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HISTOSTRUCTURAL DETAILS OF THE TONGUE IN *COTURNIX COTURNIX JAPONICA*

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Key words: tongue, salivary glands, cartilage, mucosa.

SUMMARY

The researches point to a series of microscopical particularities of the tongue in the japanese quail (*Coturnix coturnix japonica*). The structural details are analised in histological sections that concern this segment, starting from the apical anterior region until its base. In the same time, the caracteristical element for this level, age depending,were surprised, insisting on the dinamic of the development. Initially, the study presents histostructural aspects of the lingual mucosa, that espectially reffers to the epithelium, them detailing the lingual body organisation, also presenting the salivary glands and the sustaining elements.

The tongue has a pointed shape in galinaceae and has its base attached to the pharinx, from which it embriologically derives. The cross section reveals a triangular shape, following the contour of the inferior valva of the beak.

Its surface, slightly concave, presents itself covered by a keratinized stratified squamous epithelium being thicker on the ventral side and on the apical area. Particularly, the epithelium is well developed on the dorsal side, too, where the basal germinative layer appears to be flattened and presents numerous invaginations, that are occupied by a well vascularised connective tissue. Towards the base of the tongue, the epithelium forms a transversal line of tall papillae, aborally orientated. Posterior to them, the epithelium reduces its thickness significantly [2,4].

On its whole surface, the lingual epithelium is doubled by a dense connective tissue, which forms the *lamina propria*. This connective tissue increases its proportions and looseness as it goes to the posterior region of the tongue.

The body of the tongue, limited by the mucosa, contains salivary glands, the muscularis and the skeletal piece, needed for support.

The skelet of the tongue is represented by the entoglos, the oral portion of the hyoid bone in the anterior region and by the bazihyoid in the posterior region. Alongside other components, the skelet grands the tongue a different arrangement, depending on the region that is being examined [3].

In cross section, the histostructural aspect of the tongue appers different, depending on the area destined for study. Thus, structuring the apex of the tongue, only the *lamina propria* and the entoglos, covered by epithelium, can be observed. Sections made through the middle region of the tongue present in their center the entoglos bone and, ventrally, a layer of striated muscles with longitudinally oriented fibers. In the oral dorsolateral regions, the anterios lingual salivary glands are seen [5, 9]. In the same region, Toyoshima K. and col. (1993), came across, in the japanese quail (*Coturnix coturnix japonica*), subepithelial Merkel corpuscles. They contain between four and eight cells, among which free nerve endings appear. Electron-dense granules with a diameter around 120-180 nm are scattered in the cytoplasm of the cells. The Merkel corpuscles are presumed to be mechanoreceptors [7].

Posterior, near the oral cavity and the pharynx limit, the salivary glands form a compact and continuum layer, which occupies the whole dorsal suface from under the mucosa. The skelet of the tongue, represented now by the bazihyal, has a ventral layout and it is now framed with musculature in both upper and lateral sides [1,8].

In birds, the tongue is a mobile organ, although because of the poorly developed inner musculature and of the considerable thickness of the epithelium, it tends to have some rigidity. The salivary glands are well represented in the corion of the lingual body, where the anterior lingual glands are seen. The base of the tongue is occupied by the posterior lingual glands [6,10].

MATERIAL AND METHODS

For the histostructural study 12 birds have been used, belonging to *Galliformes* order, *Phasianidae* family, *Coturnix* genus, *Coturnix japonica* species, six males and six females, aged between one and ten weeks old.

The prelevated pieces have been histologically prepared, seriated and longitudinal or cross sectioned. In order to color the sections made Haematoxylin and eosin stain, van Gieson method and Trichrome technique have been used.

The examination of the histological permanent sections were made with the NIKON-LABPHOT2 optical microscope, a light filter BG-33, Nikon AFX-DX and a NIKON FX-35 DX photo camera, and the images have been processed on the computer using *Adobe Photoshop 6.0* software.

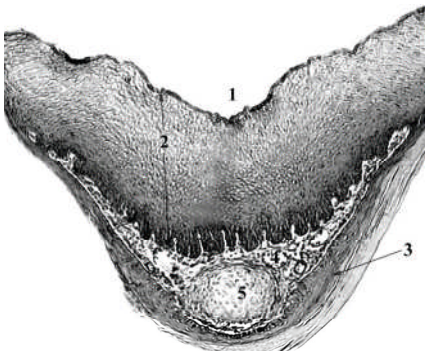
RESULTS AND DISCUSSIONS

The tongue is triangular in cross section and is composed from mucosa, cartilaginous piece of sustain and the striated muscles attached to it.

The mucosa contains a keratinized stratified squamous epithelium, with a maximum number of 25-28 layers on the dorsal side, which becomes concave due to the median groove that can be grasped only in the anterior region of the free portion. In one day old birds, the dorsal epithelium is faintly keratinized, unlike the one lining the lateral and ventral sides. These epithelia, even if they present a reduces number of layers, varying from four to eight, present an accentuated keratinization, especially towards the apex of the tongue. Here, the lamina propria, well vascularized, surrounds a dense connective tissue core, in the further sections replaced by the anterior extremity of the entoglos.

The sustaining piece of the tongue is constituted of hyalin cartilage, circular in cross sections near to the apex, in all birds considered, aged up to four weeks (Figure 1).

Figure 1. Transverse section from the tongue, which was made near the middle teritory at the japanese quail in 1 day old/ VG, ob.3x (authentic)
1. Median groove; 2. Stratified squamous epithelium, keratinized from the dorsal surface; 3.Stratified squamous epithelium, keratinized from the ventro-lateral surface; 4. Blood vessel; 5.Hyaline cartilage.



The sections made between the limit of the anterior and middle thirds of the tongue have a more complex structure, due to the appearence of the dorso-lateral lingual salivary gland grouping. The secretory units, branched tubuloacinar type, are lined by a simple columnar epithelium. The cytoplasm of the epithelial cells is foamy, abundant and the nuclei are spherical or flattened, arranged to the periphery (Figure 2). Aborally, from the middle region, the lingual cartilage widens, obtaining a reniform aspect with a ventral orientation, including, in the resulted concavity, alongside vascular formations, the first striated muscular fibers, which will laterally spread little by little (Figure 3). Regardless of the age of the subjects, the epithelium of the latero-ventral sides looses its keratinized aspect and appears to be crossed by numerous ducts of the salivary glands.

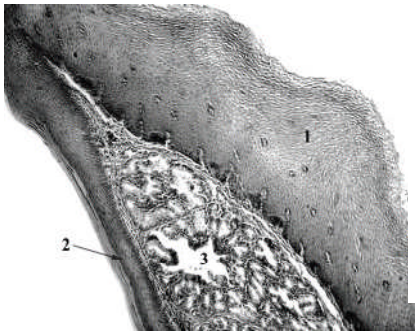


Figure 2. Apparance of dorso-lateral salivary glands in the structure of the tongue, in the aproximately middle region, at the japanese quail in 70 days old/ HEA, ob.6x (authentic)
1. Stratified squamous epithelium, superficial keratinized from the dorsal surface; 2. Stratified squamous epithelium, keratinized from the lateral surface; 3. Mucous tubulo-alveolar salivary glands.

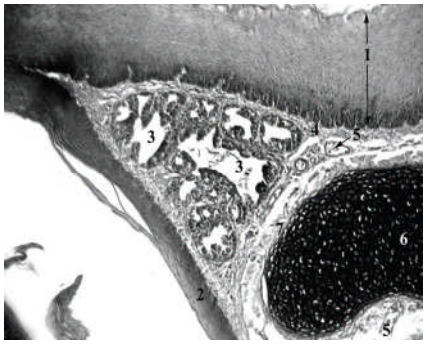


Figure 3. Transverse section from the tongue middle region, at the japanese quail in 1 day old/ HE, ob.6x (authentic)
1. Stratified squamous epithelium, superficial keratinized from the dorsal surface; 2.Stratified squamous epithelium from the ventro-lateral surface; 3. Mucous tubulo-alveolar glands; 4.Lamina propria; 5. Blood vessel; 6. Hyaline cartilage; 7. Perichondrium.

In 70 days old birds, in the glandular lobules, alongside compound tubuloacinar glands, simple tubuloacinar glands much more elongated than the ones observed until recently can be seen (Figure 4). Also, starting with this region, in the birds mentioned, the ossification of the hyalin cartilage is ascertained by the appearence of the spongy bone.

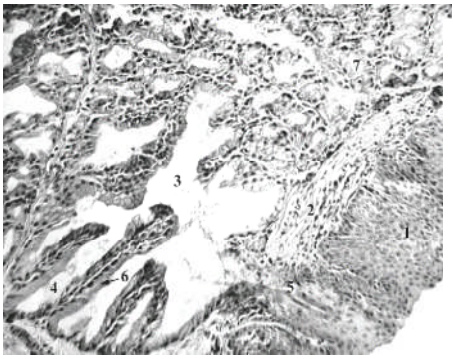


Figure 4. The opening of the salivary gland duct at the level of latero-ventral surface epithelium, at the japanese quail in 70 days old/ HE, ob.10x (authentic)
1. Stratified squamous epithelium, from the latero-ventral surface; 2. Lamina propria; 3.Glandular lumen; 4. Secretory portion; 5.Duct; 6. Mucous simple columnar epithelium; 7.Tubulo-alveolar branched gland in transverse section.

The mucosa of the dorsal side of the tongue continues with the pharyngian mucosa, which presents a stratified squamous epithelium, whose superior layers are slightly degenerating, therefore tending to transform itself in keratinized cells. At this level, in *lamina propria*, numerous compound tubulo-acinar glands, agglomerated especially around the anterior extremities of the laringian opening can be noticed.

CONCLUSIONS

3.1 In one day old birds, the dorsal lingual epithelium is weakly keratinized, unlike the epithelium on the ventral and lateral sides, where keratinization appears to be more pronounced.

3.2. From the middle region of the tongue, aborally, the lingual cartilage widens to a reniform aspect with a ventral orientation, finding

striated muscular fibers alongside the vascular formations that already are in the concavity, too.

3.3. From the age of 70 days, the ossification of the hyaline cartilage can be noticed, alongside by the appearence of the cancellous bone, in the central area of the tongue.

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HISTOSTRUCTURAL STUDY CONCERNING THE OROPHARYNGEAL CAVITY IN *COTURNIX COTURNIX JAPONICA*

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Key words: oropharyngeal cavity, epithelium, glands.

SUMMARY

The histostructural particularities of all the elements that form the oropharinx in *Coturnix coturnix japonica* have been detalied in this study. Step by step, the aspects of the opening of the oral cavity that limit the two components of the beak are presented, then the floor, the ceiling and the walls of the cavity. The accent is placed on the chronological alteration suffered by the mucosa, starting from one day old and until the birds have reached the sexual and body maturity needed.

The opening of the oral cavity is limited, in birds, by the beak (*rostrum*), that compensates the lack of lips and teeth that are found in mammals.

The two parts of the beak appear to be formed from epithelium, dermis and bony skeleton. The bony component is represented by the maxila, forming the upper beak, and the lower beak, structured by the mandibular expansion.

Both bony elements are covered by periostum, doubled by a considerably thick dermal layer, alongside an epithelium that is structurally resembling the one seen in all featherless regions of the body.

Concerning the epidermis, a clear separation cannot be made between the granular and lucidum layers [6, 8].

The oral cavity continues with the pharynx, but the limit between these two compartments is hard to distinguish, because the palatin veil is absent. For these reasons, conventionally, this line is considered to be the last row of cornificated pappilae. Still, embriologically, this limit is situated slightly ventrally, occupying the teritory around the opening of the glottis. Therefore, one can reffer to this cavity as a common cavity, named the oropharyngeal cavity [3].

The oropharyngeal cavity is covered by a considerably thick keratinized stratified squamous epithelium, that resembles the one seen in the areas with thick skin. The diferences are noticed in the corneum layer, where the cornification process is incomplete, remarking the flattened nuclei from the superficial layers.

In the anterior region of the oral cavity, but at the beak's level too, the dermis is thin, but it cannot be separated by the papillary and reticular layer. Advancing aborally, the differentiation becomes possible, because the reticular layer is not so compact, although it is formed of a fibrous connective tissue, rich in collagen fibers. In this area, the muscle fibers are lacking and some small diseminated glands can be seen [2, 7].

The salivary glands are well represented in the corion of the ceiling and floor of the oral cavity and the walls of the pharynx. These glands' structure is the compound tubular type and they open with several ducts in the oral cavity and pharynx [5].

The structure of the pharyngian wall, found in the posterior region of the oropharynx, resembles the one of the oral cavity. The mucosa of the pharynx appears to be formed of *lamina propria* and of stratified squamous epithelium, with several layers of cells. Generally, the surface of the epithelium is lacking in corneum layers.

The structures folowing the submucosa vary depending on the examined pharyngian area. Therefore, in the ceiling of the pharynx, in front of the nasal cavities, the submucosa follows the nasal epithelium, and in the lateral walls, it follows a mass of connective tissue [1].
As for the floor of the pharynx, this continuing is made with the very well represented striated muscle tissue, associated to the hyoid bone and to the base of the tongue. From the limit between the pharynx and the esophagus, the pharyngian muscle layers are to be followed by muscularis mucosae and mucosa, esophagian components.
Merkel and *Herbst* corpuscles and free nervous terminations have been observed in the pharingian wall [4].

MATERIAL AND METHODS

For the histostructural study 12 birds have been used, belonging to *Galliformes* order, *Phasianidae* family, *Coturnix* genus, *Coturnix japonica* species, three males and nine females, aged between one and ten weeks old.

The prelevated pieces have been histologically prepared, seriated and longitudinal or cross sectioned. In order to color the sections made Haematoxylin and eosin stain, van Gieson method, Alcian blue stain, Giemsa and Trichrome technique have been used.

The examination of the histological permanent sections were made with the NIKON-LABPHOT2 optical microscope, a light filter BG-33, Nikon AFX-DX and a NIKON FX-35 DX photo camera, and the images have been processed on the computer using *Adobe Photoshop 6.0* software.

RESULTS AND DISCUSSIONS

The oropharyngeal cavity or the oropharynx is covered by stratified squamous epithelium, whose degree of keratinization varies with the thikness. The differences are seen especially in the case of the oral pappillae, where the number of epithelial layers increases and the cornification process intensifies, but, as one points to the lateral walls of the anterior region where the continuing with the intensly keratinized epithelium of the valva of the beak (Figure 1). Though, comparing the mucosal epithelium from the floor and the ceiling of the rostral oropharynx, it is easy to see the median area, where the keratinization is not complete, therefore becoming posible to observe the flattened nuclei of the cells from the superficial epithelial layers.

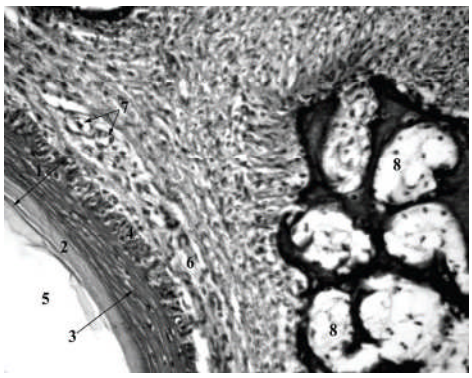

Figure 1. Oropharyngeal cavity mucosae aspect, near the lateral internal surface of the upper beak at the japanese quail in 1 day old/ HEA, ob.20x (authentic)

1. Stratified squamous epithelium, keratinized; 2. Stratum corneum; 3.Stratum spinosum; 4. Stratum basale; 5.Oropharyngeal cavity; 6. Lamina propria; 7. Blood vessel; 8. Spongy bone.

As regards to *lamina propria*, along well anchored small and medium blood vessels in the connective tissue, compound tubuloacinar salivary glands appear both in the mandibular mucosa and the palatin region (Figure 2). Starting with the age of 14 day, in the japanese quail, there is a tendency of agglomeration for the lymphoid population adjacent to the epithelium (Figure 3). In the mediolateral region, the depth of the two territories is occupied by cancellous bone, corresponding to the maxila and to the mandibula. The entire areal appears to be formed of fine trabeculae, that describe cavities with different diameters, in which red bone marrow can be found. The trabecular bone is covered by a well structured periostum, that establishes connections with the dense connective tissue nearby.

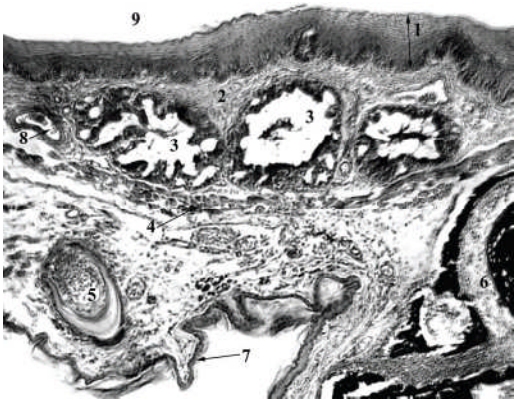

Figure 2. General view of the mandible mucosae from oropharyngeal cavity at the japanese quail in 1 day old/ HEA, ob.6x (authentic)

1. Stratified squamous epithelium, keratinized; 2.Lamina propria; 3.Tubulo-alveolar glands; 4. Skeletal muscle; 5. Feather follicle; 6.Mandible spongy bone; 7. Epidermis; 8.Blood vessel; 9.Oropharynx.

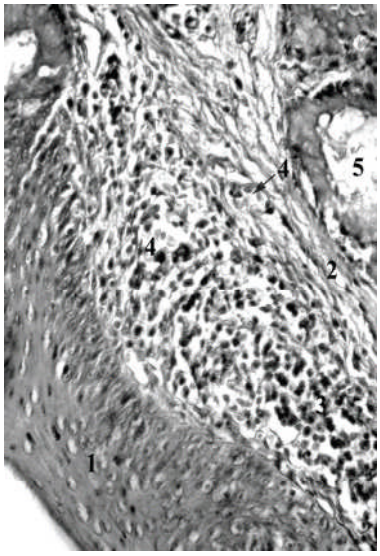

Figure 3. Started of lymphoid cells organisation in lymphoid nodules, near the palatine mucosae epithelium at the japanese quail in 14 days old/ AA, ob.20x (authentic)

1. Stratified squamous epithelium; 2. Lamina propria; 3. Lymphoid cells; 4. Blood vessels; 5.Tubulo-alveolar glands.

Aborally, the number of layers of the epithelium of the palatin mucosa grows. In the middle, it will gradually form an invagination which will continue to deepen, continuing with the vestibular epithelium of the nasal cavity in the area of the opening of the choana.

Histostructurally, the cross section highlights the tendency of migration towards the depth of the palatin glands, that will partially be separated from the *lamina propria* by the regional striated muscle fibers. On both sides of the future median fissure, where some lymphoid cells can be seen, some vascular formation coming from the medial branches of the palatin arteries can be observed. In front the last row of pappilae, the palatin epithelium is followed by ciliated pseudostratified columnar epithelium coming from the middle segment of the nasal cavity.

The sections made with the ventral median groove capture the shape of the aritenoid cartilages, lateroventrally surrounded by the laryngian striated musculature. Medially, they are contintiguous to the laryngian mucosa, that, in this territory of origin, is formed of a non-keratinized stratified squamous epithelium, with a slightly keratinization only in the territory belonging to the laryngian protuberances, and of *lamina propria* that is lacking in glands (Figure 4). In exchange, the pharyngian mucosa presents compound tubuloacinar glands.

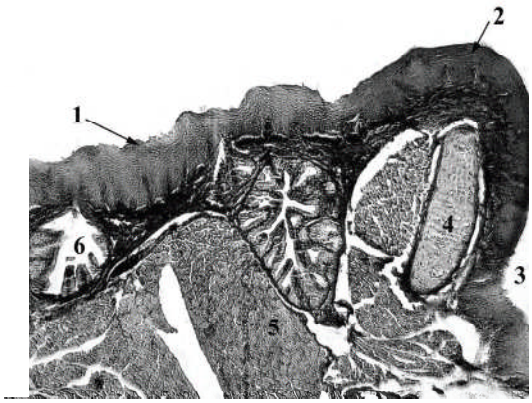

Figure 4. Transverse section from the level of the glottis, at the japanese quail in 70 days old/ VG, ob.3x (authentic)
1. Stratified squamous epithelium of the pharynx; 2. Epithelium of the larynx proeminent furrou; 3. Glottis; 4.Arytenoid cartilage; 5.Skeletal muscle tissue:

According to the median ventral groove, the infundibular groove is visible in the pharyngian floor, where the non-keratinized stratified squamous epithelium will be followed by the not-ciliated pseudostratified columnar epithelium from the auditory tubes. At this level, in the pharyngian mucosa, in *lamina propria*, it is easy to see an alteration of the aspect of the glandular formations, which are made of numerous tubuloacini, with narrowed lumina and agglomerated large cells that confere an intermediar character between the tubuloacinar glands and the mucous tubuloacinar glands. The nuclei of the glandular cells are flattened and placed to the periphery. Following the aspect of the mucosa of the infundibular groove, the presence of a dense, lacking in glands, connective tissue can be seen. The mucosal glands will have a simple tubular aspect and will be found integrated, from place to place, in the epithelium.

Back to the region of the pharingian floor, situated caudally from the median ventral groove, the characteristics of the last row of pappilae marking the limit between the oropharynx and the esophagus can be described. The pappilae are wide and short, their dimensions beeing constant, excepting the ones in the center and those placed on the sides, which are taller and sharper. Their epithelium, stratified squamous, becomes keratinized. The glands in *lamina propria* intensifies its mucosal tubuloacinar image, and appear to be surrounded in the ventro-lateral side by muscular fibers. The tendency to shape the first traheal ring is visible in the depth of the region. The glandular histostructure described is mentained even in the mucosa of the cavity's ceiling (Figure 5).

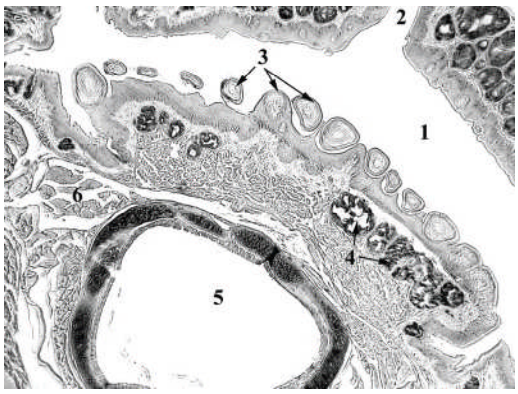

Figure 5. General view of the oropharyngeal caudal region, at the japanese quail in 1 day old/ Giemsa, ob.10x (authentic)
1. Oropharyngeal cavity; 2.Infundibular cleft; 3. Papillae; 4.Tubulo-alveolar mucous glands; 5.Origin of the trachea; 6. Skeletal muscle tissue.

The histological sections done aborally to the territory previously described, seize the characteristics of the oropharynx and esophagus.

The mucosa, with a fairly smooth look, initially will sketch rare pappilae. It consists of a very little keratinised stratified squamous epithelium and of *lamina propria*, which, starting with the pappilary region, will present numerous simple tubuloacinar glands. However, in the inmost of the connective tissue, mucosal tubuloacinar glands can be observed, that, due to the appearence of the muscularis mucosae, will clearly be separated by the *lamina propria* and the epithelium. These submucosal glands can be found, in quail, only in the area of the oropharyngoesophagian junction, where the surface epithelium maintains its eveness.

The external muscularis has itself important alterations, because the pharyngian striated muscular fibers will be replaced with smooth muscular fibers even at the esophagian origin. Hereunder, the adventitia from the anterior portion of the esophagus is imbedded with the tracheal one, establishing a strong connection between the two organs.

CONCLUSIONS

3.1. Comparing the epithelium of the ceiling's mucosa with the epithelium of the rostral oropharyngeal floor, at one day old, we observe the incomplete keratinization in the median area.

3.2. Starting with the age of 14 days old, there is the tendency of agglomeration of the lymphoid poppulation adjacent to the palatine mucosal epithelium.

3.3. In the pharyngeal ceiling, near the territory where the infundibular groove opens, an alteration of the aspect of the glandular formations belonging to the *lamina propria* was noticed; these glandular

formations are organised of many tubuloacini with narrow lumina and of agglomerated large cells which grand an intermediar aspect between the tubuloacinar glands and the mucosal tubuloacinar glands.

3.4. In the deep connective tissue, at the begining of the oropharyngoesophagian jonction, mucosal tubulo-acinar glands can be detected, glands that due to the appearance of the muscularis mucosae will clearly be separated from *lamina propria* and epithelium, therefore partly structuring the submucosa.

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DELIMITATIVE MEMBRANE STRUCTURES OF LARVAR GERMINATIVE ELEMENTS IN PULMONARY HYDATIDOSIS IN CATTLE

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Key words: echinococcosis, hydatidosis, hydatid cyst, larvar germinative elements, proligerous membrane, histopathology.

SUMMARY

This paper presents the preliminary results of a comparative cyto-histopathological study performed on membrane structures of uninfected and infected pulmonary hydatid cyst, conducted on a total of 34 lung samples from cattle, Holstein breed metis, in the age limits of 6-14years.

In the present paper are demonstrated the modifications of topohistological relationships of the delimiting membrane and of the polymorphocellular and epithelioid-giant cells layers, based on which the authors consider that the morphopathological symptoms of the infected hydatid cyst belongs to a real hydatid granulomatous diffuse proliferation, in which the specific germinative elements for the parasite Echinococcus granulosus can develop new hydatids.

This morphopathological study of the membrane structures is considered by the authors as being particularly useful to those interested in practicing the technique of surgical therapy, not without risks, especially in human lung hydatidosis, including videothoracoscopic technique.

Although echinococcosis is a disease known from the oldest times that continues to concern the Europe's and world's scientific community, taking into consideration both the major implications, especially the sanitary and economic ones, and the fact that this zoonosis has known a significant increase in cases in humans and animals (7,10,11,12,13).

In Romania, these kinds of preoccupations are entirely justified if one considers that our country is among the first in Europe concerning the incidence of hydatidosis in humans (1,4,5,6,8).

Taking into account these aspects, as well as the detail that lately it has been more and more attempted to introduce new surgical therapeutical techniques, especially in pulmonary hydatidosis in humans (12,13), techniques that are not without risks, we considered that remembering some cyto-histopathological studies previously presented (6) and attentively emphasizing on the infected hydatid cyst from the pulmonary cases seen in cattle would be adequate.

1. MATERIAL AND METHODS

The cyto-histopathologic study of the pulmonary hydatid cyst was conducted on a total of 34 lung samples from cattle, Holstein breed metis, in the age limits of 6-14years

The samples have been divided so that they would include both the whole hydatid cyst and the adjacent tissular areas, proceeding as following:

- from a number of unfixed 11 hydatid cysts, with the fine needle aspiration technique, hydatidic liquid samples have been prelevated, samples from which the sediment was obtained by centrifuging it at

1000 rotation/minute, for 2 minutes; spots between slides were made, and they have been examined under the microscope as such or vitally colored with Hematoxylin, isotonic solution 1%;

- 27 hydatid cysts have been immersed in neutral solution of formalin 10%, for 72 hours, after that they were sampled and the resulted samples have been refixed in the same fixative liquid for 24 hours; the histological pieces obtained were included in paraffin in Pathcentre Thermo Shandon and Kunz installation, after that slicing the blocks with Finesse Thermo Shandor microtome into 4-5 micrometers thick sections, deparaffined and stained with Hematoxylin-Eosin, modified Masson, Periodic Acid Schiff and Alcian blue – PAS methods. The histological slides that resulted have been examined with Olympus BX51 microscope and Zeiss Jena installation, while the image processing has been done using Camedia soft, respectively AxioPlan 2 Imaging.

2. RESULTS

The cyto-histopathological study of *uninfectious hydatidosis* allowed the observation in the cystic space, limited on the interior by the proliger-cuticular membrane, of the next components: fertile fragments of the proliger membrane; fragments of acephalocystic proliger membrane; solitary proliger daughter-vesicles; conglomerates of proliger daughter-vesicles; invaginated protoscolex united by the external canalicular excreting network; protoscolex about to devaginate; mature protoscolex with infecting potential; external canalicular excreting network fragments; rostral fragments; hooks coming from the rostral apparatus of the protoscolex; calcificated amorph masses; hydatid liquid (fig. nr. 1, fig. nr. 2, fig. nr. 3, fig. nr. 4).

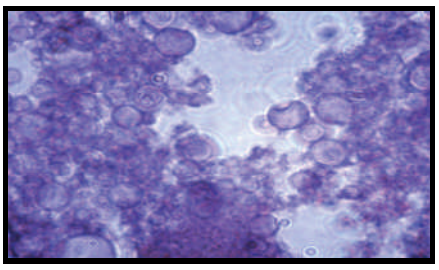

Fig. nr. 1 - Massive population of larvar germinative elements in different evolutive stages in hydatic liquid. Hydatic cyst. Cattle. Modified Masson stain, x2000.

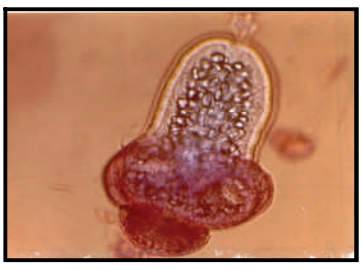

Fig. nr. 2 - Free mature protoscolex in hydatid liquid: rostrum, suckers, muscle fibers, internal canalicular excreting network, germinative-cuticular membranary system, small fragment from the external canalicular excreting network. Native. Vital Eosin stain, x160 .

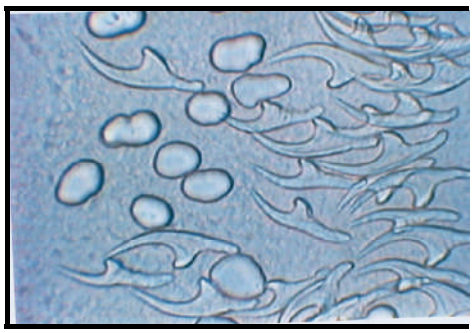

**Fig. nr. 3 - Free hooks in hydatid liquid.
Hydatid cyst. Cattle. Native. Vital Methylene blue stain, x400.**

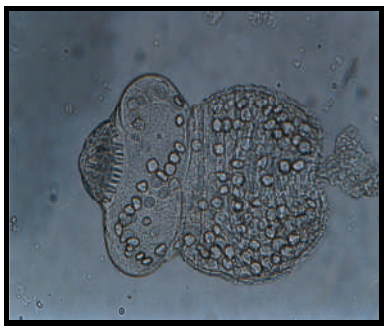

Fig. nr. 4 - Free infectant devaginated protoscolex in hydatid liquid. Hydatid cyst. Cattle. Native. Unstained, x160.

The presence of proligere vesicles is obvious in the hydatid liquid, both in solitary form, and as larger or smaller or as agglutinated and calcified groups (fig. nr. 5). In some cases, massive populations of such structures were obvious, in other cases, the proligere vesicles formed real conglomerates. Some vesicular structures presented structural configurations of pre-protoscolecs.

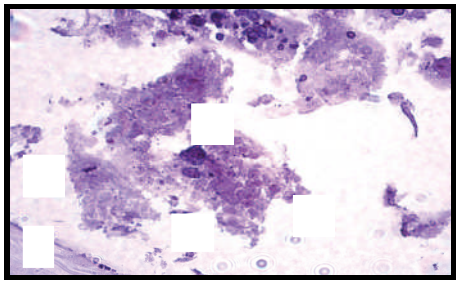

Fig. nr. 5 – Larvar germinative elements in genesis dynamic, detached from the germinative membrane. 1 – Proliger membrane; 2 – budding of the proliger membrane; 3 – cuticle; 4 – free proliger capsules in the hydatic liquid; 5 – calcification processes in different stages of evolutive parasitary elements in hydatid liquid. Hydatid cyst. Cattle. Hematoxylin-Eosin, x400.

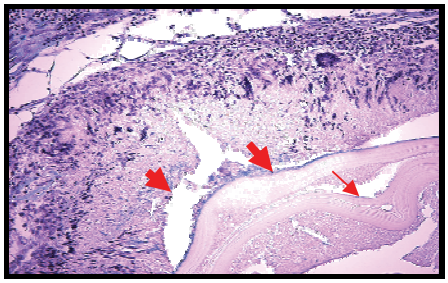

Fig. nr. 6 - Positive histochemical PAS-reaction (thin arrow) and positive Alcian blue reaction (thick arrow). Hydatid cyst. Lung. Cattle. PAS – Alcian blue stain, x200.

The protoscolecs that were observed both in the native unstained and in the histopathologic slides, have evidentiated different aspects.

The study of the protoscolecs has evidentiated the presence of different structures: rostral apparatus, suckers, internal canalicular excreting network, internal excreting network's ducts, the germinative-cuticular membranary system, the proper muscular tissue, the pedicle that connects with the external canalicular excretory network.

Both evidentiated components in the fertile, sterile and infectant hydatid liquid from the intracystic territory, as well as the cellular and fibrilar elements of the delimitating membranes of the germinative elements, including the ones of the pericystic areas, are distributively presented in table no. 1.

Table no. 1

The content of the hydatid cyst depending on the hydatidosis in cattle

No.	Components of the cystic content	Type of hydatid cyst		
		Fertile	**Sterile**	**Infectant**
1	Fragments of proliger-cuticular membrane	x	x	x
2	Fragments of proliger membranes elaborated by proliger daughter vesicles	x		
3	Proliger daughter vesicles and their conglomerates	x		
4	Hydatid liquid	x	x	x
5	Protoscolecs	x		
6	External canalicular excretory network fragments	x		
7	Rostral fragments	x		
8	Hooks from the rostral apparatus of protoscolecs	x		
9	Calcified amorph masses	x	x	x
10	Neutrophils			x
11	Eozinocytes			x
12	Plasmocyte			x
13	Macrophages			x
14	Epithelioid cells			x
15	Giant cells			x
16	Collagen fibers			x
17	Cells proper to the parenchymatose structure of the organ parasited cu Echinococcus granulosus			x

The intensely PAS-positive proliger membrane (fig. nr. 6) is presented as an internal, wavy, continuous germinal layer, with multiple nuclei that form vesicular microstructures, showing different aspects of the elaboration and development process of these larvar germinative elements. To the external side, the laminar structural aspect is remarkable obvious, the intensity of PAS reaction here being less intense.

The external laminary layer is contoured as an external membrane, sometimes hard to delimit from the proliger membrane, being amorph and acellular, uneven as thickness, sometimes unusually developed.

The pericystic area is represented mainly by connective tissue, made of collagen fibers and a mononuclear dominant cellular population, made of lymphocytes, plasmocytes, hystiocytes, macrophages, relatively uniformly distributed among connective fibers, with an inconstant abundant presence of eosinocytes and mastocytes. Sometimes, the eosinocytary reactivity is especially powerful, forming real cellular islands. The specific cellular reactivity is represented by epithelioid cells and especially giant cells that surround and limit the external acellular laminar layer of the proliger membrane.

The membranar cyto-histopathological architectonics of the *infected hydatid* is completely modified: the internal and external laminar layers of the proliger-cuticular membrane leave for the eye to notice crevasse and then empty spaces, therefore appearing fragmented, discontinuous; the integral or fragmented germinative larvar elements can be identificated not only in the hydatid liquid from the intracystic space and from the crevasses and the discontinuity of proliger-cuticular membrane, but also out of these structures, even in the structure of the pericystic membrane; many times, some proliger membrane fragments neighbor the pericystic area and even the unaltered lung tissue; the overflow of hydatid liquid among cells and fibers forms lakes that sometimes contains small germinative structure fragments from the intracystic area; the cellular reactivity in no longer arranged in columns when observed next to the proliger membrane, but presents itself as a polymorph cellular population, aleatory disseminated, in which giant cells, with eosinocytes and plasmocytes and/or neutrophilic granulocytes can be surprised; the predominantly neutrofilic inflammatory cellular infiltrate can include territories may comprise all the structures of the infected hydatid cyst and the external neighboring areas to the pericystic membrane; in unique or confluated giant cells, fragments of protoscolecs and/or phagocyte proliger vesicles can be seen; both in the restant intracystic spaces, as well as in the infected

hydatid membranar structures, numerous necrosis and calcification areas can be observed (fig. nr. 7, fig. nr. 8, fig. nr. 9, fig. nr. 10, fig. nr. 11, fig. nr.12).

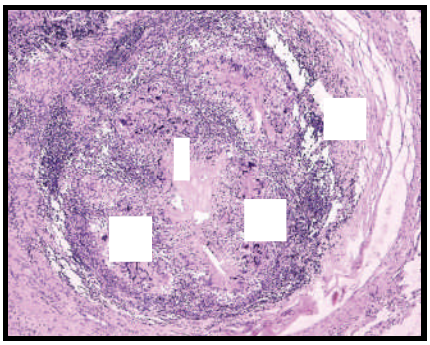

Fig. nr. 7 – Echinococcus granulomatos proliferation, with multiple areas of giant-cellular and polimorphocellular, predominant mononuclear reactivity zone. 1 – Necrosis area; 2 – giant cells; 3 – mononuclear cells; 4 – limiting peripheric fibrous tissue. Hydatid cyst. Lung. Cattle. Hematoxylin-Eosin, x50.

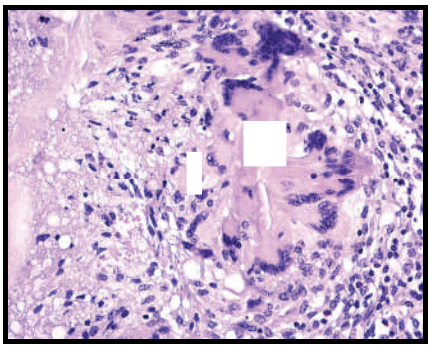

Fig. nr. 8 – Echinococcus granulomatos proliferation, with multiple areas of giant-cellular and polimorphocellular, predominant nuclear, reactivity zone. 1 – Massive necrosis of the larvar germinative elements; 2 – inactivated larvar germinative elements in the cytoplasm of giant cells. Hydatid cyst. Lung. Cattle. Hematoxylin-Eosin, x1000.

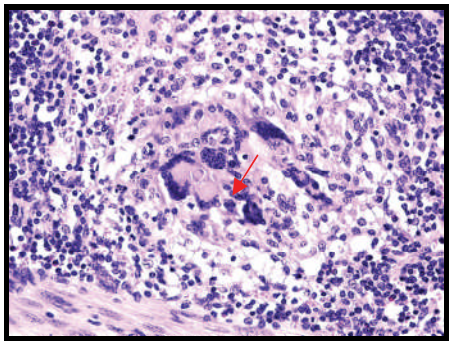

Fig. nr. 9 – Echinococcus granulomatos proliferation, with multiple areas of giant-cellular and polimorphocellular, predominant mononuclear reactivity zone. Protoscolex phagocytated by giant cells (arrow). Hydatid cyst. Lung. Cattle. Hematoxylin-Eosin, x1000.

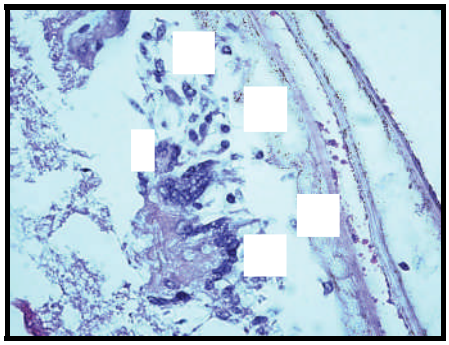

Fig. nr. 10 - Uniting process in the internal area of the pericystic membrane and in the area of proliger membrane elaboration. 1 – Elaboration of the proliger-cuticular membrane; 2 – internal continuous lacunar space; 3 – epithelioid cells; 4 – forming giant cells; 5 – giant cells. Hydatid cyst. Lung. Cattle. Pappenheim stain, x 800.

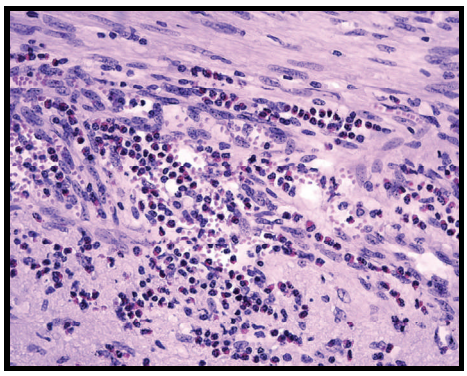

Fig. nr. 11 – Eosinocytic afflux in the pericystic area of fibroblastic proliferation and forming of lacunar spaces. Hydatid cyst. Lung. Cattle. Hematoxylin-Eosin, x400.

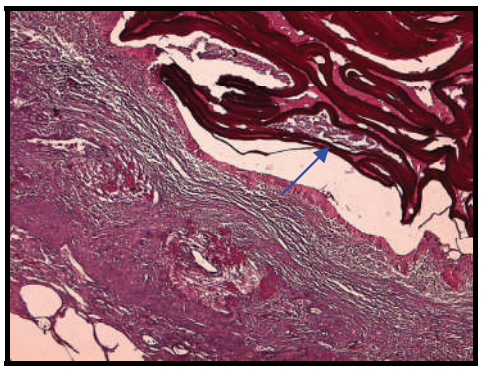

Fig. nr. 12 - Intensely PAS-positive proliger-cuticular membrane (arrow). Hydatid cyst. Lung. Cattle. PAS stain, x50.

DISCUSSIONS

It is worthy to take into account the fact that the identified germinative larvar elements that mark the echinococcic origin of the Echinococcus granulosus parasite (6) were evidentiated in the present research both in the uninfected hydatid cyst as well as in the infected cyst.

For this reason, it must be emphasised that in infected hydatide, among the germinative larvar elements destroyed by the inflammatory process, mature protoscolecs, with phonotypical aspect characteristic to the ones infected, indentified in uninfected hydatide, have been observed.

More, we consider that the structural destructions observed in the infected hydatic cyst, represented in essence by edematous and necrotic alterations, hemorrhages and calcifications, alterations of the delimitant membrane structures of the hydatic germinative elements and even by the alterations that end with affecting of the peripheric connecting sequester process, allow facile diffusion in the hydatic intermembranary spaces of these infecting forms with protoscolecs once with the cystic fluid. This way, the delimitant membranar structures of the larvar germinative elements alter their topohistological rapports, showing interpenetration both of these as well as of the polimorphocellular and epithelioido-giantcellular layers.

The structural disjunctions of the hydatid membranes determin a chaotic rearrangement of these, the intermembranary spaces newly created simply assuring the „gliding" and penetration of the larvar germinative elements in the pericystic peripheric territories.

This makes possible that the larvar germinative elements to be able to surpass the structural limits of the primary hydatid cyst, limited initially by the proliger membrane and to develop new hydatid centers in centrifuge direction in any other place of the membranary structures, including in the pericystic membrane and above this, which has bee demonstrated in the previous researches (6).

The new developed echinococcus cystic centers in the membranar structure of the infected pulmonary hydatic cyst itself and in the immediatly limitrophe areas, on one side, as well as the tissular architectonical alterations induced to it as following of superpositioning of inflammatory process, on the other hand, which can contitute a real *pulmonary granulomatos hydatid model*, which can be morphopatologically described by the following caracteristics:

- open hydatid cyst;
- inflammatory process with cellular reactivity, predomiantly mononuclear, and neutrophilic and eosinocytic granulocitar reactivity;
- Disorganization of the proliger-cuticular membrane, of the pericystic ones and of the epithelioido-giant and polimorphonuclear cell layers architectonics;
- Identification of free and phagocyted hydatid germinative elements in the intermembranary spaces;
-the peripheric secondary hydatids constituted and forming, with epithelioido-giant cellular and polimorphocellular reactivity.

As the high incidence and high mortality of the human hydatic disease in Romania is known, we consider that this *pulmonary granulomatos hydatic model* may interest the human medicine, considering the surgical techniques in therapeutical tackle of human pulmonary hydatidosis, as more as, in the present moment, there is the tendency of worldwide developing of some different non-invasive and minimal-invasive surgical techniques, including the use of videothoracoscopy.

3. CONCLUSIONS

3.1. Larvar germinative elements have been evidentiated in the bovine hydatid pulmonary infection, both in the intracystic hydatid liquid of the uninfected and infected hydatids, as well as in the lacunary

hydatid liquid from the limiting intermembranary spaces of the open infected hydatid cyst.

3.2. On the basis of the cyto-histopathological study made in the present paper, the open infected hydatid cyst, identified in lungs from cattle, can be considered, from the morphopathological point of view, as a diffuse granulomatos hydatid proliferation.

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THE TOPOGRAPHICAL CORELATIONS BETWEEN TYMPANIC BULLA AND REGIONAL CRANIAL NERVES IN DOG

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Key words: dog, topography, tympanic bulla.

SUMMARY

In the lateral or medial osteotomy of the tympanic bulla the injuries of the regional cranial nerves may be presented especially in the lateral access to the tympanic bulla due to dissections of the parotidian region where are passing by the caudal auricular and superficial temporal arteries, while the ventral access is made among the digastric, hypoglos and styloglos muscles (3).

The knowledge of the topographical relationship between tympanic bulla and surrounded nervous structures are very important to protect the extracranian structures of the 8^{th}, 9^{th}, 10^{th}, 11^{th}, 12^{th} cranial nerves and of the cranial cervical ganglion.

Tympanic bulla as a component of tympanic portions of the temporal bone is situated medially to the mandibular branch, caudally to temporo-mandibular joint, being rostrally delimitated by the retroarticular process of the temporal and caudally by the jugular process of the occipital bone.

Rostrally, tympanic bulla and the sphenoid bone delineate the carotic foramen, while caudally forms the jugular foramen with the occipital bone (1, 2, 4, 5).

1. MATERIAL AND METHODS

The dissections were made over four dogs and concerned the anatomical plans. Topographical and descriptive features were studied and the maximal important areas in the case of osteotomy of the tympanic bulla were determinate.

2. RESULTS AND DISCUSSIONS

In the subcutaneous plan there is the parotid gland which dorsally is placed on the base of auricle.

In the subparotidian plan, dorso-caudally prominences the tympanic bulla surrounded by its vascular and nervous structures (Fig. 1).

Dorsally to the tympanic bulla in the ventral part of the external acoustic duct is presented the facial nerve which leaves the petrous part

of the temporal trough the stylomastoidian foramen and lies rostrally to the maseter muscle.

In the latero-rostral part of the tympanic bulla the external carotid artery passes and caudo-dorsally the caudal auricular and superficial temporal arteries come off, accompanied by satellite veins.

Ventrally to the tympanic bulla, lingual and facial arteries and its satellite veins are detached.

The jugular foramen placed caudally to the tympanic bulla is traversed rostro-caudally by the 9^{th}, 10^{th}, 11^{th} cranial nerves and this nerves are crossed ventrally by the hypoglossal nerve and medially is in contact with the cranial cervical ganglion which cranially is placed in the angle between occipital and external carotid arteries and medially is in contact with the internal carotid artery.

From the cranial cervical ganglion leaves many postganglionar fibers for 9^{th}, 10^{th} cranial nerves and for the regional sympathetic nervous plexus.

The importance of the region comes from the various vasculo-nervous structures which surround the tympanic bulla.

The lateral wall of the tympanic bulla is placed in the base of external acoustic duct between the two branches of parotid gland, the ventral wall is covered by the digastric, hypoglossal and styloglos muscles and medially by the angular process of the mandibule.

3. CONCLUSIONS

3.1. The aspect of the tympanic bulla is in contact with the important vascular and nervous structures;

3.2. Dorsally the tympanic bulla is in contact with the facial nerve, rostrally with the external carotid artery, with the origin of the caudal auricular and superficial temporal arteries and satellite veins;

3.3. Caudo-ventrally the tympanic bulla has topographical relations with the external part of 9^{th}, 10^{th}, 11^{th}, 12^{th} cranial nerves;

3.4. Caudo-medially is presented the cranial cervical ganglion and its fibers.

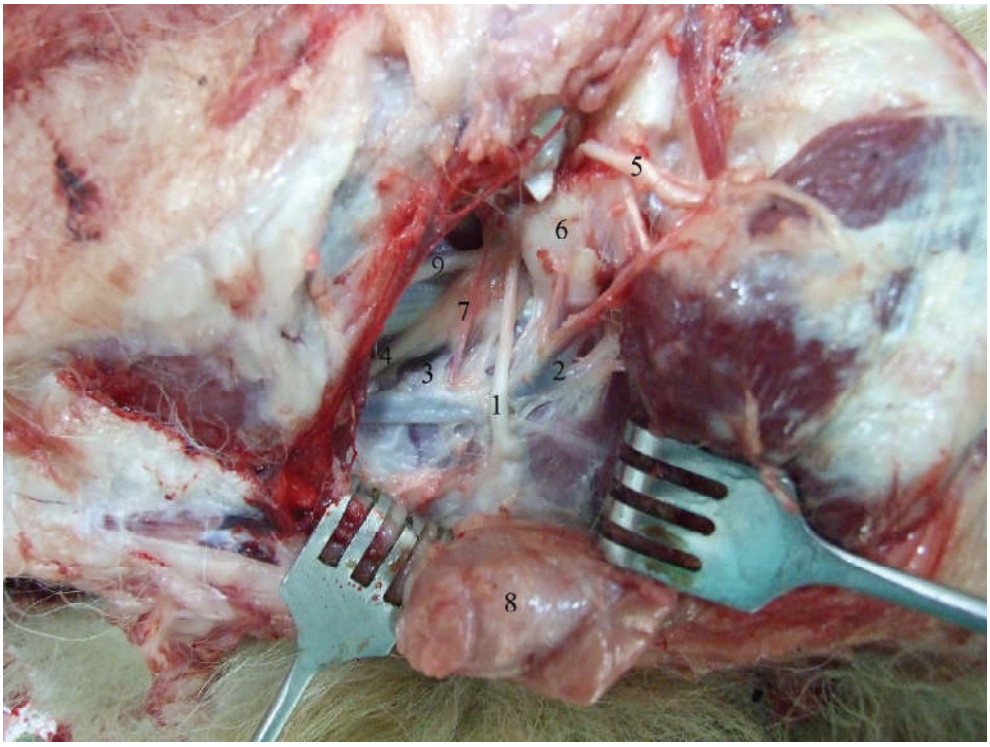

Fig. 1. Parotidian region, deep plan
1. Hypoglos nerve; 2. External carotid artery; 3. Occipital artery; 4. Vagus nerve; 5. Facial nerve; 6. Bulla tympanica; 7. Cranial cervical ganglion; 8. Parotid gland; 9. Accessory nerve.

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THE TOPOGRAPHICAL ASPECTS OF THE RIGHT AZYGOS VEIN AND ITS TRIBUTARIES IN DOG

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Key words: dog, topography, right azygos vein.

SUMMARY

The right azygos vein together with its origin and tributaries can be considerate an important venous system of the thoracic cavity.
In the lateral thoracotomies the passage of the right vein and its relationship with aorta and intercostal arteries and their affluent is very important to avoid arterial and venous hemorrhages.
The surgery of the thoracic cavity is a current practice in the veterinary field.
Knowledge as accurate of the origin and especially of the anastomoses of the affluents of the azygos vein is very important to protect venous vascularisation of the region during surgery (4).
The variation of the origin and the route of the affluents of azygos vein in the dog were determinate.

1. MATERIAL AND METHODS

The dissections were made over the thoracic cavities of four dogs and interested the successive anatomical plans and the affluents of the azygos vein. Those veins which represent the origin of the azygos vein were identified. Anatomo-topographical and descriptive features were studied.

2. RESULTS AND DISCUSSIONS

The right azygos vein passes the dorsal mediastinum on the right side and its origin is in the thinly 1st and 2nd pairs of the lumbar veins which reach over the azygos vein by overcoming the iliopsoas muscles (1, 2, 3).

In the caudo-cranial way the route of the right azygos vein is oblique cranio-dorso-laterally along the bodies of the thoracic vertebrae.

In the caudal third the azygos vein is situated to the right side of the median plan of the vertebral bodies, in the middle third is more laterally to right and in the cranial third is placed right beneath the costovertebral joints.

The end of the right azygos vein is perpendicularly to the cranial vena cava.

Was identified a difference of caliber of the vein, being thinner in the origin roughly 1 cm, increasing constantly to end, so the termination of the vein having 8-10 cm.

The right azygos vein is covered by parietal pleura and by endothoracic fascia, accompanied by the fascicle of the sympathetic trunk, by the greater dorsal splanchnic nerve and the smaller splanchnic nerve in the origin.

Ventrally, the right azygos vein is in contact with the oesophagus and being away from it in the origin.

Laterally, the right azygos vein is crossed by the right intercostal arteries, meanwhile the 9^{th} and 12^{s} intercostal arteries pass medially, hidden by iliopsoas muscle.

The costoabdominal artery passes along the caudal border of the last rib and crosses right azygos vein beneath the iliopsoas muscle. Thus, the origin of the 9^{th} and 12^{s} intercostal arteries and of the costoabodminal artery are protected by the iliopsoas muscle.

The origin of the 3^{rd} – 10^{th} right intercostals arteries are placed between right azygos vein and the correspondent costovertebral joint.

General scheme of the intercostal veins which are lying into right azygos vein shows the common orientation of the 4^{th} and 5^{th} intercostal veins especially on the right side, the 5^{th} intercostal left vein being very thin.

The 5^{th} intercostal right vein is ticker and its end perpendicularly to the right azygos vein, and the 6^{th} right intercostal vein has a very small caliber.

The 5^{th}- 8^{th} right intercostal vein are flowing perpendicularly to the azygos vein and starting with the 9^{th} intercostal vein the affluents are obliquely which became more evident to the costoabdominal vein.

In the case of surgery in the right side of the thoracic cavity most exposed are the terminations of the 5^{th} -10^{th} intercostal veins and of the azygos vein, especially in the cranial and middle third, meanwhile the caudal third is hidden between thoracic aorta and the roof of the thoracic cavity.

In the left side of the thoracic cavity the terminations of the intercostal veins are protected by the thoracic aorta.

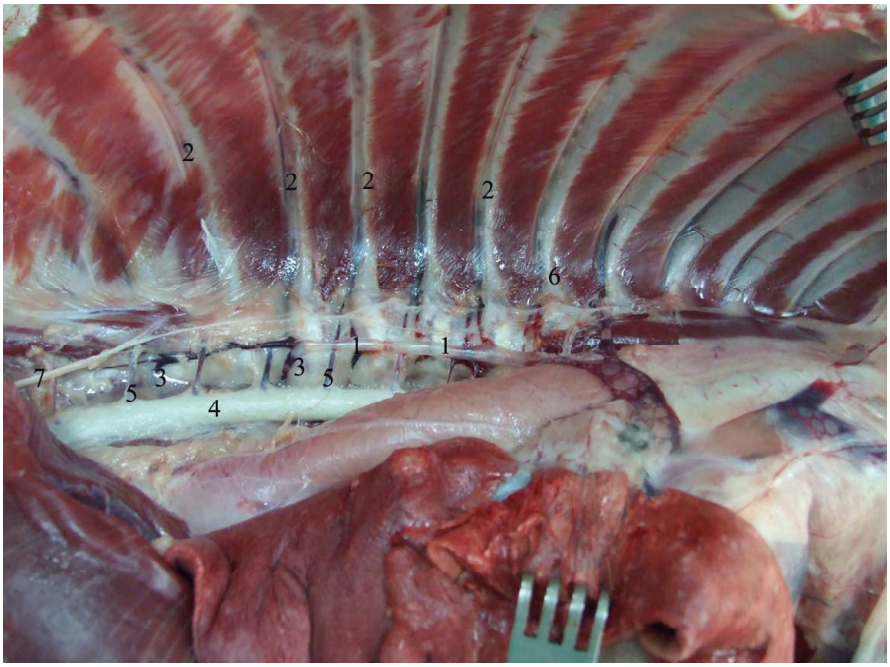

Fig. 1. Azygos venous system in dog
1. Right azygos vein; 2. Right intercostal veins which are flowing into azygos vein; 3. Left intercostal veins which are flowing into azygos vein; 4. Thoracic aorta; 5. Right intercostal artery; 6. Thoracic sympathetic trunk; 7. Greater splanhnic nerve.

3. CONCLUSIONS

3.1. The 5^{th} -12^{s} dorsal intercostal veins and the costoabdominal vein are the affluent of the right azygos vein;

3.2. The 5^{th}- 10^{th} dorsal intercostal veins are flowing perpendicularly to the azygos vein;

3.3. Starting with the 9^{th} intercostal vein the flows are obliquely cranio-laterally;

3.4. The terminations of the left intercostal dorsal veins are covered by thoracic aorta.

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RESEARCH CONCERNING THE HISTOSTRUCTURE OF ORGANS AT SWINE EMBRYO AGED OF 50 AND 60 DAYS

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Key words: embrionary developement, thymus, liver, stomach, testicle.

SUMMARY

The authors have studied the histological structure of organs of the thoracic and abdominal cavity in swine embryos aged of 50 and 60 days after having used a fixing solution of saline formol a paraffin inclusion and after the serial division into section of the organs.

The sections have been coloured with HE methods, Mallory trichromical method, silver impregnation Gömöri method and cold Giemsa method.

The thymus has no separated limphoblasts in the cortical zones of the thymal lobes and no structured Hassal corpuscles in the medular zone.

The liver has hepatical cells as Remack strings which converge towards the centrolobular vein, but have no clear delimitation of the Kiernan spaces and the perilobular conjunctive tissue. Into the hepatical sinusoids can be found eritroblasts, eritrocites and other figurative elements.

The stomach is formed only of mucosa, muscular and serous. The absence of mucosa muscularis leads to no delimitation between the chorion and the submucosa. The organ have no glandular elements.

The testicle is organised, delimited by albuginea. The seminiferous tubule are delimited well by the mesenchymal conjunctive tissue. Into the stroma the are interstitial cells. Into the seminiferous tubule the supporting cells prevail and the spermatogony are placed on the base near the membrane.

The research on the ontogenetical development of swine embryo often concentrates on the embrionary period up to 45 days old, an extremely important period in terms of creating new reproduction biotechnologies (transfer of embrios).

Most embryology studies deal with swine embryonary development from 6-10 mm up to the age of 45 days (Hill M., 2003; Schoenwolf C.G., 1973).

The fetal development of swine embryo is not presently an usual subject of research in the specific literature, a limited number of articles exist on this topic, while most studies effectuated are in fact electronomicroscopic researches on the microstructure of the organs in course of differentiation.

Therefore, we conclude that the embryonic thymus is colonized by at least two successive waves of hemopoietic progenitors during embryogenesis and that the influx of thymocyte progenitors is discontinuous (Sinkora M. et al., 2000).

The studies effectuated on 45 days old swine embryo have proved the presence of hepatic cell-strings along with sinusoidal capilars charged with embrionary sanguineous figurative elements - hemocitoblasts and erythroblasts included, proving that at this age, the liver performs hematopoietic function (Petruţ T. et al., 2006).

Also, at 45 days-old, the stomach microstructure has an epithelium in course of differentiation with PAS positive granules on its surface and in the structure of gastric epithelium cells (Georgieva R.K., K. Gerov, 1975).

Thus, studies using electron microscopy on swine embryo aged 26-27 days showed that the male gonads present differentiated components (the seminiferous tubules with the support cells and the spermatogoniae), while the interstitial tissue and testicular Leydig cells are not yet present at the this age (Pelliniemi L. J., 1990).

1. MATERIAL AND METHOD

The purpose of this research is the ontogenetic development of the thymus, liver, stomach and testicle in swine embryo 50 and 60 days old from the fecundation. The embryos were picked up from the uteri of the females sacrified by necessity and were classified by length, with special focus on the 7 and 11 cm length embryos (fig. 1, 2). This length corresponds to the age of 50 and 60 days of intrauterin development, at the limit of embrionary and fetal development.

Fig. 1 - Swine embryo-50 days

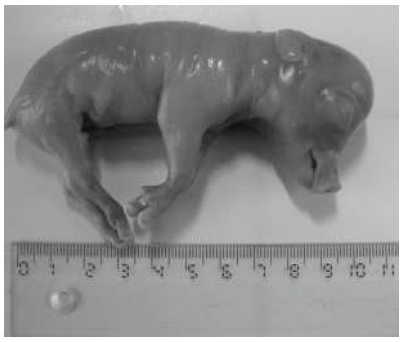

Fig. 2 - Swine embryo-60 days

The histological pieces collected were selected by dissection and fixed in saline neutral formol, being processed later for paraffin inclusion. The paraffin blocks were cut to 6 microns and coloured by the

HE methods, Mallory trichromical, silver impregnation Gömöri and Giemsa cold.

2. RESULTS AND DISCUSSION

The thymus - the thymal lobules are incompletely delimitated by mesenchymal conjunctive tissue (fig. 3).

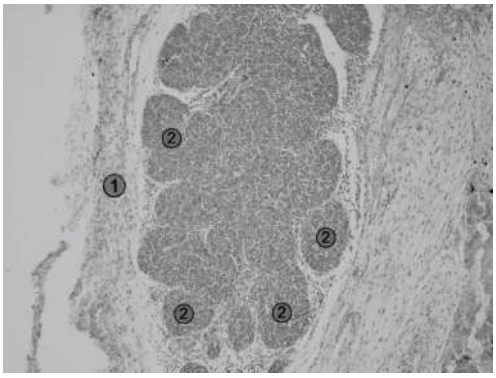

Fig. 3 - Swine embryo-50 days; section by the thymus; Col. HE; Ob. 10x
1. capsula; 2. timic lobules .

The lobules are formed of stroma and parenchyma. The parenchyma is not organized in the cortical and medular zones.Te lymphoblasts lies on the entire area of the lobe. The conjunctive capsula in course of cellular differentiation lies at the periphery. The epithelloid cells are concentrated in the central zones, without forming the Hassal corpuscles. Most of the thymal lobe's population is represented by the lymphoblasts (fig. 4).

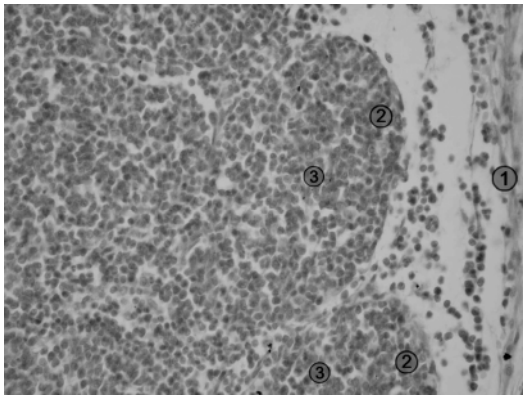

Fig. 4 - Swine embryo-60 days; section by the thymus; Col. HE; Ob. 40x
1. capsula; 2. cortical zones; 3. medular zones.

The liver - in its structure one can notice the presence of the anastomosed (Remack) hepatocytes strings, with the sinusoidal capillaries in between (fig.5).

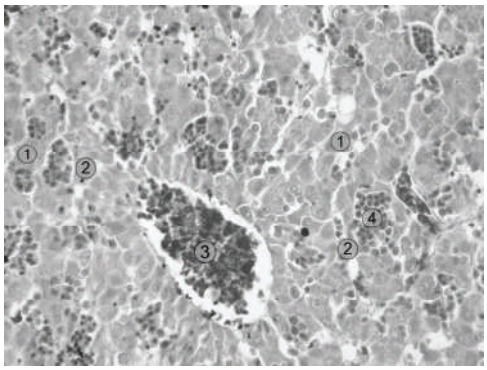

Fig. 5 - Swine embryo 50 days; section by the liver;
Col. Mallory trichromical; Ob. 40x
1. hepatocyte strings; 2. vascular sinuses; 3. the centrolobular vein;
4. undifferentiated sanguineous figurative elements.

Among the hepatocytes strings there are undifferentiated hematopoetic cells in an insular for (fig.6).

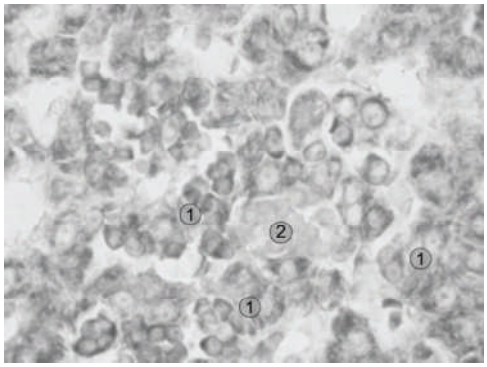

Fig. 6 - Swine embryo 60 days; section by the liver; Col.
Cold Giemsa; Ob. 100x hepatocyte strings; 2. insula
of undifferentiated sanguineous cells.

The hepatical lobules appear undifferentiated as the interlobular conjunctive openings delimitating the lobules and the interlobular Kiernan spaces which are specific for swine.

The stomach has a little differentiated gastric mucosa, with no muscularis mucosa. Thus, with no structural limit between mucosa and submucosa. The stomach has 3 tunics: mucosa, submucosa and serous (fig. 7).

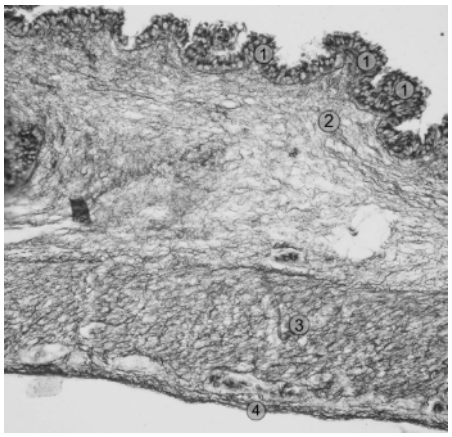

Fig. 7 - Swine embryo 50 days; section by the stomach;
Silver impregnation Gömöri; Ob. 20x
1. epithelium of gastric mucosa; 2. chorion; 3. musculous; 4. serous.

The gastric mucosa has stomachal folds that will later become conivent. The stomachal covered by a simple prismatic epithelium with agglomeration of mucin at its apical pole (fig. 8).

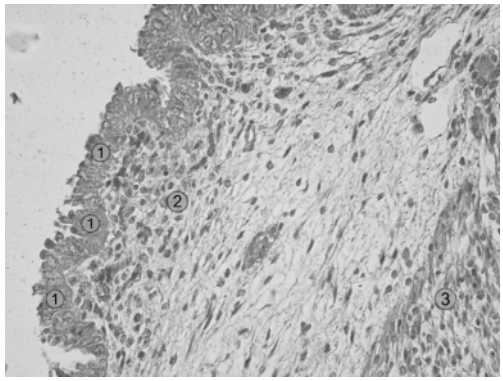

Fig. 8 – Swine embryo 60 days; section by the stomach;
Col. Mallory trichromical; Ob. 40x
1. epithelium of gastric mucosa; 2. chorion; 3. musculous.

The testicle is well organized being externally delimitated by albuginea rich in collagen fibres and populated with sanguineous

vessels (fig. 9).The testicular parenchyma has many seminiferous tubes well delimitated by the mesenchymal conjunctive tissue and the testicular lobulation is not obvious.

In the seminiferous tubes the supporting (Sertoli) cells are predominant and the spermatogonies can be noticed being disposed basally by the membrane.

In the testicular stroma there are the interstitial Leydig cells between the seminiferous tubes along with a net rich in sinusoidal capillaries.

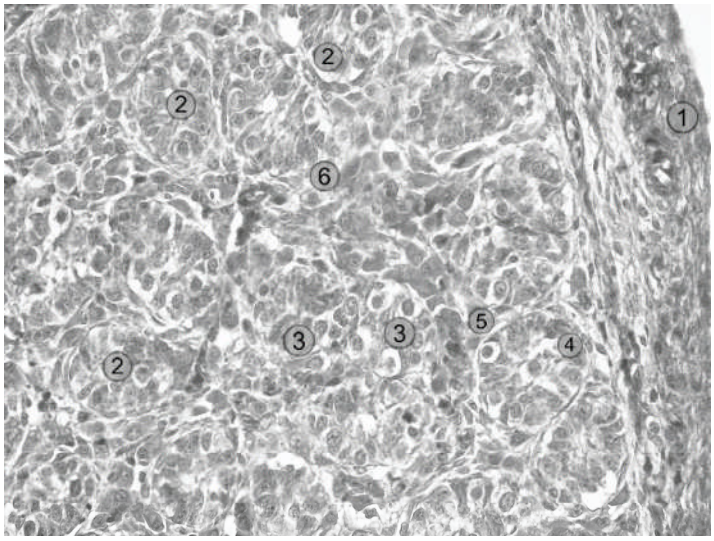

Fig. 9 - Swine embryo 60 days; section through the testicle;
Col. Mallory trichromical;Ob. 40x
1. testicular albuguinea; 2. seminiferous tubes; 3. germinative cells; 4. supporting Sertoli cells; 5. Leydig insulae; 6. testicular stroma.

3. CONCLUSIONS

3.1. The thymus has no separated limphoblasts in the cortical zones of the thymal lobes and no structured Hassal corpuscles in the medular zone.

3.2. The liver has no obvious perilobular conjunctive septae. The bile port spaces or Kiernan spaces are still not constituted. There are agglomerations of undifferentiated sanguineous figurative elements in the vascular sinuses. The Remack strings are present and the hepatocytes appear differentiated.

3.3. The stomach's as well as the intestine's tunics are in course of organization. The submucosa is not delimitated by the chorion through the musculous mucosa. The stomach has no gastric glands.

3.4. The testicle is delimitated by albuginea in course of organization. The seminiferous tubes are organized and the Sertoli cells along with the spermatogonies/spermatocytes can be noticed. The Leydig insulae are in process of organization in the testicular stroma.

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HISTOLOGICAL RESEARCHES REGARDING THE VENTRICLE AT THE PHASIANUS PHASIANUS SPECIES

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Key words: pheseant, gizzard,ventriculus, histostructure

SUMMARY

In the specialty literature the information regarding the histostructure of the common pheasant's ventricle are extremely rare, most of the dates are regarding the Gallus domesticus species.
The pheasant ventricle shows histostructural particularities witch stands at the basics of the physiological or pathological matters witch are characterizing this species.
The cuticle has a laminar organization on two lairs. The slides from the shallow lair are orientated parallel to the muscle membrane and those from the deeper lair are orientated perpendicular, entering into the muscle membrane's glands.
The muscle membrane shows a simple, prismatic epithelium with high cells and oval nucleus situated at the basics with the long axle orientated perpendicular to the basal membrane. The surface epithelium continues with another epithelium witch delimitates the lumen of the simple, tubular glands.
The ventricle (the muscular stomach) has a mucous membrane with no muscles and in the lamina propria are present many simple tubular glands witch are almost occupying it.
The ventricle's muscularis externa , witch is neat , has it's maximum thickness at the level of the organ's outer surface. Between muscle fibers we can see straps of connective tissue witch can be filled with fat .The lymphoid cells are in small numbers. The muscle fibers are disposed in 3 lairs, not very well delimitated: an external thin lair, longitudinal orientated a massive circular lair in the middle and an internal oblique lair. Between the external and the middle lair we may see a nervous plexus very well represented.
The muscle fibers are in physical continuity with a lair of dense connective tissue, existing a gradual passing from the muscle to the collagen fibers.
The lymphoid cells are in small numbers in the ventricle's structure, being placed in the sub peritoneal connective tissue.

In the specialty literature the information regarding the histostructure of the common pheasant's ventricle are extremely rare, most of the dates are regarding the Gallus domesticus species.

At the birds we can find two gastric formations: the glandular stomach or the proventriculus and the muscular stomach or the ventricle.

The ventricle is a big organ, with muscular lairs very well developed. It has an almost spherical form, easily flat on sides, with two sides and a circumference. It is placed in the back –ventral region of the abdominal cavity, being placed partially between the liver's lobs. It

communicates with the pro ventricle thru the proventriculus junction and with the duodenum thru the ventricle-duodenum orifice.

At the exterior it is covered by the visceral peritoneum, under witch we can find a dense connective lair, with aponevrosis aspect and its maximal thickness in the centre of its sides.

MATERIAL AND THE METHOD

The researches have followed the histological study of the pheasant's thin intestine, four months old. The study was made on a number of 5 subjects, experimentally sacrificed originated from a stock farm from Prahova district.

The pieces were harvested from the thin intestine, put in neutral formol, and remade to be included in paraffin.

The paraffin blocks were sectioned at 6 microns and colored through hematoxilin eosin and Giemsa method. The results were examined with a Nikon microscope, pictured and worked by computer.

RESULTS AND DISCUTIONS

The ventricle or the muscular stomach shows with the luminal surface covered with a cuticle, a dense secretion, produced by activating the mucous membrane's glands. The cuticle has a laminar organization on two lairs. The slides from the shallow lair are orientated parallel to the muscle membrane and those from the deeper lair are orientated perpendicular, entering into the muscle membrane's glands. The surface epithelium continues with another epithelium witch delimitates the lumen of the simple, tubular glands. The gland's cells are of three types: basal cells, located at the bottom of the glands, main cells disposed on the whole length of the glands; gastric endocrine cells in small numbers and sizes.

Its own lamina or the *lamina propria* of the ventricular mucous membrane shows almost occupied by the ventricle's glands. These glands have a rectilinear line and are disposed in groups, delimitated by connective tissue. We didn't see any mucous membrane muscle showing only a fine conjunctive condensation. We didn't noticed elements of the sub mucous plexus.

Muscularis externa of the ventricle, of neat type, reaches a maximum thickness at the level of the circumference of the organ, being thinner in the center of the sides where the muscular fibers are continuing with the collagen fibers witch are forming an aponevrosis in

the center of each side. Between muscle fibers we can see straps of connective tissue witch can be filled with fat. The lymphoid cells are in small numbers. The lymphoid cells are in small numbers. The muscle fibers are disposed in 3 lairs, not very well delimitated: an external thin lair, longitudinal orientated a massive circular lair in the middle and an internal oblique lair. Between the external and the middle lair we may see a nervous plexus, very well represented. The muscle fibers are in physical continuity with a lair of dense connective tissue, existing a gradual passing from the muscle to the collagen fibers. For a growing adhesion between the muscularis lairs, there are areas of connective densification, especially in the aponevrosis area.

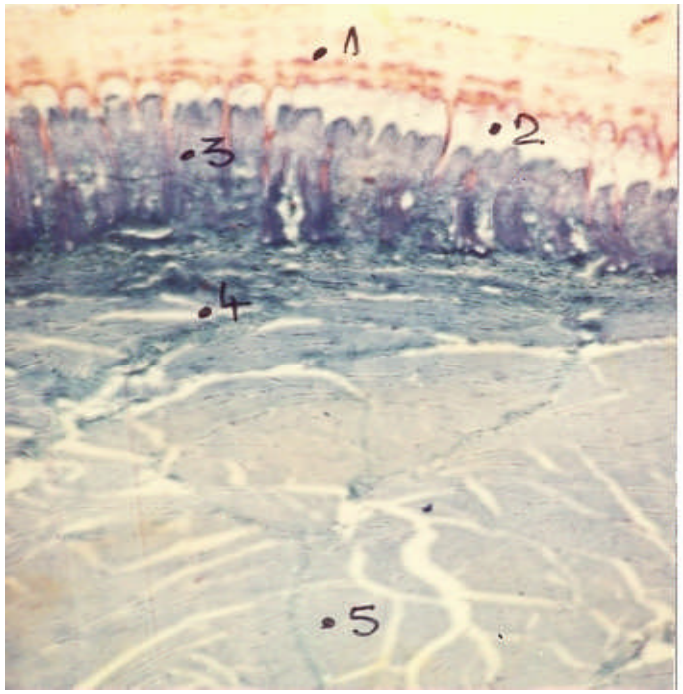

Fig. 1. Pheaseant- 4months, Ventricle Col. HE; Ob.10x
1 -Cuticle; 2 – simple, prismatic epithelium; 3 – simple tubular glands; 4 – lamina propria; 5 – muscularis externa

On the outer surface, the ventricle is covered with the mezothelial tissue of the visceral peritoneum under witch we can find a fiber lair mixed tendinous and aponevrosis disposeed in two simetrical areas twoards the each face of the organ.

The fiber lair reaches its maximum thickness in the middle. The tendinous lair is made of connective tissue with fascicles of collagen fibers, eith elonged or flat fibroblasts between the fascicles.

The peritoneum serous is very thin; at its level can be seen lymphoid infiltrations around blood vessels or lymphoid cells thru the collagen fibers.

CONCLUSIONS

The researches allowed us to come to the next conclusions:

The pheasant ventricle shows histostructural particularities witch stands at the basics of the physiological or pathological matters witch are characterizing this species.

The ventricle (the muscular stomach) has a mucous membrane with no muscles and in the *lamina propria* are present many simple tubular glands witch are almost occupying it.

The lymphoid cells are in small numbers in the ventricle's structure, being placed in the sub peritoneal connective tissue.

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HISTOLOGICAL RESEARCES REGARDING THE THIN INTESTINE AT THE PHASIANUS PHASIANUS SPECIES

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Key words: pheasant, intestine, histostructure

SUMMARY

The histostructure of the thin intestine's wall, at the pheasant, doesn't show big differences between the duodenum, the jejunum, and the ileum.
The intestinal wrinkles and villosityes are found on the whole surface of the intestinal mucous membrane. In the duodenum and in the jejunum we can see more villosityes that are very long, on the 2/3 of the thickness of the mucous membrane.
The intestinal glands (the Lieberkühn crypts) are short, little flexible and are occupying the volume, from the basics of the villosityes and the mucous membrane's muscle. Between the glands it is a little quantity of lax conjunctive tissue with blood and lymphatic vessels , nerves and lymphoid cells witch are disposed diffuse or in lymphatic nodules . This tissue accedes in the villosityes axle.
In the ileum mucous membrane is present the Peyer plates. At their level the ileum doesn't show villosityes but a reduced prominence – domul, covered with a simple, prismatic epithelium.
Sub mucous membrane is reduced being in a very thin lair of conjunctive tissue.
The muscle contains neat muscular fibers disposed in a circular internal lair and a thicker external longitudinal one. In the two lairs we can find conjunctive tissue with the nervous plexus mienteric.
At the surface of the intestine we can find the mezothelium of the visceral peritoneum subseros conjunctive tissue with vessels and nerves.

The general organization plan of the thin intestine is common for all the vertebrates including the four tunics: mucous membrane, sub mucus membrane, muscular and subserous. At the birds the thin intestine is a tubular organ with constant lumen and thin transparent wall without longitudinal taenia.

At the birds, the mucous of the thin intestine shows many long villosityes witch are constantly growing the surface of absorption of the main nutrients resulted due to the digestion. They are disposed on the whole length of the thin intestine into the duodenum, jejunum and ileum, from the ventricle duodenal orifice up to the ileo-ceco-colic orifice.

The intestinal glands or *the Lieberkühn crypts are* opening between the villosityes , are showing a tubular aspect and is occupying the

biggest part of the corrionus between the basics of the villosityes and the muscle of the mucous membrane. Sub mucous membrane is reduced and it is formed from a thin lair of conjunctive tissue.

The muscle contains neat muscular fibers disposed in a circular internal lair and a thicker external longitudinal one. In the two lairs we can find conjunctive tissue with the nervous plexus mienteric.

At the surface of the intestine we can find the mezothelium of the visceral peritoneum subseros conjunctive tissue with vessels and nerves.

MATERIAL AND THE METHOD

The researches have followed the histological study of the pheasant's thin intestine, four months old. The study was made on a number of 5 subjects, experimentally sacrificed originated from a stock farm from Prahova district.

The pieces were harvested from the thin intestine, put in neutral formol, and remade to be included in paraffin.

The paraffin blocks were sectioned at 6 microns and colored through hematoxilin eosin and Giemsa method. The results were examined with a Nikon microscope, pictured and worked by computer.

2. RESULTS AND DISSCUTIONS

The histostructure of the thin intestine, at the pheasant, doesn't show big differences between the duodenum, the jejunum, and the ileum.

The intestinal wrinkles and villosityes are found on the whole surface of the intestinal mucous membrane. In the duodenum and in the jejunum we can see more villosityes that are very long, on the 2/3 of the thickness of the mucous membrane.

The intestinal glands *(the Lieberkühn crypts)* are short, little flexible and are occupying the volume, from the basics of the villosityes and the mucous membrane's muscle. Between the glands it is a little quantity of lax conjunctive tissue with blood and lymphatic vessels , nerves and lymphoid cells witch are disposed diffuse or in lymphatic nodules . This tissue accedes inside of the villosityes.

The epithelium of the mucous membrane is simple, prismatic, with a striated plate, made of the principal cells – entrocite, calceiform, endocrine. Parts of the cells are present at the basics of the crypts.

The calceiform cells are disposed between the entrocite, being more numerous in the glands than on the surface of the velocityes. The

endocrine cells are placed at the bottom of the glands, have a pyramid shape, with the base on the basic membrane. The existence of the Paneth cells is not certain and the subject can be discussed. The leucocytes were seen between the epithelium cells and also in the glands.

In the velocityes corionului, witch have an conjunctive tissue aspect we can see blood vessels , obvious neat muscular fibers, and many lymphatic cells . We did not notice the existence of the central chyliferous. The lymphoid cells are numerous in the neighborhood of the ventricle duodenal junction, where they may form small nodes.

In the caudal part of the jejunum and in the ileum it may be seen unequal villosities, some thinner, some thicker with the villosity axel strongly infiltrated with lymphocytes and with neat fibers of the mucous membrane's muscle.

In the ileum mucous membrane is present the Peyer plates. At their level the ileum doesn't show villosityes but a reduced prominence – domul, covered with a simple, prismatic epithelium.

The plates are distinguished in the intestinal epithelium by the absence of the calciforme cells and by the neighborhood of some dilated villosityes by the presence of the germinative centers and the diffused lymphoid tissue.

The Peyer plates are known to have the part of peripherical lymphoid organs , some authors puts them in the category of the primal lymphoid organs by proliferation and differentiation of the B lymphocytes at this level.

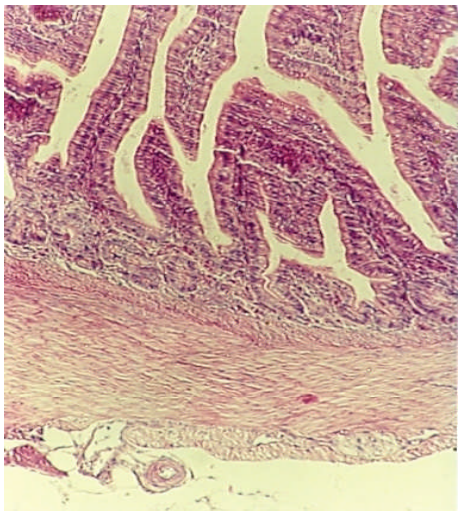


Fig. 1 – Pheasant 4months, jejunum, duodenum col HE ob.10x

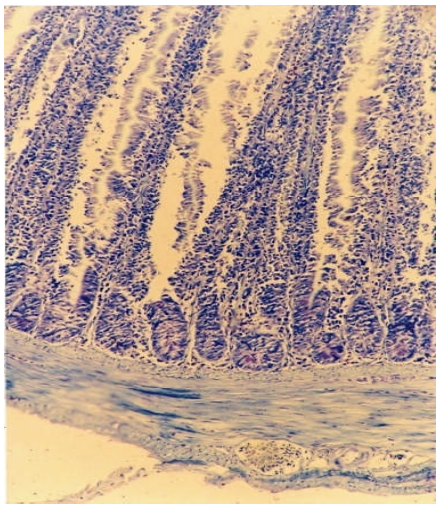
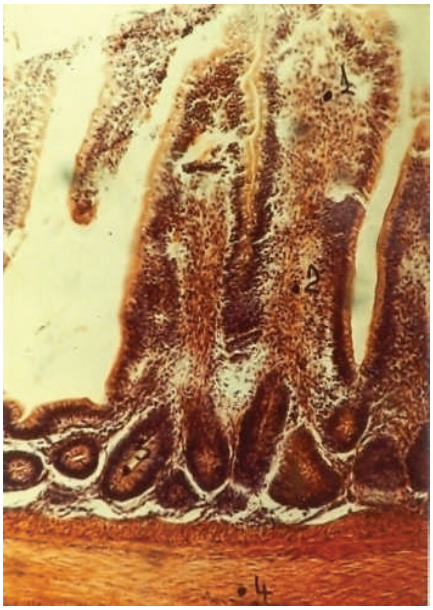


Fig.2 – Pheasant – 4 months, Col. HE ob 10 x

1 – villosity; 2 – muscularis mucosae; 1 – villosity; 2 – muscularis mucosae;

3 –Lieberkuhn glands; 4- musculoasa; 3 –Lieberkuhn glands; 4- musculoasa

The Peyer plates are lymphocyte aggregates covered by an epithelium with micro wrinkles, are containing lymphatic nodules with a sub epithelium area with B lymphocytes and a central area with T lymphocytes.



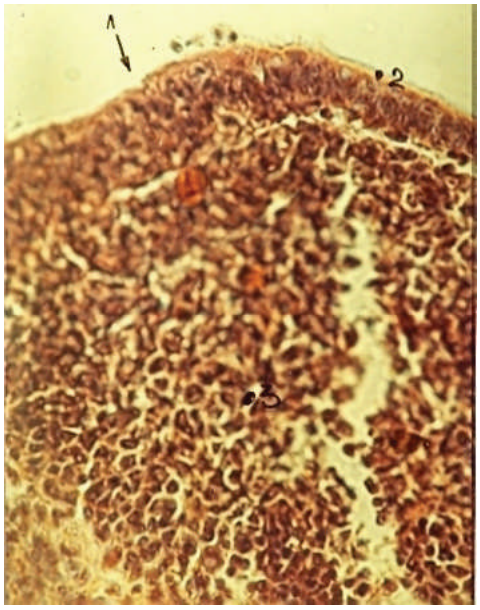


Fig.3 – Pheasant – 4 months, ileum , Col HE, ob 10 x
1 – villosity; 2 – muscularis mucosae;
3 –Lieberkuhn glands; 4- musculoasa;

Fig. 4 – – Pheasant – 4 months, Col HE, ob 10 x;
1- domul; 2- surface epithelium;
3 - folicul limfoid subepitelial;

CONCLUSIONS

The researches allowed us to come to the next conclusions:

1. The histostructure of the thin intestine's wall, at the pheasant, doesn't show big differences between the duodenum, the jejunum, and the ileum.

2. The intestinal wrinkles and villosityes are found on the whole surface of the intestinal mucous membrane. In the duodenum and in the

jejunum we can see more villosityes that are very long, on the 2/3 of the thickness of the mucous membrane

3. The intestinal glands *(the Lieberkühn crypts)* are short, little flexible and are occupying the volume, from the basics of the villosityes and the mucous membrane's muscle.

4. Between the glands it is a little quantity of lax conjunctive tissue with blood and lymphatic vessels , nerves and lymphoid cells witch are disposed diffuse or in lymphatic nodules .

5. In the ileum mucous membrane is present the Peyer plates. At their level the ileum doesn't show villosityes but a reduced prominence – domul, covered with a simple, prismatic epithelium.

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HISTOLOGICAL REACTIVITY OF DIGESTIVE MUCOSAE ASSOCIATED LYMPHOID TISSUE IN OCHRATOXICOSIS OF BROILER CHICKENS

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Keywords: ochratoxine A, immunotoxicity, chickens, mucosae associated lymphoid tissue.

SUMMARY

In 3 groups, each of 15 broiler chickens ochratoxine A (OTA) was given orally, in sunflower oil suspension, daily, for 21 days in doses of: 5µg/kg b.w. for group E1, 35 µg/kg for group E2 and 100µg/kg for E3. Control group (of 15 chickens) received only sunflower oil. 5 chickens from each group were killed after 7, 14 and 21st day of the experiment. Gut mucosa samples were prevealed from duodenum, jejunum and coecal tonsils. All samples were prepared for paraphin embeding and stained with: HEA, PAS, PAS and Alcian blue and May Grunwald Giemsa and lymphoid associated tissue was examined.

In duodenum, the cryptae from lamina propria showed numerous immature young cells, with nucleus rich in eucromatine, with 3-4 nucleoli. The enterocytes had a large nucleus rich in euchromatine, with 3-4 nucleoli. Many goblet cells were observed into duodenal mucosa. Progressively with the age and OTA dosis the lipidic droplets from the axis of the villi and proportion of degenerated enterocytes are increasing and simple epithelium is replaced by stratified epithelium, with many immature cells. The coecal tonsils in E2 group, after 14 days of OTA poisoning showed lamina propria populated by small lymph cells. Some of the lymph cells show nuclear lesions like: cariorhexis, cariolysis and other show apoptotic aspects. Into the lymphoid agglomerations some cells with nuclei rich in eucromatina, with 4-5 nucleoli were observed.

Ochratoxines, metabolites of some strains of *Aspergillus spp.* and *Penicillium spp.,* have a well known nephrotoxic and hepatotoxic effect, but also a immunosuppressive potential (2, 4, 11). The goal of this study was to demonstrate the immunotoxic effect of ochratoxine A on mucosae associated lymphoid tissue in broiler chickens.

1. MATERIALS AND METHODS

In 3 groups, each of 15 broiler chickens ochratoxine A (OTA) was given orally, in sunflower oil suspension, daily, for 21 days in doses of: 5µg/kg b.w. for group E1, 35 µg/kg for group E2 and 100µg/kg for E3. Control group (of 15 chickens) received only sunflower oil. Both control and experimental groups received identical comercial feed and

water ad libitum and were kept in identical environmental conditions. 5 chickens from each group were killed after 7, 14 and 21st day of the experiment. Gut mucosa samples were prevealed from duodenum, jejunum and coecal tonsils. All samples were prepared for paraphin embeding and stained with: HEA, PAS, PAS and Alcian blue and May Grunwald Giemsa and lymphoid associated tissue was examined microscopically.

2. RESULTS AND DISCUSSIONS

In control group, duodenum of chickens, 7 days old showed high vili and short criptae. E3 group showed lesions of the duodenum mucosa represented by disruption of intercellular jonctions, degeneration of basal membrane and sometimes of enterocytes, lipidic droplets in lamina propria, numerous intracapillary and extravased erythrocytes (fig. 1, 2). The cryptae from lamina propria showed numerous immature cells, with nucleus rich in eucromatine, with 3-4 nucleoli (fig. 3). Those imature cells were observed both in lamina propria of the mucosa and interglandulary and into the axis of intestinal villi. The enterocytes had a large nucleus rich in euchromatine, with 3-4 nucleoli. Many goblet cells were observed into duodenal mucosa.

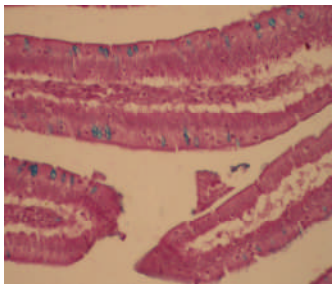

Fig.1. Duodenum. E3 group, 7th day of poisoning. High villi, disrupted intercellular jonctions, degenerated basal membrane, lipidic droplets into the lamina propria. PAS-Alcian blue x100.

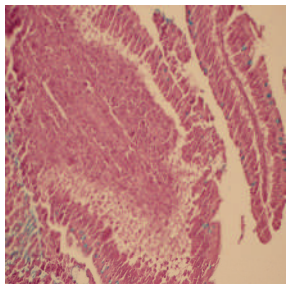

Fig.2. Duodenum E2 group 14th day of poisoning. Degenerated enterocytes, lipidic droplets into lamina propria. Numerous intracapillary and extravased erythrocytes. PAS-Alcian blue x100

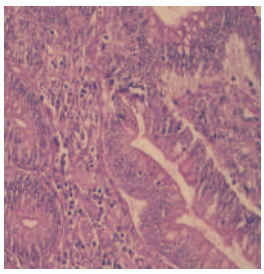

Fig.3. Duodenum. E2 group, 21st day of poisoning. Chryptae of lamina propria; numerous stratified immature cells. M. G. Giemsa x200.

The same lesions of duodenal mucosa were observed in chickens from E2 and E3 groups. Progressively with the age and OTA dosis the

lipidic droplets from the axis of the villi and proportion of degenerated enterocytes are increasing and simple epithelium is replaced by stratified epithelium, with many immature cells. Jejunum in E2 and E3 group both at 7th, 14th and 21st day of the experiment shows quite similar lesions as duodenum.

Coecal tonsils in control group, 7 days old, shows a folded mucosa, with high villi alternating with short ones. The axis of the villi contains conjonctiv tissue with few lymph cells and macrophages. In E1 and E2 group the mucosa resemble with those from control group. In E3 group (receiving 100µg OTA/kg b.w.) the coecal tonsils have into the lumen, a small lymphoid population, epithelial cells are migrating to the apex of the vill; heterophils, few eosinophils, lymph cells and macrophages were also observed. Apposite to enterocytes many goblet cells were observed. Exfoliated cells were replaced by immature round-ovalary cells generated into the cryptae of lamina propria.

In E2 group, after 14 days of OTA poisoning coecal tonsils showed a lamina propria populated by small lymph cells. Some of the lymph cells showed nuclear lesions like: cariorhexis, cariolysis and other showed apoptotic aspects (fig. 4). Into the lymphoid agglomerations some cells with nuclei rich in euchromatine, with 4-5 nucleoli were observed (fig 5). Those immature cells resemble with those from the intestine. Lymphoepithelial agglomerations are covered by enterocytes. The cryptae from lamina propria are high and rare, disposed betwen the lymphoepithelial agglomerations.

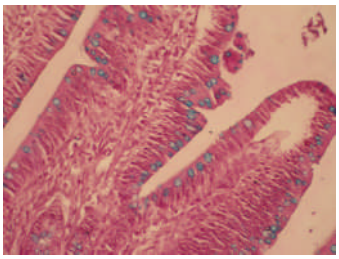

Fig.4. Coecal tonsils, E3 group, 7days. Small lymphoid population. Epithelial cells migrating to the apex of the villi and exfoliating.PAS-Alcian blue x100

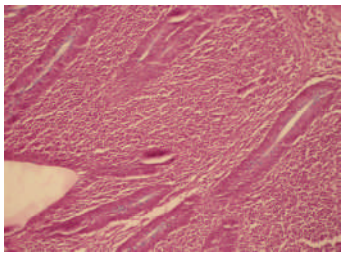

Fig.5. Coecal tonsils, E2 group, 14 days. Lamina propria populalated with small lymph cell s showing cariorhexis, cariolysis or apoptotic lesions.-x100

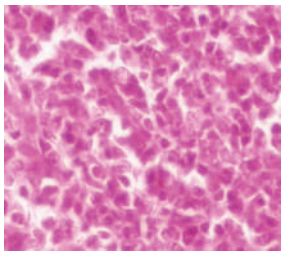

Fig. 6. Coecal tonsils, E2 group, 14 days. Into lymphoid agglomerations may be observed cells with nucleus rich in euchromatin, with 4-5 nucleoli. x400

In coecal saci lesions of mucosa associated lymphoid tissue were observed after 7th day of poisoning in E3 group and after 14 th day in E2 group. In E3 group after 7th day of poisoning lymhoid tissue is diffuse into lamina propria (Fig. 7). Lymph cells are both immature and mature with apoptotic lesions. The cryptae from lamina propria have many immature cells disposed in layeres (fig.8). Similar lesions were observed in E2 and E3 group at 14 th and 21st day of poisoning.

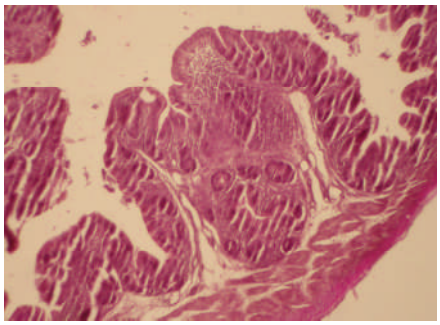

Fig.7. Cecum, E3 group, 7days. Reduced lymphoid tissue into lamina propria of the mucosa. PAS x100.

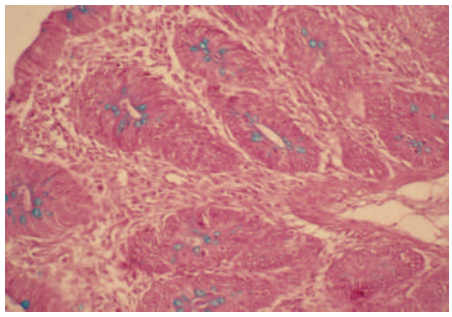

Fig.8. Coecal sac, E2 group 21 days. The cryptae from lamina propria from lamina propria have many immature cells, disposed in layers. x100

The reduction of the lymphoid population from intestinal mucosae can be explained by immunosupresive effect of OTA. OTA induces lesions, even distructions of cell membrane of the enterocytes by peroxidation of lipids induced by free radicals. Permeability of some cells increase, the brush border function is severely affected and some cells are dying. Free radicals are very reactive also inside the cells, destroying the DNA, enzymes and proteins. Kamp et all. (2005) demonstrated that OTA induces DNA degeneration, cytotoxicity and apoptosis of V79. CV1 line of kidney cells, by oxydative strees. OTA also retarded the differentation of Tcells (11).

Into the gut mycotoxins alter the functional barrier by reduction of the integrity of the epithelium, by alteration of proteins from closed jonctions. Mycotoxins are determining apoptosis, increasing of pathogens colonization, oxydative stress, cytotoxicity, inhibition of protein synthesis and lipid peroxidation of gut mucosa epithelium (4). Immunosupresive effect of OTA on intestinal mucosa increase the susceptibility to coccidia and other pathogens. OTA also increase the fragility of the intestinal mucosa in chickens by reduction of colagen synthesis (6). OTA immunosupresive action were also observed in thymus and Fabricius bursa decreasing both humoral and celular

immune response, increasing the susceptibility to bacterial, viral and parasitic diseases (8).

3. CONCLUSIONS

1. In duodenum, the cryptae from lamina propria showed numerous immature young cells, with nucleus rich in eucromatine, with 3-4 nucleoli. The enterocytes had a large nucleus rich in euchromatine, with 3-4 nucleoli. Many goblet cells were observed into duodenal mucosa. Progressively with the age and OTA dosis the lipidic droplets from the axis of the villi and proportion of degenerated enterocytes are increasing and simple epithelium is replaced by stratified epithelium, with many immature cells.

2. The coecal tonsils in E2 group, after 14 days of OTA poisoning showed lamina propria populated by small lymph cells. Some of the lymph cells show nuclear lesions like: cariorhexis, cariolysis and other show apoptotic aspects. Into the lymphoid agglomerations some cells with nuclei rich in eucromatina, with 4-5 nucleoli were observed.

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APPLICATIONS OF THE HISTOMETRIC METHOD ASSISTED BY THE COMPUTER IN THE AVIAN DIGESTIVE TUBE INVESTIGATION

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4) Alltech Biotechnology, SUA

Key words: histometry, small intestine, Bio-Mos, bird.

SUMMARY

The objective of this research consisted in the use of a histometric method by analysis of computerized image on the epithelium of the small intestine mucosa in broiler chickens for the testing of the prebiotical effect of mannanoligosaccharides (Bio-Mos, Alltech). There were made assessments about the significance of the differences between the avian groups for four parameters (height of villi, the width of villi, the number of goblets and the depth of the crypts) in fragments sampled from two areas of the ileum. The obtained values were statistical processed. The results comparison of the two types of samples (A and B) revealed significant differences on the number of goblets ($p < 0,001$), the height of the villi ($p < 0,01$) and the width of the villi ($p < 0,05$). The analysis of the obtained results between the animal groups was not conclusive because the comparison group was not known. The research conducted established the morphometric diagnostic method on the intestinal mucosa in birds, being the first paper on histometry application in veterinary medicine in Romania..

Morphometry (MM) uses methods derived from histology for the identification of the effects of some toxins, drugs, stimulant preparations and the establishment of some values of the cellular parameters in malignant and benign tumors. The MM method analyzes from the quantitative point of view different tissues and cells.

Traditional, the histologists rely on a verbal description of the tissue structure, sometimes approaching a mathematical description.

In recent years, at the international level, the measurement of the tissues microscopic images, known as histometry (HM) was used increasingly both in research and diagnostic. The histometry methods represent a wide extension of those borrowed from geology and metallurgy.

An important part of the HM technique is that it allows 3D measurements which derived from the 2D microscopic images provided

by histological sections. Some of the methods are based on complex mathematical calculations.

The development of the computer equipments, entered in the management systems of the cytopathology and histopathology laboratories, became progressively since 1990 increasingly common in industrialized countries (Morenes et al., 1992; Anderson şi Lowe, 1992; Barteles et al., 1992)

HM by computerized image is a height tech of histology, representing a relatively young field for the veterinary medicine, its impact gradually evolving in the cancer, immunopathologic and endocrine pathology, given by the height level of specificity that it offers. For example HM is more sensitive and specific than the HE or Giemsa stain for the histologic analysis of the subtypes of follicular lymphoma or for the establishment of villi height and width, the number of goblets and the depth of the crypts from the small intestine (Preston et al., 1990; Radecki et al., 1992; Bradley et al., 1994)

Internationally the HM technics are currently possible in the lab diagnosis of tumors in humans. In animals this method is used in the diagnosis of tumors and the research for the action establishment of the pre- and probiotic and immunomodulatory preparations.

The gastrointestinal tract (GI) is permanently invaded by foreign substances some of them harmful and the *lamina propria mucosae* is a place for proliferation both beneficial and disadvantageous of the bacterial flora (Mitjans et al., 1998; Adeola şi King, 2006)

The correct measurement of the absorption rate in the microvilli (brush edges) on the surface of the enterocytes and the measurement of the intestinal villi for different nutrients in mammals, birds and reptiles is important and effective in numerous research projects in terms of the digestive, ecological, nutritional function and the functional plasticity of the bowel (Starck et al., 2000; Karasov şi Diamond, 1983; Garcia et al., 2006; Dibner et al., 1996; Strong et al., 2005).

This work presents aspects of morphometry by analysis of computerized image on the epithelium of the small intestine in broiler chickens treated with mannanoligosaccharides (Bio-Mos, Alltech) for the testing of the prebiotic qualities of the product.

There were determined the number of goblets, the height and the width of the villi and also the depth of the crypts.

The researchers of the early 90 have found a component of yeast cell wall – the mannanoligosaccharidic protein – phosphorylated which is an effective alternative to antibiotic growth promoters. The oligosaccharides from yeast in which mannose is the primary

carbohydrate (MOS), proved active in the digestive tract in several animal species (Spring şi Pîrvulescu, 1998; Considine şi Spring, 2000; Spring et al., 2000; Spring, 2002, Kocher, 2006). This prevents pathogens to colonize the digestive tract and prepare and modulate the immune system of animals giving the opportunity to respond quickly to infection (Spring şi Pîrvulescu, 1998; Considine şi Spring, 2000; Adeola şi King, 2006).

The research has been aimed at the development of modern diagnostic methods and testing by histological and morphometric techniques by the analysis of computerized image applied to the epithelium and lymphoid structures associated to the mucosa (*mucosal associated lymphoid tissue* –MALT) in birds.

1. MATERIAL AND METHOD

Intestinal morphometric examinations were conducted at 40 broiler chicken, in the age of 37 days treated with an extract prepared from the outer cell wall of *Saccharomyces cerevisiae* (Bio-Mos, Alltech). 40 fragments were taken with *Meckel diverticulum* (Md) A and 40 fragments with the cranial portion of ileum 30 mm from the ileo-caecal junction (B).

The experiments were carried out at the Animal Nutrition Institute in Zurich, Switzerland, under a contract research on four lots of birds (the lots I, II, III, IV) and in each group were allocated 10 animals each. After slaughter were taken by two pieces of small intestine from all broilers and all experimental lots. The fragments were fixed in 10% neutral buffered formaldehyde solution and sent to the Pasteur Institute in Bucharest, Romania for histometric processing.

They have not submitted information regarding the treatment schedule of broilers treated with the Bio-Mos preparation and was not known how were choosen the lots.

The histological technique and the investigated parameters were similar to those used (by classical method) in works by Bradley et al., 1994 with some modifications which we present in the lines below.

- The pieces inclusion has been made in Paraplast plus (Sigma);
- The staining of the sections has made by the periodic acid-Schiff (PAS)- alcian blue (AB) reaction, pH 2,5 (Serva).
- The measurements were performed under a computer-assisted microscope.

Following the histological fixation (10% neutral buffered formaldehyde) the pieces were treated with alcohol and dehydrated in

toluene in the Citadel device 1000 – Shandom (2000), and the inclusion has been made in Histocentre 2 device – Shandon (2000).

The histological preparations were examined under the light microscope Nikon (Labophot type) fitted with a Sony color video camera (CCD-IRIS/RGB) which processed images and transmitted them to a computer equipped with software Lucia M 3.00b/2001 image processing.

This program allows measurement with an accuracy of up to 1/1000 μm of the size of various cells by transforming pixels in μm.

Before the start of the measurements the objects standardisation was performed. With the aid of a micrometer object it has been determined the number of pixels corresponding to a micrometer for the 4, 6, 10, 20, 40 objects, and the computer was programmed to read directly in the micrometer size. After the mathematical calculations, the values obtained were converted from μm in mm.

The establishment of the number of goblets, of the height and width of the villi and also the depth of the crypts were determined on 9 villi from each sample as shown in the Bradley şi col., 1994) method, to mention that reading the samples was performed with a computer-assisted microscope. For the 80 samples of small intestine there were performed 2880 measurements.

The values obtained were statistically processed in the sense that were calculated for each parameter arithmetic average ($\overline{X}$), its standard error

(E.S. $\overline{X}$) and the coefficient of variability (CV% /lot) Wardlaw, 1993.

There were made assessments about the significance of the differences between the avian groups for each parameter by the "t" test (student). It was also calculated the correlation coefficient (r) between the height of villi and the number of goblets/mm.

The research conducted established the morphometric diagnostic method on the intestinal mucosa in birds, being the first paper on HM application in veterinary medicine in Romania.

2. RESULTS AND DISCUSSIONS

The data obtained were synthesized as both individual and group mean values for the samples collected from the M. d. area (A) and for those collected from the ileo-caecal junction (B).

I. The synthesis of the results provided by the measurements performed on broiler A batch of samples are presented in Table 1 and Chart 1.

Table 1

Values obtained in batches of A samples

Groups		Goblets (no./mm)	Villi height (mm)	Villi width (mm)	Crypta depth (mm)
I	$\overline{\overline{X}}$	52 ± 10.2	1.015 ± .159	.079 ± .009	.097 ± .024
	SE$\overline{\overline{X}}$	3.2	.050	.003	.007
	VC%	19	15	11	24
II	$\overline{\overline{X}}$	55 ± 7.25	.968 ± .173	.075 ± .009	.093 ± .027
	SE $\overline{\overline{X}}$	2.29	.054	.003	.008
	VC%	13	.17	11	29
III	$\overline{\overline{X}}$	50 ± 7.6	1.070 ± .156	.073 ± .009	.097 ± .019
	SE $\overline{\overline{X}}$	2.4	.049	.003	.006
	VC%	15	14	12	20
IV	$\overline{\overline{X}}$	48 ± 10	1.055 ± .120	.087 ± .015	.098 ± .021
	SE $\overline{\overline{X}}$	3.1	.038	.005	.007
	VC%	20	11	5	21

$\overline{\overline{X}}$ = Mean of means ($\overline{X}$) VC% = Variability coefficient/group

SE $\overline{\overline{X}}$ = Standard error of $\overline{\overline{X}}$

Because it was not known a comparison group (control) the significance of the differences was done for each batch and parameter over the other three groups.

From Table 1 it results that in group 1 significant differences have not been found in comparison with the other three groups (groups II, III and IV). In lots II and III the significant differences was only towards the group IV in the villi width parameter.

These findings are given in Chart 1.

Chart 1

Significance of differences among mean values (samples A)

Lot	I	II	III	IV
I		ND	ND	ND
II	ND		ND	ND /3 parameters; SD_{vw}*/1 parameter
III	ND	ND		ND /3 parameters; SD_{vw} **/1 parameter
IV	ND	*	**	

ND = Nonsignificant difference

SD_{vw} = Standard deviation of villi width

*SD_{vw}; $p < 0.05$

$t = 2.18$ for $LD = 18$

** SD_{vw}; $p < 0.05$

$t = 2.5$ for $LD = 18$

LD = Liberty degrees

From the chart above results that the variability was the middle order for three parameters (villi height = 11-17%, villi width = 5-12 %, goblet cells number = 13-20%); as for the cryptal depth, its variability was high (20-29%).

We present some photo shoots obtained from the small intestine samples prepared for histometric exams (photo 1,2,3)

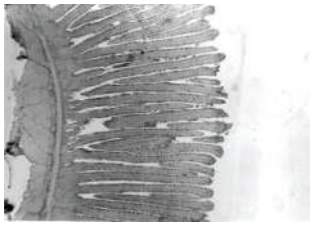

Photo1. Ileum (M.D. area). Numerous villi, intestinal crypts with submucosal glands, muscular and serous tunics. PAS – alcian reaction

Photo2. Ileum (M.D. area). Intestinal villi with numerous goblet cells. PAS – alcian reaction (x250)

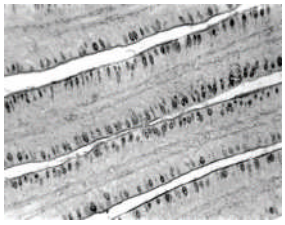

Photo3. Ileum (ileo-caecal junction). Intestinal villi with numerous goblet cells PAS – alcian reaction (x250)

As regards the correlation between the villi height and the goblets number/mm the following were found:

- Groups I and II it is distinguish a weak positive correlation (r = 0.276
and r = 0.439 respectively)

- Groups III and IV present a weak negative correlation (r = - 0.240 and
r = 0.383 respectively).

The values recorded for samples A show that in the case of Groups III and IV the total cell number remains unchanged, while the villi height is higher.

The evaluation may be that, with the Groups I and II, the total number of goblet cells rises together with the villi height increase.

II. The Summary results of measurements obtained from the portion of small intestine from the ileo-caecal junction (probele B) are presented in the Table 2 and Chart 2.

Table 2

Values per groups in samples B

Groups		Goblets (no./mm)	Villi height (mm)	Villi width (mm)	Crypta depth (mm)
I	$\overline{X}$	73 ± 13.3	.640 ± .062	.100 ± .009	.095 ± .021

	SE $\overline{\overline{X}}$	4.2	.019	.003	.006
	VC%	18	9	9	22
II	$\overline{\overline{X}}$	72 ± 15.9	.690 ± .115	.097 ± .012	.108 ± .027
	SE $\overline{\overline{X}}$	5.0	.036	.004	.008
	VC%	21	16	12	24
III	$\overline{\overline{X}}$	67 ± 11.6	.638 ± .111	.094 ± .010	.095 ± .010
	SE $\overline{\overline{X}}$	3.6	.035	.003	.003
	VC%	17	17	10	10
IV	$\overline{\overline{X}}$	70 ± 1.1	.648 ± .137	.102 ± .013	.102 ± .021
	SE $\overline{\overline{X}}$	3.5	.043	.004	.006
	VC%	15	21	13	20

$\overline{\overline{X}}$ = Mean of means ($\overline{X}$) VC% = Variability coefficient/sample

SE $\overline{\overline{X}}$ = Standard error of $\overline{\overline{X}}$

No significant difference was noticed, with samples B, on comparing each group with the other three groups.

A weak negative linear correlation was found between the villi height and goblet cells number in all the groups: Group I r = -0.375, Group II r = -0.569, Group III r = -0.592, Group IV r = -0,342.

We present some histological photo shoots obtained from the small intestine (samples B) prepared for morphometric technic (photo 4 and 5).

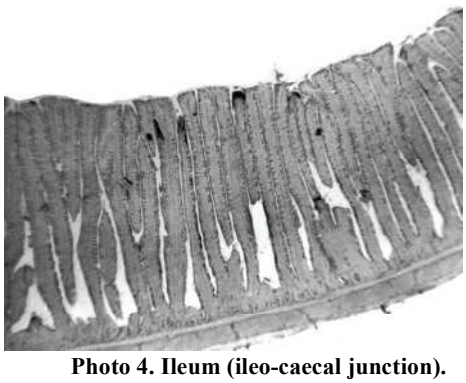

Photo 4. Ileum (ileo-caecal junction). Numerous villi, intestinal crypts with submucosal glands, muscular and serous tunics. PAS – alcian reaction (x140)

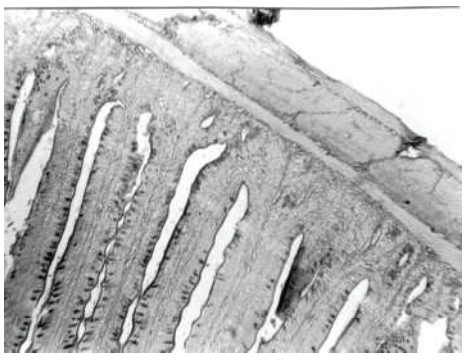

Photo 5. Ileum (M.D. area) Intestinal crypts with submucosal glands. PAS – alcian reaction (x250)

III. Samples A/B comparison

The significance of the differences among the values of the test parameters of groups A and B is presented in Chart 2.

Chart 2

The significance of the difference among means

Groups	Goblets (no./mm)	Villi height (mm)	Villi width (mm)	Crypta depth (mm)
I/IV	ND	ND	ND	ND
II/IV	ND	ND	ND	ND
III/IV	ND	ND	ND	ND
A/B	VSDt = 4.73 p< 0.001	VSDt = 7.05 p< 0.001	SDt= 2.35 p< 0.05	ND

ND = Nonsignificant difference
VSD = Very significant difference
SD = Significant difference

On comparatively analysis the results obtained the villi height was found to be lower with samples B (Table 2) than with samples A (Table 1).

With Group I, there were very significant differences ($p < 0.001$) for the following parameters: number of goblets, villi height and width.

In the Group II, distinctly significant differences ($p < 0.01$) were recorded with the goblet cell number, and very significant ones ($p < 0.001$) for the height and width of the villi.

Group III presented very significant differences ($p < 0.001$) for the goblets number, villi height and width.

In the Group IV very significant differences ($p < 0.001$) were found as far as the goblets number and villi height are concerned. Also, significant differences ($p < 0.05$) were revealed in way of the villi width.

The safety of the methods for interpreting the results of intestinal epithelium measurements is based on the integrity of the mucosal epithelium and enterocytes and on the execution quality of the histological preparations.

The ileum histological examination revealed in all the birds examined a normal aspect of villi with many goblet cells characterized by the presence of large vesicles. The epithelium of the intestinal mucosa is delicate, with long intestinal villi. In the submucosa were found many glands with normal glandular epithelium cells (photo 5). The simple prismatic epithelium covers the intestinal villi and goes down in the Lieberkühn crypts in whose lumen is lining. It has been noted many goblet cells PAS-positive or alcian positive irregularly

disseminated on the flanks of villi alternating with numerous intact epithelial absorption cells (photo 2).

In the histometrical examination no significant changes were found in the studied parameters (the villi height and width, the number of goblet cells and the crypts depth) in the sections from the birds that came from the A samples. In the B samples were recorded lower values of the villi height parameter compared with the A samples.

However, the comparative analysis of the obtained results on the analyzed parameters in A/B samples have highlighted significant differences ($p<0.001$; $p<0.01$; $p<0.05$), on the number of goblet cells, the villi height and width.

Numerous studies showed that the administration of Bio-Mos in the diet of broilers and piglets resulted in an increase in villi length and a reduction in crypts depth. The overall size of the surface absorption in the intestine and the reducing of the renewal rate of the epithelial cells in the crypts improve the nutrient availability for absorbing (Adeola şi King, 2006; Kocher,2006)

In the work that we made, we were not aware of all the elements on the experimental protocol in broilers and we could not conclude on the prebiotic action of the Bio-Mos preparation.

It results that the measurements performed on the ileum showed a significant difference in the A samples over the B samples in three parameters: goblet cells, the villi height and width

CONCLUSIONS

It were investigated by morphometric examinations fragments of small intestine from the treated broilers with the Bio-Mos preparation, Alltech.

1. The histometric test results have shown statistically significant differences of the parameters: villi height, villi width, number of goblet cells and the crypts depth on the fragments collected from the same subject with M.D. (A) compared with the obtained fragments from the cranial portion of ileum (B).

2. The registered values in the A samples demonstrates that the number of goblet cells remains the same (the Group III and IV) or increases (the Groups I and II) while the villi height is bigger.

3. In the B samples were not found significant differences between the groups, and between the villi height and the number of goblet cells it has shown a weak linear negative increase in all the groups (the Groups I, II, III, IV).

4. The comparison of the A samples results with the B samples results has shown significant differences about the number of goblet cells ($p<0.001$), the villi height ($p<0.01$) and the villi width ($p<0.05$).

5. The analysis of the obtained results in the groups of animals studied was not conclusive on the prebiotic action of the used preparation.

6. The studies have aimed the development of a diagnostic and testing method by histometric technic by analysis of computerized image on the epithelium of the small intestine mucosa.

We thank to Mrs. Lucretia Androne for the excellent quality of the histological preparations made

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MATERIALS AND METHODS USED IN CARNIVORES DERMATOLOGIC PATHOPHYSIOLOGY RESEARCH. PART OF PHD WORK

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Key words: skin, carnivores, pathology

SUMMARY

For the best therapeutical results, the etiology and the disease mechanism of the involved disease is needed. The veterinary medic needs a systematic approach, a thorough examination and appropiate diagnostic procedures. The techniques that have been used in this work were: clinical examination (including the dermatologic history records), dermatoscopic exam, cytologic exam, dermatohistopathology, electronic microscopy, serum biochemistry, endocrine tests, hemoleucograms, serum electrophoresis, allergy tests, and other. For the studies, 236 dogs and 87 cats were selected and recorded. All the exams used were useful in etiology and pathophysiology determination. Not only the skin tests are effective in dermatological diagnostic, but much more internal organs disfunctions testing can conduct us to the proper mechanism of disease. The pathological mechanisms can be multiple, more than one mechanism on the same patient.

The skin is the largest and most visible organ of the body. It is not only the anatomic and physiologic barrier between animal and environment. It is synergistic with internal organ systems and reflects pathologic processes that are either primary elsewhere or shared with other tissues (Scott, 2000).

So we can say the skin is not just an organ with its own reaction patterns; it also reflects the inside of the body and, in the same time, the surrounding environment (Gross, 2005).

For the best therapeutical results, the etiology and the disease mechanism of the involved disease is needed. The veterinary medic needs a systematic approach, a thorough examination and appropiate diagnostic procedures (Guaguere, 1999, Hill, 2002).

The techniques that have been used in our work were as follow: clinical examination (including the dermatologic history records), dermatoscopic exam, cytologic exam, dermatohistopathology, electronic microscopy, serum biochemistry, endocrine tests, hemoleucograms, serum electrophoresis, allergy tests, and other (Wilkerson, 2004).

1. MATERIAL AND METHOD

For our studies, 236 dogs and 87 cats were selected and recorded, from a total of more than 600 dermatological cases seen. The reason of selection was the possibilities of the owner to make some clinical exams for their pet. On the other hand, 37 of the dogs and of the 38 cats with dermatological problems were homeless animals, catched by us, examed, threated, and, most of them, given to adoption. The rest remained in our clinic are waiting to find a careful owner.

On all of our patients clinical examination was done, and the cases have been recorded on computer, with a case number for each patient. Where possible, pictures were taken, and lesions maps were completed (fig. 1).

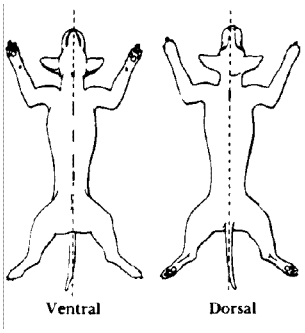

Figure no 1: Skin lesions map

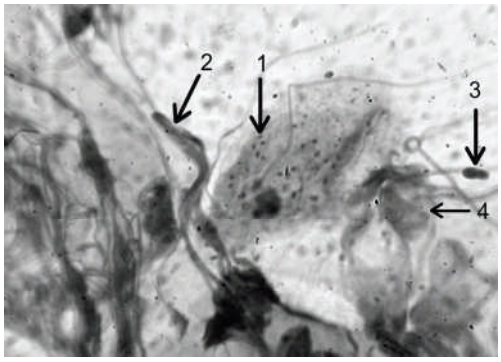

Figure no 2: Cytologic microscopical image. 1-keratinocyte with picnotic nuleus; 2-colagen fiber; 3-Malassezia spp. yeast

The dermatologic history has been taken, except, of course, the homeless patients. For the last, we were proceeding to a clinical observation in our clinic.

Dermatoscopic exam has been performed on all cases, also the cytologic exam. For the dermatoscopy the oil imersion, KaOH 10%, or lactophenol-blue have been used for sample clarifying.

For cytologic exam the Merck Hemacolor and methylene blue techniques have been used. The Hemacolor coloration has a panoptic result. The nuclei are coloured red to violet, the cytoplasm pale is blue to reddish, the keratinocytes blue, the fibers dark blue (fig. 2). Methylene blue has been used for quick examination samples. The result was dark blue to black nuclei and pale blue to blue with grannulae cytoplasm.

Dermatohistopathology was done on 135 dogs and 32 cats. The anesthesia was not usually necesary. The samples were gathered with a thumb forceps, the specimens have been put in 10% formalin. The methods used by us were hematoxylin-eosin, tricromic Mallory, toluidine blue, and anhidrous Giemsa.

Electronic microscopy has been performed on 3 dog cases. The Babes Institute Electronic Microscopy team helped us in prelevation, fixation, coloration and interpretation.

Serum biochemistry has been our choice for 200 dogs and 78 cats. The tests have been performed to the Arkray biochemical device, and to Synevo human biochemistry laboratories. The most tested parameters were liver (ALT, AST, bilirubin, total protein, albumin, glucose), and renal (urea, creatinine, phosphorus, calcium).

Endocrine tests were done for 87 dogs and 13 cats suspected to have hormonal problems. They were performed on Synevo laboratories and on Innovet Idexx device (for T4 and cortisol dosing). The parameters were growth hormone (GH), thyroid stimulating hormone (TSH), T3, T4, Adrenocorticotrop hormone (ACTH), cortisone, testosterone, estradiol, and progesterone.

Hemoleucograms have been done on all patients. The venous, or capilar blood was sampled. Panoptical coloration with Hemacolor has been used.

Serum electrophoresis has been performed on 125 dogs and 24 cats. Determinations were made using agarose gel electrophoresis machine using the Tris Barbital buffer at pH 8.6. Serum was diluted 1 / 7 with Tris Barbital buffer, protein fractions migration was accomplished at 85 V, migration time beeing 17 minutes and 30 seconds.The coloring of the protein fractions was performed with Amido Black. After drying, computerised integration and calculation of the electroforegrams was conducted, resulting in 5 distinct protein fractions: albumin, α1 globulin, α2 globulin, β globulin and γ globulin.

Allergy tests have been done on 8 dogs and 3 cats. The tests were made in Innovet veterinary clinic, using an allergy test kit. The hair had been clipped on a rectangular area in a lateral abdominal area, the injection sites were pointed with a marker, the antigens were injected with insulin needles. There were a martor (9‰ NaCl solution) and a standard histamin solution. After two hours, the injected areas were tested for oedema, and the dimension of tumefactions was measured.

2. RESULTS AND DISCUSSION

All the exams used by us have been useful in etiology and pathophysiology determination. The clinical examination, together with the patient history, has been conducting us to next level, laboratory tests, for the final result of „what's happening there". In none of the cases the clinical examination was performed as single examination.

Dermatoscopic exam was useful in the etiology of 35 cases of dog (9 cats) sarcoptic manges, in 18 cases of canine demodicosis, in 27 cases of clinical Trychophyton spp. in dogs, 7 cases of cat trychophytosis, 18 cases of Microsporum in dogs, 5 cases of Microsporum in cats.

The cytologic exam helped in 22 cases of clinical Trychophyton in dogs, 7 cases of cat trychophytosis, 17 cases of Microsporum in dogs, 3 cases of Microsporum in cats, 9 cases of Malassezia infestation in dogs, and 3 in cats. This test helped also in 7 cases of dog parakeratosis, 3 cases of canine discoid lupus, .

Dermatohistopathology helped us to discover 3 cases of canine discoid lupus, 7 pemphigus complex in dogs and 3 in cats, 2 in dogs with bullous pemphigoid, and 1 cat with indolent abcess. It also helped us to see what's happening in 9 sarcoptic mange infestation in dogs, in 5 demodicoses cases in dogs. in 7 cases of Trychophyton infestations in dogs, 5 cases of Microsporum in dog.

Electronic microscopy was helping us for 1 lupus case, and 2 pemphigus complex in dogs.

Serum biochemistry reveled us internal organs functional problems in 3 lupus affected dogs, in 3 pemphigus complex in dogs, 2 in dogs with bullous pemphigoid. It also was helpful in 7 cases of hipothyroidism, and in 25 cases of Cushing in dogs, and 1 case of yatrogen hyperprogesteronemia in 1 tomcat.

Endocrine tests helped us to confirm 7 hipothyroidism cases in dogs, 25 Cushing cases in dogs, hipogonadism in 15 male dogs, 25 female dogs, 10 cases of hipogonadism in female cats.

Hemoleucograms helped us to make an image of white blood cells evolution in all cases. Neutrophilia was observed in 12 dogs and 4 cats with immunologic diseases, eosinophilia in 62 external parasitated dogs, in 13 external parasitated cats. Monocytosis was observed in 20 dogs with clinical micosis. Lymphocytosis was observed in 23 dogs with cronical dermatologic problems.

Serum electrophoresis revealed an increasing of β globulin to 1 dog with dermatologic involving of a Walderstrom disease. Γ globulin

increased in all alergic and immunological diseases, with some particular aspects.

Allergy tests revealed skin allergy in all of 8 dogs an 3 cats tested.

3. CONCLUSIONS

3.1. The ethio-pathogenetic tests on pets are related not only with investigation possibilities, but with human society, and, why not, with the economical crysis.

3.2. The best solution for a good ethiological and pathogenical diagnostic is to have all the tests under the same roof, as much as possible.

3.3. Not only the skin tests are effective in dermatological diagnostic, but much more internal organs disfunctions testing can conduct us to the proper mechanism of disease.

3.4. The pathological mechanisms can be multiple, more than one mechanism on the same patient.

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MONITORING OF THE PHARMACO THERAPIC INTERVENTIONS IN CERCOPITECUS GROUP IN BUCHAREST ZOOLOGICAL GARDEN

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Key words: savanna cercopitecus

SUMMARY

The experiments regarding the incidence of the diseases met in Cercopitecus group in Bucharest Zoological garden were carried out during 2007-2009. There were made perodic examinations of the animals by clinic diagnosis methods, which were correlated to the results of the analyzes done in the own laboratory of the unit (blood and urine examinations, ovohelmintoscopic exams).
The entheritis cases, generally involved two patogenous microorganism species: Shigella and Salmonella and it was noticed that the maladies appeared in the individuals under stress, being considered the main pathogenetic factor in these diseases appearances.
The treatment consisted initially only in the management of nutrition and intense rehydration. The results of the treatment were positive to the alopat medicines, respective for Cotrimoxazol (trimetoprim - sulphamethoxasol), in the order of their efficiency being Colimicin, Gentamicin and Kanamicin.
Whithin all the pneumonia forms in the winter season in Bucharest Zoological Garden they optimize the hygienic and dietary conditions; the sick animals were isolated in separated cages, well aired and at 18-22 degree Celsius. The treatment were carried out before the establishing of the etiology with products based on Tetraciclin, this therapy being justified by the fact that the Tetraciclin group is active beside all the patogenous agents responsible to pneumonia incidence.
In the case of Bobita, the cercopitecus, a male of 22-23 years old, during this year were noticed alterations of the health status, it is important to mention the fact that the main reason was considered its age. The treatment were carried out with vitamins, liver trophics, calmants and rehydrating salts but they not lead to a favourable effect and when it was noticed the cardio respiratory insufficiency the euthanasy was induced , using T 61 product, in a dose of 10 ml (a sedative agreed by the whole European Community.
Some zoological gardens have a long history regarding the management and treatment needs of the animal they take care. Lately, the interest concerning the medical problems of the exotic animals increases a lot in Romania.
The setting off a medical program for animals in zoological gardens regarding prevention and treatment standard is very difficult. Although, these programs for each species have a good result in Bucharest Zoo, noticing a real improvment of the welfare and health status.

1. MATERIALS AND METHODS

The experiments regarding the incidence of the diseases met in Cercopitecs in Bucharest Zoological Garden were carried out during 2007 - 2009. There were made periodic exams by clinic diagnosis

methods, which were correlated to the results carried out in the own laboratory (blood, urine exams etc.).

It is important to mention that all the individuals in this unit are old and along the time they had especially digestive and respiratory diseases. In 2009 it was recorded the death of a male, *Bobita*, at 23 years old.

The enteritis cases, generally involved two patogenous species of microorganisms: *Shigella* and *Salmonella* and it was noticed that the disease appeared at individuals exposed to stress factors, this being considered the main pathogenetic factor in entheritis appearance. The treatment initially consisted only in nutrition management and intense hydrations. During the status period (proper diarhoeea) it was carried out an etiologic treatment due to a large number of cases determined by entheral infections. The results of the treatment were positive at Cotrimoxazol (trimetoprim – sulfamethoxazol). Ordering the efficiency, there were also the products: Colimicin, Gentamicin and Kanamicin.

During winter, the main favourable factors of pneumopathic diseases are the respiratory mucous irritation, physical debilitation and physical factors (temperature, pressure, humidity). The pneumonia diagnosis was made upon the clinic examinations completed by laboratory tests.

Within all the pneumonia cases in the Bucharest Zoological Garden the hygienic and dietary conditions are optimized, the sick animals were isolated in separate cages, well-aired and at temperature between 18 – 22^0 C. The treatments were carried out before the etiology establishing with Tetraciclin products, this therapy being justified by the fact that these medicines are active against the most of the patogenous agents responsible to pneumonia producing.

In the case of *Bobita*, a male of 22 – 23 years old, during the last year were noticed adynamia, anorexia, prostation status and decubit. It is important to mention that this male has not teeth and because the decubit there were formed skin aflictions. There were administered vitamins (Multiject -B product, Vetstar 1 cp.), liver trofics (hepatosuport 1 capsule), Nurofen ¼ tb, Aspacardin ½ tb. After a six days treatment the animal was still without dynamic, presented supplementary liver and renal insufficiency, dehydration and the lack of hunger it is isolated, noticed and treated with Baytril 5%, 0,5 ml, Dexamethason -0,3 ml, Multiject –B -0,4 ml, Novasul -0,5 ml ; glucoză s.c. 20 ml, Dufalyt s.c. 15 ml, Ringer 25 ml. The treatment was repeated at 12 hours, for 5 days but without any favourable effect. In the sixth day, it was noticed the cardio-respiratory insufficiency and it was practicted the induced

euthanasy, using T 61 products in a dose of 10 ml (this sedative medicine is agreed in the whole European Union. It was done in September 2009.

RESULTS AND DISCUTIONS

The prophylactic measures have a major importance in animal disease prevention. The main important measures are: the assurement of some optimum welfare conditions, the administering of a correct quantitative and qualitative feeding, the nutrition hygiene and also the microclimate conditions.

The ovohelmintoscopic examinations achieved during the time (23.12.2008; 21.01, 28.07, 11.08.2009) for *Bobiță* were negative.

The clinic examinations, doubled by the laboratory analyses and confirmed by the necropsic exam after euthanasy showed the difficult physiologic problems of *Bobita*, the euthanasy being the optimum solution for this animal (*Tables 1 and 2).*

Table nr. 1

Physical and chemical urine parameter in *Bobita*, the Cercopitecus male

31.03.2009		**19.07.2008**
GLU mg/dl	**NORMAL**	50 mg/dl
PRO mg/dl	**NEG.**	100 mg/dl
BIL mg/dl	**0,5**	0,5 mg/dl
URO mg/dl	**NORMAL**	2 mg/dl
PH	**8,0**	9,0
S.G.	**1,020**	< 1,005
BLD mg/dl	**0,2**	NEG. mg/dl
KET mg/dl	**NEG.**	NEG. mg/dl
NIT	++	+
LEU Leu/ul	**500**	500 Leu/ul

Legend: GLU – glucos, PRO – protein, BIL – bilirubin, URO – urobilinogen; PH – pH, S.G. –urine density, BLD – hemoglobinury, KET – cetonic corps, NIT – nitrites, LEU – leucocy.

Table nr. 2

Blood results biochemical parameters in *Bobita*, the Cercopitecus male

TEST	RESULTS	NORMAL RESULTS	UNITS
UREA	13,8		mmol/L
CA	2,02		mmol/L
TRI	0,80		mmol/L
ALT-IF	49	0 - 138	U/L
ALP	0		U/L
GLUPAP	13,8		mmol/L
TP	0,00 L*	68,86 – 86,00	g/L
CKMB	94		U/L
AST-IF	6 L*	9 - 68	U/L
CREAT	63,3		µmol/L
MG	0,00		mmol/L
GGT	80,08		U/L

L* - low value

Legend: UREA –urea, CA –calcium, TRI –triglicerids, ALT-IF –GPT, ALP –alkaline phosphatasis, GLUPAP –glucoses, TP –total protein , CKMB –cardiac creatinkinasis, AST-IF –GOT, CREAT –creatinine, MG –magnezium, GGT –gamaglutamin transferasis.

CONCLUSIONS

In the acute entheritis treatment, the maximum efficiency was obtained by the following products: *Cotrimoxazol, Colimicină, Gentamicină* şi *Kanamicină.*

3.2. In the case of pneumopaties, the treatment rapidly made with Tetraciclin products showed the fact that the animals answered positively without secondary reactions.

3.3. The clinic examinations, doubled by the laboratory analyses and confirmed by the necropsic exam after euthanasy showed the difficult physiologic problems of *Bobita*, the euthanasy being the optimum solution for this animal.

The assement of the welfare standards and also the hygiena standards and individual immunity development represent compulsory themes in the units which raise animals.

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DYNAMICS OF GROWTH PARAMETERS IN LIPITAN BREED DEPENDING ON DIFFERENT INFLUENCE FACTORS

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Key words: Lipitan, body indicators, growth coefficient, growth speed .

Summary

The researches presented in this paper were carried out during 2004 – 2008 and they proposed to underline the dynamics of the growth process of youngsters in Lipiţan breed, reared in Sâmbăta de Jos, depending on different influence factors.
These will allow the knowledge of some useful aspects regarding the youth rearing technology and also the Lipiţan horse breeding in this unit, so this breed could become a good breeder for the local horse population.

In the latest years in our country it was straighten intervened in horse breeding stimulation, insisting upon the existent material perfection but also upon founding new breeds which correspond to agricultural requiring in different areas of the country.

Conformingly the National Breeding Program, the horse breeding will have a new orientation, emphasizing the horse using for sport or performance competitions.

MATERIAL AND METHODS

The biologic material was made of the young horses in Sâmbăta de Jos unit. There were 389 individuals, 193 males and 196 females, between 0, 5 – 3 years old. The researches consisted in recording of the growth parameters as growth speed, relative and absolute and growth intensity.

The data were processed and transformed into relative values with the aid of the main body indices which emphasize the body conformation particularities.

RESULTS AND DISCUSSIONS

The study of the growing period it is made also by establishing the body weight. It is considered that between the weight of an animal and its volume there is a direct ratio, that means between its weights will show the dynamics of its sizes. The table 1 presents the values of the

growth parameters (absolute and relative growth speed, growth coefficient and intensity), for the body weight during 2004-2008 generations.

Table 1

Growth dynamics depending on sex in Lipițan breed youth

Analyzed period 2004-2008	Sex	Body weight			
		Absolute speed	Relative speed	Growth intensity	Growth coefficient
0-6 months	M	0,890	258	1,13	28
	F	0,900	331	1,30	64
	Total	0,895	310	1,25	56
6-12 months	M	0,570	46	0,37	65
	F	0,710	61	0,47	62
	Total	0,640	52	0,39	63
12-24 months	M	0,620	35	0,29	79
	F	0,640	36	0,30	36
	Total	0,630	35	0,27	67
24-36 months	M	0,100	30	0,13	10
	F	0,120	50	0,15	30
	Total	0,110	40	0,12	20

The absolute growth speed recorded decreasing values as age increases. Thus, the growth relative speed recorded very high values in the 0-6 month's category of 310%, but decreasing on age, so in the 24-36 months category it is only 40%. This reflects the fact that there is the best development of the growth process at early ages. The growth intensity in the analyzed category recorded high values in the first period of the growth period, the values decreasing till the body maturity, when the intensity is reduced to 0. The growth coefficient in the analyzed category presents the following values: 28 % in males and 64% in females. In the 6-12 months category the growth coefficient is 63%, in the 12-24 months category the higher value is recorded in males, 79%, by the whole, the coefficient is 67% in this category. In 24-36 months category the growth coefficient is only 20%.

Table 2

Body weight variation depending on generation and sex in Lipițan breed youth

Generation	Sex	No	X	±	S_X	S	V%
2004	M	36	488	±	2,23	13,35	2,74
	F	30	501	±	2,36	12,70	2,53
	Total	66	495	±	2,30	13,03	2,64

2005	M	42	457	±	1,81	12,10	2,77
	F	31	461	±	2,30	12,80	2,78
	Total	73	459	±	4,11	12,45	2,78
2006	M	33	430	±	2,50	14,38	3,20
	F	32	450	±	2,38	13,01	2,58
	Total	65	440	±	2,40	13,27	2,80
2007	M	38	430	±	2,00	12,30	2,86
	F	31	440	±	2,32	12,93	2,94
	Total	69	420	±	2,20	12,62	2,85
2008	M	36	324	±	2,50	15,00	4,62
	F	36	338	±	1,79	10,69	3,16
	Total	72	331	±	2,14	13,25	3,89

The growth relative speed recorded differences between sexes. Thus, at this growth element in males the recorded values are: 1, 13%, 0, 37%, 0, 29%, 0,13%. The females recorded higher values in the same age category: 1, 03%, 0, 47%, 0,30% and 0,15%.

The highest values in the growth coefficient are met in 6-12 months category, respectively 62% and in the 12-24 % category.

The table 2 presents the variation of the body weight depending on generation, sex and age in Lipitan youngsters.

Analyzing these data it may conclude that the females have a main of the body weight higher than the males in all the generations. The values of the variability coefficient are low and uniform in all the generations.

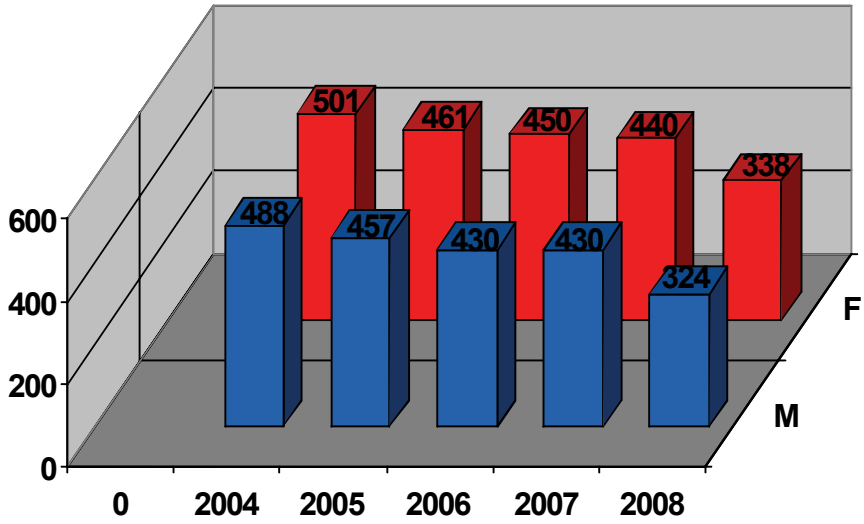
3. CONCLUSIONS

3. 1. The Lipițan breed youth have a special natural frame which assures an optimum rearing environment. There is also applied o correct breeding technology.

3. 2. The growth parameter shows a natural evolution of the body size weight which evolves more depending on age. Thus, the colts recorded an average breeding gain of 900 g, the weaned colts recorded 640 g, the 1-2 years old youngsters has the absolute speed as same as the 2-3 years old category, but an extremely low growth gain, only 110 g. The relative speed depending on age varies between 310-40%, and the growth intensity between 1,3-0,12%.

3. 3. It is certified the breed homogeneity and it is emphasized the genetic quality of this breed and the local population breeding ability.

The variation of body weight depending on generation and sex



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RESEARCHES REGARDING REPRODUCTIVE ISOLATION, INBREEDING AND PROPER RELATIONSHIP IN TWO RHODE-ISLAND HEN LINES

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Key-words: reproductive isolation range, line inbreeding, current inbreeding, non-current inbreeding.

SUMMARY

The present paper has proposed to study some aspects of the genetic history and dynamics in two Rhode - Island hen lines, under selection for many generations.
As a main goal of many researchers was the establishing of the genetic status of these lines, predicting some indicators which frame the genetic history: reproductive isolation range, total achieved inbreeding, inbreeding achieved per generation, current and non-current inbreeding, relationship and subdivision index.

MATERIAL AND METHODS

The non randomized mating during more generations has as result the interruption of the genes changing among the individuals of a mating group and also other groups. This procedure leads to the partial or total stop of genes changing among populations. So, the reproductive isolation range [1, 2, 3, and 4] represents the main criterion to consider a livestock as population. Dobzhanski i1) introduced this term which is estimated as a ratio between the indigenous reproductive animals and immigrants ones during five generations which permit the differentiation of the population.

$$I = AA - (AI + II) / AA + AI + II$$

Where:

A.A = number of individuals admitted in the reproductive livestock in the studied interval with both indigenous parents.

A.I. = number of individuals admitted in the reproductive livestock in the studied interval with one immigrant parent.

I.I = number of individuals admitted in the reproductive livestock in the studied interval with both immigrant parents.

The two lines of Rhode – Island hens were studied during twelve generations, respectively six generations and for each generation there were counted the reproducers, males and females, the indigenous, immigrant and with one immigrant parent.

Inbreeding is the mating of the more closed related individuals in the same population than the average of the inbreeding range in that population (1, 2, and 3). Within the closed population with a limited livestock, after a generation number, all of the individuals are closed related due to the fact that the ancestors number of each individual increases as a geometric progression.

Knowing the average relationship in a population at a moment is a very important thing because there is possible to estimate the possible or waited inbreeding by the random mating of the individuals. To establish the proper relationship and also the inbreeding it was used the calculus on pedigrees by sampling: choosing of the four random lines starting from grandparents, a large used procedure by Lush (two random lines) and improved by Robertson (four random lines), quoted by Draganescu, C., 1979.

Knowing the average inbreeding in a population in a moment, it could be estimated the possible ore expected inbreeding by random mating of individuals (1, 3).

After proper relationship calculation, separated by males and females, the possible inbreeding was calculated also by sexes, using the following relation:

Possible inbreeding = proper relationship/ (2- proper relationship).

In this relation, the proper relationship is expressed in decimal fractions, not in percentages. The always problem consists of keeping a livestock with a minimum inbreeding but with a limit of the population size imposed by space and other economic conditions. A current practice in this situation is the use of pedigrees, choosing reproduction mates with the lowest possible inbreeding among them. It is realized a difference between the possible inbreeding by random mates and the inbreeding achieved after choosing the mates.

The inbreeding has to be considered linked to the population it works with, understanding that the number of the common ancestors is higher and the inbreeding range between individuals' increases as the population is smaller. The populations with a small effective size have the highest inbreeding rhythm. An increasing of the effective size (Ne) did not affect the previous inbreeding, but reduces the new one.

There was identified the number of appearances of some individuals no matter the generation they take part that permitted, using some simplified methods to calculate:

Total achieved inbreeding = the number of the double lines achieved / the number of the possible double line;

Where:

- possible double line number = 4n;
- n = number of individuals.

Achieved inbreeding per generation = total achieved inbreeding/ number of generations.

Current inbreeding = the number of the double lines in the last two generations/ the possible double lines number.

Non-current inbreeding = total achieved inbreeding – current inbreeding.

The importance of the inbreeding coefficient for the genetic analyses consists in the fact that its value could be the result of subdividing the population in small lines, completely reproductive isolated, with random mating.

The average inbreeding coefficient per population is here due to relating of all the individuals in the same line and it will diminish if there is random mating between lines.

Using the formulas obtained in the previous stages the next formulas were applied to calculate the line inbreeding and the subdividing indices.

Line inbreeding = non-current inbreeding- possible inbreeding

Subdivision index = non-current inbreeding/ possible inbreeding

2. RESULTS AND DISCUSSIONS

In the tables number 1 and 2 it is presented the reproductive structure in the two studied lines.

Table 1

Reproductive structure of the paternal line

Generation	Indigenous reproducers		Immigrant reproducers	Reproducers with one indigenous parent		Total reproducers
	Males	Females	Males	Males	Females	
1	40	560	-	-	-	600
2	30	560	10	-	-	600
3	30	484	1	9	76	600

4	40	480	-	-	-	520
5	27	480	13	-	-	520
6	29	312	-	11	168	520
7	40	480	-	-	-	520
8	40	480	-	-	-	520
9	40	480	-	-	-	520
10	40	480	-	-	-	520
11	43	497	-	-	-	540
12	45	540	-	-	-	585
total	444	5833	24	20	244	6565

Of the total 6565 reproducers used along the studied generations, 24 reproducers (males) are immigrants with both parents out of the studied line. 264 reproducers, males and females have one indigenous and one immigrant parent and 6277 reproducers have both indigenous parents. By calculation results:

I males = 444 – (24 + 20)/ 444 + 24+ 20 = 0,82.

I females = 5833 – (0 + 244)/ 5833 + 0+ 244 = 0,92.

The isolation reproductive coefficient 0,82 for males and 0,92 for females permit to conclude that the studied line is a simple population, new formed, with a distinct evolution from the population it derived from.

Table 2

Reproductive structure of the maternal line

Generation	Indigenous reproducers		Immigrant reproducers	Reproducers with one indigenous parent		Total reproducers
	Males	Females	Males	Males	females	
1	40	400	-	-	-	440
2	40	400	-	-	-	440
3	40	320	-	-	-	360
4	40	400	-	-	-	440
5	40	400	-	-	-	440
6	40	400	-	-	-	440
total	240	2320	-	-	-	2560

The study effected upon the 2560 individuals admitted to reproduction in the six generations shows the fact that during the period

there were no reproducers from other populations. By calculation results:

I males = 240 – (0+0)/ 240 + 0+ 0 = 1.

I females = 2320 – (0 + 0)/ 2320 + 0+ 0 = 1.

The obtained results shows a +1 reproductive isolation coefficient that means a complete reproductive isolation of the line. After six generations of complete isolation this line is a simple population with a distinct evolution.

Studying the 90 pedigrees of the four random lines there were found 2403 appearences in the father line and also in mother's but also in the succession of the 12 generations. There were found double lines, and one of them in the first two generations. By calculations results:

Proper relationship = 2403/4 x 90 x (90-1) = 0,075 = 7,5%.

Total achieved inbreeding = 12/ (4 x 90) = 12/360 = 0,033 = 3, 3%.

Per generation achieved inbreeding = 0, 033/12 = 0, 0027 = 0, 27%.

The current inbreeding = 1/4 x 90 = 1/360 = 0, 0027 = 0, 27%.

The non-current inbreeding = 0, 033 – 0, 0027 = 0, 030 = 3%.

The subdivision index = 0,030/0,039 = 0,769.

The proper relationship of the line was 7,6% that leads to a possible inbreeding of 3,9%. The total achieved inbreeding was lower than the possible one, so it may conclude there were making procedures to avoid the related mating. The value of the subdivision index 0,769 reflects the lack of line inbreeding.

Based upon the study of the eighty pedigrees in the four random lines there were found 1266 appearences in the father line and also in mother's but also in the succession of the 6 generations. There were found 6 double lines, all of them with further ancestors.

By calculations results the following data:

Proper relationship = 1266/4 x 80 x (80-1) = 0,050 = 5%.

Total achieved inbreeding = 6/ (4 x 80) = 6/320 = 0,0187 = 1, 87%.

Per generation achieved inbreeding = 0, 0187/6 = 0, 0031 = 0, 31%.

The current inbreeding = 0

The non-current inbreeding = 0, 0187 – 0 = 1, 87%

The subdivision index = 0,0187/0,0256 = 0,730.

The proper relationship of the line was 5% that leads to a possible inbreeding of 2,56%. The total achieved inbreeding was lower than the possible one, so it may conclude there were making procedures to avoid the related mating. The value of the subdivision index 0,730 reflects the lack of line inbreeding.

3. CONCLUSIONS

3.1The reproductive isolation range of 0,82 in males and 0,92 in females in the paternal line frames this line within the valuable populations.

3.2 Regarding the maternal line, after six generations of complete reproductive isolation it is considered a distinct evolution beside other hen populations.

3.3 The 3,3% total inbreeding coefficient frames the paternal line in the category of the non inbred lines, this evolution being under the selection control, avoiding the related mating.

3.4 The value of the total inbreeding coefficient 1,87% for the maternal line shows the fact that the evolution of this line is under the total selection control.

3.5 The value of the subdivision index (0,769 for the paternal line and 0,730 for the maternal line) shows that there are not subdivisions within the lines

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RESEARCHES REGARDING INBREEDING IN A LAYING HEN LINE

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Key-words: possible inbreeding, line inbreeding, subdivision index, current inbreeding, non-current inbreeding.

SUMMARY

The present researches have proposed to study the genetic history and dynamics in a Leghorn laying hen line, under selection for many generations.
As a main goal of many researchers was the establishing of the genetic status of this line, predicting some indicators which frame the genetic history: possible inbreeding, line inbreeding, subdivision index, current and non-current inbreeding.

Inbreeding is the mating of the more closed related individuals in the same population than the average of the inbreeding range in that population (1, 2, and 3). Within the closed population with a limited livestock, after a generation number, all of the individuals are closed related due to the fact that the ancestors number of each individual increases as a geometric progression.

MATERIAL AND METHODS

The research in the present paper was made using a Leghorn laying hen line. The studied individuals were presented by their pedigrees as it follows:

- 80 pedigrees in males, corresponding to all the 80 roasters in 1998 generation;
- 1000 pedigrees in females, corresponding to all the 100 roasters in 1998 generation;

It was used a systematic choosing five by five. The pedigrees consist of 8 generations, starting from grandparents, a more used by Lush method (2 random lines) and brought to perfection by Robertson (4 random lines).

Knowing the average inbreeding in a population in a moment, it could be estimated the possible ore expected inbreeding by random mating of individuals (1, 3).

After proper relationship calculation, separated by males and females, the possible inbreeding was calculated also by sexes, using the following relation:

Possible inbreeding = proper relationship/ (2- proper relationship).

In this relation, the proper relationship is expressed in decimal fractions, not in percentages. To calculate the future possible inbreeding after a random use at reproduction of the top pedigree individuals, it was calculated the relation among males and females by half pedigrees in the two possible variants (paternal halves from males with maternal halves from females and versus) and then by total. That is why the total possible inbreeding was calculated with the previous relation specified above.

The always problem consists of keeping a livestock with a minimum inbreeding but with a limit of the population size imposed by space and other economic conditions. A current practice in this situation is the use of pedigrees, choosing reproduction mates with the lowest possible inbreeding among them. It is realized a difference between the possible inbreeding by random mates and the inbreeding achieved after choosing the mates.

The inbreeding has to be considered linked to the population it works with, understanding that the number of the common ancestors is higher and the inbreeding range between individuals' increases as the population is smaller. The populations with a small effective size have the highest inbreeding rhythm. An increasing of the effective size (Ne) did not affect the previous inbreeding, but reduces the new one.

There was identified the number of appearances of some individuals no matter the generation they take part that permitted, using some simplified methods to calculate:

Total achieved inbreeding = the number of the double lines achieved / the number of the possible double line;

Where:

- possible double line number = 4n;
- n = number of individuals in 1998 generation.

Achieved inbreeding per generation = total achieved inbreeding/ number of generations.

Current inbreeding = the number of the double lines in the last two generations/ the possible double lines number.

Non-current inbreeding = total achieved inbreeding – current inbreeding.

The importance of the inbreeding coefficient for the genetic analyses consists in the fact that its value could be the result of

subdividing the population in small lines, completely reproductive isolated, with random mating.

The average inbreeding coefficient per population is here due to relating of all the individuals in the same line and it will diminish if there is random mating between lines.

Raising animals by lines trends to separate breed into distinct groups, each group strong related with the remarkable ancestor, among them may be practiced an efficient selection. In this paper it was calculated the line inbreeding to see if there are subdivisions of it. To analyze the division in lines of a population it was calculated the subdivision indices. As it value is lower the breed is less subdivided. Using the formulas obtained in the previous stages the next formulas were applied to calculate the line inbreeding and the subdividing indices, separately by sexes:

Line inbreeding = non-current inbreeding- possible inbreeding
Subdivision index = non-current inbreeding/ possible inbreeding

2. RESULTS AND DISCUSSIONS

Possible inbreeding in males = 0, 0465 / (2-0, 0465) = 0, 0465 / 1, 9535 = 0, 0238 = 2, 38%.

Possible inbreeding in females = 0, 0412 / (2-0, 0412) = 0, 0412 / 1, 9588= 0, 0210 = 2, 10%.

Possible inbreeding by comparing the maternal halves in females with maternal halves in males = 0, 0436/ (2-0, 0436) = 0, 0436 / 1, 9564 = 0, 0222 = 2, 22%.

Possible inbreeding by comparing the maternal halves in males with maternal halves in females = 0, 0441/ (2-0, 0441) = 0, 0436 / 1, 9559 = 0, 0225 = 2, 25%.

Total possible inbreeding = 0, 0438 / (2-0, 0438) = 0, 0438 / 1, 9562 = 0, 0224 = 2, 24%.

As proper relationship, the possible inbreeding per whole population = (2, 38 + 2, 10)/ 2 = (2, 22 + 2, 25) /2 = 2, 24%.

Based upon the study carried out on the 80 pedigrees (corresponding to the 80 males in 1998 generation) of four random lines, there were found 10 double lines (common ancestors on the both sides of the pedigrees), of which two lines in the last two generations.

By calculations results the following data:

Total achieved inbreeding = 10/ (4 x 80) = 10/320 = 0,312 = 3, 12%.

Per generation achieved inbreeding = 0, 0312/7 = 0, 0044 = 0, 44%.

It was divided by 7 because there are 8 generations described into the pedigree, so 7 changes of generations.

The current inbreeding = 2/4 x 80 = 2/320 = 0, 0062 = 0, 62%.

The non-current inbreeding = 0, 0312 – 0, 0062 = 0, 0250 = 2, 5%.

Following the study upon a sample of 100 pedigrees (corresponding to 100 females in 1998 generation) of four random lines there were found 5 double lines, all of them with further ancestors than the last two generations.

By calculations results the following data:

Total achieved inbreeding = 5/ (4 x 100) = 5/400 = 0, 0125 = 1, 25%.

Per generation achieved inbreeding = 0, 00125/7 = 0, 0017 = 0, 17%.

It was divided by 7 because there are 8 generations described into the pedigree, so 7 changes of generations.

The current inbreeding = 0.

The non-current inbreeding = 1, 25%.

There are two aspects to remark: the total value lower in females and the lack of the current inbreeding.

Table 1

Possible and achieved inbreeding in a laying hen line

Specification	Males	Females	Half maternal in females with half paternal in males	Half maternal in males with half paternal in females	Total pedigrees
xxx	4,65	4,12	4,36	4,41	4,38
Possible inbreeding	2,38	2,10	2,22	2,25	2,25
Total achieved inbreeding	3,12	1,25	X	X	X

Table 2

Total, achieved and per generation inbreeding

Sex	No of generation changes	Total achieved inbreeding %	Achieved inbreeding per generation %	Current inbreeding %	Non-current inbreeding%
Males	7	3,12	0,44	0,62	2,50
Females	7	1,25	0,17	0	1,25

By calculations, in males' results:

Line inbreeding = 0, 0250- 0, 0238 = 0, 0012 = 0, 12%.

Subdivision index = 0, 0250/ 0, 0238 = 1, 05.

In females there is not line inbreeding because the possible inbreeding is higher than the non-current inbreeding. The subdivision number is under a unit. The subdivision index = 0, 0125/ 0, 0210 = 0, 60.

3. CONCLUSIONS

3.1 It may notice in table 1 that the highest proper relationship recorded in males (4, 65%), that leads to a possible inbreeding of 2, 38%. Even the achieved inbreeding was higher (3, 12%), this value (2, 38%) shows that in the breeding activity of the studied line there were measures for avoiding as possible the related mating. The selection pressure by males leads to a small increasing of inbreeding due to some common ancestors in further generations, unknown as pedigree data. That is why the mating is based upon the recording number that offers information only about the last parental generation.

3.2 Proper relationship in females (4, 17) determined a possible inbreeding of 2, 10% and the total achieved inbreeding was 1, 25%. The large number of females permitted a more efficient avoiding of the related mating.

3.3 The achieved inbreeding per generation in the studied line males maintains the population within a non inbred line.

3.4 The value of the inbreeding coefficient per generation is lower than in females (0,17%) this means a consequence of the special measures of the selection program, proving that this line is under the total control of selection.

3.5 The line inbreeding in males was 0, 12%, probably caused by the small relationship among animals.

3.6 The value of the subdivision index in males was 1, 05, that shows there are not subdivisions within the line.

3.7. In females there is not any line inbreeding and the value of the subdivision index is very low (0, 60), that means there are not subdivisions within the line.

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RESEARCHES REGARDING THE OVINE METIS YOUTH RAISED IN SEMI INTENSIVE SYSTEM

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Key words: lambs, metis youth, fattening period, daily gain, average body weight.

SUMMARY

The present paper has as aim to emphasize the role of increasing the farmers budget by the lamb fattening using an semi intensive system despite their slaugtering at a very early age, around the Easter holiday. Also, it is underlined the role of the crossing betweeen the local breed Palas Merino, a breed especially used for wool in our country and other breeds, specialized in the meat production.

INTRODUCTION

Sheep breeding represents an ancient occupation of the animal breeders in many areas of the world with a special importance mainly to the productions obtained from them but also to the fact that this species feeds along natural pastures and secondary vegetal products, ovine can use pastures with a poor vegetation and in less accesible areas for large ruminants.

The most efficient way of obtaining the ovine meat is represented by fattening. The different exploited ovine categories are established by the consummers prefferences which are influenced by the civilization range, custom, religion and geographical area.

Depending upon the animal category under fattening, slaughtering age and the quality of the carcass there are the following fattened ovine categories: lambs, lambs of 100-120 days, fattened youth,adults and emasculated males. These categories could be fattened in different systems (extensive, semi intensive and intensive), the framing in one of the systems being established upon the accomodation system, number of the animals per area unit, automatization range, feeding system and the length of fattening.

MATERIAL AND METHODS

The biologic material researched in the present paper was represented by health animals with non infectious diseases, with a good

status from the zootechnical point of view, owing to the following breeds or metis: Palas Merino, Texel-Palas Merino and Suffolk- Palas Merino.

The sheep maintening was made along 150-160 days zile on the natural pastures in Bran, where the stables were established every year.

The feeding was made with mountain hay 1,5-2 kg/animal and day; with furaje suculente, beetrot or gulii 1,5- 2 kg/animal and days; with a concentrate forage mix with the following structure: 25-30% oat, 50-60% corn, 8-12% soya cakes, 1% salt, 2% edible chalk.

When the youth under fattening achieved 35 kg body weights the fattening period was considered finished and the animals were appreciated by the biometric methods.

After the lambs weaning the experimental groups are consisted for the 120 days fattening, the animals being chosen randomly 20 animals for each group,Texel x Palas Merino group, Suffolk x Palas Merino group and PalasMerino group. The fattening control was carried out by periodic individual weighing establishing the raising speed expressed by the total gain during the three phases of the fattening period and the average gain, confirmingly the tables.

RESULTS AND DISCUSSIONS

Palas Merino lambs, fattened in semi intensive system at the beggining of the fattening period have the average body weight of 14,62 ±0,67 kg, at the final of starting period 16,71 ± 0,44 kg, and at the and of the proper fattening period 29,61 ± 1,69 kg. They finished the trial at 34,82 ± 2,01 kg body weight..

Metis lambs of Texel x Palas Merino have at the beggining of the fattening period the average body weight of 15,68 ± 0,63 kg, at the final of starting period 18,02 ± 0,58 kg, and at the and of the proper fattening period 32,52 ± 1,68 kg . They finished the trial at a body weight of 38,52 ± 2,14 kg.

Metis lambs Suffolk x Palas Merino have at the beggining of the fattening period the average body weight of 15,22 ± 0,67 kg, at the final of starting period 17,39 ± 0,45kg, and at the and of the proper fattening period 31,48 ± 1,58 kg . They finished the trial at a body weight of 36,65 ± 2,11 kg.

The evolution of the body weight in the three groups of fattened lambs may be focused in the chart below, where it may notice that they start at closed initial weights, they have almost closed values in the

starting period but at the final of the fattening and also finishing period appear body weight differences among the three studied groups.

Table 1

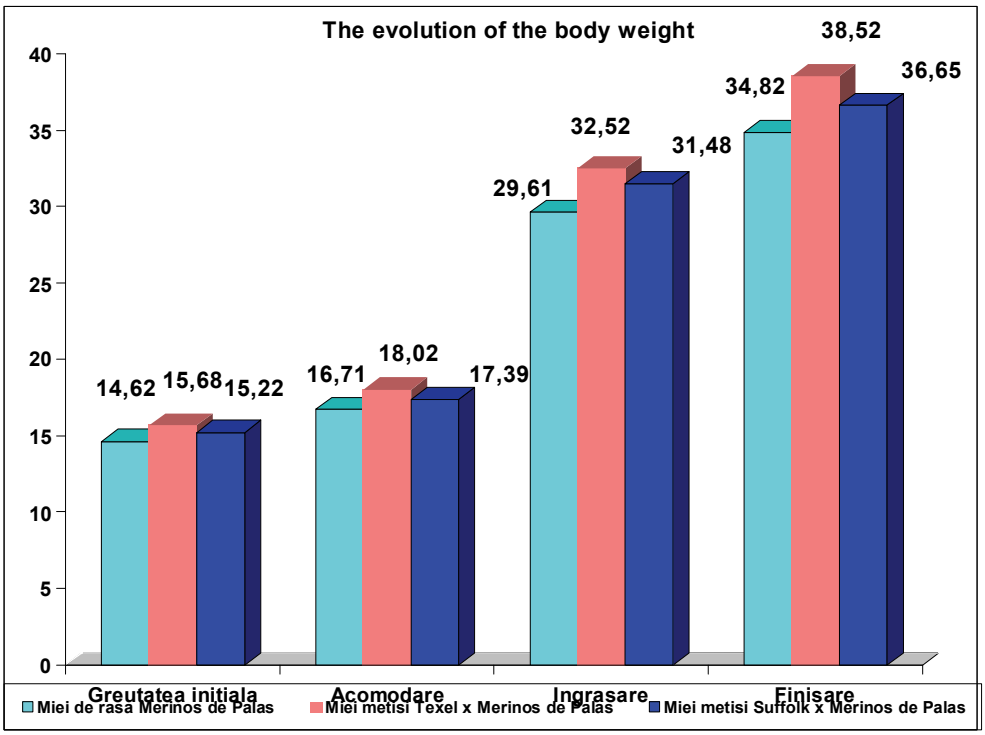
Body weight evolution in ovine youth fattened in semi intensive system

specification	Initial body weight	the fattening phases results		
		starting (15 days)	fattening (80 days)	finishing (25 days)
	- - X ± sx V%	- - X ± sx V%	- - X ± sx V%	- - X ± sx V%
Palas Merino (n=20)	14,62 ±0,67 20,49	16,71±0,44 11,77	29,61 ±1,69 25,52	34,82 ±2,01 25,81
Texel x Palas Merino (n=20)	15,68 ±0,63 17,96	18,02±0,58 14,39	32,52±1,68 23,10	38,52 ±2,14 24,84
Suffolk x Palas Merino (n=20)	15,22±0,67 19,68	17,39±0,45 11,57	31,48±1,58 22,44	36,65 ± 2,11 25,74

Table 2

Daily average gain in ovine youth fattened in semi intensive

Specification	Fattening phases and their length			Total gain (120 days)
	starting (15 days)	fattening (80 days)	Finishing (25 days)	
	- - X ± sx V%	- - X ± sx V%	- - X ± sx V%	- - X ± sx V%
Palas Merino (n=20)	139,3 ±8,15 26,16	161,2±8,39 23,27	208,4±9,17 19,67	163,3±8,56 31,90
Texel x Palas Merino (n=20)	156,0±9,29 26,12	181,2±8,34 20,58	240,0±9,19 17,12	190,3±9,24 21,71
Suffolk x Palas Merino (n=20)	144,6±8,32 25,73	176,1±9,44 23,97	206,8± 9,33 20,17	178,5±8,51 21,32



Analyzing the average body weight daily gain achieved by phases and by the total semi intensive fattening period it may notice that Palas Merino lambs have a total gain of 139,3 ± 8,15 g during starting period, 161,2± 8,39 g during the proper fattening phase, 208,4 ± 9,17 g during the finishing phase, and along the total fattening period 163,3 ± 8,56 g.

In Texel x Palas Merino metis lambs during all the fattening phases there were obtained larger gains comparatively the other lambs in the two groups Palas Merino lambs and Suffolk x Palas Merino. There was obtained an average daily gain of 156,0 ± 9,29 g during starting period, 181,2 ± 8,34 g during the proper fattening period, 240,0 ± 9,19 g during the finishing phase, but 190,3 ± 9,24 g during the whole period.

In Suffolk x Palas Merino metis youth, during all the fattening phases and the total fattening period there were obtained larger gain values comparatively the ones achieved by the Merino lambs, 144,6 ± 8,32 g during starting period, 176,1 ± 9,44 g during the proper fattening period, 206,8 ± 9,33 during the finishing phase, but 178,5 ± 8,51 g during the whole period.

CONCLUSIONS

After the researches during all our studies it may conclude the following:

1.The most performant evolution of the body weight in the ovine youth fattened in a semi intensive system at the final of the finishing phase was achieved by Texel x Palas Merino sheep with 38,52±2,14kg.

2.The daily average gain in the the ovine youth fattened in a semi intensive system recorded the minimum value in Palas Merino sheep with 163,3±8,56 g, and the maximum in Texel x Palas Merino with a value of 190±9,24 g, results achieved at the final of the 120 days.

3.The highest values of the main body siyes were recorded in Texel x Palas Merino metis youth .

4. The meat producing using this industrial crossing among the local sheep (in our case Merino sheep) and males of Texel and Suffolk breeds, assuring the fattening in semi intensive system represent a way of increasing the income of the farmers by obtaining fattened lambs at convenient prices, and also a way to an efficient sheep breeding.

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NEW APPROACHES IN THE USE OF THE MEAT *P*H VALUE IN ITS ORGANOLEPTIC CHARACTERIZATION

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Key words: meat *p*H value, meat freshness, meat organoleptic qualities.

SUMMARY

It was determined the *p*H values of the pork, beef and chicken refrigerated and frozen meat carcasses in four sausage plants of medium and small size in Bucharest city. Some differences were found according to the freezing conditions of the sausage plants. According to the obtained data, mean values of the pH were higher in the refrigerated meat vs. frozen meat, for any species (pork, beef or chicken). By comparing to the size and technological conditions, no differences were found: the percent normal *p*H values were framed in normal limits in the all the four monitored sausage plants.

1. MATERIAL AND METHODS

Determinations were performed on meat carcasses from four sausage units of medium or small capacity from Bucharest city. The sausage units were noticed as Unit A, Unit B, Unit C and Unit D. The determinations of *p*H were performed on pig, beef and chicken carcasses, refrigerated or frozen meat, between October 2007 and February 2009. According to their production capacity, units A and B are of medium capacity levels while units C and D they are of small capacity levels. *p*H measuring was performed using a HANNA *p*H meter of ±0.01 variation limits. The data were processed and the results were presented as mean ± standard error of mean ($\overline{x} \pm s_{\overline{x}}$).

2. REZULTS AND DISCUSSION

The results of *p*H determination are presented in Table 1. From the data presented in table 1 it can see that they were analyzed a number of 428 carcasses meat from the four monitored sausage units. The small number of samples from the units C and D it is due to fact that in these units processed only a little number of pork carcass meat and their production capacity is smaller then those of the units A and B.

Table 1

The results of *p*H value determinations of staple–meat used in the frame of the four studied sausage plants

Unit	Type of meat	Total number of samples[1]	Variation limits (minimal ÷ maximal values)	No and % of samples over normal values[2]	$\overline{X} \pm s_{\overline{x}}$
A	Pork refr. meat	15	5.54 ÷ 6.54	3	5.86±0.32
	Pork decong. meat	37	5.69 ÷ 6.50	2	6.08±0.22
	Beef refrig. meat	13	5.99 ÷ 6.40	5	*6.22±0.16*
	Beef decong. meat	42	5.40 ÷ 6.59	2	*5.99±0.32*
	Chicken ref. meat.	36	5.54 ÷ 6.42	4	5.89±0.76
	Chicken decon. meat	16	5.87 ÷ 6.34	-	6.16±0.21
	TOTAL	155	5.40 ÷ 6.59	17 (11.0%)	6.00±0.05
B	Pork refr. meat	3	6.20 ÷ 6.21	-	*6.20±0.11*
	Pork decong. meat	49	5.55 ÷ 6.60	3	*5.97±0.54*
	Beef refrig. meat	22	5.33 ÷ 6.21	-	5.77±0.34
	Beef decong. meat	27	5.76 ÷ 6.39	-	6.22±0.30
	Chicken ref. meat.	11	5.73 ÷ 6.56	2	6.03±0.15

[1] Each sample shows the mean of three determination values from different points of the same carcass.

[2] Normal values from V. Stănescu, *Igiena şi controlul alimentelor*, 2nd edition, 2006, p.175: refrigerated meat, max. 6.2, congealed and decongealed meat, max. 6.4.

	Chicken decon. meat	38	5.55 ÷ 6.61	6	6.03±0.31
	TOTAL	150	5.33 ÷ 6.61	11 (7.3%)	6.01±0.28
C	Pork refr. meat	-	-	-	-
	Pork decong. meat	61	5.49÷6.63	5	6.04±0.72
	TOTAL	61	5.49- 6.63	5 (14.4%)	6.04±0.10
D	Pork refr. meat	14	5.71 ÷ 6.40	2	5.94±0.44
	Pork decong. meat	30	5.75 ÷ 6.40	-	6.16±0.32
	TOTAL	44	5.71 ÷ 6.40	2 (4.5%)	6.09±0.41
TOTAL GENERAL		428		35 (8.17%)	

Normal maximal values of *p*H were 6.20 for refrigerated meat for any analyzed species.

Taking into account the variation limits of the device (±0.01), the samples smaller than 6.21 as *p*H values were considered as in normal limits. For de-congealed meat, the normal maximal values of *p*H were up to 6.40. For the same reasons as for refrigerated meat, the samples of *p*H values of 6.41 were considered in normal limits.

The data presented in table 1 show higher mean *p*H values for refrigerated meat by comparing with de-congealed meat for any species (pork, beef and chicken).

A number of 155 *p*H determinations were performed from unit A. The *p*H value of 17 carcasses from the 155 showed values of *p*H over the normal limits. This number represents 10.96% from the total number of the 155 carcasses.

From sausage unit B it was determined the *p*H values of 150 carcasses. 11 carcasses showed over-normal limits. The percent of these 11 carcasses represents 6.66% from the total number of 150 carcasses. Beef de-congealed meat *p*H values ranged between the largest limits: 5.40 ÷ 6.59.

In the case of C sausage unit, the *p*H values were determined in 61 carcasses of pork: 32 carcasses of refrigerated pork meat and 29 carcasses of de-congealed pork meat. It was found out a number of five

carcasses of those *p*H values was higher than the maximal limit. The number of five carcasses represents a percent of 14.4% from the total number of 61 analyzed carcasses from this sausage unit.

A number of 44 carcasses of pork refrigerated and de-congealed meat were analyzed from *p*H point of view in the sausage unit D. Two carcasses of pork refrigerated meat presented *p*H values over the normal limits. These two carcasses represent 4.5% from the total 44 carcasses analyzed in the frame of this sausage unit.

It is well-known the fact that the *p*H value is largely used to represent the acidity or alkalinity of certain products. Especially in food industry, the acidity and the alkalinity of any products could be very important, so the *p*H value could be an important parameter used for a lot of reasons. Food taste is one of these reasons: taste could be influenced by the *p*H value. Other organoleptic food features could be also influenced by the *p*H value: freshness, food preservation and even food bacteriological activity [6].

*p*H value in the alive skeletal muscle tissue is 7.2. When the skeletal muscle become meat (after slaughtering), the glycogen is broken in glucose which in turn is hydrolyzed to lactic acid in the anaerobic glycolise. As a consequence, *p*H value declines to values ranged between 5.2 and 7.0. The velocity of this process of *p*H declining is influenced by a lot of factors such as species, breed, or the medicament treatments before slaughtering. Beef meat reaches minimal values of *p*H of 5.4 to 5.7 in 18-24 hours after slaughtering. Pork meat reaches *p*H minimal values of 5.4 to 5.8 in 6-10 hours after slaughtering. When the minimal *p*H values have been reached, *p*H values raises again, slowly but steadily.

In PSE (*Pale*, *Soft*, *Exudative*) meat, *p*H declines suddenly under 5.8 value in the first hour after slaughtering. If the *p*H declines to the normal *p*H of 5.5-5.7 within 45 min or less, the muscle will appear very pale and soft (PSE). The color changes observed in PSE meat are mostly due to structural changes in muscle.

The rate and extent that muscle *p*H declines postmortem are both variable and have a great impact on the color of meat and meat products.

The data from autochthon literature show lower values of *p*H in refrigerated meat by comparing to decongealed meat [4, 5].

Our comparative researches revealed that in four from the seven situations from the table 1, the mean of *p*H values were higher in decongealed mead by comparing to refrigerated meat in any species (see underlined mean values in the table). The values show significant differences of *p*H according to the preserving temperature of the meat.

On the other hand, no differences were found out according to the size capacity of the sausage units. The percents of over normal limits samples were 11.0 and 7.3 in medium size unites A and B, respectively, and 14.4 and 4.5 in small size units C and D, respectively.

According to the data from the literature, for cooked (boiled) sausages the normal values of the meat must range between 5.8 and 6.3. Meat of higher *p*H values retains more water. Such a meat looses less weight during smoking and drying. Some organoleptic features, such as consistency, appearance and "bite" of the sausages from this kind of meat could be decidedly better.

Ideal *p*H value of the meat for fresh (uncooked) sausage is 5.4 to 5.8. Only meat with low *p*H value is suitable for fresh sausage manufacture.

Meat with low *p*H value avoids the development of binding similar to that in boiled sausages which arises when shredding at near to 0°C. Such binding has a negative influence on filling and dry processing. It often leads to dry edges, which causes changes in acidity leading to poor smell and taste.

In addition, a low *p*H discourages the development of undesirable micro-organisms. In turn, desirable micro-bacteria have a chance to develop, which improves the taste and keeping characteristics. A low *p*H value of the meat to start with also aids drying in the first hours after filling.

According to Baston *et al.*, *p*H monitoring of the meat offer important data on the freshness of the chicken meat. In a study on chicken meat, the authors evaluated the level of easy hydrolysable nitrogen and the *p*H and biogenic amines contents. The authors found out a progressive increase of meat *p*H from the first day (5.92) to 20th day of refrigerating (7.33). According to their obtained data, the authors calculated the freshness of refrigerated chicken meat for a maximum of three days [1].

Hassan *et al.* measured the variations of *p*H values in meat and some meat products as an indicator of the normal flora influence into Port Said markets [3]. The authors found out that maximal value of pH was 5.81 in the samples of fresh meat while the minimum value was 5.44 in the fresh meat products. The minimal values of the *p*H were assigned to the development of the different species *Lactobacillus.*

Further important uses of *p*H are as it is following:

- it is necessary to specify in the meat for vacuum packing while maturing (the meat must have a *p*H below 5.9 if it is to keep);

- *p*H values aids in the testing of the suitability of meat and meat products for sale or whether it is spoiling (*p*H value confirms the impressions gained by the senses);

- last but not list, a change to alkaline value of the *p*H in the brine (pickle) indicates that it is gone off [7].

In this case, *p*H measuring is a current determination in the meat industry, and it is obligatory in some countries [6].

An interesting study on the correlations between the evolution of *p*H value levels and the easy hydrolysable ammonium levels in the meat in a long period of preservation was performed by Danilievici [2]. The author studied the combined effects of two preservation methods upon beef meat: refrigeration and u.v. irradiation. The preservation period could be prolonged with 70% when the two methods were applied in a combined method by comparing to the only refrigerating method. Following eight days of refrigeration, the ammonium content raised from 12 to 35 mg/100 g of meat while the *p*H rose from 5.4 to 6.3. Both, ammonium and *p*H values reached the highest admitted limits.

Finally, it must to be mentioned that only the *p*H in itself is unsatisfactory to determine the freshness of the meat or meat products; its values must be correlated with a set of analyses [4,5).

3. CONCLUSIONS

3.1. *p*H values analysis allowed the identification of some differences linked by species and preservation temperature (refrigeration or congelation freezings)

3.2. Mean values of *p*H were higher for refrigerated meat by comparing to congealed meat, for any analyzed species (pork, beef, chicken).

3.3. They were not identified any differences according to the technological conditions of any sausage plants.

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THE INFLUENCE OF TIME AND TEMPERATURE STORAGE ON THE BACTERIOLOGICAL LOAD OF SEVERAL CATERING PRODUCTS

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Key words: catering products, bacteriological, contamination, time and temperature

SUMMARY

This study followed the influence of time and temperature storage on the bacteriological load of several catering products, which were storage at room temperature for 6 hours from the ending of the preparation process.
The study was performed during 01.01.2008 – 31.12.2008 in to a catering unit from Bucharest with Romanian traditional food which undergo thermical treatment: chicken soup, chicken rolls with spicy sauce, pork schnitzel, grill Bleu Shimel, chicken legs in pot, perch fingers, French fries with cheese, Serbian risotto, omlet with bacon and bell peppers, pancakes with sweet cheese and raisins.
The temperature, which is not correctly monitored, is the main factor that supports the growing of pathogenic germs responsible for foodborne outbreaks, so it is essential to have a control on the temperature in order to minimalize the risk of food contamination.

The operator's works are based on a correct producing process in so far to obtain a large variety of products in to an efficient mode.

However, if the process is not correctly done, the safety of the product can be blemished.

For this reason is very important the temperature control on the preparations maintained at the established temperature.

The preparation stage at minim 75-80°C should destroy the vegetative forms of microorganism, and without a temperature control there is a risk that the spores of *Clostridium* or/and *Bacillus* to survive, germinate and multiplicate.

The cooking, frying or backing is steps through which the raw materials suffer physical, chemical transformations in so far that the meal will gain a particular taste and nutrition value.

At the same time, the thermic treatment has the role to destroy the microorganism that may alter the final product in a short time.

It is necessary to interdict maintaining the preparations at temperature that may increase a risk on human health, e.g. being a support for bacteria grow and toxin formation.

This study followed the influence of time and temperature storage on the bacteriological load of several catering products, which were storage at room temperature for 6 hours from the ending of the preparation process.

MATERIALS AND METHODS

The study was performed during 01.01.2008 – 31.12.2008 in to a catering unit from Bucharest with Romanian traditional food which undergo thermical treatment: chicken soup, chicken rolls with spicy sauce, pork schnitzel, grill Bleu Shimel, chicken legs in pot, perch fingers, French fries with cheese, Serbian risotto, omlet with bacon and bell peppers, pancakes with sweet cheese and raisins.

From each product were sampled monthly two samples so that on the end there were 24 samples for product.

The samples were taken immediately after finishing the meal from the preparation aria (100g/sample) into a sample recipient with adequate instruments.

For each product one sample was analyzed immediately after sampling and the other one was maintained at room temperature for 6 hours.

The methods consisted of sampling a known quantity of sample, which was added to a selective enrichment medium specific to each bacteria taken in account. After that was the striking, on a solid medium of selective isolation and after incubation the developed colonies were counted and the numbers were introduced into a formula to determine the number of colonies expressed per gram or milliliter, depending on the type of product (Apostu, 2004).

The bacteriological parameters determine were Total Bacteria Count according to the SR EN ISO 4833, coliform bacteria according to SR EN ISO 4832, E. *coli* according to ISO 16649-1 and 2 and *Salmonella* spp. according to SR EN ISO 6579, coagulase positive *Staphylococcus* according SR EN ISO 6888-1 or 2, *Bacillus cereus* according SR EN ISO 7932, ISO 21781, sulfite reducing bacteria according ISO 15213 respecting the HACCP programme established by the company.

RESULTS AND DISCUTIONS

The results on the microbiological parameters listed in the HACCP programme are presented:

- Total Bacteria Count: 10.000 - SR EN ISO 4833
- coliforme bacteria: max. 10- SR EN ISO 4832

- *Escherichia coli*: absent - ISO 16649-1, ISO 16649-2
- *Salmonella* spp.: absent SR EN ISO 6579
- *Coagulase Positive Staphylococcus*: max. 1 SR EN ISO 6888-1, 6888-2
- *Bacillus cereus*: max. 1 SR EN ISO 7932; ISO 21871
- Sulfite reducing bacteria: 1 ISO 15213
– *Listeria monocytogenes*: abs.

The results are presented in the tables 1, 2, 3, 4, 5 and 6.

Table 1

Values obtained from the samples analyzed immediately after sampling at the following parameters TBC, *Listeria monocytogenes*, coliform bacteria, E.coli

Catering products	Number of samples	Total Bacteria Count /g values mean	*Listeria monocytogenes*/g	Colif./g	*E.coli*/g
Chicken soup	12	$8x10^2$	Abs.	Abs.	Abs.
Chicken rolls with spicy sauce	12	$6x10^3$	Abs.	Abs.	Abs.
Pork schnitzel	12	$2x10^2$	Abs.	Abs.	Abs.
Grill Bleu Shimel	12	$2x10^3$	Abs.	Abs.	Abs.
Chicken legs in pot	12	$7x10^2$	Abs.	Abs.	Abs.
Perch fingers	12	$5x10^2$	Abs.	Abs.	Abs.
French fries with cheese	12	$7x10^3$	Abs.	Abs.	Abs.
Serbian risotto	12	$2x10^3$	Abs.	Abs.	Abs.

Omlet with bacon and bell peppers	12	$4x10^3$	Abs.	Abs.	Abs.
Pancakes with sweet cheese and raisins	12	$8x10^2$	Abs.	Abs.	Abs.

Table 2

Values obtained from the samples analyzed immediately after sampling at the following parameters *Salmonella* spp, *Staphylococus* cp, *Bacilus cereus*, sulfite reducing bacteria

Catering products	Number of samples	*Salmonella* spp/25g	Staphy cp/g	*B.cereus*/g values mean	Sulfite reducing bacteria/g values mean
Chicken soup	12	Abs.	Abs.	Abs.	Abs.
Chicken rolls with spicy sauce	12	Abs.	Abs.	Abs.	Abs.
Pork schnitzel	12	Abs.	Abs.	$2x10^1$	Abs.
Grill Bleu Shimel	12	Abs.	Abs.	Abs.	$1x10^1$
Chicken legs in pot	12	Abs.	Abs.	Abs.	Abs.
Perch fingers	12	Abs.	Abs.	Abs.	Abs.
French fries with cheese	12	Abs.	Abs.	Abs.	Abs.
Serbian risotto	12	Abs.	Abs.	Abs.	Abs.
Omlet	12	Abs.	Abs.	Abs.	Abs.

with bacon and bell peppers					
Pancakes with sweet cheese and raisins	12	Abs.	Abs.	Abs.	Abs.

Results obtained from the samples analyzed immediately after sampling according to the microbiological parameters listed in the HACCP are presented in Table 3.

Table3

Results obtained from the samples analyzed immediately after sampling according to the microbiological parameters listed in the HACCP

Parameters	Number of samples	Compliant	Non compliant	% Non compliant samples
TBC/g	120	120	0	0,00%
Listeria onocytogenes/g	120	120	0	0,00%
colif bact./g	120	120	0	0,00%
E.coli/g	120	120	0	0,00%
Salmonella/25g	120	120	0	0,00%
cp Staf /g	120	120	0	0,00%
B.cereus/g	120	118	2	1,67%
SR bact./g	120	119	1	0,84%

According to HACCP programme the preparations are made in advance and then maintened not more then 2 hours at least 63^0 C.

The samples tacking into analyses were stored at room temperature for 6 hours.

The results on the determinate parameters are presented in tables 4, 5 and 6.

Table 4

Values obtained from the samples analyzed after 6 hours of storage at room temperature at the parameters Total Bacteria Count, *Listeria monocytogenes*, Coliforme bacteria, *E.coli*

Catering products	Number of samples	TBC/g values mean	*Listeria monocytogenes*/g	Coliforms/g	*E.coli*/g
Chicken soup	12	$7x10^3$	Abs.	$3,8\ x10^1$	Abs.
Chicken rolls with spicy sauce	12	$6x10^5$	Abs.	$3,5\ x10^1$	Abs.
Pork schnitzel	12	$3x10^4$	Abs.	$2,7\ x10^1$	$0,3\ x10^1$
Grill Bleu Shimel	12	$2x10^5$	Abs.	Abs.	Abs.
Chicken legs in pot	12	$7x10^3$	Abs.	Abs.	Abs.
Perch fingers	12	$5x10^4$	Abs.	Abs.	Abs.
French fries with cheese	12	$4x10^6$	Abs.	Abs.	Abs.
Serbian risotto	12	$2x10^5$	Abs.	$4\ x10^2$	$2\ x10^1$
Omlet with bacon and bell peppers	12	$4x10^5$	Abs.	$3\ x10^2$	$3\ x10^1$
Pancakes with sweet cheese and raisins	12	$6x10^4$	Abs.	$5\ x10^2$	Abs.

Table 5

Values obtained from the samples analyzed after 6 hours of storage at room temperature at the parameters Salmonella, Staphylococcus cp, Bacillus cereus, sulfite reducing bacteria

Catering products	Number of samples	*Salmonella*/25g	*Staphy.*cp /g	*B.cereus*/g values mean	Sulfite reducing bacteria /g values mean
Chicken soup	12	Abs.	Abs.	Abs.	Abs.
Chicken rolls with spicy sauce	12	Abs.	Abs.	Abs.	Abs.
Pork schnitzel	12	Abs.	$1,1 \times 10^1$	2×10^2	Abs.
Grill Bleu Shimel	12	Abs.	Abs.	Abs.	2×10^2
Chicken legs in pot	12	Abs.	Abs.	3×10^2	Abs.
Perch fingers	12	Abs.	Abs.	$1,1 \times 10^1$	3×10^2
French fries with cheese	12	Abs.	Abs.	Abs.	Abs.
Serbian risotto	12	Abs.	Abs.	$3,6 \times 10^1$	Abs.
Omlet with bacon and bell peppers	12	Abs.	5×10^1	Abs.	Abs.
Pancakes with sweet cheese and raisins	12	Abs.	4×10^1	Abs.	Abs.

Results obtained from the samples analyzed after 6 hours of storage at room temperature after sampling according to the microbiological parameters listed in the HACCP are presented in Table 6.

Table 6

Results obtained from the samples analyzed after 6 hours of storage at room temperature after sampling according to the microbiological parameters listed in the HACCP

Parameters	Number of samples	Compliant	Non compliant	% Non compliant samples
TBC/g	120	80	40	33,34%
Listeria onocytogenes/g	120	120	0	0,00%
Parameters	Number of samples	Compliant	Non compliant	% Non compliant samples
colif bact./g	120	90	30	25,00%
E.coli/g	120	105	15	12,50%
Salmonella/25g	120	120	0	0%
cp *Staphylococcus* /g	120	114	6	5,00%
Bacillus cereus /g	120	100	20	16,67%
sulfite reducing bacteria /g	120	113	7	5,83%

At the samples analyzed right after sampling can be noted, that according to tables 1 and 2 the parameters Total Bacteria Count, *Listeria monocytogenes*, Coliform bacteria, *E. coli*, *Salmonella* spp and *Staphylococcus* cp were compliant with the values listed in the HACCP program established by the company.

According with the provisions of the HACCP program, the values for *Bacillus cereus* on pork schnitzel from two samples and the value for sulfite reducing bacteria on grill Bleu Shimel from one sample were above of the admitting limit.

As presented in table 3 the noncompliant percentage at *Bacillus cereus* was 1, 67% and for sulfite reducing bacteria was 0, 84%.

At the samples, analyzed six hours after sampling and storage at room temperature (22°C) can be noted according to tables 3 and 4 that *Salmonella* spp and *Listeria monocytogenes* were not detected at any sample.

In table 6 are observed: 40 samples are noncompliant at the parameter TBC, 30 sample are noncompliant at the parameter coliform bacteria, 15 samples are noncompliant at the parameter *E. coli*, six samples are noncompliant at the parameter coagulase positive *Staphylococcus*, 20 samples are noncompliant at the parameter *Bacillus cereus*, seven samples are noncompliant at the parameter sulfite reducing bacteria.

According to table 6 the percentage of noncompliant samples are 33,34% at the parameter TBC, 25,00% at the parameter coliform bacteria, 12,50% at the parameter *E. coli*, 5,00% at the parameter coagulase positive Staphylococcus, 16,67% at the parameter *Bacillus cereus* and 5,83% at the parameter at the sulfite reducing bacteria.

These results prove that the samples storaged at room temperature (22°C) for six hours were of noncompliant quality.

The highest frequency of the noncompliant parameters was obtained for Total Bacteria Count followed by: coliform bacteria, *B. cereus*, *E. coli*, sulfite reducing bacteria and coagulase positive *Staphylococcus*.

3. CONCLUSIONS

3.1. The results obtain in this study shows the importance to observe the technological parameters for attaining salubrious catering products for example one of the significant factor is the operational and storage temperature. The temperature, which is not correctly monitored, is the main factor that support the grow of pathogenic germs responsible for foodborne outbreaks, so it is essential to have a control on the temperature in order to minimalize the risk of food contamination.

3.2. The results obtain from the samples that were analyzed six hours after sampling show that is indispensable to fulfill all the provisions from the HACCP programme regarding the storage of the catering products at lowest 63ºC for no more then two hours. The performed analyses on a significant number of samples reflect a high number of bacteriological noncompliant samples, which were stored at room temperature for six hours.

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EVALUATION OF THE BACTERIOLOGICAL CONTAMINATION FROM RAW MEAT USED IN OBTAINING CATERING PRODUCES

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Key words: meat, catering, bacteriological, contamination, analyses

SUMMARY

The degree of contamination of raw meat during cutting, boning and preparation of the meat used in the production of catering, depends on the hygienic conditions with which these operations are performed.
The study was performed between 01.01.2009 - 31.08.2009 in a catering establishment in Bucharest on the raw meat that originates from the four species of animals: cow, pig, sheep and poultry on 64 samples with five subunits.
The bacteriological parameters determined were TBC, *E. coli* and *Salmonella* spp and were analyzed using SR EN ISO 4833, ISO 16649-1 or 2 and SR EN ISO 6579 according to Regulation EC 2073/2005.
Following the analysis of a significant number of samples of raw meat used for the preparation of catering, the conclusion was that it was conform so that bacteriological agents incriminated in the main producing food poisoning were absent.

The degree of contamination of raw meat during cutting, boning and preparation used in the production of catering, depends on the hygienic conditions with which these operations are performed under the HACCP system implemented. During these operations, the meat is heavy manipulated and many surfaces are exposed to contamination (Barzoi and Apostu, 2002).

The contamination level reflects the hygiene in the catering establishments, as well as the composition of the contaminated microflora reflects the source of contamination and the effectiveness of the measures implemented to prevent the contamination of meat.

The meat used for the production of catering must come only from healthy animals, being known as fact that a number of infectious animal diseases may be transmitted to humans through consumption. Meat contamination may also occur during processing, which is an indicator of hygiene conditions of the unit.

The study followed the hygiene criteria compliance in the catering unit established by the processors and the safety criteria of the catering products obtained.

MATERIALS AND METHODS

The study was performed between 01.01.2009 - 31.08.2009 in a catering establishment in Bucharest on the raw meat that originates from the four species of animals: cow, pig, sheep and poultry on 64 samples with five subunits.

Monthly were sampled and analyzed two samples each with five subunits from each animal species. The samples consisted of minced meat obtained from cutted, deboned and minced pieces of carcasses received by the catering unit. The minced meat was used as raw material in various catering products.

The methods consisted of sampling a known quantity of sample, which was added to a selective enrichment medium specific to each bacteria taken in account. After that was the striking, on a solid medium of selective isolation and after incubation the developed colonies were counted and the numbers were introduced into a formula to determine the number of colonies expressed per gram or milliliter, depending on the type of product (Apostu, 2004). The bacteriological parameters determine were TBC according to the SR EN ISO 4833, *E. coli* according to ISO 16649-1 and 2 and *Salmonella* spp. according to SR EN ISO 6579 respecting the Regulation EC 2073/2005.

2. REZULTS AND DISCUTIONS

The results are presented in Tables 1, 2 and 3.

Table 1

The results obtained following the determination of TBC in minced meat

	Food category	Number of samples examined	Number of tests performed	Limits values obtained	Average values obtained
	Minced beef meat	16	90	$4x10^5$-$8x10^5$	$5x10^5$
	Minced pork meat	16	90	$3x10^5$-$6x10^5$	$5x10^5$
	Minced mutton meat	16	90	$3x10^5$-$2x10^6$	$8x10^5$
	Minced poultry meat	16	90	$2x10^6$-$4x10^6$	$3x10^6$

Table 2

The results obtained following the determination of *E.coli* in minced meat

No.	Food category	Number of samples examined	Number of tests performed	Limits values obtained	Average values obtained
1.	Minced beef meat	16	90	$2x10^1$-$6x10^1$	$4x10^1$
2.	Minced pork meat	16	90	$3x10^1$-$7x10^1$	$6x10^1$
3.	Minced mutton meat	16	90	$4x10^1$-$7x10^1$	$6x10^1$
4.	Minced poultry meat	16	90	$5x10^1$-$8x10^1$	$7x10^1$

Table3

The results obtained following the determination of *Salmonella* spp in minced meat

No.	Food category	Number of samples examined	Number of tests performed	Limits values obtained
1.	Minced beef meat	16	90	Absence
2.	Minced pork meat	16	90	Absence
3.	Minced mutton meat	16	90	Absence
4.	Minced poultry meat	16	90	Absence

The obtained results show up that there are no values exceeded according the Regulation 2073/2005.

TBC m=$5x10^5$cfu/g; M= $5x10^6$ cfu/g

E. coli m=50 cfu/g; M= 500 cfu/g

Salmonella spp.-abs/25grames.

Monthly two samples from each type of meat were sampled and the results did not show that there were major differences on the bacteriological overloads influenced by season.

Different sources of origin of the samples did not represent a significant variability factor regarding the limits of the obtained results.

Regarding the differences between animal species from which the meat was obtained, was observed small differences on the bacterial load, the largest load was noticed on minced chicken meat and the lowest on the minced beef meat.

3. CONCLUSIONS

3.1. The results from this study may indicate the level of bacterial contamination of the raw meat and after the interpretation of these results it can be concluded that the recorded values proves that the production, delivery, handling and processing of the raw materials has been achieved with respecting the hygiene criteria established at the slaughterhouse level and also in the catering unit.

3.2. Although the results of tests carried out show a relatively favorable situation on the bacterial overload, continuous monitoring both in the slaughterhouse and in the catering unit with a HACCP system is necessary in order for a quick identification of a potential source of bacteriological contamination and to minimize its effects on human health.

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MICROBIAL BIOFILM ON THE SURFACES FROM FOOD PROCESSING PLANT

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Key words: biofilm , processing plant, milk , meat

SUMMARY

In this paper we try to put into evidence the bacterial biofilm on different surfaces in two meat and milk processing plants utilizing the fluorescence microscopy. For the researches two processing plant was chosen: a milk processing plant and a meat processing plant. The surfaces with different level of polish were chosen for sampling. Also, from the equipment surfaces we sampled sanitation samples for *total number of germs*, *enterobacteria number* and *germs for Pseudomonas genus number* determination. For the biofilm identification the samples were taken by scrapping the surfaces with a knife. For each point of sampling were taken two probes in the similar manner. The first series of slides were staining with acrydine orange and the second series of probes were stained by Gram method and examined to optic microscope. In the milk processing plant the microorganisms aggregate included in biofilm were observed on following surfaces: internal surface of cooling tank, internal surface of creamer, floor surface, internal surface of pipe-line, at rubber garniture before the milk pump, the surface of paddle from milk cooling tank. In the meat processing plant, the microcolonies included in biofilm structure were observed on following structure: plastic cover of knife sterilizer, internal surfaces of knife sterilizer, worker rubber apron. In all samples the Gram positive cocci and Gram negative bacilli and cocobacili were isolated.
The major objective of food safety regulation is to assure a high level of food health consumers' protection. To achieve this, the processors are obliged to put on the market only sure and nutritive food. As follows, the control of the surfaces contamination level and the especially of those who are in contact with the food is essential. The sanitation programs made to reduction or remove the microorganisms is efficient only if the microbes are not integrated in the biofilm matrix.
The biofilm is a very complex group of microorganisms attached on different surfaces in wet medium and distinguish by structural heterogeneity, genetic diversity, complex interactions and an extra cellular matrix of polymeric substances (Characklis and Marshall, 1990; Chmielewski and Frank, 2003; Costerton, 1995; Evans, 2000; Flemming et al., 2000). The biofilm is often found on food surfaces and also on surfaces that are in contact with food. In some circumstances, the biofilm could be a reservoir of pathogen for consumers. A lot of papers show that the microorganisms benefit as matrix components by an increase of the resistance to biocide substances (Bower et al., 1996; Criado et al., 1994). The inactivation of the biofilm associated bacteria and the removal of biofilm matrix can be done much harder than the planktonic microorganism inactivation (O'Toole et al., 2000; Trachoo, 2003).
In this paper, we attempted to put in evidence the bacterial biofilms on different surfaces from the meat and milk processing plants using the fluorescence microscopy.

MATHERIAL AND METHOD

For researches, two processing plant was chosen: a milk processing plant and a meat processing plant. The surfaces with different polish levels o were chosen for sampling in order to put into evidence of microbial biofilm. Also, from the equipment surfaces we took sanitation samples for the *total number of germs*, *enterobacteria number* and *germs for Pseudomonas* genus. The sanitation samples (100 cm^2) were taken from the equipment and other surfaces. The surfaces from the meat processing plants: faience, table for cutting, hatchet, floor, plastic cover of knife sterilizer, internal surfaces of knife sterilizer, transporting cart, filling machine, worker rubber apron, table surfaces, plastic shuttle. The surfaces from the milk processing plant were: internal surface of pipeline before the milk pump, pipeline surfaces, milk heater exit, internal surface of pipeline at tampon vessel exit, the surfaces of rubber garniture before the milk pump, floor surface, internal surfaces of creamer, internal surface of milk distribution lid, internal surface of inoculation vessel, internal surface of cooling tank, the surface of paddle from milk cooling tank, table surface.

The sampling was performed using special swabs soaked in sterile physiological solution by wiping a surface of approximately 100 cm^2; later on, the tampons were placed in test tubes containing sterile saline solution, and then taken to the laboratory.

Total number of germs determination was made according to SR ISO 4833/ 2003. The test tubes were shaken and 1 cm^3 of physiological solution was drawn and inoculated in two Petri dishes and than pour PCA culture medium and mix together. After 72 hours of incubation at 30° C the colony growing on this medium were counting.

The *enterobacteria number determination* was made according to SR ISO 21528/2/ 2007. The enterobacteria isolation was made on *VRBG* medium (violet, red, bile, glucose agar). After the 24 hours at 37° C the pink, red or purple colony were selected and five typical colonies were tested for oxidase production and glucose fermentation.

The *pseudomonas germs determination* was made on the special medium for pigment production identification.

To identify the biofilm the samples were taken by scrapping the surfaces with a knife. For each point of sampling we took two samples in a similar manner. The first series of slides were stained with acrydine orange following the technique describes below: the samples were fixed on the slide with ethanol 96% for two minutes; the dehydrated samples were treated with Hanks modified solution (without D glucose and

phenol red) and than fixed with ethanol 96% for two minutes. The staining of samples with acrydine orange has been done for one minute. Microscope epifluorescence *Leica DM 2500 model* slides examination were made. Samples visualization were performed with the immersion objectives (the refraction indices of immersion liquid was 1,5180) 63x and 100x and the wave length was 488 nm. The second series of samples was stained by Gram method and examined with the optic microscope.

RESULTS AND DISCUSSIONS

In the samples taken from the milk and meat processing plant, we observed the presence of microorganisms as isolated colony or included in the biofilm matrix, were observed.

In the milk processing plant the microorganisms aggregates included in the biofilm were observed on the following surfaces: internal surface of cooling tank, internal surface of creamer, floor surface, internal surface of pipeline, at rubber garniture before the milk pump, the surface of paddle from milk cooling tank.

In the meat processing plant, the microcolony included in biofilm structure could be observed more evidently on the following structures: plastic cover of knife sterilizer, internal surfaces of knife sterilizer, worker rubber apron.

The stained images on epifluorescence microscope are presented in the figures 1-5 (for milk processing plants) and figures 6-8 (for meat processing plants).

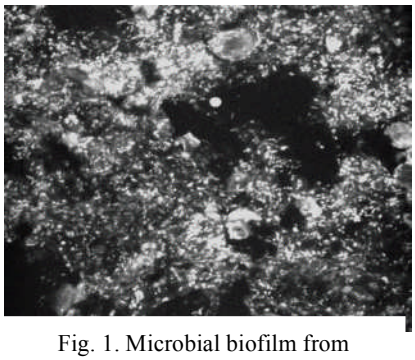


Fig. 1. Microbial biofilm from internal suface of cooling tank. Col. acrydine orange exam. epifluorescence microscope objective 63x

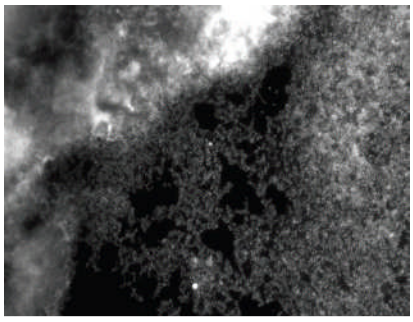


Fig. 2. Microbial biofilm from internal suface of creamer. Col. acrydine orange exam. epifluorescence microscope objective 100x

In the sanitation probes taken from the same points where we proved the presence of the microbial, from the milk processing plant, the contamination level (evaluated by standardized method) were different.

On the surfaces hardly accessible for sanitation (internal surfaces of rubber garniture before the milk pump, internal surfaces of creamer and floor surfaces), the general level of contamination was between 1 x 10^4 cfu/ cm^2, with number of *enterobacteria* of 5 x 10^2 - 5 x 10^3 cfu/ cm^2 and 8 x 10 cfu/ cm^2 of *Pseudomonas* genus. In the other probes samples taken from the paddle surface from the milk cooling tank and internal surface of cooling milk tank, the general level of contamination varied from 9 x 10^2 cfu/ cm^2 to 1 x 10^4 cfu/ cm^2, without *enterobacteria* and germs from the *Pseudomonas* genus.

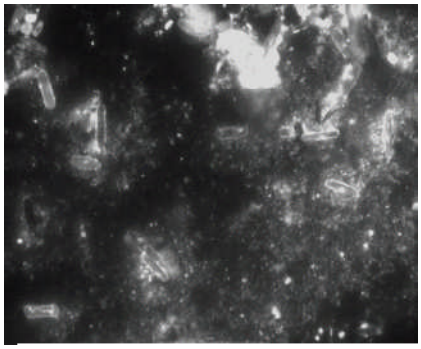


Fig. 3. Microbial biofilm from floor surface. Col. acrydine orange exam. epifluorescence microscope objective 63x

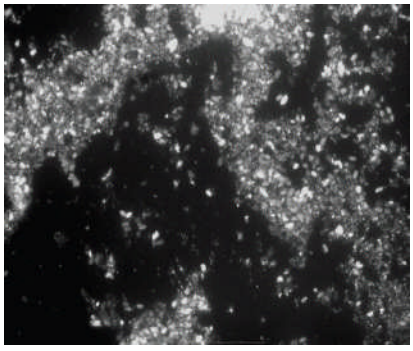
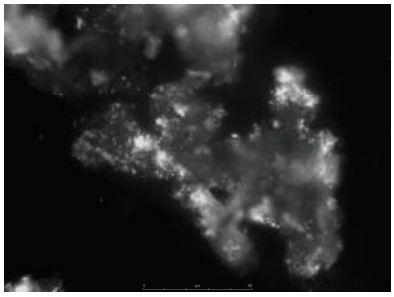


Fig. 4. Microbial biofilm from rubber garniture before the milk pump. Col. acrydine orange exam. epifluorescence microscope objective 63x

In generally, in milk processing plant we putting into evidence the microbial biofilm on those surfaces which had different roughness degrees: the surfaces of rubber garniture before the milk pump, the paddle surface from milk cooling tank, internal surfaces of creamer and floor surface.



On the whole, the milk processing plant surfaces are made of highly- polished stainless steel and the microorganisms require a longer time for attachment, so this is

the reason why we found microbial biofilm only in five sampling points.

The surfaces that made evident the presence of the biofilm are the surfaces which are in direct contact with the milk; they could be a source of microbial contamination for milk with these microorganisms which could be detached periodically from this structure and contaminate the milk.

Following the samples Gram stain examination from the same points were the microbial biofilm putting into evidence, in all samples (excepted the surface of cooling tank), surfaces which have contact with the milk before the pasteurization, the Gram positive cocci and Gram negative bacilli were isolated.

In the meat processing plant, the general contamination level was variable. The general contamination varied from 4 x 10^2 cfu/ cm^2 on internal knife sterilizer surface to 1,7 x 10^4 cfu/ cm^2 on the knife sterilizer cover and on the rubber apron worker. The contamination with *enterobacteria* of this surface varied from 1 cfu/ cm^2 to 2,1 x 10^3 cfu/ cm^2 and the germs from the *Pseudomonas* genus were present in low number (1 – 12 cfu/ cm^2).

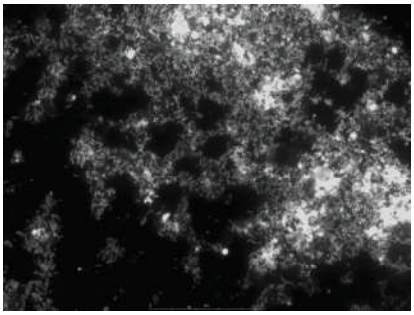


Fig. 6. Microbial biofilm on the internal surface of knife sterilizer. Col. acrydine orange exam. epifluorescence microscope objective 63x

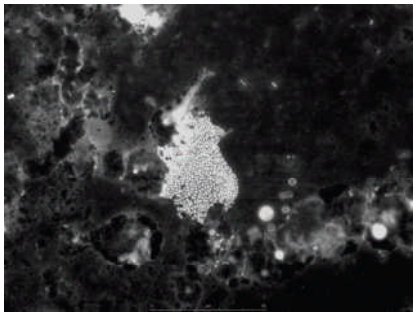


Fig. 7. Microbial biofilm on the rubber worker apron. Col. acrydine orange exam. epifluorescence microscope objective 63x

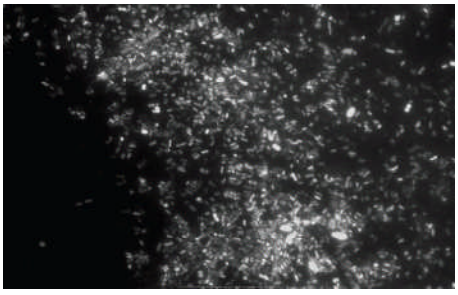


Fig. 8. Microbial biofilm on the plastic cover of knife sterilizer. Col. Acridine Orange exam. epifluorescence microscope objective 100x

Of the multitude of points where we took sanitation probes from, the presence of bacterial biofilm was evidenced only on tree surfaces: on the internal surface of knife steriliser, the plastic knife sterilizer cover and on the rubber apron surface. On these surfaces we identified microorganisms as aggregates included in biofilm-type structures.

In the meat processing plant, the biofilm was made evident, in this stage of research, only on the plastic surface of the sterilizer and on the rubber apron surfaces, although we took samples from other surfaces, too. We believe that this fail is the result of the bad method or bad staining chosen for biofilm taking and prelevation. The Holah et al. quoted by Hood and Zottola consider that the sampling of biofilm by removing from the surfaces is more accurate if the contamination level is bigger than 10^5 /cm^2 and if the contamination level is under this the direct microscope examination is more appropriate (Hood and Zottola, 1995).

Successive to the examination of the samples with evident bacterial biofilm, we observed the predominance of the Gram negative bacilli and Gram positive cocci.

The bacteria isolated on the specific culture media were stored and will be biochemically tested, including the resistance to antibiotics.

Other researchers put into evidence the microbial biofilm on surfaces from the food industry, too (Hood and Zottola, 1997; Evans et. al., 2004; Wong, 1998; Xianming Shi and Xinna Zhua, 2009). These results represent just the first step in our attempt to characterize the identified biofilm and to identify the better way for control it.

CONCLUSIONS

3.1. The presence of the microbial biofilm was made evident on five surfaces from the milk processing unit (internal surface of cooling tank, internal surface of creamer, floor surface, internal surface of pipeline, at rubber garniture before the milk pump, the surface of paddle from milk cooling tank) and three surfaces from the meat processing unit (plastic cover of knife sterilizer, internal surfaces of knife sterilizer, worker rubber apron);

3.2. The surfaces on which we identified the microbial biofilm were surfaces with a high degree roughness, stainless steel with low polishing level, plastic surfaces and rubber surfaces.

ACKNOWLEDGMENTS.

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THE MONITORING AND INSPECTION OF FOOD ADITIVES (2007)

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Key words: additives, food label, foodstuff.

SUMMARY:

Food additives are substances added intentionally in foodstuff for technological purpose The use of food additives in Romania is ruled by specific normatives. In the frame of the national activity of monitoring additives, the local public health authorities followed the presence of these substances, by looking for them on food labels, at the level of main producing companies from each county. After the verification of the additive list, the conclusion was, in all cases, that the additives were used in conformity with the law in force. The incorrect marking of additives on food labels was found 1373 times (11, 41%), drawing attention to this aspect. Hence, it must be investigated with care during the inspection activity. Respecting the normatives regarding the use of additives is a priority, being important and necessary in supervising quality of foodstuff and in preserving consumer's health.

Food additives are substances not consumed as foods and not used as food ingredients, having or having not a nutritional value. They are added intentionally in foodstuff for technological purpose, for processing, preparing, treatment, packaging, transport or storage. All the additives are noted with the letter „E", followed by a specific number. The use of food additives in Romania is ruled by the Health Ministry Order nr. 438/295/2002 that approves normatives regarding the use of food additives in foodstuff for human consume (subsequently completed and modified), which is harmonized with the European Union Standards (Directive 89/107 EC). The labeling of foodstuffs is ruled by the Government Decision nr.106/2002(subsequently completed and modified), which decides the listing of the category of additive on labels, with the name of the additive or the „E" code.

1. MATERIAL AND METHOD

In the frame of the national activity of monitoring additives, the local public health authorities followed the presence of these substances, by looking for them on food labels, at the level of main producing companies from each county of Romania. The following information were asked:

- the name of each company and the types of products made there;
- the list of the additives from the main foodstuff's labels, as found at the producing company's level;
- if the additives were listed as stated in the order nr. 438/295/2002, referring to the admissibility of the presence of an additive in a certain food (Order 438/295/2002);
- if all the additives were correctly marked (category, name or E code) on the label of the final product (Decision 106/2002).

The groups of foods had in target were: bakery (packed), confectionery (packed), diary products, meat (minced and „mititei" meat) and meat products, fish products, oils and margarines, preserves of fruit and vegetables, alcoholic and soft beverages, packed ice creams (Saltmaesh, 2000). The reporting was done every six month.

2. RESULTS AND DISCUSSIONS

In table 1 there can be seen the groups of foods and the number of companies monitored from the entire country.

Table nr.1

Food groups and number of monitored companies

Group of food	Number of companies involved in the monitoring
Alcoholic beverages	17
Soft drinks	48
Meat and meat products	143
Preserves	17
Bakery products	129
Confectionery products	62

Group of food	Number of companies involved in the monitoring
Diary products	20
Sweets	22
Ice cream	30
Fish products	4
Oils and margarines	3
Total	**495**

There have been gathered 12,029 registrations of used additives in different foodstuff, from which in the first semester – 4,811, and in the second semester – 7,218. After the verification of the additive list, the conclusion was that the use of all the additives was in conformity with the law in force.

From the entire bulk of data, the most frequently used additives, categorized on food groups, were the following:

- Alcoholic beverages:
  - Caramel (coloring)-26, 72%
  - Citric acid (acidifying stuff)-17, 05%
- Soft drinks:
  - Sodium benzoate (preservative)-12, 64%
  - Citric acid (acidifying stuff)-12, 23%
  - Aspartame (sweetener)-9, 02%
  - Sodium cyclamate (sweetener)-8, 73%
  - Saccharine (sweetener)-8, 37%
- Meat and meat products:
  - Sodium nitrite (preservative)-16, 95%
  - Sodium glutamate (***taste enhancer***)-1, 55%
  - Sodium ascorbate (antioxidant) – 8, 42%
  - Ascorbic acid (antioxidant)-7, 63%
  - ***Polyphosphate*** **(*emulsifier*)**-6, 84%
  - Fruit and vegetable preserves:
  - Citric acid (acidifying stuff)-40, 47%
  - Sodium benzoate (preservative)-13, 80%
  - Pectin (***hardening agent***)-10, 47%

- Potassium sorbate (preservative)-6, 19%
- Packaged ice creams:
- Guar gum (***hardening agent***) 15, 34%
- Mono- and diglycerides of fatty acids (emulsifier)-13, 45%
- Carruba gum (***hardening agent***)-8, 53%
- Carrageen (***hardening agent***)-8, 10%
- Tartrazine (coloring)-4, 77%
- Confectionery products:
- Lecithin (***emulsifier***)-8.67%
- Sodium bicarbonate (acidities corrector)-6%
- Ameliorators- 5, 89%
- Potassium sorbate (preservative)-4, 85%
- Ascorbic acid (antioxidant)-4, 50%
- Citric acid (acidifying stuff)-4, 39%
- Tartrazine (coloring)-2, 65%
- Bakery products:
- Ascorbic acid (antioxidant)-21, 55%
- Ameliorators-18, 66%
- Diary products:
- ***Diphosphates*** (***emulsifier***)-27, 94%
- ***Polyphosphates*** (***emulsifier***)-26, 47%

- Sweets:
  - Citric acid (acidifying stuff)-18, 19%
  - Lecithin (***emulsifier***)-11, 01%
  - Tartrazine (coloring)-6, 85%
  - Oils and margarines:
  - Potassium sorbate (preservative)-16, 66%
  - Lecithin (***emulsifier***)-13, 33%
  - Beta carotene (coloring)-15%
  - Fish and fish products:
  - Sodium benzoate (preservative)-29, 41%
  - Citric acid (acidifying stuff)-17, 64%

The incorrect marking of additives on food labels was found as follows:

- In the first semester 812 times,
- In the second semester 561 times,

representing a total of 1373 (11, 41%) from the whole bulk of analyzed products.

3. CONCLUSIONS

3.1. Respecting the normatives regarding the use of additives is a priority, being important and necessary in supervising the quality of foodstuff (ILSI 1999).

3.2. Because the incorrect marking of additives on food labels was reported in 21 counties (50% of the entire number), we recommend to give a special attention to this aspect during the inspection activity.

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USUAL MATERIALS USED IN ROMANIA FOR PACKAGING MEAT PRODUCTS AND THEIR SAFETY (2005, 2008)

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Key words: meat, package, migration, food safety

SUMMARY:

Safety in packaging is a priority in modern food science, in order to ensure the innocuity for the consumers of the materials entering in the package composition. In Romania, as in other countries from the European Union, there are several directives applicable in this field of interest. In the present paper, it is presented the monitoring of the materials used in packaging meat and meat products, in 2005 and 2008. Polyamide (PE), a very stable type of plastic, is used most frequently than other products. The conclusions of the observation are that all the materials found on market during the above period of time are stable, both the type, and the value of global migration of components respecting the requirements of the law in force. (10 mg/dm^2 or 60 mg/kg food).

Our objectives were to monitor the materials used in contact with meat a meat products and to determine the global migration of compounds in an extraction liquid, in conditions that can copy the worst situations a plastic can deal with in its practical utilization, as package for meat products (Decision Nr. 1197/2002).

1. MATERIAL AND METHOD

The study was carried out in the years 2005 and 2008, in 15 counties of Romania (Decision 357/2008), by the local laboratories from the authorities of public health. The monitoring and control of the materials in direct contact with food consisted in: identification of the type of package, defining the conditions of contact between food and package, identification of the extraction conditions and evaluation of the global migration of components (Order nr.574 /269/2008). It has been verified the value of global migration for different plastics before and after contact with meat and meat products, using as simulants distilled water and ethylic alcohol 96^0 , with a contact duration of 10 days and a

testing temperature of 5^0 Celsius and room temperature (Directive 82/711/CEE, Svenson, 2002).

2. RESULTS AND DISCUSSIONS

In 2005, from a total of 85 samples, the main materials in contact with meat and meat products were: polyamide (PA), with the highest percent, followed in a decreasing order by polyamide/ polyethylene (PA/PE), polystyrene (PS), polyethylene (PE) and plastic with a non specified composition (figure 1).

In 2008, from the total of 118 samples, the highest percent of samples in contact with meat were made of polyamide, a very stable material, followed in a decreasing order by polyamide / polyethylene, vinyl polychloride(PVC), polyethylene, no eatable membranes, multilayer materials and a plastic material with a non-identified composition (XXXX)(figure 2).

While testing the global migration of components in simulants, none of the materials showed any dangerous proprieties. All of them were innocuous for the consumer, the level of the components migrating being in the limits established by the law in force.

3. CONCLUSIONS

Polyamide was the most frequently used as package material, during the two periods of investigation. But all the materials(not only PA) showed a very good stability, the values of the global migration of components in selected simulants respecting the rules (10 mg/dm^2 or 60 mg/kg food)(Decision 1197/2002).

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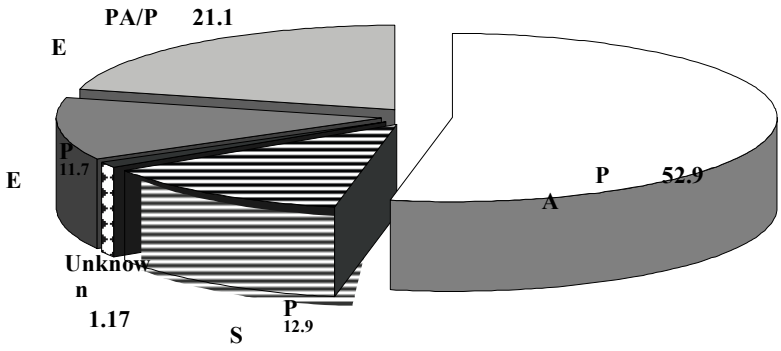

Figure 1.Materials in contact with meat, 2005 (%)

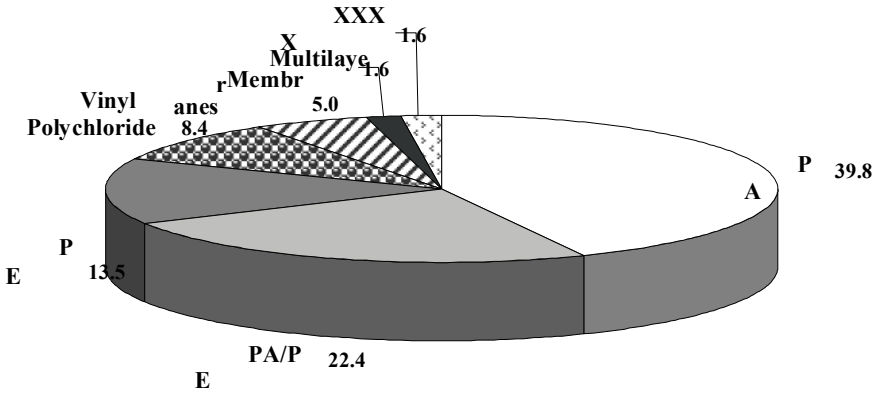

Figure 2:**Materials in contact with meat, 2008 (%**

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THE VARIANCE OF SELECTED HEAVY METALS CONTENT IN DIFFERENT MEAT TYPES DETERMINED BY ICP-MS AND DRC-ICPMS

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Keywords: meat, metallic elements, variance, microwave digestion, ICP-MS

SUMMARY

The objective of this research was to determine the levels of cadmium, lead, iron, zinc, selenium, manganese, copper and molybdenum in different cuts of beef, pork, lamb, chicken and foal collected from supermarkets and butcheries in our country. The values of concentration for manganese, copper, molybdenum, zinc, iron, selenium, cadmium and lead were determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion. From the obtained results, the calculation of the mean values and their respective coefficients of variation. The results showed for cadmium and lead ranges from 0.6 to 3.9 mg/100 g and 1.0 to 2.1 mg/100 g, respectively. The values of concentration ranged between 0.5 and 3.3 mg/100 g for iron, 0.7 and 5.1 mg/100 g for zinc, 9 and 44 mg/100 g for selenium, 3.1 and 16.7 mg/100 g for manganese, 0.3 and 132 mg/100 g for copper and 0.9 and 3.2 mg/100 g for molybdenum. The differences found in the concentration values, obtained for meat from different species as well as between the individual meat portions were noteworthy for iron, zinc, selenium and copper. The manganese concentrations had a high variability within muscles and species, while molybdenum concentrations were higher in chicken meat in comparison with the mammalian meats. The highest coefficients of variation were found for manganese (13% to 142%) and copper (13% to 224%), while the lowest was found for zinc (4% to 45%). In conclusion, in order to provide an accurate overview and to be able to calculate reliable dietary intakes, it is important to include the variability in food composition data.

Meat is considered the major source of essential trace elements such as iron (Fe), zinc (Zn) and selenium (Se) (Briggs and Schweigert, 1990). The methods used to determine these elements are therefore important in nutrition studies, particularly because meat, as a biological material, exhibits natural variations in the amounts of nutrients contained (Greenfield and Southgate, 2003). The variations can be increased taking into account the different animal husbandry and feeding systems applied. As a consequence, nutrient data, including information about trace elements, have to be updated frequently, in order to reflect the current data situation, and also to monitor possible changes. Moreover, to study the variability, there is little data available, especially for nutritive substances, including trace elements in meat, since in most food composition tables only mean values are declared (Pennington et al., 1995). In addition, information about lean meat vary to a greater extent than the nutrient composition of other food items. The data could

really help the scientists to establish the value of nutrient composition data, as a basis for dietary recommendations (Leonhardt and Wenk, 1997).

Of great interest is also the determination of certain elements like cadmium (Cd) and lead (Pb) (Bou et al., 2004). Recently much attention has been focused on the concentration of heavy metals in meat, meat products and other food in order to check for the effects of those hazardous on human health (Abou-Arab, 2001; Moeller et al., 2003; Celik and Oehlenschlager, 2004). After a long series of studies in the domain, WHO has concluded that heavy metals can cause serious damage to the human organism, due to the capacity of these metals to accumulate in living organisms. Lead, for example, bio-accumulates in the food chain from plants to animals, but its concentration in food also generally increased during the last few decades (Halliwell et al., 2000). Cadmium has a long residence time in human tissues (10 to 40 years), especially in the kidneys (Rubio et al., 2006); thus, it is of the outmost importance to monitor lead and cadmium content in the diet. The major purpose of the present study is to examine the concentration of essential elements like manganese (Mn), copper (Cu), molybdenum (Mo), Zn, Fe and Se, as well as the potentially hazardous elements Cd and Pb in different commercially available meat portions with a particular focus on the variability of these trace elements.

1. MATERIAL AND METHODS

Meat portions were purchased from local supermarkets and butcheries in order to consider different origins in terms of production system (meat labels) and anatomical location: beef (sirloin, rib-eye and braising steak), pork (neck steak, chop and loin), lamb (chop and loin), chicken (breast with and without skin and leg with skin) and foal (sirloin and filet). Beef, pork and lamb samples were purchased in two series, from June to August 2008 and from October to December 2008. Foal and chicken samples were purchased from January to March 2008. All samples were cut out of the center of the pieces of fresh meat with a ceramic knife to avoid contamination with trace metals. Samples were vacuum packaged, frozen at – 18^{o}C and stored until analysis. Two standard reference materials (SRM 1546 Meat Homogenate and SRM 1577b Bovine Liver) were prepared to test the accuracy of methods.

Sample digestion procedure - In order to determine the mentioned trace elements, a closed quartz vessels and microwave oven (MLS Ethos) digestion procedure was applied. One gram of meat sample, and

500 or 250 mg of the certified reference materials SRM 1546 or SRM 1577b, were precisely weighed into quartz digestion vessels. Two milliliters of hydrogen peroxide (H_2O_2 30%) and 8 ml nitric acid 65% (HNO_3) were added to each tube, which was then closed. For the spiking test, an appropriate amount of all measured elements (Mn, Cu, Mo, Cd, Pb, Fe and Se) was also added. Digestion was conducted by applying a four-step program as follows: 230W for 5 min, then 3 min at 330W followed by 430W for 4 min and finally 4 min at 500W. After the vessels had cooled down, the solutions were transferred into 20 ml volumetric flasks and filled to the mark using purity water (obtained from a Milli-Q water purification system, Millipore). Prior to analysis, an aliquot of these samples was spiked with the internal standard solutions and diluted further by a factor of two. Within each digestion run, two samples were randomly chosen for quality control measurements. One sample was digested in duplicate to check for reproducibility of the digestion and analysis, while the other sample was spiked with known concentrations of the elements measured before digestion to monitor potential analyte losses during digestion and further sample preparation. Furthermore, one digestion blank sample was prepared by the same procedure, which contained only the chemicals used for digestion without a sample. The reference materials – bovine liver, with certified values for Mn, Fe, Cu, Zn, Se, Mo, Cd and Pb and meat homogenate, with certified values for Mn, Cu and Zn – were used to verify the precision of the digestion and calibration protocol.

2. RESULTS AND DISCUSSION

In Tables 3-6 are shown the number of samples and the coefficient of variation (CV) for each of the meat portions, analyzed for each animal species. The results obtained after performing this study represent average values of comercially available meat portions in our country.

Cadmium and lead - The results revealed a high variance rate (CV = 60 % to 135 %) in cadmium concentration values for lamb chop and foal sirloin and fillet (Table 1). The rest of the meat portions presented mean cadmium concentration values situated between 0,6 and 0,9 mg/100 g, and lower values of CV (4-37 %). The lead concentration values, in comparison, presented a higher variance, with values between 1,7 and 2,1 mg/100 g, with CV values from 4 to 38 %. ***Iron and zinc -*** Iron concentration presented a great variability among the species considered in this study (Table 2). Among the red meat, foal meat

presented the highest iron content. Lamb and beef showed a similar range of iron concentrations, from 1.6 mg/ 100 g determined in beef (sirloin) to 2.6 mg/100 g determined in lamb (loin).Notable lower iron concentrations were determined in pork and chicken meat. The iron concentrations are in agreement with Hecht and Kumpulainen (1995), Chan et al. (1995) and Leonhardt and Wenk (1997). A feasible explanation for the main low values of CV for zinc could be that the tissue concentration values for this metallic element is determined genetically, and minimally influenced by the composition of feed (Flachowsky and Jahreis, 1995).

Table 1

The levels for cadmium and lead, in different meat portions

			Cd		Pb	
Species	Portion	Number of samples	Main value (µg/100 g)	CV (%)	Main value (µg/100 g)	CV (%)
Foal	Sirloin	3	3.9	69	1.8	3
	Filet	3	3.2	65	2.1	25
Lamb	Chop	5	1.2	127	1.9	25
	Loin	10	0.7	58	1.9	6
Chicken	Breast with skin	3	0.5	2	1.8	3
	Breast without skin	5	0.5	6	2.0	18
	Leg with skin	5	0.6	35	1.8	5
Beef	Sirloin	10	0.5	34	2.0	35
	Rib-eye	9	0.6	10	1.8	3
	Braising steak	10	0.5	15	1.8	4
Pork	Neck steak	9	0.6	8	1.8	3
	Chop	11	0.6	15	1.8	5
	Loin	11	0.6	13	1.8	4

Selenium - Selenium is considered an essential metallic element, especially for plant growth, plants not being able to control the Se uptake. In consequence, the concentration of Se in plant represent the concentration values and availability of Se in soils. The animals raised using low-Se feedstuffs form deposits with relatively low concentrations of this element in their tissues, while animals raised on a diet high in Se yield food products come with a higher Se concentration. Owing to the needs of livestock for Se in order to prevent debilitating deficiency syndromes, Se (still usually in the form of sodium selenite) is ordinarily used as a feed supplement in commercial animal agriculture in many parts of the world. This practice became widespread in our country in time, therefore meat has become a constant and an important Se source. The mean Se concentration, reported in Table 3, was higher than reported by Haldimann et al. (1999). These values indicate that Se is used more extensively as a feed supplement than the years before. The CV values considered for this element recorded in this study ranged from 13% to 82% within species due to the influence of feedstuff and supplements. For beef, the CVs for Se concentration have a variance between 65% and 82%. In the last few years, organically raised beef increased in popularity in EU. As these animals do not get any mineral supplements, the Se concentration will depend on the natural supply and therefore on the availability according to the geographic origin.

Table 2

The levels for iron and zinc, in different meat portions

		Cd		Pb	
Species	Portion	Main value (µg/100 g)	CV (%)	Main value (µg/100 g)	CV (%)
Foal	Sirloin	3.2	40	2.7	39
	Filet	3.3	24	2.0	20
Lamb	Chop	2.0	36	2.3	15
	Loin	2.6	40	2.4	15
Chicken	Breast with skin	0.6	86	0.7	4
	Breast without skin	0.5	49	0.7	5
	Leg with	1.2	54	1.4	45

	skin				
Beef	Sirloin	2.0	29	3.7	18
	Rib-eye	1.8	44	5.1	27
	Braising steak	1.7	39	3.2	26
Pork	Neck steak	1.3	46	3.3	10
	Chop	0.7	93	1.5	11
	Loin	0.7	75	1.5	14

Manganese, copper and molybdenum **-** The manganese concentrations measured within this study are lower than the values reported by Souci et al. (2000) but also show, a high unsystematic variation between animal species and different portions of meat. The copper concentrations of chicken meat differed noteworthy from the other meat portions analyzed, in which higher concentration values were found. The concentrations in meat portions coming from lamb, beef and pork are in accordance with the values reported by Hecht and Kumpulainen (1995) and Lombardi-Boccia et al. (2005).

Table 3

Levels for selenium in different meat portions

		Se	
Species	Portion	Main value (μg/100 g)	CV (%)
Foal	Sirloin	35	47
	Filet	28	30
Lamb	Chop	11	40
	Loin	11	76
Chicken	Breast with skin	12	13
	Breast without skin	19	32
	Leg with skin	28	19
Beef	Sirloin	9	82
	Rib-eye	11	65
	Braising steak	10	67

Pork	Neck steak	16	30
	Chop	17	66
	Loin	16	30

Souci et al. (2000) presented. The concentration values for molybdenum in meat are very interesting to study since these results show that chicken meat (2.4 to 3.2 mg/ 100 g) contains around twice as much as mammalian meat. This is similar to the study of Hecht and Kumpulainen (1995) who found even greater differences owing to lower Mo concentrations in beef and pork meat and higher values for chicken meat, compared with this study. The CV of molybdenum presented values of percentage from 2% to 63%, being the third lowest of all trace elements analyzed in this study.

3. CONCLUSION

The present results indicate that cadmium and lead concentrations in meat are very low and are unlikely to contribute noteworthy to the daily intake of these toxic elements.

The average values of concentration for essential metallic elements in the examined meat are generally comparable to those reported by other studies carried out in the domain.

The content in individual samples, however, may appreciably deviate from the mean values due to effects associated with animal feeding practices and concentrations in the feed.

The impact of the different elements' variability depends on their distribution in food. Nutrient variability is of more practical significance when it appears in food products that are relied upon as sources of specific nutrients. If nutrient values are not trustworthy, due to a high variation, dietary intakes calculated from mean values may be deceitful. Ergo, it is important to provide the possible variation to better appraise the incertitude of dietary intakes, which should better be reported as a range than as a single value.

Metallic elements data in food composition tables were found to be sometimes deficient especially for Se and Mo. As a consequence, it is of major importance to update and underline food composition tables with actual data.

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RESEARCHES CONCERNIG HEAVY METALS IN PARTICULAR FISH SPECIES, IN MEAT AND MEAT PRODUCTS

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SUMMARY

By using modern techniques, such as inductively coupled plasma-optical emission spectrometry (ICP-OES), Selenium, copper, nickel, zinc, cadmium, manganese, iron, copper and lead contents of several edible fish species, meat and meat products were analyzed. The results revealed various concentrations of these elements, their order in the analyzed samples being the following, in μg 100 g^{-1}: Fe (57.7–156.4) > Zn (20–159) > Ni (8.2– 24) > Pb (11.5–13.5) > Cr (8.44–9.51) > Cu (7.18–10.01) > Cd (0.77–1.04) > Mn (3.98–10) > Se (1.32–4.6).
The concentration values found in fish are very close to the ones comprised in the international standards. The highest values for the elements analyzed in this investigation was found in meat products (sausages), albeit the lowest concentration values were observed in fish species, like *Trachurus trachurus* (saurel). The iron concentration values are higher than the recommended ones.

The importance of meat and meat products in the human diet is very well known worldwide, but meanwhile, the concern about the effects of anthropogenic pollution on the ecosystems is growing. The heavy metals are continuously released into aquatic and terrestrial ecosystems, contamination with this metallic elements being considered a serious threat due to their toxicity, bioaccumulation and biomagnifications in the food chain.

Recently, a lot of attention was focused on the concentration values of these poisonous metallic elements in food products, especially in fish, thus the industrial and edible species being widely investigated. For example, samples of beef, veal, pork, chicken and horse-meat were analyzed for Ca, Cu, Fe, Mg, Mn, Ni, Zn, Cd and Pb by Hecht and Kumpulainen (1995). In addition, Mn, Cu, Zn, Fe, Cd, Hg and Pb concentrations were determined in liver, kidney and muscle meat of ducks, geese, chickens, hens, rabbits and sheep slaughtered in the northern part of Poland (Falandysz, 1991).

The Romanian meat products are well-known and consumed all over the country, the main ingredients being the meat (beef, pork), fat, salt, nitrates, sugar, garlic, spices and other series of additives, stuffed

into small intestine cases of sheep or pigs, subsequently or not to a thermal processing method. The levels of the metallic elements studied in meat and meat products are influences by factors like the pasture, the genetic traits of the living organisms and the environmental conditions, as a general rule. Moreover, the technological treatments to which the meat is subjected, are considered important when analyzing the levels of the heavy metals in the meat products, because these toxic metallic elements transfer to animals and further to the human being throughout the food chain. The main purpose of this investigation was the assessment of the concentration values of several metallic elements, as Cu, Fe, Mn, Cd, Cr, Ni, Pb, Se and Zn, in edible fish species, meat and meat products consumed in our country. Furthermore, the data were assessed in comparison with the recommended dietary allowances (RDA) suggested by WHO.

1. MATERIALS AND METHODS

1.1. Sampling

In order to accomplish the aim of this study, there have been chosen and collected a number of 34 samples, representing about 80–90% of all the types of traditional fish, meat and meat products from 11 varieties of fish, meat and meat products: salami, sausage, minced meat, *Cyprinus carpio* (carp), *Salmo trutta trutta* (trout), *Micromesistius poutassou* (mackerel) and *Engraulis encrasicholus* (anchovy). The 34 samples were analyzed individually, for example five different fish species and six meat and meat products collected from different stations were analyzed independently with three replicates per sample. The fish species, meat and meat products were sampled from the different shops and by producers in our country during 2007 and 2008. All the samples were introduced in plastic bag/containers and transported to the laboratory on the same day and stored at -18^{o}C until the moment of analysis. The internal organs and heads fish were removed because in general these organs are not consumed in our country and the edible portions (muscle) were washed with distilled water. After cutting into small pieces with a clean stainless steel knife, the small pieces were cleaned several times with demineralized water.

1.2. Analytical methods

The samples, in quantities of 0.5 mg, were weighed and exposed to digestion in 10 ml of HCl, in a microwave digestion system, with the following work parameters: max power: 1200 W, power (%): 100,

pressure (psi):100, temperature (°C): 210 and hold (min):10:00). The determinations of the elements in all samples were carried out using a Varian ICP-OES. The samples were analyzed in triplicate and statistical package for the social sciences (SPSS) statistical program was used to calculate standard deviations and means.

2. RESULTS AND DISCUSSION

The main values for cadmium, copper, lead, zinc, selenium, manganese, iron, chromium and nickel concentrations in fish and certain meat products studied are comprised in Tables 1 and 2.

Table 1

Main concentrations (µg 100 g $^{-1}$) of Cu, Fe, Cd, Cr and Mn in certain meat, meat products and fish samples and their standard errors

Products	Cu	Fe	Cd	Cr	Mn
Salami	8.4 ± 0.1	127.66 ± 11.4	0.82 ± 0.02	8.8 ± 1.7	5.7 ± 1.01
Sausages	8.83 ± 0.2	156.4 ± 14.7	0.83 ± 0.07	9.51 ± 0.99	10 ± 2
Meat	9.4 ± 0.023	136.2 ± 18.4	0.86 ± 0.07	8.8 ± 0.12	6.6 ± 1.11
Minced meat	7.19 ± 1.4	94.9 ± 11	0.79 ± 0.04	54 ± 0.99	4.23 ± 0.14
Carp	8.12 ± 1.3	130 ± 15.4	1.04 ± 0.1	8.71 ± 1.17	3.98 ± 0.99
Trout	7.18 ± 0.99	57.7 ± 0.99	0.77 ± 0.14	8.5 ± 1.16	4.55 ± 1.1
Mackerel	7.194 ± 0.17	92.196 ± 11	0.77 ± 0.2	8.7 ± 1.15	4.6 ± 1.14
Anchovy	7.19 ± 0.47	88.2 ± 0.89	0.92 ± 0.07	8.6 ± 2	4.3 ± 0.11

The concentrations of trace elements in these products presented a high variability as the following values show: Cd (0.77–1.04 µg 100 g^{-} 1), Cu (7.18–10.01 µg 100 g^{-}1), Se (1.32–4.6 µg 100 g^{-}1) and Pb (11.5–13.5 µg 100 g^{-}1). The levels obtained after carrying out this analysis can be ordered like this: Fe > Zn > Ni > Pb > Cr > Cu > Cd > Mn > Se. These results are in agreement with the data obtained by other scientists, except for lead contents found in meat and meat products, which were higher as compared with those obtained by Falandysz (1991), Khan,

Diffay, Forester, Thompson, and Mielke (1995), Zantopoulos, Antoniou, and Tsoukali (1992).

Table 2

Main concentrations (µg 100 g $^{-1}$) of Ni, Pb, Se and Zn in certain meat, meat products and fish samples and their standard errors

Products	Ni	Pb	Se	Zn
Salami	12.4 ± 0.99	12.8 ± 1.5	1.6 ± 0.98	45 ± 0.07
Sausages	16.1 ± 1.4	13.5 ± 0.99	1.8 ± 0.6	60 ± 2.1
Meat	13.02 ± 0.11	12.5 ± 1.18	3.2 ± 0.99	111 ± 0.98
Minced meat	12.1 ± 1.12	11.5 ± 1.5	1.6 ± 0.78	57,2 ± 1.4
Carp	16.2 ± 0.9	12.4 ± 0.99	3.68 ± 0.7	43,8 ± 3.4
Trout	8.2 ± 0.8	12.4 ± 0.99	2.9 ± 0.1	25.9 ± 1.78
Mackerel	8.2 ± 1.5	12.2 ± 2.1	1.32 ± 0.8	20 ± 3.1
Anchovy	18.9 ± 1.15	11.8 ± 1.7	3.3 ± 0.8	52.4 ± 0.99

The levels of cadmium in all samples analyzed ranged from 0.77 to 1.04 µg 100 g^{-}1 (average value: 0.91 µg 100 g^{-}1), this being below the codex committee on food additives and contaminants draft guideline of 0.5 mg Cd/kg. Nevertheless, cadmium concentration values obtained in this study were higher, cadmium having the ability of accumulating in the human body and inducing kidney dysfunction, skeletal damage and reproductive deficiencies (Commission of the European Communities, 2001). Cadmium concentrations in meat increase with the age of the animal and depend on the concentrations of Cd in the feed.

Lead concentration values observed in all the samples analyzed ranged from 11.5 to 13.5 µg 100 g^{-}1 (average value: 12.5 µg 100 g^{-}1). Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults (Commission of the European Communities, 2001).

The data included in Table 1 show there were no major differences in chromium concentrations between samples of fish and meat. Even though, chromium (III) is an essential element that helps the body use sugar, protein and fat, at the same time it (especially, chromium VI) is carcinogenic for organisms. Excessive amounts of chromium (III) may cause adverse health effects (Agency for Toxic Substances & Disease Registry, 2004). According to the Institute of Medicine (2002), the upper tolerable intake level for chromium for women and men aged 51–70 years is 20 and 30 mg, respectively.

The levels of manganese in the all samples ranged from 3.98 to 10 μg 100 $g^{-}1$ (average value: 6.99 μg 100 $g^{-}1$). WHO has recommended safe and adequate daily intake levels for manganese that range from 0.3 to 1 mg for children up to 1 year, 1–2 mg for children up to age 10, and 2–5 mg for children 10 and older. Additionally, the upper tolerable intake level of manganese for children (1–3 years old) and males/females (19–70 years old) is 2 and 11 mg , respectively (Institute of Medicine, 2003). Daily intake of small amounts of manganese is considered essential for growth and good health in humans otherwise deficiency of manganese can cause nervous system problems.

The highest average trace element concentrations were obtained from meat and sausage while the lowest values were observed in mackerel. This may relate to treatments of the products or addition of spices. Furthermore, the anchovy fish exhibited higher levels of Ni than the other products. Mean Ni concentrations ranged from 8.2 to 24 μg 100 $g^{-}1$. According to WHO, nickel can cause respiratory problems and is also carcinogenic. The upper tolerable intake level of nickel for children (1–3 years old) and males/females (19–70 years old) is 7 and 40 mg, respectively.

The copper concentration had an average value of 7.18-10.01 μg 100 $g^{-}1$ in the samples investigated and moreover, the highest average concentrations of Cu were observed in salami (Table 1). Copper is considered an essential element for good health but very high intakes can cause health problems such as liver and kidney damage. The upper tolerable intake level of copper for children (1–3 years old) and males/females (19– 70 years old) is 1 and 10 mg, respectively. The maximum copper concentration for fish, meat and meat products has been proposed as 900 μg–30 mg /person.

Table 3

Comparison of the dietary intakes of some elements from meat, meat products and fish with the recommended dietary allowances for a 60 kg adult, according to joint expert committee on food additives (JECFA) provisional tolerable weekly intake (PTWI) and WHO (1993)

Elements	Average concentrations (µg 100 g^{-1})		Recommended dietary allowance	Results obtained from this study (results) (µg 100 g^{-1})	
	For meat and meat products	For fish		For meat and meat products	For fish
Cadmium	0.816667	0.841667	60 µg d^{-1} person	0.8	0.84
Lead	12.61667	12.31667	210 µg d^{-1} person	12.6	12
Zinc	84.8	36.4	8 mg person	80	35.4
Copper	8.655	7.665667	900 µg-30 mg d^{-1} person	8.4	7.6
Selenium	1.6	2.55	55 µg person	2.8	2.8
Nickel	14.63667	11.66667	37-700 µg d^{-1} person	14.6	14
Iron	126.41	82.24933	10-18 mg d^{-1} person	126	92
Chromium	16.45333	8.608333	130 µg d^{-1} person	8.8	8.6
Manganese	6.738333	4.5	2-5 mg d^{-1} person	6.7	4.2

The results obtained in this study indicated that the iron concentrations in samples of fish (92 µg 100 g-1) considerably exceeded the proposed maximum concentrations (Table 2). Fish is a major source of iron for adults and children. Iron deficiency causes anemia, so the upper tolerable intake level of iron in children (0 months–8 years) and males/females (14–70 years) is 40 and 45 mg, respectively. The recommended dietary allowance (RDA) of iron for 7–12 month infants and males/females 51–70 years is 11 and 8 mg, respectively.

The zinc contents of samples were between 20 and 159 µg 100 g-1. As shown in Table 1, the lowest zinc concentrations were found in mackerel. Zinc is an essential element in human diet. Too little zinc can cause problems but on the other side too much zinc is harmful to human health (Agency for Toxic Substances & Disease Registry, 2004). The

upper tolerable intake level of zinc for children (1–3 years old) and males/females (19–70 years old) is 0.2 and 1 mg, respectively (Institute of Medicine, 2003).

The ranges of international standards for fish are; for Zn approximately 192–480, for Cu 48–480; for Pb 2.4–48 µg g^{-1}dry weight (Yamazaki, Tanizaki, & Shimokawa,1996). Selenium contents in the samples were observed to be 1.32–4.6 µg 100 g^{-1}. The concentration of both lead and manganese were higher in the sausage while cadmium was higher in the tissues of carp. However, in general, the concentrations in fish seemed to be close to the international standards. Taking into account the WHO health criteria (Table 3) for carcinogens (WHO, 1993), there are no health risks with respect to the concentrations of lead, nickel, copper and cadmium in fish, meat and meat products analyzed in this study. The body encumbrance of these metallic elements is influenced on the concentrations of the various elements in fish and meat products the frequency of consumption of these materials, the amount of food consumed and the rate of detoxification of contaminants in the human body. The choice of fish, meat and meat products consumed may vary considerably from one individual to another.

3. CONCLUSIONS

The metallic elements from edible fish species, meat and meat products from our country were determined and evaluated in comparison to levels found in samples with permissible limits stipulated by various agencies and organizations.

The results obtained confirm the fact that there are significant differences in the concentrations of elements across both fish species and meat products. This is largely related to the organism mobility, food preferences, or to other traits of behavior with respect to the environment. The estimated intakes of cadmium, lead, iron, copper and nickel from weekly consumption of the samples indicated no risk since they are lower than the permissible tolerable weekly intakes for these elements.

The analytical data obtained from this investigation show that there are no health risks from consumption of the fish species, meat and meat products when compared with levels stipulated by the health authorities in the European Union for lead, chromium, copper and nickel as carcinogens.

The levels may be reduced by more careful handling practices and processing of raw materials. In addition, a better selection of the raw material, including an analysis for toxic trace elements prior to processing, could definitely improve the situation. However the complexity of interaction between different compartments of an ecosystem makes it difficult to draw firm conclusions, the present results are within the range of normal values reported for our country.

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THE LEVELS OF HEAVY METALS DETECTED IN CANNED MEAT PRODUCTS

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Keywords: heavy metals, meat, canned products

SUMMARY

Considered very toxic and, thus, dangerous to the human health, heavy metals were assessed in this study by the purpose of the metallic container influence on the levels of such elements in the food products contained, i.e. meat products. The determinations were carried out using the flame atomic-absorption spectrophotometry with deuterium arc background correction for Zn, Cd and Pb, while the results were treated by applying the Statistical Package for the Social Sciences compiled and linked in the software of a Digital VAX/VMS 11/780 (V.4.8) computer.
As there were no significant differences revealed between samples taken from the surface and those from the interior of the products, for each of the meat products, the highest values of the concentration were found to be on Zn and Fe, (the ranges being wide), and at the same time Cd was the lowest, with mean values below 100 ppb in every product. Considering the results revealed on Mn, Ni, Cu and Zn, these show considerably higher data in comparison to the rest of the products, but no significant differences in what concerns the data observed for Cd and Pb.

The control of heavy metals in food by using analytical methods is considered of major importance, because these pollutans have the ability to accumulate in nature, therefore, can become toxic to humans once they are introduced in the human organism, along with the food products. This study comprises an analysis of seven heavy metals in semipreserved ham and shoulder pork, preserved pork meat and liver paste. The main purpose of the performed analyses is to determine the potential influence of the metallic container itself (the can) on the levels of such metals in the food products. The metallic elements involved in this study were: cadmium, lead, copper, zinc, iron, nickel and manganese. These are not considered themselves as toxic, but in high concentration values, they can cause pubic health hazards, or a decrease in the quality of the canned product, resulting in economic losses, within production sector. Recently there have been performed several studies, based on statistical and metal-to-metal correlation analysis, the mean levels of metals in different products, being compared with the maximum tolerance levels of metals allowed in the European Economic Community countries (Parker 1986).

1. MATERIALS AND METHODS

The analyzed samples (47 shoulder pork, 20 preserved lunch pork, 25 ham and 26 pork liver paste), being introduced in glass, plastic, metallic or china containers and coming from different local markets, were representative for different products habitually consumed in our country. From each product there were taken 30-35 gr samples, one from the interior of the product and the second from the surface, the latter being in direct contact with the container. Subsequently, they were dried under infrared irradiation and transformed into ash at 450 ± 10 ºC. The ash was treated with 5 ml of hot and concentrated hydrochloric acid, filtered and made up to 50 ml with deionized water in a volumetric flask. Determinations were carried out by flame atomic-absorption spectrophotometry using deuterium arc background correction for Zn, Cd and Pb. Results were treated by applying the Statistical Package for the Social Sciences compiled and linked in the software of a Digital VAX/VMS 11/780 (V.4.8) computer.

2. RESULTS AND DISCUSSION

There were no significant differences revealed between samples taken from the surface and those from the interior of the products. In Table 1, the mean, maximum and minimum level values are shown, along with the standard deviation for different meat products involved in the analysis. For each of the meat products, the highest values of the concentration were found to be on Zn and Fe, (the ranges being wide), and at the same time Cd was the lowest, with mean values below 100 ppb in every product. The mean levels of Mn, Ni, Cu and Zn found in pork liver paste were considerably higher than in the rest of the products, but no significant differences were observed for Cd and Pb.

The data for Cd, Pb and Cu fall within the ranges reported by Kirkpatrick and Coffin (1973), Tackacs et al. (1975,1976), Parolari and Pezani (1977), Cantoni et al. (1979) and Ruick and Schmidt (1982). For Mn, Fe, Ni and Zn, no data were found in the literature. Table 2 contains the mean, maximum and minimum values and the standard deviation for the seven metals studied, differentiating the four types of containers and not the products. For Mn, Cu and Ni, products in glass and china containers had higher mean values than those in plastic and metallic containers, while the concentrations of Zn and Cd were similar in the different containers.

The samples from plastic and china had lower concentrations of Fe than those from glass and metallic containers and no Pb was detected in any of the samples from china containers. From the results obtained in this study and comparisons with data for fresh pork meat and liver given by Niinivaara and Antila (1973), Hecht et al. (1973), Collet (1975), Holm (1976) Parolari and Pezani (1977), Cantoni et al. (1979), Begliomini et al. (1983), the major conclusion is that the concentrations of heavy metals studied in these pork meat and liver products came mostly from the actual products and not from changes brought about by containers.

Table 1

Main statistical data for the four types of meat products analyzed

Meat product / Element	**Cu**	**Ni**	**Cd**	**Fe**	**Mn**	**Pb**	**Zn**
Shoulder pork							
Mean	1,59	1,69	0,088	10,08	0,38	0,43	18,05
Maximum	7,11	3,62	0,220	24,22	1,84	0,75	27,13
Minimum	0,58	0,49	0,040	1,14	0,13	0,15	4,62
Standard deviation (ppm)	0,72	0,64	0,025	1,07	0,45	0,13	3,92
Ham							
Mean	1,77	1,9	0,084	8,33	0,39	0,57	14,04
Maximum	8,42	2,3	0,140	11,86	1,77	1,80	25,38
Minimum	0,83	0,80	0,020	1,96	0,08	0,30	1,24
Standard deviation (ppm)	1,65	0,44	0,026	2,42	0,46	0,21	7,09
Lunch pork							
Mean	1,84	1,75	0,070	12,62	0,65	0,49	13,85
Maximum	3,48	3,80	0,140	17,46	2,62	2,06	36,76
Minimum	0,91	0,87	0,030	4,50	0,25	0,27	7,34
Standard deviation (ppm)	0,75	0,74	0,023	2,50	0,46	0,31	6,38
Pork liver paste							
Mean	5,63	5,39	0,073	6,48	1,47	0,30	21,72
Maximum	11,87	10,63	0,190	14,23	2,22	0,38	27,98
Minimum	1,52	1,44	0,030	3,86	0,16	0,22	14,71
Standard deviation (ppm)	2,32	2,39	0,036	2,22	0,45	0,08	4,13

The E.E.C. countries legislation on meat products set only tolerance limits for Pb and two for Cd out of the seven metals studied here. The mean values for Pb in shoulder pork, ham, lunch pork and liver paste obtained during this study are lower than the tolerance limits established by Ireland (5 ppm) and Italy (1.25 ppm) and are slightly higher than the

maximum tolerance levels set by Denmark (0.3 ppm) and Holland (0.4 ppm). The mean values for Cd are lower than tolerances legislated in Denmark (0.1 ppm) and slightly higher than those established by Holland (0.05 ppm). A statistical analysis of metal binary correlation was performed on all the samples and each meat products taken into account on this study. The equations defining the most significant interrrelations found are presented in Table 3, together with their correlation coefficients. The Cu-Ni relationship is notable, because it shows the highest correlation coefficient and is, furthermore, to be found in the four types of meat products studied.

Table 2

Main statistical data obtained combining all the samples and differentiating only the four types of containers

Containers (N = 105)	**Cu**	**Ni**	**Cd**	**Fe**	**Mn**	**Pb**	**Zn**
Metallic							
Mean	2,03	1,84	0,084	10,83	0,46	0,51	16,60
Maximum	7,11	6,60	0,220	24,22	1,66	2,06	36,76
Minimum	0,77	0,80	0,030	1,96	0,08	0,27	2,42
Standard deviation (ppm)	1,42	1,00	0,028	3,31	0,31	0,31	5,80
Glass							
Mean	5,35	5,28	0,080	10,05	1,69	0,29	19,31
Maximum	11,87	10,63	0,190	14,23	2,62	0,36	24,79
Minimum	1,02	1,08	0,040	6,74	0,60	0,22	12,98
Standard deviation (ppm)	4,50	4,45	0,045	2,26	0,64	0,06	4,65
Plastic							
Mean	2,22	2,13	0,080	8,10	0,57	0,45	16,66
Maximum	8,42	5,07	0,160	19,81	1,84	0,75	16,46
Minimum	0,58	0,49	0,020	1,14	0,11	0,15	1,24
Standard deviation (ppm)	1,56	1,28	0,026	2,85	0,54	0,15	5,59
China							
Mean	5,90	5,92	0,072	6,99	1,56	ND	23,51
Maximum	7,58	7,46	0,110	7,68	2,22	ND	26,17
Minimum	1,52	1,44	0,040	6,15	0,16	ND	15,14
Standard deviation (ppm)	2,50	2,53	0,028	0,70	0,83	ND	4,71

ND – not detected.

Table 3

Most notable metal correlations

Meat Product	**Equation (ppm)** $P \leq 0,0001$	**Correlation / Coefficient**
Overall	[Cu] = 0,972 [Ni] + 0,137	0,890
Pork shoulder	[Cu] = 0,466 [Ni] + 0,708	0,467
Ham	[Cu] = 1,033 [Ni] – 0,016	0,968
Lunch Pork	[Cu] = 0,765 [Ni] + 0,502	0,758
Liver Paste	[Cu] = 0,936 [Ni] + 0,579	0,968
Overall	[Cu] = 2,306 [Mn] + 0,943	0,633
Liver Paste	[Cu] = 4,296 [Mn] – 0,623	0,823

Overall	[Ni] = 2,221 [Mn] + 0,890	0,676
Liver Paste	[Ni] = 4,477 [Mn] – 1,122	0,830
Liver Paste	[Mn] = 0,076 [Zn] – 0,091	0,690
Ham	[Ni] = 0,124 [Fe] + 0,368	0,683

Figures 1 and 2 show the computer plots for the Cu-Ni correlation in the pork liver paste and overall samples. The acclivities of the corresponding straight lines are arranged in compliance with the sequence ham>liver paste>lunch pork>pork shoulder, indicating the corresponding increase to a greater degree of the concentration of copper as compared to the increase of the nickel concentration.

The Cu-Mn and Ni-Mn correlations were found only in the overall samples and in the liver paste. In both of these cases, the slopes obtained for the liver paste were nearly two times as values than those for the overall samples. This indicates that an increase of Mn in the liver paste occurs with a greater increase in Cu and Ni concentrations than in the other meat products studied. Pork liver paste shows a larger number of intermetallic correlations, possibly due to the fact that liver, being the main detoxifying organ, is, therefore, capable of accumulating greater concentrations of metals than any other organs or tissues.

3. CONCLUSIONS

The mean values for Pb in shoulder pork, ham, lunch pork and liver paste obtained during this study are lower than the tolerance limits established by Ireland (5 ppm) and Italy (1.25 ppm) and are slightly higher than the maximum tolerance levels set by Denmark (0.3 ppm) and Holland (0.4 ppm). The mean values for Cd are lower than tolerances legislated in Denmark (0.1 ppm) and slightly higher than those established by Holland (0.05 ppm).

A statistical analysis of metal binary correlation was performed on all the samples and each meat products taken into account on this study. The equations defining the most significant interrrelations found are presented in Table 3, together with their correlation coefficients. The Cu-Ni relationship is notable, because it shows the highest correlation coefficient and is, furthermore, to be found in the four types of meat products studied.

The Cu-Mn and Ni-Mn correlations were found only in the overall samples and in the liver paste. In both of these cases, the slopes obtained for the liver paste were nearly two times as values than those for the overall samples.

This indicates that an increase of Mn in the liver paste occurs with a greater increase in Cu and Ni concentrations than in the other meat products studied. Pork liver paste shows a larger number of intermetallic correlations, possibly due to the fact that liver, being the main detoxifying organ, is, therefore, capable of accumulating greater concentrations of metals than any other organs or tissues.

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BLOOD BIOCHEMICAL PROFILE AS FISH WELFARE INDICATOR DURING TRANSPORT

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Key words: fish, welfare, transport, tanks, cages, blood biochemical profile

SUMMARY

The target of the present research was to compare the effects of two types of trout transportation upon welfare, on the basis of the blood biochemical profile. The first type of transport was one accomplished with a vehicle between two farms at 600 km distances, in 2 fish tanks of 3 m^3 each, with a density of 250 kg/fish tank (crowding conditions) and a transport period of 18 hours. The second one was made on water using immersed cages with normal fish density, the transport period being similar to the first one.
From both tanks and from the transport cage there were harvested 6-8 fish from which, after stunning, there was collected blood by caudal vein puncture (CVP), about 1-2 ml blood/fish. The blood was mixed, resulting in two samples: one from fish transported by vehicle and one from fish transported with cages. The samples were transported to laboratory, where the blood biochemical profile has been established by using Vettest 8008, being analyzed 12 blood chemistry indicators: blood urea nitrogen (BUN), phosphatemia (PHOS), calcium (CA), magnesium (MG), albumine (ALB), aspartate aminotransferase (AST), alanine transaminase (ALT), cholesterol (CHOL), triglycerides (TRIG), glucose (GLU), lactate dehydrogenase (LDH) and alkaline phosphatase (ALKP).
The results led to the following conclusions: the values of ALT, AST, ALKP and LDH were over the normal values no matter the transport type – for the trout transport with vehicles it was noticed in addition overvalues of PHOS, TRIG and GLU revealing high levels of stress; some of the overvalues (LDH, PHOS, ALKP) are due both to the dehydration and intense muscular effort during angling and restraining; the values of all analyzed indicators were significant higher for the fish transported with vehicles in comparison with values for fish transported using cages from fishnets (with a maximum 44.25 times higher for LDH) – this fact shows that welfare of trout transported by vehicle was unacceptable – the major cause of blood biochemical profile altering being transport stress and crowing.

The aquaculture industry, which has been growing at a very high rate for many years now, is projected to continue growing at a rate higher than most other industries for the foreseeable future (Lekang, O. I.; 2007; Man, C., Man A.; 2006; Decun, M.; 2004). However, the activity in this field couldn't be conceived without assuring an acceptable fish welfare level.

Similar to other animals of economical interest, fish welfare assessment could be conducted on the basis of ethological indicators, management system and practices, integrative numerical and H.A.C.C.P. systems. Fish serum biochemical profile was rarely approached due to the various difficulties which can occur in specimens' sampling and transporting, as well as due to the high costs of the analyses (Heming, T. A.; 1989; Thral, M.A.; 2004).

1. MATERIALS AND METHODS

The target of the present research was to compare the effects of two types of trout transportation upon welfare, on the basis of the blood biochemical profile as OWI (objective welfare indicator).

The first type of transport was one accomplished with a vehicle between two farms at 600 km distance. There are used 2 fish tanks of 3 m^3 each, the transport density applied being very high: 250 kg fish/tank (83.33 kg fish/m^3 of water, 2.77 times higher than the recommended value of 30 kg/m^3). The transport period was 18 hours. At unloading, the fishes were transferred with fishnets to a pond fixed on a mobile railed support, by which the fishes were brought from the unloading point to the lake into a transport floating cage (10 - 12 m height difference). As negative aspects of the destination point management, there could be mentioned the short pause period after transportation (10 - 15 minutes) and the lack of water mixing - water from the destination point (Bicaz Lake) the water from transport recipients - in order to help fish to cope with the local temperature and chemical composition. Also, water ejection from the tanks and the consecutive fish transfer generated a critical situation: fishes from the lower part of the tanks got into direct contact with the air for a long time (5 - 10 minutes).

The second type of transport was on water, using immersed cages with normal fish density (500 kg fish at 90 - 100 m3 water space, delimited by cage fishnet walls), the transport period being similar to the first one – but the transport velocity sensible lower.

From both tanks and from the transport cage there were harvested 6-8 fish which were anesthetized with Finquel (Tricaine Methanesulfonate: MS - 222) 100-200ppm concentration in water, then the blood was collected by caudal vein puncture (CVP). In order to reduce stress level during blood sampling, from each fish there were harvested maximum 2 ml of blood, then the samples were mixed - resulting two mixed blood samples: one from fish transported by vehicle and one from fish transported with cages. The samples were transported

to laboratory, then following coagulation and syneresis the serum biochemical profile has been established on 1:10 diluted samples, by using Vettest 8008. There were assessed 12 indicators: blood urea nitrogen (BUN), phosphatemia (PHOS), calcium (CA), magnesium (MG), albumine (ALB), aspartate aminotransferase (AST), alanine transaminase (ALT), cholesterol (CHOL), triglycerides (TRIG), glucose (GLU), lactate dehydrogenase (LDH) and alkaline phosphatase (ALKP).

2. RESULTS AND DISCUSSIONS

The obtained results are shown in the following table.

Table no. 1

The biochemical parameters assessed in the serum of fish harvested from the two types of transport – by vehicle and by water, in cages

Assessed parameters	Values for fish transported by vehicle	Values for fish transported in cages	Reference values for trout in specialty literature
BUN (mg/dl)	23,0	7,0	30
PHOS (mg/dl)	27,8	16,1	14
CA (mg/dl)	3,5	10,6	16-29
MG (mg/dl)	4,08	3,23	N/A
ALB (g/dl)	0,0	-	N/A
ALT (u/l)	261	54	23-43,20
AST (u/l)	1698	136,4	29-52,45
CHOL (mg/dl)	-	126	125-225
TRIG (mg/dl)	225	44	121-206
GLU (mg/dl)	161	23	20-70
LDH (u/l)	12390	280	26-60
ALKP (u/l)	137	110	46,1-60,5

As it could be noticed in the table, the blood urea nitrogen is within normal values for both types of transport; however the value is 3.29 times higher for vehicle transportation in relation with fish cage transportation.

For phosphates, the value is close to normal for fish transported in cages, while for fish transported with vehicle was recorded an 1.98 times exceeding.

For calcium, the values were within the reference interval and for magnesium the values were similar for the two different types of fish transport (we couldn't find in specialty literature an admitted limit for magnesium in trout).

The albumin analyzes results was beyond the sensibility limits of the kit used, so the results are inconclusive.

For LDH, the normal value was exceed by 206.5 times in fish transported by vehicle and by 4.66 times in fish transported by cage and for ALKP by 2.26 times, respectively 1.81. The most probable reason of the biochemical profile modification (phosphate, LDH, ALKP) is the intense muscular effort associated with fish collecting (by angling) and restraining, doubled by a mild hemoconcentration caused by maintaining the fish emersed. It can be concluded that fish welfare level is acceptable, but the harvesting and blood sampling techniques must be modified. Nevertheless, the values are higher for fish transported by vehicle compared with those for fish transported on lake in cages; facts which suggest a high level of transport stress for this particular type of transport, hence a lower level of welfare.

For alanine transaminase, the values recorded a 4.61 times exceeding of the normal values for fish transported by vehicles and a slightly exceeding for fish transported in cages (1.25 times).

For aspartate aminotransferase, there are recorded exceeding of the normal value by 32.37 times in fish transported by vehicle and by 2.6 times in fish transported with cages.

For cholesterol the values were normal, while in triglycerides and glucose were recorded overvalues only for fish transported by vehicle (1.9 times, respectively 2.3 times).

The significant increasing of cholesterol, transaminases (ALT, AST) and glucose in fish transported by vehicles could stand for hepatopancreatic disorders and subsequently very poor welfare.

3. CONCLUSIONS

3.1. The values of ALT, AST, ALKP and LDH were over the normal values no matter the transport type – for the trout transport with vehicles it was noticed in addition overvalues of PHOS, TRIG and GLU revealing high levels of stress;

3.2. Some of the overvalues (LDH, PHOS, ALKP) are due both to the dehydration and intense muscular effort during angling and restraining; the values of all analyzed indicators were significant higher for the fish transported with vehicles in comparison with the values for fish transported using cages from fishnets (with a maximum 44.25 times higher for LDH) – this fact shows that welfare of trout transported by vehicle was unacceptable – the major cause of blood biochemical profile altering being transport stress and crowing.

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WATER QUALITY AS FISH WELFARE INDICATOR DURING TRANSPORT

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Key words: transport, fish, tanks, water quality

SUMMARY

The present study aimed the water quality during a trout transport accomplished in order to repopulate a fish farm, with individuals of 90 g average weight. The transport was made between two farms at 600 km distances, in recipients with a total water volume of 6 m^3 (2 fish tanks of 3 m^3 each), with a density of 250 kg fish/tank. The transport period was 18 hours.
From the two fish tanks there were collected water samples for assessing the physical-chemical parameters (dissolved oxygen, residual chlorine, pH, phosphates, nitrites, nitrates, ammonia, phenols, copper, sulphates and detergents), as well as microbiological parameters (Aerobic plate count - APC, Fungal Plate Count - FPC and total coliforms).
The water physical-chemical parameters analyses were done by using Spectroquant Nova 60 fotocolorimeter and the microbiological ones by using Compact Dry TC (Total count), YM (Yeast and Molds) or CF (total coliforms) rapid assays.
The researches led to the following conclusions:
- dissolved oxygen shows quite high values (14.9 - 15.3 mg/l), having in view that the minimum admitted limit for trout is 6 mg/l;
- residual chlorine and ammonia exceed the maximum admitted limits for trout for 12 times, respectively 14 times;
- phosphates exceeded the admitted limit for 22 times and copper for 2.3 times;
- regarding the microbiologic properties, the water in the two fish tanks was appropriate.
The inadequate water physical-chemical parameters during the transport, the high mortality rate which was recorded and overcrowding (very high fish density) prove poor welfare of trout stock during the transport.

The transport of live fish and specifically the indigen trout (*Salmo Trutta Fario*) can be made for the following purposes: repopulation, saving juveniles during water level decreasing caused by drought or during winter and for food.

Concerning the fish transport by vehicle, the following aspects should be taken into account: water temperature must be maintained all the period at 10°C (slight variations 8 - 12°C) by permanent control using thermometers and supplying with ice cubes; fish density; vehicle's

speed, water physical - chemical and microbiological parameters (Man C., Man A, 2006; Decun M., 2004).

Right after the arrival to the destination point, it should be assured a pause of 30 - 40 minutes for calming the fishes. In order to avoid the thermical shock, the water from the transport recipients should be slowly mixed with the water from the launching farm.

If using fishnets, these should be in good state, clean and disinfected and the iron frames should be covered with cotton-cloths (Man C., Man A., 2006; Nicolescu Carmen, 2002).

1. MATERIALS AND METHODS

Water quality during the trout transport between two farms was established based on samples collected from the two fish tanks. For each tank, water volume was 3 m^3 and the trout quantity 250 kg. Each tank has an aerator which is connected to the manometer and the oxygen cylinder. The transport period was 18 hours.

From the collected water samples, there have been assessed the physical - chemical parameters (dissolved oxygen, residual chlorine, pH, phosphates, nitrates, nitrites, ammonia, phenols, sulphates, copper and detergents) and the microbiological parameters (APC, FPC and total coliforms).

Water physical - chemical parameters have been assessed by using Spectroquant Nova 60 fotocolorimeter, while the microbiological ones by running rapid assays as Compact Dry TC, YM and CF.

2. RESULTS AND DISCUSSIONS

Average values of water physical - chemical parameters during the live trout transport between two farms are shown in table no. 1.

Table no.1

Water quality during transportation by vehicle in fish tanks: physical - chemical parameters

Sampling point	*Analyzed parameters*										
	O_2 mg/l	Cl_2 mg/l	pH	P mg/l	NO_2^- mg/l	NH_4^+ mg/l	NO_3^- mg/l	Phenols ng/l	Cu mg/l	SO_4^{2-} mg/l	Deterg. mg/l
Tank 1	14,9	0,37	7,0	4,4	0,027	13,2	0	0,55	0,23	35	0,02
Tank 2	15,3	0,42	7,1	4,55	0,030	11,5	2	1,11	0,20	35	0,02
Admitted limit for trout	6 mg (min.)	0,01–0,03	6-7,2	0,2	<0,2	<1	<20	6-7	0,1	2-7	-

Analyzing the data in the table, it could be noticed that:

- dissolved oxygen shows quite high values (14.9 - 15.3 mg/l), having in view that the minimum admitted limit for trout is 6 mg/l. The excess of the dissolved oxygen in water may frequently result in unfavorable effects, intensifying the metabolism or leading in extreme cases to fish paralysis;

- the assessed residual chlorine exceeded the admitted limit for trout by 12 times in tank 1 (T1), respectively by 14 times in tank 2 (T2). The chlorine in excess in water can provoke to fish agitation, body position altering, mouth and caudal fin spasmodic movements, gills' congestion, fish body surface ischemia, asphyxia;

- phosphates had recorded overvalues by 22 times;

- nitrogen in fishponds is found as salts: nitrates and ammonia salts, coming from the dead organic substance decomposition or from nitrogen fixation in water due to nitrifying bacteria. Nitrites and nitrates recorded values way below the admitted limit in all water samples. The values for the ammonia ion exceeded by 12 times the admitted limit. Through the residual chlorine, nitrites ions enter fish' organism at gills' level. In blood, nitrites are bound to hemoglobin, resulting methemoglobin, with consecutive reduction of oxygen carrying rate, the gills and the blood color altering into brown (Heghedus Cristina, 2005);

- copper exceeded the admitted limit (0,1 mg/l) in all collected samples by 2.3 times. This metal has a role in hemoglobin and some enzymes synthesis. As salts (copper sulphates), it is used for stopping water eutrophication phenomenon. Copper overvalues in the collected samples could be justified by its existence in the transport water;

- the detergents in the water are within the admitted limits.

The results of water microbiological exams are shown in table no. 2.

Table no. 2

Quality during transportation by vehicle in fish tanks: microbiological parameters

Locul prelevării	***Analzyed parameters***		
	APC/ml	**FPC/ml**	**Total coliforms**
Tank 1	0	7	0
Tank 2	0	2	0
Admitted limit for trout	-	-	-

Analyzing the data in the table, it can be observed that at 37°C there are not developing aerobic bacteria and total coliforms. As for the fungi there has been recorded a higher number in tank 1.

Finally, there has been analyzed trout stock mortality in the two tanks which reveal a percent of 18 - 20 in T1 and 25 in T2. The causes of these high mortality consisted in the period of transport (18 hours), overcrowding and the high residual chlorine concentration (explained by tank disinfection with chloramine) and their inappropriate rinse before getting the fish in.

At unloading, the fishes were transferred with fishnets to a pond fixed on a mobile railed support, by which the fishes were brought from the unloading point to the lake into a transport floating cage (10 - 12 m height difference).

As a positive aspect of the destination point management, it could be mentioned the use of clean and disinfected fishnets, which had the iron frames covered in cotton-cloths in order to avoid injuries. As negative issues, there could be mentioned the short pause period after transportation (10 - 15 minutes) and the lack of water mixing from the destination point (Bicaz lake) with transport recipients water in order to cope with the local temperature and chemical composition.

Also, water ejection from the tanks and the consecutive fish transfer generated a critical situation: fishes from the lower part of the tanks got into direct contact with the air for a long time (5 - 10 minutes). Another negative issue was the high transport density: 250 kg fish/3 m^3 tank, having in view that the admitted limit is 30 kg/m^3. The overcrowding generated behavioral alteration before and during the unloading period: rapid swimming, agitation, frequent leaps outside the water (*** - The welfare of animal during transport, *** - The welfare of fish in European Aquaculture).

3. CONCLUSIONS

3.1. Residual chlorine exceeded the admitted limit for trout by 12, respectively 14 times.

3.2. Ammonia ion exceeded the admitted limit by 12 time.

3.3. Dissolved oxygen had high values (approximately 15 mg/dm^3 water) in correlation with the admitted limit of 6 mg/dm^3.

3.4. Phosphates recorded values which exceeded the admitted limit by 22 times and copper by 2.3 times.

3.5. Regarding the microbiologic water parameters, in the two tanks there were not isolated aerobic bacteria nor total coliforms.

3.6. Mortality percent in the two tanks was in average 22,5%.

3.7. Trouts' high mortality, the inappropriate water physical-chemical parameters during transportation, the high density indicate a poor fish welfare during transport.

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WATER QUALITY AS FISH WELFARE INDICATOR IN POTOCI FISH FARM

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Key words: fish, parameters, water, welfare, samples

SUMMARY

Water quality has a major role in fish evolution. Considering this background, from Potoci fish farm there were collected water samples at different depths in juvenile and adult trout's floating cages. From the collected samples, there were determined the following physical-chemical parameters: dissolved oxygen, residual chlorine, pH, phosphates, nitrates, nitrites, ammonia, phenols, sulphates, copper and detergents, as well as water microbiological parameters: aerobic plate count (APC), fungal plate count (FPC) and total coliforms.

Water samples were collected by using a vertical point sampler for the ones at 20 and 35 m depth and by using PSB4 portable sampler for the others. Water physical and chemical parameters assessments were made by using Spectroquant Nova 60 photocolorimeter and the microbiological ones by running Compact Dry TC (total counts), YM (Yeasts and Molds), CF (coliforms) tests.

These assessments led to the following conclusions: most of the physical and chemical parameters (dissolved oxygen, pH, nitrates, nitrites, copper and detergents) recorder values which range within the admitted limits for trout, except for the residual chlorine in the depth samples which recorded overvalues by 2.33 times, the phosphates in the adult trout floating cages by 1.5 times and sulphates in all samples by 3.2 - 4.4 times.

The water microbiological test shows a high load of total aerobic bacteria, fungi and total coliforms.

In Potoci trout farm, the water as fish environment (less the microbiological issue) ensures a good welfare of fish stock.

Animal welfare represented the subject of many argues even since the beginnings of their use as zootehnic interest species or as favorite animals. For the today society animal welfare does not longer represent an issue or a perspective, but something compulsory (Teuşdea V., 2005).

Up to recently, fish were considered subjects with no pain perception, but the scientific approach invalidated the hypothesis of subjective states absence in fish (pain, suffering, contempt), thus the welfare concept could cover also the poikilotherm species (Broom D., 1991; 2009).

There have been proved by several researchers (Kirby, 2003; Chandroo, 2004, cited in Mitranescu E. et al., 2009) that fish possess a nervous system similar to mammalian's, which transmits the pain sensibility from nociceptors to brain; as well as the fact that fish have major learning abilities revealing the existence of sentience and that the fish present stress physiological reaction similar to mammals', reactions which are implying stress hormones releasing.

In the modern aquaculture, fish optimum welfare implies ensuring rearing conditions which allow the fish to maintain homeostasis to a normal somatic development and to be protected from stress.

Fish life environment quality, as regard of physical, chemical and microbiological factor, represents a fish welfare indicator of major importance.

1. MATERIALS AND METHODS

Water quality in Potoci trout farm has been established based on water samples collected from 10, 20 and 35 m depths, as well as from juvenile and adults floating cages. From the collected samples, there were assessed water physical and chemical parameters as: dissolved oxygen (O_2), residual chlorine (Cl_2), pH, phosphates (PO_4^{3-}), nitrates (NO_3^-), nitrites (NO_2^-), ammonia (NH^{4+}), phenols, sulphates (SO_4^{2-}), copper and microbiological parameters: aerobic plate count (APC), fungal plate count (FPC) and total coliforms.

Water samples were collected by using a vertical point sampler for the ones at 20 and 35 m depths and by using PSB4 portable sampler for the others.

Water physical-chemical parameters assessments were made by using Spectroquant Nova 60 photocolorimeter and the microbiological ones by running Compact Dry TC (total counts), YM (Yeasts and Molds), CF (coliforms) tests.

Results interpretation was made depending on the reference levels for trout provided by some foreign authors' works (Hemming, T.A., cited in Dutta T., 2005).

2. RESULTS AND DISCUSSIONS

Average values of water physical and chemical parameters from Potoci fish farm are shown in table no. 1.

Analyzing the data in the table there could be noticed the following:

- dissolved oxygen recorded adequate concentration, the admitted limit for trout being of 6 mg/l;

- residual chlorine for most of the samples has recorded values at the maximum admitted limit, except for the water samples collected from 20 m depth in which it is exceeded by 2.33 times;

- water pH ranged within the admitted limits for trout in the samples collected from 10, 20 and 35 m depth and it turned to be alkaline in the samples collected at 2 m depth from adult and juvenile trout floating cages. The water with neutral pH indicates a poor buffering power and acidifying trend;

- phosphates recorded values within the admitted limit of 0.2 mg/l in most samples, except those collected from the adult trout floating cage where the limit was exceeded by 1.5 times;

- nitrites and nitrates are within the admitted limits of 0,2 mg/l respectively 20 mg/l for all the samples;

- copper recorded values bellow the maximum admitted limits of 0.1 mg/l for all samples;

- phosphates exceeded the limits provided for trout (2 - 7 mg/l) by 3.2 - 4.4 times in all samples;

- detergents have recorded values within the admitted limit;

- phenols recorded overvalues in all samples.

Table no. 1

Average values of water physical - chemical parameters in Potoci - Bicaz trout farm

Sampling point	*Analyzed parameters*										
	O_2 mg/l	Cl_2 mg/l	pH	P mg/l	NO_2^- mg/l	NH_4^+ mg/l	NO_3^- mg/l	Phenols ng/l	Cu mg/l	SO_4^{2-} mg/l	Deterg. mg/l
Bicaz lake 10 m depth	9,5	0,03	7,7	0,1	0,014	Und.	2,0	0,04	0,01	23	0,01
Bicaz lake 20 m depth	13,5	0,07	7,1	0,2	0,006	Und.	1,0	0,14	0,02	31	0
Bicaz lake 35 m depth	11,5	0,03	7,3	0,2	0,004	Und.	1	0,04	0,01	23	0
Adult trout floating cage	9,3	0,02	8,0	0,3	0,015	Und.	0	0,01	0,02	22	0,02
Juvenile trout floating cage	9,1	0,02	7,9	0,2	0,016	Und.	Undet.	0,04	0,015	24	0,02
Admitted limits for trout	6 mg (min.)	0,01–0,03	6-7,2	0,2	<0,2	<1	<20	6-7	0,1	2-7	-

Water temperature has an influence upon dissolved oxygen concentration in water, thus for each degree over the admitted value the oxygen concentration decreases by approximately 0.2 mg/dm^3. The dissolved oxygen exceeding could lead to fish paralysis.

Also, temperature and water pH are influencing the ammonia quantity in water. Ammonia concentration in water rises as a results of both water temperature and pH rising.

Chlorine in excess can produce to fish agitation, side floating; spastic movement of mouth and caudal fin, asphyxia and death.

Phosphorus is a chemical element important for fish ponds, with a major role in aquatic organisms' life and in fish units' productivity. Phosphorus organic compounds (ATP and ADP) are the main macroenergetic substances of fish metabolism.

From the collected samples there were ran microbiological analyzes as APC, FPC and total coliforms (table no. 2).

Table no. 2

Water microbiological parameters values in Potoci fish farm

Sampling point	***Analyzed parameters***		
	APC/ ml	**FPC/ ml**	**Total coliforms/ml**
Bicaz lake – 10 m depth	660	17	128
Bicaz lake – 20 m depth	594	45	136
Bicaz lake – 35 m depth	550	18	260
Juvenile trout floating cage (85)	900	1	687
Juvenile trout floating cage (104)	260	1	714
Adult trout floating cage (214)	374	16	520
Adult trout floating cage (221)	300	4	360

Analyzing the above results it can be noticed a high load of microbiologic elements, being recorded a high number of aerobic plate count, fungal plate count and total coliforms.

Microorganisms find in water appropriate conditions to develop. They can have autochthon origin, forming specific biocenoses (plnaktonical, neustonical, benthal, epibiotical) and allochthon origin (those which come accidentally in the water from soil, carried by the wind or from precipitations' water, generated by men, animals or industrial activities or ubiquitary - living everywhere: in air, on soil or in water.)

From the autochthon microorganisms, could be noticed bacteria, fungi, as well as protozoans and algae. In water are present all the physiological categories of bacteria (heterotrophic, autotrophic, chemoautotrophic etc.). The yeasts and molds from the water could result from soil or - the pathogenic ones - from ill organisms (plants or animals).

Between fish and microorganisms appear commensal relations: from the trout intestine were isolated over 200 bacterial species.

The maximum admitted density of microorganisms in the water is 10^{12} CFU/ml.

The bacteria surviving time in the water is inversely proportional with organic and microbiologic water loadup.

3. CONCLUSIONS

3.1. Dissolved oxygen, nitrites, nitrates, copper and detergents recorded values appropriate for fish rearing.

3.2. Residual chlorine exceeded the maximum admitted limit for trout for 2.33 times in water samples collected from 20 m depth.

3.3. Water pH in floating cages was alkaline.

3.4. Phosphates exceeded the maximum admitted limit for fish for 1.5 times in adult fish cages.

3.5. For all samples, sulphates exceeded the maximum admitted limit for 3.2 - 4.4 times.

3.6. Regarding microbiologic parameters, water has an intense load in aerobic germs, as well as in fungi and total coliforms.

3.7. In Potoci trout farm, the water as fish environment (less the microbiological issue) ensures a good welfare of fish stock.

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THE INFLUENCE OF POLLUANT FACTORS ON THE ERYTRONE IN MILK CATTLE FROM THE AREA OF VALCEA

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Key words: polluant factors, milk cattle, erytron

SUMMARY

The industries that developed after using the natural resources, have become the biggest pollution sources with great consequences on the environment although they represent the basis for the economics growth of Valcea county. The urban areas are the most affected by pollution, the main sources being the chemical industry, the traffic, the fuel burning (methane gas, fuel, combustible liquid) and the waste burning, etc.
The aim of the investigations presented in this paper was to follow the changes brought by pollution to the erytrone and its use in observing the welfare of milk cattle from this part of the country.
The month of June was chosen to make the hematological investigations taking into consideration that the animals have passed over the stalling period to graze where it is possible for the hematological values to be modified by the environment's state, taking into consideration the soil-plant-animal relation.

Introduction. Situated in the south-central part of Romania, Valcea county is a territory with different forms of relief organized in steps. The main environmental problems are linked to the coal exploitation in the Alunu-Berbesti area, to the lime from Bistrita-Costesti , to the exploitations of mica from Cataractele Lotrului and to salt from Ocnele Mari, to oil extractions from Madulari and Babeni areas, to the primary and secondary processing of wood, as well as to the synthesis of different chemical substances (organic and non-organic) from the industrial platforms situated in the south of Ramnicu Valcea. Due to the pedo-climatic conditions specific to Valcea county, the raising of animals is one of the oldest occupations of the inhabitants. Taking into consideration the major danger and the growing threats that the polluting substances have upon the environment, the man and the animal welfare we considered that an evaluation of the cattle's welfare is necessary. For the beginning, we chose the determination of the most important hematological parameter that constitute a barometer for

quantifying the effects of these factors on milk cattle, animals that represent a real nutrient reservoir for man (milk for example).

1. MATERIAL AND METHOD

In June, the current year, 23 blood samples were taken from milk cattle with the age between 5 and 6 years, Holstein breed, from the households in areas situated close to the industrial platforms and considered as areas with potential pollution. The samples were sent for analysis to the Diagnosis and Health Animal Institute from Bucharest where the animals' health state was monitorized in the nutritional-metabolic rate in the context of insuring their welfare, the man's and environment's protection. Blood was taken by puncture the jugular vein in the morning before giving the cereal ratio in tubes of Vacutainer type with anticoagulant (EDTA) of 3 ml. *The quantitative hematological examination* was made in the AcT5diff CP automatic analyzer, the determination of the erytrone being part of it: erythrocytes number (RBC), hemoglobin (HGB), haematocrit, (HCT) derived erythrocyte indices: Medium Cells Volume (MCV), Medium Cells Hemoglobin (MCH), Medium Concentration Cells in Hemoglobin (MCHC).

The qualitative hematological examination containing cito-morphological examination on peripheral blood smear, panopticon coloured May Grunwald Giemsa, was made at Leica DM LS2 microscope.

For analyzing the pollution factors, data was taken from the activity report of the Environmental Ministry and Environmental Protection Agency from Valcea, 2006-2008 that makes the integrated analysis of the environmental factors.

2. RESULTS AND DISCUSSIONS

The results obtained from the hematological examination are presented in table 1.

Table 1

Values of the hematological parameters (erytron) in milk cattle

Crt. nr.	RBC mil/mm^3	HGB g/dl	HCT %	MCV μ^3	MCH pg	MCHC g/dl
1.	6,02	11,5	32,5	54	19,1	35,3
2.	4,63	9,0	24,9	54	19,4	36,0
3.	5,28	9,5	32,6	50	18,1	36,1

4.	6,63	11,5	32,4	49	17,4	35,6
5.	5,68	10,9	30,2	53	19,2	36,2
6.	5,00	9,2	25,4	48	17,3	36,0
7.	5,57	10,5	29,2	52	18,8	35,9
8.	5,33	10,3	28,1	53	19,2	36,5
9.	6,99	13,1	35,7	51	18,7	36,6
10.	5,01	10,3	28,5	51	18,5	36,0
11.	5,78	10,8	30,1	52	18,7	35,9
12.	6,02	11,5	31,4	52	19,1	36,6
13.	5,28	8,9	24,0	46	16,9	37,1
14.	4,97	10,0	25,5	51	20,2	39,4
15.	5,42	10,7	30,0	55	19,8	35,8
16.	5,65	9,7	26,6	47	17,2	36,5
17.	6,03	10,8	29,5	49	17,8	36,5
18.	4,69	9,0	24,6	52	19,2	36,7
19.	6,04	10,3	26,0	43	17,0	39,6
20.	5,01	8,9	24,1	48	17,7	36,9
21.	5,57	9,6	26,0	47	17,3	37,0
22.	3,66	7,2	17,6	48	19,6	40,6
23.	6,14	11,1	30,1	49	18,1	36,9

From their analysis it can be seen that no samples come into the limits of normal reference values for age and physiological state of the investigated animals. Every animal of the 23 investigated animals show changes for at least one hematological parameter (bold values). For milk cows anemia is taken into consideration when the erythrocytes number drops under 5,1 mil/mm 3, the haematocrit under 32 % and the hemoglobin under 9,2 g/dl. In comparison with normal physiological values of reference for every animal, the following results were obtained:

The number of erythrocytes dropped to a number of 7 samples, the value of hemoglobin dropped to 5 samples and the haematocrit to 17 samples, the MEV value dropped to 10 samples from the 23, showing the presence of a *normocitare normocrome anemia* in 10 samples from 23 and a *normocrome microcitare anemia* in 10 samples, too. The cyto-morphological examination proved quantitative changes and confirmed the presence of anemia through anisocytosis ++ (*microcytes*), poikilocytosis ++ (*acantocytes, echinocytes, schistocytes*), anisocromia ++ (*hypocromic cells, target cells*).

In Valcea county systematic measures of pollution concentrations in atmosphere were made in seven areas (5 points at the Chemical level platform Ramnicu Valcea where more than half of the county's activity is concentrated and 2 points at the city's level).

From the pollution factors settled through the Kyoto Protocole (1999) the rules of taking the control the gases that determine the

greenhouse effect in Valcea county we can find the following gases: carbon dioxide, methane, azot oxide.

Table 2

Pollution concentrations in atmosphere in Valcea county

Nr. crt.	Emisia	U/M	Anul monitorizării		
			2006	2007	2008
1.	Carbon dioxide (CO_2)	Gg	1939,68	1783,25	2153,09
2.	Azot oxid (NO_2)	Mg	978,74	964,22	1001,14
3.	Methan (CH_4)	Mg	12.318,28	8442,4	5310,00

The CO_2 (carbon dioxide) contributes to the ozone layer damage, being the sub-product of the main fuel burning: fuel, coal, natural gas, wood. Deforestation is added to the CO_2 growth in concentration in the atmosphere, including the forest fires, etc.

The CO_2 resulted is absorbed by the terrestrial plants and the phytoplankton, the exceeding being added to the atmospheric one. While the CO_2 emissions have a growing tendency (owning to the use of coal from Berbesti) the methane emissions are fluctuant, the crude oil productions from the territory being different.

3. CONCLUSIONS

3.1. The month of June was chosen for making the hematological investigations, taking into consideration that the animals got out of the stalling period, to graze where it is possible for the hematological values are changed by the environmental state due to *soil-plant-animal* relation;

3.2. Even if the animals didn't show any clinical signs of anemia by making the investigations it is shown that it is present in a percentage of 86,95 %, proving its sub-clinical evolution;

3.3. It is known that the red series values (the erytrone) especially of hemoglobin and hematocrit at milk cows are closely related to milk production and their feeding and environment where they are exploited (Parvu, 1999);

3.4. Unlike other species, at cattle, the anemia etiology is very different (nutritional, toxic, parasitize, infectious, etc.) that is why, for etiopatogenetics difference we determined the derived ertrocytes indices and we made clinical investigations, epizootologics investigations,

nutritional analysis, data that will be used in a detailed study of these factors on Valcea county.

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EFFECT OF PROBIOTICS ON PERFORMANCE AND HEALTH PARAMETERS IN SWINE

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Keywords: probiotics, daily gain weight, swine mortality

SUMMARY

The aim of this study was to observe the efficacy of probiotics,based on genus Bacillus and Lactobacillus in sows ,suckling pigs and fattening pigs following the protocol: "L" Probiotical solutions containing Lactobacillus was administrated to new born piglets and "B" Probiotics premix containing Bacillus wich was administraed to pregnant and lactant sows and to fattening pigs.
The results were the significantley reduction of the mortality caused by diareea and the lowest mortality in piglets in comparision with the control batch,a raise in the daily weight gain,and the shortening of the fattening period.
The therapy and nutritional prophylaxis with probiotics,from Bacillus and Lactobacillus strains affected the health and performances of sows ,piglets,and fat pigs,being an alternative method on antibiotics as growth factors.

The use of antibiotic growth stimulators has been gradually eliminated in European Union Countries and things seem to continue that way for the treatment of bacterial intestinal or respiratory infections as well. The use of probiotics in nutritional therapy and prophylaxis has been aimed to counteract the main results of stress, disease or use of antibiotics. Various types of stress (as in nutritional, transport, birth, weaning, separating, bacterial, viral or micotic exposure) cause the inhibition of good digestive microflora, modifing the gastrointestinal pH, leading to imunosupression, pathogen microflora multiplication and, finally, the decline of the productive performances and even mortality.

1. MATERIALS AND METHODS

Obtained from various combinations of bacterian types, (like Bacillus and Lactobacillus) probiotic products are given to pregnant sows, piglets and fat pigs, following the next protocol:

„L" probiotic, oral solution, containing Enterococcus faecium, Lactobacillus acidophillus, Lactobacillus casei and Lactobacillus plantarum, was administrated to new born piglets

- Experimental batch: 600 piglets, 2 ml in the first day and 4 ml in the fourth day;

Control batch: 600 piglets received in the first and the fourth day the same amount of steril physiological serum;

-„B" probiotic, containing Bacillus licheniformis and Bacillus subtilis, was administrated to pregnant sows and fat pigs ratio like this:

-Experimental batch: 50 pregnant sows in their one hundreth day of gestation, received 0,3 kg of probiotic premix/ton until birth and 0,6 kg/ ton in the first two weeks of lactation;

-Control batch: 50 pregnant sows, without probiotics in food.

-Experimental batch of 400 fat pigs, 72 days old, fed to 95 days, on the first phase, with 450 g premix/ton and from 95 to 140 days with a 150g premix/ton;

-Control batch of 400 fat pigs, normally fed, without probiotics, of the same age and enviromental conditions as the experimental batch.

Concentration of „L" probiotic product: $3,2 \times 10^9$ UFC/ml solution

Concentration in sow's food: $3,2 \times 10^9$ UFC/g in food for lactant sows;

$1,6 \times 10^9$ UFC/g in food for pregnant sows;

$2,1 \times 10^9$ UFC/g in food for fat pigs in first phase;

$0,8 \times 10^9$ UFC/g in food for fat pigs in second phase

2. RESULTS AND DISCUSSIONS

For the sows in the experimental batch (in the last 15 days before birth and the first 4 weeks of the lactant period), the administration of „B" probiotic pointed out a very important drop in mortality by comparison with the control batch, the difference exceeding the normal situation by 62,5% (see Table I).

This is a serious argument for giving up the antibiotic and chemotherapic use and an economic reason to use probiotics in order to increase the profit in swine farms.

Table 1

The influence of „B" probiotic administration in pregnant sows

	Witness batch	„B" probiotic treated batch	Differences	Percentage differences %
Mortality in piglets	8	3	5	62,5

In fat pigs, „B" probiotic was administrated in the first phase (from 72 to 95 days) to fat pigs of 28 to 40 kg and in the second phase (from 95 to 140 days), until the weight of 70 kg.

The daily weight gain is presented in Tabel II:

Table 2

Probiotic „B" administration influence on daily weight gain in fat pigs

Production parameters	Control batch	Probiotic „B" treated batch
Period	68 days	75 days
Daily weight gain I (g)	521	480
Daily weight gain II (g)	666	600
Total weight gain (g)	610	560

Table 3

Piglets administration effects of probiotic „L" on mortality due to diarrhea and daily weight gain

Mortality / Weight gain	Control batch with antibiotic treatment	Experimental batch with „L"probiotic treatment	Difference
Mortality in piglets by diarrhea (%)	3,2	0,5	2,5
Daily weight gain (kg)	0,188	0,224	0,036

3.CONCLUSSIONS

3.1. Probiotic „B" administration in pregnant sows' food (the last two weeks) and lactant (the first four weeks) led to 62,5% drop of mortality in piglets, in comparison with the control batch.

3.2. Probiotic „B" administration in fat pigs' food, in the first and the second phase, led to the rise of daily weight gain with 50g/phase and reduced the fattening period with over 7 days.

3.3. „L" probiotic, oral solution, given to piglets in two doses, in the first and fourth day of life, significantly reduced the mortality caused by diarrhea and rised the daily weight gain by 36g/day.

3.4. The therapy and nutritional prophylaxis with probiotics, from Bacillus and Lactobacillus strains, affected the health and performances of sows, piglets and fat pigs, being an alternative method to antibiotics as growth factors.

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CARBON BALANCE AND THE ATMOSPHERE GREEN HOUSE EFFECT

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Key words: organic matter, green house gases, global heating

SUMMARY

The global heating of the earth is due to the increasing concentration of green house gases (CO_2, CH_4, ClFlC, and N2O) in atmosphere. CO_2 and CH_4 are Carbon compounds produced by live beings. There are voices claiming for decreasing the farm animal livestock in order to protect against global heating. The Carbon balance on the Earth shows that Carbon is found under ground as diamond, as lime or other mineral salts and as organic compounds forming the fossil fuel (coal, oil, and natural gases). This Carbon is blocked to having relations with the atmosphere. On the Earth surface Carbon is found as stoked in dead organic matter, as deposited in the body mass of the living beings and as mineral gas compounds in atmosphere. Only atmospheric gas compounds of carbon have green house effect. Photosynthesis uses CO_2 to format live organic matter, diminishing its quantity in atmosphere. This organic matter has to be mineralized in CO_2, H_2O and N. Nature delays mineralization by the autotrophic and by the heterotrophic nutrition nets protecting Earth from the global heating. More living beings on the Earth mean more deposited Carbon and more protection against global heating. This fact is true for the farm animals as well. Bio fuel is a shortcut in mineralizing organic matter. Emission of CH_4 results from anaerobic fermentation. Ruminant farm animals are producing CH_4 during the rumen digestion of fodder. Since anaerobic bacteria mineralizing organic matter up to H_2 and C perhaps don't exist, vaccination against methane emission seems to be utopia. Animal farming produces CH_4 when manure is collected hydraulically and is fermented anaerobic. Humans protect the Earth against global heating if increase green plans culture, if use less fossil fuel, if avoid anaerobic fermentation of animal farm manure, if improve feed conversion in useful animal products.

Measuring the external temperature along the time there has been founded that the Earth uses to have a $+15^0$ C constant temperature. It is generally accepted that the fact is due to atmosphere air composition. There are gases like Nitrogen (78%) and Oxygen (20.94%) whose molecules don't retain heat out of the infrared sun rays, but molecules of other gases as water vapours (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrogen protoxide (NO_2) and cloro-fluoro-carbones (CFC) do it. These are the green house gases.

In the last time an increase of the medium temperature of the Earth was registered. It is explained by higher concentration of one or more green gases in the atmosphere. Major contributors to increasing Earth

temperature are CO_2 and CH_4 considered responsible for more than 50% and, respectively, about 20% of the global warming.

CARBON STATES IN NATURE

The most interesting gas, out of them, is the CO_2 which is produced by all live beings trough respiration and by aerobe fermentation of part of the dead organic matter (Dom) and is consumed by the green plants trough photosynthesis. If the Carbon trace in the natural primary nutrition net is flowed (*fig. 1.)* it is found out that the C from the atmospheric CO_2 is used for biological production of first producers that becomes feed for first consumers. Further the deposited biological production of producers is feed for the secondary consumers and biological production of the secondary consumers is food for the top consumers, which usually are the humans.

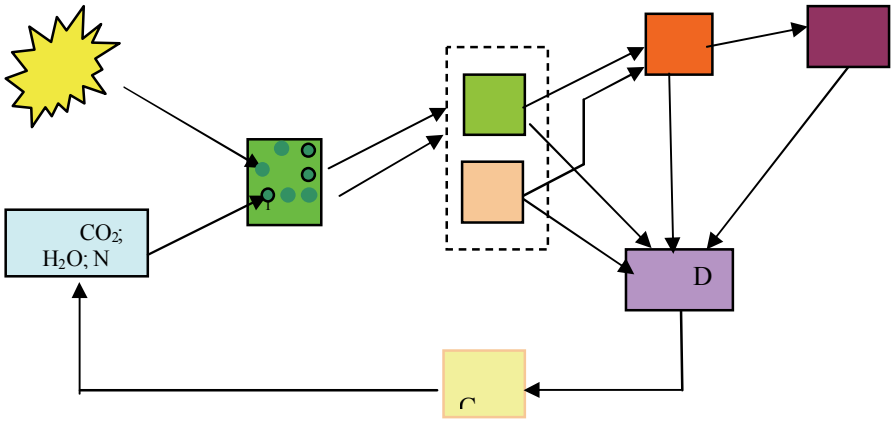


Fig. 1 Primary nutrition net

Presenting the first nutrition net in more details we must say that the atmospheric CO_2, by means of sunlight and chlorophyll granules of the green plants (P_1), is deposited in the body mass of these primary producers. Out of here part of it is sent back to the atmosphere as CO_2 by plants' respiration, part is stocked in dead organic matter formed from the excreted biological production of plants and in the body of the dead ones. The deposited biological production and the Carbon in it are ingested by the grazing animals (G) which are the first consumers. The ingested C is used in the grazing animal metabolism. So part of it is eliminated in atmosphere as CO_2 during respiration, part is deposited in the body mass of the secondary consumers and part is stocked in the

excreted biological production of the grazing animals. Part of the stocked Carbon in the dead organic matter produced by the first consumers is used as nourishment by the saprophyte plants or is mineralised by micro consumers (*fig. 2.)*. In the first case C is deposited in the body mass of the new consumers. In the second case C is liberated in atmosphere as CO_2 or CH_4 according to the fermentation that had taken place, aerobe or anaerobe. The dead organic matter that wasn't mineralised is buried under ground.

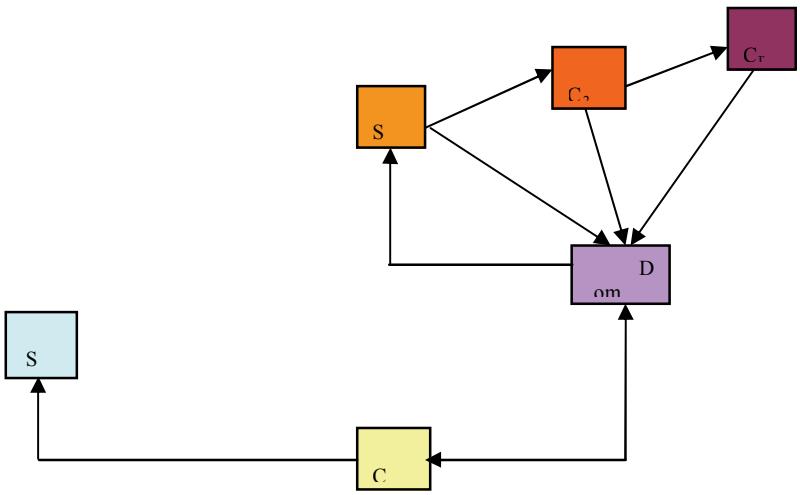


Fig. 2 Secondary nutrition net

The deposited C in the body of the secondary consumers is ingested as food by the top consumers. Again part of it is liberated in to atmosphere as CO_2, other part is stocked in the dead organic matter resulted from the top consumers (C_T) and is mineralised by fermentation or is buried underground and blocked from producing CO_2.

So, in nature, the states of Carbon related to the green house effect of the atmosphere have to be:

a) Inactive Carbon, met in nature as Diamond, that shan't produce CO_2 or CH_4;

b) Blocked Carbon in coal, oil or natural gases that can't produce atmospheric CO_2 or CH_4, while it stays

Buried underground;

c) Stocked Carbon in the dead organic matter (Dom) which could go underground and became a buried

Blocked Carbon, or undertake fermentation and mineralization as CO_2 or CH_4;

d) Deposited Carbon in the body mass of the living things on the Earth, without green house effect since it is not part of atmospheric gases molecules

e) Active, atmospheric Carbon the only one present in the molecules of the gases able to retain heat from the infrared rays of the sun.

CARBON BALANCE IN NATURE

Since the total number of Carbon atoms in nature is fixed there is a tendency that part of them to be inactivated as diamonds or blocked as buried underground organic substances. That could be explained by the necessity of blocking the energy contained by the molecules of organic substances.

The stocked carbon in the dead organic matter enriches the amount of buried underground Carbon atoms if it is not used as nutrient for the saprophyte plants or it is not mineralised by fermentation. Anyway, sooner or later, the Carbon atoms from the dead organic matter will go underground or will enter the molecules of green house gases CO_2 or CH_4.

The Carbon deposited in the body mass of the living beings is producing only CO_2. Ingested organic matter through the nutrition process is deposited in the live mass of the organism or is eliminate as CO_2, only. These Carbon becomes stocked Carbon when it is excreted or after organisms' death. At the end we can say that the more live beings on the earth the more Carbon is deposited and less Carbon is free to format molecules of the green house gases.

Some ecologists say that a higher number of farm animals contribute to the global earth heating since they expire CO_2 and eliminate CH_4 following eructation. They forgot, or maybe don't know, that the farm animal bodies are depositing carbon. What is true for the nature is true for the agriculture as well.

A study provided in 1998 by Maria Paraschivescu (2) concerning annual CO_2 emission out of pork production shows that a strong relation (see Figure 1) exists between the number of animals, and the net energy used for maintenance and for growth. The extracted data in the table 1 are based on the official statistic publications.

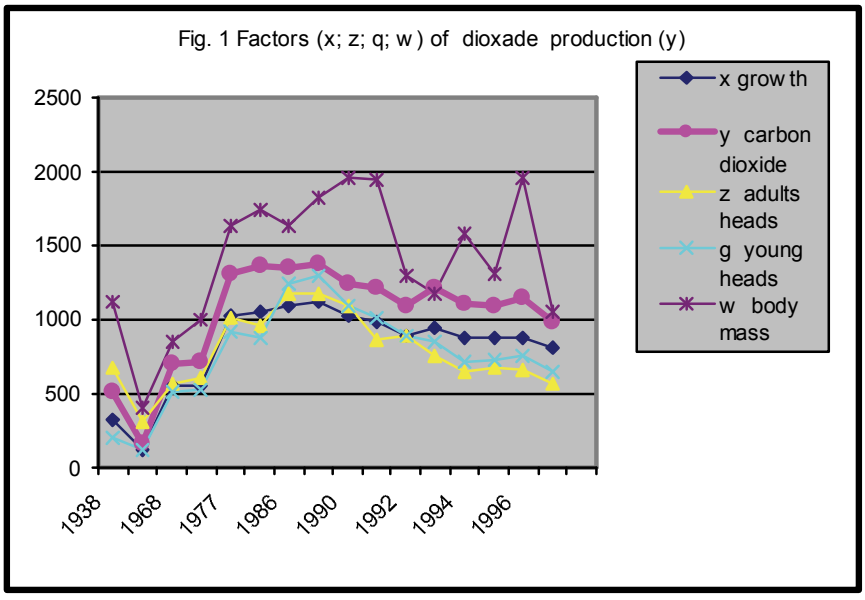
A final assumption of the study concluded that the higher are the sows' prolificacy and the daily gain of young animals the smaller is the quota of CO_2 emission per unit of useful pork production.

Table 1

Major factors of CO_2 emission in pork production

Year	Adult animals (thousand heads)	Live body mass in adult animals (thous. tones)	Slaughtered animals (thousand heads)	Growth gain (thousand tones)	CO_2 emission (thousand m^3)
1938	679.8	112.650	2080.8	322.5	512.92
1948	310.2	41.230	1280.4	120.3	160.76
1968	570.6	85.079	5181.0	556.7	709.42
1969	604.4	100.264	5255.5	547.7	721.76
1977	1014.8	163.415	9178.4	1022.4	1305.44
1978	966.3	173.880	877.4	1050.3	1363.19
1986	1169.2	163.660	12481.3	1097.2	1351.51
1987	1181.6	182.028	12913.0	1115.6	1383.69
1990	1098.0	195.444	10905.0	1031.8	1240.91
1991	870.0	194.880	10084.0	991.5	1212.51
1992	892.0	130.232	8959.0	890.2	1087.60
1993	753.0	117.468	8509.0	945.9	1213.17
1994	648.0	157.464	7110.0	878.5	1107.19
1995	670.0	131.320	7290.0	883.1	1092.81
1996	660.0	195.360	7575.0	882.4	1143.61
1997	570.0	105.450	6526.0	806.9	993.311

Concerning the assumption that CH_4 emission by cattle is increased when they are fed on concentrates, a group of research workers from the Metabolic Laboratory of the Colorado State University have published a very interesting report (1). Citation from the mentioned report says: "Major contributors to the global pool (Cicerone and Ormeland, 1988) are natural wetlands (21%), rice fields (20%), losses during fossil fuel



energy production (14%), domesticated animals (14%), burning biomass (10%) and landfills (7%). They also estimate domestic livestock likely contribute about 2% of the global warming effect. The estimate for cattle alone is about ¾ of the 2%. The importance of this contribution is being described very differently depending on the political or the economic position assumed.

According to the mentioned group methane loss by ruminants shows extremes ranging from 2 to 12 percent of global energy ingestion (GEI). They remark that comparing their results with the ones which have to be predicted from the Blaxter and Clapperton (1965) equation they didn't obtain satisfactory correlations. They also didn't find significant differences when concentrate rich diets or fiber rich diets were used. *Other than high grain diets, the majority of practical ruminant diets incur 6 ± 0.5 % methane losses.* ***For one lost calorie 8 liters of methane are emitted.***

As possible factors determining the level of methane loss they consider

Unique feedstuffs: several carbohydrate types seem to contribute to the variation of methane loss feeds. In feed mixtures based on corn, methane loss is less (7.1%) versus in barley based mixture loss (11.4%) when diets have the same energy level for maintenance. In case of equal DE intake fed to dairy cattle 40% higher methane loss was registered when a major component of a barley-based diet was replaced with beet pulp. (Beever et al., 1989). ***Ionophore effects:*** Shelling (1984) considered reducing methane loss by 4% to 31% when ionophores are used. But recent research of Colorado Metabolic Laboratory has shown a lack of persistence of methane loss. Abo-Omar (citied by Jonson E.D. and alii) found that Mo*nensin* has reduced methane emission by 35% in the day 2 and 5 of one experiment with a 95% concentrate diet, but after 16 days methane emission returned to the former level. In the same experiment *Lasalocid* (another ionophore) did not reduce methane losses. Jonson E.D. says that Saa (1991) feeding 95% roughage diet observed a similar initial reduction of methane losses and a recovery nearly the control level by day 15. He concluded that methane reduction from ionophore feeding results from reduced overall feed consumed (5-6%) rather than decreased methane per unit of feed.

Animal genotype: better genotypes for beef production and why not for milk production as well eliminate less methane per unit of daily gain. If feed conversion in products is higher methane losses per product unit is lower.

Methane emission from livestock manure: The quantity of cattle manure by anaerobic fermentation could produce more methane than the one resulting from enteric fermentation. Fortunately beef cattle are living on pasture and their feces become dry and are fermented aerobically. Dairy cows living in barns are the ones whose manure must be treated. Anaerobic lagoons of manure are source of methane emission. The fermentation taking part in these lagoons is a source of N_2O emission as well. Aerobic fermentation of manure retains nitrogen in organic compounds acting as natural fertilizer of soil.

WAYS TO REDUCE GREEN HOUSE GASES' PRESENCE IN ATMOEPHERE

In order to fight against the global Earth heating by atmospheric gases the paths to follow are:

• Reduce CO_2 concentration in atmosphere helping photosynthesis by the development of green biosphere, planting or seeding forests.

• Maintain as much as possible the quantity of underground blocked Carbon by limiting fossil fuel as energy source. Find and use alternative energy sources so the underground blocked Carbon will not come in atmosphere air.

Using of bio fuel keep the quantity of existing carbon on the Earth surface at the same level.

• Preserve the quantity of carbon stocked in the organic dead matter using this kind of matter to produce persistent goods.

• Both primary and secondary nutrition nets are sustaining biological production of organic matter deposited in the bodies of living beings. Biosphere, including plant cultivars and farm animals are acting against global heating by depositing Carbon in their bodies.

• Avoid manure lagoons and the anaerobic fermentation of manure

• Improve the genetic production of farm animals

Decreasing the farm animal livestock to reduce the concentration of green house gases in atmosphere is a mistake. That will get trouble for human food security.

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125 YEARS FROM THE DEATH OF CAROL DAVILA, FOUNDER OF THE HUMAN AND VETERINARY MEDICAL EDUCATION

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Key words: Carol Davila, founder, medicine

SUMMARY

Carol Davila holds a special place in the life and history of the Romanians. According to school records, Davila was born in 1828, in northern Italy, near Parma. Some historians believe he was born in Spain, in the small town of Avila, hence his name. Despite his activity, the sanitary state was unsatisfactory because of the lack of trained medical staff; therefore he returned to his older projects and in November 1855 he proposed to Prince Ştirbei the establishment of a **school of small surgery**, surgery assistants, within the military hospital from Mihai Vodă; in parallel he demands the discontinuation of the school of civil surgery assistants from Eforie – Colţea hospital. In 1856 was established the pharmaceutical education, as section of the School of surgery. In the same year, Carol Davila established the **School of veterinary surgery**, because he had introduced veterinary education within the curricula of the school of medicine. In 1862, Davila established the Military Hospital, a chemistry laboratory not just for educational purposes, but also for forensic, food, mineral water and beverage analyses. The 1877 independence was revealed even more the personality of Davila. With little means, sometimes starting from scrap, he manages to set up sanitary formations which amazed the reputed Russian medical personalities, such as Professors Pirogoff and Kocher, an even the Russian Emperor Alexandru II. The activity of Davila was multilateral, complex and exceptional; space and a correct interpretation of the historical phenomena are required in order to present it and to understand it. There is no medical, scientific, social, cultural, ethnographic, artistic and literary field exempt of his influence. His multilateral culture, his power to work, his brilliant mind and his prevision were too great to keep him from getting involved, usefully, in everything.

General MD Carol Davila (Carlos Antonio Francesco d'Avila) holds a special place in the life and history of the Romanians. According to school records, Davila was born in 1828, in northern Italy, near Parma. Some historians believe he was born in Spain, in the small town of Avila, hence his name (Butoianu şi Bălănescu, 1930; Simionescu, 1987).

He attended the secondary education in Limoges, France. As student of the School of Medicine from Angers he distinguished himself by knowledge and tenacity, which brought him appraisal from the teachers.

During this time, the French Government received through the means of the French Consulate in Bucharest, a special invitation from Prince Barbu Ştirbei, ruler of Walachia, asking for a physician from

France able to organise the military and civil sanitary services. The French Government recommended, with the approval of the Paris School of Medicine, MD François Charles Davila for such an important mission; he decided to go for three years, as he thought, in the country of the ruler Prince Barbu Ştirbei, entering the country through Giurgiu customs on March 13, 1853 (Diaconescu, 2005; Stancu 2002; Tomescu, 1984).

Davila was introduced to Prince Ştirbei who commissions him with the governance of the sanitary military service, giving him the rank of oberstab (major) doctor. In this quality he also received the task of running the hospital form Mihai Vodă.

Despite his activity, the sanitary state was unsatisfactory because of the lack of trained medical staff; therefore he returned to his older projects and in November 1855 he proposed to Prince Ştirbei the establishment of a **school of small surgery**, surgery assistants, within the military hospital from Mihai Vodă; in parallel he demands the discontinuation of the school of civil surgery assistants from Eforie – Colţea hospital (established by Cretzulescu in 1841 and re-established in 1842 by Polizu, discontinuation which was accomplished in 1856) in the idea of establishing and functioning of a single school of medicine within the military hospital from Mihai Vodă (Vlădescu, 1943).

Thus, the school is established on December 4, 1855, joined by museum of anatomy and the library, which he established using his own money. The school accepted initially 35 graduates of at least four elementary grades, which were enlisted with various regiments and services.

The ambition and determination of Davila was not satisfied with such a beginning; his dream was to establish a school similar to the French one. Hence, he proposed to Prince Barbu Ştirbei to establish a school for the hospital surgeons (surgery masters, same with the French sanitary officers). To this purpose, in 1856 he obtained the approval to transform the school of surgery assistants, or small surgery, into the **school of surgery**.

Also in 1856 was established the pharmaceutical education, as section of the School of surgery. In the same year, Carol Davila established the **School of veterinary surgery**, because he had introduced veterinary education within the curricula of the school of medicine (Simionescu, 1962; Simionescu şi Moroşanu, 1984).

The Princely act number 1092, of August 16, 1857, signed by Prince Alexandru Dimitrie Ghica, *caimacam* of Walachia, promulgated a new organisation of the school of medicine, turning it into the

National School of Medicine and Pharmacy. The number of students was set to 110, of which 40 for the military, 50 for the districts and 20 pharmacists.

In 1858 the National School of Medicine received the same advantages and appraisals in Italy where, by the royal decree of August 15, 1858, it obtained the same equivalence as in France (Moroşanu, 1973; Poenaru, 1931).

During this period Davila taught brilliantly several courses, of chemistry, medical clinic, general pathology etc. He didn't neglect his professional practice in the town where, due to his established reputation, was asked daily for medical advice.

As of 1859, after the union of the Romanian Principalities, the role of Davila increased and he became the chide MD of the Romanian army from both Principalities. He organised in the military camp from Floreşti several ambulances and temporary hospitals. For his activity in the medical field, the Romanian ruler Alexandru Ioan Cuza promoted him to the rank of colonel MD.

The fame of Davila's school spread over the borders and 45 students from Bulgaria and Rumelia (Constantinople, Salonic, Tracia, Macedonia, Moesia, Central Greece and the European part of Turkey) enlisted.

In 1861, Davila married Anica (Ana) Racoviță, nephew of the Goleşti family, a distinguished woman with a great soul. With the help of his wife, Davila makes philanthropic works. Ana Davila took care closely of the education and good care of the orphans from „ Elena Doamna Asylum". Davila also established a second asylum for the poor boys, with a section for the deaf and dumb (Butoianu şi Bălănescu, 1930; Zamani, 2009).

Davila had four children from Ana Racoviță: one year from their marriage, in February 1862, their first boy was born, Alexandru, followed by two girls, Elena and Zoe, and by another boy, Pia.

On January 13, 1874, his wife Aniţa (Ana) Davila died, because of the mistake of his colleague, head of laboratory, the innocent and good doctor Bernath who, by mistake gave her strychnine instead of quinine. Widower, with four children, Davila continued to work even harder, until his death on August 24, 1884.

All the children of Davila became reputed personalities who contributed to the prestige of the Romanian nation. Particularly, Alexandru Davila, the first child, (born on February 12, 1862, died on October 19, 1929) who was a reputed Romanian play writer. He started with poetry, then with several valuable patriotic and political works. His

masterpiece is the lyrical historical drama „Vlaicu Vodă" (1902) chapter of a projected trilogy, „Mirciada", one of the basic masterpieces of the Romanian drama.

In 1862, Davila established the Military Hospital, a chemistry laboratory not just for educational purposes, but also for forensic, food, mineral water and beverage analyses.

In 1864 Davila accompanied the Romanian ruler Alexandru Ioan Cuza to Constantinople, where the Romanian ruler was received with honour by the sultan.

At the end of 1865, Davila together with doctor Exarcu, initiator and director of the Athenaeum, establish the Society of Natural Sciences. He also established the "Medical Monitor", the official paper at hand for any medical doctor or sanitary agent.

The great liberal politician I. C. Brătianu, who wanted to meet Prince Carol at Düsseldorf, took Davila along because he needed a brave man, a trusted diplomat, and above all, a patriot. Thus, on April 19, 1966, I. C. Brătianu, accompanied by Davila, entered the Düsseldorf castle and proposed to Prince Carol, the throne of the Romanian Principalities.

On May 31, 1868, by a decree-law signed by Prince Carol I, Doctor Carol Davila received the Romanian citizenship.

In 1874, following the intervention of Davila, Romanian joined the Geneva Red Cross Convention. He drew up a draft statute of the Romanian Society for assistance to the military sick and wounded during the war.

Despite his preoccupations, Davila didn't neglect the „Elena Doamna Asylum", participating in his spare time at theoretical and applied classes, in his wish to improve the cultural level of the people, contributing with varied programs.

Despite his sufferings, Davila continued his activity, his major concern being the military sanitary service, which had to be endowed according to the development of the Romanian army, with the accomplished progress in science and with the experience acquired on the battlefield from Sedan (1870). Thus, on the occasion of the Sabar maneuvers, he set up medical units using the existing materials and the military doctors. Despite the shortcomings, of the insufficient available funds, Davila succeeded by repeated guiding and endowment, to organize the medical service so as it can cope with the current needs and, possibly, with the needs of the wartime.

The 1877 independence was revealed even more the personality of Davila. With little means, sometimes starting from scrap, he manages to

set up sanitary formations which amazed the reputed Russian medical personalities, such as Professors Pirogoff and Kocher, an even the Russian Emperor Alexandru II. A valuable work by the Russian doctor Kocher describes the perfect and ideal manner in which Davila was directing the sanitary services of the Romanian army, and extraordinary organiser.

In December 1877, Davila received a letter from the Romanian ruler Carol I, in whom the latter appreciated in the most eulogistic terms his activity of the battlefront, thanked him for his abnegation and activity within the sanitary services.

The activity of Davila was multilateral, complex and exceptional; space and a correct interpretation of the historical phenomena are required in order to present it and to understand it. There is no medical, scientific, social, cultural, ethnographic, artistic and literary field exempt of his influence. His multilateral culture, his power to work, his brilliant mind and his prevision were too great to keep him from getting involved, usefully, in everything.

Despite his foreign blood and despite all the misery and diseases he suffered in our country, Davila didn't spare anything to be the best, more animated and faithful son of the Romanian people.

Davila was the great hero who, 125 years ago worked for the enlightening, elevation and unification of the nation.

The memory of Carol Davila was honoured following the initiative of several organising committees as follows: his centennial was celebrated on October 17, 1928, in Bucharest, both at „Elena Doamna Asylum", at the Faculty of Medicine and at the Military Hospital „Regina Elisabeta". In the afternoon of he same day an exhibition with the works of Davila was opened at the Military Hospital „Regina Elisabeta", under the patronage of His Majesty the King of Romania. The ceremony was attended by representatives of the French medical institutes: Professor Marcel Labbé from the French Academy of Medicine, Professor Balthazar, dean of the Paris School of Medicine, Professor Boquel, director of the Angers School of Medicine.

The organising committee issued a medal with the effigy of the great French doctor. A commemorative medal in the honour of Davila and in the memory of his brilliant activity was issued in 1903, on the occasion of the anniversary of 50 years from the establishment of the School of small surgery from the Mihai Vodă hospital, the first stable medical establishment, nucleus, founding stone of the current faculties of human and veterinary medicine and pharmacy, as Davila himself foresaw and proclaimed in 1856 in a enthusiastic speech. On the 50

years jubilee of the Faculty of Medicine, a statue of Carol Davila was set in front of the building of the Faculty of Medicine, as a final and permanent confirmation of the true paternity of one of the highest cultural and medical educational institutions in Romania (Curcă, 2001 – 2002, 2002 b, 2002 c; Curcă şi colab., 2001, 2002 a, 2003, 2004, 2006).

The military sanitary corps, in acknowledgement of his activity, a bust of Doctor Carol Davila was unveiled in 1909 in the courtyard of the Military Hospital „Regina Elisabeta".

Aware of his merits and of their public acknowledgement, Doctor Carol Davila was presenting himself simply and strikingly by his name card which was printed with a single word: „Davila".

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MATHEMATICAL MODEL OF ENERGY AND PROTEIN METABOLISM IN POULTRY

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SUMMARY

The paper presents a new approach regarding the computer simulation of the metabolic processes in poultry. It provides a way to evaluate the feeding norms in terms of the rate of weight gain and of carcass quality.

Traditionally, the energy and protein norms aim to obtain the highest possible productions (we will refer subsequently to the weight gain). There are few systems evaluating the energy and nutrient requirements which also allow controlling the quality of the obtained carcass, quality which is expressed mainly by the lipid to protein ratio.

The mathematical modelling of the metabolism processes gives the possibility to evaluate in dynamics the feeding norms related both to the speed of weight gain and to carcass quality. This paper presents a point of view in this respect.

Because the quality of a given weight gain may range between $\left(\min \frac{Lr}{\Pr} \le \frac{Lr}{\Pr} \le \max \frac{Lr}{\Pr}\right)$, where Lr – lipids retained daily and Pr – protein retained daily, it results that the feeding norms will be expressed as [$norm_{min}$, $norm_{max}$] intervals. Thus, there is infinity of values possible for the feed requirement.

The choice of one or other among the possible values can be done according to the rearing technology that is used, according to the purpose expressed in economic terms and/or in terms of a proper human feeding. In this paper we will present a way to calculate intervals for the energy and protein requirements, next to formulations which characterise, in our opinion, the physiological evolution of the weight gain for a specific lipid to protein ratio of the carcass.

Calculation of the energy and protein requirement using the mathematical model method.

For the calculation of the energy and protein requirements of broiler chickens, the following parameters should be taken into account:

- body weight (W, expressed in grams);
- body weight gain intended to be reached (ΔW, grams);
- ratio of the lean gain (protein + water) to the retained protein (α);
- ratio of the lipids and protein retained daily $\left(\frac{Lr}{\Pr} = \beta\right)$;
- ratio of the daily gain of ash and protein $\left(\frac{Ashr}{\Pr} = \gamma\right)$;
- biological value of the diet (BV);
- ambient temperature (Ta);
- digestible energy per Kg DM of the diet (MJ/Kg DM);

The standard body weight can be evaluated with an equation which expresses the net weight (Wn, g) as a time-variable (t, time) Gompertz function (Burlacu et al., 1995):

- for males:

(1) $$\mathrm{Wn} = 37.96 \times e^{\frac{0.16232}{0.03288} \times \left(1 - e^{-0.03288 \times t}\right)}$$

- for females:

(2) $$\mathrm{Wn} = 37.96 \times e^{\frac{0.15501}{0.03223} \times \left(1 - e^{-0.03223 \times t}\right)}$$

The (gross) body weight is calculated for both genders with the following formulation:

(3) $$\mathrm{W} = \frac{Wn}{0.973}$$

From (1) and (2) we can obtain by derivation the expression of the standard daily net weight gain ($\Delta Wn, g/day$).

- for males:

(4) $$\Delta Wn = \frac{dWn}{dt} = 0.16232 \times Wn \times e^{-0.03288 \times t}$$

- for females:

(5) $$\Delta Wn = \frac{dWn}{dt} = 0.15501 \times Wn \times e^{-0.03223 \times t}$$

The value of the body weight can be measured directly and used as such in the model. Likewise, the weight gain has not a single value. For each body weight there is an interval $[\Delta W_{\min}, \Delta W_{\max}]$ of the daily weight gain. Equations (4) and (5) propose maximal values for it.

The ratio of the lean gain (protein + water) and the retained protein (α) was calculated function of the evolution of the net body weight (Wn):

- for males:

(6) $$\alpha = 7.51245 \times Wn^{-0.07287}$$

- for females:

(7) $$\alpha = 7.65598 \times Wn^{-0.0784}$$

Unlike the parameters discussed previously, the value of α is single for a given weight.

Ratio of the daily retained lipids and protein $\left(\frac{Lr}{\Pr} = \beta\right)$.

This parameter has the highest variability for a given weight gain and a set daily weight gain. This ratio is the key parameter in setting, through the feeding norm, the quality of the daily weight gain. Experimental determinations have shown that $\beta \in [0,2;1,2]$. The physiological value of β, which we define as the lowest value specific to each age is calculated function of the body protein (Pt) and of the age expressed in days (t):

- for males:

(8) $$\beta = \frac{34.6 + 11.78 \times t^{1.06} \times e^{(0.00198 \times t - 0.0017 \times t^2)}}{23.6 \times 0.1765 \times Pt \times e^{-0.03425 \times t}} - 1$$

- for females:

(9) $$\beta = \frac{33.97 + 14.14 \times t^{0.94} \times e^{(0.00536 \times t - 0.0001 \times t^2)}}{23.6 \times 0.17 \times Pt \times e^{-0.03425 \times t}} - 1$$

The ratio of the daily gain of ash and protein $\left(\frac{Ashr}{\Pr} = \gamma\right)$ was calculated function of the total body protein (Pt) using the following equations:

- for males:

(10) $\gamma = 0.14518 \times Pt^{0.02523}$

- for females:

(11) $\gamma = 0.13646 \times Pt^{0.03438}$

Similar to the case of α, the value of γ has a single value for a given total body weight.

Calculation of the requirement for digestible crude protein and digestible amino acids.

We begin this calculation by determining the total body protein (Pt,g) (also necessary for equations (8) and (9)).

(12) $Pt = \frac{W}{100} \times (15.7651 + 0.00302W)$

If the growing conditions are not limited by the environmental factors, we propose the following Gompertz-type equation, function of time (t), for the calculation of the maximal total body protein (at a give age).

- for males:

(13) $$Pt^m = 5.99375 \times e^{\frac{0.17646}{0.03425}\left(1 - e^{-0.03425 \times t}\right)}$$

- for females:

(14) $$Pt^m = 5.99375 \times e^{\frac{0.17006}{0.03449}\left(1 - e^{-0.03449 \times t}\right)}$$

From equations (13) and (14), by derivation, we obtain the formula for the maximum amount of daily retained protein.

- for males:

(15) $$\Pr^m = \frac{dPt^m}{dt} = 0.170 \times Pt^m \times e^{-0.034525 \times t}$$

- for females:

(16) $$\Pr^m = \frac{dPt^m}{dt} = 0.170 \times Pt^m \times e^{-0.034495 \times t}$$

For all the other cases, the determination of the value calculated for the retained protein (Pr^c,g) is done according to the following e face conform reasoning:

$\Delta Wn = lean.gain + Lr + Ashr$, but

Lean gain = $\alpha \times \mathrm{Pr}^c$, $Lr = \beta \times \mathrm{Pr}^c$, $Ashr = \gamma \times \mathrm{Pr}^c$

therefore, $\Delta Wn = \alpha \times \mathrm{Pr}^c + \beta \times \mathrm{Pr}^c + \gamma \times \mathrm{Pr}^c = \mathrm{Pr}^c \times (a + \beta + \gamma)$

$(\Delta Wn = 0.973 \times \Delta W)$

It results:

(17) $$\mathrm{Pr}^c = \frac{\Delta Wn}{\alpha + \beta + \gamma} = \frac{0.973 \Delta W}{\alpha + \beta + \gamma}$$

Finally, the rate of the daily retained protein (Pr,g) is determined as follows:

$Pr = \min (Pr^c, Pr^m)$

Once we have obtained the value of Pr we can calculate the available protein (PA,g)

(18) $$PA = Pm + \frac{Pr}{0.837}$$

where (Pm,g) is protein requirement for maintenance which is calculated function of Pt, as follows:

- for males:

(20) $$Pm = 0.038 \times Pt^{0.744}$$

- for females:

(21) $$Pm = 0.036 \times Pt^{0.761}$$

Using the available protein we can now calculate the norm of digestible crude protein and that of digestible amino acids:

(22) $$PBD = \frac{PA}{BV}$$

where BV $\in$[0.45; 0.85] is the biological value of the dietary protein.

(23) Digestible lysine = 0.066 $\times$AP

(24) Digestible Met. + Cyst. = 0.056 $\times$ AP

(25) Digestible tryptophan = 0.012 $\times$ AP

For the other amino acids we can calculate in a similar way, having in view that AP is multiplied with the proportion of amino acid existing in the broiler meat.

Calculation of the metabolisable energy requirement (ME, KJ).

We can use the following formula to calculate the requirement of metabolisable energy:

(26) $ME = ME_m + Epr + Elr + Q' + Q''$ where:

ME_m is the requirement of metabolisable energy for maintenance, which is calculated as follows:

- for males:

(27) $$EM_m = 546 \times \left(\frac{Wn}{1000}\right)^{0.700}$$

- for females:

(28) $$EM_m = 518 \times \left(\frac{Wn}{1000}\right)^{0.646}$$

Epr is the requirement of metabolisable energy for protein synthesis and retention. The same formula applies for both sexes:

(29) $Epr = 50 \times Pr$

Elr is the requirement of metabolisable energy for lipids synthesis and retention. The same formula applies for both sexes:

(30) $ELr = 56 \times Lr$, where $Lr = \beta \times Pr$

The requirement of energy for thermoregulation was noted with Q' and it is given by the following formula:

(31)

$$Q' = \begin{cases} (Tci - Ta)\left(31.7215 - 2.042 \times 10^{-2} \times W + 6.7 \times 10^{-6} \times W^2 - 6.561 \times 10^{-10} \times W^3\right) \times W^{0.75}, \\ if Tci > Ta \\ 0 \text{.....} otherwise \end{cases}$$

We noted with Ta the ambient temperature and with Tci – the critical lower temperature which is determined with the following formula:

(32) $Tci = 37.5784 - 2.014 \times 10^{-2} \times W + 8.242 \times 10^{-6} - 1.231 \times 10^{-9} \times W^3$

Q'' is the requirement of energy spent for the daily physical activity:

(33) $$Q'' = EMm \left(\frac{140.34 - 0.4034x}{100}\right) - 1$$

where x is the feeding level (% of ad libitum).

The maximal amount of ingested dry matter is calculated with the following formula.

- for males:

(34) g DM max/day = $(5.686 + 1.904 \times t^{1.234} \times e^{-0.01159 \times t}) \times \frac{1}{\sigma}$

- for females:

(35) g DM max/day = $(5.686 + 2.273 \times t^{1.149} \times e^{-0.01073 \times t}) \times \frac{1}{\sigma}$

where σ is a coefficient between 0.7 and 1 whose value depends on the level of genetic improvement of the broilers.

The amount of ingested dry matter is currently calculated with the following formula:

(36) $DM = \frac{ME(norm)}{ME / KgDM}$

The value such calculated must be lower or, at most, equal with the DMmax given in (33) and (34), which don't have single values, expressing variations of both the diet (level of ME/Kg DM, biological value of the diet), and of the quality of the gain $\left(ratio \frac{Lr}{\Pr}\right)$ involving the existence of norms expressed by intervals and not by single values, as it is now the custom.

In conclusion, the model allows calculating the requirement of energy and protein starting from readily observable inputs (body weight, age etc.).

weight (g)	**DM intake (g)**	**ME (KJ)**	**DCP (g)**	**Lysine (g)**	**Met.+Cys. (g)**
100	14.5-22.2	232-300	4.7-3.1	0.24-0.22	0.15-0.13
200	23.3-37.4	373-504	7.5-5.3	0.38-0.37	0.23-0.22
500	42.0-67.4	673-908	13.2-10.9	0.67-0.66	0.41-0.40
1000	66.6-100.3	1066-1354	22.0-14.3	1.12-0.99	0.68-0.60
1500	83.9-125.8	1342-1698	28.0-18.2	1.43-1.26	0.87-0.77
2000	92.3-135.8	1476-1834	29.0-19.0	1.48-1.32	0.90-0.81

In table 1 we present the energy and protein requirement calculated according to the model proposed in broilers, feeds with diets with

variable content of metabolisable energy and biological value, between 13.5 and 16 MJ/Kg DM, respectively 0.55 and 0.75 and with a retained fat: retained protein ratio(β) between 0.2 and 1.0.

Table 1

Energy and protein requirement in broilers per animal and day

Based on the norms of DM (35) and DCP (21) we can calculate easily the total amount of the diet and the amount of dietary crude protein.

(37) $TCI = \frac{DMin..the..diet}{0,001 \times \delta}$, where δ = g DM/Kg diet

(38) $CP = \frac{DCP}{dCP}$ where dCP = digestibility of the dietary crude protein

Figure 1 shows the amount of compound feed dry matter which can be given in order to archive body weight gain ranging between first and 8th weeks, depending on the CP percentage in the diet (dCP was taken to be 0.8) taking in account the maximum rate of protein retention and the limits of the ratio between fat and protein retention (β).

It is to remark that body weight gain/day can be obtained for instance on 28th day only by giving compound feeds in amounts ranging within wider limits, from 62 to 93 g, but with protein concentration between 45 to 18 g/100 g DM, a β ratio from 0.2 to 1.0 and a biological value of protein from 0.45 to 0.65.

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A NEW METHOD TO CALCULATE THE FEEDING VALUE OF THE FORAGES FOR PIGS

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Keywords: pigs, forages, feeding value, mathematical model

SUMMARY

The paper presents a new method to calculate the feeding value of the forages used in pig rearing. This model has a better accuracy of the productive potential of forages. Compared to other models, this model makes correction for the biological value of the protein and for the bacterial fermentescible matter, also making a dissociation of the net energy into protein energy and lipid energy, which can be produced by the analysed forage.

INTRODUCTION

The feeding value of the forages for pigs was calculated from the gross chemical composition in nutrients of the forages and from their digestibility coefficients. The feeding value is expressed in corrected metabolisable energy (ME_c), in available protein (AP) and in digestible limiting amino acids (digestible lysine, digestible methionine + cystine, digestible tryptophan and digestible threonine).

DIGESTIBLE ENERGY (DE)

From the gross chemical composition in nutrients and of their digestibility coefficients, we calculated the digestible nutrients and the amount of digestible energy (DG) with the following formula:

DE= 24.2 DCP + 39.4 DEE + 18.4 DCF + 17.0 DNFE
(MJ/Kg DM)

where DCP is the digestible crude protein; DEE are the digestible ether extractives DCF is the digestible crude fibre and DNFE are the digestible nitrogen-free extractives, determined by Weende.

BACTERIAL FERMENTESCIBLE MATTER (BFM)

The bacterial fermentescible matter results from the difference between the sum of the digestible crude fiber + the digestible nitrogen-free extractives and the sum of the starch + sugar, entirely digested (in g):

$$SFB = (DCF + DNFE) - (St + Su) \qquad (g/Kg\ DM)$$

Available protein

The available protein (AP) is the amount of protein which can be used for maintenance and for protein synthesis calculated from its biological value (BV) according to the formula:

$$AP = DCP \times BV \qquad (g)$$

where BV (biological value) was calculated using the DCP content in dietary digestible essential amino acids (AAdig), which related to the amino acids of the retained protein (or secreted in milk) shows the lowest value:

$$BV = (AAdig/DCP)/(X/100)$$

where X = is the amino acid content corresponding to 100g protein in meat or milk.

We accepted the fact that the available protein is used entirely to cover the net requirement of protein for maintenance, and with a yield of 81.3% when the protein is retained in the meat or milk of the sow..

Retained protein (Pr)

The retained protein Pr, (in meat, or secreted in the milk) results from the difference:

$$Pr = (AP - Pm)\ 0.813 \qquad (g)$$

where: AP- available protein, Pm – protein for maintenance

$$Pm = 4Pt \qquad (g)$$

and Pt – total body protein

$$Pt = c \times G \quad 0{,}103 < c < 0{,}113 \qquad (kg)$$

where G – body weight (kg).

CORRECTED METABOLISABLE ENERGY (ME_C)

Knowing the starch and sugar content of the forages, which are considered to be entirely digestible, we calculated the amount of corrected metabolisable energy (ME_c) taking into account the corrections for the bacterial fermentescible matter (BFM), sugar (Su)

content and the consumption of energy during deamination, according to the model for energy and protein metabolism simulation in fattening pigs (Burlacu et al. 1998).

BFM correction is done only for the forages with more than 100 g BFM per kg DM, the loss of energy by fermentation being 6.8 KJ/g BFM, which means 40% (17 KJ $\times$ 0.40 = 6.8 KJ).

The second correction in ME calculation is done when the forage has more than 80 g sugar per kg DM forage. This correction is of 1.4 KJ/g sugar, which means 8.24% (17 KJ $\times$ 0.824 = 1.4 KJ) and it represents the difference gross energy content between 1 kg starch and 1 kg sugar.

The third correction is done when the digestible protein is deaminated in excess of the requirement for maintenance, the expenditure of energy being evaluated to 4.9 MJ/Kg deaminated protein (DP).

Thus, the corrected metabolisable energy of the forages is calculated with the following formula:

MEc = DE – (UE + Edeam.+ 0,0068 BFM + 0,0014 Su) (MJ Kg DM)

where DE is the digestible energy, UE is urine energy Edeam is the deaminated energy expressed in MJ, while BFM and Su are expressed in grams.

The formula for urine energy calculation is:

UE = 7.2 DP (MJ)

where DP –deaminated protein, which is calculated with the formula:

DP = DCP - Pr (g)

The deaminated energy is calculated with the following formula:

Edeam = 4.9(DP-Pm) (MJ)

(all notations from the formula have been explained earlier in the text).

The corrected metabolisable energy (EMc) of the forages with the above formula can be done only within the context of the diet because the biological value of the diet, BV, which occurs in the calculation of the available protein (AP), as well as the BFM and Su content, are not the average value of each single forage, and Pm which appears indirectly in the formulation varies with the age and physiological state of the animal.

When calculating the corrected metabolisable energy (EMc) and the net energy (NE) of the forages with this model we took into

consideration the standard capacity of feed utilization of a 70 kg pig, with a maximal protein retention of 140 g Pr/day, with an average daily gain of 600 g, housed under thermally neutral conditions (Q` and Q" = 0).

For this standard pig we calculated with the model a requirement of metabolisable energy for maintenance (MEm) of 10.448 MJ/day and a requirement of protein for maintenance (Pm) of 36.3 g, which can be supplied by a diet with 11.117 MJ DE and 70 g DCP, which is metabolised at maintenance level (protein and energy balance = 0) with a loss of 0.504 MJ as energy urine and 0.165 MJ as deamination energy.

Simulating the administration of different types of supplements to the diet for the standard pig, which should not exceed the maintenance diet as digestible energy, we calculated the efficiency of using these supplements as corrected metabolisable energy and as net energy using the model. For the forages with higher protein contents, the simulation of using them as supplements to the diet was done taking into consideration the maximal protein retention level (140 g/day), so that the supplemental feeds don't exceed the amount of available protein (AP) corresponding to this maximal retention level and also taking into consideration a maximal 1/0.5 ratio of the protein gain to lipid gain. Also, for the bulk forages we calculated the supplements to the maintenance diet, given by the practice, which should not exceed 10% CF for overall diet. Following are several examples of such calculations.

The feeding value of the forages was evaluated in crude protein (CP), digestible crude protein (DCP), corrected metabolisable energy (MEc), net energy (NE) and feed units (oats), knowing that one FU contains 8.067 MJ NE (or 1928 Kcal).

We gave up this the feed unit of 3500 Kcal, or 14.65 MJ, which was less accepted by the specialists in animal feeding from Romania.

The feed unit of 8.067 MJ NE, or 1928 Kcal is in a 1.22/1 ratio with the feed unit used for ruminants, whose energy content id 6.78 MJ NE milk and 6.59 MJ, NE meat, the difference being justified by the better efficiency of using the same feed (normally used for both category of animals) by the monogastric animals versus the ruminant animals; the ratio of 8.067/6.68 (average value if NEmilk and NEmeat) corresponding to this difference of percentage.

CONCLUSIONS

The calculation of the feeding value of the forages for pigs with the mathematical model for energy and protein metabolism simulation

offers a better accuracy when the productive potential of the forages is evaluated because it provides the following additional operations compared to the current (local or foreign) systems:

- correction for the biological value of the protein, which shows the amounts of energy in energy and during the deamination process, which vary with the protein content in essential amino acids;
- correction of the bacterial fermentescible matter and of the sugar function of the forage content of crude fibre and mono and disaccharides;
- calculation of the nutritive value of each feed under standard conditions, taking into account the daily requirement of energy and protein for gain;
- dissociation of the net energy into protein energy and lipid energy, which can be produced by the analysed forage.

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ASSESSMENT OF BACTERIAL COUNT FLUCTUATIONS FOR *STREPTOCOCCUS THERMOPHILUS* FROM YOGHURT DURING ITS SHELF LIFE

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Key words: Streptococcus thermophilus, yogurt, probiotic

SUMMARY

The investigated yoghurt samples revealed a decrease with approximately 1 log in bacterial count for *Strptococcus thermophilus,* during the shelf life of this product. The bacterial count was maintained above the minimal level of 10^6UFC/ml, which is considered the minimal probiotic bacteria concentration that could still have benefits for consumer's organism.

Yoghurt is considered the most important probiotic bacteria „carrier". The yoghurt microflora is consisted of two main thermophilic lactic bacterias, namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus.* Other probiotic bacteria, belonging to genuses such as *Bifidobacterium* and *Lactobacillus* have also been recently reported in yoghurt. The viability of probiotic microorganisms in yoghurt is influenced by numerous factors, such as the product initial acidity, the additional acidity occurred during refrigeration (post-acidification), the oxygen level in the product, the oxygen packaging penetration degree, the lysogenic feature of starter bacteria, the sensitivity to antimicrobials developed by the starter cultures, the product's concentration of nutrients etc.

Some authors consider that a product has probiotic qualities when the probiotic bacteria concentration is equal or higher than **10^6 UFC/ml or g product** (Rafan Irkin-2008).

1. MATERIALS AND METHODS

The present study used 10 yoghurt samples of 5 different sources (T, P, D, M and A). All yoghurt samples were taken immediately after manufacturing and were subjected to refrigeration during the previously established shelf life (4°C). A *Streptococcus thermophilus* count

(CFU/ml yoghurt) was performed right after sampling and every 5 days throughout the investigation period, using the poured plates method. Consequently, decimal dilutions of bacteria in peptonated water were prepared for each sample, followed by inoculation of 1 ml of each concentration in two Petri dishes. In each Petri plates, melted *Streptococcus thermophilus* agar was poured after being cooled down to 45°C. After solidification, all Petri plates were turned upside down and subjected to incubation at 37°C, for 24-48 hours.

The plates were enumerated and the bacterial count was considered only for the plates with a bacterial count between 20 and 200. An average number was calculated for each plate and was afterward multiplied by the inverse dilution value.

2. RESULTS AND DISCUTIONS

The results are listed in tabel 1 and illustrated in figure 1.

Table 1

***Streptococcus thermophilus* population dynamics (CFU/ml yoghurt) during shelf** life

Sample	*Streptococcus thermophilus* CFU/ml yoghurt						
Trials	1 Initial	2 After 5 days	3 After 10 days	4 After 15 days	5 After 20 days	6 After 25 days	7 After 30 days
T1	$1,6x10^9$	$1,1x10^9$	$8,9x10^8$	$7,6x10^8$	$6,4x10^8$	$5,1x10^8$	$3,6x10^8$
T2	$5,8x10^8$	$4,5x10^8$	$3,1x10^8$	$1,9x10^8$	$1,2x10^8$	$8,9x10^7$	$6,7x10^7$
P1	$4,0x10^8$	$2,1x10^8$	$1,8x10^8$	$9,3x10^7$	$8,5x10^7$	$6,9x10^7$	$5,2x10^7$
P2	$7,1x10^8$	$5,8x10^8$	$4,3x10^8$	$2,9x10^8$	$1,3x10^8$	$9,8x10^7$	$8,5x10^7$
D1	$1,2x10^9$	$9,8x10^8$	$6,7x10^8$	$5,6x10^8$	$4,3x10^8$	$2,9x10^8$	$1,6x10^8$
D2	$1,9x10^9$	$1,3x10^9$	$9,8x10^8$	$6,8x10^8$	$5,7x10^8$	$4,3x10^8$	$1,9x10^8$

M1	4,5x 10^8	3,2x 10^8	1,6x 10^8	9,7x 10^7	8,5x 10^7	7,1x 10^7	5,6x10^7
M2	2,1x 10^9	1,5x 10^9	9,3x 10^8	7,9x 10^8	6,8x 10^8	5,2x 10^8	3,1x10^8
A1	1,3x 10^9	1,1x 10^9	8,9x 10^8	7,3x 10^8	5,3x 10^8	3,8x 10^8	2,4x10^8
A2	8,2x 10^8	7,5x 10^8	6,1x 10^8	4,8x 10^8	2,1x 10^8	1,2x 10^8	9,3x10^7

The initial *Streptococcus thermophilus* concentration in yoghurt was different from one sample source to another, but also from one sample to another, the count differences ranging from 10^8 to 10^9 CFU/ml yoghurt. A decreasing shift in viable germs with up to 1 log, was revealed for all investigated samples.

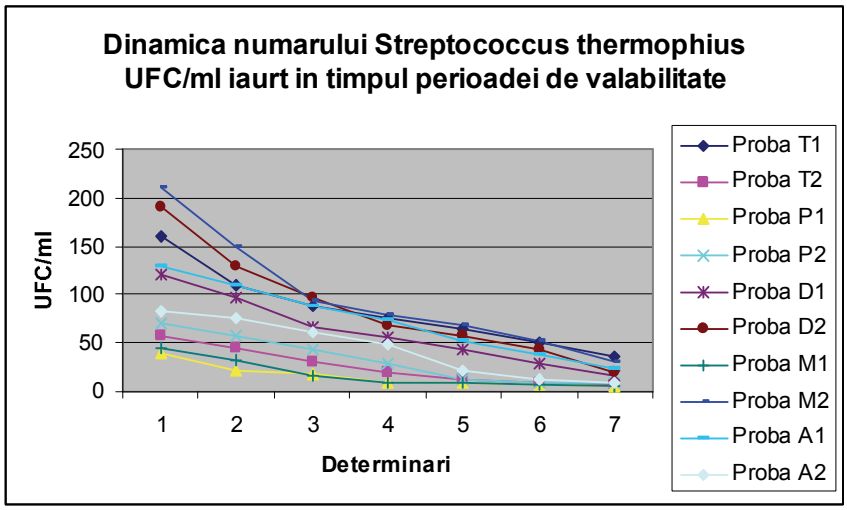

Fig. 1.*Streptococcus thermophilus* population dynamics (CFU/ml yoghurt) during shelf life (the count values reveal multiplications with 10^7)

After 30 day of refrigeration, the number of *Streptococcus thermophilus* was over 10^7CFU/ml, with more than 1 log higher than the minimum value considered for the occurrence of benefits for the organism due to probiotic germs (10^6CFU/ml).

The results were statistically processed using SPSS program.

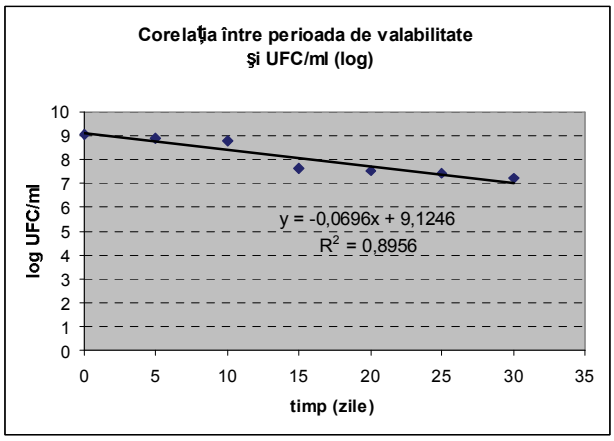

Fig. 2. Correlation between the shelf life, log CFU/ml yoghurt and preservation time (dispersion diagram)

In order to calculate de correlation coefficient between the value of log CFU/ml and the preservation time, the average values were first calculated and then transformed to log, for each of the ten samples.

The value of correlation coefficient between CFU/ml count and preservation time was of -0.8956. The closeness of -1, reveals a high degree of correlation between the two variables, the correlation being linear and decreasing (Fig. 2).

The dispersion diagram has a decreasing tendency. The positive correlation between log CFU/ml and time at 4 ^{o}C is negative: increasing the preservation time in optimal conditions, will lead to a decrease in *Streptococcus thermophilus* population (CFU/ml). The value of determination coefficient d = r^2 = 0,9 indicates the high degree of correlation between the number of preservation days at 4^{o}C and CFU/ml. CFU/ml residual variation is 10%.

3. CONCLUSIONS

3.1. The present study revealed a decrease of viable *Streptococcus thermophilus* cells for all analyzed yoghurt samples, with approximately 1 log, during the product's shelf life (from 10^8 and 10^9 CFU/ml yoghurt initially, down to 10^7CFU/ml yoghurt, after 30 days of conservation).

3.2. There is a significant, linear, decreasing correlation between CFU *Streptococcus thermophilus*/ml and the preservation time at 4°C.

3.3. The final level of bacteria was approximately 1 log above the minimal admitted value of 10^6CFU/ml, considered for a product to have probiotic effect.

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THE PURIFICATION AND THE CHARACTERIZATION OF PIG'S IMMUNOGLOBULIN G (IGG) AND ALSO OF PIG'S ANTI-IMMUNOGLOBULIN G SERUM FOR USING THEM IN DIAGNOSIS TESTS

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Key words: pig's G immunoglobuline, purification

SUMMARY

The pig's G immunoglobuline purifying was made by ammonium sulfate precipitation and chromatography on ion changers (DEAE cellulose). The purity assay of the obtained IgG was made by ion exchange chromatography and immunoelectrophoresis (IEF) toward a rabbit serum total pig antiserum and sodium dodecyl sulfate poliacrylamide electrophoresis (PAGE-SDS). A single precipitation arch was obtained with cathodic migration to IEF and a fraction with the molecular mass of 150 KDa at PAGE-SDS suitable to IgG. The anti-IgG serum was obtained by rabbits hyperimmunization. The IgG antibodies level quantification was made by the immunoenzymatic technique (ELISA) and we obtained raised values of the optic densities which showed an high level of antibodies. The rabbit's anti-IgG pig serum obtained will be used as reagent in the immunodiagnosis tests of high sensibility and specificity.

The immunoglobulins (Ig) is the main isotope at the porcine species, and it represents 80-85% from the hole amount of serum immunoglobulin and from colostrums. Five subclass of immunoglobulin G were described in the pig: IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgG_4 (Novotny, 2005 and Metzger, 2005). The pig's IgG has a similar structure and ability with the ones from other species. The IgG synthesis lie under the control of some structural genes represented for C and V part of the two types of chains through independent sets. (Vior C, 1980).

Murakamy and Kazu isolated the IgG for pig's serum using the ammonium sulfate precipitation and the chromatography on ion exchange (DEAE cellulose) using phosphate buffer with different molarity values (0,01-0,04). The IgG was eluted using the first two buffers with 0,01 and 0,02 molarity. The fractions were merged and after that they were purified using columns wih CM cellulose and Sephadex G200 (Murakami and Kazu, 2005).

Pham and Paraj isolated IgG, IgA and IgM from the sow's milk combining the gel filtration with chromatography on ion chngers. The

immunoglobulins purity (Ig) was checked up using SDS-PAGE and immunodiffusion and they obtained 85% purity degree for IgG, 54% and 32% for IgA and IgM. The authors recommend using these techniques also for the IgG class purification and for other biological liquids (Pham and Paraf, 1987; Bhanushali et al, 1994).

During some experiments done by Bokhout et al., pig's IgG subclasses purification was made using the afinity chromatography. The results obtained showed 2 picks which contained antigenic determinatives partial different, for IgG_1 and IgG_2 subclasses. The analysis of the 2 IgG underclasses was done usind immunodiffusion tests and ELISA (Bokhout et al, 1986; Kim et al, 1994).

Walker and Jhon have done the IgG purification through affinity chromatography using anti-IgG antibodies joined on Sepharose (Walker and Jhone, 2002).

In this paper we present the research results regarding the purification and the characterization of pig's anti IgG serum for using them in diagnosis tests.

1. MATERIAL AND METHOD

The obtaining of pig total gamma globulin was done using the precipitation technique of the pig's normal serum with a saturated ammonium sulfate solution neutralized up to pH=7. Three successive precipitations were done and the final amount of immunoglobulin was re-engaged with a small quantity of distilled water. The obtained gamma globulin obtained was then dialyzed at 4^oC with a NaCl solution 0,15 M.

The purification of pig's IgG was made using ion exchange chromatography (DEAE – cellulose). For obtaining the IgG we used a chromatographic column (K 2,5/30 cm) with DEAE-cellulose (with a change ability of 0,009+0,1mEq/g) balanced with phosphate buffer 0,075M and pH=6,3. The gamma globulin solution was inserted in the column, keeping in mind that at 3-4 g DEAE-cellulose we add at 200mg protein. The sample elution was done with phosphate buffer 0,0175M, pH=6,3 gathering 2mL/tube sample. The gathered fractions were put together and they were tested for purity and specificity using the electrophoretic technique in poliacrylamide gel in denaturated system (SDS-PAGE) and immunoelectrophoresis (IEF).

The fraction's electrophoresis in poliacrylamid gel was done in denaturated conditions (SDS-PAGE) using a SCIE-PLAS TV 100 machine proceeding from the technique described by Laemmli. The proteins separation was done in two gels with different concentration

and pH values: one for proteins concentration using 4% acrylamid gel in Tris-HCl 0,5M buffer, pH=6,8 and another one for them separation (7,5%) acrylamide gel in Tris-HCl 1,5M buffer, pH= 8,8. After the gels migrated they were coloured with a 0,1% solution Coomassie Brilliant Blue G-250. The molecular mass markers that were used are: ovalbumin (45kDa), bovine serum albumin (66kDa), galactozid 116 kDa and myosin (205kDa).

The immunoelectrophoresis was performed in agarose gel 1,2% which was hurried on a glass mount (7,5cm long/2,5cm wide) prepared in veronal buffer with an ionic force of 0,05. The fractions that had to be studied were put in the gel wells, and then after their electrophoretic migration, in the split created between two gel wells the rabbit's serum pig's antiserum was put. The mounts were maintained in a wet room, at the lab temperature for 18-24 hours. The precipitation arch was seen using a down-up highlight source. The proteins were colored using a 0,1 solution of Amido Black 100.

The rabbits immunization (n=3) to obtain the pig's monospecific anti-IgG serum was done with 4 mg IgG/ml put in complete Freund adjuvant. Three subcutaneous inoculations were done, in several places, on the body's sides. The bleeding was done after seven days, after the last inoculation. The quantification of pig's antibodies anti-IgG level was made using the immunoenzymatic technique.

The immunoenzymatic technique.

- *The antigen*: to catch and to quantize the pig's anti-IgG serums we used as antigen pig's IgG diluted at 10 µg/ml in a NaOH solution 0,1N. In the coated stage we added 100µL IgG in every hole. After the plates were put in the incubator for two hours at 37^{o}C, they were washed up using PBS/Tween in a Mindray MV-12A plate cleaner. The pig's anti-IgG serums were diluted in a ratio of 1/25, 1/50, 1/200, 1/400, 1/800, 1/1600 and 1/3200 in a PBS/Tween buffer with an addition of 0,5% bovine serum albumin. The plates incubation was done at 37^{o}C, for 60 minutes.
- *The conjugate*: we used a sheep anti-IgG conjugate IgG marked with peroxidase. It was diluted in a ratio of 1/100 in PBS/Tween buffer to which we added bovine serum albumin 1% and then we put 100µl in each well.
- *The substratum*: it contained 0,005 hydrogen peroxide and 0,6mg/mL ABTS in citrate buffer, pH=4. We used 100µl substratum in each well and after one hour the reaction was stopped with 50 µl sodium florure 1,5%.

- *The reading*: the optical densities (DO) were read at 405nm with a multichannel spectrophotometer on ELISA Apollo LB 911 plates (Berthold Technologies).

2. RESULTS AND DISCUSSIONS

The total gamma globulin fraction on a DEAE-cellulose column showed only one proteic pick. The elution profile obtained after the chromatography is presented in figure 1.

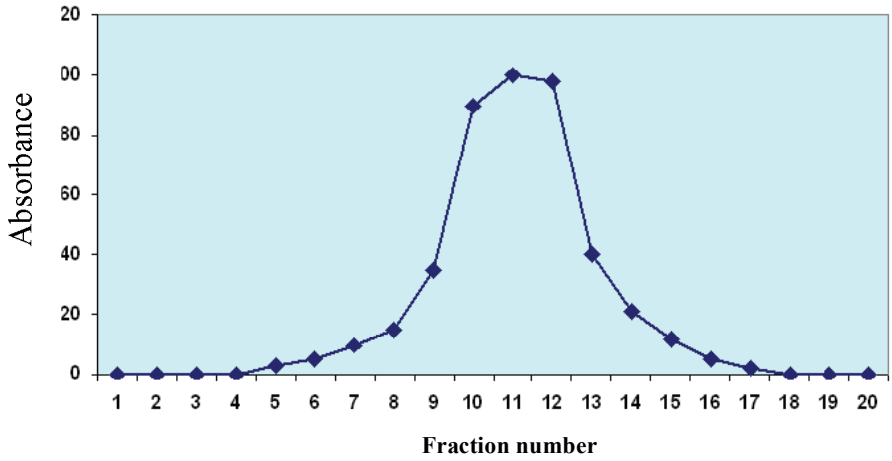

Figure 1. The elution profile of pig's IgG obtained on a DEAE cellulose column

As the data show, the fractions that are in the pick's maximal area were put together, concentrated and analyzed for their purity using IEF towards the pig's anti-serum. In the diluted part the IgG appeared after collecting 30ml buffer. From the total gamma globulin, the IgG was diluted in the next 20-25 ml buffer. The elution of the others proteins left in the column was done with a NaCl solution 0,25 M.

Using the electrophoretic analyses for the fractions suitable to the pick obtained, we could see a precipitation arch characteristic to IgG with electroforetic migration in the cathodic area of the mount (figure 2).

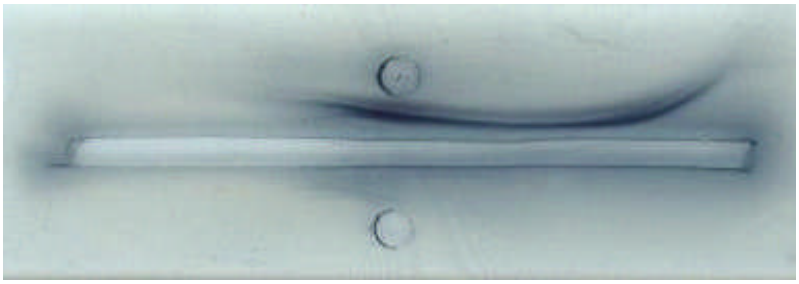

Figure 2. The purity checking of pig's IgG using immunoele trophoresis towards the rabbit's serum pig's antiserum.

It was proven that IEF is a qualitative method very useful in estimating the G immuno globulin purification degree. Using the SDS-PAGE electroforetic study of the molecular fractions obtained through separation on DEAE cellulose we could see the presence of a single band with the molecular mass of 150kDa (figure 3).

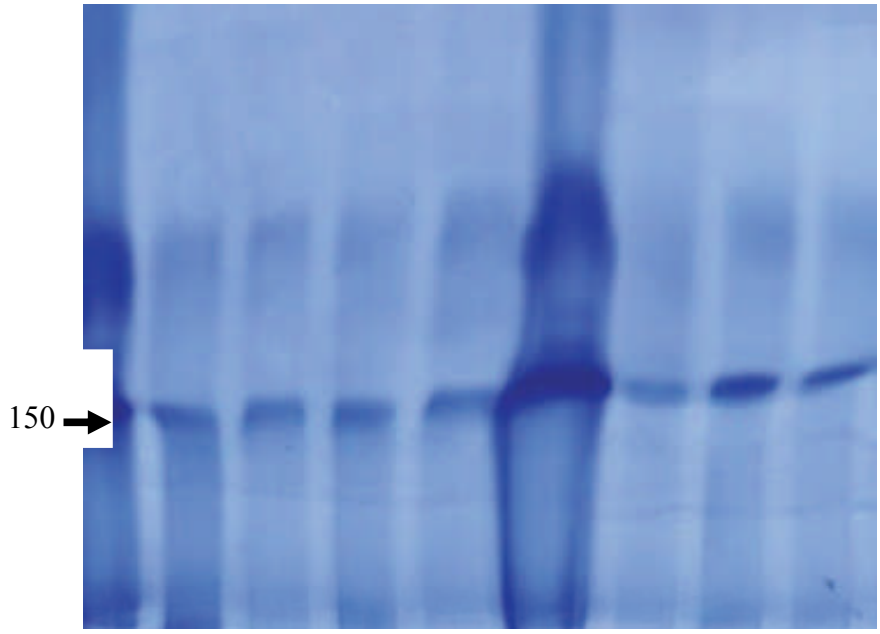

Figure 3. Electrophoretic analysis of pig's IgG using SDS-PAG

This band is a match to IgG which shows that using the DEAE-cellulose splitting we can do this purification.

The titers of the pig's anti-IgG serum (n=3) between 1/25 -1/3200 (values of concentrations and dilutions) are presented in table 1 and figure 4.

Table 1.

The DO values of pig's anti-IgG serum obtained using the immunoenzymatic technique

Serum dilution		Optic density (DO)		
		Serum 1	Serum 2	Serum 3
	1/25	1,987	1,993	1,872
2	1/50	1,695	1,701	1,602
3	1/100	1,347	1,394	1,297
4	1/200	0,897	0,912	0,836
5	1/400	0,548	0,607	0,517
6	1/800	0,428	0,489	0,407
7	1/1600	0,224	0,256	0,211

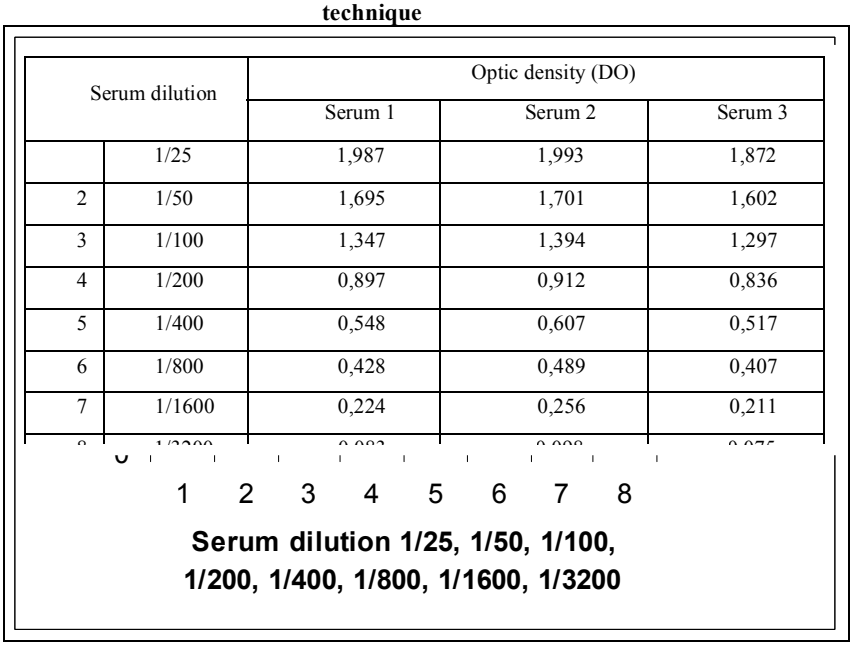

Figure 4. The pig's anti-IgG serum titration using immunoenzymatic technique

The level's quantification of the pig's antibodies anti-IgG using ELISA showed high titer of antibodies up to a dilution of 1/800 (DO serum 1=0,428; DO serum 2=0,489 and DO serum 3=0,407). These values show the ELISA test sensitiveness and the possibility to determine the titer of the analyzed anti-sera (1/800 in all 3 sera).

The pig's anti-IgG serum specificity was studied using the immunoenzymatic technique for the evaluation of antigen-antibody reactions towards other species immuno globulin: ox's IgG, sheep's IgG, rabbit's IgG and dog's IgG (10µl/ml).

In figure 5 we showed that DO were negative comparative to the ox's IgG (DO=0,226), sheep's IgG (0,173), rabbit's IgG (DO=0,011) and dog's IgG (DO=0,005) and this fact proves that the pig's anti-IgG serum have a raised specificity.

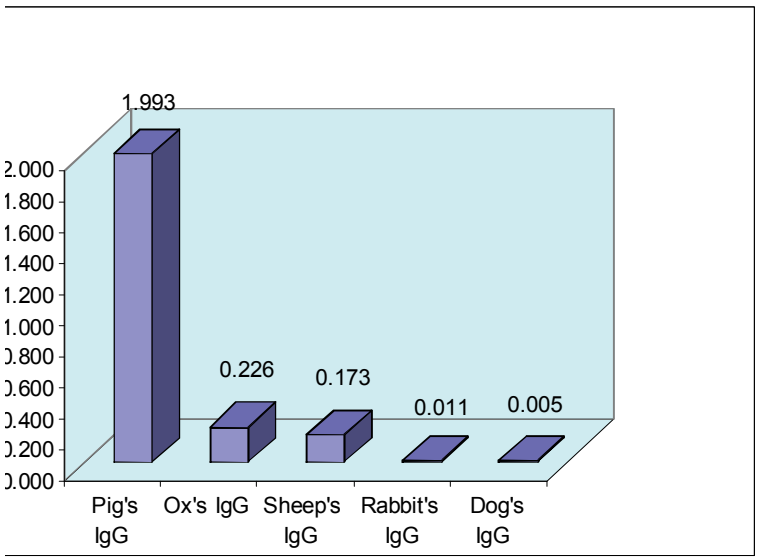

Figure 5. The pig's anti-IgG serum specificity testing

The pig's anti-IgG serum presented a weak reaction towards the ox's IgG. The DO=0,226 value was in the negative reaction area (figure 5).

The proven qualities of the pig's serum helped them became valuable reagents used in diagnosis and therapy. Their applicable area can be expanded in the animals pathology, especially in the scientific research to achieve immunodiagnostic techniques of high efficiency (immunoenzimatic, immunofluorescence), structural and ultrastructural immunocitochemistry with marked antibodies, the antibodies research using blotting tests, immunobiosensors, immunoelectronomicroscopy).

3. CONCLUSIONS

3.1 The pig's IgG purification was done using the precipitation reaction with ammonium sulfate and ion exchange chromatography (DEAE –cellulose).

3.2 The IgG's purity checking was done using the IEF technique towards the rabbit's anti-serum and using also SDS-PAGE.

3.3 After using IEF we obtained a single precipitation arch with migration in the chatodic area and a fraction with the molecular mass of 150KDa using SDS-PAGE.

3.4 The pig's anti-IgG serum were obtained from the rabbit's hyperimmunization.
3.5 The sensitiveness of the pig's anti-IgG serum tested using ELISA showed high antibodies titers.
3.6 The pig's anti-IgG serum testing was done using ELISA test comparative to their species Ig and the results obtained are in the negative area.
3.7 The pig's anti-IgG serum prepared will be used in the immunodiagnostic tests of great sensitiveness and specificity.

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THE DETERMINATION OF THE CORRELATION BETWEEN NEWBORN PUPPIES BLOOD BIOCHEMICAL PARAMETERS

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Key words: pup, neonate, biochemistry, biostatistics

SUMMARY

The neonatology in domestic carnivores in veterinary medicine is weak development. Neonatal illnesses in the dog have less specific clinical signs.
Very few experimental studies have been conducted in canine neonatology. The difficulty lays mainly in the problems occurring in the canine neonatal. Clinical expression of neonatal disease generally reduces the "dehydration - hypothermia – hypoglycaemia" group.

This study suggets that complementary test analysis of the dynamics of certain blood biochemical parameters in the pup during its first eight weeks of life.

The results observed showing usual values in pups are completely different from those observed in the adult.

Using biostatistics methods to examine the correlation between parameters and target values were obtained by comparing the biochemical profile of adult dogs.

The discussion around the study includes assumptions of explanation of the actual values and approaches the breed differences observed.

1. MATERIAL AND METHODS

In the study nine newborn puppies of Beagle breed, in apparently good health coming from two nests were followed for eight weeks.

0.5 ml of blood was collected from each puppy in the first day of life, blood necessary for biochemical determinations. Blood was also collected during the 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th}, 42^{nd}, 49^{th} and 56^{th} day of life. The determination of biochemical parameters was done using spectrophotometric method kits, with the purpose of saving as much samples as possible (μL). Prior to the blood collecting procedure, the puppies were weighted.

Biostatistics Analysis of the data obtained was performed with SPSS version 15.

2. RESULTS AND DISCUSSION

The data on the dynamics of biochemical parameters followed, depending on age and weight as are presented in Table 1.

Hypoglycemia is part of the triad "fading puppy syndrome": hypoglycemia, hypothermia, dehydration.

A normal value in healthy adults is 70 to 160 mg/dL. The puppies are very fluctuating values, averaged between 89.7 ± 12.7 and 135.3 ± 14.3 mg/dL. Slight decrease of blood glucose after weaning can be caused by changing diet and exposure to risk of parasitism.

Table 1

Changes in blood biochemical parameters of age and weight of newborn puppies

Age (weeks)	Weight (g)	Glycemia (mg/dL)	Alkaline phosphatase (IU/L)	Uremia (mg/dL)
0	290,2 ±23,6	94,6 ± 10,8	3337 ± 1084	0,78 ± 0,13
1	461,6 ± 33,9	116,3 ± 11,8	521 ± 192	0,49 ± 0,09
2	667,9 ± 46,7	115,4 ± 9,3	382 ± 101	0,33 ± 0,06
3	843,8 ± 80,4	120,4 ± 11,5	407 ± 98	0,27 ± 0,08
4	1033,4 ± 145,3	135,3 ± 14,3	158 ± 94	0,16 ± 0,04
5	1539,3 ± 137,6	116,6 ± 8,4	329 ± 88	0,26 ± 0,03
6	2011,6 ± 163,1	89,7 ± 12,7	241 ± 86	0,38 ± 0,05
7	2355,1 ± 133,4	115,4 ± 13,8	397 ± 122	0,41 ± 0,04
8	2585,3 ± 210,5	123,3 ± 6,1	420 ± 116	0,28 ± 0,06

Alkaline phosphatase (ALP) enzyme activity in healthy adults is below 80 IU/L. ALP activity in puppy's plasma is very particular. Its values are very high in the first weeks of the life of the dog and especially in the first days. Scientific works explain the high levels of ALP in the infant dog as a consequence of increased osteoblast activity of puppies. Isoenzymes present in osteoblasts may be the cause of this intense activity levels. Hoskins is associated with increased activity of the colostrum, rich in ALP.

Another explanation is that during pregnancy, in the placenta is a large amount of ALP. Rapid decrease of enzyme activity of ALP in the first weeks of life may be related to a malfunctioning liver.

Ureas are a good marker of renal function and allow a picture of glomerular function.

In adults neonatal uremia should be between 0.20 and 0.50 mg / dL. We noticed the puppies in the study a higher maximum uremic adults (0.78 ± 0.13). Furthermore, uremia decreases gradually after four or five weeks, reaching close to the limit minim adults (0.16 ± 0.04). Then it increases gradually.

Urea found in clove essential after birth comes from the mother. High levels at birth may be related to installed volume depletion between dehiscence placenta during birth and the first meal. A possible explanation of the kinetics and minimum observed in four to five weeks (weaning period) may be given by examining the growth curve (Figure 1).

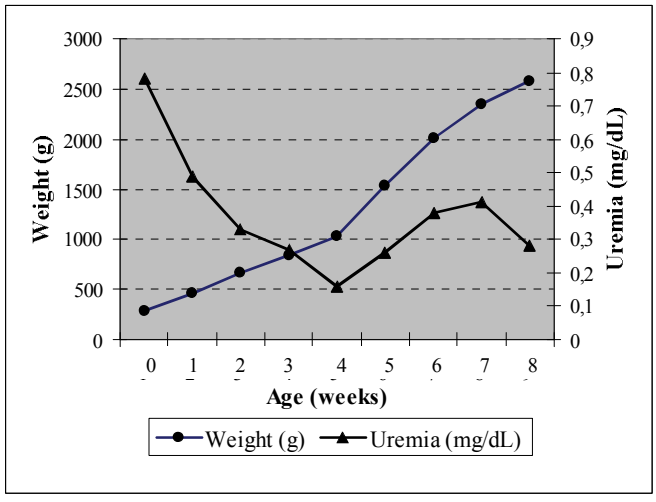

Figure 1: Uremia puppy's dynamics of age and weight

The puppies generally prevails with respect to protein anabolism over catabolism was maximum in this period the daily weight gain.

Catabolism of protein is low in this period and logical amount of urea formed. Another possible explanation can be found by examining the sources of protein. Milk proteins are highly digestible and high quality, thus the amount of urea formed is reduced. Turning to dry food, quantities of urea are formed much higher than in milk diets.

Urea kinetics can be explained by the dog renal physiology, renal function being immature at birth.

Results of statistical analysis on the correlation between age, weight and biochemical parameters specified for newborn puppies are presented in Table 2.

Table 2

Correlation Analysis age-weight-blood sugar-uremic alkaline phosphatase in newborn puppies

	Age (weeks)	Weight (g)	Glycemia (mg/dL)	Phosphatase (IU/L)	Uremia (mg/dL)
Age (weeks)	1				
Weight (g)	0,983999	1			
Glycemia (mg/dL)	0,185368	0,051829	1		
Phosphatase (IU/L)	-0,56655	-0,46338	-0,52162	1	
Uremia (mg/dL)	-0,54435	-0,39466	-0,7024	0,878142	1

Analyzing the data in Table 2 we see that age and weight are strongly (positively) correlated, the correlation coefficient being 0.98. Significant (negative) are age related and alkaline phosphatase activity (correlation coefficient 0.57) and plasma urea and age (correlation coefficient 0.54).

Alkaline phosphatase is strongly (positively) associated with uremia (coefficient of variation 0.87) and very much (negative) associated with glucose (coefficient of variation 0.52). These correlations are given and the right of regression (Figure 2 and Figure 3).

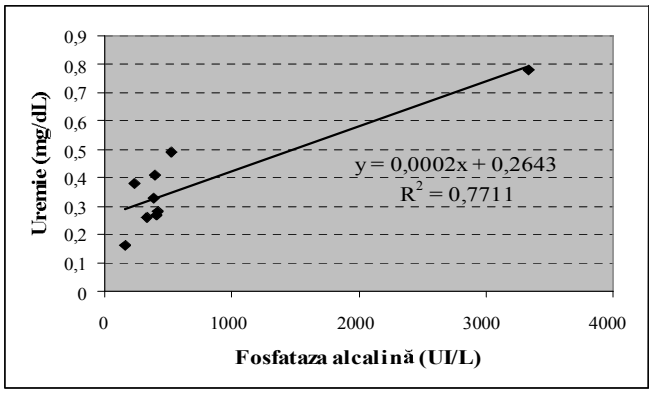

Fig. 2: Right Graph regression correlation associated uremia - alkaline phosphatase.

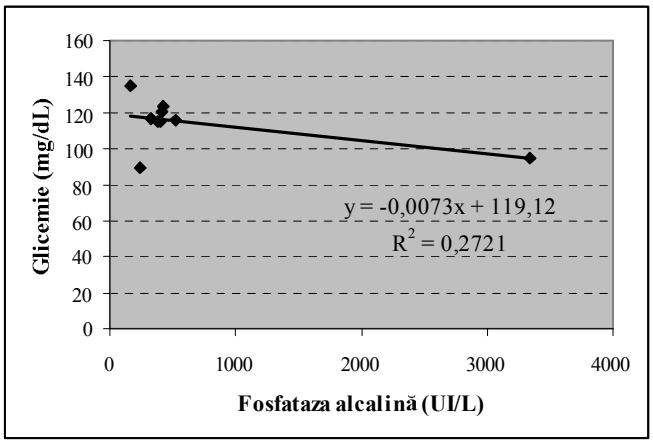

Fig 3: Right Graph regression correlation associated blood sugar - alkaline phosphatase.

3. CONCLUSIONS

3.1. The results observed for some blood biochemical parameters showed normal values in puppies that are totally different from those of adults.

3.2. These parameters should be interpreted with caution. Some are intrinsic parameters for the dog (breed, waist) others are extrinsic (type of weaning food, motherhood ambience). They can modify the normal values of biochemical parameters.

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THE EFFECTS OF ENRICHING THE RAISING ENVIRONMENT ON PIG WELFARE

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Key words: pig, welfare, enriched environment

SUMMARY

The purpose of this study was to track the effects of environmental enrichment on welfare growth for fattening pigs, as evidenced by the response induced physiological and behavioral indicators of welfare. The enriched environment for pigs may determine different responses of some welfare indicators.
While in the collection fold, the behaviour of pigs raised in boxes located in stimulating environment (A) was different from the ones exhibited by pigs raised in plain boxes (B) when they were moved in order to be mixed and later on transported to the slaughter house. The group B pigs spent more time on the move (9%) compared to group A pigs (6%), and manifested more aggressive behaviour (4%). Salivary cortisol levels also showed high values for pigs in-group B after being removed from their growth environment, as compared to its concentration for group A pigs.
These results indicate that a stimulating, enriched environment of the boxes may influence the animal welfare level even when these are taken out of their environment.

Animal welfare is dependent on growth conditions, especially on sheltering place, type of microclimate, feeding and watering manner (De Jong et al, 1998). Physiological and behavioural welfare indicators may suffer obvious modifications subsequent to raising factors and especially to box environment (De Jong et al. 2000). Pigs raised in a dry, bare environment exhibit a more aggressive, less social behaviour (Beattie et al., 1999) compared to the ones raised in enriched environment boxes. Following research (2001), Hauser and Mayer state that alternative farming systems in which the pigs have outdoor access offer more beneficial raising conditions regarding the animal welfare than conventional farming systems as well as more economically productive output (Bockich et al. 1998). This is due to pigs' physiological and morphological adaptation mechanisms to the environment they live in, which react by means of behavioural manifestations (Andersen et al.1998).

Movement, mixing and transport of pigs to the slaughter house may determine physiological stress and the induced response of some indicators such as plasmatic and saliva cortisol (Grandin, 2006).

MATERIALS AND METHOD

Research was carried out in two intensive farming system units on 68 fattening pigs (n:68) of Landrace breed, of similar weight and age until their slaughtering moment in the slaughter house. The animals were not taken out of the production cycle and were identified by means of a marker spray for easy observation.

The pigs were placed in two shelters similar as construction and organization, equipped with monitored microclimate and ventilation systems, automatically controlled and regulated. Pigs accommodation was performed differently, namely: A in collective boxes with continuous flooring on 75% of the surface, 25% discontinuous out of metal bars and B collective boxes with discontinuous flooring on 50% of the surface (plastic material). (A) box environment was enriched with various materials (plastic/wooden sticks and barracks) whereas no enriching material was present in box B. The animals were fed age and weight specific recipes and all pigs had free access to water.

Pigs were transported to the slaughterhouse with specialized, trip lasts three o'clock. One hour prior to loading, the animals were mixed in a collecting fold. All the manoeuvres were performed according to applicable legislation concerning animal protection during transport.

Saliva was collected from all animals participant to the research in order to determine cortisol concentration, in shelter boxes prior to loading, following loading, upon reaching the destination - slaughterhouse and prior to their slaughtering. Sampling was done on cotton pads soaked in saliva after each animal had chewed the pad for 45-60s. The soaked pads were then centrifugated, stored at -20^0C according to the working protocol and analysed by means of the Elisa test.Animal behaviour (table 1) was monitored the entire period by direct observation of behaviour manifestations for 30 minutes, 3 times/day (at 8, 1pm,18 pm) weekly, and during transport.

Table 1.

The behaviour of pigs participant to the research, monitored throughout the period

Observed behaviour	Description of behaviour manifestations
Walking	Movement around the box
Investigation	Examination of box walls and flooring
Rest	Lying down, eyes closed
Feeding	Feeding from the feeder
Aggressiveness	Fight between two animals from the same box
other manifestations	Short interactions with other congeners, watering, open eyed decubitus, etc.

Statistical analysis of data included the student t test to compare the data obtained.

RESULTS AND DISCUSSION

The research carried out has demonstrated the influence of raising conditions on pigs' welfare by different behavioural and physiological modifications recorded in the animals.

In pigs raised in enriched environment boxes (A), the level of saliva cortisol was higher (10,2 ng/ml), compared to only 4,7 ng/ml in the pigs raised in non-enriched environment boxes B (Fig.1).

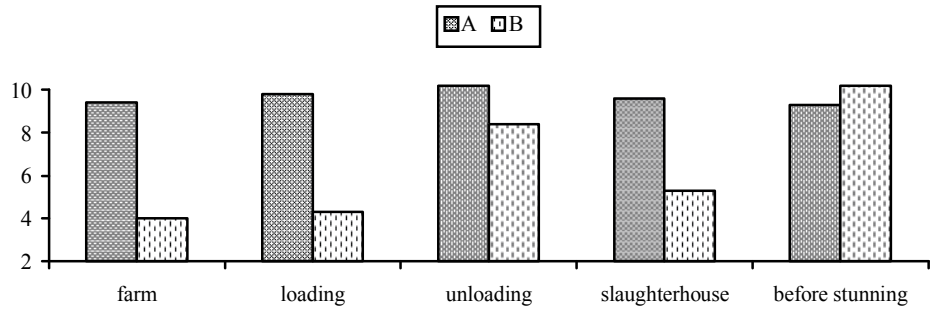

Fig. 1. Cortisol level (ng/ml) of pigs during the study

Following mixing and loading onto vehicle, saliva cortisol level stayed within the same trends both for group A (10,8 ng/ml) where concentration did not vary significantly compared to the one recorded in the shelter boxes, and for group B (4,9 ng/ml). Upon unloading, transport related stress induced higher responses in B group pigs (8,1 ng/ml) compared to the level recorded in raising boxes, but lower than those in group A pigs (11,30 ng/ml). This indicates the fact that

hypothalamus-hypophysis-adrenal glands axis is more reactive in lot B than in group A pigs.

While in the slaughter house, prior to their entering the asomation box, pigs movement on transit lanes determined an increase in saliva cortisol levels in all group B pigs (11,80 ng/ml), compared to the ones in group A (10,30 ng/ml).

Following research (De Jong, 1998, 2000), state that pigs raised in a non-enriched environment responded by a low cortisol level compared to the pigs raised in an enriched revironment. Our study confirmed this result, additionally pigs responses were different regardless of the materials used to enrich the environment (fig.2). The level of saliva cortisol was therefore lower in pigs whose box environment was enriched with plastic/wooden sticks compared to the one enriched by barracks in other boxes.

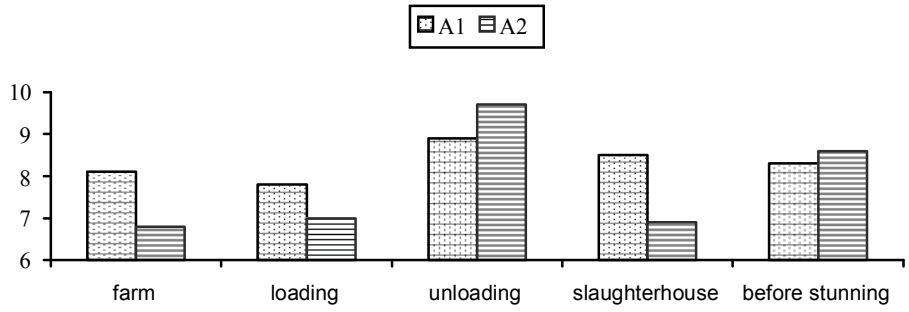

Fig. 2. Cortisol level (ng/ml) **measured by the material used to enrich the environment**

Parrott et al., state (1989) that fodder deprivation prior to slaughtering may lead to an increase in cortisol levels. De Jong et al 2000 consider that enriching box environment for pigs prior to their loading onto transport vehicles may also lead to an increase in saliva cortisol levels as a result of behavioural display enhancement such as straw consumption following fodder deprivation to which the pigs had been subjected to.

Geverink et al. (1996), have shown that cortisol levels increases in direct relation with the intensity of pigs mixing activity and aggressive behaviour display during mixing, while in 1999, the same author did not find behavioural differences during transport of animals that had not been previously mixed.

Our research indicates that pigs have displayed different behaviours following their mixing before the travel depending on the environment they were raised in. The methods used to mix and handle the animals towards loading – although in compliance with regulations – constituted a stress factor especially in pigs raised in a bare, non-enriched environment compared to the group A pigs. Thus, during the first part of the journey group B pigs showed a tendency to spend more time moving around, standing and even aggressing other pigs (a few cases) on the vehicle (fig. 3), compared to group A pigs who preferred to sit down from the beginning.

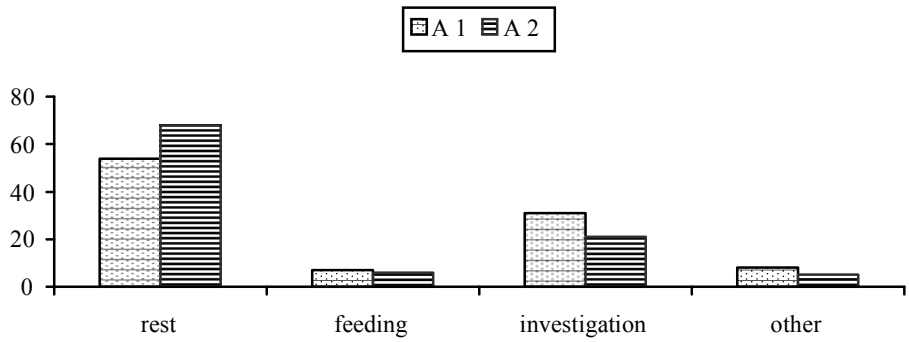

Fig. 3. Behavioral manifestations (%) of pigs depending on the environment in which increased

This aspect determined an even greater anxiety in group B as opposed to group A. Pigs raised in a non-enriched environment were more active and spent more time investigating the new environment during transport compared to the pigs raised in an enriched environment. Pigs' behaviour in group A and B did not vary concerning their willingness to leave the shelter boxes (26,8 sec, compared to 18,4 sec for A and B pigs) and be loaded onto the transportation means (19,2 sec, compared to 17,6 sec for A and B pigs).

Previous research has shown that an enriched raising environment facilitates the enhancement of pigs social behaviour, as they display a reduced to virtually non existent aggressive behaviour towards pigs raised in a bare, non-enriched environment, (Beattie, 2000). Following unloading, group A pigs spent more time moving about in the slaughter house folds (9%) as compared to the group B pigs (6%), while group B pigs diplayed more aggressive behaviour (4%) as opposed to those from group A (2%).

CONCLUSIONS

3.1. Results have proved that various enriched raising conditions impacted the pigs physiological and behavioural responses during their transport to slaughter house. Different levels of saliva cortisol and behavioural displays between the two groups were due to the fact that pigs raised in a bare, non-enriched environment could experience more stress during mixing and loading procedures than those raised in an enriched environment.

3.2. Study results indicate mixing and loading related stress in all pigs transported to the slaughter house regardless of the raising conditions, thus impacting their welfare.

3.3. Raising conditions of fattening pigs may influence the quality level of their welfare.

3.4. Research has shown that in a shelter whose box environment has been enriched, pigs behavioural displays were more numerous and various compared to those of the pigs raised in a bare, non-enriched environment.

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A COMPARATIVE RISK ANALYSIS BETWEEN AVIAN INFLUENZA AND NEW HUMAN INFLUENZA OF SWINE ORIGIN

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key words: swine influenza, human influenza, comparative risk analysis

SUMMARY

Diseases caused by influenza viruses classified in Orthomyxoviridae family, of any type, are very common for human and animal populations, some of them being related to seasonal incidence. Influenza A strains that can infect mammals (eg, pigs and humans) may undergo genetic reassortment through contact with avian strains. The reassortment of an avian strain with a mammalian strain may produce a chimeric virus that is transmissible between mammals; such mutation products may contain haemaglutinins and/or neuraminidase proteins that are unrecognizable to the immune systems of mammals. This antigenic shift results in a much greater population of susceptible individuals in whom more severe disease is possible. Such antigenic shifts can cause a pandemic event. The most striking of these pandemics was the 1918 Spanish influenza, which infected one third of the world's population and caused approximately 50 million deaths. The next reassortment of influenza viruses between H2N2 of avian origin and H1N1 human origin caused a new pandemic trend in 1957, followed by Hong-Kong influenza attack in 1968 inside a new reassortment of H2N2 of human origin and H3 avian origin. A new type of H5N1 avian influenza virus caused, starting with 2003, dramatic losses in avian livestock all over the word but a real psychosis in a virtual event of its pandemic development. Since April 2009 a new strain of influenza A virus with unknown origin caused dramatic events in North America, than in Europe, Africa and finally Asia by its highest morbidity and sometimes lethality in humans. A comparative risk analysis has been performed by our specialists in order to identify the similarities and differences between these five influenza waves. This comparative study reveals important differences regarding the origin of outbreaks, way and mode of virus transmission for different reassortant strains as well as the pattern of risk emission and risk exposure, involving different risk consequences and so different management approaches based on particular selected measures for preventions and control.

Since 6,000 years ago when evidence of a disease similar with nowadays seasonal human influenza exists and this type of disease was assigned to the astral and cold influenza on human health, till the last decade of the last century, this kind of disease occurring in human, mammals and birds have been assess as unitary pattern. Development of genetic engineering and especially PCR techniques gave to scientists and workers in diagnostic laboratory the opportunity to relive the differences between different types of influenza viruses and establishing the hierarchical relations among them. Pointing out the drift and shift mechanisms and appearance of reassortant strains of influenza virus of different origin constituted the most useful tool to make differences

between influenza diseases in human, mammals and birds. These particularities complete the other sort of differences expressed during clinical development of each kind of influenza as well as through epidemiological studies or morfopathological investigations. Taking into account the updated scientific information available on different influenza diseases, this paper constitutes an attempt to draw up a comparative risk analysis among them in order to provide to making-decision operators the most useful tools to design strategies and policies for different type of influenza in human, mammals and birds, also to implement specific measures making population aware from risk raised from the new pandemic trend induced by new swine origin human influenza.

MATERIALS AND METHODS

In order to perform such a comparative study the main particularities of each kind of influenza diseases have been selected using the same criteria. The model Corvello Merckhofer for animal and public risk analysis in relation with influenza diseases affecting animals and public health has been used with corrections made by specialists from French Agency for Sanitary Security of Food (AFFSA) in 2008 and 2009. The pattern of risk identification, risk assessment, risk management and risk communication have been followed, structured as identification of risk origin, transmission ways of identified risks, risk emission, risk exposure and risk consequences. Inside of second major stage of this kind risk analysis, risk assessment has been done, against the international standards, technical and scientific knowledge related to animal or human influenza. This comparative study gave to involved institutions in managing human influenzas or animal influenzas, the principles to design appropriate measures to prevent interspecies transmission so, more accurate to prevent reassortation between influenza strain virus of different origin (human, mammals or avian origin) being able to prevent pandemic influenzas and to decrease the consequences of epizootic influenza.

RESULTS AND DISCUSSIONS

Till advanced microbiological or virological techniques having developed the differences between human, mammals and avian influenzas were done only based clinical features, morfopathological findings and epidemiological reveals. Sequencing and genotyping of

influenza strains of different origin allowed, not only their very detailed characterization and classification in A, B, C and D but at the same time the genetic interrelations among them, pointing out that mainly influenza A viruses have the ability of reassortation, B, C, D influenza types causing smooth influenza outbreaks in humans. Concerning the **genetic origin** of compared influenzas there a strong differences among them. In this comparative risk analysis we took into consideration the following kinds of influenza: seasonal human influenza, avian influenza caused by low and high pathogen virus, swine influenza, human influenza caused by a reassortant virus of avian origin (H5N1, H7N7 and H7N2 or H2N2), new swine origin human influenza caused by a reassortant virus resulted from four reassortation mechanisms including avian, swine and human origin viruses. All these influenzas belong to type A. In order to understand these differences it should be better to assess the following pictures.

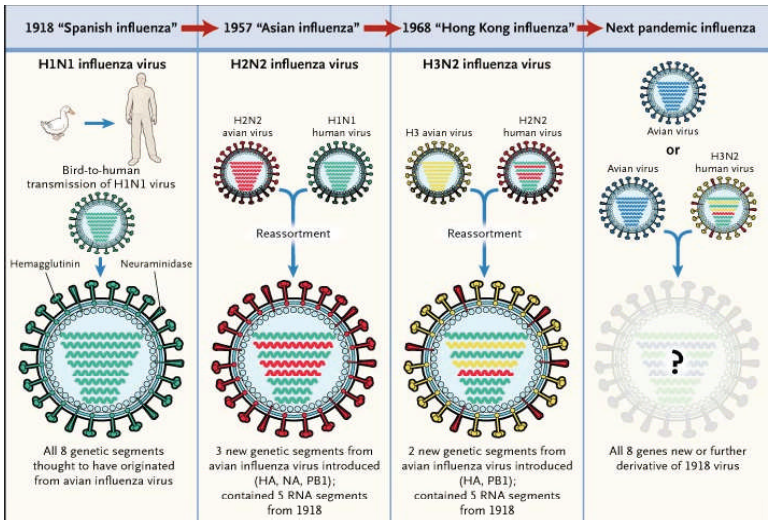

Fig.1. Reassortant mechanisms of influenza strains A of different origin (Johan Giesecke, Chief Scientist European Centre for Disease Prevention and Control, Stockholm, 1 May 2009)

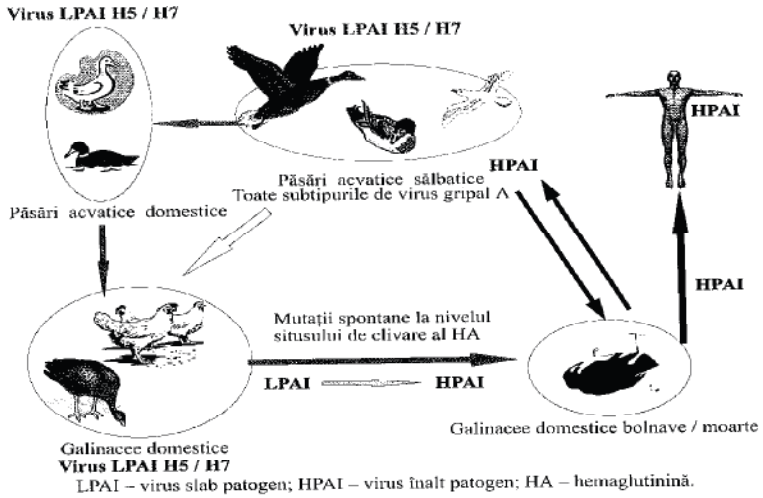

Fig. 2. Ecology of avian influenza and human influenza of avian origin

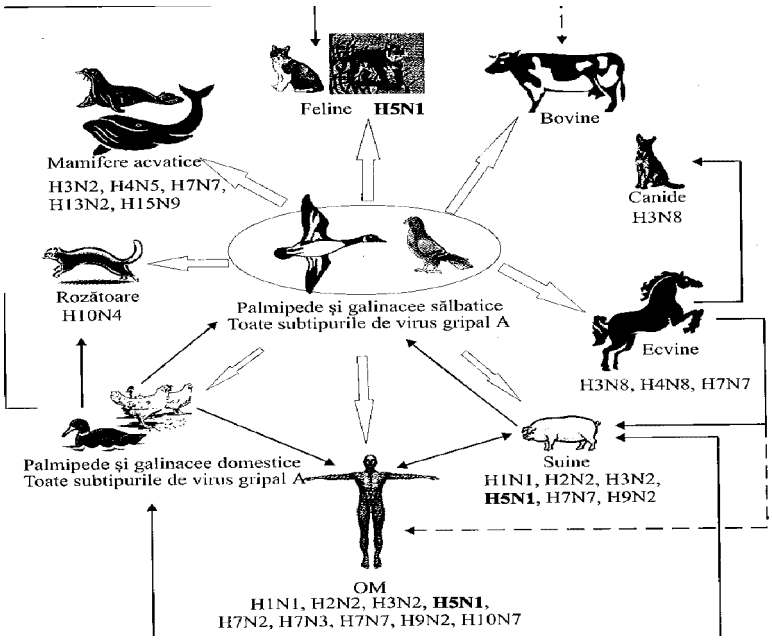

Fig. 3. The ecology of influenza A viruses

The main difference between human influenza of avian origin and new swine origin human influenza consist in the fact that the outbreaks

of H5N1 human influenza was caused by a reassortment mechanism between a non-reassorted virus H5N1 and human genome, while the outbreaks occurred started with April (new swine origin human influenza) resulted through a reassortation mechanism between an already reassorted virus including human/swine/avian genetic components and a human origin N1H1 virus influenza, resulting a new reassorted virus influenza, with enhancing pathogenity, causing several outbreaks in humans all over of the world and two outbreaks of influenza in pigs in Canada and Ireland mentioned in a previous paper, being transmitted from human to pigs. The formal reassortant virus has circulating among swine and human populations with out any severe complication. The second aspect of risk origin is referring to the **origin place** of starting epidemics. It is suggesting the focus of main reassortments have historically been in Southeast Asia, the proposed "influenza epicenter," because agricultural practices in this region brought pigs, people and domestic and water birds into close contact with one another. Concerning the way of transmission, there are also clear differences. Since H5N1 human influenza is transmitted almost by close relation between human and some bird species affected by H5N1 avian influenza, as well as with products or by-products originated in these affected birds, new swine origin human influenza was spread mainly by human – to – human relation. No human – to – human transmission has been notified for H5N1 avian influenza. Even if both human influenzas aforementioned are related with seasonal aspect, the carrier for virus is totally different: new swine origin human influenza is less related with season, by H5N1 human influenza are related with H5N1 avian influenza in water birds then domestic birds outbreaks, but this outbreaks are closely related to the migration flows of migratory birds. Referring to **emission risk**, in the case of new swine origin human influenza this is quite peoples and the **exposure risk** consists in humans and pigs, for H5N1 human influenza, the emission risk are H5N1 avian influenza affected birds and exposure risk reflects both human and avian populations. Seasonal human influenza has genetic and site origin in humans, also the emission risk and exposure risk consist in human and sometimes few mammalian species. New swine origin human influenza had almost no serious consequences, excepting the sharp engagement of sanitary services, while H5N1 human influenza had terrific social, economical, commercial and religious consequences. At the same time H5N1 human influenza deeply involves both sanitary and sanitary veterinary services. The seasonal human influenza is very accustomed event for sanitary services. The **management** of influenza A diseases

took into consideration has some different elements, started with the national surveillance programs, the level of impose restrictions and the involvement of national sanitary and/or sanitary veterinary services or nongovernment organizations.

CONCLUSIONS

3.1. This paper plays as first national attempt to perform a comparative risk analysis study.

3.2. Using specific clinical, morfopathological and epidemiological particularities and findings resulted from genotyping, pointing out the main differences among influenza A diseases in play have been stipulated.

3.3. The results of this study are to be used by authorities in order to design strategies and policies for each of them in a different approaches and mentioning specific measures besides general approaches of influenza A.

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AN ATTEMPT TO PERFORM A RISK ANALYSIS FOR NEW SWINE ORIGIN HUMAN INFLUENZA

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key words: swine influenza, human influenza, risk analysis

SUMMARY

Diseases caused by influenza viruses classified in Orthomyxoviridae family, of any type, are very common for human and animal populations, some of them being related to seasonal incidence. Based on type of haemaglutinins and neuraminidase the influenza viruses are classified in A, B, C, D influenza viruses affecting different animal species or human beings. A new type of H5N1 avian influenza virus caused, starting with 2003, dramatic losses in avian livestock all over the word, but at the same time, a real psychosis in a virtual event of its pandemic development. Since April 2009 a new strain of influenza A virus with unknown origin caused dramatic events in North America, than in Europe, Africa and finally Asia by its highest morbidity and sometimes lethality in humans. The sequential genetic analyses of this new type of viruses isolated in Mexico and USA denote that combinations of the 8 influenza gene segments in the novel strain had not been previously described in either human or swine viruses and revealed the genetic reassortment of three low pathogenic strains viruses of human, swine and avian origin. An attempt to draw up a risk analysis for this new human influenza A of swine origin, called "new human influenza" has been performed by our specialists. In this framework the model Corvello Merckhofer risk analysis has been performed in order to define the risk level for Romania and to design national policies and strategies in the field. The crucial step of this pattern is to clearly define risk identification especially to settle the risk factors with can contribute to spreading of virus. Following this step the part of risk management has been specified, to be able to elaborate and presents practical options to the making-decision operators giving them the possibility to select the most accurate measures to prevent and control this new human swine origin influenza virus.

Swine influenza viruses were first isolated in the United States in 1930.Since that time; they have become an economically important cause of respiratory disease in pigs throughout the world, and a human public health risk. The clinical signs/symptoms of influenza in pigs and people are remarkably similar, with fever, lethargy, lack of appetite and coughing prominent in both species. Furthermore, influenza viruses can be directly transmitted from pigs to people as "zoonotic" disease agents, and vice versa,

from people to pigs. These interspecies infections are most likely to occur when people are in close proximity to pigs, such as in swine production barns, livestock exhibits at fairs, and slaughterhouses. Finally, because of their unique susceptibility to infection with influenza viruses of both mammalian and avian species, pigs can serve as intermediaries in the transmission of influenza viruses from birds to

people. The birds of greatest concern are wild waterfowl, because these species provide an immense natural reservoir of influenza viruses. Replication of avian influenza viruses in pigs may allow them to adapt to and be able to efficiently infect mammals, and ultimately be transmitted to people. In addition, pigs can serve as hosts in which two (or more) influenza viruses can undergo "genetic reassortment." This is a process in which influenza viruses exchange genes during replication. The influenza viruses responsible for the worldwide 1957 and 1968 "pandemics" of human influenza were reassortant viruses with genes from both human and avian influenza viruses.

There are 15 different subtypes of haemaglutinins and 9 different subtypes of neuraminidase among influenza A viruses. Of practical significance, there is no cross-protective immunity mediated by antibodies from one HA subtype to another. In the course of history, relatively few haemaglutinins and neuraminidase combinations have consistently circulated among pigs or people (predominantly H1N1, H1N2 and H3N2 in pigs, and H1N1, H1N2, H2N2 and H3N2 in people). Influenza viruses carry their genes on 8 separate pieces ("segments") of nucleic acid (RNA), rather than on one long single molecule. This structural feature has very important implications for virus evolution, because if two (or more) influenza viruses simultaneously infect cells in the same individual, then during replication, these viruses can exchange RNA segments with one another, thereby creating viruses with entirely new combinations of genes and pandemic potential.

The latest pandemic trend influenza started this year includes human/swine/avian virus reassortant H3N2 viruses that have spread widely within the American pig population since their emergence in 1998, as well as "second generation" reassortant H1N2 and H1N1 viruses derived by genetic mixing between the reassortant H3N2 and classical swine H1N1 viruses. In order to draw up the national and global strategies and policies, a risk analysis must be performed, designing such approach being the aim of this paper.

MATERIALS AND METHODS

The model Corvello Merckhofer for animal and public risk analysis in relation with diseases affecting animal and public health has been used with corrections made by specialists from French Agency for Sanitary Security of Food (AFFSA) in 2008 and 2009. The pattern of risk identification, risk assessment, risk management and risk

communication have been followed, structured as identification of risk origin, transmission ways of identified risks, risk emission, risk exposure and risk consequences. Inside of second major stage of this kind risk analysis, risk assessment has been done, against the international standards, technical and scientific knowledge related to animal or human influenza. Taken into account the features obtained from risk identification and risk assessment procedures, a real framework for risk management concerning new swine origin human influenza has been settled with the aim to be able to identify the most realistic and beneficial options for prevention and cure the diseased cases. After this step of performing risk analysis, the decision makers (private or institutional bodies) have the possibility to select the most suitable approaches to design national and global strategies and policies in the field, also to implement specific measures making population aware from risk raised from the new pandemic trend induced by new swine origin human influenza.

RESULTS AND DISCUSSIONS

In the framework of risk identification precisely establishing of **risk origin** is of crucial importance. In March and early April 2009, **Mexico** experienced outbreaks of respiratory illness and increased reports of patients with influenza-like illness (ILI) in several areas of the country. On April 12, the General Directorate of Epidemiology (DGE) reported an outbreak of ILI in a small community in the state of Veracruz to the Pan American Health Organization (PAHO) in accordance with International Health Regulations. On April 17, a case of atypical pneumonia in Oaxaca State prompted enhanced surveillance throughout Mexico. On April 23, several cases of severe respiratory illness laboratory confirmed as swine-origin influenza A (H1N1) virus (S-OIV) infection were communicated to the PAHO. Sequence analysis revealed that the patients were infected with the same S-OIV strain detected in two children residing in California. On April 13, 2009, Center for Diseases Control of USA (CDC) was notified of a case of respiratory illness in a boy aged 10 years who lives in San Diego County, **California**. The patient had onset of fever, cough, and vomiting on March 30, 2009. He was taken to an outpatient clinic, and a nasopharyngeal swab was collected for testing as part of a clinical study and investigational diagnostic device identified an influenza A virus, but not a human influenza subtypes H1N1, H3N2, and H5N1. On April 14, 2009, CDC received clinical specimens and determined that the virus

was swine influenza A (H1N1). On April 17, 2009, CDC received an influenza specimen from a girl that had been forwarded as an unsubtypable influenza A virus from the Naval Health Research Center in San Diego, **California**, but identified as a swine influenza A (H1N1) virus on April 17, 2009. On 27 April, 2009 **Canada** reported to the World Health Organization (WHO) six cases of influenza of new swine origin human influenza, with no deaths and **Spain** one case, with no deaths. Between April 28 and 1 May, 2009 the following countries reported to the WHO new cases of new swine origin human influenza: **New Zealand** (3), the **United Kingdom** (2), **Israel** (2), **Austria** (1), **Germany** (3), **Netherlands** (1), **Switzerland** (1), **Austria** (1), **Hong Kong** (1). In the decade 1 - 11 May the disease was spreaded in: **Denmark** (1), **France** (1), **Republic of Korea** (1), **Costa Rica** (1), **Ireland** (1), **El Salvador** (2), **Colombia** (1), **Portugal** (1), **Italy** (1), **Guatemala** (1), **Sweden** (1), **Poland** (1), **Brazil** (4), **Argentina** (1), **Australia** (1), **Brazil** (6), **Japan** (3), **Panama** (2), **China** (1), **Norway** (2). The first case was reported in **Romania** on 23 May and in Africa, in **Egypt,** on June 3, 2009.

Regarding the **transmission ways** of identified risks it is very clear that the main way of virus spreading is human-to-human contamination. This is supported by following evidence. Despite the presumption the present virus may have swine origin, is now clearly spreading mainly between humans. Even so, the rate of transmission does not seem to be extremely high and it cannot be explained why the disease seems to be more virulent in Mexico than in other parts of the world. Also the lack of known exposure to pigs in the two cases increases the possibility that human-to-human transmission of this new influenza virus has occurred. The other thing is based on the fact that on September 4, 24 primary and secondary schools in USA announced that they had sent home a total of 25,000 students with flu-like illness thought to be caused by the H1N1 strain. The virus clearly has a pandemic potential, but it should be noted that the word 'pandemic' strictly just describes a disease that spreads around the world - it does not necessarily imply a severe disease. ECDC Threat Assessment - internal decision stated 30 April 2009 the large majority of cases in Europe, USA or Canada have a history of travel to Mexico or USA. The settlement of WHO's phase 5 alert level installed on 29 April, 2009 characterize human-to-human spread of the

virus into at least 2 countries in a single WHO region. The final phase - 6, announced on June 11, 2009 indicates that a global pandemic is under way and is characterized by community-level outbreaks in at least 1 other country in a different WHO region in addition to the

criteria defined in phase 5. Dr. Fukuda from WHO described 3 types of transmission that are taking place. In North America, there is widespread community transmission, in Europe and Asia, there is a "mixed" picture, including both travel-related cases as well as community transmission and the third level consists of travel-related cases only. By the other way, two famous incidents

in Canada and Ireland conform the transmission of A(H1N1) virus from human to pigs. On 2 May, 2009 Canada reported the identification of the A(H1N1) virus in a swine herd in Alberta and proved that the pigs were exposed to the virus from a Canadian farm worker recently returned from Mexico, who had exhibited flu-like symptoms and had contact with the pigs.

Similar, clinical signs commenced in a pig farm in Ireland, on 25 September, with sows off feed and laboured breathing, following the contact with a ILL affected farm worker on 22 September and was proved that he was source of the outbreak or origin of infection. Significantly, WHO stated there is also no risk of infection from this virus from consumption of well-cooked pork and pork products or by-products. Regarding influenza A(H1N1) **risk emission**, the role played by traveling human is crucial for a pandemic trend, but, at the same time, close contacts between communities consist the second reason for virus emission. It is supported by casual boy aged 10 years and his family, who lives in San Diego County, California, with onset of fever, cough, and vomiting on March 30, despite that the child had had no exposure to pigs. Critically the boy's mother had respiratory symptoms without fever in the first few days of April and a brother aged 8 years had a respiratory illness 2 weeks before illness onset in the boy. The second support is a girl aged 9 years who resides in Imperial County, California, adjacent to San Diego County, with onset of cough and fever on March 28. The girl and her parents reported no exposure to pigs, although the girl did attend an agricultural fair where pigs were exhibited approximately 4 weeks before illness onset. The girl's brother aged 13 years had influenza-like symptoms on April 1, and a male cousin aged 13 years living in the home had influenza-like symptoms on March 25, 2009, 3 days before onset of girl's symptoms. There is a strong evidence of disease transmission between families living in two neighboring counties. Referring to risk exposure, the aforementioned cases are relevant for children exposure risk to A(H1N1). CDC updated guidelines on A(H1N1) management reveal that human categories at risks are children younger than 5 years, adults aged 65 years and older, pregnant women, persons with certain chronic medical or

immunosuppressive conditions, and persons younger than 19 years who are receiving long-term aspirin therapy. According to Dr. Schuchat, from WHO, 57% of the cases have occurred in people aged 5 to 24 years, and 41% of hospitalizations were also within that age range. The highest rates of hospitalizations are in children younger than 5 years. Of the hospitalizations, 71% have occurred in people with respiratory problems or pregnant women. Surprisingly, people born before 1957 may be less susceptible than younger people to the A(H1N1) swine influenza, being experienced from other waves of influenza. Those two pig farms affected A(H1N1) linked to human cases reflect the possibility of disease transmission from human to pigs. There is now evidence, till now, of disease transmission from pigs to human. The risk consequences of A(H1N1) influenza virus are unpredictable, despite its pandemic trend and the fact that over 343,298 cases and 4108 human death have been registered. The major consequence is on national sanitary systems especially hospitalization capacity and new reassortments with influenza virus of different origin. The second main stage of risk analysis is **risk assessment.** At least 10 major risk factors have been identified and their impact on public health and pig health have been scored taking into account the WHO guidelines for A(H1N1) influenza virus: animal-human interface, clinical care, healthcare management and facilities, laboratory and virology, pandemic preparedness and response, meting reports, surveillance and epidemiology, travel and work, vaccines, candidate vaccine viruses. The main risk factors identified are the following, influenza virus - level 3, influenza virus reassortments – level 5, spreading speed among humans – level 4, virus transmission to pigs – level 2, now vaccine available – level 4, antiviral resistance development – level 3, specific age groups at risks – level 4, traveling globalization – level 3, preparedness of sanitary systems – 3, poor sanitary systems in more than 60% of affected countries – level 3.

In order to determine the level of risks the classical formula was applied

$$RiW = \frac{\sum_{i=1}^{10} r_i \times R_i}{\sum_{i=1}^{10} r_i} = \frac{1(5x5) + 3(4x4) + 5\,(3x3) + 1(2x2)}{1x5 + 3x4 + 5x3 + 1x2} = \mathbf{3,58}$$

resulted an high intolerable unacceptable risk for A(H1N1), being necessary corrective measures to decrease the risk level to a tolerable acceptable risk in order to be successfully managed.

The risk factors for Romania have the following different level: now vaccine available – level 2, poor sanitary systems in more than 60% of affected countries – level 1.

$$RiRo = \frac{\sum_{i=1}^{10} r_i \times R_i}{\sum_{i=1}^{10} r_i} = \frac{1(5x5) + 2(4x4) + 4(3x3) + 2(2x2) + 1(1x1)}{1x5 + 2x4 + 4x3 + 2x2 + 1x1} = \mathbf{3,4}$$

The real risk was calculated based on Corvello formula

$$\mathbf{RrW} = \frac{\sum Ri}{\sum nRi} + Rc = \frac{10}{100} = 0,1 + 3,58 = 3,68$$

$$\mathbf{RrRo} = \frac{\sum Ri}{\sum nRi} + Rc = \frac{10}{100} = 0,1 + 3,4 = 3,50$$

$$Rcomp = \frac{RrRo}{RrW} = \frac{3,50}{3,68} = 0,95$$ so this is a sub-unitary risk involving slighter measures than those practiced at world level

Risk management procedures have been established at global level inside the WHO in colaboration with Global Influenza Surveillance Network (GISN) and consist in official guidelines aforementioned. WHO advises no restriction of regular travel or closure of borders. It is considered prudent for people who are ill to delay international travel and for people developing symptoms following international travel to seek medical attention, in line with guidance from national authorities. Individuals are advised to wash hands thoroughly with soap and water on a regular basis and should seek medical attention if they develop any symptoms of influenza-like illness. Corrective measures established following risk assessment and management include Global Influenza Preparedness Plan consisting in: national preparedness and response as a

whole-of-society responsibility (government leadership, health sector, non-health sectors, communities, individuals, and families), WHO responsability under International Health Regulations (IHR 2005) (the designation of the global pandemic phase, switching to pandemic vaccine production, rapid containment of the initial emergence of pandemic influenza, providing an early assessment of pandemic severity on health), recommended actions before, during and after a pandemic (phases 1-3, phase 4, phases 5-6, the post-peak period, the post-pandemic period).

3. CONCLUSIONS

3.1. A basic risk analysis for new swine origin human influenza has been performed for the first time in Romania.

3.2. A clearly identification of H1N1 influenza risk and its particularities have been established as well as the major risk factors involved.

3.3. The national and world risk levels have been calculated and due to their unacceptable level the risk management and corrective measures have been defined granting to authorities real tool to prevent and control the disease.

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RESEARCHES CONCERNING THE EFFECT OF CERTAIN NUTRITIONAL FACTORS ON THE CHEMICAL COMPOSITION OF THE CHICKEN SKELETAL MUSCLE TISSUE

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Key words: feeding, chicken broiler, skeletal muscles, chemical constituents

SUMMARY

Several researches targeted the effects exerted by the usage of certain diet types on chickens growing performances, while meat composition has been neglected. Our researches evaluated the effect induced by the usage of certain kinds of diets (standard, protein enriched, starch enriched, fat enriched) onto the skeletal musculature composition in chickens.
120 "COBB-500" chicken broilers have been used as biological material and raised within the standard intensive technological system, divided in 4 groups (according to the used diets, 30 chickens/group). At 42 days old, the broilers have been slaughtered and muscular samples have been detached from carcass, in order to run chemical assessments (water and dry matter content, ashes and organic matter-fats, proteins content). The musculature issued from breast, wings, thighs and shanks have been thus analyzed for chemical composition. Other tests have been also run, such as: Nessler, Kreiss and H_2S identification.
High dietary fat concentrations induced the production of meat reach in fat and poor in other compounds like water and proteins. Concerning the composition of certain carcass parts, it has been found that breast musculature is richer in nitrogen than limbs, while the shanks and thighs had the highest fat percent. Enriched protein diet did not affect the muscular composition in nitrogenous matters but influenced the growing process, resulting in higher weight gain

Previous researchers stated that "nutrition quality, consequently the nutrients level and quality in food, is a main influential factor of the consumers' health status. Therefore, the food industry should consider all possible ways to improve nutritional value of aliments. Despite this, the data related to some quality indexes of the poultry meat, that could contribute to establish an overall image of the nutritional and dietetic facts of this product are poorly represented in the scientific literature, mainly when the consumer might be interested by the meat produced and commercialised nowadays in Romania" [7, 10].

Other scientific issues showed the opportunity in usage of certain local or even cheaper feedstuffs in formulating broiler diets, with the appropriate consequences on meat quality [4, 6]. Moreover, the chickens nutrition could be used to improve the chemical composition of skeletal musculature, to create the so-called designed food, especially projected to provide quality added, safer and healthier aliments [3, 5]. Several

researches revealed different meat chemical composition values, depending on broiler genotype, rearing technology and nutrition, as main influential factors [1, 7, 8].

1. MATERIAL AND METHOD

The biological material comprised 120 chicken broilers belonging to the „COBB-500" hybrid, reared within the halls of „Avicola Buzău", using the intensive exploitation system (accommodation on the shelter floor) [9]. The chickens have been separated in 4 groups of 30 individuals each one, according to the nutritional features of the feed they received during the three feeding periods (starter – 0-10 days; grower – 11-34 days; finisher – 25-42 days) (feed composition in tab. 1 to 3).

Table 1

Nutritional composition of the STARTER forage used in the 4 broilers groups

Nutritional Feature	M.U.	Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Moisture	%	12.07	11.74	12.15	11.67
Dry matter	%	87.93	88.26	87.85	88.336
Metabolisable energy (ME)	Kcal/ Kg	3030	3030	3046	3202
Crude protein (CP)	%	24.09	27	19.25	24.07
Raw fat	%	5.18	5.52	2.80	8.18
Ash	%	3.90	4.78	3.69	3.92
Raw fibber	%	3.70	3.64	2.65	3.61
Digestible protein	%	20.82	23.33	16.68	20.79
Calcium	%	1.01	0.99	1.00	1.02
Av. phosphorus	%	0.51	0.50	0.51	0.52
Ca/P	Ratio	1.97	1.98	1.96	1.96
Natrium	%	0.17	0.16	0.18	0.17
Chloride	%	0.23	0.26	0.31	0.23
Acid:Base	Meq/ 100	22.26	23.03	13.94	22.02
ME/CP	Ratio	125.77	112.22	158.31	133.05

Table 2

Nutritional composition of the GROWER forage used in the 4 broilers groups

Nutritional Feature	M.U.	Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Moisture	%	11.94	11.65	11.94	11.56
Dry matter	%	88.06	88.35	88.06	88.44
Metabolisable energy (ME)	Kcal/ Kg	3152	3152	3150	3321.4
Crude protein (CP)	%	21.83	25	18.70	21.78
Raw fat	%	6.64	7.47	4.28	9.64
Ash	%	3.35	4.16	3.63	3.34
Raw fibber	%	3.53	3.64	2.55	3.47
Digestible protein	%	18.79	21.57	16.14	18.74
Calcium	%	0.92	0.91	0.95	0.92
Av. phosphorus	%	0.45	0.45	0.45	0.45
Ca/P	ratio	2.04	2.01	2.11	2.04
Natrium	%	0.16	0.16	0.20	0.16
Chloride	%	0.23	0.23	0.35	0.23
Acid:Base	Meq/ 100	19.33	22.19	12.95	19.30
ME/CP	ratio	144.36	126.08	168.47	152.50

Table 3

Nutritional composition of the FINISHER forage used in the 4 broilers groups

Nutritional Feature	M.U.	Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Moisture	%	11.86	11.49	12.12	11.44
Dry matter	%	88.14	88.51	87.88	88.56
Metabolisable energy (ME)	Kcal/ Kg	3220	3200	3200	3406.25
Crude protein (CP)	%	20.01	23	15.19	20.00
Raw fat	%	7.68	8.13	5.86	11.00

Ash	%	2.76	3.34	2.03	2.76
Raw fibber	%	3.53	3.62	2.96	3.48
Digestible protein	%	17.17	19.67	13.02	17.16
Calcium	%	0.87	0.85	0.87	0.87
Av. phosphorus	%	0.42	0.42	0.42	0.42
Ca/P	Ratio	2.06	2.02	2.07	2.06
Natrium	%	0.16	0.16	0.16	0.16
Chloride	%	0.23	0.23	0.23	0.23
Acid:Base	Meq/ 100	17.82	18.99	12.55	17.82
ME/CP	Ratio	160.91	139.13	210.66	170.31

Thus, four categories of mixed feed have been used, one of them being the standard recipe for broilers and fed to the control group (LC), the other three diets being characterized by an peculiar excess of nutrients: high protein content (HP group), high starch content (HS group) and high fat content (LF group). The nutritional features of the used feeds are presented in tables 1 to 3.

Dietary crude proteins levels varied between 15.19% (starch enriched feed, finisher version) and 27% (protein enriched feed, starter version), while the energy level was maintained at close levels, except for the feed recipes whose fat content exceeded.

In the 40^{th} day of the experiment we sampled blood for the serum biochemical assessments then, in the 42^{nd} day of life, the chickens have been slaughtered. From each group, 5 individuals have been randomly chosen ~~for necropsy~~, in order to take muscular samples (breast, wings, thighs and shanks). The meat pieces have been processed through dehydration at +105°C, to find out the water and dry matter proportions then through calcinations at +550°C to assess the minerals participations. Total nitrogen content was analyzed through the Kjeldahl method, adapted to the VelpScientifica DK-6 digestor and VelpScientifica UDK 127 distiller then the issued results have been multiplied by 6.25, in order to estimate meat protein content. The direct Soxhlet method was used for lipids extraction, within a VelpScientifica SER-148 extraction system, using the petroleum ether 40-60°C as reagent. The data achieved through analytic chemistry have been statistically analyzed, the mean, the standard mean error and the variation coefficient being calculated.

RESULTS AND DISCUSSIONS

While the Nessler, Kreis and H_2S identification methods gave negative results for all test we run, the data issued from chemical assessments, run on samples taken from 42 days old chickens are presented in table 4 to 8.

Thus, the meat issued from pectoral muscles proved to have water contents between 71.72±0.13 g% (diet with high fat level) and 73.17±0.8 g% (diet with high protein level). The other groups, who received control and high starch feeds, realized water proportion around 72.9 g%. The uniformity of the analyzed treat could be considered well. The dry matter content conversely varied, while the minerals were poorer represented in the meat ob broilers which received high caloric feed (based on fat or starch) (tab. 4).

The lipids from breast muscles were found within the 1.19±0.1 g% (high protein diet)… 2.65±0.13 g% (high fat diet) interval, while the best protein content (24.29±0.21g%) was achieved by the chickens which consumed enriched protein diet. Close values have been achieved by the group received high starch content (tab.4). Highest variability was calculated for the ashes and fat contents in all treatments.

Table 4

Chemical composition of the breast muscles issued from chicken broilers fed with different nutrients level

Chemical components		Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Water (g/100g)	$\bar{x}$	72.98	73.7	72.8	71.72
	$\pm s_{\bar{x}}$	0.16	0.18	0.14	0.13
	$v\,\%$	0.87	0.91	0.69	0.94
Dry matter (g/100g)	$\bar{x}$	27.02	26.26	27.2	28.28
	$\pm s_{\bar{x}}$	0.14	0.13	0.1	0.15
	$v\,\%$	2.72	2.49	3.02	2.82
Ashes (g/100g)	$\bar{x}$	1.08	1.08	0.97	0.83
	$\pm s_{\bar{x}}$	0.05	0.02	0.06	0.04
	$v\,\%$	9.74	10.2	9.05	8.84

Proteins (g/100g)	$\bar{x}$	24.13	24.29	24.26	24.19
	$\pm s_{\bar{x}}$	0.21	0.23	0.21	0.19
	v %	3.42	4.19	2.96	3.18
Fats (g/100g)	$\bar{x}$	1.21	1.19	1.42	2.65
	$\pm s_{\bar{x}}$	0.09	0.1	0.08	0.13
	v %	7.51	8.39	8.63	9.48

When wings muscles have been chemically examined, it was found that the water content was a little bit higher than in pectoral muscles, varying from 73.12±0.15g% (fat excess) till 74.27±0.14g% (starch excess) (tab. 5).

Considering the metabolic specialization of the limbs muscles, no mater the upper or the lower ones, it was expected higher fats content than in breast muscles. Therefore, lipids varied from 4.38±0.07g% (control diet) till 5.87±0.11g% (fat enriched diet), while proteins content amplitude was less wide (19.35…19.49 g%). The homogeneity was average, knowing that in some situations the variation coefficient reached almost the 10% edge (tab. 5).

Table 5

Chemical composition of the wings muscles issued from chicken broilers fed with different nutrients level

Chemical components		Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Water (g/100g)	$\bar{x}$	74.27	74.1	74	73.12
	$\pm s_{\bar{x}}$	0.14	0.17	0.11	0.15
	v %	0.62	0.67	0.58	0.71
Dry matter (g/100g)	$\bar{x}$	25.73	25.92	26	26.88
	$\pm s_{\bar{x}}$	0.13	0.16	0.12	0.17
	v %	2.39	2.84	2.71	2.93
Ashes (g/100g)	$\bar{x}$	1.27	1.27	1.08	1.13
	$\pm s_{\bar{x}}$	0.04	0.03	0.05	0.02

	v %	10.06	9.87	10.2	9.34
Proteins (g/100g)	$\overline{x}$	19.38	19.49	19.46	19.35
	$\pm s_{\bar{x}}$	0.22	0.19	0.23	0.16
	v %	3.51	3.28	3.67	3.19
Fats (g/100g)	$\overline{x}$	4.38	4.47	4.91	5.87
	$\pm s_{\bar{x}}$	0.07	0.09	0.12	0.11
	v %	8.13	8.28	8.59	8.42

Table 6

Chemical composition of the thighs muscles issued from chicken broilers fed with different nutrients level

Chemical components		Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Water (g/100g)	$\overline{x}$	72.18	72.3	71.9	70.63
	$\pm s_{\bar{x}}$	0.11	0.15	0.19	0.13
	v %	0.54	0.71	0.75	0.63
Dry matter (g/100g)	$\overline{x}$	27.82	27.66	28.1	29.37
	$\pm s_{\bar{x}}$	0.14	0.12	0.15	0.14
	v %	2.28	2.09	2.41	2.37
Ashes (g/100g)	$\overline{x}$	1.06	1.06	1.12	1.04
	$\pm s_{\bar{x}}$	0.06	0.05	0.04	0.06
	v %	9.91	9.76	9.69	9.95
Proteins (g/100g)	$\overline{x}$	19.59	19.6	19.5	19.15
	$\pm s_{\bar{x}}$	0.26	0.21	0.29	0.18

	v %	3.82	3.41	3.65	3.23
Fats (g/100g)	$\bar{x}$	6.47	6.28	7.06	8.65
	$\pm s_{\bar{x}}$	0.09	0.14	0.19	0.15
	v %	8.02	8.34	8.38	8.21

The rearlimbs (tab 6&7) musculature proved to be richer in fats than in other analyzed muscles (breast and wings). This could be explained through the natural trend of fat supply formation between the red muscles of thighs and shanks. Thus, the fat enriched diets generated highest fat contents (8.65±0.15g% in thighs and 7.36±0.14g% in shanks).

Table 7

Chemical composition of the shanks muscles issued from chicken broilers fed with different nutrients level

Chemical components		Control (LC)	High protein (HP)	High starch (HS)	High fat (HF)
Water (g/100g)	$\bar{x}$	72.45	72.9	72.6	71.16
	$\pm s_{\bar{x}}$	0.19	0.16	0.17	0.14
	v %	0.96	0.83	0.74	0.91
Dry matter (g/100g)	$\bar{x}$	27.55	27.13	27.4	28.84
	$\pm s_{\bar{x}}$	0.15	0.12	0.09	0.13
	v %	2.61	2.43	2.97	3.02
Ashes (g/100g)	$\bar{x}$	1.14	1.14	1.12	0.98
	$\pm s_{\bar{x}}$	0.03	0.04	0.04	0.02
	v %	9.82	10.2	9.19	8.73
Proteins (g/100g)	$\bar{x}$	20.35	20.4	19.9	19.89
	$\pm s_{\bar{x}}$	0.25	0.27	0.18	0.24
	v %	3.45	4.27	2.81	3.23

Fats (g/100g)	$\bar{x}$	5.46	5.19	5.83	7.36
	$\pm s_{\bar{x}}$	0.08	0.06	0.12	0.14
	$v\,\%$	7.68	8.19	8.91	9.23

In opposition with the previous state of facts, the lowest fat contents were observed at the meat provided by the chickens fed with high protein diet. The homogeneity of this treat was found toward average (v% varied between 7.68% and 9.23%) (tab. 6 and 7).

Protein content in the rearlimbs was situated between 19.15±0.18 g% (thighs from chickens fed with high fat diet) and 20.40±0.27 g% (shanks from chickens received high protein feed). The treat could be considered as homogenous, as the variation coefficient did not exceed 4.5%.

CONCLUSIONS

3.1. Among the main chemical constituents of the meat, proteins are the most stable ones as proportion, while fats are subject to change, as consequence of alteration in dietary fat concentration.

3.2. Both starch and fat enriched feeds led to higher levels of fat in the studied muscles.

3.3. Higher protein levels occurred in breast and wings muscles and, overall in groups which were fed with control and high protein diets.

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RESEARCHES CONCERNING THE CORRELATIONS BETWEEN THE ALTERATION OF MUSCLE TISSUE COMPOSITION AND THE MORPHOLOGIC BLOOD TRAITS, UNDER THE INFLUENCE OF CERTAIN NUTRITIONAL FACTORS

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Key words: nutrition, chicken broiler, blood, biochemistry

SUMMARY

The paper emphasizes on the changes induced in the blood serum parameters of the chicken broilers, by the usage of certain types of diet (standard, protein enriched, starch enriched, fat enriched).
We used as biological material 120 "COBB-500" chicken broilers reared within the standard intensive technological system. Blood has been sampled when they turned 40 days old. The data issued from automatic biochemistry assessments (BS-200 unit analyzer) have been statistically processed.
Several biochemical blood parameters were examined: blood glucose concentration, cholesterol and total lipids level, certain proteins level (albumins and globulins), uric acid content and calcium content.
Glycemia was higher in the group fed with increased starch levels. Cholesterol and lipids were increased at high dietary energy and fat levels, while serum albumins, globulins and uremia were found in higher concentrations in those blood samples issued from the chickens fed with proteins in excess. Lowest calcium quantity in blood serum was observed in samples from the group which received fat enriched diet, possibly suggesting an inverse correlation between calcium absorption in gut and the dietary fat levels.

The nutritional effect on serum blood parameters, on carcass traits and skeletal muscles in poultry has been previously investigated by other researches. Therefore, more than alterations of nitrogen equilibrium or of other biochemical parameters, changes in diets concentrations in nutrients lead to certain changes on the carcass development and on meat chemical composition and nutritional value [1, 5].

Chicken broilers blood biochemical parameters were found in wide ranges by other researchers [6, 7, 8]. Thus, glucose was detected between 90-215 mg/dl, cholesterol was specified between 80 and 190 mg/dl, albumins from 1.2 till 1.7 g/dl, while triglycerides have been assessed between 243 and 430 mg/dl. The data suggest that there are many influential factors on the blood serum biochemistry, such as

chicken bloodline, exploitation technology, stressor factors and especially the diet [2, 4, 6].

The aim of this study was to evaluate the influence exerted by feeding differentiations (excesses of dietary proteins or starch or fats) onto the broilers serum blood parameters, using the feedstuffs common met in our country feed factories.

1. MATERIAL AND METHOD

The biological material comprised 120 chicken broilers belonging to the „COBB-500" hybrid, reared within the halls of „Avicola Buzău", using the intensive exploitation system (accommodation on the shelter floor) [9]. The chickens have been separated in 4 groups of 30 individuals each, according to the nutritional features of the feed they received during the three feeding periods (starter – 0-10 days; grower – 11-34 days; finisher – 25-42 days). The nutritional features of the used feeds are presented in tables 1 to 3.

Table 1

Nutritional features of the STARTER feed used in the 4 broilers groups

Nutritional Feature	M.U.	Control group feed (LC)	High protein feed (HP)	High starch feed (HS)	High fat feed (HF)
Moisture	%	12.07	11.74	12.15	11.67
Dry matter	%	87.93	88.26	87.85	88.336
Metabolisable energy (ME)	Kcal/ Kg	3030	3030	3046	3202
Crude protein (CP)	%	24.09	27	19.25	24.07
Raw fat	%	5.18	5.52	2.80	8.18
Ash	%	3.90	4.78	3.69	3.92
Raw fibber	%	3.70	3.64	2.65	3.61
Digestible protein	%	20.82	23.33	16.68	20.79
Calcium	%	1.01	0.99	1.00	1.02
Av. phosphorus	%	0.51	0.50	0.51	0.52

Ca/P	ratio	1.97	1.98	1.96	1.96
Natrium	%	0.17	0.16	0.18	0.17
Chloride	%	0.23	0.26	0.31	0.23
Acid:Base	Meq/ 100	22.26	23.03	13.94	22.02
ME/CP	ratio	125.77	112.22	158.31	133.05

Thus, four categories of mixed feed have been used, one of them being the standard recipe for broilers and fed to the control group (LC), the other three diets being characterized by an peculiar excess of nutrients: high protein content (HP group), high starch content (HS group) and high fat content (LF group).

Table 2

Nutritional features of the GROWER feed used in the 4 broilers groups

Nutritional Feature	M.U.	Control group feed (LC)	High protein feed (HP)	High starch feed (HS)	High fat feed (HF)
Moisture	%	11.94	11.65	11.94	11.56
Dry matter	%	88.06	88.35	88.06	88.44
Metabolisable energy (ME)	Kcal/ Kg	3152	3152	3150	3321.4
Crude protein (CP)	%	21.83	25	18.70	21.78
Raw fat	%	6.64	7.47	4.28	9.64
Ash	%	3.35	4.16	3.63	3.34
Raw fibber	%	3.53	3.64	2.55	3.47
Digestible protein	%	18.79	21.57	16.14	18.74
Calcium	%	0.92	0.91	0.95	0.92
Av. phosphorus	%	0.45	0.45	0.45	0.45
Ca/P	ratio	2.04	2.01	2.11	2.04
Natrium	%	0.16	0.16	0.20	0.16
Chloride	%	0.23	0.23	0.35	0.23
Acid:Base	Meq/ 100	19.33	22.19	12.95	19.30
ME/CP	ratio	144.36	126.08	168.47	152.50

Table 3

Nutritional features of the FINISHER feed used in the 4 broilers groups

Nutritional Feature	M.U.	Control group feed (LC)	High protein feed (HP)	High starch feed (HS)	High fat feed (HF)
Moisture	%	11.86	11.49	12.12	11.44
Dry matter	%	88.14	88.51	87.88	88.56
Metabolisable energy (ME)	Kcal/ Kg	3220	3200	3200	3406.25
Crude protein (CP)	%	20.01	23	15.19	20.00
Raw fat	%	7.68	8.13	5.86	11.00
Ash	%	2.76	3.34	2.03	2.76
Raw fibber	%	3.53	3.62	2.96	3.48
Digestible protein	%	17.17	19.67	13.02	17.16
Calcium	%	0.87	0.85	0.87	0.87
Av. phosphorus	%	0.42	0.42	0.42	0.42
Ca/P	Ratio	2.06	2.02	2.07	2.06
Natrium	%	0.16	0.16	0.16	0.16
Chloride	%	0.23	0.23	0.23	0.23
Acid:Base	Meq/ 100	17.82	18.99	12.55	17.82
ME/CP	Ratio	160.91	139.13	210.66	170.31

Dietary crude proteins levels varied between 15.19% (starch enriched feed) and 27% (protein enriched feed), while the energy level was maintained at close levels, except for the feed recipes whose fat content exceeded.

At 40 days old, the chickens were blood sampled, in order to run biochemical assessments. The blood serum has been automatically processed, using a BS-200 biochemical analyzer. Samples have been statistically analyzed, the mean, the standard mean error and the variation coefficient being calculated.

RESULTS AND DISCUSSION

The data issued from biochemical blood serum analyses, run on samples taken from 40 days old chickens are presented in table 4 and illustrated in fig. 1 (a-g).

Glucose concentration in blood serum was detected between 112.0±3.27 mg/dl (control group, standard broiler diet) and 118.2±0.58 mg/dl (starch enriched diet, HS). The glycemia of the chickens fed with excess of proteins or fat was measured at close levels (115.0-114.8 mg/dl). The best uniformity of the assays was provided by the high protein (HP) and high starch (HS) samples (tab. 4 and fig. 1-a).

Cholesterol and total lipids levels varied proportionally and the highest concentrations have been found in the samples issued from the chickens fed with fat enriched diet (cholesterol=151.6±3.57 mg/dl and total lipids=404.00±13.67 mg/dl), while the lowest levels were found in the control and HP (high protein) groups. The best uniformity was also observed in control group (v = 0.74% in cholesterol and 5.30% in total lipids concentration). The other groups tended to become more heterogeneous, reaching even 12.87% variability in HP group (tab. 4, fig. 1, b&c).

Higher concentrations of protein in HP group diets (27% starter, 25% grower and 23% finisher) induced increased serum proteins and urea levels. Thus, at the HP group blood samples, the greatest values for albumins reached 1.51±0.03 g/dl, for globulins 1.18±0.06 g/dl respectively 22.8±1.93 mg/dl for urea. The lowest serum protein and urea levels were observed in the blood sampled from the groups fed with starch and fat excess. The highest variability for urea content occurred in the group which consumed high starch feed. However, every analyzed group proved to be strong heterogeneous for this parameter (tab. 4, fig. 1-d, e&f).

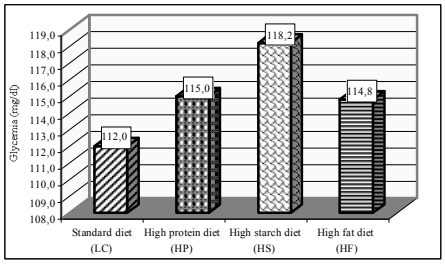
Table 4

Blood serum biochemical parameters, issued from the chicken groups fed with certain levels of nutrients concentrations

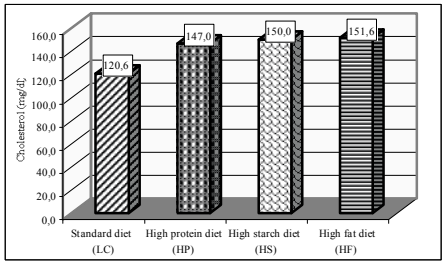
Chicken group	Sample No.	Glyc.* (mg/dl)	Chol.* (mg/dl)	Tot. lip.* (mg/dl)	Albs.* (g/dl)	Globs.* (g/dl)	Urea (mg/dl)	Calcium (mg/dl)
LC (standard broiler feed)	1	103	120	374	1.56	0.86	25	12.8
	2	120	122	348	1.25	1.14	16	11.8
	3	119	120	324	1.12	1.21	18	12.2
	4	108	121	362	1.38	0.98	22	11.4
	5	110	120	356	1.21	1.18	20	11.9
	$\bar{x}$	112	120.6	352.8	1.304	1.074	20.2	12.02

	$\pm s_{\bar{x}}$	3.27	0.40	8.36	0.08	0.07	1.56	0.23
	v %	6.53	0.74	5.30	13.11	13.87	17.29	4.34
HP (high protein feed)	1	117	155	322	1.42	1.04	29	12
	2	115	132	317	1.44	1.1	18	12.2
	3	115	164	414	1.58	1.38	25	11.9
	4	112	141	338	1.55	1.25	22	12.4
	5	116	143	403	1.56	1.15	20	12
	$\bar{x}$	115	147	358.8	1.51	1.184	22.8	12.1
	$\pm s_{\bar{x}}$	0.84	5.61	20.66	0.03	0.06	1.93	0.09
	v %	1.63	8.54	12.87	4.91	11.31	18.97	1.65
HS (high starch feed)	1	117	158	340	1.32	0.98	15	11.8
	2	119	163	316	1.33	0.95	12	11.7
	3	120	144	358	1.26	1.03	29	11.9
	4	117	136	303	1.25	1.04	14	11.8
	5	118	149	310	1.25	0.99	28	11.5
	$\bar{x}$	118.2	150	325.4	1.282	0.998	19.6	11.74
	$\pm s_{\bar{x}}$	0.58	4.83	10.25	0.02	0.02	3.67	0.07
	v %	1.10	7.20	7.05	3.09	3.71	41.86	1.29
HF (high fat feed)	1	110	157	430	1.28	0.83	20	10.8
	2	119	155	396	1.23	0.87	23	10.9
	3	112	138	388	1.18	0.96	12	11
	4	117	151	366	1.2	0.99	28	10.9
	5	116	157	440	1.36	0.96	22	11.1
	$\bar{x}$	114.8	151.6	404.00	1.25	0.922	21	10.94
	$\pm s_{\bar{x}}$	1.66	3.57	13.67	0.03	0.03	2.61	0.05
	v %	3.22	5.27	7.56	5.77	7.41	27.77	1.04

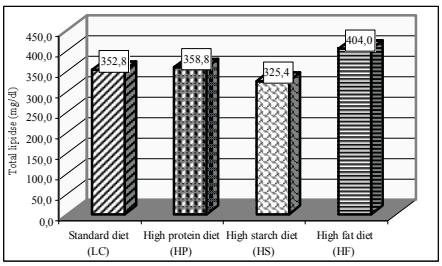
*Glyc.=Glycemia; Chol.=Cholesterol; Tot.lip.=Total lipids; Albs.=albumins; Globs.=Globulins



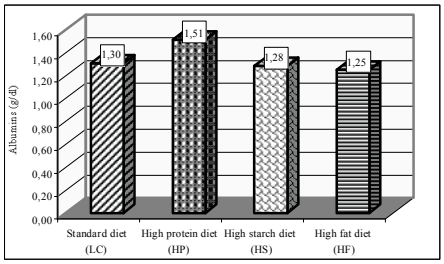
a) Blood serum glycemia



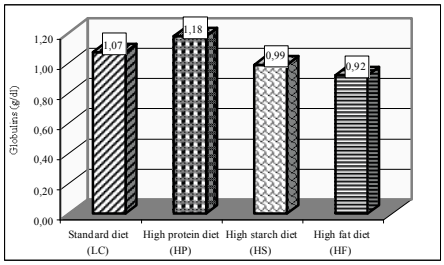
b) Blood serum cholesterol



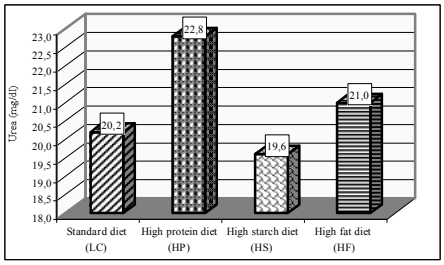
c) Blood serum total fats



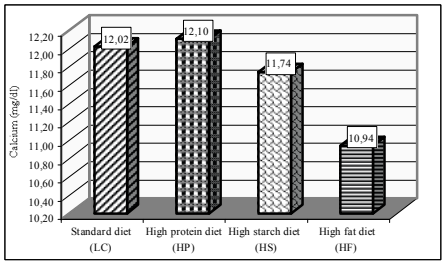
d) Blood serum albumins



e) Blood serum globulins



f) Blood serum uremia



g) Blood serum calcemia

Fig. 1 – Blood serum biochemical parameters in chicken broilers, in relationship with the diet type

Calcium assessments revealed maximal values in protein enriched and control diets (12.1±0.09 mg/dl and 12.02±0.23 mg/dl), while the minimal ones were observed in the blood taken from chickens fed with high fat diets (10.94±0.05 mg/dl).

CONCLUSIONS

Feeding unbalanced diets to chicken broilers could lead to certain alterations of their metabolic profile, assessed through the blood serum biochemical parameters.

The starch enriched feed induced higher glucose levels in blood, while cholesterol and total lipids from blood were significantly increased at those chickens fed with high fat proportions.

Proteins serum content and especially urea level were higher in the group which received protein enriched diets. The protein balance should be very well managed, knowing the issues linked to environment pollution through excessive nitrogen excretion.

Blood calcium has not been strongly influenced by dietary changes. However, the lowest level was measured in the blood issued from the chickens fed with the fat enriched diet, possible due to difficulties in this macroelement intestinal absorption.

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RESEARCHES CONCERNING THE EFFECTS OF EXPERIMENTAL CORTICOSTEROID TREATMENT UPON THE EVOLUTION OF SOME METABOLIC ENZYMES IN PREGNAND SHEEP

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Key words: cortisol, metabolic enzymes, pregnant sheep.

SUMMARY

It was determined the activity of some enzymes involved in the protein metabolism (TGO – glutamate oxaloacetate transaminase, TGP – glutamate piruvate transaminase, GGT – gamma glutamil transpherase), ALP – alkaline phosphatase, ACP – acid phosphatase and a glicolytic enzyme (LDH – lactic dehydrogenase) in cortisol treated pregnant sheep. The results showed differences according to the physiological status and the type of the metabolic enzyme: involved in protein metabolism, mineral or in glycolitic metabolism.

Glucocorticoids constitute one of the most frequently prescribed medicines during pregnancy. Their use is the mainstay for a variety of maternal and fetal indications, both in acute and chronic settings. The pharmacokinetics of corticosteroids during pregnancy remain poorly understood. A number of works shows the large metabolic effects of the glycocorticoid hormones, but differences according to the species and the physiological status (pregnant, milking etc.) still remain [2, 3, 9]. The present work aimed the effects of the experimental cortisol treatment upon the activity of the main metabolism via main metabolic enzymes in pregnant sheep.

1. MATERIAL AND METHODS

The researches were performed on two groups of one year old Merinos ewes:

1. one control pregnant ewes group and
2. one experimental cortisol treated pregnant ewes group;

All the ewes were diagnosticated for pregnancy before the beginning of the experiment.

Cortisol (as hydrocortisone) was administered at two days intervals, i.m., for four times in dose of 80 μg/kg body weight to the experimental

group. Control group was injected by the same volume of saline solution.

Day before administration and then, from two to two days intervals during cortisol treatment blood from both, experimental and control groups, was collected and the serum was separated after coagulation, decantation and centrifugation at 1500 rpm for 15 minutes and serum samples were stored at 1-4°C for no more than two hours before analysis.

Serum samples from control and experimental groups were analyzed for the activity of the main metabolic enzymes. It was determined the activity of the following metabolic enzymes: TGO (glutamate oxaloacetate transaminase), TGP (glutamate piruvate transaminase), GGT (gamma glutamil transpherase), ALP (alkaline phosphatase), ACP (acid phosphatase), LDH (lactic dehydro-genase). The analyses were performed according to Manta *et al*. [5]. The results were processed from statistical point of view and expressed as mean ± standard error of mean ($\bar{X} \pm s_{\bar{X}}$).

RESULTS AND DISCUSSION

Table 1 shows the effect of consecutively four two days interval i.m. administrations of 80 μg/kg body weight cortisol on the metabolic enzymes in three months pregnant ewes by comparing with non hormonal-treated pregnant ewes. The data from the table 1 show an increased activity of TGO, GGT and LDH, while TGP activity remained relatively constant. These activities reflect an increasing of the proteic metabolism, explained by the physiological status of the sheep (late pregnancy). A number of authors revealed a physiological rise of the glucocorticoid levels and, consecutively, an increase of the enzymes involved in proteic metabolism, according to the stage of pregnancy in different species [6, 7, 11].

The most raised activity showed ALP: from 132 to 246 U/L ($P<0.01$) in four days of experimental treatment while in the control group, the increase was only from 149 to 221 U/L. This increase of the ALP activity in the mother blood may be induced by the intensified metabolism of calcium and by bone development in fetus. Cortisol involvement in this process may be explained by the physiological interferences of adrenal and endocrine glands as parathyroid glands [3].

In the same period, the LDH activity raised steadily from 664 to 806 U/L ($P>0.05$), showing an intensified metabolism of carbohydrates,

especially by glycolise. An increase of LDH activity was found out in control sheep, but this time only it was a no significant increase, from 598 to 621 U/L (P>0.05).

Table 1

Effect of cortisol treatment on the activity of some metabolic enzymes in three months pregnant sheep (expressed as mean ± standard error of mean, $\overline{X} \pm s_{\overline{x}}$)

Enzyme	UM	Sheep group	Times of cortisol administration			
			1st admin.	2nd admin	3rd admin	4th admin
TGO	U/L	Control (5)	90±12	90±20	94±21	99±15
		Hormonal treated (5)	84±11	87±7	101±11	109±38 ↑*
TGP	U/L	Control (5)	12 ±1	12 ±1	14 ±4	13 ±2
		Hormonal treated (5)	15±5	10±2	11±4	13 ±5 ↔
GGT	U/L	Control (4)	47±8	54±14	50±11	69±21
		Hormonal treated (4)	59±21	91±13	102±31	98 ± 17↑*
ALP	U/L	Control (6)	149±42	166±48	190±55	221±64
		Hormonal treated (6)	132±32	258±121	293±65	246± 49 ↑**
ACP	mU/L	Control (4)	5,245±254	5,861±211	5,271±94	5,253±182
		Hormonal treated (4)	5,940±245	6,670±322	5,190±632	5,380±242 ↓*
LDH	U/L	Control (5)	598±32	610±72	631±34	621±40
		Hormonal treated (5)	664±72	771±211	788±134	806 ±162 ↑*

Legend:
TGO – Glutamate oxaloacetate transaminase
TGP – Glutamate piruvate transaminase
GGT – Gamma glutamil transpherase
ALP – Alkaline phosphatase
ACP – Acid phosphatase
LDH – Lactic dehydrogenase
1st , 2nd , 3rd , 4th - values following first, second, third and fourth consecutive administrations of cortisol in two days intervals.
*P<0.05; **P<0.01.
Numbers in parentheses indicate number of sampled animals.

Numerous studies revealed the cardinal role of cortisol in fetus development and parturition. All these effects are by the means of the metabolic process development.

Damjanovic *et al.* [2008] found that in human, cortisol rise during pregnancy is connected with fetal maturation. Together with the increase of insulin resistance it might have a role in allocation of nutrients between mother and fetus. Cortisol and insulin secretion

during pregnancy seems to be induced by fluctuations in basal metabolism rate [1].

In all the stress situations there is a rapid and high increase of ACTH secretion. This ACTH secretion is followed by a significant adrenal secretion of cortisol [8].

Wood demonstrated that acutely stimulated fetal ACTH secretion is not regulated by cortisol negative feedback in the last few days of fetal life in sheep. Reduction in negative feedback efficacy may allow the preparturient rise in cortisol secretion that is responsible for stimulating parturition in these species [11].

3. CONCLUSIONS

3.1. In pregnant sheep, cortisol induced an increase of the proteic and glycolitic metabolic processes and an increase of the calciu metabolism, reveled by the intensified activity of the main enzymes involved in these processes.

3.2. The increasing of the metabolic processes under cortisol treatment doubles a lesser steady natural increase of the same processes in pregnant sheep.

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AGAROSE GEL ELECTROPHORESIS CHANGES IN RABBITS IMPLANTED WITH HELLEBORE ROOT (HELLEBORUS PURPURASCENS)

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Key words: Helleborus, rabbit, electrophoresis.

SUMMARY

They were studied the results of serum electrophoregrams in rabits of different races and sexes, aged 6 months. An subcutaneous implant has been performed, Blood testing was performed before the implant and on 3, 5 and 7 days after implant.

The results of serum electrophoregrams distinguished:
- to the experimental batch the rate variation of albumin was from 53.65% before implanting, to a decreasing to 46.81% 5 days after implanting, and a return to 52.35% after 7 days from implanting;
- the rate variation of α1 globulin was from 7.47% before implanting, to 4.61% 5 days after implanting, and 7.15% 7 days after implanting;
- the rate variation of α2 globulin was from 8.3% before implanting, an increase to 11.63% 5 days after implanting, and a return to 8.62% 7 days after implanting;
- the evolution of β globulin was from 12.74% before implanting, 18.62% 5 days after implanting, and 8.62% 7 days after implanting;
- γ globulin registered values of 17.82% before implanting, 18.31% 5 days after implanting, and 18.52% 7 days after implanting;
After the results we can see that the γ globulin from blood serum doesn't sustain a meaningful increasing after hellebore implanting.

The hellebore (Helleborus purpurascens, fam. Ranunculaceae) is a herbaceous plant, spread in the broadleaf forests and in the wet hayfields on the hilly and mountaineous areas (Bruneton, 1993; Mohan, 2001).

It contains 3 types of active principles:

- bufanolide-steroidal glycosides (the most important is the helebrin which generates helebrigenin, glycosis and ramnosis);
- 2 saponosides: heleborin and heleborein
- an unsaturated lactone: protoanemonin (Grigorescu et al, 2001; Mozăceni, 2003).

Due to the bufanolide glycosides, the hellebore has a cardiotonic action, similar to that of digital and strophantin and also vomitive and purgative effects (Nueleanu, 2006). Helleborus purpurascens also has an irritant, severe purgative and oxytocic effects, due to its saponosides. Apud Neagu et al., heleborin has effects on the nervous system, too.

The active principles of the hellebore (H. purpurascens) are found mostly in the rhizome, roots and in the seeds.

Electrophoresis is the migration of electrically charged particles (eg protein molecules) in an electrolyte under the influence of the electric field. The father of the protein electrophoresis is Tiselius.

He developed the method of separation of proteins in solution. Following further development appeared a type of electrophoresis called „zonal" electrophoresis, by which different protein molecules are separated into distinct zones in stabilized environment. In 1955 Smithies introduced starch gel as a enviroment for the migration of protein molecules.

In 1957 Kohn reported the use of cellulose acetate as a good support of electrophoretic migration (Manchenko, 2003). Agarose gel began to be used for electrophoretic migration in the late 1970.

The purpose of this study was to test the rabbits seric albumin and globulin changes after subcutaneus injections of helleborus rhizome.

1. MATERIAL AND METHOD

Research has been conducted on a total of 30 rabbits of different sexes and races aged 6 months, in the following batches:

Batch I: 15 rabbits on which implanted with hellebore s.c. (1 cm piece of rhizome);

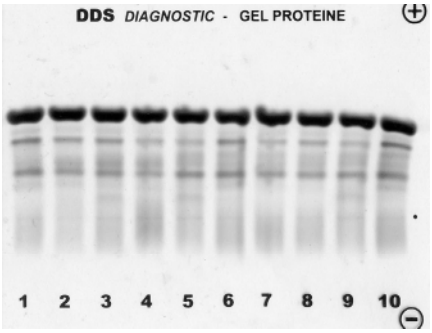
Batch II: control.

Blood testing was performed before the implant and on 3, 5 and 7 days after implant. Processing of samples was performed by centrifugation, expressed blood serum was stored in tubes Ependorf at a temperature of + 4ºC for 24 hours.

Determinations were made using agarose gel electrophoresis machine using the Tris Barbital buffer at pH 8.6.

Serum was diluted 1 / 7 with Tris Barbital buffer, protein fractions migration was accomplished at 92 V, migration time beeing 17 minutes and 30 seconds.

The coloring of the protein fractions was performed with Amido Black (fig. 1). After drying, computerised integration and calculation of the electroforegrams was conducted, resulting in 5 distinct protein fractions: albumin, $\alpha 1$ globulin, $\alpha 2$ globulin, β globulin and γ globulin (fig 2).



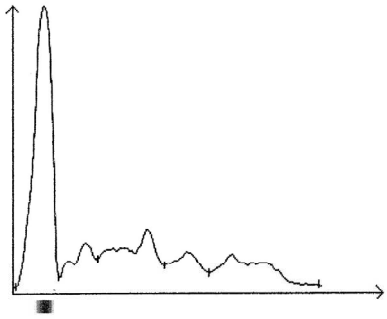


Fig. 1 - Seric protein migration **Fig. 2 - Aspect of seric protein migration curve**

In parallel with electroforegrams, total protein was determined with the refractometric method with Zeiss Abbé refractometer.

2. RESULTS AND DISCUSSION

The results of cumputer integration of the electrophoregrams on experimental and control batch before and in different time after helleboro rhizome implanting are revealed in the tables 1, 2, and 3, and the figure 3.

Table 1

The results of cumputerised electrophoregrams of the rabbits used in the experiment before hellebor implanting

Batch	Samples/lot	Total protein g/dl	Albumin %	α1 globulin %	α2 globulin %	β globulin %	γ globulin %
I	1/15	5.30	52.12	3.92	13.42	15.17	15.36
	1/15	6.00	60.91	6.01	11.19	6.26	15.63
	1/15	6.20	48.57	9.12	7.69	13.75	20.86
	1/15	6.00	51.21	3.18	9.87	19.66	16.07
	10/15	6.10	54.22	8.65	7.13	12.03	17.97
	1/15	6.40	49.75	3.42	11.05	15.98	19.80
Average		6.06	53.65	7.47	8.30	12.74	17.82
II	8/15	4.60	55.74	10.24	12.62	6.35	15.05
	7/15	7.00	45.78	3.29	6.30	16.24	28.39
Average		5.72	51.09	6.99	9.67	10.96	21.27

Table 2

The results of cumputerised electrophoregrams of the rabbits used in the experiment 5 days after hellebor implanting

Batch	Samples/lot	Total protein g/dl	Albumin %	α1 globulin %	α2 globulin %	β globulin %	γ globulin %
I	**1/15**	**5.80**	**47.53**	**4.30**	**11.52**	**19.35**	**17.30**
	1/15	**5.20**	**52.42**	**3.37**	**8.05**	**17.14**	**19.02**
	1/15	**5.30**	**55.13**	**4.57**	**7.77**	**19.10**	**13.43**
	1/15	**5.60**	**50.45**	**3.94**	**8.78**	**18.01**	**18.82**
	10/15	**5.80**	**44.96**	**4.06**	**13.06**	**19.35**	**18.57**
	1/15	**5.40**	**47.11**	**12.46**	**7.76**	**12.24**	**20.43**
Average		5.68	46.81	4.61	11.63	18.62	18.31
II	**7/15**	**5.40**	**55.41**	**8.50**	**15.83**	**8.81**	**11.45**
	8/15	**5.80**	**47.54**	**9.38**	**8.77**	**10.88**	**23.42**
Average		5.61	51.21	8.96	12.06	9.91	17.83

Table 3

The results of cumputerised electrophoregrams of the rabbits used in the experiment 7 days after hellebor implanting

Batch	Samples/lot	Total protein g/dl	Albumin %	α1 globulin %	α2 globulin %	β globulin %	γ globulin %
I	**1/15**	**5.80**	**57.44**	**3.01**	**9.88**	**19.93**	**9.75**
	1/15	**5.20**	**56.87**	**7.75**	**9.03**	**8.91**	**17.43**
	1/15	**6.20**	**50.90**	**2.99**	**9.42**	**17.20**	**19.49**
	1/15	**5.00**	**57.54**	**8.32**	**9.85**	**9.57**	**14.72**
	10/15	**5.40**	**51.38**	**7.57**	**8.31**	**12.95**	**19.79**
	1/15	**5.40**	**48.84**	**9.54**	**8.11**	**14.89**	**18.63**
Average		5.44	52.35	7.15	8.62	13.33	18.52
II	**7/15**	**5.00**	**60.84**	**2.36**	**8.18**	**21.03**	**7.60**
	8/15	**5.40**	**51.03**	**7.40**	**7.10**	**11.26**	**23.22**
Average		5.21	55.60	5.04	7.60	15.81	15.93

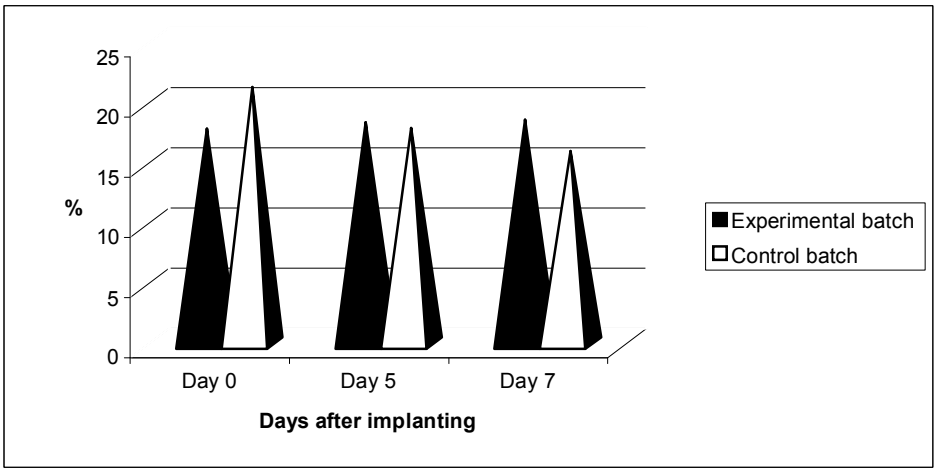


Fig. 3 – Graphic representation of percentile dynamic of gamma-globulins in experimental and control batch

From results of the analysis next topics emphased:

- to the experimental batch the rate variation of albumin was from 53.65% before implanting, to a decreasing to 46.81% 5 days after implanting, and a return to 52.35% after 7 days from implanting;
- the rate variation of α1 globulin was from 7.47% before implanting, to 4.61% 5 days after implanting, and 7.15% 7 days after implanting;
- the rate variation of α2 globulin was from 8.3% before implanting, an increase to 11.63% 5 days after implanting, and a return to 8.62% 7 days after implanting;
- the evolution of β globulin was from 12.74% before implanting, 18.62% 5 days after implanting, and 8.62% 7 days after implanting;
- γ globulin registered values of 17.82% before implanting, 18.31% 5 days after implanting, and 18.52% 7 days after implanting;

After the results we can see that the γ globulin from blood serum doesn't sustain a meaningful increasing after hellebore implanting.

3. CONCLUSIONS

3.1. Agarose gel electrophoresis can be used with great succes in analysis and dosage of high molecule proteins from blood serum.

3.2. The average of initial values of albumin was 53.65%, α1 globulin was 7.47%, α2 globulin was 8,30%, β globuline was 12.74%, and γ globulin was 17.82%.

3.3. The changes of most of the fractions were observed in 5 days after implanting. Theese returned to roughly initial values in 7 days after implanting.

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INVESTIGATIONS ON THE IMMUNOLOGICAL PROFILE AND GRAVIMETRICAL DETERMINATION IN CHICKENS SUBJECTED TO SUPPRESSION FACTORS

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Key words: cyclophophosfamide treatment, lymphopoetics organs, imunosuppresing.

SUMMARY

The investigations concerned the suppressing effects of cyclophosphamide administred three times, and of some stressing factors (restricted alimentation) on some parameters of the immume status of 50 days old chickens, at the beginning of the experiment. The birds were inoculated against infections bursitis and Newcastle disease and observed along 40 days.
The modifications induced by vaccines and the other treatments were evaluated conncerning the weight of some lymphoid organs, the leucocytes and the antibody levels against infectious bursitis and Newcastle diseasc viruses. The birds' weight was significantly diminished in the group treated with cyclophosphamide, in comparasion with the non inoculated and the stressed group. The weight of the bursa was diminished only in the group treated with cyclophosphamide; the bursal index was diminished in comparasion with the control group.
The weight of the spleen and the spleenic index were significantly smaller at both the experimental groups.
Experimental vaccination against infectious bursitis had determined a sensible increase other anti - Newcastle virus antibody level, al control groups chickens. On the contrary, the restricted alimentation and the inadequate environment conditions induce a diminuation of the anti Ncwcastle virus antibody level.
The results confirm the suppressing effect of cyclophosphamide treatment and of inadequate environment conditions on the immune status of birds.

Structural and functional parameters of the immune system are influenced by a number of endogenous and exogenous factors that can cause stimulation, amplification, suppression and even the immune response.

The numerous experimental data has shown the action of the most diverse external factors on the ability of immunological defense in animals. The most significant and important of these are the environmental, nutritional, microbial, stress related (Chandra, 1998).

Thus, several authors have found that protein-energy malnutrition is an immunosuppressive factor for humans and all species of domestic animals (Kim et all, 1998).

External conditions that trigger complex manifestations of stress are also involved in determining immunopathological changes (Rojs, O.Z, M. Cerne, 2000).

Kim and collaborators (Mast, J., BM Goddeeris, 1999) showed that the birds may develop some specific diseases, particularly those caused by viruses whose development is accompanied by changes in immunological parameters suppression. Such viruses are: chicken infectious anemic virus, Marek disease virus, Reoviruses and, in particular infectious bursitis virus (Şofei D.M., E. Bucur, 1999).

1. MATERIALS AND METHOD

The research was carried out on 40 coventional chickens, heavy breed (hybrid Rosso) aged 50 days, assigned to 4 groups (A, B, C, D), each group containing 10 chicks, which were subjected to drug immunosuppression , stress by starvation and restriction of light, vaccination against avian infectious bursitis (BIA), and control infection, according to experimental scheme (table 1).

Table 1

Experimental scheme

No.	Batch	No. chicken	Immunosuppression Endoxan	Starving stress	Vaccination against BIA	Control infection
1.	A(whitness)	10	-	-	-	*
2.	B	10	-	-	*	*
3.	C	10	*	-	*	*
4.	D	10	-	*	*	*

Within each phase were taken blood samples for hematological and serological examinations. The experiment was conducted over a period of 42 days.

Administration of cyclophosphamide (Endoxan - 200 mg active substance / vial) was performed by intramuscular inoculation (im), 3 days in a row, with booster at week 2 and 4.

Starvation stress was produced by the r4eduction of the daily ration (21 / 2) to half and the light restriction was made by reducing the period of light to 8 hours / day.

Vaccination against avian infectious bursitis (BIA) was performed with a lyophilized live vaccine strain PA embrioadapted I - 93 (103.5 DIE 50 / vaccine dose), administered subcutaneously into 2 halves. The first vaccination was performed at the beginning of the experiment with booster in 2 weeks.

Control infection was achieved with standard pathogenic strain of the virus BIA, 52/70 (2.50 X 102 CID50), inoculated im.

Before starting the test, chickens were vaccinated against Newcastle disease by the vaccination schedule.

Quantified parameters: body weight, weight of the Fabricius bursa, spleen weight, gravimetric indices for bursa (IB) and spleen (IS), determination of the total number of leukocytes, the concentration of antibodies against Newcastle disease, produced by the reaction of haemagglutination inhibition.

2. RESULTS AND DISCUSSIONS

The results on body weight are summarized in table 2 and figure 1. It decreased *significantly distinct* from cyclophosphamide-treated group ($P < 0.025$) compared to unvaccinated control group, and from batch to be *highly significant* food stress ($P < 0.005$) compared to unvaccinated control group, the latter the final weight.

Table 2

Body weight values – statistical evaluation

Batch	A	B	C	D
Media ± ΔS	1,46 ± 0,061	1,51 ± 0,039	1,29 ± 0,077**	1,18 ± 0,145***

**** = statistically significant difference; *** = highly significant difference.**
ΔS – standard deviation.

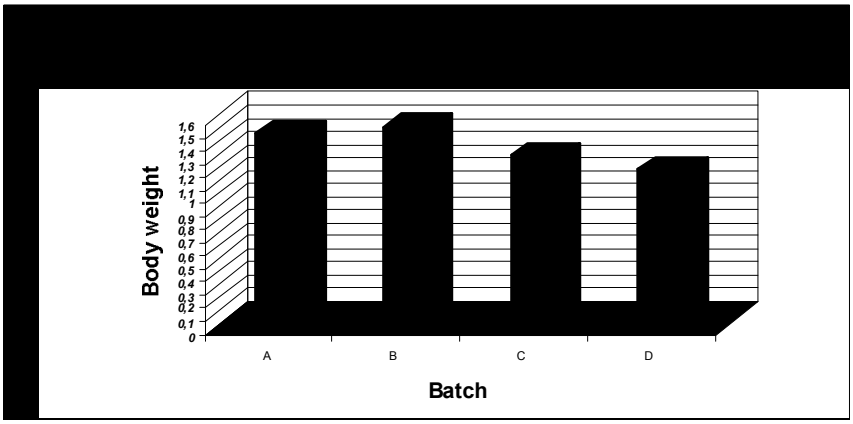

Fig. 1 – Body weight at the end of the experiment

Gravimetric determinations were performed on spleen and cloacal bursa. Spleen weight (Table 3 and Figure 2) decreased significantly high

in group C (P <0.005) and significantly distinct from group D (P <0.01) than the control group, which evolved similar in body weight.

Weight of the cloacal bursa decreased significantly distinct only in group C, treated with cyclophosphamide (P <0.01) compared to the witness batch. (Table 3 and Figure 2).

Table 3

The average weight of the cloacal bursa and the spleen- statistical evaluation

Batch	A	B	C	D
Spleen weight ± ΔS	3,48±0,50	3,62±0,60	2,34±0,53***	1,98±0,34**
Cloacal bursa weight ± ΔS	2,06±0,63	1,98±1,15	0,74±0,31**	1,40±0,99

** = statistically significant difference; *** = highly significant difference;

Splenic index (Table 4 and Figure 2) decreased significantly distinct from group C (P <0.005) and significantly high in group D (P <0.025) compared to unvaccinated control group. BET (Table 4 and Figure 2) decreased only in group C were significantly distinct (P <0.005) from a statistical viewpoint.

Table 4

Splenic and bursal index- statisctical evaluation

Batch	A	B	C	D
Splenic index ± ΔS	0,23±0,037	0,23±0,046	0,17±0,041**	0,16±0,036***
Bursal index ± ΔS	0,13±0,043	0,13±0,076	0,05±0,022**	0,13±0,084

** = statistically significant difference; *** = highly significant difference;

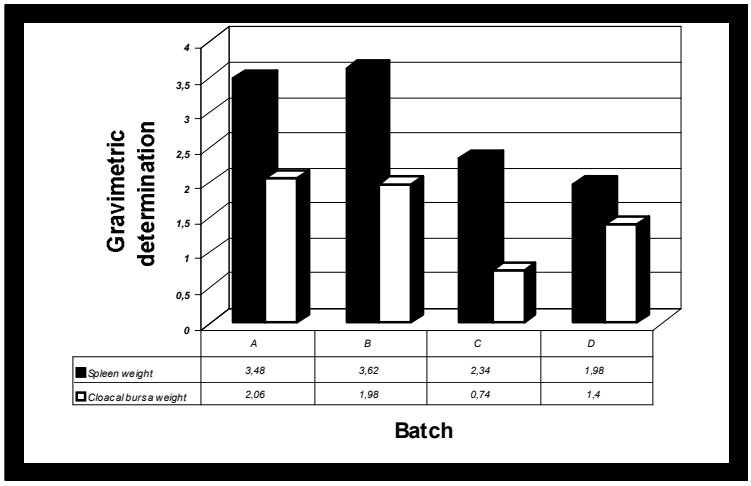

Fig. 2 – Weight of the spleen and cloacal bursa at the end of the experiment

Table 5

Splenic and bursal index – statistical evaluation

Batch	A	B	C	D
Splenic index ± ΔS	0,23±0,037	0,23±0,046	0,17±0,041**	0,16±0,036***
Bursal index ± ΔS	0,13±0,043	0,13±0,076	0,05±0,022**	0,13±0,084

**** = statistically significant difference; *** = highly significant difference;**

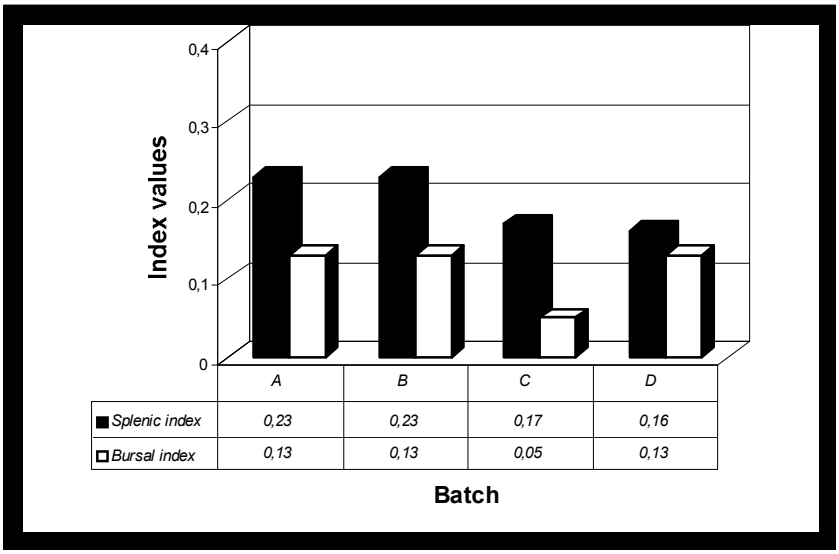

Fig. 3 Splenic and bursal index

Results of the total number of leukocytes from the leukogram (Table 6, Figure 4) show a moderate decrease in the number of leukocytes in the immunosuppressed group (24,4 ± 0,8 thou cells/mm^3) **and food stress group** (23,24 ± 2,65 thou cells/mm^3) **versus** 27,3 ± 2,4 thou cells/mm^3 in witness batch.

***Table* 6**

Leukogram –medium values (thou /mm^3)

Blood draw	Stage 1	Stage 2	Stage 3
Batch A	**24,74±1,78**	**26,40±2,25**	**27,30±2,40**
Batch B	**27,35±2,10**	**28,92±2,07**	**28,74±1,25**
Batch C	**26,40±3,20**	**25,36±1,96**	**24,40±4,08**
Batch D	**26,08±2,24**	**25,82±3,62**	**23,24±2,65**

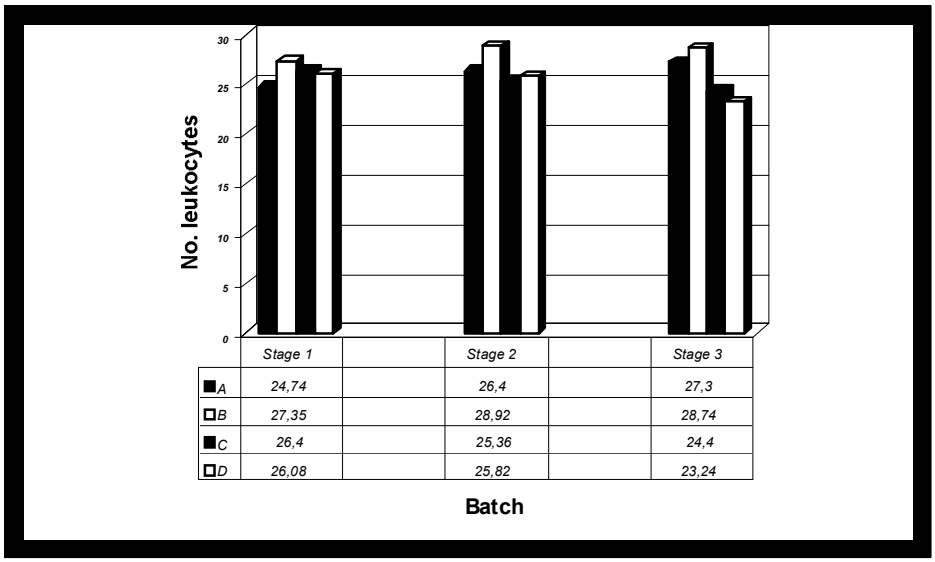

Fig. 4 - Number of WBC (thousands / mm3)

Results of virus titration of antibodies against Newcastle disease (ND) are shown in Table 7, Figure 5. This evaluation was performed by haemagglutination inhibition test.

Table 7

Concentration dynamics of antibodies against Newcastle disease virus (UIHA)

Blood draw day	Day 1	Day 14	Day 28	Day 42
Batch A	**43,94**	**39,98**	**12,62**	**10,08**
Batch B	**52,78**	**105,60**	**106,60**	**60,64**
Batch C	**20,00**	**105,10**	**69,64**	**45,95**
Batch D	**13,29**	**121,30**	**80,00**	**45,57**

Vaccination against one embrioadapted avian infectious bursitis strain caused a sharp increase in levels of antibodies against ND in the control group (group B) who was not immunosuppressed.

Stress produced by cyclophosphamide (group C) and feed restriction and improper environment (group D), were dimmed by the end of the experiment the effect of the vaccine BIA, leading to a reduction of antivirus-ND antibody titres.

Treatment with cyclophosphamide, restriction of feed and light stress caused significant changes in immunological profile that fits the characteristic manifestations of secondary imunodeficit.

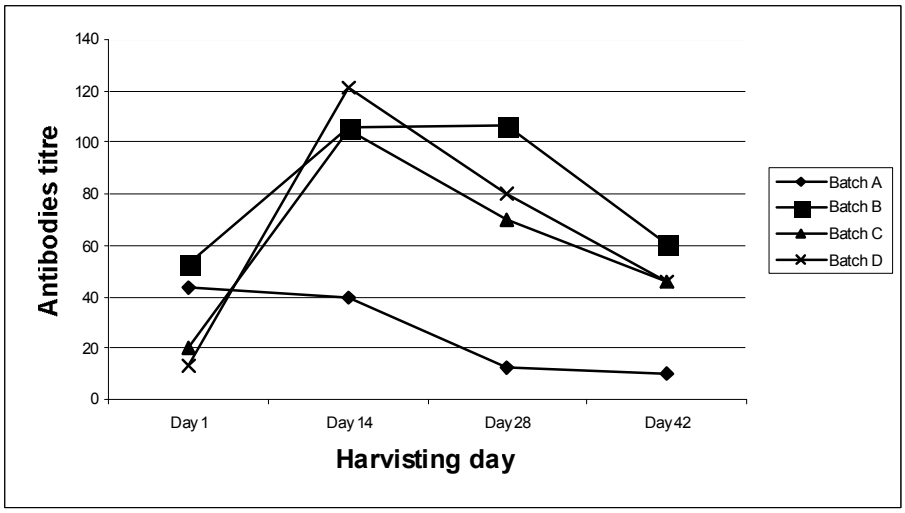

Fig. 5 – Antibodies titre against Newcastle disease virus

In lots of heavy breed chicks that were subjected to adverse environmental conditions observed significant reductions of key parameters of immune status as splenic and bursal index.

Also restructuring of leukocytes were observed (at levels that ensure the statistical viewpoint). All these changes are similar to those induced by treatment with immunosuppressive drugs (cyclophosphamide).

An important finding in practical terms refers to the positive influence of vaccination against infectious bursitis in the immune response by antibodies induced by the vaccine against Newcastle disease.

This result is explained by the positive regulatory influence of the antibursitic vaccine to the Fabricius bursa, implemented by performing a corresponding function of differentiation and maturation of B lymphocytes involved in antibody synthesis.

3. CONCLUSIONS

3.1. It was made an immunological suppression response ability in chickens by administration of cyclophosphamide and by keeping birds under stress (reduction in feed ration, poor light exposure).

3.2. Induced suppression resulted in reduction of the body weight, reduced indices of lymphoid organs (cloacal bursa, spleen) and moderate changes of the leukogram.

3.3. Experimental vaccination against avian infectious bursitis caused an increase in antibody titres induced by the previous vaccination against the Newcastle disease virus.

3.4. The vaccine against avian infectious bursitis can be used as immunostimulator, to increase the antibody titer prior to vaccination.

3.5. The results obtained confirm the existence of suppressor action after treatment with cyclophosphamide and unfavorable environmental conditions on immune status in birds.

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COMPARATIVE STUDIES AND RESEARCH TO DETERMINE THE SCRAPIE RESISTANCE OF SHEEPS IN RUSETU AREEA AND HATEG COUNTRY

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Key words: scrapie, ,genotype, resistance, selection intensity

SUMMARY

Breeding for scrapie resistance is still being encouraged, although obligatory use of ARR/ARR rams was suspended in 2007. The Animal Breeding and Genomics Centre (ABGC) has produced a report presenting general recommendations on how to continue breeding for scrapie resistance. The recommendations depend on the population size and the percentage of ARR in the population. In breeds with too few ARR animals, there is low selection intensity; otherwise there is a risk of excessive inbreeding in the population. In breeds with a great deal of double ARR, the selection can be stronger. In our study and research, the best genotype class G1 (ARR/ARR) and G2 (ARR/ARH) it was display in the samples of Hateg Country in percentage of 37,37%. In Rusetu area the genotype class G2 it was display in the samples of 42,85%.

For scrapie diagnostic three major mutations are associated with sheep susceptibility or resistance to classic scrapie and BSE: at codons 136 (A or V), 154 (R or H), and 171 (R, Q, or H). Animals with genotypes $V^{136}R^{154}Q^{171}$/VRQ, ARQ/VRQ, ARQ/ARQ, and VRQ/ARH PrP are most susceptible to scrapie.

Natural scrapie and bovine spongiform encephalopathy (BSE) infections have essentially not been diagnosed in sheep homozygous for the $A^{136}R^{154}R^{171}$ haplotype of the prion protein. This genotype was therefore assumed to confer resistance to BSE and classic scrapie under natural exposure conditions.

MATERIALS AND METHODS

The technologies for sample collection in entire populations by DNA tagging and for the high throughput DNA preparation and isolation were developed and patented by Prof. Brem and colleagues at the Agrobiogen GmbH and nexttec. These higly-performant analyses are

conducted at the well-known AGROBIOGEN laboratories in Germany, by Prof. Brem and his team, with their patented methods.

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) labelled 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 eartag by a plug and socket and is easily removed after the eartag has been affixed and the tissue sample simultaneously collected. If desired, the sample container can also be used without the eartag.

Tissue collection with TypiFixTM –System

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 pipetting robot and a special one-step procedure (Nexttec technology). PCR reactions with these samples can also be prepared automatically. The results of the multiplex PCR analyses are linked with the scanned identification number and saved in the animal data bank. This aspect is very important for studying traceability and domestic animal biodiversity.

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. These higly-performant analyses are conducted at the well-known AGROBIOGEN laboratories in Germany, by Prof. Brem and his team, with their patented methods.

2. RESULTS AND DISCUSSION

The Animal Breeding and Genomics Centre (ABGC) has produced a report presenting general recommendations on how to continue breeding for scrapie resistance. The recommendations depend on the population size and the percentage of ARR in the population. In breeds with too few ARR animals, there is low selection intensity; otherwise there is a risk of excessive inbreeding in the population. In breeds with a great deal of double ARR, the selection can be stronger.

Tab 1

DNA analyses of resistance/susceptibility genes for Scrapie in Rusetu (partially results)

No.	Lab no.	Typifix no.	Resuls AS Codon animal ID 136		154	171	Genotype²
1	JS080042	80	RO1072726994	AA	RR	RQ	ARR/ARQ
2	JS080043	81	RO1072353152	AA	RR	QQ	ARQ/ARQ
3	JS080044	82	RO1072353154	AA	RR	RQ	ARR/ARQ
4	JS080045	83	RO1074538405	AA	RR	QQ	ARQ/ARQ
5	JS080046	84	RO1074538440	AA	RR	RQ	ARR/ARQ
6	JS080047	85	RO1072726972	AA	RR	RQ	ARR/ARQ
7	JS080048	86	RO1072726973	AA	RR	QQ	ARQ/ARQ
8	JS080049	87	RO1072727018	AA	RR	RQ	ARR/ARQ
9	JS080050	88	RO1072726932	AA	RR	RQ	ARR/ARQ
10	JS080051	89	RO1072726928	AA	RR	QQ	ARQ/ARQ
11	JS080052	90	RO1074538450	AA	RR	QQ	ARQ/ARQ
12	JS080053	91	RO1074538450	AA	RR	QQ	ARQ/ARQ
13	JS080054	92	RO1074538447	AA	RR	QQ	ARQ/ARQ
14	JS080055	93	RO1074538404	AA	RR	RQ	ARR/ARQ

In ours results of analysis (Table 1) in Rusetu half the probes present the arginine (R) at codon 171 of the prion protein who confers resistance to the prion protein undergoing the structural change.

The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. All the probes have in the 136 codon the alanine. The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep.

The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep.

In results of analysis in Hateg country (Tab 2) 41,4% the probes present the arginine (R) at codon 171 of the prion protein who confers resistance to the structural change of prion scrapie.

Tab 2

***DNA analyses of resistance/susceptibility genes for Scrapie in Hateg* – partially results**

			Results				
			AA Codon[1]				Genotype-
No.	Animal ID	sex	136	154	171	PrP Genotype[2]	class[3]
1	RO146819946	m	VA	RR	RQ	ARR/VRQ	G4
2	RO FS 1	m	AA	RR	QQ	ARQ/ARQ	G3
3	RO 1075862576	m	AA	RR	QQ	ARQ/ARQ	G3
4	RO 146052131	m	AA	RR	RQ	ARR/ARQ	G2
5	RO 146819988	m	AA	RR	QQ	ARQ/ARQ	G3
6	RO 1041701026	m	AA	RR	QH	ARQ/ARH	G3
7	RO 1061463250	m	VA	RH	QQ	VRQ/AHQ	G5
8	RO 146052072	m	AA	RR	RQ	ARR/ARQ	G2
9	RO 146051967	m	AA	RR	RQ	ARR/ARQ	G2
10	RO FS 2	m	AA	RR	RQ	ARR/ARQ	G2
11	RO 146052082	m	AA	RR	QQ	ARQ/ARQ	G3
12	RO 1061459553	m	AA	RR	RQ	ARR/ARQ	G2
13	RO 1061463223	m	AA	RR	QQ	ARQ/ARQ	G3
14	RO 146052017	m	AA	RR	RQ	ARR/ARQ	G2
15	RO 146052023	m	AA	RR	RQ	ARR/ARQ	G2
16	RO 1044743731	m	VA	RR	QQ	VRQ/ARQ	G5
17	RO 1047102583	m	AA	RR	RQ	ARR/ARQ	G2
18	RO 146052050	m	AA	RR	QQ	ARQ/ARQ	G3

19	RO 106143340	m	AA	RR	QQ	ARQ/ARQ	G3
20	10614459607	m	AA	RR	QQ	ARQ/ARQ	G3
21	RO 146052035	m	AA	RR	RQ	ARR/ARQ	G2
22	RO FS 3- 022	m	VA	RR	QQ	VRQ/ARQ	G5
23	RO 146052034	m	AA	RR	QQ	ARQ/ARQ	G3
24	RO 106146331	m	AA	RR	RQ	ARR/ARQ	G2
25	RO 1061463185	m	VA	RR	QQ	VRQ/ARQ	G5
26	RO 146052004	m	AA	RR	QQ	ARQ/ARQ	G3
27	RO 146052069	m	AA	RR	RQ	ARR/ARQ	G2
28	RO FS 4	m	AA	RR	RQ	ARR/ARQ	G2
29	RO 1041701602	m	AA	RR	QQ	ARQ/ARQ	G3
30	RO 1046052027	m	AA	RR	QQ	ARQ/ARQ	G3

The coding for alanine (A) by codon 136 confers resistance to the prion protein undergoing the structural change associated with scrapie. All the probes in Rusetu Center have in the 136 codon the alanine. In results of analysis in Hateg country 86,8% the probes present the alanine (A) at codon 136 of the prion protein who confers resistance to the structural change of prion scrapie. The presence of glutamine (Q) or histidine at site 171 may convey some resistance, because has not detected scrapie in thise sheep. In results of analysis in Hateg country 90,9% the probes present the glutamine (Q) at codon 171 of the prion protein who confers resistance to the structural change of prion scrapie.

But for genotype class (genotype classifications by the German Society of Animal breeding-DGfZ in Hateg country (Tab.2) we detected 4 probes with genotype G4 (ARR/VRQ) and 5 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease.

The best genotype class G1 (ARR/ARR) and G2 (ARR/ARH) it's presents in the probes of Hateg Country in percentage the 37,37%.

3. CONCLUSION

3.1.It was analysis the prion protein for scrapie resistance genotyping *as codon*- amino acid at codon 136, 154, 171 from 5 known haplotypes resulting PrP Genotype.

3.2. The TypiFix™ ear tag system is simple, one-step collection and preservation of tissue samples.

3.3. The TypiFix™ ear tag system is fast, fully-automated and economical preparation of DNA.

3.4 This method is to be performed much more quickly and economically than is currently possible with the traditional methods of sample preparation.

3.5. In results of analysis in Hateg country 86, 8% the probes present the alanine (A) at codon 136 of the prion protein who confers resistance to the structural change of prion scrapie. In Hateg country (Tab.2) we detected 41,4% probes with genotype G4 (ARR/VRQ) and 5 probes with -G5 (VRQ/ARQ) who is susceptibility from scrapie disease.

3.6. In results of analysis in Rusetu half the probes present the arginine (R) at codon 171 of the prion protein who confers resistance to the prion protein undergoing the structural change.

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THE HOG CHOLERA EPIDEMIOLOGY AND DIAGNOSTRIC

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Keywords: Classical swine fever virus, CSF epidemiology, CSFV diagnostic, animal infectious disease

SUMMARY

The classical swine fever (CSF) is an infectious disease, recorded in the WAHO database which causes major losses among the pig populations in some member states of the European Union and in other countries of the world. At the end of the eighties, the CSF was considered nearly eradicated in the European Union. In 1990, concomitantly with the creation of the Common Market, the countries which have still used anti-CSF live vaccines were required to stop the vaccination, for the creation of a common status in the all member states. During the past decade, several major CSF epidemics occurred in the European Union, and the Romanian CSF outbreaks were 843 in 2005, 1458 in 2006, and 159 in 2007.

The swine represents the only natural CSFV reservoir. The virus is present in the blood, the tissues, the secretions and the excretions of the infected animal. The transmission occurs mainly by oral way, but also through conjunctivae, mucous membranes, cutaneous abrasion, insemination and percutaneous blood transfer (for example: by means of a usual needle, of contaminated instruments). The introduction of infected swine into areas uncontaminated with CSF represents the main source of infection. Some activities performed in the farms (such as public auction, livestock exhibitions, visits by food suppliers and by trucks delivering animals) are also potential contamination sources. Another significant CSF transmission possibility is the feeding with raw or insufficiently boiled household residues [4]. During the warm season, the virus may be transmitted mechanically by insect vectors which are usually present in the farm environment. The management methods play also an important role in the virus transmission. The main cause of infection in the households of the population is the direct contact with

the infected swine or the supplying of virus contaminated food (for example: hogwash). The transmission of the disease through the sperm of boars may occur in the large units in which mating is practised in a continuous system, the low virulence strains being indefinitely perpetuated until the cycle is interrupted by means of a stopping procedure, as well as by through cleaning and disinfection. The incubation period lasts usually 3-4 days, but it may also have duration of 2-14 days [8].

Clinical signs of CSF depend on the strain virulence and on the susceptibility of the swine host. The virulent strains are responsible for the acute form, while the low virulence CSFV strains induce a relatively small proportion of chronic infections, which may be unapparent or atypical. The low virulence strains are responsible for the "syndrome of carrier sow", which farrows persistently infected piglets. Under field conditions, the symptoms may become obvious in a household only 2-4 weeks after the introduction of a virus or later. In the case of older breeding pigs, the course of the infection is usually mild or subclinical. The same CSF isolate may produce different forms of disease, in dependence on the age, the breed and the immune state of the animal host [6]. Acute, chronic and prenatal (congenital) CSF forms may be distinguished, but a complex of classical symptoms invariably associated to the disease does not exist.

1. EPIDEMIOLOGICAL DATA

In 1990, concomitantly with the creation of the Common Market, the countries which have still used anti-CSF live vaccines were required to stop the vaccination, for the creation of a common status in the all member states. The vaccination was replaced by rigorous control measures, such as sacrificing of the infected and suspected animals and of those which are in contact with affected animals, as well as movement restrictions. In spite of these measures, during the subsequent years a series of severe epidemics occurred in several European Union countries and in many cases neither the responsible authorities nor the industry were thoroughly prepared for the implementation of strict control strategies [3]. The first outbreak appeared after the interdiction of vaccination in Belgium in 1988, followed by other epidemics in 1990, 1993 and 1994. Almost all the isolates belonged to the subgroup 2.3 [11].

In Germany, 424 CSF epidemics among domestic swine and a great number of cases among boars were recorded between 1990 and 1998

[7]. The genetic typing of isolates from these epidemics showed that this virus was very probably recently introduced into the EU as it belonged to the subgroup 2.1, which previously has been only sporadically detected in the EU. This phenomenon caused a severe epidemic of 429 outbreaks of disease, which ravaged during 14 months and finally led to the death of approximately 1 million swine.

From the Netherlands, the virus spread into Italy and Spain through the shipping of the infected swine at the beginning of February 1997 [5]. The genetic typing of the virus isolates revealed that all these outbreaks belonged to the same epidemic. The viruses of the subgroup 2.1 occurred only sporadically in the European Union in 1989, 1993, 1997 and 2000.

In North of Italy, the viruses belonging to the subgroup 2.2 occurred in the boar population and caused sporadic outbreaks among domestic swine. Viruses of the subgroup 2.2 caused also in Austria epidemic outbreaks but these viral isolates differed from the Italian isolates [1, 5, 9]. In the Central and East European countries, with the exception of Russia, the viral isolates belong to the subgroups 2.2 and 2.3.

The Romanian CSF outbreaks listed in WAHID were 843 in 2005, 1458 in 2006, and 159 in 2007. After October 2007 (1 outbreak) the WAHO database don't listed other similar events.

2. THE DIAGNOSTIC OPTIONS IN CSF

Comprehensive studies were performed by using monoclonal antibodies against the viral protein, for the differentiation of pestiviruses [10]. The result of these studies was the elaboration of a reference list of these antibodies. Two lists of monoclonal antibodies were used: 12 directed against the glycoprotein E2 and 11 against the ERNS glycoprotein for the typing of 126 CSFV strains and isolates, permitting to establish 21 antigenic types [7]. The technological advances regarding the polymerase chain reaction (PCR) and the automation DNA sequencing have significantly favored the genetic typing, while the antigenic typing, which is more laborious, was left on a second place [5].

A standardized protocol was elaborated for the typing of new CSFV isolates, involving the genomic fragment which should be evaluated, the algorithms for the calculation of phylogenetic trees and the nomenclature of genetic groups [10]. This protocol was elaborated in order to improve the prospects of preventing new infection epidemics

and to assure that the results obtained in different laboratories could be comparable [2].

Three regions of the viral genome were evaluated: the fragments at the 3' end of the polymerase chain (NS5B) [2], 150 nt of 5' NTR and a fragment (190nt) of the gene coding the E2 glycoprotein [3]. As many data referring to the E2 fragment are available permitting an accurate elucidation, this fragment is at present used for the phylogenetic analysis of viral CSF isolates.

The nomenclature of the genetic groups [8] was adapted to some additional virus groups from Asia. Thus, the CSF viruses were divided into three groups, each of them composed of three or four subgroupes: 1.1-1.3; 2.1-2.3 and 3.1-3.4 [10]. The phylogenetic analyses performed during the past years have clearly demonstrated that the CSFV isolates which were found to be different, seem to be characteristic by the genetic typing of certain geographic regions and their utilization has become a useful tool for the characterization of the strains of individual epidemics. The database regarding CSF viruses is kept at the Reference laboratory of the European Community in Hanover, Germany [3]. The genetic typing was successfully used for the characterization of viruses of the epidemic occurred during the years 1994-1998 in several states of the EU, of the virus newly introduced into the population of boars of Switzerland as well as of the virus of the outbreaks produced in the year 2000 among domestic swine in Great Britain.

Numerous laboratory techniques for the CSF diagnosis were described, but only a few of them were accepted on the international scale and integrated in national control programs: the fluorescent antibody test on tissue sections (FATTS), the direct fluorescent antibody technique is used for the detection of CSFV antigens in frozen tissues of organs taken from dead swine in the biopsy material or in smears performed by imprenting [6]. In Romania, the immunological tests utilized are the direct immunofluorescence assay, the agar-gel seroprecipitation, the quantitative immune electroprecipitation and the seroneutralization on cell cultures. Theoretically, a diagnosis may be confirmed within a few hours after the reception of the results. The serologic tests are essential for an efficient control and for a final eradication program.

3. RECOMANDATIONS

The control of the introduction of meat and meat products from countries situated outside the EU should be intensified. The controls

should be performed on a large quantity of infected meat products used for commercial purposes, as well as on individual imports made for the personal consumption.

The swine trade at great distances favors the spread of the virus. The reduction of the swine density in high risk regions will contribute to the avoidance of the local disease dissemination.

The markets and the animal distributors mix a large number of animals of different origins, permitting the spread of the virus also on the local level. These contacts should be reduced to a minimum and it is preferable to acquire the new animals from a limited number of suppliers.

Rigorous hygiene measures should be taken by all the visitors of swine farms as well as for the vehicles used for the transport of animals.

The boar population should be monitored for the presence of CSF.

The CSF infection should be taken into account in any differential diagnosis in the earliest stage by the veterinary practitioners and by the sanitary veterinary inspectors.

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SEROLOGICAL AND ENTOMOLOGICAL MONITORING OF BLUETONGUE IN ROMANIA

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Keyword: Bluetongue, Culicoides, Surveillance, Epidemiology, Animal Infectious Disease

SUMMARY

Bluetongue (BT) is an infectious disease, noncontagious, that is transmitted through the prick of some midge species from the Cullicoides genus. It has been found at the ruminant species especially in sheep and, with a lower frequency, in bovines and goats. Serological monitoring in 2008 demonstrated that the Romanian status for BTV is the free country. Although the presence of BTV vectors was confirmed in Romania, the vectors are still BTV-free. The risk of transboundary contamination by vectors or animals recommend maintaining of actual BT control measures in Romania.

The bluetongue virus (BTV), the type species of the *Orbivirus* genus, *Reoviridae* family, is a viral particle with sizes ranging from 50 to 100 nm, icosahaedral symmetry and 32 capsomeres. The viral genome consists in 10 bicatenar RNA sequences. The viral genome undergoes mutations in a higher percent which explains the antigenic variability of the virus (so far 24 serotypes) [4]. Each serotype confers a solid immunity against itself, but frequently low and sometimes null against others. The pathogenicity is variable; from spontaneously attenuated strains to high pathogen strains (e.g. Cyprus strain induces 90% mortality).

The disease was first detected in Africa and further in Asia, North America and Europe. Today the virus is present in states situated into a band from 40°N to 35°S latitude. In the Mediterranean region, the first BT cases were reported in Cyprus and Israel in 1943, but are thought to have occurred in this region as early as 1924. Six serotypes (2, 4, 6, 9, 10, and 16), recurred at varying intervals. At this moment, in Turkey,

Syria, Israel and Egypt, BT is considered enzootic and thus countries may act as source areas for the spread of the disease westwards. The outbreaks of bluetongue are mediated by the transport of infected hematophage *Cullicoides* genus midges (the main route of BTV transmission) through winds. That may seasonally prevail from enzootic to non enzootic areas and this is the principal way in which a fractural, but eternal cycle of BT disease is being perpetuated around the Mediterranean Sea [2]. Starting from the autumn 1999 the infection spread across the Mediterranean basin involving several countries (Israel, Turkey, Greece, Cyprus, Bulgaria, Albania, the Former Yugoslavian Republic of Macedonia, Bosnia-Herzegovina, the Republic of Serbia, Croatia, Italy, the French Island of Corsica, Tunisia, Algeria, Spain, Morocco), with the diffusion of the following serotypes: BTV-1, BTV-2, BTV-4, BTV-9, BTV-16. In the summer 2006, the presence of BTV-8 was detected in Central Europe, in the Maastricht region [1].

Incubation period is 5-20 days. Acute form (malign BT) is met at sheep and some deer species. In bovine and goats, the course of the disease is usually benign or unapparent. Lesions described in BTV infections are: congestion, edema, hemorrhages and ulcerations of digestive and respiratory mucosa (mouth, esophagus, stomach, intestine, pituitary mucosa, tracheal mucosa) [3]; congestion of hoot lamina and coronary band; hypertrophy of lymph nodes and splenomegaly; severe bilateral broncholobular pneumonia (when complications occur) [5]. For those reasons the differential diagnosis must be performed against contagious ecthyma, foot and mouth disease, photosensitization, pneumonia, polyarthritis, footrot, foot, abscesses, plant poisonings, peste des petits ruminants, coenurosis, epizootic haemorrhagic disease of deer.

Laboratory diagnosis procedures for isolation of the agent are inoculation of sheep or intravascular inoculation in 10-12-day-old embryonated chicken eggs, and identification of the agent by plaque reduction serum neutralisation (for serotyping - many cross-reactions). The serological tests used in BT are virus neutralization, complement fixation, competitive ELISA and agar gel immunodiffusion (the last two recommended by WAHO) [8].

1. MATERIAL AND METHODS

The study was performed in The Institute of Animal Diagnosis and Health, Bucharest, Romania, The Faculty of Veterinary Medicine Bucharest and The Romanian Academy. All data were reported to the

National Laboratory of Reference for BT. The monitoring was performed monthly, in the first 5 days of the month, consecutive to the month established by methodology of BT surveillance.

The methods used were in concordance with the Romanian program of surveillance, prevention and control of the BT. In the year 2008, the major component of the entomological surveillance was represented by the weekly monitoring of the vectors at the level of at least one location in each district.

2. RESULTS AND DISCUSSION

The most susceptible species at BTV is the sheep, the other species being characterized by inapparently infection, but the Romanian surveillance programs involved bovines, sheep and goats. The risk of contamination is higher in Merinos sheep breed, males after 3 years of age [6]. The control measures chose by Romania for BT are: notifiable disease, precautions at the borders, monitoring, screening, general surveillance, targeted surveillance, movement control inside the country, stamping out, zoning, no vaccination prohibited, no routine vaccination, no treatment, and control of arthropods. The serological monitoring and control of arthropods performed in 2008 at county level are described below.

Serological Monitoring

In the first trimester of the year 2008, 6644 domestic ruminants were tested for Bluetongue, which represents 0.048% from the total livestock of domestic ruminants at the national level. The repartition by species was: bovines 4175 (0.16%); sheep 2063 (0.021%); goats 406 (0.025%).

The monitoring percentage planned for this period of the year was of ~1% of the domestic ruminants. From the view point of the rhythmicity of the serological monitoring for Bluetongue at the level of the districts, the situation is the following:

1. A very good monitoring performed monthly in 9 districts: Arges (AG), Braila (BR), Neamt (NT), Hunedoara (HD), Satu Mare (SM), Suceava (SV), Teleorman (TR), Tulcea (TL), and Vrangea (VN);

2. A satisfactory monitoring performed not done regular, but in at least 2 months from quarter in 4 districts: Botosani (BT), Covasna (CV), Dambovita (DB), and Harghita (HR).

Control of Arthropods

The principal vector known in the Mediterranean pond is *Cullicoides imicola*. Species belonging to *Cullicoides obsoletus*

complex have been highly suspected to play a role in the transmission of BTV in Cyprus in 1977, Bulgaria in 1999 and Italy in 2002. Similarly, midges belonging to *Cullicoides pulicaris* complex have been considered as potential vectors in Sicily during 2002 [7].

The number of captures of vectors planned to be achieved in the first trimester of 2008 was 504. Practically there have been achieved 244 captures, which represent a percentage of performance of 48.41% from the analyzed period.

From the viewpoint of rhytmicity of the entomologic monitoring for the vectors of the Bluetongue virus at the level of the districts, situation is the following:

1. proper monitoring performed through weekly capture of the vectors and their identification in 13 districts: AB, BC, BN, BT, CS, CJ, DJ, GR, MM, SM, VS,VL,VN;

2. satisfactory monitoring through sporadic captures of vectors and not through the systematic monitoring in 7 districts: BR,BZ,CV,GL,NT,OT,GJ.

3. 22 districts which so far, no monitoring of vectors was performed. In this situation are the districts: AR, AG, BH, BV, Bucharest, CL, CT, DB, HR, HD, IL, IS, IF, MM, MS, PH, SJ, SB, SV,TR, TM, TL.

2. CONCLUSIONS

Serological monitoring in 2008 demonstrated that the Romanian status for BTV is the free country. Although the presence of BTV vectors was confirmed in Romania, the vectors are still BTV-free. The risk of transboundary contamination by vectors or animals recommend maintaining of actual BT control measures in Romania.

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THE EFFECT OF POLYPHENOLS FROM SOME PLANTS ALCOHOLIC EXTRACTS ON LIPID PEROXIDATION AND NONENZYMATIC HAEMOGLOBIN GLYCOSYLATION

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Key words: polyphenol content, DPPH•, lipid peroxidation, non-enzymatic haemoglobin glycosylation.

SUMMARY

The purpose of this study was to evaluate mistletoe (*Viscum album*), birthwort (*Aristolochia clematitis*) and greater celandine (*Chelidonium majus*) as new potential sources of natural antioxidants. The alcoholic extracts were assessed for total phenolic content by Folin-Ciocalteu reagent, total flavonoids content, DPPH free radical scavenging, inhibition of lipid peroxidation and inhibition of haemoglobin glycosylation. The amounts of total phenolic compounds were higher in *Aristolochia clematitis* ethanolic extract (21.04 ± 3.39 mg/g) than in *Viscum album* ethanolic extract (11.33 ± 1.35 mg/g). *Aristolochia clematitis* and *Chelidonium majus* alcoholic extracts showed DPPH scavenging activity higher than *Viscum album* alcoholic extract. All the extracts presented significant results for lipid peroxidation inhibition in brain and liver homogenate, but the most important results were obtained for *Chelidonium majus* alcoholic extracts. Inhibition percentages of nonenzymatic haemoglobin glycosylation of alcoholic extracts were: *Aristolochia clematitis* 56.09 % ± 12.00 %, *Chelidonium majus* 68.26 % ± 18.19 % and *Viscum album* 55.34 % ± 13.17 %.

It has been reported that phenolic acids and flavonoids are distributed in different solvents depending on the polarity and ethanolic extracts contain the most polar compounds (Marnett, 2000). Herbals and herbal extracts, which contain different classes of polyphenols, are very attractive for modern phytotherapy. The antioxidant activity of medicinal plants could be attributed to the presence of phenols, especially flavonoids (Braca *et al.*, 2002; Ebrahimzadeh *et al.*, 2008).

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer and it is involved in aging (Kumar *et al.*, 2008). Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention those diseases, by free radical scavenging activities (Aruoma *et al.,* 1991). Nowadays, there is a growing interest towards natural antioxidants from herbal sources (Crivineanu *et al.*, 2009; Durdun *et al.*, 2009).

1. MATERIALS AND METHODS

Preparation of plant extracts

Dried plant material was extracted with ethanol for 3 hours, allowed to cool and filtered using Whatman no. 1 filter paper. The obtained filtrates were centrifuged at 5000 rpm for 20 min at 5ºC. The filtrates were kept at 4ºC until the tests.

Phenolics content

The total phenolics content was determined by adding the ethanolic extract to Folin–Ciocalteau's reagent and 20% sodium carbonate. The reaction mixture was completed by adding distillated water up to 10 mL, then incubated at 25ºC for 20 min, and the absorbance was measured at 725 nm using an UV – VIS spectrophotometer. Caffeic acid was used as a standard phenol (Conrad *et al.*, 2001). The total phenolics content was expressed as mg of caffeic acid equivalents/ g dried plant.

Flavonoid Content

The total flavonoid content was measured using a colorimetric assay (Jay and Gonnet, 1975), with minor modifications. The pink colored solution was measured at 430 nm using an UV-VIS Jasco spectrophotometer V670. Quercetin was used as a standard for the calibration curve.

DPPH radical-scavenging

Scavenging of the stable radical DPPH was assayed in vitro (Burits and Bucar, 2000). The extract was added to 4 mM alcoholic solution of DPPH. The mixture was shaken and allowed to stand in dark, at room temperature for 30 min and the absorbance was measured at 517 nm in a UV – VIS spectrophotometer. Inhibition percentage was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Animal experiment

Adult Wistar albino rats (170–200 g) of both sexes were used for the pharmacological activities. The rats were kept at 26±2°C and relative humidity 44 - 55%, light and dark cycles of 10 and 14 h respectively for 1 week before the experiment. Animals were fed with standard animal feed and water *ad libitum*. The rats were starved overnight and were sacrificed by cervical dislocation, and then whole brain and liver were removed and weighed. All the experimental protocols respected E.U. legislation for experimental animals.

Lipid peroxidation in brain and liver homogenates

Production of thiobarbituric acid reactive substances (TBARS) was determined using a modified method of Kizil (Kizil *et al.*, 2008; Park,

2008). Quantities of 1 g tissues were homogenized in cold 100 mM Tris-buffer pH 7.4 (1:10 w/v). The homogenates were centrifuged for 10 min at 5000 rpm at 4ºC to yield a supernatant used for the assay. The homogenates were incubated in the presence of 15 mM $FeSO_4$ with or without plant extracts for 5 hour at 37ºC. The color reaction was carried out by adding 10 g /100 mL sodium dodecyl sulphate (SDS). After incubation for 60 min at room temperature, 1 g/100 mL thiobarbituric acid (TBA) was added to the reaction mixture. The mixture was heated in a water bath at 85°C for 30 min in a boiling water bath to complete the reaction. After cooling, 1 ml of n-butanol was added and tubes were centrifuged after strong agitation. The intensity of the formed pink colored complex was measured at 532 nm in a UV – VIS spectrophotometer. The percentage inhibition of lipid peroxidation was calculated by the following formula:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Non-enzymatic haemoglobin glycosylation assay

The assay was performed by adding 2 g / 100 mL glucose solution, 60 mg/100 mL haemoglobin solution, 20 mg / 100 mL gentamicin and plant alcoholic extracts in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 h (Pal and Dutta, 2006). The degree of glycosylation of hemoglobin in the presence and in the absence of different concentrations of extracts was measured colorimetrically at 520 nm. Inhibition of hemoglobin glycosylation was calculated by the following formula:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Data analysis

The results were expressed as mean values (±SD) of 3 determinations. The mean values and standard deviation were calculated with EXCEL program from Microsoft Office package.

2. RESULTS AND DISCUSSIONS

Phenolic content

Phytochemical analysis of the plants showed the presence of high contents of phenolics, which may be responsible for the plants' antioxidant activity, besides other phytochemicals. In table 1, the results were expressed as caffeic acid equivalent**.** Phenolics content ranged between 11.33 – 21.04 mg/g dried plant. The amounts of total phenolic compounds were higher in *Aristolochia clematitis* ethanolic extract (21.04 ± 3.39 mg/g) then in *Viscum album* ethanolic extract (11.33 ±

1.35 mg/g). *Chelidonium majus* ethanolic extract had a content in phenolics of 16.95 ± 2.67 mg/g.

Flavonoid Content

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral and anticancer activities.

Table 1

Phenolic and flavonoid contents of alcoholic plant extracts

Sample	Phenolics content (mg equivalent caffeic acid/ g dried plant)	Flavonoid Content (µg equivalent quercitin/ g dried plant)
Aristolochia clematitis	21.04 ± 3.39	1191.5 ± 78.29
Chelidonium majus	16.95 ± 2.67	678.5 ± 37.12
Viscum album	11.33 ± 1.35	486.00 ± 43.24

Flavonoid contents of plant alcoholic extracts were proportional to phenolics content (table 1). *Aristolochia clematitis* presented 1191.5 ± 78.29 µg quercitin equivalents/g dried plant, *Chelidonium majus* 678.5 ± 37.12 µg quercitin equivalents/g dried plant and *Viscum album* 486.00 ± 43.24 µg quercitin equivalents/g dried plant.

DPPH radical scavenging

The results of DPPH radical scavenging assay revealed that the extracts, by hydrogen and/or electron donation, might prevent reactive oxygen species from attacking biomolecules such as lipoproteins, poly unsaturated fatty acids (PUFAs), DNA, amino acids and proteins (Halliwell, 1995).

The DPPH radical scavenging activity of the studied plant extracts is shown in Fig. 1. *Aristolochia clematitis* alcoholic extracts presented an inhibition percentage of 49.59 % ± 9.12 %, *Chelidonium majus* 37.55 % ± 7.43 % and *Viscum album* only 28.23 % ± 5.98%. The alcoholic extract of *Aristolochia clematitis* and *Chelidonium majus* showed a scavenging activity higher than *Viscum album* at the same concentration of polyphenols.

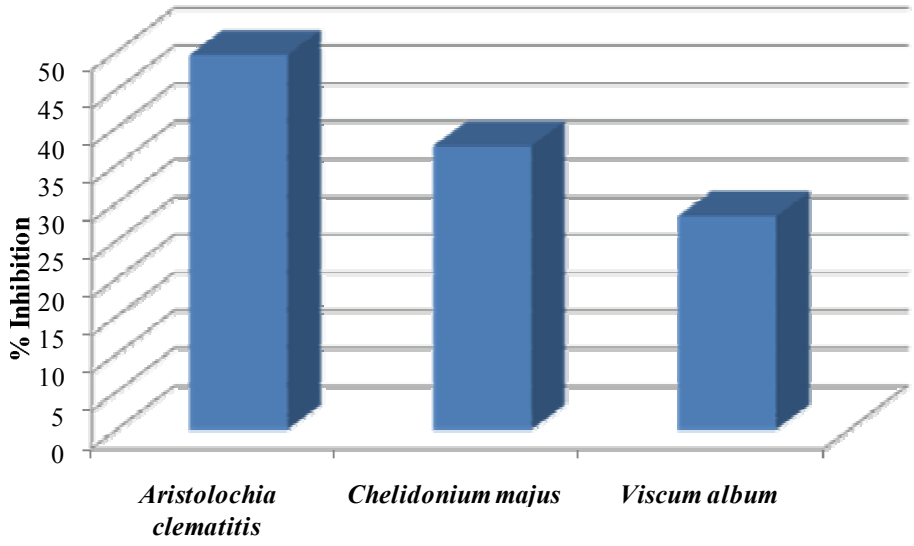

Fig. 1. DPPH radical scavenging activity of the studied alcoholic extracts

Lipid peroxidation in brain and liver homogenates

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and protectors of biomolecules from oxidation (Rice-Evans and Miller, 1997). Iron is an extremely reactive metal and it catalyzes oxidative changes of lipids, proteins and other cellular components. Thus, it was studied the ability of *Aristolochia clematitis*, *Chelidonium majus* and *Viscum album* alcoholic extracts to inhibit lipid peroxidation. Figure 2 shows the results, which indicate the inhibition of lipid peroxidation by the ethanolic fractions.

Mistletoe, birthwort and greater celandine alcoholic extracts inhibited lipid peroxidation induced by $FeSO_4$ upon rat brain lipids. Addition of alcoholic extracts to rat brain and liver homogenate significantly inhibited TBARS formation. The results showed that alcoholic extracts of *Aristolochia clematitis*, *Chelidonium majus* and *Viscum album* have the capacity to prevent oxidative deterioration of membrane lipids. The beneficial effect of tested alcoholic extracts on lipid peroxidation is attributed to their phenolic contents.

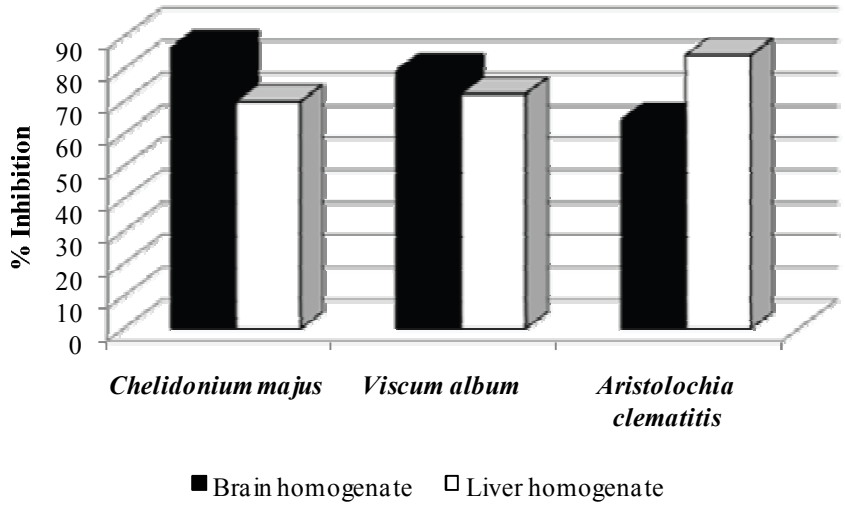

Fig. 2. Inhibition of lipid peroxidation on brain and liver homogenate by the studied plant extracts

Inhibition of peroxidation in brain lipids homogenate presented by the studied plant extracts was: *Chelidonium majus* 86.72 ± 12.21%, *Viscum album* 79.53 ± 20.18%, *Aristolochia clematitis* 64.35 ± 18.78%. In the presence of alcoholic extracts obtained from medicinal plants, the results of lipid peroxidation inhibition from liver homogenate were: *Aristolochia clematitis* 84.34 % ± 21.57 %, *Chelidonium majus* 70.00 ± 19.21%, *Viscum album* 72.34 % ± 20.81 %.

Non-enzymatic haemoglobin glycosylation assay

The antioxidant activities of *Aristolochia clematitis*, *Chelidonium majus* and *Viscum album* alcoholic extracts were also investigated by estimating the degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically. For instance, in diabetes, increased oxidative stress which co-exist with reduction in the antioxidant status has been postulated: oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication of diabetes (Kumar et *al*., 2008). The three tested plant extracts presented important results for the inhibition of haemoglobin glycosylation non–enzymatic induced. Inhibition of nonenzymatic haemoglobin glycosylation by the studied alcoholic extracts was: *Aristolochia clematitis* 56.09 % ± 12.00 %, *Chelidonium majus* 68.26 % ± 18.19 % and *Viscum album* 55.34 % ± 13.17 %. Among the three extracts, greater celandine show the highest

inhibition of haemoglobin glycosylation. A lower inhibition capacity was observed for mistletoe (Fig. 3).

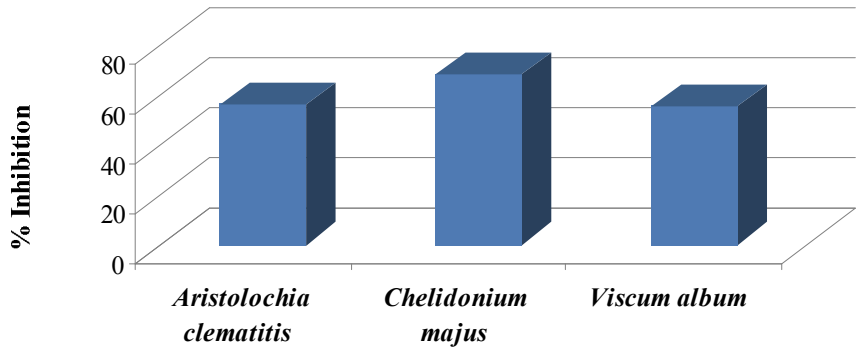

Fig. 3. Inhibition of non-enzymatic haemoglobin glycosylation by the studied alcoholic extracts

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3. CONCLUSIONS

3.1. The obtained results in the present study indicate that *Aristolochia clematitis*, *Chelidonium majus* and *Viscum album* alcoholic extracts contain important amounts of polyphenols and flavonoides.

3.2. The presence of high flavonoid contents in the extracts of *Aristolochia clematitis, Chelidonium majus* and *Viscum album* contributes directly to their DPPH free radical scavenging activity.

3.3. Alcoholic extracts obtained from *Aristolochia clematitis*, *Chelidonium majus* and *Viscum album* showed important inhibition of lipid peroxidation on rat brain and liver homogenates.

3.4. *Aristolochia clematitis*, *Chelidonium majus* and *Viscum album* alcoholic extracts represent potential sources of natural antioxidants that could inhibit haemoglobin glycosylation.

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REDUCING POWER, FE(II) CHELATING ABILITY AND ANTIOXIDANT ACTIVITY OF SOME MEDICINAL PLANTS

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Key words: polyphenols, flavonoids, lipid peroxidation, reducing power, antioxidant activity.

SUMMARY

Polyphenols are the most abundant antioxidants in human diets and they are widely found in medicinal plant. In this study, alcoholic extracts of nettle (*Urtica dioica*), wild pansy (*Viola tricolor*) and stag's-horn club moss (*Lycopodium clavatum*) were investigated for polyphenols and flavonoids contents. Ethanolic extracts were tested for reducing power, Fe (II) chelating ability and inhibition of lipid peroxidation in rat brain. Alcoholic extracts obtained from *Viola tricolor* showed the highest concentration on flavonoid compounds, while *Urtica dioica* alcoholic extracts showed the highest pilyphenols concentration. The Fe^{2+} chelating abilities of the alcoholic extracts obtained from the three tested plants were 63.35 % ± 15.09 % for *Viola tricolor*, 61.55 % ± 14.39 % for *Urtica dioica* and 14.55 % ± 4.55 % for *Lycopodium clavatum*. Fe^{3+} reducing capacity was higher for ethanolic extract of *Viola tricolor* (1.3 ± 0.19 mM $FeSO_4$ equivalent/g of dry plant). In this study, Mo(VI) reducing power of the extract was found to be 179.52 ± 49.91 µg ascorbic acid/g (*Urtica dioica*), 159.32 ± 39.07 µg ascorbic acid/g (*Viola tricolor*) and 72.64 ± 23.18 µg ascorbic acid/g (*Lycopodium clavatum*). *Urtica dioica* inhibited the most lipid peroxidation (74.24% ± 8.41%), compared to the alcoholic fraction of *Viola tricolor* (68.91% ± 7.23) and *Lycopodium clavatum* alcoholic fraction (29.24% ± 3.81 %).

In traditional societies, nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes (Pieroni, 2000). The consumption of non-cultivated botanicals plays a central role in the diet, but very few ethnopharmacological and phytopharmacological studies have dealt exhaustively with the potential health benefits of such diets (Trichopoulou *et al.*, 2000). On the other hand, the number of studies on the antioxidant properties of specific plant foods and their phenolic constituents has become very impressive (Crivineanu *et al*., 2009; Durdun *et al*., 2009).

Aerobic organisms have developed many types of antioxidant defense. An antioxidant is defined by Gutteridge *et al.* (1994) as "any substance that delays or inhibits oxidative damage to a target molecule". Phenolic compounds also function as free-radical scavengers, reducing and chelating agents, and quenchers of reactive oxygen species.

Antioxidant compounds that scavenge free radicals help protect against degenerative diseases (Amin and Tan, 2002).

1. MATERIALS AND METHODS

Preparation of ethanolic extracts

In this study there were used dried aerial parts of nettle (*Urtica dioica*), wild pansy (*Viola tricolor*) and stag's-horn club moss (*Lycopodium clavatum*). The parts of plants were powdered and extracted with ethanol 60% (1:10 ratio, w:v) for 3 hours at 60ºC. These crude extracts were used for further investigation for potential antioxidant properties (Papuc *et al*., 2007; Papuc *et al*., 2008).

Determination of total phenolic content

The total phenolic content of the ethanolic extracts was determined by Folin-Ciocalteu method, using caffeic acid as a standard (Conrad *et al*., 2001). Folin-Ciocalteu reagent was added to ethanolic extract solutions, after dilution, then 20% aqueous sodium carbonate solution was added and the tubes were homogenized and left to stand for 20 minutes. A blue color appeared and the absorbance was measured at 725 nm with an UV-VIS Jasco spectrophotometer V670. All measurements were made in triplicate and the results were expressed as mg caffeic acid / g of dried sample.

Determination of total flavonoids

Total flavonoid contents were determined using the method described by Jay and Gonnet (1975). A yellow color indicated the presence of flavonoids. After ten minutes at room temperature, the absorbance was measured at 430 nm using an UV-VIS Jasco spectrophotometer V670. Total flavonoid content was calculated as µg equivalent quercetin per gram of dried sample.

Fe^{3+} reducing power

The reducing power was determined according to the method described by Oyaizu (1986). Diluted plant extracts were mixed with 0.2 M phosphate buffer pH 6.6 and 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min and then 10% trichloroacetic acid was added to the mixture. The upper layer of the solution was mixed with distilled water and 0.1% ferric chloride and then the absorbance was measured at 700 nm in UV-VIS spectrophotometer. $FeSO_4$ was used as reference standard; the results were expressed as mM $FeSO_4$ equivalent/g of dry plant material.

Mo(VI) reducing power

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm (Jayaprakasha *et al.,* 1999). Medicinal plant extracts dissolved in ethanol were homogenized with 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes with reaction solutions were capped and incubated in a water bath at 95ºC for 90 min. After cooling to room temperature, the absorbance of the aqueous solution in each tube was measured at 695 nm using an UV-VIS Jasco spectrophotometer V670. Ascorbic acid was used as standard and the Mo(VI) reducing power of nettle (*Urtica dioica*), wild pansy (*Viola tricolor*) and stag's-hom club moss (*Lycopodium clavatum*) ***ethanolic extracts*** was expressed as equivalents of ascorbic acid.

Determination of Fe^{2+} chelating ability

The ability of alcoholic extracts to chelate Fe^{2+} was determined with a modified method described by Minotti (Minotti *et al*., 1993). Fresh prepared 500 µM $FeSO_4$ was added to a reaction mixture containing 0.1 M Tris-HCl pH 7.4 and diluted alcoholic extract. The mixture was incubated for 5 minutes, before adding 0.25% 1,10 o-phenantroline (w:v). The absorbance was measured at 510 nm using UV-VIS spectrophotometer. The chelatation percentage was calculated using the following formula:

$$\% \text{ Fe (II)Chelatation} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Lipid peroxidation (LPO) assay

LPO was induced and assayed in rat brain (Park, 2008). The reaction muddle, in a total volume of 1.0 ml, contained 0.58 ml phosphate buffer (0.1 M, pH 7.4), brain homogenate (10%, w/v), 100 mM ascorbic acid and 100 mM $FeSO_4$ and was incubated at 37ºC in a shaking water bath for 1 h. The reaction was stopped by the addition of 1.0 ml trichloracetic acid (TCA) (10%, w/v). Then, 1.0 ml thiobarbituric acid (TBA) (1%, w/v) was added and all the tubes were introduced in a boiling water bath for 20 min. At the end, the tubes were centrifuged at 5000 rpm for 10 min. The amount of thiobarbituric acid reactive substances (TBARS) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm by an UV-VIS spectrophotometer.

2. RESULTS AND DISCUSSIONS

Determination of total phenolic content and total flavonoids

The concentration of total phenolics (mg/g dried plant) are shown in table 1. *Urtica dioica* presented the highest phenolics content (18.57 ± 2.79 mg/g) and a total flavonoid content of 1145 ± 98 µg/g. The other investigated extracts (*Viola tricolor* and *Lycopodium clavatum*) presented values of 14.54 ± 2.57 mg/g, respectively 8.89 ± 2.01 mg/g for polyphenolic compounds and 1212.5 ± 101 µg/g, respectively 334 ± 58 µg/g for flavonoid compounds.

Table 1

Total phenolics and flavonoid contents of the studied alcoholic plant extracts

Sample	*Phenolics content (mg equivalent caffeic acid/g dried plant)*	*Flavonoid Content (µg equivalent quercitin/g dried plant)*
Viola tricolor	14.54 ± 2.57	1212.5 ± 101.32
Urtica dioica	18.57 ± 2.79	1145.03 ± 98.41
Lycopodium clavatum	8.89 ± 2.01	334.34 ± 58.56

Fe^{3+} reducing power

It has been reported by Gutteridge (Gutteridge *et al*., 1994) that the Fe^{3+} reducing power of plant alcoholic extracts is associated with flavonoid content. Thus, it is necessary to determine the reducing power of phenolic constituents. Fe^{3+} reducing capacity was higher for ethanolic extract of *Viola tricolor* (1.3 ± 0.19 mM $FeSO_4$ equivalent/g of dry plant). *Urtica dioica* presented a reducing power of 0.9 ± 0.1 mM $FeSO_4$ equivalent/g of dry plant and *Lycopodium clavatum* presented 0.4 ± 0.1 mM $FeSO_4$ equivalent/g of dry plant (Fig. 1).

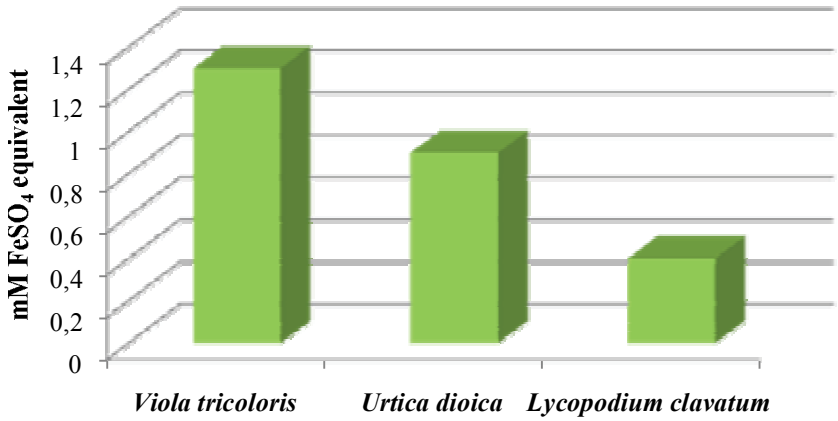

Fig. 1. Fe^{3+} reducing power of the tested plant alcoholic extracts

Therefore, by correlating these results, we can suggest that there may be a relationship between the amount of total flavonoids content and reducing power.

Mo(VI) reducing power

Mo (VI) reducing power of the extracts was found to be 179.52 ± 49.91 µg ascorbic acid/g for *Urtica dioica*, 159.32 ± 39.07 µg ascorbic acid/g for *Viola tricolor* and 72.64 ± 23.18 µg ascorbic acid/g for *Lycopodium clavatum.* Mo (VI) reducing power of the alcoholic extracts, expressed as the number of µg ascorbic acid equivalent, is shown in Fig. 2.

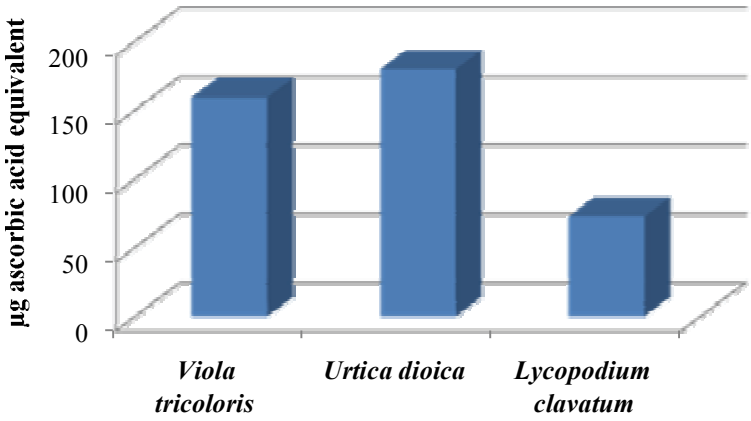

Fig. 2. Mo (VI) reducing power of alcoholic extracts

Determination of Fe^{2+} Chelating Ability

The Fe^{2+} chelating ability of the alcohol extractable phytochemicals of the studied plants (*Lycopodium clavatum, Urtica dioica* and *Viola tricolor*) was determined and the results are showed in Fig. 3. The alcoholic extracts of *Viola tricolor* (63.35 % ± 15.09 %) and *Urtica dioica (*61.55 % ± 14.39 %) had a higher Fe^{2+} chelating ability than *Lycopodium clavatum* (14.55 % ± 4.55 %) at the tested concentrations. However, the alcoholic extracts of the nettle and wild pansy had a significantly higher Fe^{2+} chelating ability than the alcoholic extract of stag's-horn club moss.

The use of iron chelation is a popular therapy for the management of Fe^{2+} associated oxidative stress in brain. Iron chelating ability of the plants under study is an indicator of the neuroprotective property of the plants because iron is involved in the pathogenesis of Alzeimer's and others diseases by multiple mechanisms (Velioglu *et al*., 1998).

Viola tricolor was found to have the highest chelating power, followed by *Urtica dioica* and *Lycopodium clavatum* (Fig. 3).

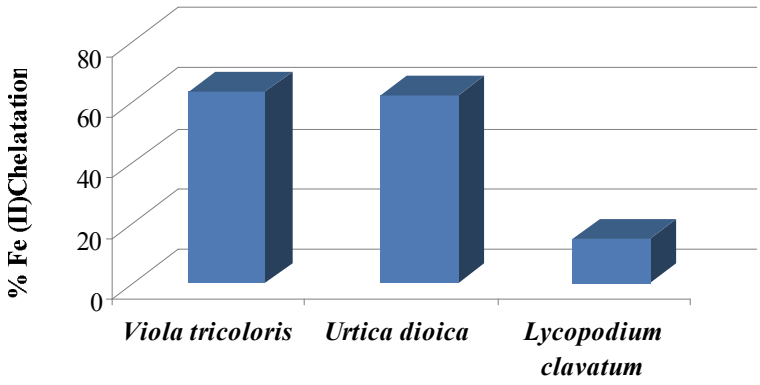

Fig. 3. Fe^{2+} chelating ability of the tested alcoholic extracts

Lipid peroxidation assay

In this study, it was measured the potential of alcoholic extracts of *Viola tricolor*, *Urtica dioica* and *Lycopodium clavatum* to inhibit lipid peroxidation in rat brain homogenate, induced by the ascorbic acid - $FeSO_4$.

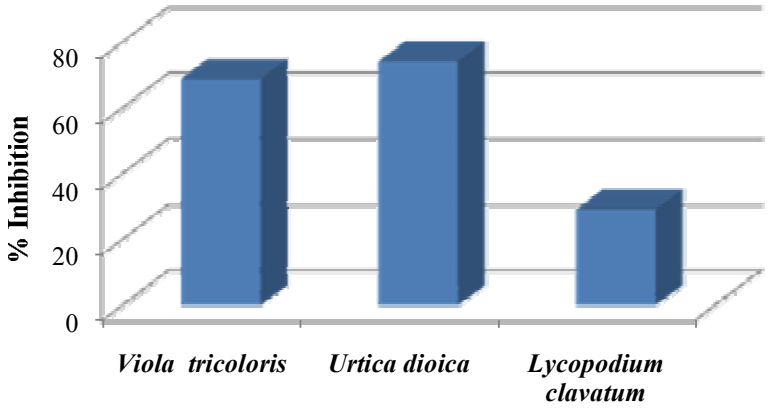

Fig. 4. Total antioxidant capacity and inhibition of lipid peroxidation by alcoholic extracts

Fig. 4 shows that the alcoholic fraction of *Urtica dioica* inhibited lipid peroxidation in a percentage (74.24 ± 8.41%) higher than *Viola tricolor* (68.91 ± 7.23%) and *Lycopodium clavatum* (29.24 ± 3.81%).

There is a correspondence between table 1, fig. 2 and 4: alcoholic plant extracts with the highest total phenolics content (*Urtica dioica*), had the highest Mo (VI) reducing power and the highest inhibition of lipid peroxidation.

3. CONCLUSIONS

3.1. Alcoholic extracts obtained from nettle (*Urtica dioica*), wild pansy (*Viola tricolor*) and stag's-horn club moss (*Lycopodium clavatum*) presented important amounts of polyphenol compounds.

3.2. Alcoholic extracts obtained from *Viola tricolor* showed the highest concentration of flavonoid compounds.

3.3 Alcoholic extracts of *Urtica dioica*, *Viola tricolor* and *Lycopodium clavatum* presented significant Fe^{3+} reducing power and Mo (VI) reducing power.

3.4 Fe^{2+} chelating ability of the alcohol extractable phytochemicals from Urtica dioica, Viola *tricolor* and *Lycopodium clavatum* explain the inhibition of lipid peroxidation on rat brain homogenate.

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