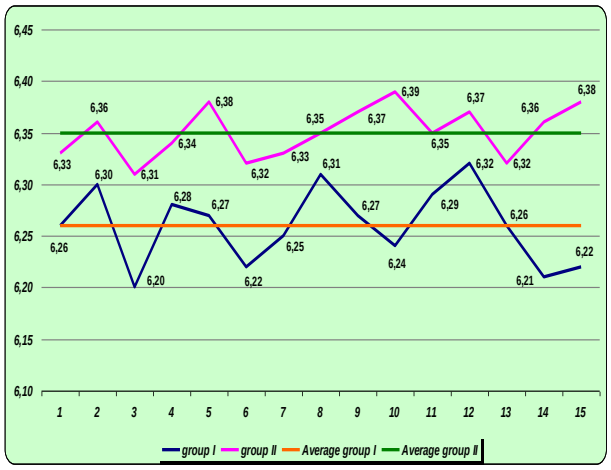
Table 1

Meat chemical reaction values

Sample no	Group I	Group II
1	6,26	6,33
2	6,30	6,36
3	**6,20**	**6,31**
4	6,28	6,34
5	6,27	6,38
6	6,22	6,32
7	6,25	6,33
8	6,31	6,35
9	6,27	6,37
10	6,24	**6,39**
11	6,29	6,35
12	**6,32**	6,37
13	6,26	6,32
14	6,21	6,36
15	6,22	6,38
Average	6,260	6,351

Figure 1

Meat chemical reaction graphic values



Water holding capacity recorded lower values in young animals meat and higher in adult animals'. As it can be seen from the data presented in Table no. 2 and Figure no. 2, goatling meat has a lower water holding capacity than the one from the adult group. Water holding capacity ranged from 60,28 to 62,18% (with an average value of 61,23%) for group I and from 63,65 to 64,69% (with an average value of 64,17 %) for group II.

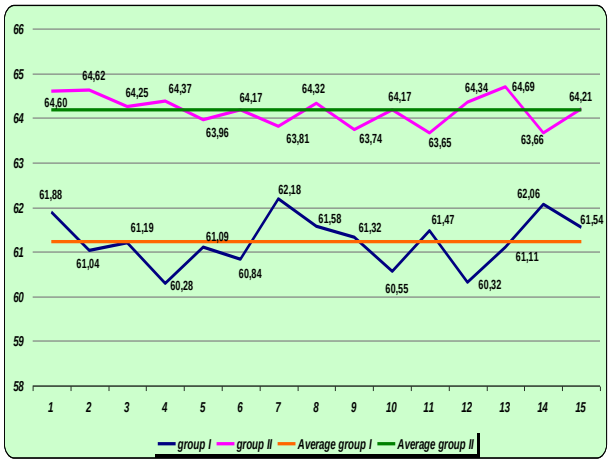
Table 2

Water holding capacity values

Sample no	Group I	Group II
1	61,88	64,60
2	61,04	64,62
3	61,19	64,25
4	**60,28**	64,37
5	61,09	63,96
6	60,84	64,17
7	**62,18**	63,81
8	61,58	64,32
9	61,32	63,74
10	60,55	64,17
11	61,47	**63,65**
12	60,32	64,34
13	61,11	**64,69**
14	62,06	63,66
15	61,54	64,21
Average	61,230	64,171

Figure 2

Water holding capacity graphic values



Cooking loss: after cooking different types of goat meat, depending on their age, there were recorded values shown in Table no. 3 and Figure no. 3. As it can be

noticed, the effect of age on the meat cooking loss varied along with the two studied age groups. Cooking losses for meat from young animals (group I) was higher – 37,62% (from 34,76 to 40,48%), comparing with meat samples from group II (adult goats) – 32,28% (between 31,14 to 33,42%).

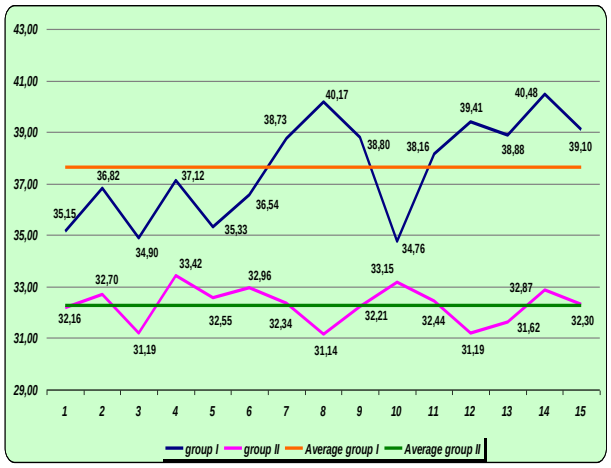
Table 3

Coking loss test values

Sample no	Group I	Group II
1	35,15	32,16
2	36,82	32,70
3	34,90	31,19
4	37,12	**33,42**
5	35,33	32,55
6	36,54	32,96
7	38,73	32,34
8	40,17	**31,14**
9	38,80	32,21
10	**34,76**	33,15
11	38,16	32,44
12	39,41	31,19
13	38,88	31,62
14	**40,48**	32,87
15	39,10	32,30
Average	37,623	32,283

Figure 3

Coking loss test graphic values



Drip loss: goat meat from the two age groups analyzed recorded values presented in Table no. 4 and Figure no. 4. Drip loss in the samples collected from young animals showed an average of 4,88% (min. 4,64% and max. 5,12%) and in the samples collected from adult animals an average of 4,06% (min. 3,82% and max. 4,30). Analysis of the obtained data indicated that the values for goatling were greater than the values for adult goats.

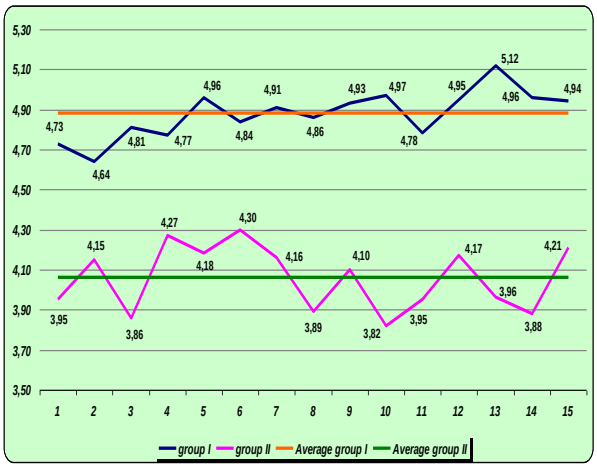
Table no. 4

Dip loss test values

Sample no	Group I	Group II
1	4,73	3,95
2	**4,64**	4,15
3	4,81	3,86
4	4,77	4,27
5	4,96	4,18
6	4,84	**4,30**
7	4,91	4,16
8	4,86	3,89
9	4,93	4,10
10	4,97	**3,82**
11	4,78	3,95
12	4,95	4,17
13	**5,12**	3,96
14	4,96	3,88
15	4,94	4,21
Average	4,878	4,057

Figure 4

Dip loss test graphic values



CONCLUSIONS

The results obtained from measurements presented in this study indicate the presence of minor differences between technological parameters of meat from goatling and meat from adult animals. These differences support the findings of other authors who have analyzed goat meat in terms of technological efficiency and who have not recommeded the slaughter of young animals because technological properties of the obtained raw material are lower compared with those obtained from slaughtered adult animals.

Water holding capacity is lower for young animals' meat compared with the one from adult animals, which makes the return in the products increase proportionally with the age of slaughtered goats.

It was noticed that cooking loss decreases with age from 37,62% in goatling to 32,28% in adult, which may be associated with the increasing of the pH from 6,26 to 6,35.

Drip loss recorded a significant decrease in adults' meat (4,06%) compared to the young animals' meat (4,88%), which could be associated with the observation that along with the age the sarcomeres are shortening and the myosin filaments are contracting, appearing grouped as bundles.

REFERENCES

AOAC, 2000. Meat and Meat products. In: Official Methods of Analysis. Association of Official Analytical Chemists Inc. Gaithersburg, U.S.A.

Norman, G.A. 1991. The potential of meat from the goat. Developments in Meat Science. Lawrie, R.A. Elsevier Applied Wcience, London.

Memisi N, Bauman F, 2007. Goat Nutrition. Admiralbooks, Belgrad.

Sen, A.R., A. Santra and S.A. Karim, 2004. Carcass yield, composition and meat quality attributes of sheep and goat under semiarid conditions.

Shrestha JNB, Fahmy MH, 2005. Breeding goats for meat production: a review. 1. Genetic resources, management and breed evaluation. Small Rumin.

Tăpăloagă Dana, 2012. Tehnologii de obţinere a laptelui şi a cărnii - Ed. Granada, Bucureşti, ISBN 978-606-8254-16-6.

STUDY ON THE CHEMICAL COMPOSITION OF GOAT MEAT SAMPLES CORRELATED WITH THEIR AGE

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Abstract

The research has been conducted in order to determine the values of main chemical components in goat meat and establish a link between animal age and the values of these parameters. Age groups considered in the study have been goatling and adult goats. Samples were collected in 2012 from an approved European slaughterhouse involved in intra-authorized veterinary trade, fulfilling all specific legal requirements. For determinations were used following methods: moisture content by drying in an oven, protein content by Kjeldahl method, total fat content used Soxhlet extraction unit and ash percentage was determined by using calcination method. The mean values for the results obtained from the measurements made were: 77,6% moisture for goatling to 73,5% for adults goats,14,8% protein for goatling to 19,8% for adults, 1,74% fat for goatling and 2,88% for adults, 1,14% ash for goatling to 1,52% for adults goats samples. As it can be seen, as age increases, the major components with important nutritional role occupy a higher share of the goat carcass, resulting in superior technological and organoleptic characteristics compared to the slaughtered youth.

Keywords: *goat meat, goat age, goat slaughter, meat composition, meat quality*

INTRODUCTION

Meat, regardless of the animal from which it comes (beef, mutton, pork, poultry), has a composition correlated with the age and nutritional status of the animal. Meat contains approximately 20% proteins. Fat meat content depends on species and nutritional status. According to some authors, the lowest fat content is founf in beef or veal meat (6-8%) and the richest in pork (30%). Meat (especially of young animals) contains a small amount of carbohydrates and a high amount of extractive substances (purine, creatine, creatinine) and minerals (phosphorus, iron). Viscera (liver, kidney, heart) contain copper and cobalt and increased amount of iron. The other minerals (calcium, sodium, chlorine, sulfur, magnesium) are found in meat in small concentrations. Chlorine, phosphorus and sulfur ions cause acid action in the body. Meat is rich in soluble vitamins - B complex. In addition, viscera are rich in fat-soluble vitamins (A, D). Given its large content of proteins, vitamins and minerals, meat nutritional value is high.

Goats are not as effective in terms of meat production as other slaughtered livestock. Their rearing is encouraged by the advantage of the efficiently use of poor quality fodder such certain plants or shrubs. Many studies have highlighted major differences between variants within the same breed, gender difference and even between individuals concerning the fodder use. Growth rate and meat chemical composition are influenced both by the animal physiological state and by microclimate and rearing conditions (Tăpăloagă Dana, 2008).

Goat meat has a fat content with 50-65% lower than beef, while similar proteins content. It also has 42-59% less fat than lamb meat and respectively 25% less fat than veal. Moreover, saturated fats in goat meat are lower than in chicken's (without skin) with 40%, with 850% than in beef's, with 1100% lower than in pork's and with 900% in lamb meat (U.S.D.A., 2000).

From the numerous studies on goat meat, it results that regardless of race, age or region, goat is an important source of high quality proteins, healthy fats (based on a proper unsaturated fats - saturated fats ratio), and with a low cholesterol intake. Goat meat is low cal and sodium, but the high levels of iron, potassium and essential amino acids should range it within the category of high quality meat (Argüello A., 2005; Lee H.J., 2008).

MATERIALS AND METHODS

The study has been conducted on two age groups: gloating and adult goats. There were collected and analyzed 30 samples, 15 for each group. Samples were taken during 2012 from an approved European slaughterhouse involved in intra-authorized veterinary trade, fulfilling all specific legal requirements. The analyzes were conducted in the Animal Source Food Quality Control Laboratory belonging to the Animal Productions and Public Health Department - Faculty of Veterinary Medicine Bucharest.

The samples' **moisture content** has been established by using the heating and water evaporation method from a certain amount of product at a temperature of 103±2°C until a constant mass was reached, which means that the results of two successive weights did not change by more than 0,0001g (AOAC, 2000). For the same sample processed for analysis, there were performed two parallel determinations; the final result was given by the arithmetic mean. The percentage of water was calculated using the formula:

$$\% \text{ moisture} = (W_1 - W_2) * 100 / W,$$

where:

W = weight of the analyzed product (in grams);

W_1 = weight of the capped vial + stick + sand + sample: before drying (in grams);
W_2 = weight of the capped vial + stick + sand + sample: after drying (in grams).

Determination of **total protein** was done by establishing the total nitrogen with the Kjeldahl method, which consists in extracting the total nitrogen from a mineralized sample [as ammonium sulphate - $SO_4(NH_4)_2$], then expressing it as ammonia (through distillation and caption on acid) and converting the total ammonia into protein with a correction factor. The percentage of total proteic substances was calculated using the formula:

$$\% \text{ total protein} = [(V - V_1) \times 0{,}0014 \times 6{,}25 \times 100] / W$$

where:
V = n/10 sulphuric (hydrochloric) acid volume in the capture cup (in milliliters);
V_1 = n/10 natrium hydroxide volume for titer acid excess (in milliliters);
0,0014 = nitrogen equivalent in grams for 1 milliliter of n/10 sulphuric (hydrochloric) acid;
6,25 = correction factor for converting total nitrogen into protein;
100 = for percentual representation of total protein;
W = weight of analyzed sample (in grams).

Determination of **total lipids** was done by using Soxhlet extraction method, which consists in the extraction of fat from analyzed meat samples with an organic solvent (by repeated siphoning in a closed system), its quantitative accumulation and percentual expression. The extraction is considered complete after approximately 6-8 hours of consecutive siphonings (10-12 siphonings/hour). Once the extraction complete, the ether from the flask is evaporated and the drying can be done at 95-100^{o}C. The difference between the initial flask weight and its weight after the ether evaporation represents the amount of fat in the sample (AOAC, 2000). Fat percentage was calculated using the formula:

$$\% \text{ fat} = W \times 100 / w$$

where:
W = weight of fat extracted from the analyzed sample (in grams);
w = weight of the analyzed sample (in grams);
100 = for percentual representation of the result.

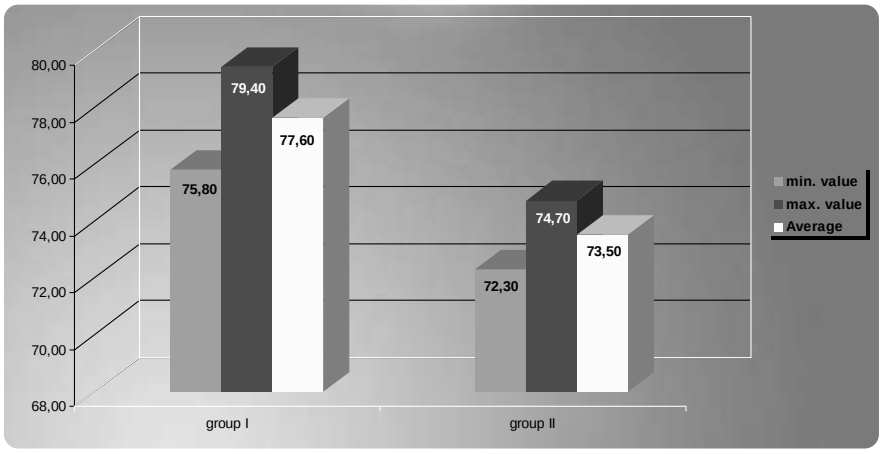
Ash percentage was determined by calcinating the sample. This consists in a complete transformation of organic substances in the sample, resulting in simple inorganic compounds which cannot be reduced, at a temperature of 525 ± 25°C, for 16-18 hours. Calculation was done using the formula:

$$\% \text{ ash} = W \times 100 / M$$

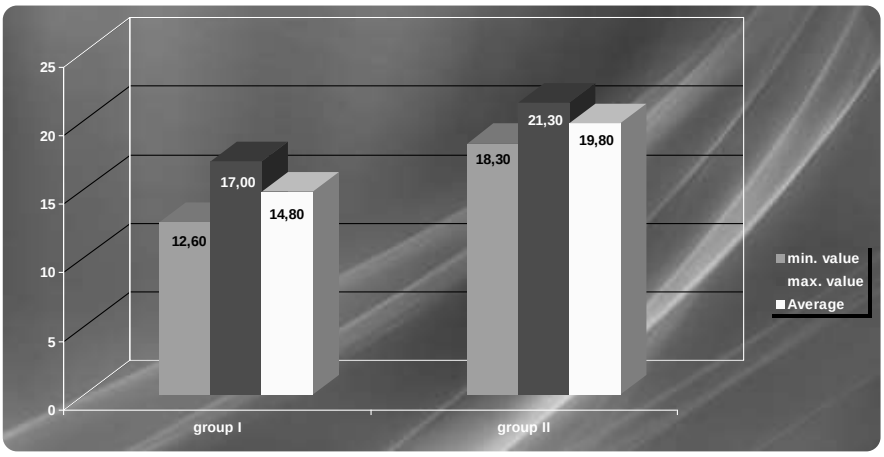
where:
W = weight of the ash after calcination (in grams);
M = weight of the analyzed sample (in grams);
100 = for percentual representation of the result.

RESULTS AND DISCUSSIONS

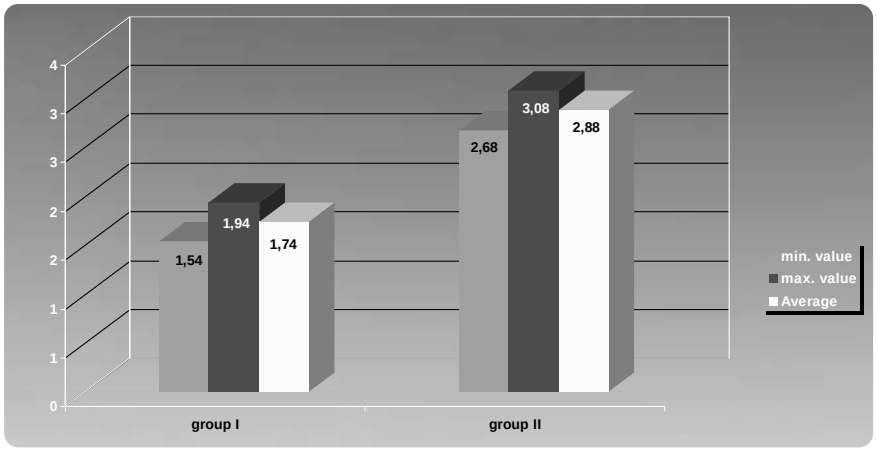
Regarding water content of the analyzed samples there were recorded average values of 77,6% in the samples collected from goatling, respectively of 73,5% in the samples from adult goats. The average values ranged within the following values: 75,8-79,4% for goatling, 72,3-74,7% for adult goats.



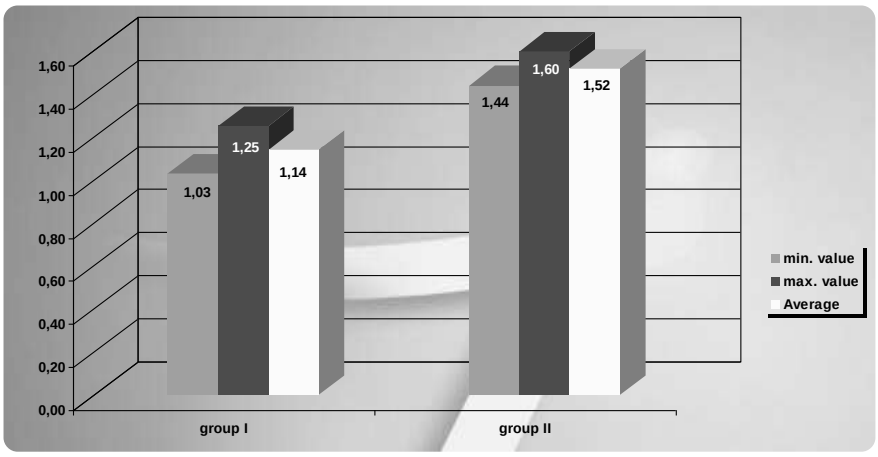
Average values for protein content were 14,8% (min. 12,6% and max. 17,0%) in goatling and 19,8% (min. 18,3% and max. 21,3%) for adult goats, which shows that age leads to a slightly increasing of protein content.



Fat percentage of the analyzed samples showed similar values for the two age groups, the difference being about 1%. Thus, the percentage of fat in goatling' meat samples was 1,74%, while in adult goats meat was 2,88%. These values were ranged between 1,54% and 1,94% in goatling, respectively between 2,68% and 3,08% in adult goats.



The difference between average values recorded for ash percentage was only 0,38%. The values obtained were 1,03 to 1,25% for goatlings (1,14% mean value) and 1,44 to 1,60% in adult goats (1,52% mean value).



CONCLUSIONS

As it can be seen, along with age the major components with nutritional role occupy a higher share in goat's carcass, leading to superior technological and sensory properties compared to the slaughtered goatling.
It is also noted that the values of these components are not close to the maximum ones recorded in other slaughtered livestock, which makes goat meat to be considered a healthy meat, regardless the age of animal's slaughter.
The only analyzed parameter which recorded decreased figures with increasing of age was water content, but the values were not as low as to describe a low digestibility or a decrease of pleasantness of the products.

REFERENCES

AOAC, 2000. Meat and Meat products. In: Official Methods of Analysis. Association of Official Analytical Chemists Inc. Gaithersburg, U.S.A.
Argüello A, Castro N, Carote J, Solomon M., 2005. Effects of diet and live weight at slaughter on kid meat quality. Meat Sci.
Babiker, S.A., I.A. El Khider and S.A. Shafie, 1990. Chemical composition and quality attributes of goat meat and lamb. Meat Sci.
Babji, Y., T.R.K. Murthy and A.S.R. Anjaneyulu, 2000. Microbial and sensory quality changes in refrigerated minced goat meat stored under vacuum and in air. Small Rum.
Lee HJ, Kouakou B, Kannan G., 2008. Chemical composition and quality characteristics of chevon from goats fed three ifferent postwaning diets. Small Rumin.
Tăpăloagă Dana, 2008. Sisteme de productie animaliera, Editura Vox, Bucuresti, ISBN 978-973-158-010-4.

FOOD SECURITY AND ADAPTATION TO CLIMATE CHANGE - GENOTYPING FOR RESISTANCE OF DISEASE

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ABSTRACT
Adaptation to climate change is essential for any efforts to promote food security, poverty alleviation, or sustainable management and conservation of natural resources. Many countries are already dealing with climate change impacts. The dynamic of food's world population projection to 2050 year and consumption dynamic of cereals, animal products (meat, milk, eggs) 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 2050 year 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 projections for the future socio-economic environment and the assessment of the situation and prospects of the natural resource base raise the question as to whether and under what conditions the estimated future food demand can be met and how food security can be achieved.

Keys Words: *food security, climate change, traceability*

INTRODUCTION

Long-term climatic models predict that the present breeds of low heat tolerance face serious risks for the stability of their production. Beside changes in management technologies the introduction (or selective breeding) of heat tolerant populations seems to be the most obvious step in adaptation to the new climatic conditions.Climatic changes indicate a scalable stressor for both farm animals and natural habitat. In farm animals the impacts can generally be alleviated by appropriately selected management technologies but in some cases these effects may even be amplified, as well. Changes in climatic environment for farm animals can be expressed not only in function of temperatures but temperatures, humidity

and circadian temperature threshold values combined in the Temperature Humidity Index (THI). Values above show that 72 THI can be considered as stressors in cattle. Based on mean values heat stress monthly distribution is heterogeneous characteristic regarding the seasons, but based on the gloomiest scenarios almost 60% of the territory will be affected by this change. Heat stress impairs several physiological, production and reproduction functions in cattle.

The severity of the impacts caused by heat stress of equal magnitude depends primarily on the genotype. Cattle breeds in tropical regions (Bos taurus types including N'dama, Senepol, Romosinuano, Carora, Bos indicus types with zebu genetic background) possess traits which allow for the regulation of body temperature with the maintenance of production and reproduction during times of heat stress. At the same time, thermo tolerant cattle breeds were not subjected to milk and meat production traits selection at the same rate as the European and North American cattle breeds.

Currently, humanity is in a new phase of economic and social development at the beginning of the XXI century - the century and millennium III - century, with numerous and varied characteristics knowledge society based on science and education. At the same time manifests successive economic-financial crisis, this requires integrated prevention and control of risk management and crisis situations. Knowing the food problem in complex relationships with explanations of population dynamics (10.6 billion-2050) issued with maximum credibility of organizations such as: NATO, the World Bank, FAO, and recognized experts in scientific forecasts and projections of long-term main focus of the United Nations Summit, held between 20-22 September 2010. Eradicating poverty and hunger are clear targets of global strategies. Globalization of the food chain causes constant new challenges and risks to health and consumer interests. 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).

The EU has developed a comprehensive body of legislation on food safety, which is continually monitored and adapted as new developments. Thus, 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. 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.The dynamic of food ‘s world population projection to 2050 year and consumption dynamic of cereals, animal products (meat, milk, eggs) 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 2050 year perspective also.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.

MATERIAL AND METHODS

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 origin. This scientifically paper presents results of research concerning recognition of genotypes by microsatellites genetic markers collecting and preserving the tissue samples by TypiFix method. Concerning traceability of animal products there are hopes as well. 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 TypiFixTM –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 pipe ting robot and a special one-step procedure (Nexttec technology). PCR reactions with hese 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.

In the future, many developed countries will see a continuing trend in which livestock breeding focuses on other attributes in addition to production and productivity, such as product quality, increasing animal welfare, disease resistance and reducing environmental impact. 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.

CONCLUSION

Adaptation to climate change is essential for any efforts to promote food security, poverty alleviation, or sustainable management and conservation of natural resources.The future of agriculture and the ability of the world food system to ensure food security for a growing world population are closely tied to improved stewardship of natural resources. Major reforms and investments are needed in all regions to cope with rising scarcity and degradation of land, water and biodiversity and with the added pressures resulting from rising incomes, climate change and energy demands. 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.

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

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.

ACKNOWLEDGMENTS

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REFERENCES

Bogdan, AT, DL, Constantinescu; Amalia, Străteanu; S. Chelmu; I. Surdu; M.Th. Paraschivescu (2009) "Solutions for livestock crisis by providing food independence European Romania"(Romanian)- intervention in the debate on "What can we learn from the current economic crisis?", Romanian Academy, Romanian Academy Publishing House, Bucharest,

Lutz, W.; W., Sanderson; S.,Scherbov (2001). The end of world population growth. Nature, 412, 543-545

*** European Commission (2005b), Prospects for Agricultural Markets and Income 2005 – 2012: Update for the EU-25, Brussels (December).

*** European Commission (2005c), Biomass Action Plan, [SEC(2005) 1573], Brussels.

Ipate Iudith, Brem G., A.T.Bogdan, Monika Gutscher, I.Seregi, G.F.Toba , L. Zoldag, A. Maroti-Agots (2009)- *Analiza microsatelitilor pentru studiul biodiversitatii si trasabilitatii la suine* , Lucrari Stiintifice Zootehnie si Biotehnologii – vol 42 (1) USAMV-Timisoara, pg.563,

UN, 2007. World Population Prospects: the 2006 revision. United Nations, Department of Economic and Social Affairs, Population Division, New York.

THE EVALUATION OF THE ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* AND *SALMONELLA* SPP. STRAINS ISOLATED FROM RAW MEAT

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Abstract
The antimicrobial resistance of the most probable pathogen germs isolated from raw meat has not been tested thoroughly yet in our country. The transmissibility of this resistance from food to human has been previously described in the foreign literature that is why such a complex study is mandatory given the current situation. For the isolation and antimicrobial resistance assessment, the classical method was used, the confirmation being performed through molecular methods (simplex PCR). In the antimicrobial evaluation the automatic system TREK was used. The majority of the Escherichia coli strains isolated from the meat samples were confirmed as non-pathogenic ones but revealing a high number among them as being resistant to ampicillin and enrofloxacin. The Salmonella spp. bacteria isolated was found in a lower amount and with a high prevalence of resistance to cefazolin, cefuroxime and tetracycline. Although a lot of food poisoning episodes are treated in the infectious diseases hospitals with ampicillin, enrofloxacin and tetracycline it has been shown in this study that these antibiotics might not have the wanted effect. We recommend the antibiogram in every case given the fact that these bacteria have become more and more resistant due to improper use of antibiotics in animal feed, animal illnesses and human diseases.

***Key words*:** antibiogram, antimicrobial, raw meat, resistance.

INTRODUCTION

The improvements in antimicrobial treatments along with the sanitation, nutrition and immunization have finally lead to a decrease in the number of deaths and a major gain in life expectancies (WHO, 2007). But along with the increase use of antimicrobials this phenomenon of resistance (AMR – antimicrobial resistance) has proved to be one of the most serious threats to human health (WHO, 2007) and a major concern for public health, anima health and also food safety authorities (Tenover, 2006; Talbot, 2006; Courvalin, 2005; O'Brien, 2002; Marchese, 2007). In order to minimize the risk of occurrence, a few steps must be taken into account,, like the

antibiotic prescription control and use in animal growth, in veterinary practices, improving hygiene, epidemiological studies' making and applying measures for decreasing the probability of cross-contamination among resistant strains and healthy individuals. The control of this phenomenon implies sustained efforts from the involved authorities, directed towards the identification of resistant bacteria as well as towards the transmissibility pathways to humans. The purpose of this study was to evaluate the antimicrobial resistance of *E.coli* and *Salmonella* spp. bacterial strains isolated from pork and chicken meat and to evaluate the possible effect of their spread in meat destined for public consumption.

MATERIAL AND METHODS

This study was conducted on 40 samples of pork meat collected from the carcass surface after the protocol previously described by Dragomir (2012) and 30 samples of poultry carcasses according to the methods and working procedures described in the National and Comunity legislation, respectively Regulation (CE) no.2073/2005 regarding the microbiological criteria for food products modified with Regulation. (CE) no.1441/2007.
The *in vitro* antimicrobial susceptibility was tested by agar difussion test using the Muller-Hinton method and also by Trek automatic system method. The Trek Diagnostic System is a rapid testing waqy of the bacteria strains to a large variety of antibiotics. This system use the Sensititre MIC plates, each of them being dosed with antimicrobials agents in corresponding dilutions. The results have been read automatically using the fluorescence captured by the ARIS/ AutoReader apparatus.
The inoculation procedure:
With a sterile loop, 3-5 freshly obtained colonies have been emulsified in sterile demineralized water, adjusted to 0.5 McFarland standards. After this step, 30µl from the suspension was transferred in a Muller Hinton broth of 11 ml that contains TES tampon. Afterwards, 50µl were transferred in Trek plates and then incubated for 24h at 37°C.

RESULTS AND DISCUSSIONS

The antimicrobial susceptibility of the *E.coli* strains isolated from pork and poultry meat are shown in figure1:

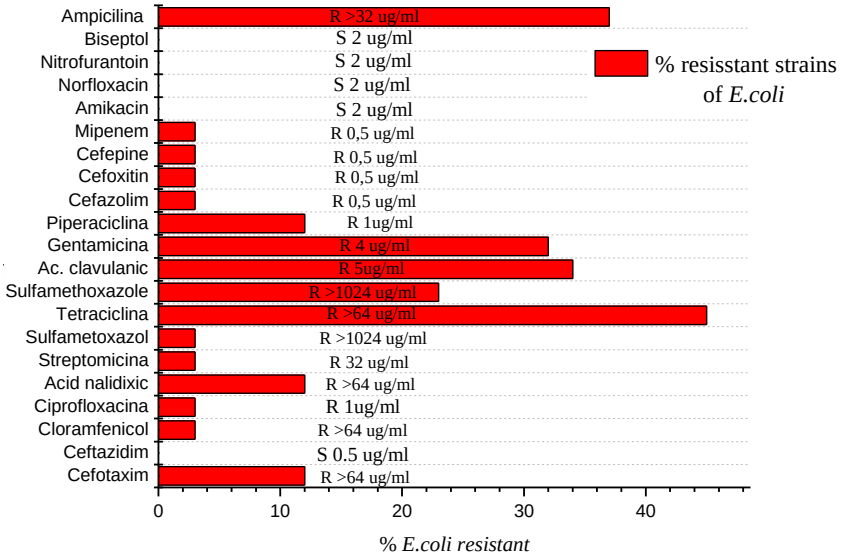

Figure 1. The percent of resistant *E.coli* isolated from pork and poultry meat

The number of *E.coli* strains resistant to some classes of antibiotics was relatively high given the fact that all the bacteria isolated came from raw meat samples destined for public consumption. The highest prevalence was revealed in the case of *E.coli* strains resistant to tetracycline and ampicillin. Thus, fig.1 shows that the *E.coli* strains were sensible to a series of antibiotics, the field literature stating the fact that there is a high prevalence of resistant among *E.coli* especially to fluoroquinolone (Andraud M. et al., 2011). The antibiotics that showed the highest percent in the case of resistant bacteria have proved to be the ones most frequently used in animal therapy at swine as well as birds.

The highest prevalence of *E.coli* resistant strains was found at poultry meat some authors claim that this is most commonly seen in pork meat (Kim et al., 2011). Our explanation for such a high prevalence in poultry meat is the fact that farmers still use in this intensive broiler growth systems antibiotics to reduce their mortality in the first days.

In case of *Salmonella* spp. identification, there was a low incidence of its occurrence in pork and poultry meat. Only one sample of pork meat was

identified with *Salmonella* spp. group C, that was sensible to all the antibiotics tested.

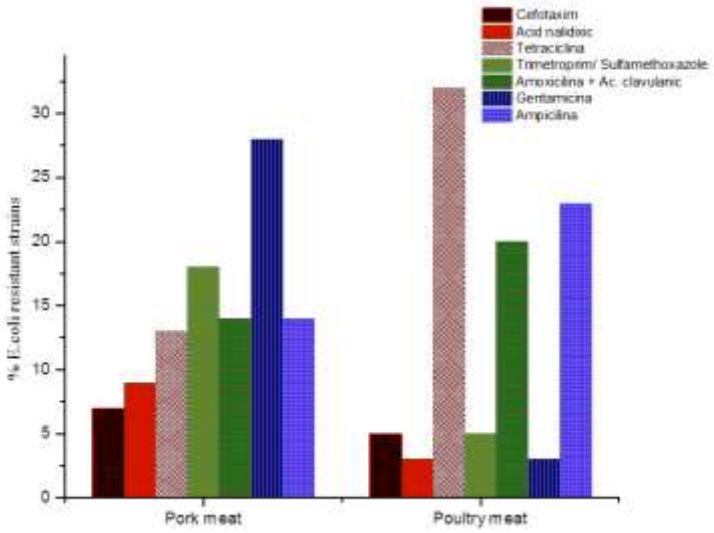

Figure 2. The frequency of *E.coli* resistant isolation among the two species studied

At the morphological exam of the selective media for the identification of *Salmonella* genus there were revealed red colored colonies with black center due to H_2S production on Rambach (Figure 3) but also on XLD media. In a study made by Marculescu et al. (2007) on *Salmonella* spp. prevalence at pig carcasses, the antimicrobial susceptibility was also evaluated. This study showed that there was a high prevalence of sensitive strains to ampicillin and amoxicillin (81.25%), with a low prevalence of resistant ones (18.75%). None of the poultry meat samples tested was found *Salmonella* positive which means that there is a low prevalence of occurrence in the studied slaughtering units.

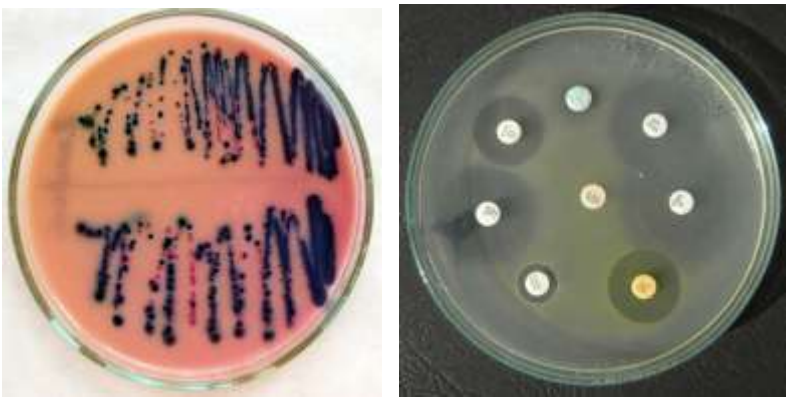

Figure 3. Identification of *Salmonella* spp. colonies on Rambach media and respectively the antimicrobial susceptibility tested by agar difussion

CONCLUSIONS

The prevalence of *E.coli* strains resistant to certain classes of antibiotics is relatively high in poultry meat. The most common resistance was observed at tetracycline (45%), over 30% of the poultry meat samples tested revealing *E.coli* resistant to tetracycline. The prevalence of *Salmonella* spp. in pork and poultry meat is low and the one investigated being sensible to every antibiotic tested. This study shows that there is an imperative need of keeping under control the transmissibility of *E.coli* resistant strains through food consumption given the great risk of contamination.

REFERENCES

Andraud M., Rose N., Laurentie M., Sanders P., Le Roux A., Cariolet R., Chauvin C., Jouy E., 2011. Estimation of transmission parameters of a fluoroquinolone-resistant Escherichia coli strain between pigs in experimental conditions. Vet Res., 42, 44-47.

Courvalin P., 2005. Antimicrobial Drug Resistance: "Prediction Is Very Difficult, Especially about the Future". Emerg Infect Dis.,11,1503-6.

Kim H., Baek H., Lee S., Jang Y., Jung S., Kim A., Choe N., 2011. Prevalence and antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolated from pigs at slaughterhouses in Korea. African Journal of Microbiology Research, 5(7), 823-830.

Marchese A, Schito G.C., 2007. Recent results of multinational studies on antibiotic resistance: should we have "PROTECTion" against these resistances. Med Mal Infect. 37, 2-5.

Mărculescu A., 2007. The methodology of setting up and the management of antibiotic treatments in veterinary medicine. Medicamentul veterinar/ Veterinary drug 1:2.

O'Brien T.F., 2002. Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. Clin Infect Dis., 34, S78-84.
Talbot G.H., Bradley J., Edwards J.E., Gilbert D., Scheld M., Bartlett J.G., 2006. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis., 42, 657-68.
Tenover F.C., 2006. Mechanisms of antimicrobial resistance in bacteria. Am J Med., 119, 10-17.
WHO. 2002, Antimicrobial resistance. Fact Sheet No 194.
WHO. 2007, A safer future: global public health security in the 21st century. Chapter 2: Threats to public health security. World Health Organizationed.
***Regulation (CE) no.2073/2005
***Regulation. (CE) no.1441/2007.

COMPLIANCE GENERAL AND SPECIFIC HYGIENE RULES FOR FOOD

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Abstract

Official controls should be carried out through appropriate techniques therefor, including checkups and a more intensive controls such as inspections, verifications, audits, sampling and sampling controls.
Community rules should not apply to primary production for private domestic use or preparation, handling and storage of food for private domestic consumption.
Relevant risk assessment takes into account the probability of their occurrence, severity and reproduction at every stage technology.
Food business operators shall develop, implement and maintain a permanent procedure or procedures based on HACCP principles.

Key words: *conformity, food, hygiene.*

Regulation (EC) no. 852/2004, the European Parliament and the Council shall lay down general rules on food hygiene to be respected by food business operators.
Certain foodstuffs may present specific hazards to human health and is necessary to lay down specific hygiene rules. This is particularly the case for food of animal origin that have been frequently observed microbiological and chemical hazards.
In addition, the direct supply to the final consumer or to local retail, small quantities of primary products or certain meats by the food business operator producing these foods, it is necessary national legislation to protect public health taking especially into account the close relationship between producer and consumer.
Controls in food producing units should be based on risk category involved in activities in that unit.
Food business operators placing on the market of animal products produced in the Community only if they were prepared and handled exclusively in establishments:
a) comply with relevant requirements of Regulation (EC) no. 852/2004 and other requirements that apply to food;

b) which have been registered or authorized by the competent authority.
At all stages of production, processing and distribution are interactions of food business operators, including animal feed sector and considerations links between animal health, animal welfare and public health. Should therefore have been a proper communication between the various parties involved in all processes of the food chain, from primary production to retail.
Food safety is a result of several factors: legislation should lay down minimum hygiene requirements, it should be held official checks to see whether food business operators comply with these requirements and food business operators must establish and implement food safety programs and procedures based on HACCP principles.
To ensure food safety is necessary to consider all aspects of the food production chain as a continuum, starting from and including primary production and feed production, up to and including sale or supply of food to consumer because each element may have a potential impact on food safety.
Traceability of food and food ingredients along the food chain is essential to ensure food safety.
Food business operators shall, as appropriate, the following specific hygiene measures:
a) compliance with microbiological criteria for foodstuffs;
b) the procedures necessary to achieve the objectives set out in community legislation;
c) compliance with temperature control requirements for foodstuffs;
d) the cold chain;
e) sampling and analysis.
If a food business operator considers or has reason to believe that a food which it has imported, produced, processed, manufactured or distributed does not satisfy food safety requirements, it shall immediately initiate procedures to withdraw the food in question from market where the food has left the immediate control of that initial operator and inform the competent authorities.
Where the product may have reached the consumer, the operator shall, efficiently and accurately the consumers of the reason for its withdrawal, and if necessary, recall from consumers products already supplied to them when other measures are not sufficient to achieve a high level of health protection.

Food business operators shall ensure that all stages of production, processing and distribution of food under their control satisfy health requirements. Also, they must ensure, insofar as possible, that products are protected against contamination, having regard to all the processing that products will subsequently undergo.
Food business operators may use national and community guides designed to help them meet their obligations.
Guides to good practice are developed and disseminated by food:
a) in consultation with representatives of parties whose interests may be substantially affected, such as competent authorities and consumer groups;
b) in compliance with the codes of practice of the Codex Alimentarius.
Food premises are to be kept clean and maintained in good condition. The layout, design, construction, siting and size of food premises are to:
- permit adequate maintenance, cleaning and / or disinfection, avoid or minimize air-borne contamination and ensure sufficient and adequate working space for the hygienic performance of all operations;
- to be such as to prevent the accumulation of dirt, contact with toxic materials, shedding of particles into food and the formation of condensation or undesirable mold on surfaces;
- to permit good hygiene practices, including protection against contamination and, in particular, pest control;
- to ensure adequate storage and handling conditions, with temperature control and sufficient capacity for maintaining foodstuffs at appropriate temperatures can be monitored and, if necessary, recorded.
In premises used for food must be: a sufficient number of toilets with running water, a sufficient number of sinks, suitable and sufficient means of natural or mechanical ventilation, natural lighting system and / or artificial appropriate, personal lockers .
Drainage facilities must be appropriate to. They must be designed and constructed so as to avoid the risk of contamination.
If drains are discovered, in whole or in part, they must be designed to prevent the discharge of waste water from a contaminated area clean areas, especially those where food is handled likely to present a high risk to the final consumer.
Cleaning agents and disinfectants should not be stored in areas where food is handled.

CONCLUSIONS

Food business operators may use national and community guides designed to help them meet their obligations.
Conformity of a food with specific provisions applicable to that food shall not prevent the competent authorities to take appropriate measures to impose restrictions on the placing on the market or to require its withdrawal from the market, where there is reason to suspect that, despite such compliance, foodstuff risk in terms of food safety.
Controls in food producing units should be based on risk category involved in activities in that unit.

REFERENCES

Regulation (EC) no. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs;
Regulation (EC) no. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin;
Regulation (EC) no. 882/2004 of the European Parliament and of the Council on official controls performed to ensure the verification of compliance with feed and food law and animal health rules for animal welfare;
Regulation (EC) no. 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety;

TRANSFER FACTORS FOR ENDOCRINE DISRUPTING COMPOUNDS FROM FEED TO MILK

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Abstract

Toxic compounds, such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are ubiquitous in the environment, thus indirectly in animals feed. The transfer of these compounds from animal feed to food products contributes to the human exposure, through consumption. OCPs and PCBs are highly persistent, biocumulative and exercise a wide range of toxic effects, including endocrine system disrupting, being considered as a new class of nonsteroidal xeno-estrogens. In this study, the transfer factors (TFs) for 19 OCPs and 7 PCBs have been evaluated for the quantification of their transfer from cows' fodder to milk. The samples (milk and feed from cow's diet) were collected in two villages from Maramures County, Romania. The compounds OCPs and PCBs were analyzed using gas-chromatography coupled with electron capture detection after solvent extraction. Among the OCPs, all the HCH isomers were detected in all the investigated samples, the highest concentration were obtained for α-HCH. All the isomers DDTs (except 2,4'-DDD) were determined, with highest concentration for 4,4'-DDE. The obtained results showed that the TFs ranged between 0.03-0.28, the highest value was recorded for hexachlorobenzene and the lowest for lindane. High values were determined also, for dieldrin (0.25) and for heptachlor (0.23).

Key words: *endocrine disruptors, feed, milk, transfer factor.*

INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent, bioaccumulative and toxic compounds (Tajkarimi et al., 2008). PCBs have been produced for many applications since 1929 and OCPs have been extensively used in agriculture and public health since the Second World War. Due to their wide range of toxic effects, the use and the production of PCBs and OCPs have been regulated worldwide, but they are still present in the environment (Herceg Romanic and Krauthacker, 2006).
Due to the lipophilic characteristic of organochlorine compounds (OCCs), they tend to accumulate and persist in fat tissues through dietary intake. Ingestion of contaminated food, especially with fat content (dairy products, meat and fish) is the main intake route through which OCPs and PCBs enter the human body (Huang et

al., 2008). Milk-producing animals, such as cows, accumulate residues of OCCs through contaminated feed and inhaled air (Heck et al., 2007).
There are evidences that OCCs exhibit a wide range of toxic effects and pose a serious risk to human health, especially for infants, since their enzymatic and metabolic systems are not fully active (Falandysz et al., 2004). Some OCPs (aldrin, dieldrin, DDT, 4,4-DDE, endosulfan, heptachlor, heptachlor-epoxide) and PCBs may interfere with the endocrine system and produce adverse effects in human and wildlife, these chemicals are referred as endocrine disruptors (Prins, 2008). DDT and its principal metabolites (p,p′-DDE, p,p′-DDT, p,p′-DDD, o,p′-DDT) can be involved also in human reproductive toxicity, cancer development, neurodevelopment and intellectual dysfunction in infants (Wang et al., 2009).
Different parameters can be used to describe the potential of various contaminants to be transferred from feed to milk (eggs, tissue) in order to assess the risk of these contaminants. The most used parameter is Transfer Factor (TF), based on long-term exposure of animals to a particular contaminant, so the contents in the milk (eggs, tissue) reach a constant value over time. The TF is calculated according to equation 1 (MacLachlan, 2011):

$$FT = C_i/C_{feed} \quad (1)$$

where: C_i is the residual concentration of the compound or element in animal product (milk, eggs, tissue),
C_{feed} is the residual concentration of the compound or element in the diet of the animal, including the contribution of soil ingestion (mg/kg dry weight).
In this study, the (TFs) for 19 OCPs and 7 PCBs were evaluated for the quantification of their transfer from cows' fodder to milk.

MATERIALS AND METHODS

Sampling
This study was conducted on animals that were exposed to food (grass/hay) with relatively fixed concentrations of contaminants/day for long periods. It was assumed that the main source of contamination of milk is the feed (grass/hay), eliminating from the study the water intake, inhalation of air and skin exposure. In July 2012, cow milk and grass/hay samples were collected in two villages from Maramures County: Satu Nou de Sus and Ocolis (Table 1). The grass/hay samples were collected from the investigated animals' diet, on the land where they are grazed.

Table 1. Sampling points and investigated samples' type

Village	Milk	Grass/hay
Satu Nou de Sus, nr. 179	1 sample (raw milk)	1 sample grass/hay
Ocolis, nr. 57	1 sample (raw milk)	1 sample grass/hay

Raw milk was collected in sterilized glass, previously cleaned with nitric acid 1:1 v/v and distilled water. The samples were refrigerated at -20°C until analysis procedure (Heck et al., 2007).

Reagents

For OCCs analysis, the used solvents (n-hexane, acetonitrile and ethanol) were gas-chromatography grade of quality (Merck, Darmstadt, Germany). Anhydrous sodium sulphate, silica gel and Florisil were acquisitioned from Merck (Darmstadt, Germany). Standard solution (NE7550) for OCPs and PCBs was purchased from LGC Promochem (Germany) and contained: α-, β-, γ-, δ-, ε-isomers of hexachloro-cyclohexane (expressed as HCHs), 4,4′-DDE, 2,4′-DDE, 4,4′-TDE, 2,4′-TDE, 2,4′-DDT and 4,4′-DDT (expressed as DDTs), aldrin, dieldrin, heptachlor, heptachlorepoxide (isomer A and B), alfa-endosulfan, beta-endosulfan, hexachlorobenzene (HCB) and the following PCB congeners: tri (28), tetra (52), penta (101), hexa (138, 153), hepta (180) and octa (194), at 10 μg/mL each analyte and working standard solutions were prepared diluting accurate volumes of mix standard solution in dichloromethane.

Instrumentation

In order to separate, detect and quantify the OCCs, gas chromatography electron-capture detection (GC-ECD) was used (Agilent Technologies 6890N GC-μECD) equipped with a DB-608 capillary column, 30 m L×0.32 mm ID×0.50 μm, (J&W). Helium was used as carrier and nitrogen as make up gas. For solvents evaporation was used a rotary evaporator, Laborota 4010 (Heidolph, Germany) coupled with a vacuum pump (Ilmvac, Germany).

Sample preparation

For OCCs analysis, the extraction of milk samples was carried out according to the method described by Ennaceur et al. (2007). The method consists of extraction with n-hexane, acetonitrile and ethanol, clean-up on Florisil column and elution with dichloromethane and n-hexane, evaporation with rotary evaporator and then GC-ECD analysis. The grass/hay samples were analysed according to the method reported by Yenisoy-Karakas (2006), using ultrasonic extraction with dichloromethane, clean-up on Florisil column and elution with cyclohexane–ethyl acetate mixture, evaporation with rotary evaporator and then GC-ECD analysis.

Sample analysis

In order to separate and quantify the organic compounds, subsequently the extraction and evaporation, 1 μL of purified extract was injected in the GC-ECD at 280°C. The oven temperature program consists of 4 stages, from 80°C to 275°C.

Three independent replicates of each sample were measured, and the concentrations were calculated using the average of each value.

RESULTS AND DISCUSSIONS

Organochlorine compounds in milk

The obtained values of the organochlorine compounds for raw milk samples collected from two villages in Maramures County are given in Table 2. Values are expressed in µg/L wet weight (ww) and represent mean ± standard deviation of three replicate determinations.

Table 2. Average concentrations of organochlorine compounds (µg/L ww) with standard deviations, in raw milk samples

Compound	**Milk, Satu Nou de Sus**	**Milk, Ocolis**
Hexachlorbenzene (HCB)	0.125 ± 0.044	<LQ
Aldrin	<LQ	0.030 ± 0.005
Dieldrin	0.362 ± 0.089	0.023 ± 0.007
α-HCH	1.010 ± 0.36	1.410 ± 0.315
β-HCH	0.116 ± 0.012	0.288 ± 0.081
γ-HCH (Lindane)	0.453 ± 0.101	0.173 ± 0.024
δ-HCH	0.128 ± 0.036	0.038 ± 0.004
ε-HCH	<LQ	0.283 ± 0.012
Heptachlor	<LQ	0.282 ± 0.054
Heptachlor epoxide β	<LQ	0.146 ± 0.0038
Heptachlor epoxide α	<LQ	0.035 ± 0.007
Endosulfan α	<LQ	0.012
Endosulfan β	<LQ	<LQ
2,4'-DDE	0.005 ± 0.001	0.012 ± 0.003
4,4'-DDE	0.501 ± 0.121	0.567 ± 0.108
2,4'-DDD	<LQ	<LQ
4,4'-DDD	0.034 ± 0.019	<LQ
2,4'-DDT	0.018 ± 0.008	0.007 ± 0.002
4,4'-DDT	0.093 ± 0.006	<LQ
PCB28	0.125 ± 0.341	<LQ
PCB52	1.010 ± 0.151	1.410 ± 0.76
PCB101	0.116 ± 0.031	0.288 ± 0.069
PCB138	0.453 ± 0.078	0.173 ± 0.008
PCB153	0.128 ± 0.024	0.038 ± 0.007
PCB180	<LQ	0.283 ± 0.086
PCB194	<LQ	0.030 ± 0.005

QL – quantification limit

The analysis of the obtained results allows the following observations:

All the investigated compounds recorded low concentrations.
In the milk sample collected from Satu Nou de Sus, the following analytes were detected: HCB, dieldrin, α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, endosulfan-beta, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, PCB28, PCB52, PCB101, PCB138, PCB153.
In the milk sample collected from Ocolis, the following analytes were detected: aldrin; dieldrin; α-HCH; β-HCH; γ-HCH; δ-HCH; ε-HCH; heptachlor; heptachlor epoxide β; heptachlor epoxide α; endosulfan alfa; 2,4'-DDE; 4,4'-DDE; 2,4'-DDT; PCB52; PCB101; PCB138; PCB153; PCB180; PCB194.
Among the OCCs, the highest concentrations were determined for α-HCH, both in sample collected from Satu Nou de Sus (1.010 µg/L ww) and Ocolis (1.410 µg/L ww). All HCH isomers were determined in relatively high concentrations, especially in the sample collected from Ocolis (α-HCH – 1.410 µg/L ww; β-HCH – 0.288 µg/L ww, γ-HCH (lindane 0.173 µg/L ww, δ-HCH – 0.038 µg/L ww; ε-HCH – 0.283 µg/L ww; heptachlor – 0.282 µg/L ww). In general, the average concentrations of OCCs were higher in milk samples from Satu Nou de Sus, for: dieldrin (15.74 times); γ-HCH (2.62 times); δ-HCH (3.37 times); 2,4'-DDT (2.57 times); PCB138 (2.62 times) and PCB153 (3.37 times).
For the following compounds the average concentrations recorded in Ocolis were higher than those of Satu Nou de Sus: α-HCH (1.40 times); β-HCH (2.48 times); 2.4'-DDE (2.40 times); 4.4'-DDE (1.13 times); PCB52 (1.40 times); PCB101 (2.48 times). Compared with the sample of Ocolis, only in the sample from Satu Nou the following compounds were determined: HCB (0.125 µg/L ww); 4,4'-DDT (0.093 µg/L ww); PCB28 (0.125 µg/L ww).

Organochlorine compounds in grass

The average concentrations of organochlorine compounds (mg/kg dry weight, dw) in grass samples from both studied villages are shown in Table 3.

Table 3. Average concentrations of organochlorine compounds (mg/kg dw) with standard deviations, in grass samples

Compound	**Satu Nou de Sus**	**Ocoliş**
Hexachlorbenzene (HCB)	0.13 ± 0.08	0.06 ± 0.02
Aldrin	0.05 ± 0.01	0.03 ± 0.01
Dieldrin	0.39 ± 0.12	0.02 ± 0.01
α-HCH	1.09 ± 0.32	1.59 ± 0.08
β-HCH	0.13 ± 0.08	0.32 ± 0.06
γ-HCH (Lindane)	0.51 ± 0.14	0.19 ± 0.05
δ-HCH	0.14 ± 0.07	0.04 ± 0.02
ε-HCH	0.08 ± 0.02	0.31 ± 0.10
Heptachlor	0.09 ± 0.03	0.32 ± 0.07
Heptachlor epoxide β	0.05 ± 0.01	0.16 ± 0.05
Heptachlor epoxide α	0.02 ± 0.01	0.04 ± 0.02

Compound	Satu Nou de Sus	Ocoliş
Endosulfan α	0.07 ± 0.03	0.01 ± 0.005
Endosulfan β	0.06 ± 0.02	0.05 ± 0.02
2,4'-DDE	0.01 ± 0.002	0.01 ± 0.004
4,4'-DDE	0.55 ± 0.07	0.64 ± 0.12
2,4'-DDD	0.06 ± 0.01	0.05 ± 0.02
4,4'-DDD	0.04 ± 0.02	0.04 ± 0.01
2,4'-DDT	0.02 ± 0.01	0.01 ± 0.003
4,4'-DDT	0.10 ± 0.03	0.01 ± 0.005
PCB28	0.14 ± 0.04	0.01 ± 0.003
PCB52	1.14 ± 0.42	1.62 ± 0.68
PCB101	0.12 ± 0.06	0.31 ± 0.06
PCB138	0.51 ± 0.11	0.19 ± 0.07
PCB153	0.14 ± 0.08	0.00 ±
PCB180	0.06 ± 0.02	0.32 ± 0.08
PCB194	0.05 ± 0.01	0.03 ± 0.01

QL – quantification limit

In the grass samples from the two investigated localities all the analysed endocrine disruptors were determined.

The highest concentrations were recorded for α-HCH (1.09 mg/kg dw in Satu Nou de Sus and 1.59 mg/kg dw in Ocolis) and PCB52 (1.14 mg/kg dw in Satu Nou de Sus and 1.62 mg/kg dw in Ocolis).

Dieldrin concentration recorded in Satu Nou de Sus is 16.04 times higher than in sample taken in the Ocolis. Similar behaviour was recorded also for 4,4 '-DDT (of 10.95 times higher in Satu Nou de Sus) and PCB28 (by 17.50 times higher in Satu Nou de Sus). Also, the following OCCs: alpha-endosulfan (5.30), beta-endosulfan (1.20), PCB138 (2.69) and PCB194 (1.49) present the same type of evolution, but with a lower ratio values was observed.

Transfer factors of organochlorine compounds

The transfer factors for organochlorine compounds from grass to milk are shown in Figure 3.

Regarding the obtained results, the followings can be concluded:

The grass-milk TFs ranged between 0.03 and 0.29, the highest value was recorded for HCB and the lowest for lindane (δ-HCH). High values were also obtained for dieldrin (0.25) and heptachlor (Σ(heptachlor+epoxide) (0.23).

Depending on the obtained values, the Transfer Factors increased in the following order: lindane>δ-HCH>ΣPCBs>DDT (Σ(DDTs+DDEs+DDDs))> heptachlor (Σ(heptachlor+epoxide)>dieldrin>HCB.

Compared to lindane, considered as reference, the TFs of the OCCs recorded the following valus: ΣPCBs were with 433% higher; DDT (Σ(DDTs+DDEs+DDDs)) with 633% higher, heptachlor (Σ(heptachlor+epoxide) with 766% higher; dieldrin with 833% higher, and HCB 966% higher.

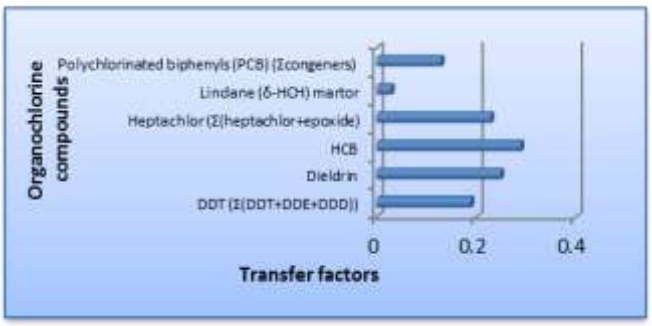

Figure 3. Transfer factors for organochlorine compounds

The grass-milk TFs ranged between 0.76 and 0.94, the highest values were recorded for Co (Satu Nou de Sus) and Pb (Ocolis) and the lowest, for Zn (Satu Nou de Sus) and Cu (Ocolis). Relatively high values were also obtained for all the investigated elements. The TFs for Ocolis samples were higher than TFs for Satu Nou de Sus, for all the elements.
The obtained values are comparable with those reported by MacLachlan (2011) with some exceptions, taking into consideration the particularities and the specificities of the investigated areas.
In the risk assessment it is important to determine the transfer factors of contaminants compounds, taking into consideration also the differences in animal physiology and the growth rates.

CONCLUSIONS

All the investigated compounds recorded low concentrations. Among the OCCs, the highest concentrations were determined for α-HCH, in both samples. HCH isomers were determined in relatively high concentrations, especially in the sample collected from Ocolis. In general, the average concentrations of OCCs were higher in milk samples from Satu Nou de Sus, for: dieldrin, γ-HCH, δ-HCH, 2,4'-DDT, PCB138 and PCB153.
In the grass samples from the two investigated localities, all the analysed endocrine disruptors were determined. The highest concentrations were recorded for α-HCH and PCB52.
The grass-milk TFs ranged between 0.03 and 0.29, the highest value was recorded for HCB and the lowest for lindane (δ-HCH).

Depending on the obtained values, the Transfer Factors increased in the following order: lindane>δ-HCH>ΣPCBs>DDT (Σ(DDTs+DDEs+DDDs))> heptachlor (Σ(heptachlor+epoxide)>dieldrin>HCB.

REFERENCES

do Nascimento, I., de Jesus, R. M., dos Santos, W. N. L., Santos, S. A., Fragoso, W. D., dos Reis, P. S., 2010, Determination of the mineral composition of fresh bovine milk from the milk-producing areas located in the State of Sergipe in Brazil and evaluation employing exploratory analysis, *Microchemical Journal*, 96, 37–41.

Ennaceur S., Gandoura N., Driss M.R., 2008, Distribution of polychlorinated biphenyls and organochlorine pesticides in human breast milk from various locations in Tunisia: Levels of contamination, influencing factors, and infant risk assessment, *Environmental Research*, 108, 86– 93.

Falandysz J., Wyrzykowska B., Warzocha J., Barska, I., Garbacik-Wesolowska A., Szefe, P., 2004, Organochlorine pesticides and PCBs in perch Perca fluviatilis from the Odra/Oder river estuary, Baltic sea, *Food Chemistry*, 87, 17-23.

Heck M.C., J. Sifuentes dos Santos, S. Bogusz Junior, I.Costabeber, Emanuelli T., 2007, Estimation of children exposure to organochlorine compounds through milk in Rio Grande do Sul, Brazil, *Food Chemistry*, 102, 288-294.

Herceg Romanic S., Krauthacker B., 2006, Organochlorine pesticides and PCB congeners in human milk from two population groups in Croatia, *Bulletin of Environmental Contamination and Toxicology*, 76, 705-711.

Huang, M., Zhou, S., Sun, B., Zhao, Q., 2008, Heavy metals in wheat grain: Assessment of potential health risk for inhabitants in Kunshan, China, *Science of the Total Environment*, 405, 54-61.

MacLachlan, 2011, Estimating the transfer of contaminants in animal feedstuffs to livestock tissues, milk and eggs: a review, *Animal Production Science*, 51, 1067–1078.

chain, *The International Journal of Biochemistry and Cell Biology*, 41, 1665–1677.

Prins G.S., 2008, Endocrine disruptors and prostate cancer risk, *Endocrine-Related Cancer*, 15, 649-656.

Tajkarimi M., Faghih M.A., Poursoltani H., Nejad A.S., Motallebi A.A., Mahdavi H., 2008, Lead residue levels in raw milk from different regions of Iran, *Food Control*, 19, 495-498.

Wang Y.-R., Zhang M., Wang Q., Yang D.-Y., Li C.-L., Liu J., Li J.-G., Li H., Yang X.-Y, 2009, Exposure of mother–child and postpartum woman–infant pairs to DDT and its metabolites in Tianjin, China, *Science of the Total Environment*, 396, 34-41.

Yenisoy-Karakas, S., 2006, Validation and uncertainty assessment of rapid extraction and clean-up methods for the determination of 16 organochlorine pesticide residues in vegetables, *Analytica Chimica Acta*, 571, 298–307.

THE IMPORTANCE OF PIG TONSILS REMOVAL FOR THE FINAL ASSESSMENT OF THE CARCASSES' HYGIENE QUALITY

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Abstract

Although the tonsils removal at pigs is mandatory in conformity with the European Union Regulation 854/2004, there are slaughtering units that do not respect this rule. The importance of their complete removal is shown in this study through a thorough assessment of the bacteria load found in tonsils and afterwards in the carcasses where the tonsils have not been removed. For the isolation of these bacteria the classical method was used and the confirmation being performed through biochemical microtest systems (API) and molecular methods (simplex PCR). Also, there were two other automatic ways of bacteria identification: the microscan WALKAWEY system and the Trek system. The results revealed a polymorphic microflora, with a predominance of Gram negative bacteria in the majority of the tonsil samples examined. The bacteria prevalence in the pharyngeal tonsils was represented by: Gram negative bacilli (32.1%), diplococcic (19.75%), streptococci (16.05%), Gram positive bacilli (13.58%), polymorphic non spore forming Gram positive bacilli (8.64%), spore forming Gram positive bacilli (1.24%) and Candida (8.64%). Following the bacteriological exam, a large variety of bacteria species were revealed at the carcasses also, worth mentioning are Staphylococcus, Proteus, Streptococcus, Listeria and Salmonella and the prevalence of these species was significantly higher ($p<0.05$) during the warm season than the cold one in both units studied. The pharyngeal tonsils at pig represent a deposit area for some pathogen bacteria (Yersinia enterocolitica, Staphylococcus aureus, Escherichia coli, Salmonella spp.), that can contaminate the carcasses during slaughtering and can jeopardize the consumers' health.

Keywords: carcasses, hygiene, tonsils, quality.

INTRODUCTION

The lymphoid tissue of the pharyngeal tonsils plays a very important role in the immune response against oral and nasal cavity bacteria (Belz and Heath, 1996; Horter et al., 2003). A large number of studies have high lightened an asymptomatic prevalence with commensal bacteria like *Actinobacillus pleuropneumoniae, Actinobacillus suis, Streptococcus suis, Haemophilus*

parasuis or *Mycoplasma hyopneumoniae* and some viruses: the respiratory and reproductive syndrome virus, the classical swine fever (Marois et al., 2008). The pharyngeal tonsils can represent the focus of some pathogen bacteria like *Salmonella spp*., *Campylobacter spp*., *Listeria monocytogenes, E. coli* or *Yersinia enterocolitica* (Swanenburg et al., 2001; Bucher et al., 2008). The purpose of this study was to make a complex evaluation of the bacteria prevalence through bacterioscopic and bacteriologic methods combined also with molecular ones for accurate confirmation in pigs' pharyngeal tonsils and to correlate them to the hygiene quality of the carcasses obtained.

MATERIALS AND METHODS

The study was conducted on 81 pig pharyngeal tonsils samples collected from two slaughterhouses ("A" and "B") found in Maramureş county and respectively 40 samples taken from the carcasses obtained in the same units.
The methodology of examination followed the steps stated in the National and Comunity legislation, respectively Regulation (CE) no.2073/2005 regarding the microbiological criteria for food products modified with Regulation. (CE) no.1441/2007. The cultural and morphological aspects of the bacteria were characterized on regular and blood media. The morphological characters were revealed on smears made from broth test tubes BHI stainnned by Gram method. For differentation, colonies developed on broth were passed on differentiaton on selective media: Chapmann or Baird-Parker for staphylococci, Mac Conkey and XLD for Enterobacteriaceae. The colonies were incubated for 24 hours at 37º C. for confirmation the following API galleries were used: API 20 STAPH, API 20 STREP, API 20 E and ID32 E.
The bacteria isolation was performed also by using the automatic system WALKAWEY which identifies the susceptibility patterns *in vitro* of the isolated bacteria from the clinical specimens. It is used along with the Microscan dilution plates that contain media, antimicrobials in seriate dilution and selected chemical reagents. The confirmation was performed also by molecular testing (PCR) following the protocol described previously by Lăpuşan A. (2012). The reaction was performed in a 25µl in which it was added: 12,5 µl MyTaq (Bioline), 1µl primer Forward; 1µl primer Reverse, 4 µl DNA and 6,5 µl pure grade water PCR (Sigma). In order to confirm the *Listeria* bacteria we used the Listeria Genus Primer Set (BioScientific) which contains the Foward and Reverse primers common for all the *Listeria*

species. For the confirmation of *Salmonella* spp. the following primers previously used by Malorny et al. (2004), were selected:

F: 5'- CTCACCAGGAGATTACAACATGG -3

R: 5' – AGCTCAGACCAAAAGTGACCATC – 3'

The *E.coli* confirmation was performed using the following sequences that amplify the common region (23S) of all *E.coli* strains, having a molecular weight of 736 bp.

F: 5' – AAGGAATCACCTTGCAGATAAACTC – 3'

R: 5' – TTTCCGAGTACATTGGCATCGT – 3'

The amplification protocol was: Hot start: 95°C–5min.; Denaturation: 94°C – 00.45''; Alignment: 55°C (*Listeria* spp.); 62°C (*Salmonella* spp.); 57°C (*E.coli*) – 00.45''; Elongation: 72°C – 1min. These cycles were repeated 35 times after which the final elongation was made at 73°C for 4 minutes. The statistic analysis was performed in Windows 7, program Origin 8.5, ANOVA test.

RESULTS AND DISCUSSIONS

Following the bacterioscopic exam of the 81 smears made from the analyzed tonsils the following prevalence was revealed: Gram negative bacilli (32.1%), dyplococci (19.75%), streptococci (16.05%), Gram positive bacteria (13.58%), Gram positive bacilli non-sporulated (8.64%), sporulated Gram positive bacilli (1.24%) and *Candida* yeasts (8.64%). Results are shown also according to the unit in figure 1:

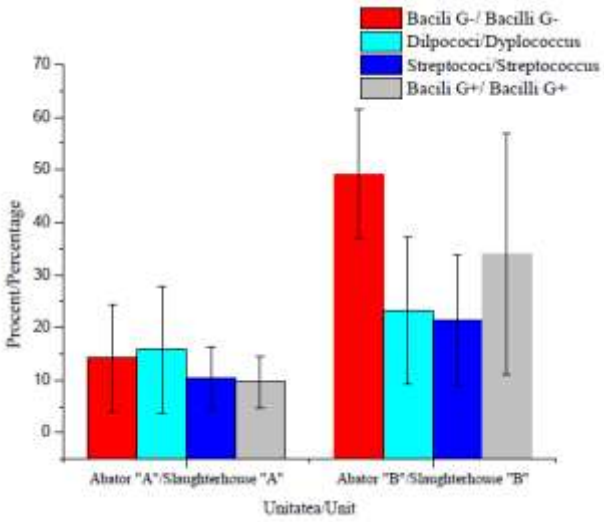

Figure 1: The bacteria prevalence in the units studied

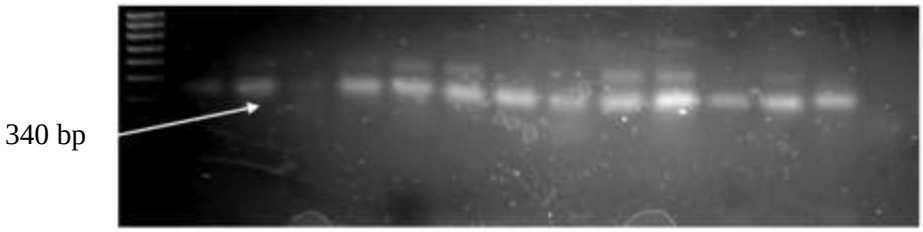

Figure 2: The electrophoresis profile of the 340 bp fragment corresponding to *E.coli*

Along the *E.coli* strains identified we had samples were we found *Streptococcus* spp. associated with *Klebsiella pneumoniae.* In the samples harvested from the small unit "B" in 16 of them there were identified germs belonging to *Staphylococcus* genre. With the help of Walkawey system it was confirmed with a certainty of 85% that the strain identified was *Staphylococcus aureus* in 3 of the samples tested (18%). The rest of the positive samples belonged to *S. chromogenes* (44%), *S. Intermedius* (22%), *S. Werneri* (16%).

It is well-known that the palatin tonsils are an entrance gate and a multiplication area for a number of microorganisms (Salles and Middleton, 2000). In this study the number of bacteria and especially *E.coli* was very high. In the samples harvested from unit "B", the bacteria load was the highest, and the percent of *E.coli* reported a prevalence of 76%. Some of these samples (12%) were confirmed also for *Staphylococcus* spp. The

E.coli prevalence in this study is much higher from the one previously reported by Salles and Middleton (2000).
Most of the bacteria strains isolated from the tonsils were identified also at the carcasses samples examined. The prevalence was again revealed for *E.coli* (56%) and *Staphylococcus* spp (34%). In previous studies concerning pork carcasses obtained in different slaughtering units the Enterobacteriacaeae bacteria were revealed in a high percent (75%) (O'Brien et al., 2007, Quirke et al., 2001). Similar results have been reported by other authors like Dorsa et al., 2000, Gill et al., 2000, Pearce et al.,2005).
Another study made on the prevalence of *Salmonella* spp. in three processing steps of pig slaughtering (after the bleeding, at higiene and refrigeration) has shown that from a total of 182 positive samples, 24% were confirmed as *Salmonella* spp. after bleeding and 3% after refrigeration (Bouvet et al., 2003). The most common serovars were S. Typhimurium (27%) and S. Dersy (40.5%)**.**

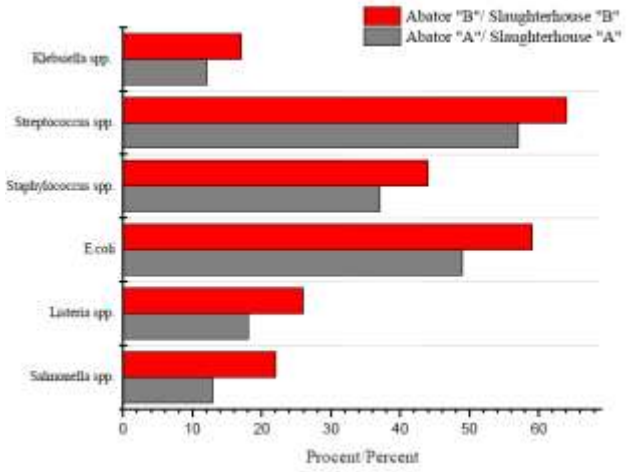

Figure 3: The comparative analysis of the bacteria prevalence in tonsils and carcasses between the two units studied

The highest prevalence of bacteria in tonsils and carcasses was found in the small unit "B" where it was seen at a risk analysis performed that the tonsils are not being removed completely or not at all. It was not surprising the fact that in this slaughterhouse the hygiene quality of the carcasses was much lower as it can be seen in figure 3. Concerning for public health is the presence in both units of the *Salmonella* spp. and *Listeria* spp. at the

carcasses analyzed which represent a great risk for food poisonings. All the *E.coli* strains identified were non-pathogenic.

CONCLUSIONS

In the small capacity unit, the bacteria prevalence was significantly different ($p<0.05$) than in the large unit, identifying strains that were present also in the carcasses.

The pharyngeal tonsils at pig, represent a deposit area for some pathogenic bacteria (*Yersinia enterocolitica*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp.) that can contaminate the carcasses during the slaughtering process if the tonsils' removal process is not correctly performed and can jeopardize the consumer's health.

REFERENCES

Belz G.T., Heath T.J., 1996. Tonsils of the soft palate of young pigs: crypt structure and lymphoepithelium. Anatomy Researches, 245, 102-113.

Bouvet J., Bavai C., Rossel R., Le Roux A., Montet M.P., Mazuy C., Vernozy-Rozand C., 2005. Evolution of pig carcass and slaughterhouse environment contamination by Salmonella 2003. Journal of Aplied Microbiology, 98, 896-900.

Bucher M., Meyer C., Grotzbach B., Wacheck S., Stolle A., Fredriksson-Ahomaa M., 2008. Epidemiological data on pathogenic Yersinia enterocolitica in Southern Germany during 2000-2006. Foodborne Pathog Dis., 5, 273-280.

Dorsa W.J., Cutter C.N., Siragusa G.R., 2000. Evaluation of six sampling methods for recovery of bacteria from beef carcass surfaces, Letters in Applied Microbiology, 22, 39–41.

Gill C.O., Jones T., 2000. Microbiological sampling of carcasses by excision or swabbing, Journal of Food Protection, 63, 167–173.

Horter D.C., Yoon K.J., Zimmerman J.J., 2003. A review of porcine tonsils in immunity and disease, Animal Health Researches, 4, 143-155.

Lăpuşan A. (2012) PhD thesis, Researches concerning the certified quality and authenticity markers in the traceability of buffalo milk and dairy products.

Malorny B., Paccassoni E., Fach P., Bunge C., Martin A., Helmuth R., 2004. Diagnostic real-time PCR for detection of Salmonella in food, Applied and Environmental Microbiology, 70, 7046–7052.

Marois C., Cariolet R., Morvan H., Kobisch M., 2008. Transmission of pathogenic respiratory bacteria to specific pathogen free pigs at slaughter. Veterinary Microbiology, 129, 325-332.

O'Brien S., Lenahan B., Sweeney M., Sheridan T., 2007. Assessing the hygiene of pig carcasses using whole-body carcass swabs compared with the four-site method in EC Decision 471, Journal of Food Protection, 70, 432–439.

Pearce R.A., Bolton D.J., 2005. Excision vs. sponge swabbing a comparison of methods for the microbiological sampling of beef, pork and lamb carcass. Revue Med. Vet, 154, 775-779.
Quirke A.M., Leonard N., Kelly G., Lynch P.B., Rowo T., Quinn P.J., 2001. Prevalence of Salmonella serotypes on pig carcasses carcasses from high- and low-risk herds slaughtered in three abattoirs, Berl Munch. Tierarztl., 114, 360-362.
Salles, M. W. S., & Middleton, D. M. (2000). Lymphocyte subset in porcine tonsillar crypt epithelium. Veterinary Immunology and Immunopathology, 77, 133–144.
Swanenburg, M., P.J. van der Wolf, H.A.P. Urlings, J.M.A. Snijders, F. van Knapen, 2001, Salmonella in slaughter pigs: The effect of logistic slaughter procedures of pigs on the prevalence of Salmonella in pork. International Journal of Food Microbiology, 70, 231–242.
***Regulation (CE) no.2073/2005
***Regulation. (CE) no.1441/2007.

WELFARE ASSESSMENT IN DAIRY COWS IN A FARM FROM PRAHOVA COUNTY

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Abstract

Assuring a good animal welfare level is not only a mean of increasing livestock production, but also a moral duty of human society. As a major concern, of general interest, animal welfare is covered by numerous governmental or nongovernmental organizations and bodies: Food and Agriculture Organization of the United Nation, World Trade Organization, European Council, European Union, Intergroup on the Welfare and Conservation of Animals, Eurogroup for Animals, World Organization for Animal Health, Codex Alimentarius, World Veterinary Association, World Society for the Protection of Animals.

The present paper aims to assess the welfare level in a farm from Prahova County, respectively in 2 houses for dairy cows with capacities of 520 and 480 animals, reared in collective pens. Because our country hasn't an official welfare assessment system, we used an integrative numerical system from Austria organic farming: Animal Needs Index 35. This system consists in the study of welfare indicators included in 5 areas of influence: locomotion, social interaction, flooring, light and air, stockmanship, for each parameter points being awarded. The sum of all scores gives the overall ANI score. The research was based on metric measurements, data from health records, body hygiene score, gait score or were done by using specific equipment (Dräger MiniWarn portable gas analyzer, LM8010 multifunction device, SL4012 sound level meter, Hill catathermometer).

The overall ANI score was 24 for the first house and 23.5 points for the second - with a weighted average of 23.76 points. As critical issues stand out: lack of outdoor access, draughts' high velocity, poor hygiene of pens, feeding and drinking areas, as well as poor body hygiene of animals (soiled animals).

Based on obtained scores, the welfare of dairy cows in the farm can be rated as average.

Key words: *dairy cows, score, welfare.*

INTRODUCTION

Donald Broom defines the welfare of an animal as "its physical and psychological state as regards its attempts to cope with its environment". According to this definition, animal welfare could largely vary from very poor to very good and, most important, can be measured (Broom, 1996).

Assuring a good animal welfare level is not only a mean of increasing livestock production, but also a moral duty of human society. As a major

concern, of general interest, animal welfare is covered by numerous governmental or nongovernmental organizations and bodies: Food and Agriculture Organization of the United Nation, World Trade Organization, European Council, European Union, Intergroup on the Welfare and Conservation of Animals, Eurogroup for Animals, World Organization for Animal Health, Codex Alimentarius, World Veterinary Association, World Society for the Protection of Animals (Teusdea, 2005).

MATERIALS AND METHODS

The present paper aims to assess the welfare level in a farm from Prahova County, respectively in 2 houses for dairy cows with capacities of 520 and respectively 480 animals.

Cows are reared in open fronted houses with 200 m length, 50 m width and pitched roofs. The lighting and natural ventilation are assured by 2 openings in the longitudinal walls limited by tarpaulins of equal length with the walls and adjustable height (60 -150 cm), as well as by a shed of 40 cm width. For completing the natural light, it was used artificial lighting: 12 light bulbs of 450 W and 12 of 250 W.

The houses' inner space division consists in 4 collective pens of 97,5 m/21,5 m with deep litter bedding, each pen for a group of maximum 130 cows. The milk is collected in a dairy hall linked with both houses by covered passage ways.

Due to the fact that our country hasn't an official welfare assessment system, we used an integrative numerical system from Austria organic farming: Animal Needs Index 35 (Bartussek et al., 2000). This system consists in the study of welfare indicators included in 5 areas of influence: locomotion, social interaction, flooring, light and air, stockmanship, for each indicator points being awarded. The sum of all scores gives the overall ANI score.

The research was based on metric measurements, data from health records, body hygiene score, gait score and specific equipment (Dräger MiniWarn portable gas analyzer, LM8010 multifunction device, SL4012 sound level meter, Hill katathermometer).

RESULTS AND DISCUSSIONS

For the first area of influence – locomotion – there were assessed 2 indicators: floor area (space allowance) and resting area comfort (lying down, lying and rising possibilities) respectively.

The indicators regarding outdoor access could not be taken into account, because the animals did not have access to paddocks or pasture.

Regarding *space allowance* (obtained by dividing the total surface of the four collective pens by the value of animal weight unit (500 Kg), the results were 11.16 sqm for the first house and 12.09 sqm for the second, much higher than the reference optimum value for dehorned dairy cows which is 8 sqm. Therefore, for this indicator it was awarded a maximum score of 3

points.
The *resting area* is confortable – the surface is large, the floor is covered with deep bedding (figure 1) and the pen walls didn't restrict any animal behavior (there were not noticed abnormal lying down/rising behaviors, repetitive head swinging etc.), so for this indicator the score was also the maximum one: 3 points.
The general score for the first area of influence was 6 points.

Figure 1. Different aspects from one of the studied houses for dairy cows
Left: deep bedding area, Right: walking, feeding and manure collection area

For the second area of influence – social interactions – there were assessed 2 indicators: space allowance and herd structure.
Similar to the indicator from the first area of influence, *space allowance* received a maximum score of 3 points.
Regarding *herd structure*, it was practiced stock division in production groups and not in family herds, the last being known for better promoting animal behavioral manifestations. Therefore, it was awarded 1 point for this indicator.
The general score for social interaction was 4 points.
For the third area of interest – flooring – the following four indicators were assessed:
Softness of the lying area: because it was used straw bedding with more than 60 mm thickness and the floor was continuous, with first layer of concrete, this indicator was scored with 2.5 points.
Cleanliness of the lying area: it was rated as medium and scored with 0.5 points.
Slipperiness in the lying area: the floor had a proper grip, preventing cows' sliding or falling, the given score being 1 point.
The type and characteristics of activity areas (passage ways, feeding and drinking areas, manure collecting areas): The activity areas are quite clean, with low risks of slipping; the incidence of lameness is also reduced. The score was 0.5 points.

The general score for flooring was 4.5 points.

For the fourth area of influence – light and air – there were assessed four indicators: light, air quality, draughts in lying area and noise.

Because the houses were open, the *light* (both the intensity and uniformity) was optimum and was awarded with 2 points (percentage of transparent openings relative to floor area being 16% and artificial light intensity of 1.68 W/sqm). The light intensity, measured with LM 8010 light sensor, varies between 167 and 368 Lx, the coefficient of light uniformity having the value of 0.454 (higher than 0.3, the reference minimum value for an even light).

Air quality was very good, the ammonia level reaching maximum 1 ppm in the first house and 2 ppm in the second. However, it can be noticed that the temperatures often recorded values outside the thermal comfort interval for cows (10-14 ˚C). The score was 1.5 points.

Regarding *draughts in the resting area*, the scores were 1 point for the first house (which had a better wind protection) and 0.5 points in house 2. For the second house, the maximum air draught velocity was 1.15 m/s measured at 1 meter distance from longitudinal wall and 0.3 m/s in the center of the house (reference values being 0.5 m/s).

Noise was awarded with 0.5 points, corresponding to some noise. In fact, the noise level is reduced, but it becomes much higher (even 86 dB) when manure are collected by the tractor blade.

The general scores for light and air were 4.5 points for the first house and 4 points for the second.

In the last area of influence – stockmanship – all indicators could be approached.

The hygiene of resting and activities areas was rated as insufficient, so the given score was 0 points.

The technical condition of equipment was good, being awarded with 1 point.

The condition of integument was rated as medium and scored with 0.5 points. There were observed minor lesions in 12% from total number of cows.

The cleanliness of animals was poor and had received 0 points. For an increased objectivity, it was used body hygiene score (Cook and Reinemann 2007).

Condition of hooves was rated as good and received 1 point. Lameness affected 9% of the stock, with mild symptoms. There was applied gait score in a batch of 50 cows, resulting 2 animals with 2 points, 1 animal with 3 points, the others being scored with 1 – normal (Cook, 2005).

The technopaties incidence (mastitis, osteoarticular disorders, abnormal behaviors) was very low (1%), this indicator being scored with 1.5 points.

On the basis of farm records, the *health status* was rated as good and received 1 point. The bronchopneumonia had an incidence of 1-2 cases/year, placental retention an incidence of 2-3%, endometritis of 10%, abomasal displacement of 0.1-0.5%.

The general score for stockmanship was 5 points.

The general scores obtained for the five areas of influences in the two studied houses for dairy cows are shown in figure 2.

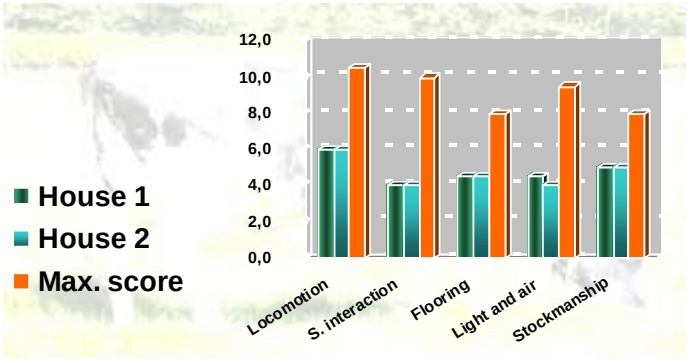

Figure 2. General scores for ANI 35 areas of influences in the studied houses of dairy cows

The overall ANI score was 24 for the first house and 23.5 for the second, with a weighted average of 23.76 points.

CONCLUSIONS

Based on obtained scores, the welfare of dairy cows in the farm can be rated as average.

As critical issues, stand out the lack of outdoor access, the draughts' high velocity, the poor hygiene of pens, feeding and drinking areas, as well as a poor body hygiene (soiled animals).

REFERENCES

Bartussek H., Leeb Ch., Held S., 2000. Animal needs index for cattle (ANI 35L/2000 - cattle). Federal Research Institute for Agriculture in Alpine Regions BAL Gumpenstein, Austria

Broom D. M., 1996. Animal welfare defined in term of attempts to cope with the environment. Acta Agric. Scand, Sect. A, Animal Sci. Supplementum, no. 27/1996: 22-28

Cook N.B., Reinemann D.J., 2007. A toolbox for assessing cow, udder and teat hygiene, In: Proc. Natl. Mastitis Counc. Annu. Mtg. Natl. Mastitis Counc. Inc., Verona, WI, 31–43

Teusdea V., 2005. Bunastarea si protectia animalelor, Editura Omega Print, Bucuresti

*** vetmed.wisc.edu/dms/fapm/fapmtools/6lame/New5point_locomotionscoreguide.pdf (Cook, N.B., 2005)

EVALUATION OF FOOD ESTABLISHMENTS

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Abstract

Units that produce, process, store, transport and / or distribute products of animal origin may carry out veterinary checked only after obtaining the veterinary authorization.
Before entering the legal representative catering units required to obtain, where appropriate, the veterinary authorization, conditional authorization or authorization veterinary intra-community trade in food of animal origin from the Veterinary Direction and Food Safety.
This unit gives authorization for the activities concerned only if the food business operator has demonstrated that the unit complies with the relevant requirements of Regulations (EC) no. 852/2004 and (EC) no. 853/2004 and other relevant requirements of food law.
Establishments handling products of animal origin may perform activities only if they have been approved by the competent authority.
After rectification of deficiencies, the legal representative of the unit applies for veterinary direction and food safety county reevaluation unit.

Key words: *authorization, evaluation, health, safety.*

An establishment subject to approval can operate only if the competent authority has granted the establishment approval to carry out or permit granted conditional approval.

This gives final approval only if a new visit at home, made three months after granting conditional approval proves that the unit complies with all applicable requirements.

If clear progress has been made, but the unit still does not meet all these requirements, the competent authority may prolong conditional approval but not more than six months in total.

For activities in the catering units veterinary and food safety county shall grant authorization approval number and veterinary health.

To obtain, where appropriate, authorization of veterinary, veterinary authorization conditional or veterinary authorization for intra-community trade in foodstuffs of animal origin, the legal representative of the unit submitted to the veterinary and food safety county documents will include:

a.request authorization from the unit, specifying activities;

b.unit plan project highlighting the technological flows and equipment;

c.plan with target location in the area;
d.The technical evidence;
e.single copy of the certificate of registration;
f.copy of the certificate issued by the Trade Register on the tribunal whose jurisdiction is based;
g.receipt stating that the legal fees were paid.
Veterinary Direction and Food Safety county is required to address the authorization request within 15 working days from the date of registration, the expert veterinary staff within the department / office and veterinary hygiene epidemiology.
Specialized veterinary staff is required to complete, after completing evaluation unit requiring authorization, notice of finding and evaluation form.
The evaluation form is determined by the Veterinary Hygiene and Epidemiology Division of the National Sanitary Veterinary and Food Safety Authority for each type of unit and includes conditions that must be fulfilled units in accordance with Community legislation, namely Regulation (EC) no. 882/2004 of the European Parliament and of the Council as amended, Regulation (EC) no. 853/2004 of the European Parliament and of the Council as amended, and Regulation (EC) no. 854/2004 of the European Parliament and of the Council as amended; To the evaluation form attached note finding, which includes details of the evaluation results.
Where are veterinary requirements in catering establishments, the veterinary and food safety county issued within 5 working days after completing the evaluation and finding the note, the veterinary authorization.
Where are detected nonconformities, official veterinarians recorded in the evaluation form, and finding note deficiencies and measures taken.
Evaluation form and note the findings will be made known to the legal representative of the unit.
If during official inspections shows that units no longer qualify veterinary operating under the laws in force, the veterinary and food safety county will suspend or, where appropriate, prohibiting conduct business and communicate in this writing the National Sanitary Veterinary and Food Safety.
If non-compliance is not remedied within the time entered in the order of suspension work done under veterinary authorization, the veterinary and food safety county, withdraw the unit owned and issued the order prohibiting the activity, which shall notify the Trade Register on the tribunal whose jurisdiction is based, within three days from the date of its issuance.

After veterinary authorization issued by the veterinary and food safety county, the entire responsibility on the production, processing, storage, transportation and / or distribution of food business operator is obtained.
When units are authorized sanitary veterinary requesting authorization of additional activities that meet the veterinary requirements is through a new permitting procedure for the veterinary work for which authorization is requested veterinary health, animal health to withdraw the previously held and released a new authorization animal health, which includes all the licensed activities.
If, following official controls, the veterinary and food safety county finds that the conditions laid down by the veterinary legislation, shall notify the legal representative unit tested and may order sanctioning in accordance with the legal provisions effect.
In addition to sanctioning, the veterinary and food safety county may order:
a.suspension of work performed under veterinary authorization for activities for which the veterinary conditions;
b.prohibiting the activity, the activity taking place without fulfillment directive.
In the assessment report the veterinary and food safety county check:
General information:
-unit activity;
-year built;
-year and during the last renovations / repairs / modifications;
-period when the unit is operational;
Production capacities:
-total annual production capacity;
-destination and type of products;
Cutting capacity;
Cooling and storage capacities;
Processing raw materials:
-origin of raw materials;
-number processing areas;
-production capacity by product type;
-number of storage spaces finished;
Additional information on the different types of meat products;
Design construction and equipment unit;
-Drinking water supplies;
-channeling;
-Heat source;

-type of refrigerant;
General conditions space in establishments meat products factory;
General conditions of space and facilities in meat establishments manufacturing;
Special conditions for approval of establishments meat products factory;
-Hygienic conditions;
-Documents, records veterinary;
-Seized goods.
Veterinary authorization issued for public catering establishments remain valid as long as the conditions under veterinary legislation.

CONCLUSIONS

Where legislation requires that units be authorized, the competent authority shall conduct a site visit.
The competent authority may grant conditional approval if the site visit allows to conclude that the unit meets all requirements for infrastructure and equipment.

REFERENCES

Order of the President of the National Sanitary Veterinary and Food Safety Authority no. 57 of 24 June 2010 approving the sanitary veterinary norm on animal health permitting of facilities that produce, process, store, transport and / or distribute products of animal origin;
Regulation (EC) no. 854/2004 of the European Parliament and of of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption;
Regulation (EC) no. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

INTEGRATED USE OF CARBOHYDRATES AND PHENOLIC STRUCTURES FOR THE FRACTIONING OF LIGNOCELLULOSIC RESIDUES

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Abstract

The paper presents the results of fractioning the biomass towards its bioconversion to ethanol for both – the carbohydrates resulting from hemicelluloses and from the ones of the lignocellulosic complex. Therefore not only methods of chemical hydrolysis were applied, but enzymatic ones too, using products developed in our own laboratories. Also, the possibility of turning into account the phenolic compounds – with high antioxidant potential – was closely looked into, as the phenols are the result of the enzymatic cleavage of the lignocellulosic edifice, rigidly structured. This treatment, unlike the acid hydrolysis, does not cause degradations in the aromatic structures, when submitted to high temperatures.
The distillation process, as well as the correction one for the ethanolic solutions that came both from the pretreatment phase and from the celluloso – lythical saccharification phase were performed in the same fashion, using the identical methodology. The results were, therefore, comparable and compared as considering the bioconversion yields and the „INVENTA AG" protocol, applied in Switzerland, through which a quantity of 95% ethylic acid is obtained, that is of 240 liters/tone ligneous dried residue.

Keywords*: lignocellulosic residues, ethylic alcohol, bioconversion*

INTRODUCTION

Obtaining ethylic alcohol out of lignocellulosic residues in most advantageous conditions is a sensitive standard of all progress recorded in all areas of research, mainly throughout the last decades[3].
This is due to the fact that the disassembly of the rigid macromolecular well consolidated of the biopolymers that are found throughout the biomass` composition, yet with a minimal degradation of the fermentable monomers was the beneficiary of unconventional technologies, such as the water

vapors explosion, gamma irradiation, microwave irradiation and was also subjected to high pressures 300-500 Mpa. On the other hand, when using genetic engineering, mutants were obtained, with a high bioconversion potential, capable of simultaneously fermenting, not only pentoses, but hexoses too, at temperatures of 60 - 70°C, thereby allowing the continuous elimination of alcohol as vapors, therefore substantially lowering the energetic costs in the distillation process and when correcting the alcohol obtained through bioconversion. Had been studied, at a micro pilot level the possibility of recovery the lignocellulosic residues in integrated phases of chemical pretreatment, enzymatic saccharification and oligophenol recovery as a result with sulfuric acid 4% pretreatments. The sugars resulted in the pretreatment phase were fermented with *Pachysolen tannophilus* yeast, strain CBS-4044 NRRLY-2460 (Genencor Co. – internal patent). The remaining lignocellulosic residue was treated with cellulases obtained from *Trichoderma viridae*, strain 3196 ICA (according to the Gh. Mencinicopschi technique, Institute of Food Chemistry (ICA) internal standard)[3,4].

MATERIALS AND METHODS

Materials

Biotransforming cellulosic materials using either commercial enzymatic products or microorganisms which produce cellulases leads to obtaining simple chemical compounds from which, through numerous fermentative processes (aerobic or non – aerobic) a wide variety of compounds might be obtained, with value of dietary supplement, of fodders, biofuels, solvents, enzymes etc. Considering these opportunities, the present study targets the analysis and the optimization of the biotechnological conditions of bioconverting carbohydrate polymers of lignocellulosic residues into ethanol.

Microorganisms

- ✓ *Pachysolen tannophilus* strain CBS-4044 NRRLY-2460
- ✓ *Trichoderma viridae*, strain 3196 Institute of Food Chemistry (ICA)
- ✓ *Trichoderma reesei* QM9414 (Merk – Germany)
- ✓ *Saccharomyces cerevisiae* strain ATCC-42368

Substrates

Corn cobs, beech shavings (of Brasov County), fir shavings (of Brasov County)

Methods

Preparations

The lignocellulosic residues were processed, made of 1 – 1.5 kg batches, with an average density of 0.2 – 0.7 gr/v.

Corn cobs (after removing the corn grains), dried by warm air ventilation, with an average contents of dry substance of 88.92% were chopped in a mill until the particles` sizes reached 20 to 40 meshes. The beech chips and the fir ones, the result of the beech or of the fir trees` filling for various purposes (for either timber or furniture production) was dried and chopped in the same fashion[1,2].

The residues were further introduced into the autoclave, in a ratio of 1:10, dry residue: sulfuric acid 4% g/v, at 1.5 atm for 45 minutes.

The acid hydrolysis was performed with sulfuric acid, therefore separating the liquid fraction from the residue, using a screw press, after which the raw hydrolyzate was introduced into a centrifugal separator, in order to separate the particles of the suspension. The clear filtrate was then neutralized with calcium carbonate, through intermittent agitation.

The purification and the concentration of the acid hydrolyzate was performed by removing the calcium sulfate suspension by filtering and, thus, a liquid fraction was obtained, that mainly contains the sugars of the pentosan hemicelluloses, with a concentration in between 1.7% and 4.2%, according to each substrate, and with a dry substance content of 3 to 7%[2,5].

The calcium sulfate is removed by filtering, thus obtaining a liquid fraction which contains the sugars of the pentosan hemicelluloses, with an average content of 1.7% to 4.2% (according to each substrate) and with a dry substance content of 3%-7%.

The solid residual fraction that resulted after the hemicelluloses` hydrolysis was washed in the filter, in order to remove the hydrolytic agent, and until the pH value reached 4.8.

The solid residual fraction is mainly composed of the lignocellulosic residue (cellolignin) with a variable degree of destructuration, quite convenient to be candied by treating with celluloso – lytical enzymes.

The liquid fraction was then concentrated using two methods: evaporation in vacuum and microwave concentration. In both cases a 5% to 15% concentration of dry substance was obtained, while the reducing sugars were in between 4% to 8%.

The concentrated hydrolyzate was then purified by adding active coal 0.3%, considering the liquid phase`s volume, with maintaining it at the room`s temperature for about 2 – 3 hours, continuously agitating. Later, the

filtration phase followed, which led to a liquid with „0" transparency, while the sediment mainly kept the phenolic compounds and some colored substances, the result of some compounds` degradation throughout the acid hydrolysis phase. At a sulfuric acid concentration of only 4% g/v the degradation level is not significant.

I. The pentosan sugars` fermentation

The filtrate was submitted to fermentation, using the *Pachysolen tannophilus* yeast, strain CBS-4044 NRRLY-2460. The fermentation was performed with both microaeration and continuous agitation, at 240 rpm, in a bioreactor at 28°C for 72 hours. The densities of the yeast cells of the *Pachysolen tannophilus* was 1,5 x 10^8 /ml per inoculum. The *Pachysolen tannophilus* yeast was used, that has the ability to perform the bioconversion to ethanol of pentosic sugars and of some derivatives. The bioconversion yield, evaluated with xylose as a standard, was of a 30,6% average[3].

Distillation of the ethanol obtained from the pentosanic sugars

The gathered ethanol was after distilled and corrected according to the classical procedure, therefore a mash was obtained, in which proteins, macro and micro mineral nutrients, lipids, vitamins and some volatile compounds are found. The remaining solid residue, after the pretreatment phase was introduced into a bioreactor and further treated with an acetate buffer solution to a 4.8 pH. The ratio: solid residue:buffer was 1:7. The cellulosic residue, the result of the pressing operation, was of 26 to 27% humidity.

II. The enzymatic hydrolysis of the lignocellulosic substrate

The cellulolytic product was added, obtained before in our own laboratories. Therefore, the ICA 3196 *Trichoderma viridae* strain was used, that produced enzymes with the specific activity of 20.000 - 22.000 FPU/kg residue dry substance. The enzymatic hydrolysis was performed at 50°C, at a pH=4.8 for 40 hours, at 200 rpm. The enzymatic hydrolysis was performed with an enzymatic concentrate prepared in the Institute of Food Chemistry (ICA) laboratories, with 21.42 FPU/ml enzymatic product, and 1 FPU was used for treating 46.68 mg of lignocellulosic substrate. The added quantity of enzymatic product was calculated considering the substrate`s mass, in FPU/kg dry residue units[4].

The cellulozolytic product came from *Trichoderma viridae* (ICA), strain 3196, cultivated onto the potato dextrose – agar medium, after growing it

for 10 days at 30°C.
The carbon source is the filter paper. The nitrogen source is a peptone enriched with urea and with macro and microelements (Mg, Ca, Fe, Zn, Mn).
The culture medium was concentrated in vacuum, using Simax equipment, thus obtaining a cellulosolytic concentrate with an activity of 10.2 FPU/ml – 21.42 UPF/g. For 5g of residual substrate, 10.5 ml of enzymatic concentrate was added, that is the equivalent of 107.1 FPU units.
In a similar fashion the experiments with the *Trichoderma reesei* QM9414 (Merk-Germania) were carried out.

Alcoholic fermentation of the enzymatically hydrolizated cellolignin

After the enzymatic hydrolysis, the liquid fraction, separated by pressing does contain glucose and its oligomers. Further, the solution of sugars is to be concentrated in vacuum or with microwaves to half its initial volume. The concentrated fraction was submitted to fermentation, using the classical technique and the *Saccharomyces cerevisiae* strain ATCC-42368 yeast, at 30°C.

Determining the ethylic alcohol

The ethylic alcohol concentration was around 2% - 2.3%.
The bioconversion yield was in between 28% to 32%. Then, the distillation and the correction of the gathered ethylic acid took place. At the same time, a mash was obtained which contains the yeast biomass, along with proteins, phenolic compounds, vitamins, residual carbohydrates and others. The mash, as a residual waste of the ethylic alcohol distillation process, was dried up to 80% dry substance.
The distillation and the correction was performed through bringing together, in the same installation the ethanol that was the result of the hemicellulosic pentosans` bioconversion and the one that was the result of the cellulosolytical saccharification. By drying the mash with concentrated solutions of sodium hydroxide 2N, the remaining lignin throughout the lignocellulosic structures was separated through saponification and filtration, further submitted to acidification to release the Na^+ ion as a salt and finally extracted with organic solvent, with ethyl ether or ethyl acetate.
Through the distillation of the organic phase a brown powder was collected, with a variable content of oligophenols. Determining the phenolic compounds was performed out of the liquid fraction of the hydrolyzate after the sulfuric acid was neutralized with calcium carbonate, up to a pH of 5.4 – 5.8, and after separating the liquid phase from the remaining organic residue and from the calcium sulphate precipitate.

Evaluating the total phenols was performed by the Folin – Ciocâlteu technique [1, 2, 5], at a wavelength of 765 nm (standard – galic acid) as shown in Table 3.
After separating, by pressing, the lignocellulosic residues of the oligophenolic structures solubilized in organic solvents, the remaining biomass shows a microporous structure with potential applications as a natural fertilizer, for improving the degraded soils or as insulation biomass[1,5].

RESULTS AND DISCUSSION

The percentages of reducing sugars, the hemicellulosic ones, released by the pretreatment phase, at a hydromodule of 1:10 lignocellulosic substrates: sulfuric acid 4% g/v are detailed in table 1.

Table 1: Fermentable sugars distribution, in various lignocellulosic substrates, in the pretreatment phase

Features	Beech chips	Fir chips	Corn cobs
Reducing sugar, % of the lignocellulosic	18.7 - 20.7	14 - 16	33 - 38.5
Reducing sugar, % in the initial liquid fraction	1.3 - 1.7	0.9 - 1.05	3.5 - 4.2
Reducing sugar, % after microwave treatment	3.8 - 4.5	2-2.2	7.8 – 8.2

Experimental data regarding the levels for the total amount of sugars and for the quantity of reducing sugars are displayed in figures 1 and 2.

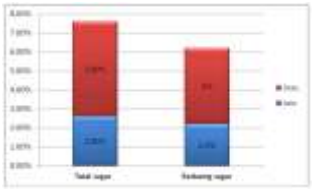

Fig.1. Total sugars level and reducing sugars level after enzymatic saccharification

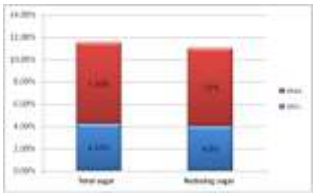

Fig.2. Total sugars level and reducing sugars level after vacuum concentration

After the *Saccharomyces cerevisiae* yeast, strain ATCC-42368 fermentation of the solution of sugars, the concentration in ethylic alcohol was in between 2 and 2.3%. The bioconversion yeast, of sugars from the enzymatic cleavages of the beta – glycozidic bonds from the lignocellulose into ethanol, the values were in between 28-32%.
The liquid fraction of the pretreatment phase, performed wih sulfuric acid 4% g/v, separated from the lignocellulosic residues contains the sugars from heteropolysaccharides and the phenolic oligomers and represent 10-18% of the total amount of lignin from the initial material. Therefore, the UV absorption levels of the mentioned hydrolyzates reaches values of 0.62 – 1.2. The oligophenols that can be recovered out of the heteropolysaccharidic hydrolyzate are 2.5 to 4.5% of the whole amount of dry substance.

Compared hydrolysis

Table 2 shows the amounts of sugars that were the results of the enzymatic treatment with the ICA cellulosolytic product, in comparison to the *Trichoderma reesei* QM9414 (Merk – Germany) one, with an enzymatic activity declared of 1U/mg.
One unit of cellulosic activity is the quantity of enzyme which releases 1μM of reducing carbohydrates per one minute (DNS method with 3,5 dinitrosalicylic acid), in the following conditions, of a pH = 4.5, at a temperature of 45^0C).

Table 2: The concentration of the fermentable resulted in the enzymatic hydrolysis, when using the I.C.A. enzyme, in similar conditions to the enzymatic product of *Trichoderma reesei* (QM 9414)

Substrate	Enzymatic product	Reducing carbohydrate (mg/ml)
Beech shaves, Brasov County	*Trichoderma reesei* QM9414	6.4
Fir shaves, Brasov County	*Trichoderma reesei* QM9414	43
Beech shaves, Brasov County	Celluloso – lytical Product I.C.A.	6.1 – 6.7
Fir	Celluloso – lytical Product ICA.	3.9-4.9

shaves, Brasov County		
Corn cobs	*Trichoderma reesei* QM9414	22.5
Corn cobs	Celluloso – lytical Product ICA.	14.4-16.2

The tested I.C.A. cellulosolytical product is *Trichoderma viridae*, strain 3196 ICA[3].
The values of the global phenols, solubilized in aqueous medium, expressed in percentages (%) of the mixture`s dry substance are shown in Table 3.

Table 3: The total phenol content, released after the fungal attack took place

Experimental version	Total phenols (% of d. S.)
Beech shaves, Brasov County + *Trichoderma reesei* QM9414	3.46
Fir shaves, Brasov County + *Trichoderma reesei* QM9414	4.02
Corn cobs + *Trichoderma reesei* QM9414	2.82
Beech shaves, Brasov County + celluloso – lytic product of I.C.A	3.14
Fir shaves, Brasov County + celluloso – lytic product of I.C.A	3.84
Corn cobs + celluloso – lytic product of I.C.A	2.56

CONCLUSIONS

The sulfuric acid 4% g/v pretreatment was performed for a hydromodule of 1:10, for 45 minutes at 1.5 atm. In these conditions, from the beech chips and from the corn cobs, sugars in concentrations mos adequate for bioconversion to ethanol, in the yeast fermentation of *Pachysolen tannophilus* are released, with yields of about 30.6%, (reported to xylose).
The enzymatic hydrolysis with the ICA product, at a concentration of 21.42 FPU/g substrate is comparable to the one performed with *Trichoderma reesei* QM9414 (Merk – Germany), in similar enzymatic processing conditions.
Considering the bioconversion technologies of the lignocelluloses currently applied, the technique submitted by the present paper is to be easily accomplished using materials and equipments quite accessible (as presented by Scheme 1), for when they are turned into account in an integrated manner, along the fermentable sugars and the phenolic compounds with

high antioxidant potential and with the proteic biomass of the mash.
The submitted version offers the possibility of a sequential turning into account of the hemicellulosic polysaccharides into ethylic alcohol, along with the recovery of the polyphenols with antioxidant potential.
The recovery level for the phenolic structrures with high antioxidant potential is in between 2.5% - 4% of the dry substance for the processed residue.
The obtained fermented product is substantially enriched with organic nitrogen to be used as natural fertilizer in order to remedy degraded soils, or even as insulation biomass.

ACKNOWLEDGMENTS

Institute of Food Chemistry, Bucharest, Research Department.

REFERENCES

Box, J.D. Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. In: *Water Res*, 1983, vol. 17, pp. 511-525.
Ikawa, M., Schaper, T.D., Dollard, C.A., Sasner, J.J. Utilization of Folin-Ciocalteu phenol reagent for the detection of certain nitrogen compounds. In: *J. Agric. Food Chem.*, 2003, vol. 51(7), pp. 1811–1815.
Mencinicopschi, G., Atudosiei, L., Nicolae, F. Informational food matrix sustained by nutrigenomics. In: *ESNA Annual International Conference*, Krakow, Poland, 2008.
Mencinicopschi, G., Iosif, K. *Biotehnologii în prelucrarea produselor agroalimentare.* București: Ceres, 1987.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: *Methods Enzymol*., 1999, vol. 299, pp. 152-178.

NONCOMPLIANCES WHICH LEADS TO AN INEFFICIENT PEST CONTROL IN MEAT PROCESSING PLANTS

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Abstract

The aim of this study is to avoid the mistakes which lead to an inefficient pest control in meat processing plants. Pest control programme are an essential part of maintenance and sanitation. Pest poses a major threat to the safety of the food products.
Unfortunately, in many cases I found that the pest control programmes were not correctly created and applied, so I met a lot of mistakes which should be avoided for an efficient prevention.
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.
The target of pest control is to prevent the pest ingress in the plant and not to combat them inside. Prevention is critical in pest control.

Keywords: *contamination, hazards, pest control.*

INTRODUCTION

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.
Food business operators have the responsibility to control pests:
„Adequate procedures are to be in place to control pests. Adequate procedures are also to be in place to prevent domestic animals from having access to places where food is prepared, handled or stored (or, where the competent authority so permits in special cases, to prevent such access from resulting in contamination)" - reg. 852/2004, Annex II, Foodstuffs, Chapter IX, point 4.

MATERIAL AND METHODS

The study was carried out since 2004 to 2012. During this time, I worked with many plants from meat industry (slaughterhouses, deboning plants, meat processing plants). A chapter of the foods safety assessments in these plants was the pest control. Unfortunately, in many cases I found that the pest control programmes were not correctly created and applied, so I met the following mistakes which shall be avoided for an efficient prevention:

The pest control has not been taken into account in the layout, design and construction of the premises, for example:
Many storages designed for auxiliary materials were built without a sluice or a receiving area. The existence of warehouse doors that open directly outside it is not appropriate because of the high risk contamination through pests. In the receiving area shall be made the control regarding the integrity or packaging or the presence of any trace of pest infestation in dry auxiliary materials. If the pests (insects, mice) are present in these materials, the materials will be rejected from the beginning and they will not be sent into the storage;
The external doors (especially in the receiving and delivery areas) were not tight fitting, the light was visible around the frame when closed;
The dock shelters were to big comparing with the trucks sizes. In this situation, the rainwater, dust, insects could enter very easy into the plant and also cannot prevent interior cold air from leaking out;
Doors without self-closing devices, if the doors are left open the pest can go inside;
External windows without insect proof screens or the screens were fitted to the windows but these were not cleaned and not maintained in a good state of repair;
Holes, drains and other places where pests are likely to gain access, were not be kept sealed;
The fence was not adequately built, without concrete foundation, so the animals (rats, dogs) made holes and channels to can enter in the yard;
The garbage platform was not enclosed and was not provided with water source for sanitation and drainage, so whole area including the garbage containers cannot be cleaned properly. The accumulated filth generates unpleasant smells which are attractive for pests.
The yard was not kept clean and tidy, for example:

The vegetation was not removed, in many outside areas were found bushes, tall grass, weeds and other plants which give to pest the possibilities for harbourage. Some plants and bushes were planted too close to the factory building;
Many objects are inadequate store (construction materials, wooden pallets, broken crates and bins etc.). Such materials may provide nesting places for rodents;
The yard drainage for rain water was not cleaned from leaves, sand and other wastes. Not all effluent drains were properly covered;
The garbage was put in inadequate containers, the garbage was not disposed quickly and correctly, so this attracted the pests. In some plants, the containers from the garbage platform did not have lids and because of wastes from the canteen, the animals and insects were attracted.
There were problems regarding pest monitoring, detection and eradication procedures, for example:
The location and the number of the bait stations were established without the neighbourhood assessment;
Bait stations made from inadequate materials ex. cardboard stations which were destroyed after first rain. Or the baits were not put into the stations, so birds or other animals had access of them;
The small amount of baits were too low (the amount of toxic substances) and the monitoring frequency of the bait stations was also too low;
Boxes with toxic baits inside of the plant (in the storages rooms, receiving and delivery areas etc.). The baits station must not be placed in the plant, they should be placed outside, along walls and fence, near main entrance, doors and loading docks;
Fly-killers devices installed in the production areas (food handling areas), that mean that insects are present there. Fly-killers devices should be sited in a good position, at each entrance and each exit from the plant, to prevent the insects access in the production areas;
Other problems were lack or poor personnel training regarding the food safety hazards associated with pest infestation, so:
The dogs were fed by the personnel and other dogs were attracted to come in the premises area, more than that, the peoples from the neighbourhood were encouraged to abandon the puppies near the factory;
The personnel from security services allowed the dogs access in the factory yard;
The personnel responsible for outside cleaning changed the location of the baits stations;

In many situations the personnel did not report the signs of pests presence to the pest operator, in other situations they were not trained to recognize and to report any kind of sign which can be pests associated;
The absence of control regarding the integrity of packaging or the presence of any trace of pest infestation in dry auxiliary materials;

RESULTS AND DISCUSSIONS

All situations described above leads to an inefficient pest control. The problems regarding the design and the construction of the premises, an inadequate maintenance of the building and facilities, an inadequate cleaning and tidiness of the yard, poorly executed pest control programmes and lack or poor personnel training regarding the food safety hazards associated with pest infestation, leads to an inefficient pest control.

CONCLUSIONS

The target of pest control is to prevent the pest ingress in the plant and not to combat them inside.
If it is waiting until there is evidence of pests within an establishment, this may already have a major infestation.
Prevention is critical in pest control.

REFERENCES

Bonne R., Wright N., Camberou L., BoccasF., 2005, Guidelines on HACCP, GMP and GHP for ASEAN Food SMEs, EC-ASEAN Economic Cooperation Programme on Standards, Quality and Conformity Assessment;
Codex alimentarius, 2003, Recommended international code of practice, general principles of food hygiene, CAC/RCP 1-1969, Rev. 4-2003;
FSA UK, 2006, Guide to food hygiene & other regulations for the UK meat industry, Part two, Chapter 5, MIG, UK;
Leliveld H. L. M., Moster M.A., Holah J., 2003, Hygiene in food processing, Woodhead Publishing Limited, Cambridge England;
Leliveld H. L. M., Moster M.A., Holah J., 2005 Handbook of hygiene control in the food industry, Woodhead Publishing Limited, Cambridge England;
Piscoi P., Rusen G., Tudor L. ,2007, Good manufacturing and hygiene practices for the meat processing plant, 2 nd edition , Agricola Publishing House, Bucharest.
Regulation 178/2002 EC;
Regulation 852/2004 EC.

THE INCIDENCE OF SALMONELLA BACTERIA IN MEAT AND MEAT PRODUCTS DURING THE PERIOD 2009 - 2011 IN DOLJ COUNTY

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ABSTARCT

During the period 2009-2011, 3971 meat samples and meat preparations collected both from agri-processing units and from alimentary cold stores and food marketing network were analysed within the Sanitary-Veterinary Laboratory of Dolj County, thus seeking to isolate and identify the serotypes of Salmonella spp. incriminated in triggering food poisoning.
Of the total number of samples examined, six samples were positive representing 0.15 percent. The positive samples were composed of pork minced meat (3), a mixture of prepared beef and pork meat (cattle-swine) (1), refrigerated minced pork meat rolls (1) and turkey wings (1).
All strains of Salmonella spp. isolated in the Sanitary-Veterinary Laboratory of Dolj County were submitted for serotyping of relevant isolates of Salmonella spp in the reference laboratories within the Institute for Diagnosis and Animal Health (IDSA) and the Institute of Hygiene and Public Veterinary Health (IISPV). Four strains belong to the Salmonella Typhimurium serotype, one strain belongs to Salmonella Goldcoast and one strain belongs to Salmonella Hadar. The dominant serovariant identified among tested isolates is Salmonella Typhimurium (66.33 %).
Of the four serovariants of isolated Salmonella Typhimurium, three serovariants presented the classical antigenic structure and one serovariant exhibited a different antigenic structure, lacking the 1,2 factors – initially denominated as Salmonella Typhimurium-like; it has been recently denominated as Monophasic Salmonella Typhimurium.

Key words: *Salmonella spp., antigenic structure, food poisoning.*

INTRODUCTION

The Salmonella genus is a member of the family Enterobacteriaceae comprising over 2400 serotypes; and bacteria contained within the genus Salmonella have a worldwide proliferation therefore affecting the entire fauna, including the human being (4). Due to its high pathogenicity for human beings through contamination of animal products especially, it is of specific interest for food microbiology (2) Primary Salmonella infections determine high economic damages namely by seizing the products and by-

products derived from animals slaughtered (5).
The spreading of Salmonella infections to human beings is increasing subsequent to handling of food products contaminated through raw materials or during their preparation, manipulation, storage and commercialization. The storage inside cold stores prevents the multiplication of Salmonella strains but this does not destroy them.
In most cases disorders in humans are a consequence of the consumption of potentially contaminated food resulted from the meat of animals slaughtered upon requirement which were carrying and spreading germs of the genus Salmonella, or the consumption of animal products which were sterilized insufficiently or preserved inadequately.
In addition, it must not be forgotten that food products could be contaminated by people carrying Salmonella or infected with Salmonella during the handling of food products as raw materials or finished products.
The key feature of food products contaminated with Salmonella is the fact that no organoleptic defects emerges which might draw the attention of the potential presence of germs, which means that the appearance, colour, consistency, smell and taste of food products will remain unchanged (1, 3).
The HACCP plan should include control measures for prevention, destruction or elimination of this bacteria and in order to avoid recontamination (1).

MATERIAL AND METHODS

Meat samples and meat preparations and products collected from slaughterhouses, processing units, cold stores and food marketing network have been analyzed.
The detection and identification of bacteria of the genus Salmonella was carried out according to the reference method SR EN ISO 6579/2003/AC/2009, but alternative methods (Vidas and Vitek methods) were also used; these methods offers the additional advantage of getting the result within a shorter period of time. However, in the event of a positive result obtained through these alternative methods confirmation by means of classical methods should also be carried out.
According to the reference method the detection of bacteria of the genus Salmonella requires four successive stages, namely: pre-enriching in non-selective liquid media; isolation and identification; confirmation.

The detection of Salmonella represents the determination of the presence or absence of Salmonella in a certain mass or product bulk when tests are carried out according to the classical method.
The pre-enriching is performed by means of buffered peptone water (225ml) which has been pre-warmed to room temperature and has been inoculated to the sample to be analysed (25g), then it is incubated at 37°C ±1°C for a period of 18h±2h.
The following have been used as liquid selective media: Rappaport media - Vassiliadis cu soia (bulion RVS) şi bulionul Muller - Kauffmann tetrationat/novobiocină (bulion MKTTn). These media are incubated at specific temperatures according to the working standard procedure, for a period of 24h±3h.
For the purpose of isolation and identification two solid growth media are innoculated: agar with xylose - lysine - deoxycholate (XLD) – the first selective medium and the second selective medium – any other solid growth medium complementary with the XLD agar and appropriate especially for the isolation of lactose positive Salmonella and the genus Salmonella Typhi and Salmonella Paratyphi (Istrati Meitert - IM or Edel and Kampelmaker - EK); the selective growth medium Edel and Kampelmaker is currently used in the Sanitary-Veterinary Laboratory of Dolj County. The XLD agar is being incubated at 37°±1°C and it is examined after a period of 24h±3h. The second selective agar is being incubated according to the manufacturer's regulations.
The typical colonies of Salmonella grown on XLD agar have a black spot in the middle and a transparent bright reddish area due to the change of the indicator colour.
The confirmation of the supposed Salmonella colonies is carried out by means of biochemical or serological tests using identification kits.
The recognition of Salmonella colonies is mainly an issue related to experience and their appearance may vary anyway, not only from serovar to serovar but also from a certain selective growth media batch to another selective growth media batch used.

RESULTS AND DISCUSSIONS

Throughout the survey period 3971 samples on different matrices were analyzed within Dolj Sanitary Veterinary and Food Safety Laboratory in order to detect bacteria of the genus Salmonella in meat and meat preparations and products.

Table1

Samples collected on different matrices during the period examined

MATRIX	Samples examined 2009	Samples examined 2010	Samples examined 2011
Bovine, sheep, caprine carcases	34	47	30
Pigs carcases	41	42	40
Poultry carcases	38	69	21
Fresh meat and comestible by-products	293	286	38
Minced meat and prepared meat derived from poultry, meant to be cooked	25	33	27
Minced meat and prepared meat derived from species other than poultry, meant to be eaten after being cooked	389	563	330
Processed chicken meat meant to be prepared (cooked)	56	40	35
Heat treated processed meat	500	434	560
Total	1376	1514	1081

Subsequent to the carrying out of analyses and data processing it results the fact that in 2009 two samples were found positive out of a total number of 1376 samples representing a 0.15 percentage of meat and meat preparations' samples. In 2010, two samples were found positive out of a total number of 1514 samples representing a 0.14 percentage; in 2011, two samples were found positive out of a total number of 1081 samples examined representing a 0.19 percentage.

Table2

Positive cases and percentage of positive samples reported to the total examined

Period / Samples	2009	2010	2011
Examined	1376	1514	1081
Positive	2	2	2
% positive	0.15	0.14	0.19

The six positive confirmed samples derive from different matrices, namely: three samples are pork minced meat, one sample was collected from a mixture of prepared beef and pork meat (cattle-swine), one sample was collected from refrigerated minced pork meat rolls and one sample was collected from turkey wings.
It should be specified that Salmonella spp. strains, dated 2009, were isolated in the following assortments: mixture of prepared beef and pork meat (crude frozen product), sample collected from a cold store; the pork minced meat was taken from a supermarket. Salmonella group OC was isolated from the pork minced meat sample according to the reference method SR EN ISO 6579/AC/2006. This strain was confirmed at IDSA and IISPV in Bucharest as being a Salmonella Goldcoast strain. Subsequent to the carrying out of the product traceability it has been determined that the manufacturer that had supplied the pork minced meat to the supermarket had purchased the raw material from a swine farm endowed with its own slaughterhouses which had a past history with this type of Salmonella (S. Goldcoast) detected in its own head.
All strains of Salmonella spp. isolated in LSVSA Craiova were submitted for serotyping in the reference laboratories within IDSA and IISPV.

Table 3

Correspondence between positive samples and matrices examined

Period / Matrix	2009		2010		2011	
	Samples examined	Positive samples	Samples examined	Positive samples	Samples examined	Positive samples
Fresh meat	293	-	286	1	38	-
Minced meat and prepared meat	389	2	563	1	330	2

Out of the six isolated strains, four strains belong to the Salmonella Typhimurium serotype, one strain belongs to Salmonella Goldcoast and one strain belongs to Salmonella Hadar.
The dominant serovariant identified among tested isolates is Salmonella Typhimurium representing 66.33 percentage of the total number of isolated germs of Salmonella species.
Among the serovariants of isolated Salmonella Typhimurium, three serovariants presented the classical antigenic structure and one serovariant

exhibited a different antigenic structure, lacking the 1,2 factors – initially denominated as Salmonella Typhimurium-like; it has been recently denominated as Monophasic Salmonella Typhimurium.

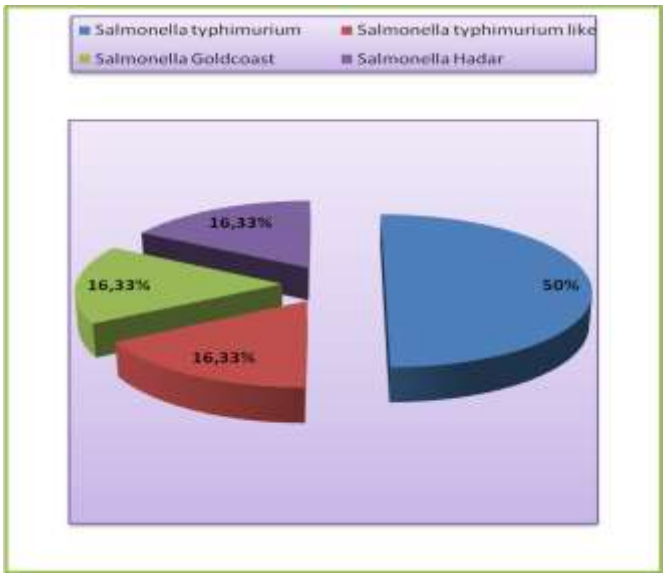

Chart 1- the percentage of the four genii isolated from the total number of samples examined

CONCLUSIONS

Bacteria of the genus Salmonella identified, isolated and confirmed throughout the period examined were six; they did not exceed the said 1 percentage of the samples examined each year.

Subsequent to the carrying out of this study, it can be concluded that the majority serovariants isolated were Salmonella typhimurium.

The highest percentage of germs of the genus Salmonella was detected in meat half-cooked products: minced meat, prepared meat and minced meat rolls paste.

The isolation of germs of the genus Salmonella spp. in meat and meat products is mainly the consequence of intense processing and handling labours carried out by people as the highest percentage was isolated from sorts enduring multiple operations.

The epidemiologic surveys performed led to the conclusion that contamination with Salmonella inside the slaughterhouses cannot be neglected when slaughtering animals carrying and spreading bacteria of the

genus Salmonella and in cases when principles regarding products handling, instruments sterilisation are disregarded as well as in cases of non-compliance with the principle of the two knives.

REFERENCES

Constantin Savu, Carmen Daniela Petcu, Laurentiu Tudor, Mara Nicolaescu, Victor Butean (2007) – General Sanitary Requirements and Enforcement of HACCP Principles on Food Safety Guarantee. 56-57
Dobre Bărzoi, Sorin Apostu (2002) - Food Microbiology. Risoprint, 71.
Savu Constantin, Mihai Gabriela (1997) – Food Sanitary-Veterinary Control. Ceres, 41.
Simona Ivana (2002) – Special Veterinary Bacteriology. Ceres, 236.
SR EN ISO6579/2003/AC2009.
Tudor Perianu (1996) - Infectious diseases in animals – bacterioses. Chemarea Iaşi, 175.

DETECTION OF LISTERIA MONOCYTOGENES IN FOOD PRODUCTS USING A POLYMERASE CHAIN REACTION-BASED METHOD IN COMBINATION WITH A STANDARD REFERENCE ENRICHMENT STEP

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Abstract

Listeria monocytogenes remains one of the many foodborne pathogens that require continuous monitoring. The detection and tracing methods are quickly developed for a better improvement and a quicker identification of this foodborne pathogen that may be present in food samples.
A total number of samples consisting of different types of food products were collected from local markets in Bucharest, subjected to enrichment steps (according to EN ISO 11290-1 and ISO 11560) and further on to a PCR analysis.
This resulted in eight samples with positive results, among them goat cheese, smoked fish, sausages and raw chicken meat. The identification was possible and quick, no matter the bacterial load of the samples, due to the existence of a virulence gene pertaining to the internalin gene family: InlB, which is specific to L. monocytogenes. This method ensures a high sensitivity and specificity in a very short period of time, reducing the work time for this analysis with five days concerning the negative results and seven days for the positive ones.

Key words: *food products, Listeria monocytogenes, PCR-based methods, standard enrichment.*

INTRODUCTION

Listeria monocytogenes is a pathogenic bacterial species that causes illness but without a high prevalence and low mortality rates, like other foodborne pathogens (Kaclikova et al., 2003). It frequently contaminates food products, particularly cheese and ready-to-eat meat-containing products (Farber and Peterking, 1991). Due to the necessary period of 7-10 days for a

thorough examination considering the detection and tracing *L. monocytogenes* (EN ISO 11290-1 for food and animal feeding stuff and ISO 10560 for milk and milk products) (Kaclikova et al., 2003), new methods emerge, in a trial to shorten the period of analysis.
Growth rates during enrichment vary among *Listeria* species due to interactions with food matrices, production of inhibitors by the organism (monocins, bacteriophages) or the competing background microflora (Besse et al., 2005, Curtis and Lee, 1995, Jasson et al., 2009).
When using nucleic acid amplification based methods, the recovery of *L. monocytogenes* is problematic if food samples containg accompanying non-pathogenic *Listeria* spp. (Aznar and Alarcon, 2002; Churchill et al., 2006). Polymerase chain reaction (PCR) presents a great potential to speed-up the detection process, especially for *L. monocytogenes* (Olsen, 2000). However, nowadays this method is used frequently on highly contaminated samples (Bansal et al., 1996), therefore comparable methods to the standard ones were developed in order to shorten the analysis time, one of them including a three days PCR-based method (Kaclikova et al., 2003). The inclusion of a non-selective post-enrichmnt step to obtain a useful detection limit for PCR ($\geq 10^4$ CFU/mL of *L. monocytogenes*) was used to improve the sensitivity of the PCR assay. Also the targeting of *inlB*, which is 100 % specific for this bacterial species facilitates the detection of 10^0 CFU in a sample. The presence of internalin genes, associated with the virulence cycle of *L. monocytogenes* represents a valuable indicator for their virulence potential (Barocci et al., 2007; Liu et al., 2007).
This paper aims to use this shortened PCR-based method for the detection and tracing of *L. monocytogenes* in several food products.

MATERIALS AND METHODS

Food samples. A total number of 90 samples consisting of smoked fish (10), goat cheese (10), sausages (10), tuna salad (10), ground chicken meat (10), ready-to-eat hamburgers (10), pastry products containing meat (10), mayonnaise salad (10) and pepperoni pizza (10) were obtained from local supermarkets. For the determination of the detection limit, 10 g of food samples were homogenized in 90 ml of half Fraser or *Listeria* enrichment broth (AES Chemunex) using a stomacher for homogenization. Further on, they were inoculated with decimal dilutions (10^1-10^2 CFU/sample) obtained from a culture of *L. monocytogenes* prepared in brain heart infusion broth (AES Chemunex), at 37^oC, for 24-48 h.

The enrichment. The food samples quantities of 10 g were homogenized in 90 ml of Fraser broth and incubated for 24h at 30^{o}C. A volume of 0.1 ml of the primary-enriched sample was introduced in 10 ml of Fraser broth and incubated 24 h at 37^{o}C. A volume of 0.1 ml of the secondary-enriched sample was introduced in 10 ml of BHI broth and incubated for 5 h at 37^{o}C (EN ISO 11290-1).
DNA extraction. For this step, a volume of 1 ml of the enriched sample was introduced to centrifuge at 13000g for 10 min. Afterwards, the sediment was subjected to washing with 0.85% NaCl, and re-suspended in 200 µl of buffer, that contained 20 mM Tris-HCl (pH 8.0) and 50 mM KCl. This mix was incubated at 95^{o}C for 25 minutes. The sample was afterwards centrifuged at 13000g for 3 minutes, and the supernatant was further on used.
Internal control preparation. For the internal control preparation, DNA of *Enterococcus faecalis* was used, by the method described by Pangallo et al. (2001a). The DNA was amplified using PCR, with primers inlB-L and inlB-R (Pangallo et al., 2001b) and a fragment of 400 bp was selected.
PCR. A quantity of 25 µl of mixture for the reaction, containing 200 µM of dNTP, 250 nM of each primer (inlB-L: ctggaaagtttgtatttgggaaa, inlB-R: tttcataatcgccatcatcact; Roche), 1.5 U of *Taq* DNA polymerase, 2.5 µl of the buffer supplied with the polymerase, 2.5 µl internal control and 2.5 µl of the sample lysate. The reaction was performed in a Roche Light Cycler 2.0 (Roche) using an amplification program consisting of initial denaturation at 94^{o}C for 2 min, 35 cycles with a denaturation at 94^{o}C for 45 s, annealing at 60^{o}C for 45 s and polymerization at 72^{o}C for 90s, followed by the final polymerization at 72^{o}C, for 8 min.
Detection of the amplification product. After the PCR, the LightCycler includes a step that replaces the use of electrophoresis, but instead uses the UV-light in toder to identify the amplified product, with a molecular weight control of 100 bp analyzed at the same time with the samples.
Interpretation of the PCR results. The presence of fragments consisting of DNA with less than 400 bp was interpreted as positive result, while those with fragments of approx. 400 bp were interpreted as positive results.

RESULTS AND DISCUSSION

PCR-based methods are considered very reliable and having a great potential for fulfilling the requirements fast enough, while being specific and sensitive for the detection, even though they require a specific prior

preparation step for the samples. In this case, we used the enrichment step described in EN ISO 11290-1 and ISO 10560, while the last steps were performed using the PCR-based method. This approach has been previously studied by Kaclikova et al. (2003), proving its efficiency. The results were positive for the samples artificially contaminated by $\geq 10^0$ CFU for 10 g of *L. monocytogenes*. However, among the 90 samples studied only eight samples were positive for the presence of the foodborne pathogen (table 1).

Table 1. Results of the analysis of *L. monocytogenes* in naturally contaminated food samples by PCR-based methods

Sample	Number of positives/number of analyzed samples
Tuna salad	0/10
Goat cheese	1/10
Smoked fish	1/10
Sausages	4/10
Ready-to-eat hamburgers	0/10
Pastry containing meat	0/10
Raw chicken meat	2/10
Mayonnaise salad	0/10
Pepperoni pizza	0/10

The used method was advantageous due to the wider detection window. This facilitates the detection of 10^0 CFU of *L. monocytogenes* per sample, without a danger of false positivity when detection of dead cells may happen. This is due to the effective enrichment step, performed as required in EN ISO 11290-1 and ISO 10560. Also, the dilution of the food samples by a factor of 10^5 was an effective method for the high sensitivity. The cell lysis step was done shortly after the enrichment step, the latter ensuring an exponentially growing cells that can be easy to lyse.

CONCLUSIONS

The presented PCR-based method combined with a selective enrichment proved to be very effective and the necessary period was therefore reduced from 7-10 days to only three. The results are considered equivalent to the reference methods (EN ISO 11290-1 and ISO 10560) as stated by Kaclikova et al. (2003). Dead cells were not detected by this method due to the multiple dilutions performed on each sample. However, the application of this combination between reference enrichment step and PCR-based method reduced with five days the period in case of the negative results and with seven days in case of positive results, as confirmation is not needed anymore. This study consisted also as a confirmation of the use of this sample as a faster alternative for the detection of *L. monocytogenes* in food samples of different types.

REFERENCES

Aznar R., Alarcon B. 2002. On the specificity of PCR detection of *Listeria monocytogenes* in food: a comparison of published primers. Sys Appl Microbiol 25(1):109-119.

Bansal N.S., McDonell F.H.Y., Smith A., Arnold G., Ibrahim G.F. 1996. Multiplex PCR assay for the routine detection of *Listeria* in food, Int J Food Microbiol 33:293-300.

Barocci S., Calza L., Blasi G., Briscolini S., De Crutis M., Palombo B., Cucco L., Postacchini M., Sabbatini M., Graziosi T., Nardi S., Pezzotti G. 2007. Evaluation of a rapid molecular methods for detection of *Listeria monocytogenes* directly from enrichment broth media. Food Control 19(6):750-756.

Besse N.G., Audinet N., Kerouanton A., Colin P., Kalmokoff M. 2005. Evolution of *Listeria* populations in food samples undergoing enrichment culturing. Int J Food Microbiol 104(2):123-134.

Churchill R.L.T., Lee H., Hall C. 2006. Detection of *Listeria monocytogenes* and the toxin listeriolysin O in food. J Microbiol Met 64:141-170.

Curtis G.D.W., Lee W.H. 1995. Culture media and methods for the isolation of *Listeria monocytogenes*. Int J Food Microbiol 26:1-13.

Farber J.M., Peterkin P.I. 1991. *Listeria monocytogenes*, a foodborne pathogen. Microbiol Rev 55:476-511.

Jasson V., Rajkovic A., Debevere J., Uyttendaele M. 2009. Kinetics of resuscitation and growth of *L. monocytogenes* as a tool to select appropriate enrichment conditions as a prior step to rapid detection methods. Food Microbiol 26:88-93.

Kaclikova E., Pangallo D., Drahovska H., Oravcova K., Kuchta T. 2003. Detection of *Listeria monocytogenes* in foo, equivalent to En ISO 11290-1 or ISO 10560 by a three-days polymerase chain reaction-based method. Food Control 14:175-179.

Liu D., Lawurence M.L., Ainsworth A.J., Austin F.W. 2007. Towards an improved laboratory definition of *Listeria monocytogenes* virulence. Int J Food Microbiol 118(2):101-115.

Olsen J.E. 2000. DNA-based methods for detection of foodborne bacterial pathogens. Food Res Int 33:257-266.
Pangallo D., Kuchta T., Drahovska H. 2001a. Preparazione di un controllo interno mimic per una multiplex PCR per la rivelazione di *L. monocytogenes.* Ind Alim 40:152-154.
Pangallo D., Kaclikova E., Kuchta T., Drahovska H. 2001b. Detection of *Listeria monocytogenes* by polymerase chain reaction oriented to *inlB* gene, New Microbiol 24:333-339.

OCCURRENCE OF *CAMPYLOBACTER* SPP. IN ROMANIAN BROILER CHICKEN PRODUCTION SECTOR

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Abstract

Due to the well-known potential of Campylobacter spp. to determine illness in humans, its detection and occurrence, especially in poultry meat (due to the frequent contamination of this food product) are considered highly important for the consumer's health point of view.
In order to determine the occurrence of Campylobacter spp. in the Romanian broiler chicken production sector, a number of five units were selected and samples were collected as it follows: a total number of 600 samples consisting of chicken skin neck, 600 samples of intact intestines and 1200 samples of fresh chicken carcasses.
The results were different from one unit to another, but overall, 54.7 % of the caecal material samples, 48.5 % of the neck skin samples and 31,5 % of the carcass samples were contaminated with Campylobacter spp., with an overall percentage of positive samples reaching 60,2 %. This high occurrence opens the opportunity for future research in order to determine the causes leading to contamination, while also identifying the species of this genus, for a better understanding of this mechanism through which this foodborne pathogen contaminates broiler meat.

***Keywords*:** broiler *carcass, Campylobacter, food safety, slaughterhouse.*

INTRODUCTION

Campylobacter spp. are well known to be able to determine illness in humans, especially the thermotolerant strains, such as *Campylobacter jejuni* and *C. coli*, the most commonly reported bacterial causes of human infections in the European Union (EU) (Hermans et al., 2011; Habib I. et al., 2012; Kovalenko et al., 2013). In 2010, the campylobacteriosis cases per 100,000 EU inhabitans were confirmed to reach 48.6 % of the total (EFSA, 2012). Fica et al. (2011) mentions gastroenteritis cases produced through *Campylobacter* infection and the possibility that this pathogen may also determine several complications, such as Miller-Fisher and Guillain-Barré syndromes.

C. jejuni and *C. coli* are Gram-negative rods, with a characteristic motility, but compared to several other foodborne pathogens, more fragile and requiring microaerobiosis for multiplication (Park, 2002; Rodgers et al., 2012).
It is well known that this foodborne pathogen is a frequent factor of contamination for poultry meat (Friedman et al., 2004; Malher et al., 2011), in 2010 the frequency of *Campylobacter*-contaminate broiler meat samples varied from one Member State to another, with ranges of 3.1 % and 90 % (EFSA, 2012). This is usually caused by the poor or insufficient biosecurity in and around the poultry farm (Newell and Fearnley, 2003; Thakur et al., 2012). Van Gerwe et al. (2005) showed that in a flock comprising 20,000 broilers, the prevalence of *Campylobacter* can increase from 5 % to 95 % in only six days after the inoculation. Also, in the slaughterhouse, the chances of cross-contamination are also increased. Contamination has been proven to happen in steps such as scalding, evisceration and water chilling (Hue et al., 2010; Jacobs-Reitsma, 2000).
The aim of this presence is to determine the occurrence of *Campylobacter* spp. in broiler chicken production at slaughterhouse and retail level in Romania, for 2011.

MATERIALS AND METHODS

Sampling
A total number of 600 broiler chicken neck skins, 600 number of broiler chicken intact intestine an 1200 fresh broiler chicken carcasses were collected from five different units, during the year 2011. All the samples were collected monthly, 10 samples monthly from each unit, consisting of broiler chicken neck skins, another 10 samples of intact intestines and 20 samples of fresh broiler chicken carcasses. The intact intestines samples were taken during evisceration step, and placed all together in a single sterile plastic bag. Neck skin samples were collected separately and placed each in sterile plastic bags. During the same day, carcass samples were collected, but not from the same slaughter batch as the other two categories of samples. All chicken carcasses produced in the five selected units included in this study were usually sold in tight, sealed plastic bags. The samples were introduced in a cooler, in order to be kept at a temperature of 4-6^{o}C and transported to the laboratory for microbiology analyses.

Isolation and identification of Campylobacter spp.

A quantity of 10 g of chicken back skin from the carcass and another 10 g of neck skin were aseptically taken and introduced in sterile bags, for the enrichment step. Afterwards, the sterile bags were filled with 90 mL sterile Bolton Broth and introduced in a stomacher for one minute. Further on, they were incubated under microaerobic conditions at 37^{o}C, for 46 h, followed by 41.5^{o}C for 44 h (Kovalenco et al., 2013). After enrichment, 10 µl of the enriched broth was plated on mCCDA agar and incubated for 48 h at 42^{o}C under microaerobic conditions. From the colonies on mCCDA agar, several typical ones were streaked on Columbia blood agar, the plates being further on incubated for 24 h at 41.5^{o}C in microaerobic conditions.

Concerning the intestines, the caeca was selected for the identification. Caecal material from the 10 samples of intestines was analyzed separately, 1 g of content being selected for each, for further analysis.

For the identification, according to ISO 10272-1:2006, bacteria isolated from broiler chicken material that showed typical growth on mCCDA, were Gram-negative, with specific corkscrew motility, oxidase positive and without any growth at 41.5^{o} C in aerobic conditions and growth at 25^{o}C in microaerobic conditions, were considered as *Campylobacter* spp.

RESULTS AND DISCUSSIONS

The results showed a high *Campylobacter* colonization for caecal samples and a high contamination of neck skin samples in the five units chosen for this study (Table 1).

The present results showed that between the chosen units, there are differences concerning the contamination for the different collected samples. Concerning the samples of caecal content, the highest contamination percentage was shown for unit D, while the lowest for unit A. Overall, from the total number of analyzed samples, 328 showed positive results. Concerning the neck skin samples, overall 48.5 % of the total numbers of samples were shown to be contaminated with *Campylobacter* spp, the highest number of positive results among the analyzed ones pertaining to unit C. For the carcass samples, the highest number of samples with positive results was shown in the samples collected from unit D, while the lowest among the ones collected in unit A. Overall, 31.5 % of the total number of samples were contaminated with *Campylobacter* spp. (Figure 1).

Table 1. The percentage of *Campylobacter* spp. positive broiler chicken samples in five Romanian units in 2011

Unit	*Campylobacter* spp. positive samples percentage (no. positive/total no.)			
	Separate caecal material	**Neck skin samples**	**Carcass samples**	**Total number**
A	48,3 (58/120)	45,8 (55/120)	59,2 (142/240)	53,1 (255/480)
B	53,3(64/120)	40,0 (48/120)	72,9 (175/240)	59,8 (287/480)
C	49,2 (59/120)	55,8 (67/120)	67,5 (162/240)	60,0 (288/480)
D	62,5 (75/120)	51,7 (62/120)	78,3 (188/240)	67,7 (325/480)
E	60,0 (72/120)	49,2 (59/120)	66,3 (159/240)	60,4 (290/480)
All	54,7 (328/600)	48,5 (291/600)	31,5 (757/1200)	60,2 (1445/2400)

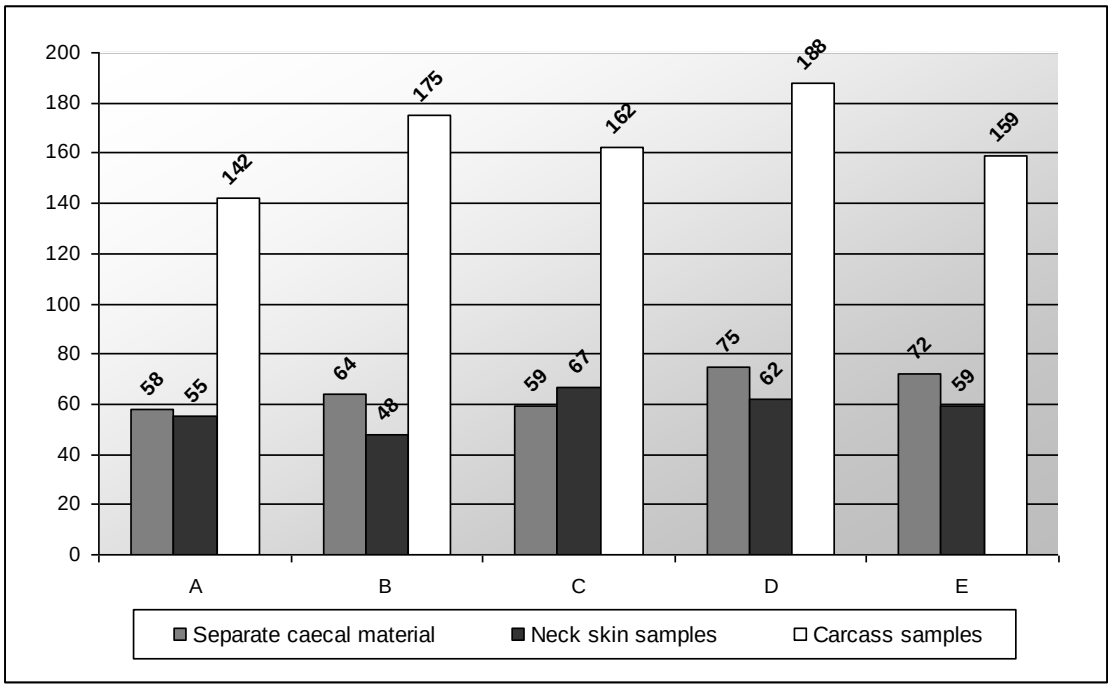

Fig. 1. The number of positive results for *Campylobacter* spp. identification, for separate caecal material, neck skin samples and carcass samples in each unit included in the study

For the monthly evolution for the entire year of analysis, data are presented in figure 2. During the months of 2011, the total number of positive results for the carcass samples as well as for the caecal material showed several variations, with the highest number for April and August, while the lowest were observed for January.

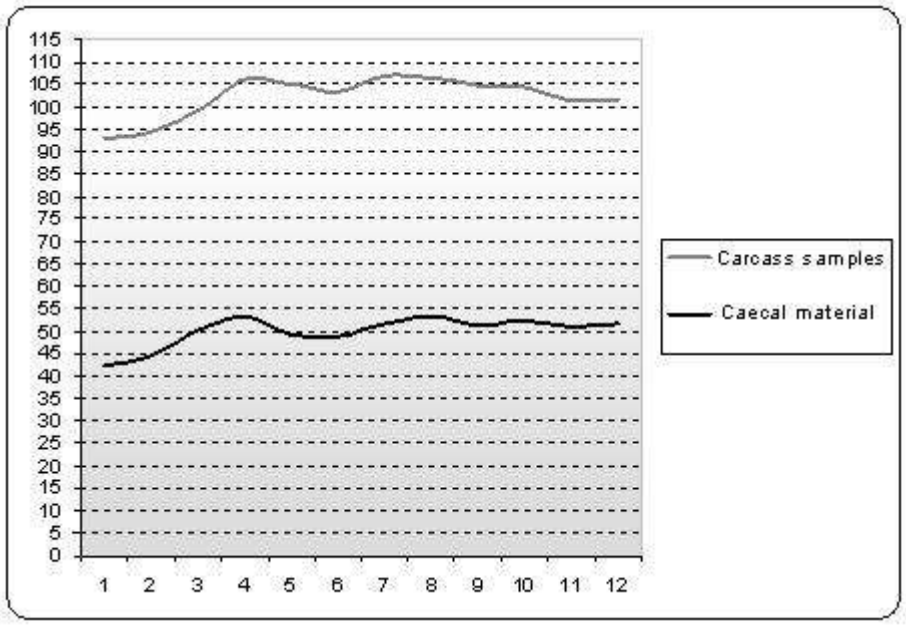

Fig. 2. The monthly percentage of positive samples for the presence of *Campylobacter* spp. – caecal material and carcass samples analysis

CONCLUSIONS

In conclusion, this study revealed a high *Campylobacter* spp. contamination in the slaughterhouses and also on retail level. There is a need for a state *Campylobacter* monitoring and control programs. Further on, research could show the causes for this high contamination, as well as the mechanisms needed for *Campylobacter* spp. to survive during the processing.

REFERENCES

EFSA, European Food Safety Authority. 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010, EFSA J 10:2597.

Fica C.A., Porte T.L., Braun J.S., Veas P.N., Pavez A.C., Dabanch P.J. 2011. Bacteremia and endarteritis cases secondary to *Campylobacter* spp. in a metropolitan hospital: our experience along a quarter of a century. Rev Chi Infect 28:211-216.

Friedman C.R., Hoekstra R.M., Samuel M., Marcus R., Bender J., Shiferaw B. et al. 2004. Emerging infections program foodnet working risk factors for sporadic *Campylobacter* infection in the United States:a case control study in foodnet sites. Clin Infect Dis 38:285-290.

Habib I., Berkvens D., De Zutter L., Dierick K., Van Huffel X., Speybroeck N., Geeraerd A.H., Uyttendaele M. 2012. *Campylobacter* contamination in broiler carcasses and correlation with slaughterhouses operational hygiene inspection. Food Microbiol 29:105-112.

Hermans D., Van Deun K., Messens W., Martel A., Van Immerseel F., Haesebrouck F., Rasschaert G., Heyndrickx M., Pasmans F. 2011. *Campylobacter* control in poultry by current intervention measures ineffective: Urgent need for intensified fundamental research. Vet Microbiol 152:219-228.

Hue O., Le Bouquin S., Laisney M.J., Allain V., Lalande F., Petetin I. et al. 2010. Prevalence of and risk factors for *Campylobacter* spp. contamination. Food Microbiol. 27: 992-999.

Jacobs-Reitsma W. 2000. *Campylobacter* in the food supply. In I Nachamkin, M.J. Blaser, Jacobs-Reitsma W. (editors) *Campylobacter* (second edition) pp. 467-481. Washington D.C. U.S.A.: American Society for Microbiology Press.

Kovalenko K., Roasto M., Liepins E., Maesaar M., Horman A. 2013. High occurrence of *Campylobacter* spp. in Latvian broiler chicken production, Food Control 29:188-191.

Malher X., Simon M., Charnay V., Danguy des Deserts R., Lehebel A., Belloc C. 2011. Factors associated with carcass contamination by *Campylobacter* at slaughterhouse in cecal-carrier broilers. Int J Food Microbiol 150:8-13.

Newell K., Elias P., Tamme T., Kramarenko T., Lillenberg M., Karus A. et al. 2010. The occurrence of *Campylobacter* spp. in Estonian broiler chicken production in 2002-2007. Food Control 21:272-275.

Park S.F. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int J Food Microbiol 74:177-188.

Rodgers J.D., Lawes J.R., Vidal A.B., Ellis-Iversen J., Ridley A., PLeydell E.J., Powell L.F., Toszeghy M., Stapleton K., Clifton-Hadley F.A. 2012. Characteristics and comparative performance of direct culture, direct PCR and enumeration methods for detection and quantification of *Campylobacter* spp. in broiler caeca. Vet Microbiol 159:390-396.

Thakur S., Brake J., Keelara S., Zou M., Susick E. 2012, Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks. Res Vet Sci http:// dx.doi.org/10.1016/j.rvsc.2012.07.014.

OPERATION AND HYGIENE OF FOOD ESTABLISHMENTS

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Abstract

Audit is a systematic and independent examination to determine whether activities and results comply with planned programs and whether these programs are implemented effectively and are suitable to achieve objectives.
Food business operators shall have in place systems and procedures to identify other businesses to which their products have been supplied.
Food business operators must identify any person from whom they have been supplied with a food, a pet food to food or any substance intended or expected to be incorporated into a food product.
Food business operators shall cooperate with the competent authorities on action taken to avoid or reduce risks posed by a food which they supply or have supplied.

Key words: *audit, business operator, hygiene.*

Catering units operating under veterinary authorization issued by the Veterinary Direction and Food Safety.
Represents the veterinary authorization document issued by the veterinary and food safety county, attesting that the activities of the units that produce, process, store, transport and / or distribute products of animal origin, veterinary meet operating conditions established by veterinary legislation European Union and national;
If it is found during official inspections of food establishments that no longer meet operational requirements veterinary, veterinary inspectors have suspended or, where appropriate, prohibiting conduct business.
Suspension or, where appropriate, prohibiting conduct veterinary business units to have the veterinary and food safety county, or upon verification by expert veterinary staff of the National Sanitary Veterinary and Food Safety.
Veterinary Hygiene and Epidemiology Division of the National Sanitary Veterinary and Food Safety Authority, in view of risk group classification unity, performed the first audit of authorized units in the first 6 months of authorization and thereafter at least every 2 years.
The audit is a comprehensive evaluation of a food business operator. Audit is performed by one or more auditors, who have the power to conduct audit.

Veterinary direction and food safety county audit performed by specialized personnel whenever necessary but at least once every six months, catering establishments licensed veterinary.
The audit report will include details on the following:
a) general provisions and scope of the audit;
b) issues found during the audit, compliance, non-compliance with the legislation in force;
c) recommendations of the audit team to remedy nonconformities;
d) measures ordered by the audit team when deviations from the laws in force;
e) the conclusions of the audit team, including a proposal to authorize the establishment or maintenance of the suspension / ban of its activity;
f) the names and signatures of the persons who participated in the audit, official stamps direction from veterinary and food safety county and food business operator.
Depending on the outcome of the audit, expert veterinary staff of the Directorate Veterinary Hygiene and Epidemiology of the National Sanitary Veterinary Food Safety, which conducted the audit, we propose maintaining authorization or, where appropriate, suspension or ban the activity and will make recommendations to ensure safe products.
Regulation 852/2004 EC, lays down general rules for food business operators on the hygiene of foodstuffs, taking particular account of the following principles:
a) primary responsibility for food safety rests with the food business operator;
b) is necessary to ensure food safety along the food chain, from primary production;
c) is important to maintain the cold chain, especially for foods that can be stored at room temperature in a safe food, especially frozen foods;
d) general implementation of procedures based on HACCP principles, together with the application of good hygiene practice, should reinforce food business operators' responsibility;
e) guides to good practice are a valuable instrument to aid food business operators at all stages of the food chain, to comply with food hygiene and HACCP principles apply;
f) is necessary to establish microbiological criteria and temperature control requirements based on scientific risk assessment.

To this end, such operators shall have in place systems and procedures which allow for this information to be made available to the competent authorities on request.
Foods placed on the market are adequately labeled or identified to facilitate its traceability, through relevant documentation or information.
If a food business operator considers or has reason to believe that a food which it has imported, produced, processed, manufactured or distributed does not satisfy food safety requirements, it shall immediately initiate procedures to withdraw the food in question from market and inform the competent authorities.
A food business operator shall immediately inform the competent authorities if it considers or has reason to believe that a food which it has placed on the market may be injurious to human health.
European and international legislation on food production provides for all units involved in the production, transportation, storage and marketing of foods, principles of food quality assurance system, the principles of hygienic quality assurance system based on risk assessment and prevention, so kind of a HACCP system.
This is a preventive system used by food business operators to help ensure food safety.
Properly applied, the system controls the dangerous elements of food production systems, such as contaminants, pathogens, foreign bodies, chemicals, ensuring that the raw materials, processing conditions, instructions on the use of consumer data or storage conditions are appropriate.
HACCP concept is an appropriate tool for controlling hazards in food and especially the food in those establishments which use techniques that favor the introduction of hazards, if the operations are not carried out properly.
Veterinary inspectors verify the existence, implementation and operation of HACCP program so that the specific levers to ensure the smooth flow of production as a finished product and getting healthy and good quality.
Successful implementation of procedures based on HACCP requires the full cooperation and participation of employees in the food sector.
HACCP is a tool to help food business operators to achieve a higher level of food safety.
Regulation (EC) no. 852/2004 provides that the HACCP requirements should provide sufficient flexibility in all situations, including for small businesses. The main objective of implementing a HACCP-based procedures is to control hazards in food.

This can be done using various means, given that hazard control procedures should be based on risk, priority and focus on what is important for food safety in the food drive.

CONCLUSIONS

Food safety is a result of several factors: legislation should lay down minimum hygiene requirements, it should be held official checks to see whether food business operators comply with these requirements and food business operators must establish and implement food safety programs and procedures based on HACCP principles.
This information is made available to the competent authorities on request.
Operators shall inform the competent authorities of the action taken to prevent risks to the final consumer and shall not prevent or discourage any person from cooperating, in accordance with national law and legal practice, with the competent authorities, where this may prevent, reduce or eliminate a risk arising from a food.

REFERENCES

Regulation (EC) no. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs;
Regulation (EC) no. 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety;
Order of the President of the National Sanitary Veterinary and Food Safety Authority no. 57 of 24 June 2010 approving the sanitary veterinary norm on animal health permitting of facilities that produce, process, store, transport and / or distribute products of animal origin;