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INCIDENCE OF MOBILE SEROVARS OF *SALMONELLA SPP.* ISOLATED FROM BROILER CHICKENS IN 2009

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Key words: chickens broiler, salmonellosis, serovar

SUMMARY

The objective of this work was to establish the incidence of mobile Salmonella from broiler farms in the Romania. As the results of this study conducted in 2009, there were isolated 400 strains of mobile *Salmonella*, placed in 15 serovars, and the serovar *S. infantis*, having the highest incidence (63.3%). There were identified more exotic serovars for Romania. It was also, isolated *S. anatum* serovar (specific to palmipedes). Regarding the two relevant serovars the incidence was 4.5% for *S. enteritidis* and 1% for *S. Typhimurium* (specific to palmipedes).

Infections caused by mobile *Salmonella*, also known as salmonellosis, at the species *Gallus gallus*, are commonly found in poultry intensive worldwide. The incidence of these infections is variable depending on serovars assets, structure of flocks and presence of predisposing factors in livestock holdings (1).

Serovars that cause these diseases on birds, mainly on the species *Gallus gallus*, are pathogenic for humans. Due to reason for their zoonotic risk should be monitored continuously (1).

Poultry products, represented by eggs, poultry meat and poultry meat products are the main source of mobile *Salmonella* infection, producing foodborne disease at human population.

In Romania, the National Program was developed to control mobile *Salmonella* infections, with zoonotic risk, at the species *Gallus gallus*, during 2009-2011. The program objective is to reduce to 1% or less, until 31 December 2011, the percentage of broiler flocks which remaining positive for *S. enteritidis* and *S. typhimurium*.

1. MATERIALS AND METHODS

For the development of this work an epidemiological study was carried out, regarding to incidence of mobile serovars *Salmonella spp*., isolated from broiler farms in the Romania, namely in 2009.

The primary data were collected, graphically processed to be interpreted.

Sampling in broiler farms is made as following:

- Self control (at the initiative of farmers);
- Official control (performed by the county Sanitary Veterinary and Food Safety Directorates).

Refrigerated samples are sent in county accredited laboratories within 24 hours, where they are processed within 48-96 hours from harvest. Bacteriological examinations are conducted in accordance with ISO 1:2007 6579-2002/ Amendment 1:2007 - Horizontal method for detection of *Salmonella spp.* developed by the Community Reference Laboratory for *Salmonella spp.* isolated from birds existing in Bilthoven in the Netherlands.

The samples are cultured in enriched BPW (buffered peptone water) broth, after that the subcultures being cultured in modified semisolid Rappaport-Vassiliadis medium. The next stage after obtaining pure cultures is biochemical identification and determination of antibiotic sensitivity in county accredited laboratories.

Isolated strains are phenotypically studied and are sent for final identification (serotyping, molecular typing and phage typing) to the National Reference Laboratory for Salmonellosis in Animals within Institute for Diagnosis and Animal Health from Bucharest.

2. RESULTS AND DISCUSSION

In 2009, have been isolated 400 mobile strains of Salmonella from broiler flocks existing in our country, which were assigned in 15 serovars presented in Table 1. Analyzing the incidence of serovars and isolated strains it can be observed that this is variable. Thus, the highest incidence had the serovar *S. infantis* (253 strains being isolated and identified). The lowest incidence was manifested by the serovars *S. bovismorbificans* and *S. Montevideo,* only one strain being isolated from each serovar.

All isolated serovars are mobile and they are considered serovars with zoonotic risk. Analyzing mobile serovars incidence, two relevant serovars were identified, which were included in the National Control

Program for mobile *Salmonella* in broilers, represented by *S. enteritidis* and *S. typhimurium*. It was also observed that *S. typhimurium* considered as relevant serovar, in the investigation had a low incidence, only four strains being isolated.

Table 1

Incidence of *Salmonella* serovars in broiler farms, in 2009

No.	Serovars	Number of isolated strains
1	*S. amsterdam*	4
2	*S. anatum*	2
3	*S. bovismorbificans*	1
4	*S. enteritidis*	18
5	*S. hadar*	6
6	*S. infantis*	253
7	*S. livingstone*	43
8	*S. montevideo*	1
9	*S. mbandaka*	9
10	*S. senftenberg*	29
11	*S. tennessee*	12
12	*S. thompson*	10
13	*S. typhimurium*	4
14	*S. taksony*	4
15	*S. uganda*	4

Some serovars considered exotic for Romania, such as *S. livingstone*, *S. mbandaka*, *S. senftenberg*, *S. thompson* and *S. tennessee* had a high incidence due to imports of eggs and breeding hens from third countries, where the incidence of these serovars is increased. Introduction of exotic serovars in free countries, including Romania, is the result of *Salmonella* epidemiological circuit worldwide, provided primarily by the sale of poultry.

In this study, it was isolated the serovar *S. anatum* too, pathogenic serovar of web-footed, which has entered into a farm, perhaps from backyard. Analyzing, in time, the incidence of mobile *Salmonella* serovars, isolated in our country, we see that it is variable in recent years, increasing both the number of isolated strains and the number of isolates serovars within them.

Volintir in 1975, quoted by Daneş (2) shows within a study that *S. typhimurium* was isolated in 63-93% proportion at broilers and hens, and

other serovars proportion was much lower. Sicoe in 1988, quoted by Daneş (2) showed that serovars *S. typhimurium* and *S. enteritidis* had the highest frequency.

After trade liberalization for poultry material in our country Drăghia *et al*. (3), showed that 6% out of broiler chickens were mobile *Salmonella* carrier, dominant serotypes beeing *S. enteritidis* (47,4%) and *S. typhimurium* (18,6%).

Tatu-Chițoiu *et al.* (5), between the 2001-2005 studied 2807 mobile *Salmonella* strains, of which 2402 were isolated from birds, the main serovar beeing *S. enteritidis*, with a frequency of 43.3%, and lowest frequency for serovar *S. djugu* (1.25%). In this study were identified 57 serovars of which seven were considered new serovars for our country.

The mobile serovar frequency, worldwide, isolated from broiler chickens is variable, changing periodically, depending on many factors. In USA, in *Gallus gallus* species, the frequently isolated serovars are *S. heidelberg*, *S. kentucky*, *S. enteritidis*, *S. seftenberg*, and in EU were isolated 21 mobile *Salmonella*, and five may be considered relevant serovars because of high-frequency and high zoonotic risk (4).

In 2009, isolated serovars from broiler chickens, under the National Program are frequently found in serovars isolated both in USA and European Union.

3. CONCLUSIONS

3.1. In 2009 there were isolated 400 strains of mobile *Salmonella*, falling in 15 serovars, with highest incidence for the serovar *S. infantis*, and the lowest incidence for serovars *S. montevideo* and *S. bovismorbificans*.

3.2. There were identified two relevant serovars that are included in the National Program of Control of mobile *Salmonella* in broilers, represented by *S. enteritidis* and *S. typhimurium*.

3.3. There were identified more exotic serovars due to the sale of poultry and animal feed in community and non-community countries.

3.4. It was also isolated *S. anatum* serovar, which is specific to palmipedes.

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INCIDENCE OF MOBILE SEROVARS OF *SALMONELLA SPP.* ISOLATED FROM BREEDING HENS

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Key words: breeding hens, salmonellosis, serovar

SUMMARY

The faeces samples were collected from various parts of the hall with a frequency established by the community legislation, to cover 1% incidence in the flock with a 95% confidence interval. Number of places where it was harvested, is correlated with the number of birds in the flock. Following the study undertaken were isolated 21 strains of mobile *Salmonella. Salmonella eneteridis* serovar had the highest incidence, other relevant serovars represented by *S. infantis*, *S. hadar, S. typhimurium* and *S. Virchow,* were not identified in breeding hens.

In intensive aviculture has been an increased incidence of mobile *Salmonella* infections, respectively salmonellosis, associated with the development and circulation of new serovars involved in the etiology of these diseases. The emergence and evolution of salmonellosis generates losses by mortality, through control measures required and restrictions on the sale of poultry (1, 4).

Besides the economic aspect, mobile *Salmonella* infections presents a sanitary importance due to the special zoonotic risk of the serovars involved. Poultry production (eggs, meat and their derivatives) represents the major source of mobile *Salmonella* infection for humans (1, 4).

In our country, based on existing EU legislation in this field, the National Program was developed to control mobile *Salmonella* infections, with zoonotic risk, for *Gallus gallus* species, in holdings of reproduction, laying hans, broilers and hatching stations (1).

The aim of this study was to analyze, by epidemiological point of view, the effectiveness of existing measures under the program conducted in 2009.

1. MATERIAL AND METHODS

To preparing this paper was performed an epidemiological study of longitudinal type, held during one year, respectively 2009. This year is the first year of the National Programme for control of mobile *Salmonella* infections, with zoonotic risk, on *Gallus gallus* species. This study, longitudinal type, was based on primary data collected from breeding hens holdings at country level.

In this study was supervised the incidence of some serovars isolated from breeding hens farms.

The primary data collected at country level have been given in table 1, they have been compiled and presented graphically in order to be interpreted.

Legislative framework for breeding hens holdings provides two types of controls:

- Self control (at the initiative of farmers);
- Official control (performed by county Sanitary Veterinary and Food Safety Directorates).

In case of the self control the samples are harvested one time in two weeks, from hatchery or from the farm. Currently, in Romania, the sampling is done at the farm level.

In terms of official control, the samples were harvested at the farm level, three times during the production cycle:

- four weeks after the starting of laying;
- eight weeks before the end of the laying period;
- in the middle of the laying period.

Samples are represented by faeces and disposable shoes (boot swab) made of absorbent material.

Faeces samples are collected in order to cover 1% incidence in the flock with a 95% confidence interval (Table 1). Samples are represented by at least 1 g of fresh faeces collected from several points of the flock.

Persons designated to use the disposable footwear (boot swab), walk inside of the shelter on the well-established route with surface corresponding (permanent bedding, grids).

Shoes used is moistened with dilute solutions previously recommended by the National Reference Laboratory (0.8% sodium chloride, 0.1% peptone, double-distilled or distilled water, pH = 7). Persons

designated running routes must be between 20-50% of the shelter.

Refrigerated samples were sent to the accredited county laboratories within 24 hours and they were processed within 48-96 hours from harvest.

Bacteriological examinations are conducted in accordance with ISO 1:2007 6579-2002/ Amendment 1:2007 - Horizontal method for detection of *Salmonella spp.* developed by the Community Reference Laboratory for *Salmonella spp.* isolated from birds existing in Bilthoven in the Netherlands. This methodology is used in veterinary county authorized laboratories.

Table 1

Number of locations from which the samples are prelevated

Number of birds kept in the breeding flock	Number of points that are collected faeces samples to be taken from the breeding hens flock
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

2. RESULTS AND DISCUSSION

During the National Program to detection of mobile *Salmonella* from breeding hens holdings, existing in our country, in 2009, were isolated 21 strains of *Salmonella* belonging to the five mobile serovars, the results are presented in Table 2.

Table 2

Serovars of mobile *Salmonella* isolated from flocks of breeding hens in Romania, in 2009

No.	Serovar	Number of isolated strains
1	*S. enteritidis*	7
2	*S. mbandaka*	5
3	*S. montevideo*	3
4	*S. senftenberg*	4
5	*S. thompson*	2
TOTAL		21

Frequency of serovars and strains isolated was variable. Thus, the highest frequency had *S. enteritidis* serovar, in which they were isolated and identified seven strains, and the lowest frequency had the serovars *S. montevideo* and *S. thompson,* in which were isolated only three respectively two strains.

Analyzing the frequency of mobile serovars notice that was identified only the serovar *S. enteritidis,* out of the five relevant serovars of breeding hens, represented by *S. enteritidis, S. infantis, S. hadar, S. typhimurium* and *S. virchow.* Both breeding hens and broiler chickens was not identified the serovar *S. virchow*.

Some serovars considered exotic, for Romania, such as *S. senftenberg* and *S. thompson,* arose due to imports of young replace and day-old chicks from the third countries, where frequency of those serovars is increased.

Intrusion of exotic serovars in free countries, including Romania, is the result of salmonella epidemiological circuit, worldwide, provided first by the sale of poultry from third countries where the law is more permissible at the mobile *Salmonella* infection.

From breeding hens farms unlike the broilers farms, were isolated much less serovars because the imported flocks are lower, and biosecurity and control rules are very strict. Also, *S. amsterdam, S. anatum., S. livingstone, S. taksony, S. tennessee* and *S. uganda,* was not isolated.

Frequency of mobile *Salmonella* serovars, isolated in our country has been variable in recent years, increasing both the strains number and the serovars number isolates. The results provided by other authors were influenced largely by the developments of intensive poultry farming and sale of poultry.

Volintir (1975), quoted by Verdes (7) showed that *S. typhimurium* was isolated in 63-93% proportion from broilers and hens, and other serovars proportion was much lower, and Sicoe (1988), quoted by Daneş (2) mentioned that the highest frequency had serovars *S. typhimurium* and *S. enteritidis*.

After liberalization of trade with poultry material in our country Drăghia *et al*. (3), showed that 5% of breeding hens were mobile *Salmonella* carrier, dominant serotypes were *S. enteritidis* (47,4%) and *S. typhimurium*

(18,6%).

Tatu-Chițoiu *et al.* (6), between the 2001-2005 studied 2807 mobile *Salmonella* strains, of which 2402 were isolated from birds, the dominated serovar was *S. enteritidis*, with a frequency of 43.3%, and lowest frequency had serovar *S. djugu* (1.25%). In this study were identified 57 serovars of which seven were considered new serovars for our country.

The mobile serovars frequency, worldwide, isolated from breeding hens are variable, changing periodically, depending on many factors. In USA, at *Gallus gallus* species, frequently isolated serovars are *S. heidelberg*, *S. kentucky*, *S. enteritidis*, *S.seftenberg*, and in EU were isolated 21 mobile *Salmonella*, five may be considered relevant serovars because of high-frequency and of high zoonotic risk (4, 5).

The isolated serovars in 2009, from breeding hens flocks, under the National Program are found in high frequency serovars isolated both in USA and European Union, but their number and the isolated strains number, are much lower than in broilers flocks.

Frequency of serovars was between 9.4% and 33.3%. Through the intervention of favorable factors, the mobile *Salmonella* circuit is complex promoting the movement of certain serovars.

Thus, in 2009 , in our country was dominant serovar *S. enteritidis*, both breeding hens and broiler chickens flocks.

3. CONCLUSIONS

3.1. In 2009, 21 strains of mobile *Salmonella* were isolated from the breeding hens, falling in five serovars and serovar *S. enteritidis*, was with the highest incidence.

3.1. From breeding hens was isolated only one relevant serovar represented by *S. enteritidis*, out of the five relevant serovars breeding hens, represented by *S. enteritidis, S. infantis, S. hadar, S. typhimurium* and *S. virchow*. Also, in Romania, were isolated serovars considered exotic, such as *S. senftenberg* and *S. thompson.*

3.1. Most of serovars isolated in 2009 from breeding hens are often isolated from EU or non-EU countries, they appearing in Romania due to the trade with poultry material and feed.

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RESEARCH ON THE DYNAMICS OF BIOCHEMICAL INDEXES CAUSED BY STREPTOZOTOCIN EXPERIMENTAL DIABETIC IN RATS

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Key words: experimental diabetes, biochemistry, streptozotocin, rats.

SUMMARY

Following research carried out on 63 male rats, aged 2 months, Wistar breed, divided into five groups, experimental diabetes was induced with streptozotocin. Two groups were treated orally with Eridiarom and Diavit phyto-therapeutic products and one lot with Siofor. Monthly samples were collected for monitoring blood biochemical parameters (for 7 months). It appears that after the first month, serum calcium begins to recover, and after two months also the magnesium, glucose, serum urea and creatinine. After 5-7 months of observation, track parameters return to normal within the treated groups, but still changed in the untreated patient group.

Diabetes is a complex metabolic disease, which evolves as alarming in humans and pets.

The work shows that biochemical parameters followed for a long time are normalized after administration of the original phyto-therapeutic products.

The work is part of a long series of observations begun in 1993, which our team has shown experimentally that the beta cells of Langerhans island recover, stimulated with phyto-therapeutic products (patent 2001). Biochemical results indirectly prove this.

1. MATERIAL AND METHODS

The investigations were made on 63 male rats of Wistar breed, aged 2 months, elected in weight (150 g), age and size should be as uniform as possible. At first they were taken, randomized, seven heads, and we formed the first batch:

Lot 1 healthy - 7 heads —not diseased, healthy controls.

Other animals were injected with streptozotocin, 4 mg/100 g body weight intraperitoneally. The animals were monitored for 3 days, and then we formed the experimental groups as follows:

Lot 2 consists of 14 animals - sick and the untreated control group.

Lot 3 consists of 14 animals - group treated daily with one tablet of SIOFOR 1000, equivalent to 12 mg/kg body weight and 4 g of lactose to correct taste.

Lot 4 consists of 14 animals - group treated daily with 5.4 g ERIDIAROM/lot/day.

Lot 5 consists of 14 animals treated daily with DIAVIT, 6 g/ batch/day.

Housing, feeding and watering, for all of the animals were the same (standard food), microclimate conditions as well.

The investigations were made during 2007 - 2008, on a group of 63 rats.

After the first, 2nd, 3rd, 5th and 7th month experiment, blood samples were collected (from each 7-8 animals/group) for serological and biochemical tests. Samples were taken individually, from the internal angle of the eye, were collected in specially prepared containers, kept on ice and transported within an hour, at the biochemical research laboratory.

All tests were made using specific enzymatic methods, colorimetric, samples were taken in special containers and were processed with automatic analyzer KONELAB, biochemical and immunological.

2. RESULTS AND DISCUSSION

Total serum calcium: It is the mineral most important and most abundant in the body, because it forms the bones and teeth.

Table 1

Average calcium serum values in the study groups (mg/dl)

Month	No. Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
1	6	4.5 –5.5	4.42	4.88	5.18	5.12	4.76
2	5	4.5 –5.5	4.17	5.38	5.26	5.4	5.3
3	7	9 –9.5	7.99	5.36		9.33	9.60
5	6	9 –9.5	8.725	8.083		9.38	9.56
7	6	9 –9.5	8.068	7.974		9.18	9.389

Evolution of calcium was increasing in groups treated with Eridiarom and Diavit, growth is observed after the first month of treatment, but also in the untreated patient group and the group treated with Siofor.

After 2 months of treatment, serum calcium increased significantly in the group treated with Eridiarom.

After 3 months of treatment and by the end of experience, there is significant loss of calcium in the untreated patient group, but at the upper limit of normal values in Eridiarom and Diavit treated groups.

Magnesium: is a chemical element essential for the proper functioning of organs in the body of animals: liver, muscle, nervous system, etc.

Table 2

Average magnesium values in the studied groups (mg/dl)

Month	No Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
1	6	1.8 –2.4	2.34	2.3	2.36	2.42	2.38
2	5	1.8 –2.4	2.227	2.423	2.6	2.453	2.603
3	7	3 –4.5	2.917	2.936		3.871	4.197
5	6	3 – 4.5	3.125	3.210		4.185	4.578
7	6	3 – 4.5	3.366	2.946		4.27	4.243

From the third month of observation, and by the end of the experiment, the magnesium increases for the groups treated with Eridiarom and Diavit, reaching the upper limit of normal, healthy control and untreated sick group magnesium values remaining at 2.917 to 2.936 mg/dl.

Glucose (GOD / POD): is the most important saccharides, normally found in organs and blood of animals, and is the most precious fuel to obtain energy needed for various activities.

Glucose during the experimental period was maintained at the lower limit for healthy control group.

Table 3

Average values of glucose in the studied groups (mg/dl)

Month	No Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
2	5	75 - 135	77,66	122,33	82	140,33	152
3	7	50 - 135	83,57	127,85		130,57	136,97
5	6	50 -135	72	183,2		124,25	121,4
7	6	50 - 135	80	203,5		129,28	126,4

For the group treated with Eridiarom, glucose decreases progressively from 140.33 mg/dl at 2 months of induced diabetes, to 130.57 mg/dl at 3 months and then at 124.25 mg to 5 months, down to normal, 129.28 mg/dl in 7 months, similar to the group treated with Diavit.

We note that the decrease of glucose, is constant in groups treated with Diavit and Eridiarom.

Serum Urea: Urea is the nitrogen excretion form of protein**.**

Table 4

Average values of urea, for groups followed for 1 - 7 months

Month	No Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
1	6	10 - 50	38,06	48,9	50,1	45,6	45,9
2	5	10 - 50	37,77	45,97	50,63	47,93	49,17
3	7	15 - 26	29,75	48,2		21,04	21,87
5	6	15 - 26	21,5	24,95		21,77	22,72
7	6	15 - 26	21.94	24,22		21.234	19.28

After the first month, the average urea increased significantly in diseased and untreated group, as well as in the group treated with Siofor.

Lower values are found in groups treated with Eridiarom and Diavit.

After 2 months of treatment, urea remains constant and then rises above the normal value, in the group treated with Siofor, and maintained at

the upper limit for the groups treated with Eridiarom - 47.93 mg/dl and with Diavit - 49.17 mg/dl.

After 3 months, and by the end of research, urea remains low in the groups treated with Eridiarom and Diavit.

Creatinine - is synthesized in muscle tissue through a process of creatine transformation.

Table 5

Average values of creatinine in groups followed for 7 months (mg/dl)

Month	No. Indiv.	Normal value	Healthy control group	Untreated sicked group	Group treated with Siofor	Group treated with Eridiarom	Group treated with Diavit
2	5	0.2 - 0.8	0.553	0.593	0.450	0.463	0.497
3	7	0.2 - 0.8	0.619	0.427		0.581	0.577
5	6	0.5 —1.1	0.515	0.493		0.555	0.518
7	6	0.2 - 0.8	0.401	0.468		0.485	0.386

After 2 months of treatment, it is within normal range in all groups. The highest values are recorded in the untreated patient group, for other groups the values are very close, the lowest recorded value is in the group treated with Siofor, followed by Eridiarom and Diavit.

After 3 months its values increased slightly in all groups reaching the highest in untreated healthy group, but even this group does not exceed the average of the literature. For the diseased and untreated group, there is an average of 0.427 mg/dl, and the lots treated with Eridiarom and Diavit have similar values, normal.

3. CONCLUSION

3.1. Total serum calcium: For the groups treated with Diavit or Eridiarom, calcium dynamics is growing even after the first month of treatment; after 2 months the increase is significant, and reaching after 5-7 months the normal upper limits, of literature data and healthy control group. The untreated patient group, after 3 months there is a drastic decrease of calcium that has not been normalized thereafter.

3.2. Magnesium: after 2 months of treatment in all groups values are low, below the lower limit of normal, even for the healthy group; after 3 months of treatment increases until the upper limit of normal, in the groups treated with Diavit and Eridiarom.

3.3 Glucose is constantly increasing, in the untreated patient group, and also increases as the disease progresses. For the groups treated with Eridiarom or Diavit, glucose decreases gradually, reaching normal values. Similar data were obtained for treated children.

3.4. Serum urea: After the first month of treatment in all diseased groups, serum urea was elevated above the maximum allowed, even in healthy control. After 3 months of treatment, they decreased to normal values and remain low in treated groups with Diavit or Eridiarom, reaching average literature data in the healthy control group.

3.5. Creatinine: After 2 months of treatment, the highest values recorded belong to the untreated patient group, but lower values in the groups treated with Siofor, Eridiarom and Diavit. After 7 months of observation, the highest values are recorded in the untreated patient group and lower values in the groups treated with Eridiarom, Diavit and healthy controls, by reaching the limits of normal.

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IMMUNOLOGICAL DETERMINATION IN RABBIT AFTER IMMUNE RESPONSE POTENTATION BY USING IMMUNOMODULATORS

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Key words: non-specific immunomodulation, Leporidae, hemorrhagic disease.

SUMMARY

Immunological responses of the body was followed ,as a result of potentiation with unspecific immunomodulators, to the immune response induced by vaccination against the rabbit haemorrhagic disease (specific immunomodulation) in rabbits reared in semi-intensive system. There were tested 30 rabbits in the form of three lots from the age of 180 days.

Lot 1 was used only as a witness being subjected to vaccination against rabbit haemorrhagic disease. In group 2, the animals received vitamin E and selenium, Romselevit product using a dose of 0.1 ml / kg. In group 3,animals received vitamin E and selenium using Romselevit product, the double dose. The two experimental groups were subjected to vaccination against the rabbit haemorrhagic disease.

The results obtained confirm the existence of an immunomodulatory action after treatment with vitamin E and selenium in normal doses, with positive influence on the immune status in rabbits. WBC counts presented at the end of the experiment, a significantndecrease in statistical terms, in the group that received E and selenium, and the results confirmed the existence of an immunomodulatory action after treatment with vitamin E and selenium in normal doses, with positive influence on immune status in rabbits with high dose, compared with other groups. Immunostimulation by vitamin E and selenium increased the percentage of lymphocytes, in inverse proportion to the percentage of neutrophils, which fell from the same batch, the effect is an increase of the percentage of antibodies against the rabbit haemorrhagic disease.

Animal organism can increase resistance by using immunomodulating products (Amici A. et. all, 2000). They may act specifically to produce different effects (destruction of pathogens, or blocking their activities); in this category fit vaccines, immune sera and even antibiotics (Gonzales, S.R.,et. all, 1998; Ijaiga, 2000,). Intensity of immune response may be increased in non-specific way (Rivera J.D., and Duff G.C, 2003) for a particular type of aggression, using a diverse range of cellular structures, organic or inorganic substances. It was well demonstrated the participation of mineral elements to the proper functioning of the immune system - is standing out above all the observations showing the participation of compounds of selenium, iron, copper and zinc. Bodies deficiencies in these elements have imunodeficite

complex at cellular and humoral levels (Uko O.J et. all, 2000). Within the research it has been aimed the immunological responses of the body, after potentiation with non-specific substances acting on the immune response induced by vaccination against the rabbit haemorrhagic disease (specific immunomodulation), in rabbits reared in semi-intessive system.

1.MATERIALS AND METHOD

There were tested 30 rabbits in the form of three groups (group 1, group 2, and group 3), from the age of 180 days; testing was performed on animals reared in semi-intensiv system.

Group 1 was used only as a witness being subjected to vaccination against rabbit haemorrhagic disease. In group 2, the animals received vitamin E and selenium using the product Romselevit inoculated sc at a dose as the experimental scheme (table 1). In group 3,the animals received vitamin E and selenium using the product Romselevit inoculated sc in double dose, according to the experimental scheme (Table 1).

Also the two experimental groups were subjected to vaccination against the rabbit haemorrhagic disease.

The experiment was conducted over a period of 45 days, during which three samples were made of blood.

Table 1

Experimental Scheme

Stage	Batch	Day	Vitamin E + Se	Vaccination	Sampling blood
Stage 1	1	Day 1	-	0,5ml sc/anim	*
		Day 3	-		-
	2	Day 1	0,1ml sc/kg	0,5 ml sc/anim	*
		Day 3	0,1ml sc/kg		-
	3	Day 1	0,25 ml sc/kg	0,5 ml sc/anim	*
		Day 3	0,25 ml sc/kg		-
Stage 2	1	Day 15	-	-	*
		Day 17	-		-
	2	Day 15	0,1ml sc/kg	-	*
		Day 17	0,1ml sc/kg		-
	3	Day 15	0,25 ml sc/kg	-	*
		Day 17	0,25 ml sc/kg		-
Stage 3	1	Day 45	-	-	*
	2	Day 45	-	-	*
	3	Day 45	-	-	*

* = Group who carried out blood sampling.

Quantified parameters:
1.Antibodies concentration against the rabbit haemorragic disease virus carried by heamagglutination inhibition reaction;
2. The total number of leukocytes;
3. The percentage of lymphocytes;
4. The percentage of neutrophils;

Blood examinations were conducted by electronic means in an analyzer Coulter - Counter CBC - 5. Data were statistically processed.

2. RESULTS AND DISCUSSION

Three experimental blood sampling were taken in the three experimental groups before vaccination on 14th day and 45th day after the beginning of the experiment. In tables 2, 4 ,3 it can be noticed that both groups 2 and 3 in (nonspecific modulated with different doses of vitamin E andselenium) antibody concentrationagainst the rabbit haemorr hagic disease (VBHI) increases substantially compared with group 1 (control), the phenomenon being more obvious at the last harvest.

Table 2

The concentration of antibodies against VBHI - harvesting I

Batch	Batch titre of haemagglutinating inhibition						
	1/32	1/64	1/128	1/256	1/512	1/1024	Average
Batch 1	-	-	5	4	1	-	1/218
Batch 2	-	-	2	4	4	-	1/332
Batch 3	-	-	2	3	5	-	1/358

Table 3

The concentration of antibodies against VBHI - harvesting II

Batch	Batch titre of haemagglutinating inhibition						
	1/32	1/64	1/128	1/256	1/512	1/1024	Average
Batch 1	4	4	2	-	-	-	1/64
Batch 2	3	5	2	-	-	-	1/80
Batch 3	3	4	3	-	-	-	1/72

Table 4

The concentration of antibodies against VBHI - harvesting III

Batch	Batch titre of haemagglutinating inhibition						
	1/32	1/64	1/128	1/256	1/512	1/1024	Average
Batch 1	-	-	1	8	1	-	1/268
Batch 2	-	-	1	-	7	2	1/576
Batch 3	-	-	-	2	6	2	1/563

After statistical processing of the leukocyte count there was a significant decrease in statistical terms, the third blood collection , in group 3, receiving the double dose E and selenium ($p < 0.05$), their number being of 7.77 ± 2.77 thousands/mm3, compared to first blood collection where it was obtained a total of 8.90 ± 3.93 thousands/mm3 leukocytes (Table 5, Fig. 1). For this constant no other changes were found statistically significant.

Table 5

Batch	Stages		
	I	II	III
1	8,53±3,94	7,99±3,66	8,10±4,94
2	9,03±5,09	8,03±2,84	8,16±4,38
3	8,90±3,93	8,11±4,12	7,77±2,77*

*** WBC count —absolute values**

* Mean + standard deviation
* = Significant difference

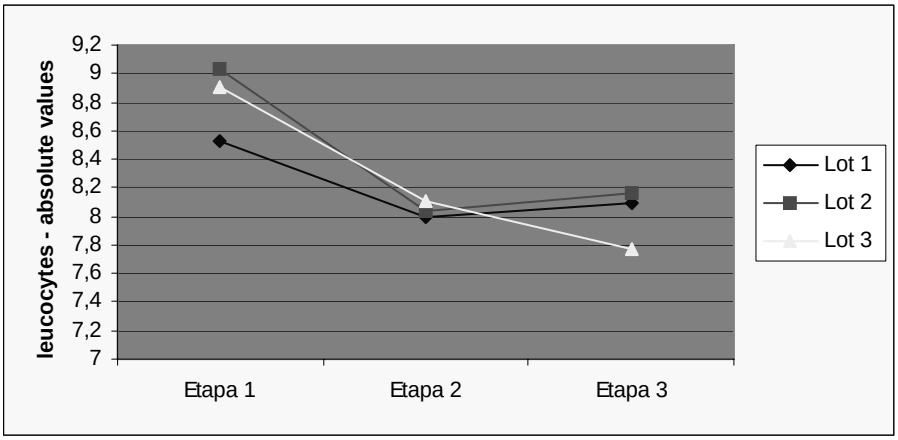

Fig. 1 - Graphical representation of leucocytes - absolute values

Table 6

Percentage of lymphocytes

Batch	***Stages***		
	I	II	III
1	55,61±0,81	54,14±1,89	52,27±2,26
2	53,33±0,7	62,11±0,81***	59,13±1,14**
3	55,37±1,06	60,2±0,61**	56,54±0,32

* Mean + standard deviation
** = Significant difference *** = Higher significant difference.

In Table 6 and Fig. 2 is shown the percentage of lymphocytes, which occurred statistical changes only in group 2, which increased significantly ($p<0.01$) after blood collection II (62.11 ± 0. 81%), compared to blood collection I (53.33 ± 0.7%).

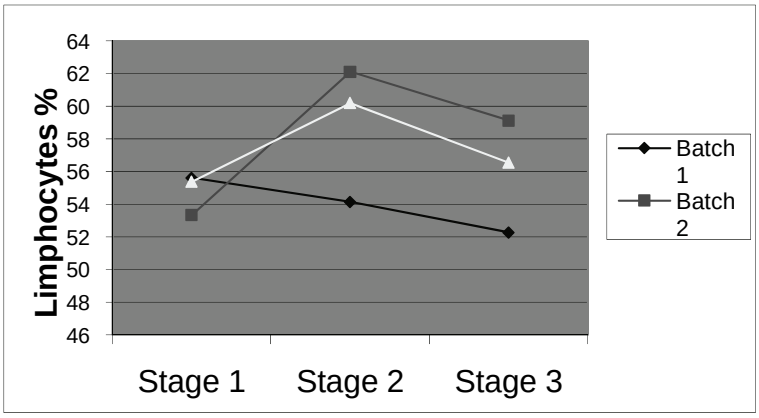

Fig. 2 - Graphical representation of percentage of lymphocyte evolution

In contrast to the percentage of lymphocytes, the percentage of neutrophils (table 7, graph 3) changed in group 2 and 3, decreasing significantly distinct ($p < 0.01$) at the second blood collection compared to first blood collection.

Table 7

The percentage of neutrophyls

Batch	Stages		
	I	II	III
1	40,83±2,28	44,27±5,74	43,05±6,58
2	42,85±2,04	34,17±2,69*	37,43±3,26
3	44,07±2,83	35,17±2,46**	35,73±12,09

* = Significant difference, ** = significant distinct difference;

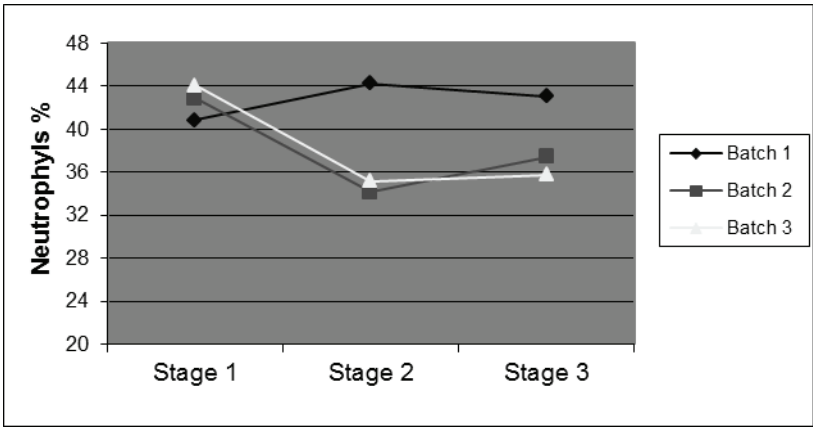

Fig. 3 - Graphical representation of the percentage of neutrophyl evolution

3. CONCLUSIONS

3.1. Testing was performed in a semi-intensive growth system, animals were subjected to normal feeding conditions and microclimate throughout the experiment.
3.2. There was a significant increase of antibody against rabbit haemorragic disease (VBHI), the phenomen is more obvious at the last harvest.
3.3. WBC counts (absolute values) presented at the end of the experiment, a striking decrease in statistical terms,in the group who received high-dose E and selenium compared with other groups.
3.4. Immunostimulation by vitamin E and selenium increased the percentage of lymphocytes, in inverse proportion to the percentage of neutrophils, which fell in the same group, the effect being the increasing percentage of antibodies against the rabbit haemorrhagic disease.
3.5. The results obtained confirm the existence of an immunomodulatory action after treatment with vitamin E and selenium in normal doses, with positive influence on immune status in rabbits.

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OSTEOLOGICAL FEATURES OF THE THORACIC LIMB IN THE REINDEER (*RANGIFER TARANDUS*)

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Key words: osteological features, thoracic limb, reindeer,

SUMMARY

Osteological features of the thoracic limb in reindeer are in general similar with those from the other domestic ruminants.

There are some specific particularities at the level of the bones of the thoracic limb, such as scapula, humerus, radius and ulna, carpal and metacarpal bones, phalanges.

The morphology of the bones of the thoracic limb in reindeer (Rangifer tarandus) has been studied related to the bones of the thoracic limb of the domestic ruminants (3, 4, 5, 6). Based on different features the bones of the thoracic appendicular skeleton were identified within the specie (1, 2).

1. MATERIAL AND METHODS

The study was performed on the bones of the thoracic limb from three reindeers; one female and two males.

The bones were prepared in the laboratory of Anatomy, Faculty of Veterinary Medicine Timişoara. The bony features of the appendicular skeleton were identified according to N.A.V. 2005 and compared with those from the domestic mammals. The most important differences are point out.

2. RESULTS AND DISCUSSIONS

Scapula (Fig. 1)

On the lateral surfaces (*Facies lateralis*) there is a distinct spine (*Spina scapulae* which has a smooth start and it ends in an abrupt angle, a hook-shaped-like acromiom.

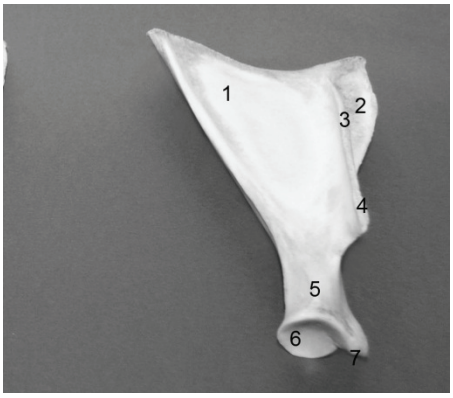

Fig. 1 Dorsal view of the reindeer bony head

1. Fossa infraspinata; 2. Fossa supraspinata; 3. Spina scapularis; 4. Acromiom; 5. Collum scapulae; 6. Cavitas gelnoidalis' 7. Processus coracoideus.

Humerus (Fig. 2)

In the proximal extremity, the greater tubercle (*Tuberculum majus*) is divided into a cranial part (*Pars cranialis*) and caudal part (*Pars cranialis*) which are equal and convex proximally.

The cranial part of the greater tubercle presents an apex slightly curved medially. The greater and lesser tubercle are separated by a large intertubercular groove (*Sulcus intertubercularis*) with a medially low ridge (*Tuberculum intermedium*) described in horses and old bovines.

The *facies musculi infraspinata* lies latero-median on the greater tubercle, ventrally to notch that divides the two parts of the greater tubercle.

Radius and ulna (Fig. 3)

The proximal interosseous antebrachial space (*Spatium interosseum antebrachi*) is long and extended proximally to the joint between the radial notch of ulna (*Incisura radilis ulnae*) and the articular circumference of the radius (*Circumferentia articualris*).

The olecranon (*Olecranon*) is quadrate-like shape and expanded proximally to form a convex tuber (*Tuber*).

The accessory carpal bone (*Os carpi accesorium*) has a disc-shaped aspect with a single articular surface on its outline (Fig. 4).

Fig. 2. Proximal extremity of humerus in reindeer

1. Greater tubercle, cranial part; 2. Greater tubercle, caudal part; 3. The notch of great tubercle; 4. Facies infraspinata; 5. Lesser tubercle; 6. Intermedium tubercle; 7. Intertubercular groove

Metacarpal bones (Fig. 5)

The large metacarpal bone (*Os metacarpale III et IV*) bear medio-palmar a deep palmar longitudinally groove (*Sulcus longitudinalis palmaris*) bordered laterally and medially by a prominence.

Distal phalanx (Fig. 6)

The abaxial surface of the distal phalanx (*Facies abaxialis*) is large and convex, being extended axially. The dorsal border (*Margo dorsalis*) is perpendicular on the solear axial border (*Margo soleare axiale*). The apex of the distal phalanx is rounded and moved axial. The rudimentary dew-claw usually bearing only a distal one.

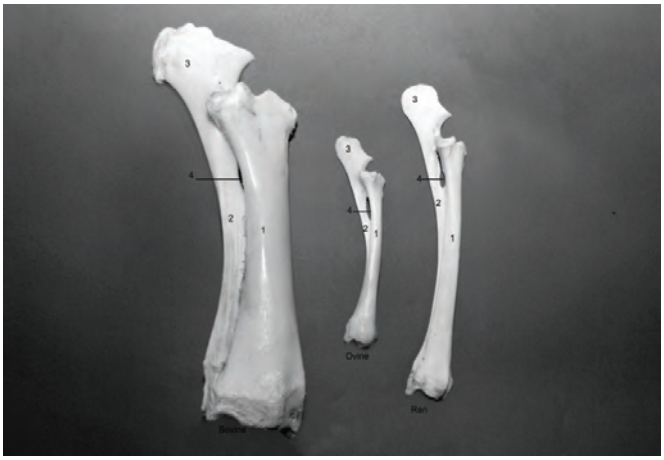

Fig. 3 Radius and ulna of the reindeer

1. Radius; 2. ulna; 3. Olecranon; 4. Proximal antebrachial space; 5. Trochlear notch; 6. Anconeus process; 7. Olecranon tubercle; 8. Radial trochlea; 9. Styloid process.

Fig. 4 Accessory carpal bone in reindeer

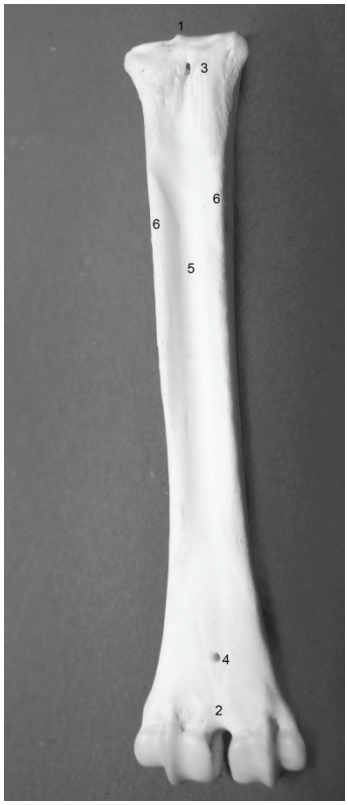

Fig. 5. The 3rd and 4th metacarpal bones
1. Base of metacarpal bones; 2. Head; 3. Proximal metacarpal channel; 4. Distal metacarpal channel; 5. Palmar longitudinaly groove.

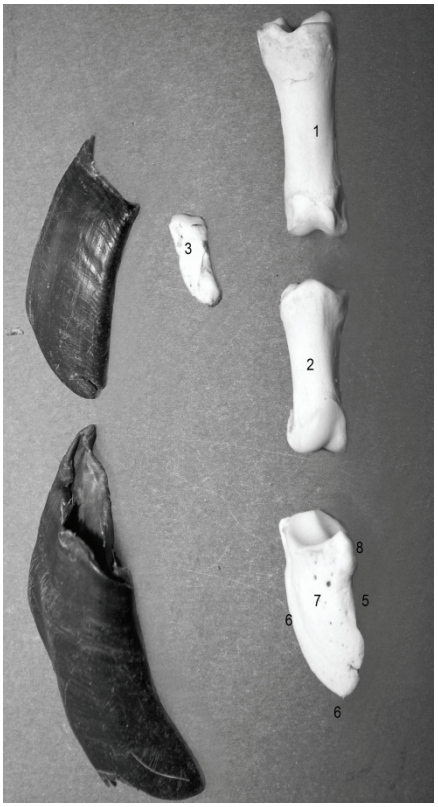

Fig. 6. Phalanges in reindeer
1. Proximal phalanx; 2. Middle phalanx; 3. Os ungulare II s. IV; 4. Distal phalanx; 5. Distal border; 6. Apex; 7. Abaxial surface; 8. Extensor process; 9. Solear border; 10. Epidermis ungulae.

3. **CONCLUSIONS**

3.1. The scapula presents a hook-shaped-like acromiom.

3.2. The two parts of the greater tubercle are equal and convex; in the intertubercularis fossa is presented a slightly intermediary tubercle.

3.3. The antebrachial interosseum space is long, the tuberosisty of the olecran is unique and convex.

3.4. The accessory carpal bone has a disc-shaped aspect.

3.5. On the palmar surface of the metacarpal bones a long proximo-distal groove is presented.

3.6. The axial surface of the distal phalanx is perpendiculary on the ground and the dorsal bordel moved axially.

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VARIATION OF SOME HEMATOLOGICAL INDICATORS, HEMATOLOGICAL INDICES AND WHITE BLOOD CELL COUNT IN CHICKS FED WITH ORGANIC SELENIUM SUPPLEMENTED FODDER

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Key words: selenium, oxidative stress, chicks, hematological indicator, hematological indices.

SUMMARY

Selenium is known to be an antioxidant mineral element that prevents free radical formation. It has its own biochemical role, by mediating the glutathione activity, thus indirectly protecting the hemoglobin from peroxidation.

In the beginning of egg laying period, the chicks are found in full growing and process, thus their organism must satisfy both the metabolic needs oriented to a harmonious development and the ones needed for egg production. It is likely that, by intensifying the metabolic processes, to generate more free radicals, so that it is possible that the organism is exposed to greater oxidative stress than normal.

This study researched the beneficial effects of supplementing the fodder ratio with Se on some hematological indicators and hematological indices in chicks: erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and also the leukocyte count (WBC). All the values obtained were between the normal range for the specie, hybrid, age, sex and production category.

The erythropoiesis increase can be attested by the decrease of the mean erythrocyte volume (MCV), concluding that a large number of young erythrocyte with a smaller volume, are released into the blood circulation. Concomitantly, because of its antioxidant role on the red blood cell membrane, the selenium prevents the degradation of the mature erythrocytes.

Because of the intensification of the erythropoiesis, the red blood cell count and the hemoglobin value increase, therefore the MCHC also increases, and the tissue oxygenation rate is amplified, it is justified to believe that by supplementing the fodder ratio with Se the health status of the livestock, but also their reproductive performances.

The decrease of the leukocyte count (WBC) could be explained by the increase of the erythrocyte count, the percentage by which these values are altered being sensibly equal.

Selenium is known to be an antioxidant mineral element that prevents free radical formation by mediating the glutathione activity, thus indirectly protecting the hemoglobin from peroxidation, through three antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase

(GSH-Px) and catalase (Avanzo *et al.*, 2001; Bartholomew *et al.*, 1998; Combs and Combs, 1986).

Stressful conditions, associated with free radicals over-production, may cause cellular lysis. These cases are often seen when animals are fed with polyunsaturated fatty acids, vitamin E, selenium, zinc, or manganese deficient fodder, or on the contrary, iron or vitamin A excessive in the fodder, alongside with toxins or toxic compounds (Curcă, 2005).

The non-enzymatic defensive system includes molecules that are able to neutralize free radicals generated by excess oxygen, both from the lipophilic media (vitamin E and vitamin A), as well as from the hydrophilic media (ascorbic acid, reduced glutathione, uric acid).

The selenium deficit, even insignificant, affects the activity of reduced glutathione (GSH-Px) and leads to cellular and intracellular membrane peroxidation, thus affecting the cellular permeability and increasing the levels of prostaglandins (inflammatory factors) that also influence blood pressure (Curcă, 2008b). The structure of numerous molecules (e.g. the DNA molecule) is also affected once with the peroxidation of the cellular membranes, and the molecules continue their physiological functions, even if the induced alterations would gradually lead to various states of cancer. Also, selenium deficiency could cause erythrocyte hemolysis and/or anemia in primates, dogs, rats, and chickens as well (McDowell, 1992).

The animal organisms have far better to gain from organic selenium forms (selenoaminoacids, e.g. selenomethionine), which have a better biodisponibility than the inorganic compounds. The amount of selenium assimilated in the organism increases, because these amino-acids are not rapidly excreted in the urine, and may be used in every cell that capture them (Surai, 2000, 2002, 2006).

1. MATERIAL AND METHODS

This study noted the values of some hematological indicators, of some hematological indices and white blood cell count after supplementing the fodder ratio with selenium (2 g/kg, selenium yeast) for four weeks, in eight Rosso hybrid chicks (experimental group), and compared them with the values of the same hematological indicators, indices and white blood

cell count from twelve Rosso hybrid chicks (control group), the age of all the animals used in the present experiment averaging between 18 and 20 weeks.

The combined fodder recipe (Fig.1) was as follows: protein-vitamin-mineral complex 10%, maize 48%, wheat 27%, soy 8%, fishmeal 1%, sunflower schrot 6%, and it had following nutritional characteristics: assimilable energy 2870.15 kcal/kg, protein 15.60%, methionine 0.29%, lysine 0.70%, fat 2.68%, calcium 1.07%, phosphorus 0.70%.

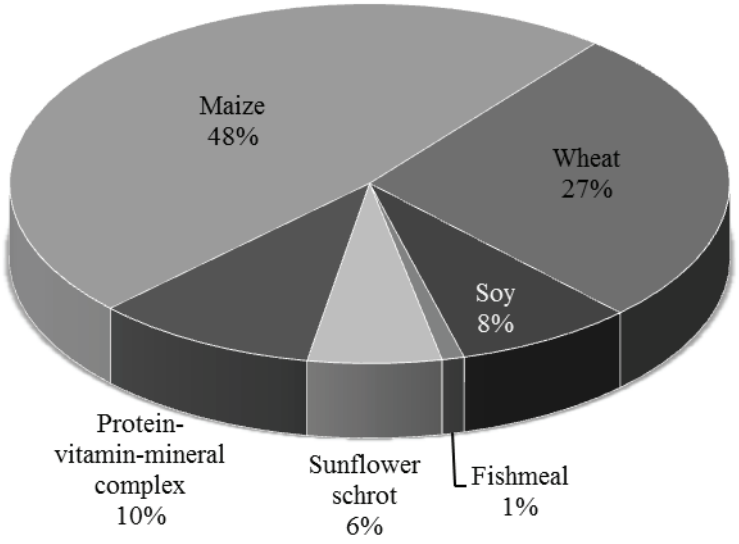

Fig. 1 The combined fodder recipe

This experiment used organic selenium (Sel-Plex®, AllTech Kentucky). Each tablet contains selenium yeast 50 mcg (selenomethionine 50%, selenocysteine 20% and selenoprotein, and selenium organic compounds 30%), magnesium stearate 225 mcg (6%), lactose, microcrystalline cellulose and sodium laurylsulphate.

Four weeks after the first supplementation with selenium, blood samples were collected by venipuncture of the cubital vein, which were the object of hematological investigations, while the organs and skeletal muscles were macroscopically observed after the control sacrifice.

The blood was collected on anticoagulant (EDTA 1-2 mg/ml blood), and the hematological indicator were determined with the automated machine Coulter-Counter (ACT 5 diff CP-Beckman analyzer).

A complete blood count (CBC) has been determined: red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), and hematological indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

2. RESULTS AND DISCUSSION

After performing control sacrifices, it was concluded that the findings in the carcasses, in the organs of the thoracoabdominal cavity and in the brain were insignificant**.**

The results of the hematological investigations concerning hematological indicators and hematological indices, as the leukocyte count, are presented in Table 1, while Fig.2 suggestively illustrates the tendency of increasing or decreasing.

Table 1

The average values of some hematological indicators, hematological indices and leukocyte count in chicks from the control group and from the experimental group, and the percentage by which the ones in the experimental group increases/decreases in value

Specification	Hematological indicators			Hematological indices			WBC (x 10^3/µl)
	RBC (x10^6/µl)	Hb (g/dl)	Hct (%)	MCV (μ^3)	MCH (pg Hb/E)	MCHC (g Hb/dl E)	
Control group	3,125	7,10	34,65	110,88	22,72	20,52	28,375
Experimental group	3,43	7,69	31,92	93,57	22,50	24,09	25,77
Increase/ decrease (%)	↑ 9,76	↑8,30	↓7,87	↓15,61	↓0,96	↑17,39	↓9,21

The tendency of the RBC count to increase with 9.76% in the experimental group as the result of the acceleration of the erythropoiesis has also been mentioned by Curcă, 2005.

Meanwhile, the hemoglobin value indicates an increase of only 8.30%, consecutive to the increase in number of young erythrocytes, with

less cytoplasm than in a mature erythrocyte, therefore explaining the discrete tendency to decrease of the mean corpuscular hemoglobin with 0.96%, as the total quantity of hemoglobin found in the blood stream decreases, as mentioned by Curcă, 2008a; Curcă, 2008b; Maysa, M.Hanafy *et al.*, 2009.

The result of the erythropoiesis acceleration is that more young erythrocytes are released into the blood stream by the hematopoietic bone marrow, but because of their smaller volume, the value of the hematocrit decreases. But the hematocrit value may decrease also due to a better hydration of the animals, so an increase of the plasmatic mass in the detriment of the corpuscular mass, an event also noted by Yahav *et al.*, 1997.

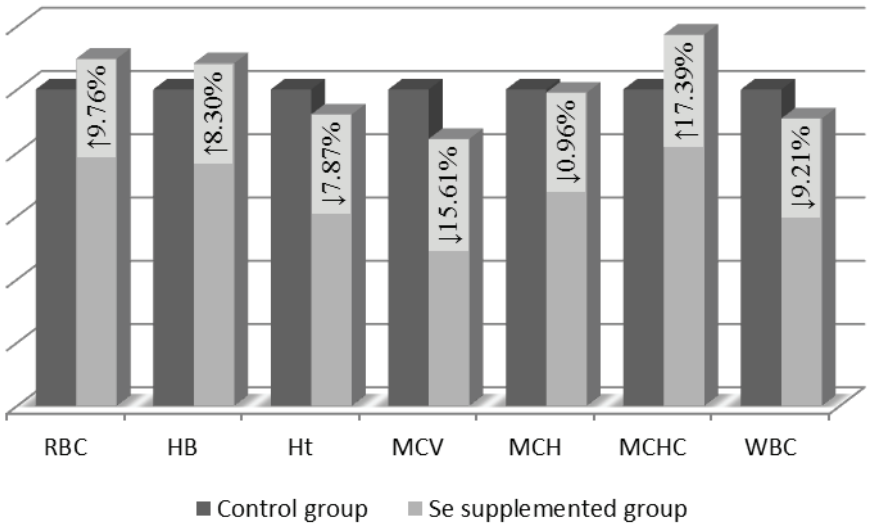

Fig. 2 Variation of some hematological indicators, hematological indices and WBC count in chicks from the control group and from the experimental group

Because of the large number of young erythrocytes with a smaller volume than that of mature erythrocytes, the mean corpuscular volume has decreases, similar to the results of McDowell, 1992; Răduță, 2011; da Silva *et al.* 2010.

Once with the increase of the value of hemoglobin and with the decrease of the mean corpuscular volume, the mean corpuscular hemoglobin concentration increases by 17.39%

The tendency to decrease of the white blood cell count is comparable to the percent of red blood cell count increase.

3. CONCLUSIONS

3.1. In this experiment one of the biological roles of selenium could be observed, that of its implication in the acceleration of the hematopoietic bone marrow activity, and so its role in the formation of new red blood cells.

3.2. The anti-anemic role of selenium is pointed out, first by the hematopoiesis acceleration, and second by its antioxidant effect that prevent hemolysis.

3.3. By stimulating the erythropoiesis, increasing the red blood cell count, and the hemoglobin, selenium may help to a better tissue oxygenation, so to an increase of the basal metabolism, therefore promoting the growing processes, but also optimizing the productive parameters.

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VARIATION OF SOME HEMATOLOGICAL INDICATORS AND INDICES IN CHICKS FED WITH L-CARNITINE SUPPLEMENTED FODDER

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Key words: L-carnitine, chicks, hematologic indicators, beta-oxidation, amino-acids.

SUMMARY

L-carnitine and its compounds were discovered at the beginning of the 20th Century, but their effects in the animal organisms have yet to be elucidated.

L-carnitine is a quaternary amine with a very important role in the activity of both the cardiac and the skeletal muscles and many more tissues. It protects the cellular membrane from the ischemic reperfusion by increasing the resistance of the cellular membrane from the action of free radicals, and so prevents the installing of oxidative stress.

This study researched the beneficial effects of supplementing the fodder ratio with L-carnitine on some hematological indicators and hematological indices in chicks: erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). It must be mentioned that all the values obtained were in normal range for the specie, hybrid, age, sex and production category.

After feeding the experimental group of chicks with L-carnitine supplemented fodder, increases of the erythrocyte count (RBC) and of the hemoglobin (HGB) due to the L-carnitine effects on the erythrocyte cellular membrane, have been noted, but also L-carnitine effect on the hematopoietic bone marrow, by increasing the number of colony-forming unit erythrocyte (CFU-E).

The hematocrit (HCT) also has the tendency to increase, as the plasmatic mass increases in the detriment of the cellular mass. The mean corpuscular volume (MCV) decreases because the L-carnitine stimulated hematopoietic bone marrow releases young erythrocytes with a smaller volume. Because of the same reason, but also because of erythrocytes life-span prolonging effect, the values of the mean corpuscular hemoglobin (MCH) and of the mean corpuscular hemoglobin concentration (MCHC) increase, therefore a better transportation of hemoglobin and a better tissular oxygenation occurring.

Carnitine was discovered in the first half of the previous century, but its major role in the metabolism of fatty acids effect has been recently discovered. Carnitine and its natural esters have an essential role in the normal development of the cardiac and skeletal muscle activity, although it is not a vitamin. The two-step endogenous synthesis is based on lysine and methionine, while food rich in proteins of animal represents the exogenous source. It is predominantly found in the mitochondrial membrane, and it

facilitates the transfer of long and medium chain fatty acids inside the mitochondria, being the main way of energy production (ATP) in the cardiac and skeletal muscles. Fatty acids are used in the beta-oxidation cycle, under normal circumstances ensuring the most of the energetic needs of the heart and skeletal muscles (Curcă, 1998; Curcă and Codreanu, 2003; Răduță, 2011).

L-carnitine and its short chain acil-derivates could protect against ischemic reperfusion, by increasing the resistance of cellular membranes to the disturbing action of free radicals or other toxic agents. L-carnitine has a stabilizing effect on the cellular membranes, along with other compounds. The indirect proof of this stabilizing effect shows that the L-carnitine positively enriched molecules may interact with the cardiolipin negatively charged molecules in the mitochondrial membranes of the hepatocyte (Batteli *et al.*, 1992; Curcă, 2005; Thiemel and Jelinek, 2004; Uchendu Chidiebere *et al*., 2011).

The L-carnitine and acetil-carnitine role of increasing the stability of the mature erythrocyte membrane is achieved by the specific interaction with one or more cytoskeletal proteins (Abbas *et al*., 2009; Curcă, 2008).

Acetil-carnitine is a vital co-factor in the mitochondrial oxidation of fatty acids that results from the production of ATP in the peripheral tissues. It has antioxidant activity and it may be implicated in the diminishing of cellular disfunction by inhibiting the lipidic hydroperoxidation (Curcă, 2003).

Because L-carnitine stimulates the kidney to elaborate erythropoietin (De Jong and Ferrari, 1995), it has an indirect role in erythropoiesis. This role can be observed in the hematopoietic bone marrow, where L-carnitine stimulates erythropoiesis by increasing the number of colony-forming unit erythrocyte (CFU-E), by stimulating the erythrocyte diapedesis and by increasing the hemoglobin indicator (Karadeniz et al., 2008). Also, it seems that L-carnitine administration increases the life-span of the erythrocyte by its effect of decreasing the transmembranary flux of calcium, so L-carnitine is implicated in the renewal of the erythrocyte membrane, by ensuring its stability and normalizing the osmotic resistance, by delaying hemolysis (Lorenzo *et al.*, 2005).

1. MATERIAL AND METHODS

This study noted the values of some hematological indicators and of some hematological indices after supplementing the fodder ratio with selenium L-carnitine (2 g/kg) for four weeks, in eight Rosso chicks (experimental group), and compared them with the values of the same hematological indicators and indices from twelve Rosso chicks (control group), the age of all the animals used in the present experiment averaging between 18 and 20 weeks.

The combined fodder recipe (Fig. 1) was as follows: protein-vitamin-mineral complex 10%, maize 48%, wheat 27%, soy 8%, fishmeal 1%, sunflower schrot 6%, and it had following nutritional characteristics: assimilable energy 2870.15 kcal/kg, protein 15.60%, methionine 0.29%, lysine 0.70%, fat 2.68%, calcium 1.07%, phosphorus 0.70%.

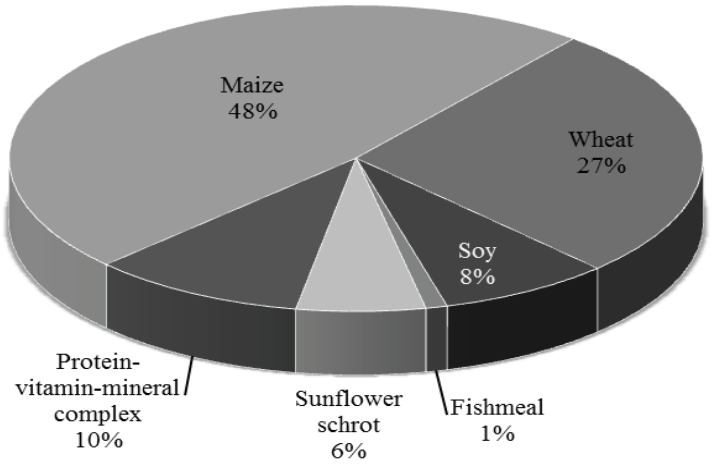

Fig. 1 The combined fodder recipe

This experiment used L-carnitine (*L-carnitine formula 985*, Wonder Laboratories). Each tablet contains L-carnitine fumarate 500 mg/tablet, gelatin, cellulose, magnesium stearate and silica.

Four weeks after the first supplementation with L-carnitine, blood samples were collected by venipuncture of the cubital vein, which were the object of hematological investigations, while the organs and skeletal muscles were macroscopically observed after the control sacrifice.

The blood was collected on anticoagulant (EDTA 1-2 mg/ml blood), and the hematological indicators were determined with the automated machine Coulter-Counter (ACT 5 diff CP-Beckman analyzer).

The blood count has been determined: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), and hematological indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

2. RESULTS AND DISCUSSION

After performing control sacrifices, it was concluded that the findings in the carcasses, in the organs of the thoracoabdominal cavity and in the brain were insignificant.

The results of the hematological investigations concerning hematological indicators and hematological indices are presented in Table 1, while Fig. 2 suggestively illustrates their tendency of increasing or decreasing.

Table 1

The average values of some hematological indicators and hematological indices in chicks from the control group and from the experimental group, and the percentage by which the ones in the experimental group increases/decreases in value

Specification	Hematological indicators			Hematological indices		
	RBC ($x10^6$/μl)	Hb (g/dl)	Hct (%)	MCV (μ^3)	MCH (pg Hb/E)	MCHC (g Hb/dl E)
Control group	3,125	7,10	34,65	110,88	22,72	20,52
Experimental group	3,21	8,043	33,65	104,82	25,05	23,90
Increase/ decrease (%)	↑ 2,72	↑ 13,23	↓ 2,89	↓ 5,47	↑ 10,85	↑16,47

In chicks fed with L-carnitine supplemented fodder, both the red blood cell count and the hemoglobin value have the tendency to increase, due to the erythrocyte membrane osmotic resistance increase, therefore maintaining the membrane functions by diminishing the calcium flux in the erythrocyte, also noted by Karadeniz, 2008. As well, L-carnitine protects erythrocyte membrane lipids, by preventing their peroxidation and so preventing the installation of oxidative stress.

In the hematopoietic bone marrow, L-carnitine stimulates erythropoiesis, and inducing erythrocyte diapedesis and increasing the hemoglobin quantity per deciliter, thus increasing the mean corpuscular hemoglobin concentration.

Hematocrit value has a small drop in value, due to the increase of plasmatic mass in the detriment of the cellular mass.

Due to the accelerating effect of L-carnitine on the hematopoietic bone marrow and the increase of younger erythrocytes with smaller volume, the mean corpuscular volume decreases.

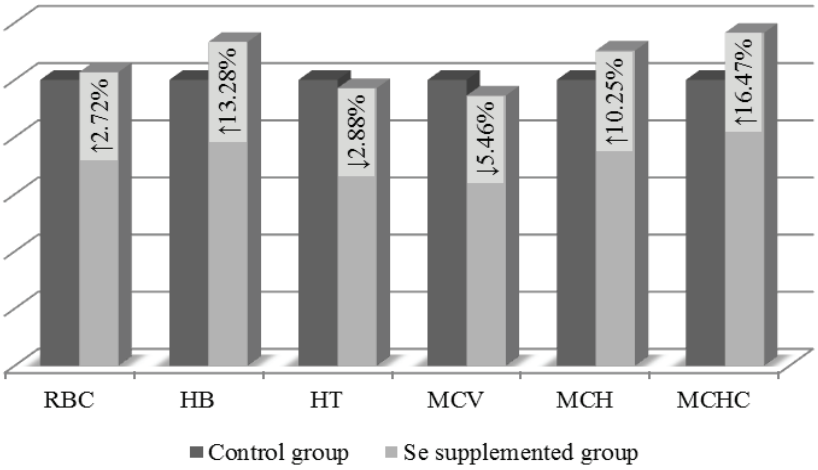

Fig. 2 Variation of some hematological indicators and hematological indices in chicks from the control group and from the experimental group

Because L-carnitine increases the hemoglobinogenesis and prolongs the life-span of erythrocytes, it indirectly increases the mean corpuscular hemoglobin. For the same reasons, the mean corpuscular hemoglobin concentration also increases.

3. CONCLUSIONS

3.1. One of the roles of L-carnitine is that of ensuring a greater resistance for erythrocyte membrane, by reducing the calcium flux in the cell. Also, because it prevents the peroxidation of the membrane lipids and the occurrence of oxidative stress, it protects them, thus prolonging the life-span of red blood cells.

3.2. L-carnitine has beneficial effects on the basal metabolism, by stimulating the erythropoiesis, by determining the erythrocyte diapedesis and a better loading of these cells with hemoglobin.

3.3. L-carnitine has a supporting role in stimulating the erythropoiesis and erythropoietin, therefore generating greater values of red blood cell count and mean corpuscular hemoglobin concentration, meaning a larger quantity of hemoglobin per blood unit.

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EFFECT OF SOME POLYPHENOLIC EXTRACTS FROM MEDICINAL PLANTS ON ANTIOXIDANT ENZYMES SYSTEMS. *IN VIVO* STUDIES.

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Key words: polyphenols, antioxidative enzymes, glutathione, thiobarbituric acid reactive substances (TBARS).

SUMMARY

The aim of this study was to determine the effect of *Viscum album and Aristolochia clematitis* alcoholic extracts on the liver reduced glutathione (GSH) level and the activities of two enzymes involved in glutathione metabolism, as well as the activity of catalase (CAT), superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G6PDH). The concentration of thiobarbituric acid reactive substances (TBARS) was determined as lipid peroxidation index.

Mice were divided into six groups: unstressed group mice (G1), which received distilled water, unstressed groups mice (G2 and G3), which received *Viscum album* and *Aristolochia clematitis* alcoholic extracts, stressed group mice (G4), which received distilled water, stressed groups mice (G5 and G6), which received mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)* alcoholic extracts. The experimental groups, where treated with alcoholic extracts in doses of 100 mg/kg. After 21days, the livers were quickly removed and placed in iced 0.9% NaCl containing 0.16 mg/ml heparin.

The changes of examined parameters of antioxidative system as well as lipid peroxidation index were found. The correlation between TBARS concentration and the elements of enzymatic and non-enzymatic antioxidative system was determined. A statistically correlations were found between investigated parameters.

Oxidative stress occurs when antioxidant balance is disrupted by excessive production of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, hydrogen peroxide, singlet oxygen (Cadenas and Cadenas, 2002; Mcdonald *et al.*, 2003). ROS constitute a major part of biologically important free radicals, and current knowledge indicates that free radical damage plays a key role in induced endotoxic shock (Cadenas and Cadenas, 2002; Salvemini and Cuzzocrea, 2002). SOD catalyses the reaction between two molecules of superoxide into hydrogen peroxide and molecular oxygen. GPX catalyses the reduction of hydrogen peroxide or lipid peroxides with reduced glutathione. CAT catalyses the breakdown of hydrogen peroxide to molecular water and oxygen. GSH has a very

important role in the protection against free radical damage by providing reducing equivalents for several key enzymes. On the other hand, GSH is a scavenger of hydroxyl radicals and singlet oxygen (Cadenas and Cadenas, 2002). When antioxidant capacity is not sufficient against ROS, lipid peroxidation occurs in the cell. TBARS are formed during lipid peroxidation, and they are accepted as a marker of lipid peroxidation (Mcdonald *et al.*, 2003).

In the present study, the antioxidant effect of *Viscum album* and *Aristolochia clematitis* alcoholic extracts in liver was investigated. For this reason, SOD, CAT, GPX, GST, G6PDH and GSH were measured as antioxidants, and TBARS were measured as a biomarker of lipid peroxidation. The liver was selected because it is one of the tissues showing a high rate of free radical generation.

1. MATERIALS AND METHODS

Preparation of ethanolic extracts

In this study, there were used dried aerial parts of mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)*. The interest parts of plants were powdered and extracted with ethanol 60 % (1:10 ratio, w:v) for 3 hours at 60ºC. The homogenates obtained were filtered using filter paper Watman no. 1 and the filtrates were then centrifuged for 20 min at 5000 rpm and 5ºC. After the ethanol was evaporated, the aqueous residues were utilized.

Animals

The animals used in this study were purchased from Cantacuzino Institute. In this study, thirty adult female albino mice (25-30 g weight, 9 weeks old) were used as experimental animals. They were kept in polypropylene cages under standard laboratory conditions of 12 h/12 h light/dark, 22 ± 2°C temperature, fed with a normal rodent diet one week before the experiment. Starvation was used prior to all assays because polyphenols extracts were always administered orally (by gavage) using distilled water as vehicle. Oxidative stress was induced in experimental albino mice by keeping them in special lighting conditions (6 hours of daylight and 18 hours of darkness). The administration of plant extracts to mice began seven days before inducing oxidative stress. All the pharmacological experimental protocols respected European legislation for experimental animals.

Experimental protocol

The mice were randomly divided into 6 groups of 5 animals each, as follows:

- Group 1: Normal control mice treated with 0.9% NaCl: mice were orally administered 1 mL 0.9% NaCl, for 21 days.
- Group 2: Normal control mice treated with mistletoe extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.
- Group 3: Normal control mice treated with birthwort extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.
- Group 4: Control mice group was stressed and treated with 0.9% NaCl: mice were orally administered 1 mL 0.9% NaCl, for 21 days.
- Group 5: Stresed mice were treated with mistletoe extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.
- Group 6: Stresed mice were treated with birthwort extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.

Twenty-four hours following last administration, the animals were sacrificed by cervical dislocation. The abdomen was excised and the liver was removed immediately by dissection, washed in ice-cold isotonic saline and blotted between two filter papers. The liver was transferred into preweighed vials to determine the wet weight. A 10% (w/v) liver homogenates was prepared in ice-cold 0.1 M potassium phosphate buffer, pH 7.5.

Determination of lipid peroxidation

The measurement of liver lipid peroxide by a colorimetric reaction with thiobarbituric acid was done as described by Ohkawa et al. (Ohkawa *et al.*, 1979), and the determined lipid peroxide is referred to as malondialdehyde. Briefly, in a test tube, 20% trichloroacetic acid solution and 0.67% thiobarbituric acid solution were added to the homogenate. The color of thiobarbituric acid pigment was developed in a water bath at 100°C for 20 min. After cooling with tap water to room temperature, 2mL *n*-butanol was added and shaken vigorously. After centrifugation, the color of

butanol layer was measured at λ_{max} 532 nm. The TBARS concentration of the sample was calculated using the extinction coefficient of MDA (1.56×10^5 $M^{-1}cm^{-1}$) and the values were expressed as nmol/mg protein.

Determination of superoxide dismutase activity (SOD)

The activity of superoxide dismutase in liver was measured using a commercial kit (Fluka Analytical). This method uses xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4- iodophenyl)- 3- (4- nitrophenol)- 5- (2, 4- disulfophenyl)-2H-tetrazolium, monosodium salt to form a water soluble formazan dye. The values are expressed as Units/mg of protein in liver tissue.

Determination of catalase activity (CAT)

Catalase activity was measured by the method described by Aebi (Aebi, 1984). Supernatant was added to cuvette containing 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as Units/mg of protein.

Determination of glutathione peroxidase activity (GPx)

The method is based on the oxidation of gluthatione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate, Reduced) (Gupta and Baquer, 1998). Final reaction mixture (3 ml) was 65 mM KH_2PO_4/K_2HPO_4, pH 7.5, 2 mM GSH, 1 U glutathione reductase, 0.12 mM NADPH and 8 mM tert buthyl hydroperoxide. Activity of glutathione peroxidase was expressed as Units/mg of protein.

Determination of liver glutathione S-transferase (GST) activity

Estimation of the liver GST activity was carried out by the method of Habig *et al.* (Habig *et al.,* 1974). The reaction mixture consisted of 1.425 mL 0.1M phosphate buffer (pH 6.5), 0.2 mL of 1 mM reduced glutathione, 0.025 ml 1mM 1-chloro-2,4-dinitrobenzene (CDNB) and 0.3 ml 10% liver homogenate in a total volume of 2.0 ml. The changes in absorbance were recorded at 340 nm and enzymatic activity was calculated as nmol CDNB conjugate formed / min / mg protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Determination of liver glucose-6-phosphate dehydrogenase (G6DH) activity

Estimation of glucose-6-phosphate dehydrogenase (G6DH) of liver homogenate was performed using the method described by Kornberg, A. and Horecker, BL (Kornberg, A. and Horecker, BL, 1955). Glucose-6-phosphate dehydrogenase (G6FD) catalyzes the first reaction of pentozo-phosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6-phosphogluconat and reducing $NADP^+$ to NADPH. Coenzyme nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to nicotinamide adenine dinucleotide phosphate reduced form (NADPH) in the presence of G-6-P. NADPH formation rate is directly proportional to the activity and assessed spectrophotometrically G6DH at a wavelength of 340 nm.

Determination of liver reduced glutathione (GSH)

Reduced glutathione in liver was determined by the method of Jollow *et al.* (Jollow *et al.*, 1974). An aliquot of liver homogenate (10% in 0.1M phosphate buffer) was precipitated with sulfosalicylic acid (4%). The samples were kept at 4°C for 1h and then subjected to centrifugation at 4000 rpm for 15 min at 4°C. The assay mixture contained 0.1 mL aliquot from the supernatant, 0.1M phosphate buffer (pH 7.4) and dithionitrobenzene (DTNB) in a total volume of 3.0 ml. The optical density of the yellow color developed was read immediately at 412 nm in a spectrophotometer. GSH was expressed as mg / 100 g tissue using a GSH standard curve.

Determination of total proteins

Total proteins were determined according to the method of Lowry, using bovin seric albumin (BSA) as a standard (Lowry *et al.*, 1951).

Statistical data interpretation

Statistical data interpretations were calculated with EXCEL program from Microsoft Office package. All the data are shown as mean value ± standard deviation (SD). Number of mice per group n = 5. Statistical data interpretation considered the corresponding differences for a given significance threshold: $p>0.05$ statistically insignificant; $*p<0.05$ statistically significant; $**p<0.01$ strong statistical significance; $***p<0.001$ very strong statistical significance.

2. RESULTS AND DISCUSSION

Determination of lipid peroxidation

When the antioxidant capacity is insufficient against ROS, lipid peroxidation occurs and TBARS is formed. In the present study, stress induced oxidative damages in liver characterized by increased MDA concentration. In the present study, when mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)* alcoholic extracts were administered in normal mice, were founded ($p<0.001$) decreased TBARS levels in liver. Pretreatment of normal mice with mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)* alcoholic extracts decreased TBARS level in the liver; however the polyphenolic treatment in stressed mice have the same effects on TBARS in this tissue (Fig. 1). It was evidenced that the treatment of stressed mice with mistletoe extracts significantly ($p < 0.001$) decreased the amount of TBARS in the liver (Fig. 1).

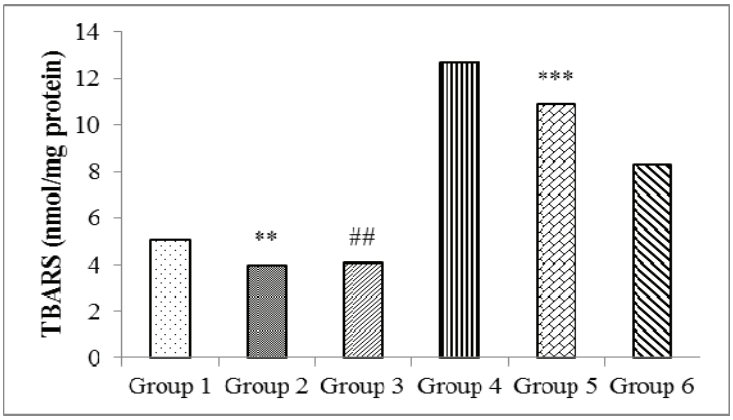

Fig. 1. The influence of oral administration of plant polyphenols (100 mg/kg) on lipid peroxidation in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. ** $p < 0.01$ and # # $p < 0.01$ vs 1st mice group; *** $p < 0.001$ vs 4th mice group.

Determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6DH) activities

SOD, GPx, CAT, GST and G6DH activities of liver mice in the experimental groups are given in Table 1. As shown in Table 1, hepatic superoxide dismutase (SOD) and gluthation peroxidase (GPx) activity were unchanged and gluthation S transpherase (GST) was increased in mice

treated with polyphenolic extracts. When stressed mice were treated with mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)* extracts, SOD CAT GPx GST and G6DH activities were found to be increased from those of the control group (G4). In the present study, mistletoe *(Viscum album)* extract administration caused a significant ($p<0.05$) increase of SOD and G6DH activities in liver, a strong significant ($p<0.01$) increase of liver GPx and GST activity and a very strong significant ($p<0.001$) increase of CAT activity in liver. In the present study, the significant increase of CAT and GPX activities was mainly observed in liver stressed mice treated with mistletoe polyphenolic extracts. Also, significant increase of liver SOD and GPX activities was observed in stressed mice treated with birthwort polyphenolic extracts.

Table 1

The influence of oral administration of plant polyphenols (100 mg/kg) to mice on antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6DH)]

Animal groups	**SOD U/mg protein**	**CAT U/mg protein**	**GPx U/mg protein**	**GST U/mg protein**	**G6DH U/mg protein**
Group 1	9.2±0.2	4.2±0.2	5.1±0.5	8.4±0.8	11.5±0.3
Group 2	9.1±0.3	4.3±0.5	5.2±0.3	8.6±0.4	11.4±0.5
Group 3	9.3±0.3	4±0.4	5.1±0.3	8.7±0.5	11.2±0.6
Group 4	4.8±0.8	1.4±0.2	1.8±0.4	3.9±0.3	5.9±0.7
Group 5	5.5±0.4	2.4±0.4	3.2±0.5	5.1±0.5	6.7±0.4
Group 6	7.2±0.4	2±0.6	2.8±0.7	4.9±0.5	6.9±0.5

Determination of liver reduced glutathione (GSH)

The significant ($p<0.001$) reduction in the liver non enzymatic antioxidant system (GSH) in stressed mice (G4) as compared to the control unstressed group (G1) could be responsible for increased lipid peroxidation levels observed during oxidative stress induced.

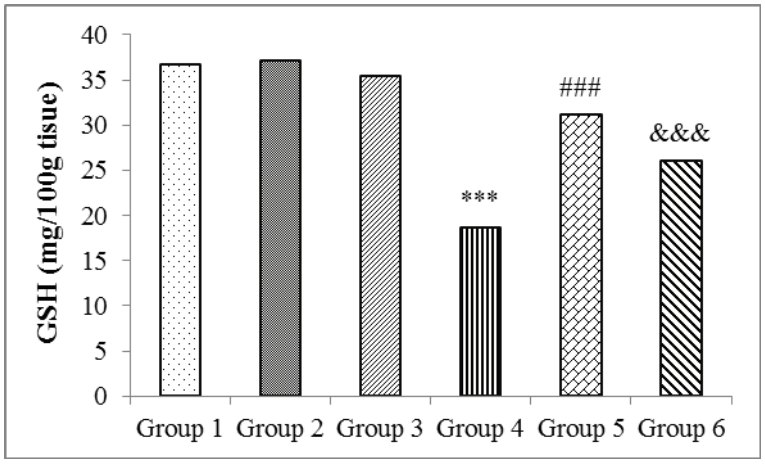

Fig. 2. The influence of oral administration of plant polyphenols (100 mg/kg) on GSH levels in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 1st mice group; ### $p < 0.001$ and &&& $p < 0.001$ vs 4th mice group.

Mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)* extracts caused a statistically significant ($p<0.001$) increases in GSH levels when stressed mice were treated.

3. CONCLUSIONS

3.1. Polyphenols supplementation to stressed mice decreased the level of TBARS, compared to control group.

3.2. The treatment of stressed mice with plant polyphenols in quantity of 100 mg/kg significantly improved the levels of SOD, CAT, GPx, GST and G6DH activities in liver homogenates.

3.3. Polyphenols extracted from mistletoe *(Viscum album)* and birthwort *(Aristolochia clematitis)* improved the levels of GSH in stressed mice liver homogenates when compared with stressed untreated mice.

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ANTIOXIDANT POTENTIAL OF *LYCOPODIUM CLAVATUM* AND *CNICUS BENEDICTUS* HYDROETHANOLIC EXTRACTS ON STRESSED MICE

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Key words: *Lycopodium clavatum, Cnicus benedictus*, lipid peroxidation (LPO), reduced glutathione (GSH), antioxidant enzymes.

SUMMARY

The study was planned to evaluate the efficacy of club moss (*Lycopodium clavatum*) and blessed thistle (*Cnicus benedictus*) hydroethanolic extracts in preventing oxidative stress damage in liver tissue of mice. Stressed and unstressed mice randomly divided into 6 groups were treated with and without plant extracts for 21 days.

The oxidative stress was measured by lipid peroxidation (LPO) level, reduced glutathione content, total protein level and by enzymatic activities of SOD, CAT, GPx and GST in liver tissue homogenate. Oxidative stress induced in mice by maintaining them in special lighting conditions (6 hours light and 18 hours of darkness) enhanced lipid peroxidation with concomitant reduction in SOD, CAT, GPx, GST, GSH and total protein content. Treatment of mice with plant polyphenols resulted in marked improvement in most of the studied parameters.

Several studies have shown that, at the cellular level, oxidative stress often involved in the production of reactive oxygen species (ROS). ROS includes the superoxide radical (O2•−), hydrogen peroxide (H2O2), and the hydroxyl radical (•OH), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids (Damien et al., 2004). The importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses by scavenging of ROS. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST) and glutathione peroxidase (GPx). Superoxide radicals that are generated are converted to H2O2 by the action of SOD, and the accumulation of H_2O_2 is prevented in the cells by CAT and GPx. It has been demonstrated that the activities of SOD, CAT, and GPx activity are modified by some external factors in response to stress (Fornazier et al., 2002a; Lee and Shin, 2003).

Medicinal plants are commonly used for the treatment of various ailments, as they are considered to have advantages over the conventionally used drugs that are much expensive and known to have harmful side effects.

1. MATERIALS AND METHODS

Preparation of hydroethanolic extracts

In this study, there were used dried aerial parts of club moss (*Lycopodium clavatum*) and blessed thistle (*Cnicus benedictus*). The interest parts of plants were powdered and extracted with ethanol 60 % (1:10 ratio, w:v) for 3 hours at 60ºC. The homogenates obtained were filtered using Watman no. 1 filter paper and the filtrates were then centrifuged for 20 min at 5000 rpm and 5ºC. The extracts were used after ethanol was evaporated.

Animals

The animals used in this study were purchased from Cantacuzino Institute. In this study, thirty adult female albino mice (25-30 g weight, 9 weeks old) were used as experimental animals. They were kept in polypropylene cages under standard laboratory conditions of 12 h/12 h light/dark, 22 ± 2°C temperature, fed with a normal rodent diet one week before the experiment. Starvation was used prior to all assays because polyphenols extracts were always administered orally (by gavage) using distillated water as vehicle. Oxidative stress was induced in experimental albino mice by keeping them in special lighting conditions (6 hours of daylight and 18 hours of darkness). The administration of plant extracts to mice began seven days before inducing oxidative stress. All the pharmacological experimental protocols respected European legislation for experimental animals.

Experimental protocol

The mice were randomly divided into six groups of five animals:

- Group 1: Normal control mice treated with distillated water.
- Group 2: Normal control mice treated with club moss extract: mice were orally administered polyphenols in a dose of 100 mg kg^{-1}, for 21 days.
- Group 3: Normal control mice treated with blessed thistle extract: mice were orally administered polyphenols in a dose of 100 mg kg^{-1}, for 21 days.

- Group 4: Control mice with induced oxidative stress: mice were treated with distillated water, for 21 days.
- Group 5: Mice with induced oxidative stress treated with club moss extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.
- Group 6: Mice with induced oxidative stress treated with blessed thistle extract: mice were orally administered polyphenols in a dose of 100 mg kg−1, for 21 days.

Twenty-four hours following last administration, the animals were sacrificed by cervical dislocation. The abdomen was excised and the liver was removed immediately by dissection, washed in ice-cold isotonic saline and blotted between two filter papers. The liver was transferred into preweighed vials to determine the wet weight. 10% (w/v) liver homogenates were prepared in ice-cold 0.1 M potassium phosphate buffer, pH 7.5.

Determination of lipid peroxidation

The measurement of liver lipid peroxide by a colorimetric reaction with thiobarbituric acid was done as described by Ohkawa et al. (Ohkawa H. *et al.*, 1979). Briefly, in a test tube, 20% trichloroacetic acid solution and 0.67% thiobarbituric acid solution were added to the homogenate, in a final volume of 2 mL. The color of thiobarbituric acid pigment was developed in a water bath at 100°C for 20 min. After cooling with tap water to room temperature, 2 mL *n*-butanol was added and shaken vigorously. After centrifugation, the color of butanol layer was measured at λ_{max} 532 nm. The TBARS concentration of the sample was calculated using the extinction coefficient of MDA (1.56×10^5 $M^{-1}cm^{-1}$) and the values were expressed as nmol/mg protein.

Determination of superoxide dismutase activity (SOD)

The activity of superoxide dismutase in liver was measured using a comercial kit (Fluka analytical). The values are expressed as Units/mg of protein in liver tissue.

Determination of catalase activity (CAT)

Catalase activity was measured by the method described by Aebi (Aebi, 1984). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as Units/mg of protein.

Determination of glutathione peroxidase activity (GPx)

The method is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate, Reduced) (Gupta and Baquer, 1998). GPX activities were measured by linking the reaction to that of glutathione reductase and following the decrease in NADPH at 340 nm (extinction coefficient of 6.22×10^3 $M^{-1}\times cm^{-1}$) at 25°C, for 5 min. Activity of glutathione peroxidase was expressed as Units/mg of protein.

Determination of liver glutathione S-transferase (GST) activity

Estimation of the liver GST activity was carried out by the method of Habig *et al.* (Habig *et al.*, 1974). The changes in absorbance were recorded at 340 nm and enzymatic activity was calculated as nmol CDNB conjugate formed / min / mg protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Determination of liver reduced glutathione (GSH)

Reduced glutathione in liver was determined by the method of Jollow *et al.* (Jollow *et al.*, 1974). GSH was expressed as mg / 100 g tissue using a GSH standard curve.

Determination of total proteins

Total protein was determined according to the method of Lowry *et al.* using bovine seric albumin (BSA) as a standard (Lowry *et al.*, 1951).

Statistical data interpretation

Statistical data interpretation was calculated with EXCEL program from Microsoft Office package. Statistical data interpretation considered the corresponding differences for a given significance threshold: $p>0.05$ statistically insignificant; $*p<0.05$ statistically significant; $**p<0.01$ strong statistical significance; $***p<0.001$ very strong statistical significance.

2. RESULTS AND DISCUSSION

Determination of lipid peroxidation

In the present study, considerably rise in the TBARS level was observed for stressed mice group when compared with unstressed mice,

which suggests that enhanced lipid peroxidation was a consequence of tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with polyphenolic extracts lipid peroxidation was strongly significant ($p<0.01$) prevented when the source was club moss (*Lycopodium clavatum*) and very strongly significant ($p<0.001$) prevented when the source was blessed thistle (*Cnicus benedictus*), indicating its antioxidant action. The concentration of TBARS and was significantly higher in liver of stressed untreated mice, as compared to normal control animals (G1) (Fig. 1).

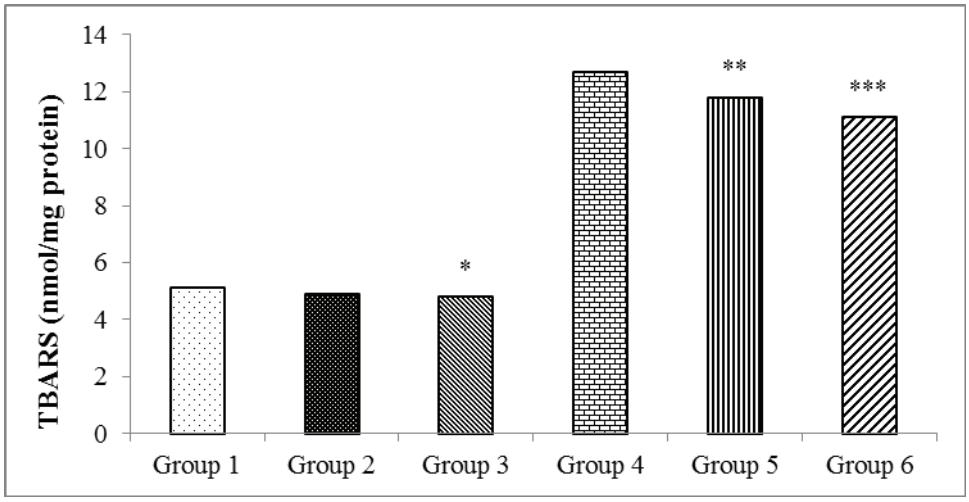

Fig. 1. The influence of oral administration of plant polyphenols (100 mg/kg) on lipid peroxidation in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. * $p < 0.05$ vs 1st mice group; ** $p < 0.01$ and *** $p < 0.001$ vs 4th mice group.

Determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities

Antioxidant enzyme levels are applied as markers of oxidative stress. Based on the present study stress induced toxicity might result in decreased tissue activities of enzymatic antioxidants SOD, CAT and GPx. The decrease of SOD and CAT activities might predispose the examined tissue of mice to oxidative stress, because these enzymes catalyze the decomposition of ROS. The levels of these antioxidants might provide a

clear indication on the extent of cytotoxic damage that occurs in hepatic tissue. Diminished or inhibition in the activities of these antioxidants upon stress exposure may led to increased oxidative modifications of cellular membrane and intracellular molecules. Activities of antioxidant investigated enzymes are presented in Table 1.

The potential mechanism for stress induced alterations in superoxide dismutase and glutathione peroxidase activities might be inhibition of their functional groups or through binding to their metal enzyme cofactors. The GPx catalyses the oxidation of GSH to GSSG and this oxidation reaction occurs at the expense of (H_2O_2). GST is a family of the enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates, which have electrophilic functional groups. They play an important role in detoxification and metabolism of many xenobiotic and endobiotic compounds (Hsu and Guo, 2002).

Table 1

The influence of oral administration of plant polyphenols (100 mg/kg) to mice on antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST)].

Animal Groups	**SOD U/mg protein**	**CAT U/mg protein**	**GPx U/mg protein**	**GST U/mg protein**
Group 1	9.2	4.2	5.1	8.4
Group 2	9.2	4.2	5	8.5
Group 3	9.1	4.1	4.9	8.5
Group 4	4.8	1.4	1.8	3.9
Group 5	5	1.5	2.1	4.2
Group 6	5.7	1.7	2.7	4.8

Determination of liver reduced glutathione (GSH)

Stress induced decrease in GSH concentration in the liver. This may be one of the mechanisms of peroxidative action of stress in this organ. Enzymes, such as GPx and GST, take part in maintaining GSH homeostasis in tissues. The mutual relations between GST and GSH in the redox system, the simultaneous decrease in both GST activity and GSH concentration and the positive correlation between these parameters may suggest that the decrease in the hepatic GSH concentration might result, at least partly, from

the decrease in GST activity. GSH content in liver of group 4 animals showed a significant decline when compared with control group (G1) (Fig. 2).

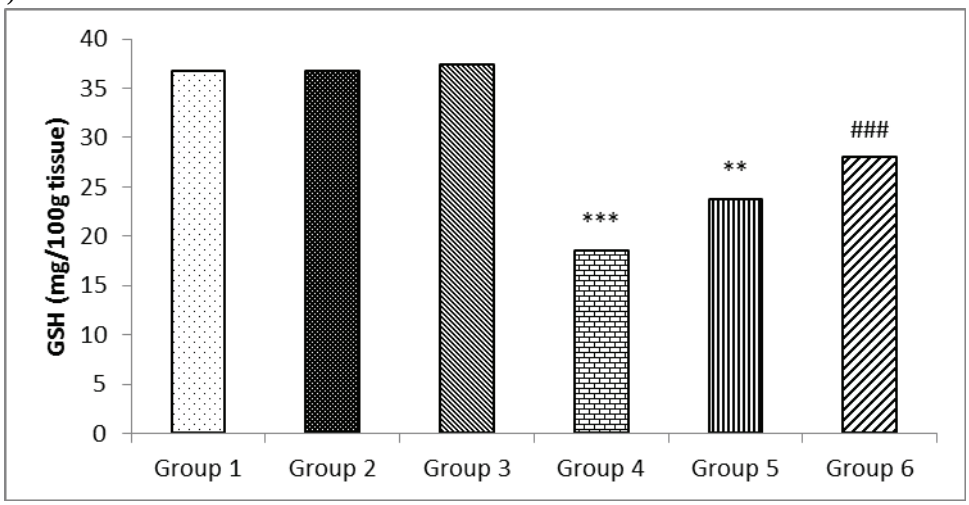

Fig. 2. The influence of oral administration of plant polyphenols (100 mg/kg) on GSH levels in the liver of normal and stressed mice. Data are expressed as mean ± S.D. Number of mice per group n = 5. *** $p < 0.001$ vs 1st mice group; ### $p < 0.001$ and ** $p < 0.01$ vs 4th mice group.

3. CONCLUSIONS

3.1. Polyphenols supplementation to stressed mice groups improved the level of TBARS, compared to control untreated stressed mice.

3.2. The treatment of stressed mice with plant polyphenols (100 mg/kg) significantly improved the levels of GST activities in liver homogenates.

3.3. Polyphenols extracted from club moss (*Lycopodium clavatum*) and blessed thistle (*Cnicus benedictus*) improved the levels of GPx activity in liver homogenates.

3.4. The CAT activities in liver homogenates obtained from stressed mice groups treated with plant extracts were almost similar to normal control mice group.

3.5. Club moss (*Lycopodium clavatum*) and blessed thistle (*Cnicus benedictus*) polyphenols administration improved GSH concentration in stressed mice liver homogenates when compared with untreated stressed mice liver.

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CAMPYLOBACTER, FROM UNKNOWN TO FAMOUS

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Key words: *Campylobacter*, zoonosis, morbidity, poultry

SUMMARY

Campylobacter species have been the focus of growing attention for the past 30 years because of the increasing frequency with which they have been isolated from humans, animals, food and water.

Found frequently in animals, particularly in bovines and ovines, *Campylobacter* has been known for more than 40 years as a cause of disease.

After its successful isolation from stools in the 1970s, *Campylobacter jejuni* has rapidly become the most commonly recognised cause of bacterial gastroenteritis in humans. Campylobacteriosis is a zoonosis, the reservoir of infection comprising wild and domestic animals, particularly birds. Chickens constitute by far the largest potential source of human infection. Campylobacteriosis is mainly a food-borne infection in which foods of animal origin, particularly poultry, play an important role. Any raw meat bred for consumption may be contaminated with *Campylobacter* organisms.

Although several *Campylobacter* species (*C. jejuni*, *C. coli*, *C. upsaliensis*, *C. lari*, *C. concisus*, *C. fetus* subsp. *fetus*, *C. jejuni* subsp. *doylei*, *C. hyointestinalis*) and *Arcobacter butzleri* have been shown to cause diarrhoea, *C. jejuni* is by far the most frequent species isolated from humans. *C. jejuni* is a frequent cause of morbidity, in both industrialised and developing countries, and represents a considerable drain on economic and public health resources.

In industrialised countries, most infections are acquired through the handling and consumption of poultry meat. In developing countries, where the disease is confined to young children, inadequately treated water and contact with farm animals are the most important risk factors.

The first description of a bacteria now belonging to the genus *Campylobacter* is attributed to Theodore Escherich, at the end of the nineteenth century (Vandamme, 2000). At the beginning of the twentieth century, a *Campylobacter* species, described as a related *Vibrio*, was recognized as causing abortions in sheep.

Despite their widespread occurrence, *Campylobacter* species were not understood as a cause of diarrhoea in humans until 1957 (King, 1957), and their impact in terms of sheer numbers of human infections emerged only in the past 30 years. Only after a suitable isolation medium was developed in the 1970s, two closely related pathogens, *C. jejuni* and *C. coli*, were recognized to be common human enteric pathogens (DeKeyser *et al*., 1972). *C. jejuni* accounts for approximately 90% of human *Campylobacter* infections (Kramer *et al*., 2000).

The family *Campylobacteraceae* consists of two genera, *Campylobacter* and *Arcobacter* and occur primarily as commensals in humans and domestic animals

(Vandamme, 2000). The genus *Campylobacter* contains more of 18 species of which *Campylobactecter jejuni*, *Campylobacter coli* and *Campylobacter lari* are pathogens for humans; these microorganisms are small (0.2–0.8μm × 0.5–5μm), Gram-negative, slender spirally curve rods. When two or more bacterial cells are grouped together, they form an "S" or a "V" shape of gull-wing. The majority of the species have a corkscrew-like motion by means of a single polar unsheathed flagellum at one or both ends of the cell. The only exceptions are *Campylobacter gracilis*, which is non-motile, and *Campylobacter showae*, which has multiple flagella (Debruyne et al., 2005). Oxidase activity is present in all species except for *C. gracilis*. They neither ferment nor oxidize carbohydrates; instead, they obtain energy from aminoacids or tricarboxylic acid cycle intermediates (Vandamme, 2000).

Campylobacter jejuni hydrolyzes hippurate, indoxylacetate and reduces nitrate. Most strains are resistant to cephalothin, and also resistance to fluoroquinolones, a category of antibiotics normally used to treat animal and human illness (Koenraad et al., 1995). Under unfavourable growth conditions, these microorganisms have the ability to form viable but non-culturable cells (VBNC) (Porter et al., 2007). It has been observed, under laboratory conditions, that *Campylobacter* strains isolated from the soil around broiler house, may have been transformed into viable but non-cultivable forms and might have become cultivable after passing through the intestinal tract of chickens. Many questions have been raised on whether non-culturability equates to non-viability, whether it is possible to convert the VBNC form to a culturable form, and whether, indeed, a VBNC form of *Campylobacter* actually exists (ACMSF, 2010).

MICROBIOLOGY

Bacteria belonging to the genus *Campylobacter* are non-spore forming, oxidase-positive, Gram-negative rods. Thermophilic *Campylobacter* species are able to grow between 37 and 42˚C, but incapable of growth below 30˚C, with an optimum temperature of 41.5˚C. *C. jejuni* and *C. coli* are distinguished from most other *Campylobacter spp*. by their high optimum growth temperature (41,5°C). Levin (2007) suggested that these organisms should be referred to as "thermotolerant" since they do not exhibit true thermophily (grow at 55˚C or above). However, a study by De Cesare et al. (2008) revealed that *C. jejuni* survived for more than 4h at 27˚C and 60–62% relative humidity on some common clean or soiled food contact surfaces. These characteristics reduce the ability of *Campylobacter* to multiply outside of an animal host and in food during their processing and storage (Park, 2002). Growth does not occur in environments with water activity (*a*w) lower than 0.987 (sensitive to concentrations of sodium chloride (NaCl) greater than 2%w/v), while optimal growth occurs at *a*w =

0.997 (approximately 0.5% w/v NaCl). Freezing–thawing also reduces the population of *Campylobacter spp.* (Engberg, 2006). In pure cultures, *Campylobacter* spp. are normally inactivated by frozen storage at -15˚C for 3 days (Engberg, 2006); however, freezing does not eliminate the pathogen from contaminated foods (Lee et al., 1998). Hazeleger et al. (1995) revealed that aged *C. jejuni* cells survived the longest at 4˚C. *Campylobacter* spp. may persist for prolonged periods in chilled and frozen products; though a reduction in the concentration and viability has been recorded after several weeks of storage at 4°C and in frozen poultry after several months (Solow et al., 2003). *Campylobacter* spp. will not survive below a pH of 4.9 and above pH 9.0 and grow optimally at pH 6.5–7.5. These non-spore-forming and fastidious bacteria are essentially microaerophilic, growing the best in an atmosphere with low oxygen tension (5% O_2, 10% CO_2, and 85% N_2) (Garénaux et al., 2008).

LABORATORY METHODS FOR THE ISOLATION OF *CAMPYLOBACTER*

The sensitivity of *Campylobacter spp.* to oxygen and oxidizing radicals has led to the development of several selective media containing one or more oxygen scavengers, such as blood, ferrous iron, pyruvate, etc., and elective agents, particularly antibiotics. In some protocols, in order to ameliorate the inhibitory effects of these selective agents on potentially damaged cells, initial suspension of samples is made into a basal broth without selective agents, with the latter being gradually added after a short period of incubation. In order to permit recovery of damaged cells, the incubation temperature may also be gradually increased from 37˚C to the final incubation temperature of 41.5˚C. This methodology is the basis for one of the ISO standard methods (ISO 10272/1/2, 2006). Several of the selective broths, e.g. Bolton broth (BB), *Campylobacter* enrichment broth (CEB), Park and Sanders broth and Preston broth (PB), have been compared for their efficacy (Baylis et al., 2000; Nicorescu and Crivineanu, 2008). Of them, Bolton broth has inhibited the best the microflora of association and has stimulated the growth of *Campylobacter*.

The incorporation of the enzyme oxyrase in selective broths is particularly effective in reducing the levels of oxygen and improving the isolation of *Campylobacter spp.* from naturally contaminated samples. As

reported by Wonglumson et. al (2001), enrichment broth with added oxyrase incubated under aerobic conditions was as efficient as microaerophilic incubation for recovery of *Campylobacter jejuni* from artificially inoculated food samples.

A comparison by Federighi et al. (1999), of Karmali, Butzler, and Skirrow isolation agars after enrichment of a large number and wide range of samples in Preston or Park and Sanders broths, showed that Park and Sanders broth followed by isolation on Karmali agar was the most effective combination. In a study performed by Nicorescu (2009), four selective media for isolation (mCCDA, Karmali agar, Skirrow agar and Preston agar) were tested using reference strains; of them mCCDA and Preston agar provided a better selectivity for isolation of *C. jejuni* and *C. coli* than other media tested.

The most recent standard method (ISO 10272-1) for detection and isolation, and a direct plating method for enumeration of campylobacters (ISO 10272-2) use mCCDA as the selective agar. Bolton broth is used for the enrichment step and the suspension is incubated at 37˚C in a microaerophilic atmosphere for 4–6h, followed by 41.5˚C for 40–48 h and plating on mCCDA and an other agar medium of the operator's own choice. However, the isolation of *Campylobacter spp.* is difficult because its sensitivity of oxygen and its fastidious nature.

Several alternative and rapid methods have been developed for detecting and confirming *Campylobacter spp.*, including immuno-enzimatic fluorescent test (ELFA), fluorescence *in situ* hybridization (FISH) (Lehtola et al., 2006), latex agglutination, and a physical enrichment method (filtration) that permits the separation of *Campylobacter* from other organisms present in the food matrix. Perhaps the most effective confirmation methods are those based on the polymerase chain reaction (PCR), since the phenotypic reactions are often atypical and difficult to read, e.g., the hippurate hydrolysis test for differentiating *Campylobacter coli* from *C. jejuni.* The PCR reaction has been combined with immuno-separation with some success (Waller and Ogata, 2000) in detecting low numbers. However, some components of both food samples and selective broths can be inhibitory to the PCR reaction. More recently, real-time PCR methods have been developed, having the potential of detecting as few as 1 cfu in chicken samples, and in less than 2h (Debretsion et al., 2007).

Epidemiological studies have been benefited from the use of molecular typing techniques such as PCR, random amplification of polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE). In the present, PFGE technique is the most often used for typing of Campylobacter.

SOURCES OF ILLNESS AND RISK FACTORS

Campylobacter is a leading cause of zoonotic enteric infections in humans in most developed and developing nations (WHO, 2002). The sources and incidences of illness differ, sometimes quite dramatically, between developed and developing countries. The reported incidence of *Campylobacter* infections has markedly increased in many developed countries within the last 20-year period. Under-reporting of *Campylobacter* infections is an issue in most countries and incidence rates only reflect the number of laboratory-confirmed cases. According to the latest report of EFSA (European Food Safety Authority), *Campylobacter* continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in EU. The highest numbers and notification rates of *Campylobacter* cases in humans were reported during the summer months, from June to August, and started gradually decreasing from September to December. Cases are usually caused by *Campylobacter jejuni,* and to a lesser extent by *Campylobacter coli* (EFSA, 2011). The most frequently reported *Campylobacter* species in 2009 was *C. jejuni* (36.4 %) and accounted for 90% of the cases characterised at species level (EFSA, 2011).

Most *Campylobacter* infections in humans are classified as sporadic cases, or as part of small, family-related outbreaks, and identified outbreaks are relatively uncommon.

Direct transmission is mainly occupational (farmers, butchers, abattoir workers, poultry processors), but pets can bring infection into ordinary homes. Inter-human transmission has been described infrequently in young children. Perinatal transmission, from a patient who is not necessarily symptomatic, may occur following exposure *in utero*, during passage through the birth canal, or during the first days of life. Many cases of campylobacteriosis are associated with foreign travel, accounting for 3—50% or more of all cases, and usually result from the consumption of contaminated food or water in the visited countries. In developing countries where campylobacters are hyper-endemic, the disease is confined to young

children who, through repeated exposure to infection, develop immunity early in life (Oberhelman and Taylor, 2000; Coker et al., 2002). In these countries, *Campylobacter* plays an important role, and its effects are particularly acute during weaning. Consequently, campylobacteriosis contributes significantly to malnutrition in infants, who represent an at-risk group. In developing countries, exposure in the household to the faeces of live chickens infected with *C. jejuni* is the predominant risk factor for childhood diarrhoea. Exposure to inadequately treated water is also assumed to be an important risk factor.

Overall, chicken, poultry and other foods are thought to be the most likely potential sources of infection in developed countries (Friedman et al. 2004; Neiman et al., 2003). It is generally accepted that *C. jejuni* colonizes the avian gut as a commensal and colonized broilers carry a large number of bacteria in their ceca (generally around 10^6 to 10^8 cfu/g), the predominant site for colonization (Harvey et al., 2003). Ingestion of *C. jejuni* numbers as few as 35 cfu can be sufficient for successful colonization of chicks. After ingestion, the bacterium reaches the cecum and multiplies, resulting in an established colonizing *Campylobacter* population within 24 hours after entrance.

During the slaughter operations (scald, de-feather, evisceration, wash, chill) is possible the dissemination of *Campylobacter* from ceca to skin and meat; these products became so contaminated, representing a potential danger.

Many studies have identified handling of raw poultry and the consumption of poultry products as important risk factors, accounting for a variable percentage of cases (Neimann et al., 2003; Wingstrand et al., 2006; Kapperud et al. 2003; Studahl and Andersson, 2000).

The incidence of *Campylobacter* in raw chicken is different between the countries of Europe, varying from 0% to 95,8%. In Romania, this incidence was in 2010 about 40% (EFSA, 2011). In addition, cross-contamination of *Campylobacter* from raw chicken to prepared food has been identified as a risk factor (Kapperud et al., 2003).

Other food-related risk factors that have repeatedly been identified include consumption of other meat types, and undercooked or barbecued meat raw seafood, drinking untreated surface water, unpasteurized milk or dairy products (Neimann et al., 2003; Studahl and Andersson, 2000)

Although the meat represents the principal vector for gastroenteritis produced by *Campylobacter*, the milk can be implicated in production of illness. Surveys of bulk tank milk indicate that unpasteurized milk is a source of *C. jejuni*. In one study, approximately 10% of unpasteurized milk specimens from dairy bulk tanks were contaminated with *C. jejuni* (Jayarao and Henning, 2001). In 2011, in Sweden was recorded an outbreak of gastroenteritis, consequently the consume of milk from a farm family (Hansson, 2011). To minimize the risk for illness associated with milk borne pathogens, unpasteurized milk and milk products should not be consumed. Good milking hygiene and properly working milking machines reduce the risk of faecal contamination of raw milk.

Surface waters are often contaminated with campylobacters. In a Norwegian study, 32 of 60 water specimens from the Bo River contained campylobacters, and *C. coli* was detected more often than *C. jejuni* (Rosef et al., 2008). In developing countries, waterborne transmission, direct contact with animals, and environmental sources are thought to be the major routes of human infection (WHO, 2002; Coker et al., 2002).

The clinical manifestations of *Campylobacter* infections in humans also differ between developed and developing countries, both in the ages of the affected populations and in the severity of illness. In developed countries, *Campylobacter* enteritis often affects older children and young adults and can be severe, characterized by fever, abdominal cramping and bloody diarrhoea that may require treatment with antimicrobials. In contrast, *Campylobacter* infections in developing countries tend to affect children under one year of age, with more severe symptoms and illness. In older children, the illness and symptoms are often milder. Strain differences could be one explanation for these observed epidemiological differences, with fewer yet more severe infections in developed countries compared with a larger number of milder infections in young children in developing nations. *C. jejuni* is responsible for a majority of *Campylobacter* infections in both developing and developed countries, although strains such as *C. coli*, *C. lari*, *C. upsaliensis* and *C. hyointestinalis*, and others, may be responsible for a larger proportion of infections in developing compared to developed countries.

In the industrialized world, acute self-limited gastrointestinal illness, characterized by diarrhoea, fever and abdominal cramps, is the most

common presentation of *C. jejuni* infection, but symptoms and signs are not so distinctive that the physician can differentiate this infection from illness caused by other organisms (WHO, 2002). The incubation period is commonly 2–5 days, but estimates have extended up to 10 days. In about 50% of patients, diarrhoea is preceded by a febrile period with malaise, myalgia, abdominal pain and fever of about 40°C; fresh blood may appear in the stools by the third day. Faecal samples show an inflammatory exudate with leukocytes on microscopic examination; moreover, it is usually possible to recognize numerous *Campylobacter* organisms from their characteristic morphology. Vomiting is rare. The diarrhoea continues for about 2–3 days, but abdominal pain and discomfort may persist after the diarrhoea has stopped. In a significant proportion of patients, the stools contain fresh blood, pus or mucus, which suggests that colorectal inflammation is not uncommon in *Campylobacter* infection. Sigmoidoscopy usually reveals abnormalities ranging from mucosal oedema and hyperaemia, either with or without petechial haemorrhage, to mucosal friability. Severe abdominal pain may mimic acute peritonitis. Occasionally, these patients, especially teenagers or young adults, develop peritonitis from acute appendicitis, but in most patients, it is the inflammation of some part of the ileum and jejunum with mesenteric adenitis (Mishu-Allos, 2001; Crushell, 2004). Local complications such as cholecystitis, pancreatitis and peritonitis occur rarely. Recently, immunoproliferative small intestinal disease has been associated with *C. jejuni* (Lecuit et al., 2004). Bacteriemia is detected in less 1% of patients with *Campylobacter* enteritis, and occurs most often in patients whose immune system is severely compromised (Crushell, 2004). Some patients develop *erythema nodosum* or reactive arthritis. Extra-intestinal infections, including meningitis, osteomyelitis and neonatal sepsis, are rare.

It has been recognized that the paralytic condition, Guillain–Barré syndrome (GBS), is the most serious complication of *Campylobacter* infection, with an incidence of 1/1000 infections (Ang 2002; Gilbert, 2004). *C. jejuni* is the most frequently observed antecedent infection in cases of GBS. In general, one in three GBS patients has suffered from a preceding infection with *C. jejuni*. Symptoms of GBS usually occur 1–3 weeks after the onset of *Campylobacter* enteritis. GBS cases associated with *Campylobacter* infection are usually more severe and can require intensive

hospital treatment, with possible long-term disability. Molecular mimicry has been proposed as an attractive concept to explain the pathogenesis of GBS (Ang, 2002). Starting with food-borne *Campylobacter* diarrhoea, antibodies and/or T-cells are induced by the infection and are directed initially against *Campylobacter*, leading to eradication of the organism. However, because of the strong resemblance between the microbial antigens and the self-antigens, in this example the peripheral nerve cells, the tissue is destroyed, leading to GBS (Gilbert, 2004).

Until a few years ago, if antimicrobial therapy was indicated for *Campylobacter* infection, fluoroquinolones were considered the drugs of choice. This approach was the simplest for physicians and patients alike because the symptoms of *Campylobacter* enteritis (fever, abdominal cramps, and diarrhoea) are clinically indistinguishable from those of bacterial gastroenteritis caused by other organisms, such as *Salmonella* or *Shigella* species. Because these other pathogens were also generally susceptible to fluoroquinolones, empirical treatment with these drugs could be used without waiting for the results of stool cultures. Fluoroquinolones were especially apt to be used for the treatment of traveler's diarrhoea.

However, in the past few years, a rapidly increasing proportion of *Campylobacter* strains all over the world have been found to be fluoroquinolone-resistant. Primary resistance to quinolone therapy in humans was first noted in the early 1990s in Asia and in European countries such as Sweden, The Netherlands, Finland, and Spain. Not surprisingly, this coincided with initiation of the administration of the fluoroquinolone, enrofloxacin, to food animals in those countries (Endtz, 1991). A similar increase in rates of resistance to fluoroquinolones in *Campylobacter* isolates from humans was observed in the United Kingdom after the approval of the use of fluoroquinolones in animals as well (Sam, 1999). In the United States, the licensure of sarafloxacin in 1995 and enrofloxacin in 1996 for use in poultry flocks contributed to an increase in the number of domestically acquired fluoroquinolone-resistant *Campylobacter* infections in Minnesota (Smith et al., 1999).

High rates of resistance make tetracycline, amoxicillin, ampicillin, metronidazole, and cephalosporins poor choices for the treatment of infections with *C. jejuni*. All *Campylobacter* species are inherently resistant to vancomycin, rifampin, and trimethoprim.

Erythromycin is considered the optimal drug for treatment of *Campylobacter* infections. Despite decades of use, the rate of resistance of *Campylobacter* to erythromycin remains quite low. Other advantages of erythromycin include its low cost, safety, ease of administration, and narrow spectrum of activity.

Campylobacter species also are generally susceptible to aminoglycosides, chloramphenicol, clindamycin, nitrofurans, and imipenem.

PREVENTION

European Food Safety Authority has emphasized the importance and recommended the establishment of an active surveillance of campylobacteriosis in all MS, including efforts to determine the uncertain and unreported campylobacteriosis cases. In addition, storage and genotyping of human and putative reservoirs of isolates in all MS have also been recommended. Thereafter, it would be important to identify the *Campylobacter* properties of virulence, survival characteristics and ecology (EFSA, 2011).

As the major source of human campylobacteriosis in the industrialised world is poultry, prevention should aim at reducing infection at all stages of poultry production. It is difficult to control *Campylobacter* during poultry processing because of the high incidence of this pathogen in poultry flocks and the high levels in chicken intestines. More information is needed about the effectiveness of biosecurity measures for poultry farms, and about the impact of these measures on human campylobacteriosis. *Campylobacter* is relatively sensitive to low-dose radiation treatment and could be eliminated readily from poultry meat products by this means, but there is still considerable resistance among consumers to this method of disinfection. Appropriate precautions in the handling and preparation of foods of animal origin will reduce cross-contamination. Raw meat and poultry should be cooked adequately. Hands should be washed thoroughly with soap after handling raw foods of animal origin and before touching anything else. Chopping boards used for raw meats should not be used for preparing other foods. Chopping boards and utensils should be cleaned with soap and hot water after preparation of raw food of animal origin.

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THE LEVEL OF THE INDUSTRIAL TOXIC POLLUTANTS IN THE AREA TURNU MĂGURELE

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Key words: heavy metals, soil pollution, Turnu Măgurele

SUMMARY

There were determined by specific methods nonferrous heavy metals (nickel, lead, copper, zinc, cadmium) in Turnu-Magurele area, polluted by a Chemical Company specialized in agricultural fertilizer products, and the obtained resuls were compared with an unpolluted area.

On samples collected to a depth of 5 cm., it was found that the threshold is exceeded, even to the threshold for intervention for the Pb, Cu, Zn, and Cd, in the southeast area to the Company which is on wind direction; at the soil samples prelevated to a depth of 30 cm., thresholds were exceeded only for Pb and Cd, in the southwest direction from the Company area. These data demonstrate a permanent risk of toxicity of heavy metals in the corresponding with Chemical Turnu Magurele Company.

The pollution, generally defined as „any introduction of some substances or energy in the environment, directly or indirectly, by the humans , with damaging effects, which can endanger the human, animal and plant health, can prejudice the biological resources, the ecosystems and the material property, can reduce the blessings or stop other legitimate uses of the environment", constitutes a special interest for the hunting fauna, because, starting with the development of the chemical industry for the production of the fertilizers used in agriculture, the negative influences in wild animals health were observed. This reason determined us to start a study regarding the level of the heavy metals toxic industrial pollutants in the area around the Agriculture Fertilizers Chemical Plant of Turnu Magurele

1. MATERIALS AND METHODS

The statistic data obtained at the automatic stations to monitor the impact of pollution were used, respectively TR-T1, situated at theTurnu Magurele City Hall, and TR-T2, situated in the storage area for the cribs resulted from the Plant; for comparison the statistic data taken from the Calmatui Station, situate dat a distance of 40 km north were also used.

The determination of the heavy, non-ferrous metal pollution (Ni, Pb, Cu, Zn and Ca) was made on ground samples, harvested at 100 m distance and depths of 0-5 cm and 6-30 cm in the directions S-E and S-W of the wind, compared to the source.

2. RESULTS AND DISCUSSION

The results presented in Table 1, in which we mentioned : the harvesting and depth points, the heavy, non-ferrous metals indicators-, the value in mg on ground kg. (admitted, normal and obtained), alert situations for sensitive and less sensitive ground and as a last section, the intervention threshold in mg./kg. of dry field substance, also on sensitive and less sensitive ground.

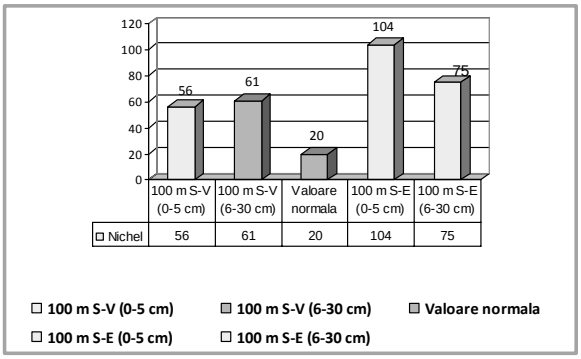
Table 1

The pollution with heavy, non-ferrous metals in the Călmățui and Turnu Măgurele area

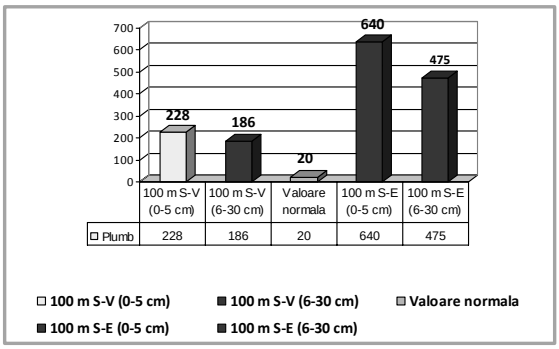
Harvesting point and depth	Indicator	Value in mg/kg		Alert		Interv. threshold mg/kg dry subst.	
		Normal	Obtained	Sensit. ground	Less sensit. ground	Sensit. ground	Less sensit. ground
Călmățui E. R. Roşiori — Alexandria — Bucharest Milestone 114 0,5 m	Ni	20	12,244	75	200	150	500
	Pb	20	3,174	50	250	100	1000
	Cu	20	8,616	100	250	200	500
	Zn	100	26,301	300	700	600	150
	Cd	1	SLD	3	5	5	10
S. C. DONAU	Ni	20	104	78	200	150	500

Harvesting point and depth	Indicator	Value in mg/kg		Alert		Interv. threshold mg/kg dry subst.	
		Normal	Obtained	Sensit. ground	Less sensit. ground	Sensit. ground	Less sensit. ground
CHEM SRL T. Măgurele pyrite ash dumps 100 m S - E. 0 —5 m	Pb	20	640	50	250	100	1000
	Cu	20	550	100	250	200	500
	Zn	100	1142	300	700	600	1500
	Cd	1	13,8	3	5	5	10
S - E 6 —30 cm	Ni	20	75	75	200	150	500
	Pb	20	475	50	250	100	1000
	Cu	20	351	100	250	200	500
	Zn	100	700	300	700	600	1500
	Cd	1	9,5	3	5	5	10
100 m S - V 0 —5c m	Ni	20	56	75	200	150	500
	Pb	20	228	50	250	100	1000
	Cu	20	191	100	250	200	500
	Zn	100	547	300	700	600	1500
	Cd	1	6,2	3	5	5	10
S —V 6 —30 cm	Ni	20	61	75	200	150	500
	Pb	20	286	50	250	100	1000
	Cu	20	167	100	250	200	500
	Zn	100	554	300	7000	600	1500
	Cd	1	5,8	3	5	5	10

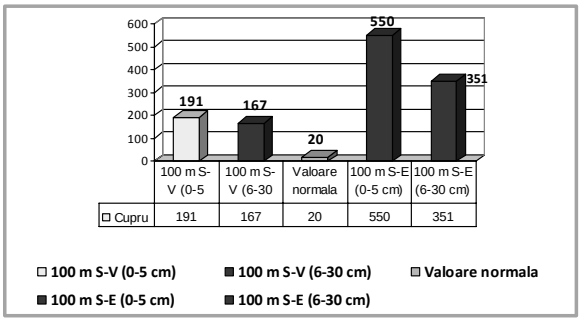
The obtained values are represented in the following graphics:



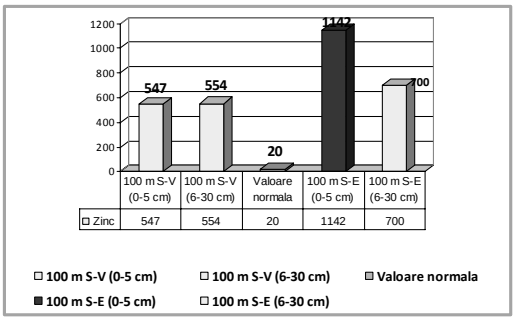
For Nickel, the values are in the admitted limits at the depths of 0-5 and 6-30 cm in the S-W wind direction and alert for the depths situated in the S-E wind direction



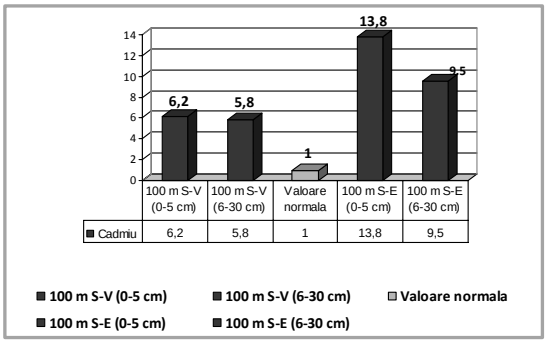
For Lead (Pb) the values are on the intervention level for the samples in the points on the dominant S-E wind direction and alert for the samples in the S-W wind direction



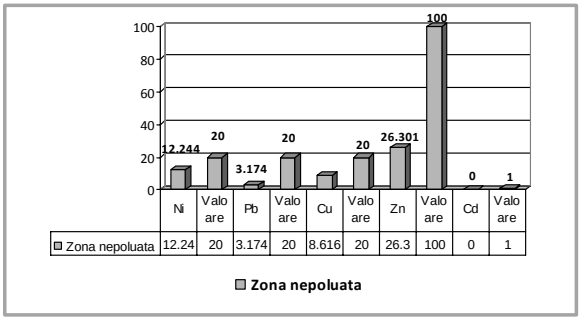
For Copper (Cu) the values are on the intervention threshold for the samples in the points on the dominant S-E wind direction and alert for the samples in the S-W wind direction



For Zinc, the values are on the intervention threshold for the samples in the harvesting points 0-5 cm in S-E direction and on the alert threshold in the other harvesting points, respectively 6-30 cm S-E and 0-5 S-W



For Cadmium, an element often quoted in literature as „a risk factor for Cancer development", the values are extremely high in all the four samples, respectively in the cases 0-5cm on the S-E, S-W directions.



The values for heavy metals in the non-polluted area are under the admitted limits.

3. CONCLUSIONS

3.1 Pollution with heavy-non-ferrous metals is more intense at the ground samples harvested from the dominant S-E wind direction.

3.2 For alert risk at depths of 0-5 cm, Ni values were presented in the S-E direction and Ni, Pb, Cu, Zn in the S-W direction; for the 6-30 cm depth were the samples of Pb, Cu, Zn; at 0-30 cm were registered admitted values of Ni.

3.3. The intervention threshold in samples harvested at 0-5 cm depth and 6-30 cm was exceeded for Pb, Cu, Z nat the samples on the S-E direction.

3.4 The Cd levels exceeded the intervention threshold of 5-10 mg/kg dry substance, in all the four situations afferent to the Turnu Magurele Chemical Plant, presenting a high risk of toxic pollution.

3.5 In the witness area of „Calmatui", the obtained values were normal for all elements.

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MACROSCOPIC AND MICROSCOPIC LESIONS RECORDED AT THE HARES (*Lepus europaeus*) FROM THE POPULATED REGION OF TURNU MĂGURELE

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Key words: rabbit, histology, pollutants

SUMMARY

Rabbits with poor welfare were sacrificed for detect lesions consecutive pollutants action from first zone (0-5 km) for Turnu Magurele Chemical Company. We have been described macroscopic and microscopic pathological tissue structures in the heart, lungs, liver and kidney. There were found hemorrhagic lesions and dystrophic ones - some subtle- at the level of analyzed organs.

The work described on the tissue sections from analyzed organs: capillary congestion, vacuolar degeneration, granular, hyaline, lyses of cardiomiocytes and epitheliocytes from the proximal convoluted glomerular tube,etc. which demonstrate a permanent toxic state with slow evolution. The paper is commented on 5 macroscopic images and 35 microscopic images, very enlightening.

In a detailed study regarding the influence of the chemical pollutants in the 5 km area around the Agricultural Fertilizers Chemical Plant of Turnu Măgurele, macroscopic and microscopic anatomico-pathological exams of hares (*Lepus europaeus*) were conducted in order to establish the influence of the chemical pollutants on the health status, with influences on the weight increase.

1. MATERIALS AND METHODS

Having in view the discovery of some lesions consecutive to the action of the emissions from the area of 5 km no I, two hares with precarious health were sacrificed, in which the macroscopic and microscopic anatomical modifications of the heart, lungs, liver and kidney were described, in cooperation with the specialists of the Institute of Diagnosis and Animal Health.

2. RESULTS AND DISCUSSION

2.1. ***Macroscopically,*** when opening the thoracic-abdominal cavities, discreet haemorrhagic dystrophies were observed at the level of the organs analized;

At the „a" hare (Fig. 1) on the background of the hepatic dystrophy were noticed characteristic lesions and generalized congestions in the „b" hare.

a b

Fig. 1. Difference in weight between the two hares

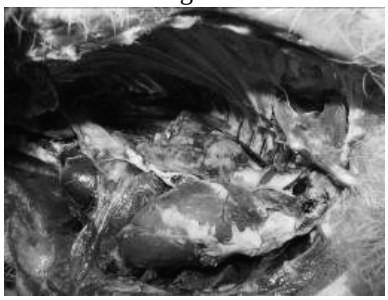

Fig. 2. Haemorrhagic lesions on serous and discreet dystrophies

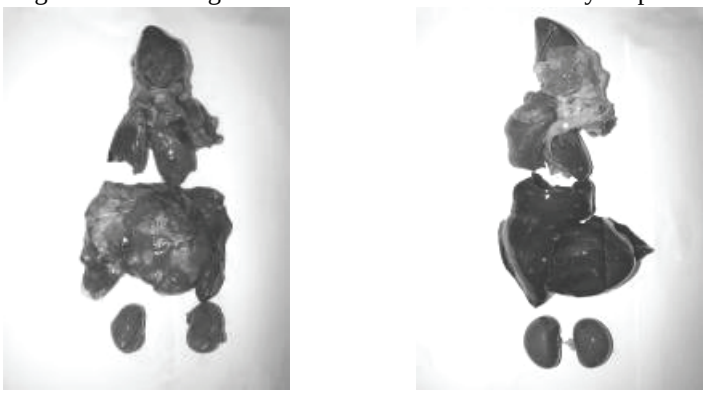

Fig. 3. Liver dystrophy due to liver coccidiosis (original)

2.2. ***Microscopically,*** on organ sections were evidenced the following:
- **heart**: vacuolary degeneration, hialin, myocardical fibres granulary, congestions and heart oedema, necrosis.

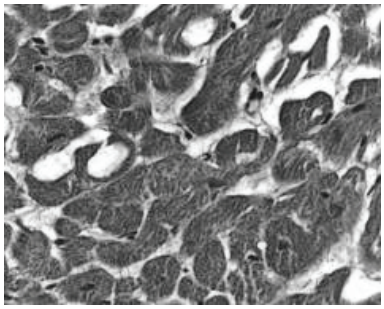

Fig. 4. Myocardial vacuolary degeneration. Hare. HEA, x 1000 (original)

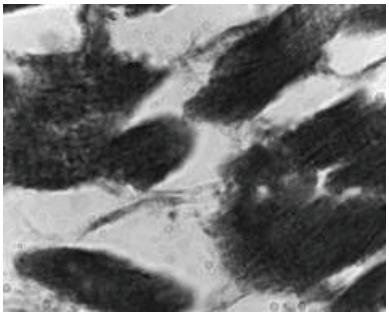

Fig. 5. Destructions of myocardial fibers and interfibrilary oedema. Hare. Met HEA, x 2000 (original)

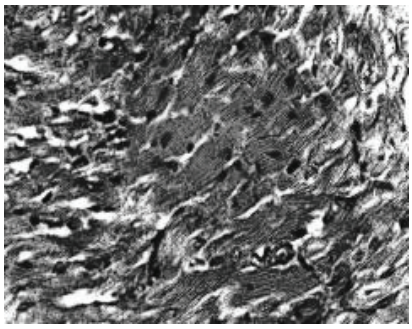

Fig. 6. Hialin degeneration of myocardial fibers, with cardiomyocitary lysis Heart. Hare MNet. HEA, x 630 (original)

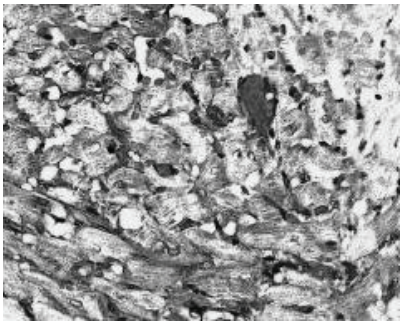

Fig. 7. Congestion of the interfibrilary capilaries, with degeneration of the granulary lysis of the cardiomyocytes. Heart. Hare. Met. x 400 (original)

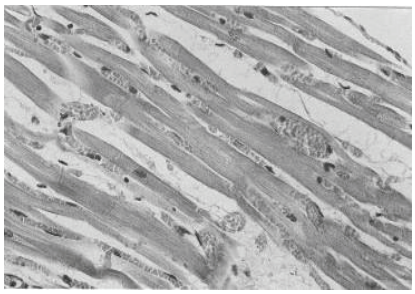

Fig. 8 Heart congestion and oedema (original)

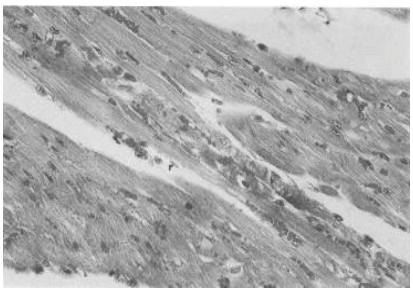

Fig. 9. Heart muscle fiber necrosis (original)

- **lung:** oedema, emphysema; stasis and haemosiderosis; bronchiolis; pulmonary hepatization; siderocytes in the vasculary and alveolary lumen.

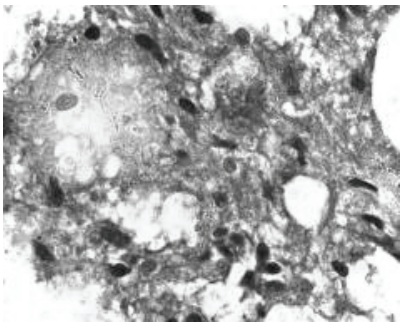

Fig. 10. Septal oedema and pulmonary emphysema. Hare. Met. HEA, x 1000 (original)

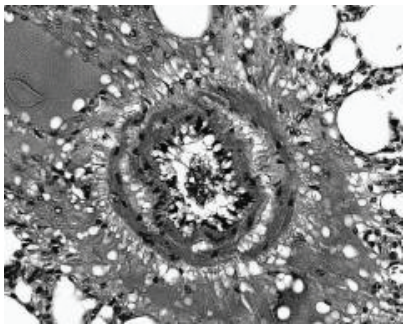

Fig. 11. Interstitial Hialinosis, with obvious plasmexody. Lung. Hare.Met HEA, x 400 (original)

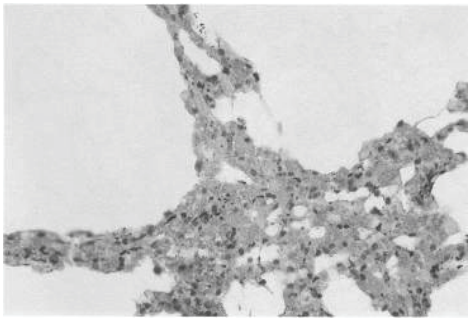

Fig. 12. Stasis and hemosiderosis in alveolar walls (original)

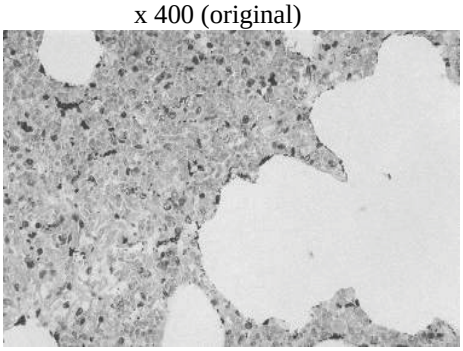

Fig. 13. Stasis and hemosiderosis (original)

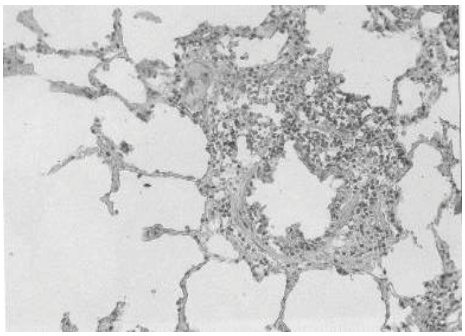

Fig. 14. Bronhiolitis and emphysema (original)

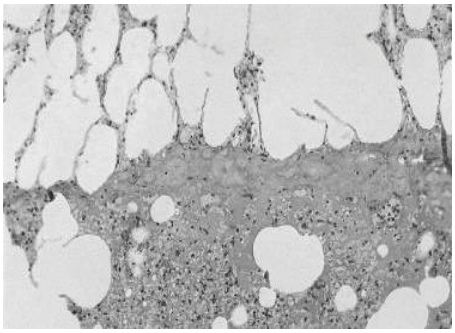

Fig. 15. Pulmonary oedema and emphysema(original)

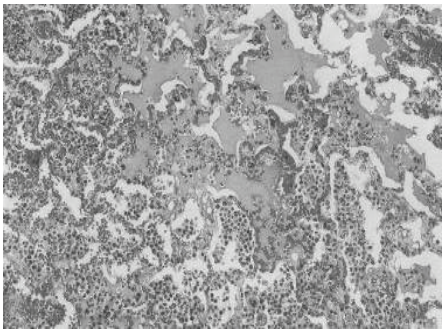

Fig. 16. Pulmonary hepatization area (original)

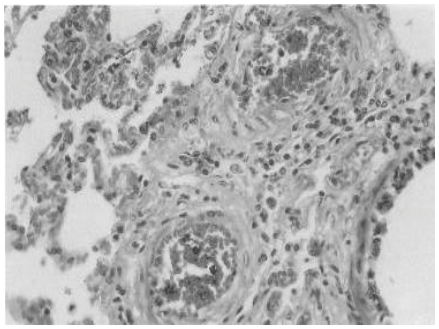

Fig. 17. Siderocytes in the vascular lumen and alveola (original)

- **liver:** necrosis and hepatocitosis lysis; intercellulary oedema; vacuolary, granulary degeneration,; hemosideurie pigment; fibrosis of the Disse space.

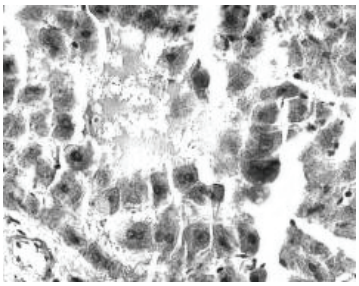

Fig. 18. Hepatocitosis areactiva necrosis. Hare. (original)

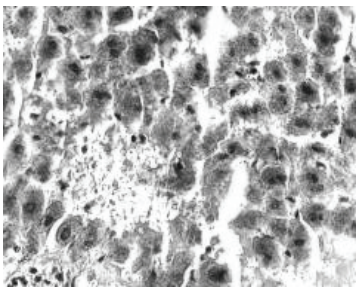

Fig. 19. Focal hepatocyte lysis. Hare. Met. HEA, x 630 (original)

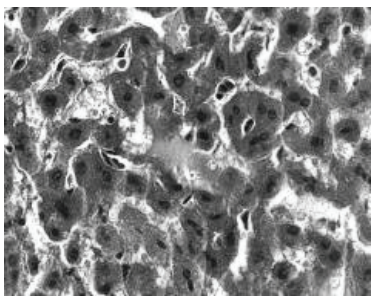

Fig. 20. Tumefied hepatopcytary and intercelulary oedema Hare. (original)

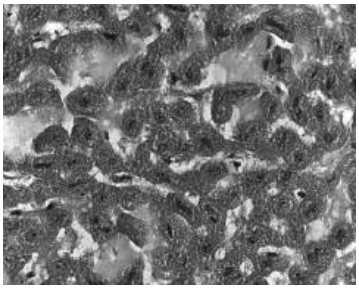

Fig. 21. Hepatic oedema. Hare (original)

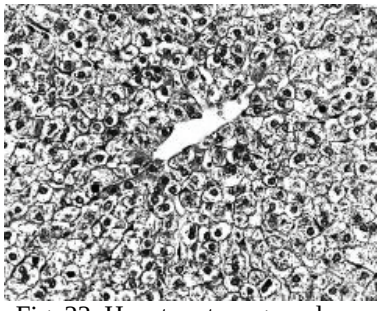

Fig. 22. Hepatocytary granulary degeneration and hepatocytolysis. Liver. Hare. (original)

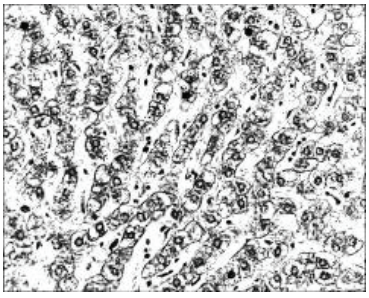

Fig. 23. Vacuolary degeneration of hepatocytes. Liver. Hare. (original)

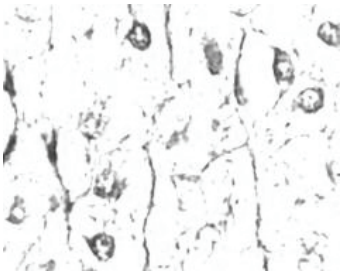

Fig. 24. Hepatocyte lysis. The liver. Hare. Met HEA, x1300. (original)

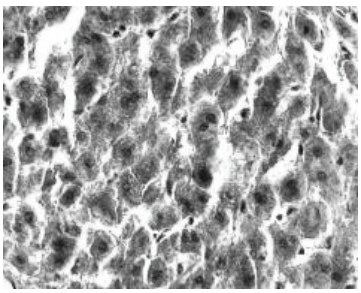

Fig. 25. Vacuolary degeneration with hepatocytolysis. Hare (original)

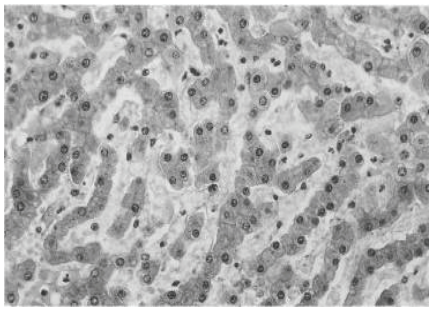

Fig. 26. Hepatic oedema (original)

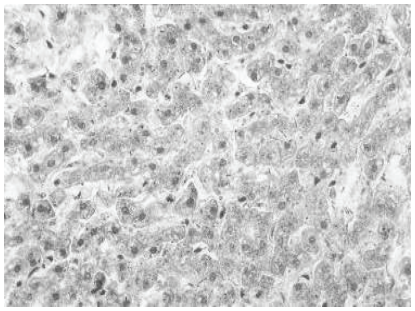

Fig. 27. Hepatocitary with vacuole degeneration in cytoplasm (original)

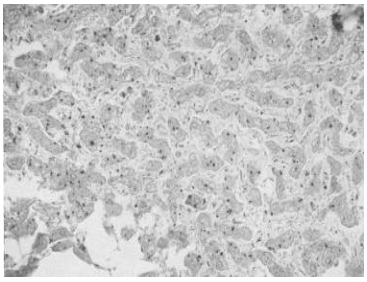

Fig. 28. Distrophic hepatocyte and hemosiderinic pigment accumulation, fibrosis Disse space (original)

- **kidneys:** glandulary and periglomerulary oedema; inter-glomerulary bleedings, hialinisation of the epiteliocytes of the urinal tubes; glomerulonefritis with necrosis areas, generalized glomerulary stasis.

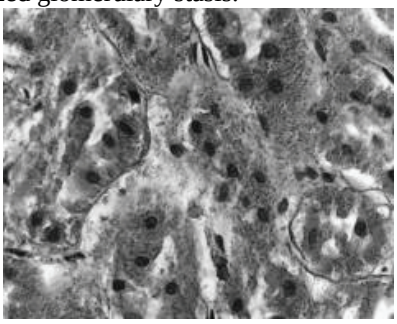

Fig. 29. Areactive lysis of the urinal tubes. Hare. Met HE, x 1000 (original)

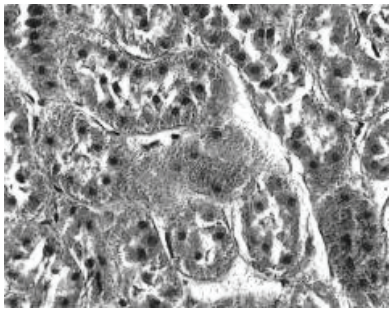

Fig. 30. Swelling of the renoepithelia and lysis tube. Hare. Met HE, x 630 (original)

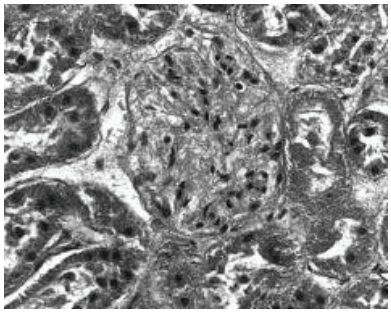

Fig. 31. Glomerulus and periglomerulary. Hare oedema Met. HEA, x 630 (original)

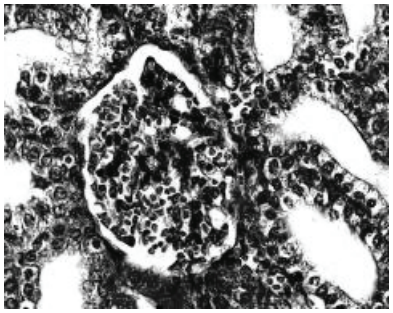

Fig. 32. Intraglomerulary bleeding. Kidneys. Hare. HEA, x 630 (original)

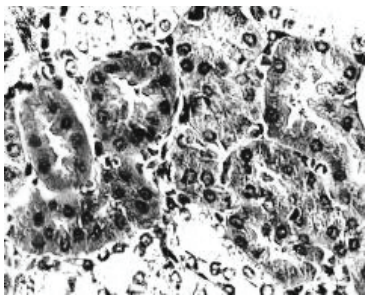

Fig. 33. Hialinis of the urinal renoepithelia tubes. Kidneys. Hare. Met. HEA, x 630 (original)

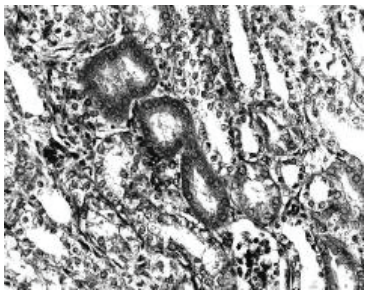

Fig. 34. Hialinis of the urinal epitheliocytes tube. Kidneys,Hare HEA, x 400 (original)

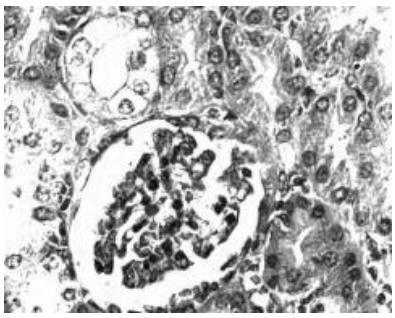

Fig. 35. Epitheliocytes lysis a tube proximal convoluted and glomerular congestion. Kidneys. Hare. Met. HEA, x 630.jpg (original)

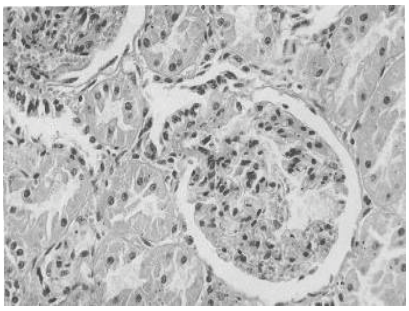

Fig. 36. Glomerulonephritis, with areas of necrosis of the capillaries, in the degenerated renal tubes, nefrocytes outlined some cariolysis stage. (original)

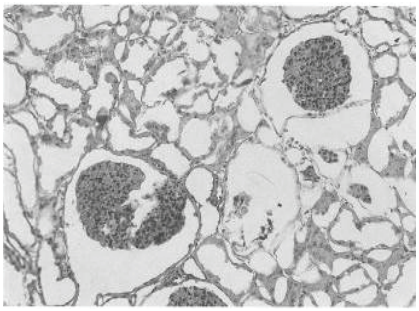

Fig. 37. Stasis glomerular and renal tubule epithelial lysis (original)

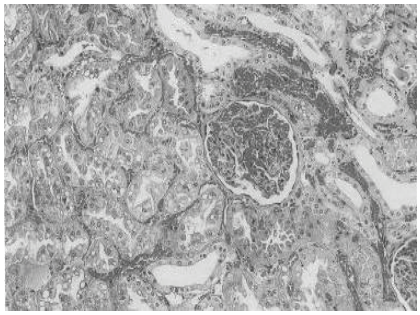

Fig. 38. Stasis and generalized glomerular interstitial (original)

3. CONCLUSIONS

3.1. The lesions described above demonstrate the toxicity of the habitat medium for *Lepus europaeus* in the area around the Chemical Plant of Turnu Măgurele.

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THE INFLUENCE ON THE WEIGHT INCREASE OF HARE (*LEPUS EUROPAEUS*) IN THE TURNU MĂGURELE REGION COMPARED WITH THE NON-POLLUTED AREA

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Key words: rabbit, toxicity, weight

SUMMARY

For an appreciation of the influence of toxic substances in the chemical industry Turnu Magurele area, weight determinations were made at rabbits by two years, in four areas related to the Company, an area witness. All determinations were reported to a 3800 g standard weight.

There was a 41.4% reduction in average weight comparative with standard weight in rabbits from the first area of 5 km, 32% in the second area with 5-10 km, 29, 8% in the third of 10 -15 km, 21.2% in the fourth area of 15-20 km, and in the fifth area, unpolluted 22.4%, compared to the standard. The conclusion in this case it is that the risk of toxicity is maintained up to 20 km distance from the source of pollution.

The effects of pollutants on the health of plants, animals and humans constitute subjects of study in many scientific research institutes, and the environmental protection represents an international problem, for which the states constituted themselves in organizations with world, regional, national character meant to elaborate laws, ordinances for environment protection of the air, then ground and through them, of animal and human health.

The pollutants have irritating, asphyxiating, toxic systemic, fibrosis, carcinogenic, allergenic, infecting actions which determin on animals and humans diseases with acute, sub-acute, cronic evolution and also with sub-clinical, occult evolution.

If in the hunting domain the diseases with clinical evolution determin the survival incapacity, the sick animals becoming an easy mark to the „sanitaries" of the silvatica environment, such cases being uncontrollable, the diseases with an occult evolution determin a delay in growth, unfulfillment of the weight increase.

In the present paper we intended to study the difference in weight of the hare (*Lepus europaeus*) in the area chemically polluted with heavy, non-ferrous metals related to the Chemical Plant of Turnu Măgurele

1.MATERIALS AND METHODS

In order to fulfill this objective, when harvesting the hares for export were weighed five two years old hares from four regions situated in a range of 5 km, related to the Chemical Plant of Turnu Măgurele and from the Calmatui non-polluted region, (40 km from the Plant.

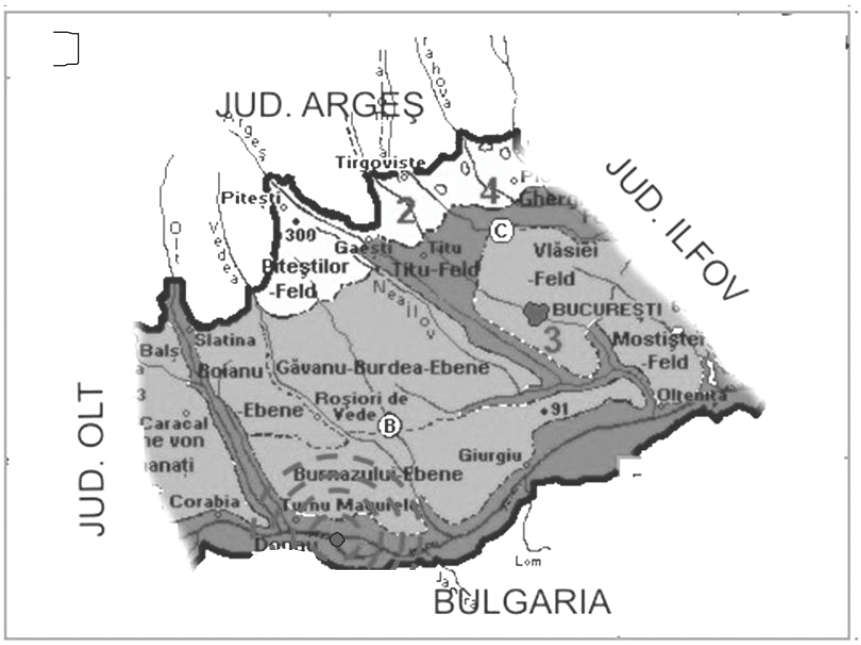

Fig. 1.

2.RESULTS AND DISCUSSION

Table 1

Table with the difference in weight of *Lepus europaeus* compared with the minimum standard values (quoted by Cotta et. al., 2008)

Region	Standard weight in grams	Medium weight in grams	Difference from the standard	Deviation in less %
I	3800	225,5	1574,5	41,4
II	3800	2502,5	1237,5	32,5
III	3800	2667,2	1132,8	29,8
IV	3800	2994,4	805,5	21,2
M. (Călmățui)	3800	2950,0	850	22,4

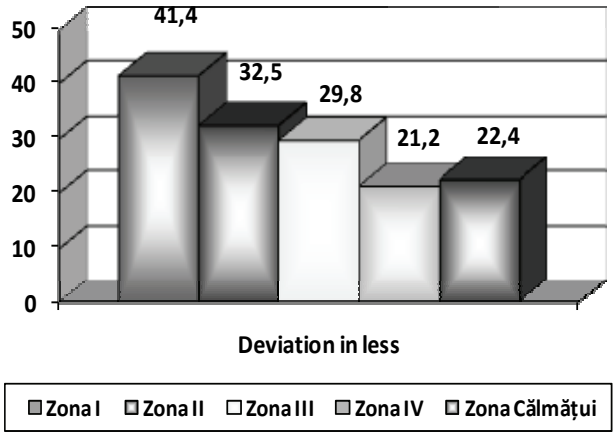

Fig. 2

We mention that the action took place at the beginning of the winter season, which excludes the weight decrease determined by the scarce food during winter, granting more motivation to the negative effects determined by the pollutants.

A decrease in the average weight was observed, compared with the standard weight with 41,4% at the hares in the first 5 km region, with 32% in the second 5-10 km region, with 29,8% in the third 10-15 km region, with 21,2%in the fourth 15 —20 km region and in the fifth, non-polluted region with 22,4% from the standard.

3. CONCLUSIONS

3.1. The chemical pollutants from the Turnu Măgurele Chemical Plant region proved their toxic action on the hunting fauna, in the present example *Lepus europaeus*, through the gradual increase of the deviation in less of the body weight from 21,2% in the farther region from the source of pollution to 41,4% in the 5 km perimeter of the polluting source.

3.2 The toxicity risk is maintained up to 20 km from the polluting source in the S-E dominant wind direction.

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EFFECT OF A MIXTURE OF OREGANO (*ORIGANUM VULGARE*) AND CRANBERRIES (*VACCINIUM MYRTILLUS L.)* ADDED TO WEANED PIGLET DIETS ON FE AND MN BALANCE

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Key words: oregano, cranberries, iron, manganese, piglets

SUMMARY

A 4-week study conducted on 8 weaned piglets (average initial weight 15 kg) evaluated the effects of the mixture of oregano (*Origanum Vulgare*) and cranberries (*Vaccinium Myrtillus L.)* used in the presence of phytase on Fe and Mn balance, while eliminating their inclusion in the diet as inorganic salts. Oregano and cranberries were harvested from the wild flora and the Fe and Mn concentrations that were taken into consideration (for oregano: Fe 1832.3 ppm, Mn 77.92 ppm and for cranberries : Fe 79.15 ppm, Mn 203.00 ppm) were the consensus values obtained in an interlaboratory study. The piglets were assigned to 2 groups (C and E), housed in individual metabolic cages and fed on corn-soybean meal-based diets. The diet of the control group (C) contained 1% inorganic mineral premix. The determination of the total polyphenols and of the flavonoids, has shown that the cranberries and oregano extracts have about the same concentrations of polyphenols (570.02 mg equivalents gallic acid/100 ml for cranberries and 564.10 mg equivalents gallic acid/100 ml for oregano), but the oregano extract had almost two times more flavonoids (396.41 mg equivalents catechin/100 ml). The experimental diet differed from C diet as follows: 3% oregano, 1% cranberries and 0.01 % phytase. The balance was performed for 5 days every week. The Fe and Mn were determined by FAAS in the samples (weekly samples/piglet) of ingesta, faeces and urine. Due to the higher concentration of Fe in the oregano, the absorption coefficients are comparable between the groups (absorption % for C was 35.91 and for E was 40.39), and so are the utilization coefficients (98.30 % for C and 99.82 % for E). For the Mn were obtained the following results: absorption coefficient for C was 35.79 % and for E was 45.26 %, and utilization coefficients were 99.87 % for C and 99.74 % for E.

A balanced feeding is necessary for an as god as possible development and productivity of the piglets, which to contain all necessary nutrients and an optimal ratio of them.

Rincker *et al*., 2005, show that although the presence of trace minerals in pig diets is indispensable for the proper growth and health of the piglets, the problem of requirement versus supplement gets more acute instead of getting solved.

Minerals should not be added at hazard in the diet. If minerals are added without reason, more harm than good can occur because all minerals have a toxic level. Iron (Fe), manganese (Mn), copper and zinc are among the most important trace elements for pigs.The maximum content of trace elements in the feeds, currently regulated by EC no. 1334/ 2003, decreased continuously.

The study of phytogenic additives (derivatives of plant compounds included in the diets) as alternative to the mineral premixes in animal feeding may be a solution and a modern approach to the modern trend of return to the nature.

Oregano and cranberries, from the wild flora, can be used as phytogenic additives. Many studies have been conducted on their use in veterinary and human therapy. Sarac and Ugur, 2008, studied the antimicrobial activity of the oils obtained from several varieties of oregano and observed that their efficacy depends on the location of harvesting.

Walter and Bilkei, 2004, conducted a study on fattening pigs with a supplement of 3000 ppm commercial additive enriched in oregano (*Oregpig Pecs*). Comparing the performance of the pigs treated with this additive with a control group of similar weight but with no supplement of oregano, it was noticed that the oregano group had an average daily weight gain significantly ($p \leq 0.05$) higher than the group without oregano treatment .

The extract of cranberry leaves decreases blood glucose and triglycerides (Petlevski *et al*., 2001). The cranberry has been included in different plant mixtures as antioxidant (Szentmihalyi K *et al*., 2005).

Within this context, we conducted an experiment on recently weaned piglets in order to the effect of a mixture of oregano (*Origanum vulgare*) and cranberries (*Vaccinium myrtillus L.*) added to weaned piglet diets on Fe and Mn balance under the conditions of absence of a dietary mineral premix.

1. MATERIALS AND METHODS

The experiment used 8 weaned half-brother Landrace × Large White castrated male piglets. The animals were assigned to 2 randomized groups (C and Exp) according to their body weight. All piglets were housed in individual metabolic cages in an experimental hall under controlled environmental conditions (temperature 24°C; humidity 50-60%). At the age of 2 days the piglets were treated according to the standard procedures and received further 200 mg injectable Fe as Fe-dextran (produced by the Pasteur Institute, Bucharest).

At the beginning of the experiment, weekly, and at the end of the experiment, the piglets were weighed individually and their growth rate calculated. The feed was supplied ad libitum and was weighed before administration. During the 21 experimental days there were 3 periods of mineral balance (5 days each). During these periods the intake, the amount of excreted faeces and the volume of urine were recorded for each piglet. Individual/piglet/weekly samples of ingesta, faeces and urine were collected.

After the amount of faeces excreted by each pig was weighed, 10% from the production of each day was retained and stored in the refrigerator (4°C). At the end o each balance period the samples of faeces were weighed, homogenized, dried at 65°C, ground until processing for Fe and Mn determination. The volume of urine was recorded daily and 10% was retained for the average weekly sample. The stored urine was treated with 10 mL 10% sulphuric acid. The samples were stored at -180°C until processing for Fe and Mn determination.

The coefficients of apparent absorption, retention and utilization of the dietary minerals were calculated using the Fe determinations corroborated with the data on intake and excreta.

The coefficient of apparent absorption was calculated as the ratio of the absorbed amount of metal to the amount of ingested metal, multiplied by

100, where the absorbed amount is the difference between the ingested amount and the amount excreted through faeces.

The coefficient of retention was calculated as the ratio of the retained amount and the amount of ingested metal, multiplied by 100. The amount of retained metal is the difference between the ingested amount and the amount excreted through faeces and urine.

In the end of the experiment (experimental day 21), blood samples were collected from each piglet. After blood sampling the piglets were slaughtered according to the provisions of the Law on animal protection and welfare (Dir. Cons. 93/119/CCE, Order 180/11.08.2006, M. Of. 721/23.08 2006).

The coefficient of utilization is the ratio of the used amount to the absorbed amount, multiplied by 100. The amount of used metal is the difference between the absorbed amount and the amount excreted through urine.

The two experimental diets (C and Exp) had the same basal structure (Table 1), but differed by the supply of oregano, cranberries and phytase. The differences of the diets were as follows: the control diet contained 1% vitamin-mineral premix *ZOOFORT* (produced by IBNA Balotesti, Romania); the experimental diet was without *ZOOFORT* but it contained 3% oregano, 1 % cranberries, 0.1% phytase. The diets were calculated using the mathematical model for energy and protein metabolism simulation (Burlacu, 1983). The diets were isoproteic and isocaloric and contained corn and soybean meal as basic ingredients.

Table 1

Diets given to piglets

	C	Exp
Corn (8% CP)	641,5	611.5
Sunflower meal (34% CP)	80	80
Soybean meal (46% CP)	140	140
Gluten	20	20
Powder milk	50	50
Oil	18	18
Monocalcium phosphate	14	14
Calcium carbonate	17.5	17.5
Salt	2	2
Methionine	1	1
Oregano(*Origanum vulgare*)	0	30
Cranberries (*Vaccinium Myrtillus L.*)	0	10
Phytase	0	0.1
Lysine	5	5
Choline	1	1
Premix ZOOFORT	10	0
Analysed		
Gross energy (kcal/kg)	3250	3250
Crude protein (%)	20,16	19.27
Fe (mg/kg)	301.75	308.84
Mn (mg/kg)	65.95	42.95

Sovarvul or oregano (*Origanum vulgare*) was harvested in the state of vegetation —anthesis, from rural localities in Valcea County: Păuşeşti-Otăsău (lat-45° 4' 0" N, long- 24° 8' 0" E); the altitude of the harvesting area was 200 m; we also collected from Valea Mare (lat. 44°62' N, long 23°95' E); the altitude of the harvesting area was 400 m. Harvesting was done in the morning, because at this moment the plan has the highest content of etheric oil. The harvested part was *Origani herba* which consists if the straight, branched stems, leaves and inflorescence. The plants were dried in thin layers, on wood frames or in bunches hanging on strings. From 2 to 3 kg fresh plant we obtained 1 kg dried product.

The cranberries (*Fructus myrtillus*) were harvested by the botanists from Craiova University from northern Oltenia, Tidvele Mountain, Parâng Massive (Gorj County). The fruits were collected at physiological maturity in August. The plant was harvested from Ranca (lat-45°17′29″N, long-23°41′20″E) at an altitude of 1600 m.The fruits were dried on mesh of galvanized wire, in aerated rooms, properly warmed. The artificial drying was done at 60 to 70°C. The fruits are kept in tissue bags of paper bags.

Phytase (*Natuphos*) was produced by the recombination of *Aspergillus niger* (activity 500 PU/Kg). One phytase unit is defined as the amount of enzyme necessary to release 1 μmol of inorganic P/minute from the sodium phytate, at pH 5.5 and 37°C.

The ratio between the concentration of Fe and Mn, in the experimental diet and the concentrations from the control diet are shown in Table 2. Mn is in lower concentration in the experimental diet than in the control diet, which is normal because no *ZOOFORT* was used. Fe concentration was comparable in the control and experimental diets (3% oregano and 1% cranberries) because of the high Fe concentration in oregano (1832.3 ppm).

Table 2

Trace minerals concentration of the experimental diet, in percent compared to the concentration from the control group

Group	Fe %	Mn %
C	100	100
Exp	102.34	71.31

We used several biochemical analytical methods. We determined the fry matter by removing the water by evaporation at 103°C (stove Ecocell BMT); crude protein by Kjeldahl (FOSS: Kjeltec 2300); the minerals were analysed with an atomic absorption spectrometer Thermo Electron—SOLAAR M6 Dual Zeeman fitted with deuterium lamp for background correction. Class A glassware was used for transvasation, dilution and storage; porcelain crucibles with lids were used for calcinations. Seven mL of blood was collected from each piglet, from the jugular vein, in tubes with heparin. The blood tubes were centrifuged at 2500 RPM for 10 minutes. The: haemoglobin (HGB), erythrocytes (RBC) and haematocrit (HCT) were determined MINDRAY BC 2800 VET, AUTO HEMATOLOGY

ANALYZER (China); P, Fe and the alkali phosphatase were analysed with the BS-130 Chemistry Analyzer (Bio Medical Electronics Co LTD China).

The evaluation of phytogenic additives antioxidant capacity was made as follows: total polyphenols content was done using Folin Ciocalteu reagent; flavonoid content was estimated by a colorimetric method; absorbances were measured at λ=510 nm and the results, expressed as %Fe(II) chelation; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity - was determined by the method described by Burits *et al*, 2000; absorbances of the samples were measured at λ=517 nm. The scavenging of DPPH radical was expressed as %. The initiation of the reaction was made by the addition of phenazine methosulfate (PMS); the capacity of the extracts to annihilate superoxide anion was expressed as % Inhibition; hydroxyl radical (OH•) scavenging activity assay. The ability of the extracts to annihilate hydroxyl radical was evaluated according to the method described by Halliwell *et al*, 1987. The obtained results were expressed as % Inhibition; nitric oxide (NO•) scavenging activity was determined by the method described by Marocci *et al.*, 1994. The obtained results were expressed as % Inhibition; hydrogen peroxide scavenging activity assay (H_2O_2). The ability of the extracts to annihilate H_2O_2 was determined by the method described by Ruch *et al.*, 1989. The obtained results were expressed as % Inhibition.

StatView software was used to calculate the evolution of mineral intake and excretion as well as the correlations during the balance period.

2. RESULTS AND DISCUSSIONS

Table 3 shows that the experimental group (oregano, cranberries and phytase) had comparable performances of growth with the control group, but the intakes were significantly lower ($p \leq 0.05$) compared to the control group, C.

The results obtained regarding the total polyphenols and flavonoids shows that the cranberry and oregano extracts have almost similar concentrations of polyphenols, but the oregano extract contains almost two times more flavonoids (Table 4). The antioxidant capacity of these phyto-additives contributed to the good health state of the pigs from the experimental group, although they didn't have any mineral premix in their diet. Three piglets from the control group had 3 days of diarrhoea, while no piglet from the experimental group had diarrhoea.

Table 3

Pig performance

Specification	C	Exp
Experimental period (days)	21	
Temperature (to °C)	23.65 ± 0.50	
Weight (kg) - initial; - final;	 13.80±1.54 27.73±2.52	 13.35±1.35 26.68±4.02
Average daily gain (g gain/pig)	663.32±35.6	634.52±15.5
Average daily feed intake, (g CF/pig/day)	1327.28± 88.45a	1188.17±95.23b
Feed conversion ratio	2.00[a]	1.87[b]

a,b= significantly ($p \leq 0.05$) different

Table 4

Polyphenols and flavonoids concentration

Alcohol extract	Total polyphenols (mg equivalent gallic acid /100 ml)	Flavonoids (mg equivalent catechins/100ml)
Cranberry	570.02	204.26
Oregano	564.10	396.49

The results of the blood panel assessing the status of animal health are given in Table 5.

Table 5

Blood parameters

	MU	C	E3
Erythrocytes count	mil/mm^3	6.09	6.28
Haemoglobin	g/dL	10.8	10.13
Haematocrit	%	31.61	33.2
Iron	mg/L	0.8	0.96
Alkali phosphatase	mg/dL	146.5	201.5
Phosphors	mg/dL	8.8	9.8

The haematological parameters that were determined (erythrocytes count, haemoglobin and haematocrit) show that the values at the end of the

experiment are within the normal range for the species and category of animals (Parvu *et al*., 2003).

Table 6

Fe and Mn balance (average values/group)

	Iron		Manganese	
	C	Exp	C	Exp
Ingested (mg/day)	410.7	274.39	85.49[a]	38.87[b]
Faeces (mg/day)	300.2	187.99	49.06[a]	24.37[b]
Urine (mg/day)	1.90	0.17	0.06	0.03
Absorbed (mg/day)	110.5	86.40	36.43[a]	13.65[b]
Absorption %	35.91	40.39	35.79[a]	45.26[b]
Retention %	35.45	40.33	35.74[a]	45.17[b]
Use %	98.30	99.82	99.87	99.74

a,b= significantly ($p \leq 0.05$) different

Table 7

Percent, compared to the control group,of Fe and Mn balance data (average values/group)

	Iron		Manganese	
	C	Exp	C	Exp
Ingested (mg/day)	100	66.81	100	45.47
Faeces (mg/day)	100	62.61	100	49.67
Urine (mg/day)	100	8.79	100	57.24
Absorbed (mg/day)	100	21.04	100	15.97
Absorption %	100	112.49	100	126.47
Retention %	100	113.77	100	126.41

Corroborating the balance data shown in Tables 6 and 7, we may notice that Fe and Mn ingestion in the experimental group was 66.81% of the Fe ingested by the control group, even though in the diet of the experimental group, Fe concentration was slightly higher than in group C (Table 2). The explanation is in the significantly lower daily intake of the experimental group compared to the control group (Table 3). Although in the experimental group the amount of ingested Fe was lower than in the control group, the absorption and retention coefficients for Fe were slightly (not significant) higher than in group C, which proves a good availability of the Fe from the diet with oregano, cranberry and phytase.

In the case of Mn, the amount ingested by group Exp was significantly ($p \leq 0.05$) smaller than in group C, but the same significant difference was also observed for the excreta. The absorption and retention coefficients for Mn were significantly ($p \leq 0.05$) higher in group Exp than in group C, which proves that this traced element too had a better bioavailability in the diet with oregano, cranberry and phytase.

3. CONCLUSIONS

3.1. The use of oregano and cranberries harvested from the wild flora, in the diets for weaned piglets, under the conditions of our experiment, was efficient to maintain Fe and Mn status within the normal parameters specific to this category.

3.2. This study supports the hypothesis of replacing the inorganic sources of minerals with natural sources, which reduces the excreta of minerals and thus the environmental pollution due to animal husbandry.

3.3. The alcohol extracts of oregano and cranberries contain polyphenols with antioxidant activity. The oregano and cranberries proved their antimicrobial properties because no piglet from the experimental group had diarrhoea.

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BIOCHEMICAL AND HEMATOLOGICAL BLOOD MODIFICATIONS DUE TO CONSUMPTION OF DIFFERENT TYPES OF DRY GRAULATED FOOD IN DOGS

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Summary

A long-debated issue of dog breeders, veterinarians and producers of industrial food for dogs, worldwide, is related to the nature of protein component, carbohydrates and fat needed to feed dogs. Until now it has not been established with certainty what type of diet is indicated for dogs: "natural" food (prepared in the household) or dry food produced in industrial regime (7, 8, 9). Basic nutrients essential to dogs are proteins, lipids, carbohydrates, vitamins and minerals (1, 2, 3). Dogs need dietary protein to meet the needs of essential amino acids that the body can not synthesize in a sufficient quantity of an optimal performance. Moreover, these amino acids are used in the synthesis of new proteins that are essential cellular constituents by regulating metabolic processes (as enzymes) and are used for growth and tissue repair (10, 4, 5, 6).

Through the present study we sought to identify changes occurring on biochemical and hematological constants that can arise from the consumption of different types of dry granulated dog food and to establish the effects induced by prolonged consumption of this type of diet on health.

1. MATERIAL AND METHOD

To determine the effects induced by prolonged consumption of granulated dry food and mixed food on the biochemical and hematological constants, three groups of dogs were set up, each group having a different diet. Thus, a lot was fed on mixed food, especially food prepared in the household. Lot II was fed with dry granulated food containing 18% protein, and the third group was fed with granulated dry food containing 30% protein.

Sampling of blood for the analysis was made in 30 days and 60 days after commencement of food consumption.

2. RESULTS AND DISCUSSIONS

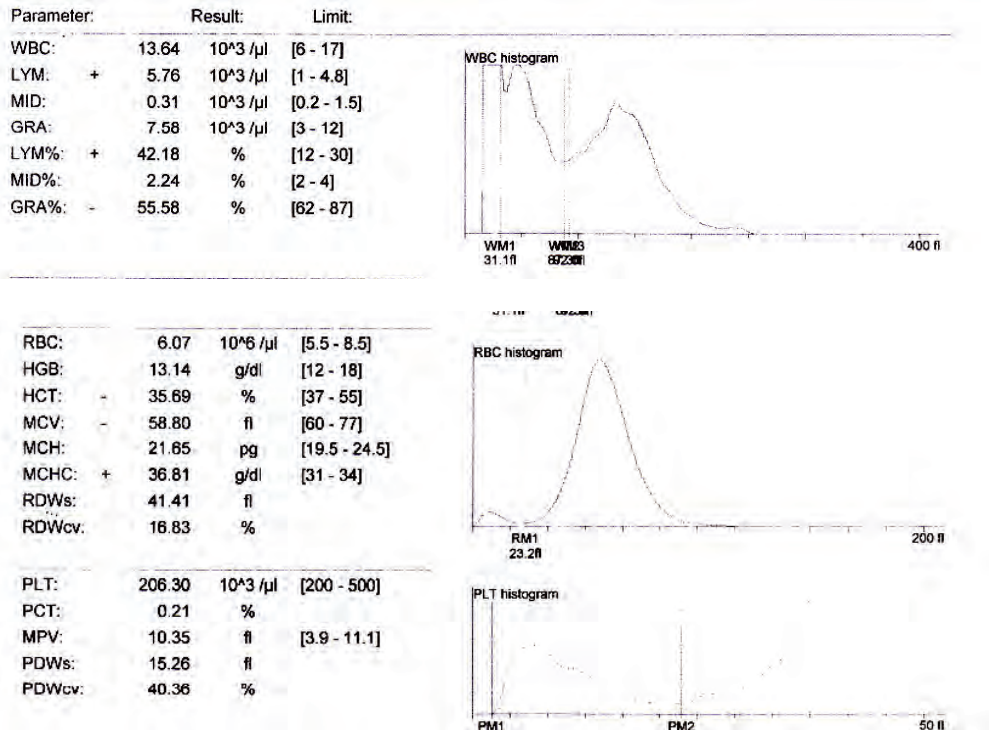
The results are presented in table form, as histograms and graphs depending on the type of food consumed, at 30 and 60 days from consumption, compared to the three experimental groups.

The group fed with mixed food, especially food prepared at home, the values of biochemical examination within 30 days of consumption are included in Table 1, and blood values are presented as histograms. Interpretation of data obtained is presented in graphical form (Figure 1). The values of biochemical examination within 60 days of consumption are included in Table 2, and blood values are presented as histograms. Interpretation of data obtained is presented in graphical form (Figure 1).

Table 1. Biochemical examination in 30 days from mixt food lots

TEST	RESULT	UNIT	MIN	MAX
CHOL	131	mg/dl	120	250
TRIGL	103	mg/dl	10	150
TOTAL CA	8.78	mg/dl	8.7	11.8
MG	1.73	mg/dl	1.7	2.7
ALP	750	U/l	10	150
GAMMA GT	7	U/l	0	9
UREA	32	mg/dl	10	30
GLYCEMIA	101	meldl	62	110
CREA	0.95	me/dl	0.5	1.3
GOT	46	U/l	10	50
GPT	58	U/l	0	55
ISE-Na	149	mEq/l	140	154
ISE-K	4.3	mEq/l	3.8	5.6
TOT PROT.	6.1	g/dl	6.0	8.0
AMILAZN EP	750	U/l	260	l500
TOT BILIRUB.	0.51	mg/dl	0	1.00
P	4.95	mg/dl	2.9	6.2

Histogram of hematological results in 30 days from food administration



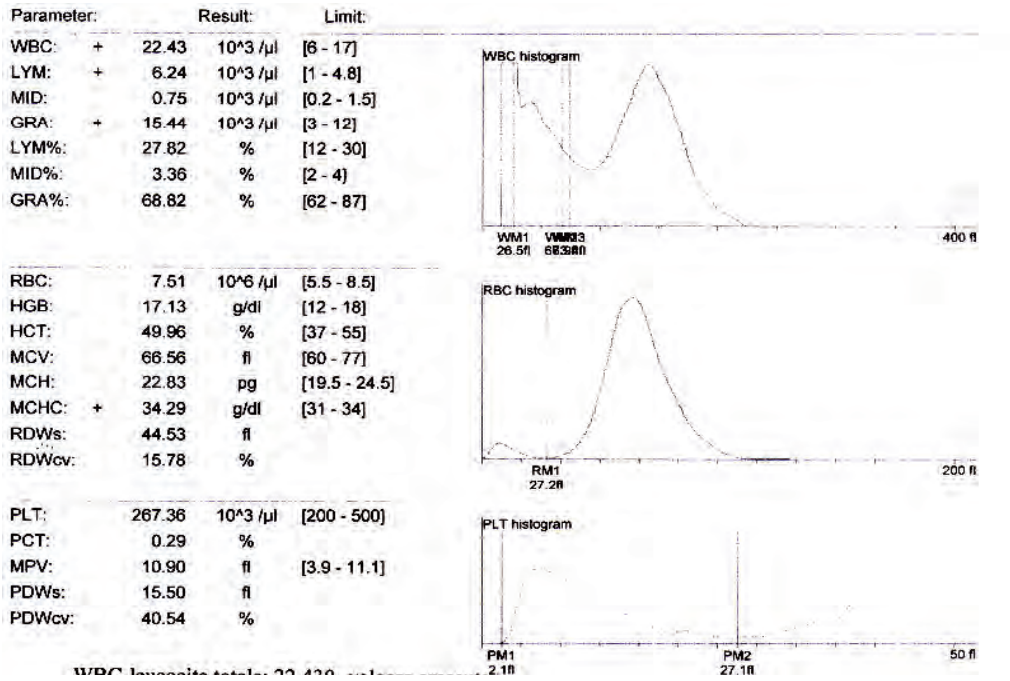
Hematological values in 30 days from mixed food adminsitration: bloodwork - PMN=53%; L=42%; M=2%; Eo=3%.

Table 2. Biochemical examiation in 60 days of mixed food consumption

TEST	**RESULTS**	**UNIT**	**MIN**	MAX
CHOL	128	mg/dl	120	250
TRIGL	111	mg/dl	10	150
TOTAL CA	8.80	mg/dl	8.7	11.8
MG	1.75	mg/dl	1.7	2.7
ALP	714	U/I	10	150
GAMMA GT	9	U/I	0	9
UREA	25	mg/dl	10	30
GLYCEMIA	92	mg/dl	62	110
CREA	0.84	ms/dl	0.5	1.3
GOT	45	U/I	10	50
GPT	53	U/I	0	55

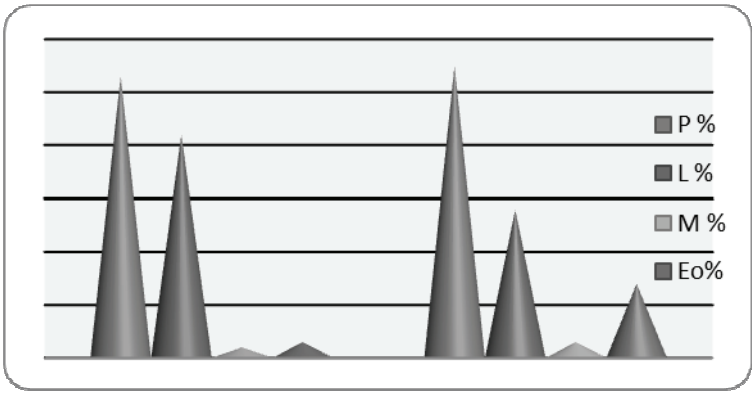
ISE-Na	147	mEq/l	140	154
ISE-K	4.2	mEq/l	3.8	5.6
TOT PROT	6.4	g/dl	6.0	8.0
AMILASES EP	606	U/I	260	1500
TOT BILIRUB	0.44	mg/dl	0	1.00
P	5.2	mg/dl	2.9	6.2

Histogram of hematological constants in 60 days within food consumption



Hematological constants in 60 days within mixed food consumption: bloodwork - PMN=55%; L=28%; M=3%; Eo=14%.

In 30 days after the start of the mixed food consumption decreases in hematocrit value of 35.69% and platelet count of 206,300, indicating a slight dehydration of the body, appears. Bloodwork values indicate mild neutropenia and limphocytosis. At 60 days of consumption of mixed food, hematocrit and platelet counts return to normal, but the bloodwork shows leukocytosis, eosinophilia and moderate lymphocytosis.



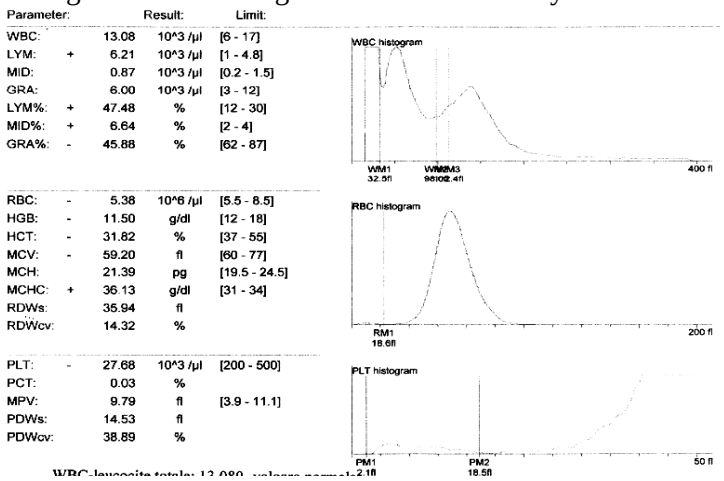
Graph 1. Values in bloodwork in 30 and 60 days of mixed food consumption

In the groups fed with granulated dry food containing 18% protein, the values of biochemical examination within 30 days of consumption are included in Table 3, and blood values are presented as histograms. Interpretation of data obtained is presented in graphical form (Graph 2). The values of biochemical examination within 60 days of consumption are included in Table 4, and Blood constant values are presented as histograms. Interpretation of data obtained is presented in graphical form (Graph 2).
La lotul hrănit cu hrană granulată usactă cu un conținut de 18% proteină, valorile examenului biochimic la 30 de zile de consum se regăsesc în tabelul 3, iar valorile constantelor hematologice sunt prezentate sub formă de histograme. Interpretarea datelor obținute este prezentată sub formă grafică (Grafic 2). Valorile examenului biochimic la 60 de zile de consum se regăsesc în tabelul 4, iar valorile constantelor hematologice sunt prezentate tot sub formă de histograme. Interpretarea datelor obținute este prezentată sub formă grafică (Grafic 2).

Table 3. Biochemical examination in 30 days de within mixed granulated food with 18% protein content

TEST	RESULTS	UNIT	MIN	MAX
CHOL	123	mg/dl	120	250
TRIGL	88	mg/dl	10	150
TOTAL CA	8.72	mg/dl	8.7	11.8
MG	l.7l	mg/dl	1.7	2.7
ALP	618	U/I	l0	150
GAMMA GT	l0	U/I	0	9
TIREA	28	mg/dl	10	30
GLYCEMIA	73	ms/dl	62	110
CREA	0.20	mg/dl	0.5	1.3
GOT	7l	U/I	l0	50
GPT	64	U/I	0	55
ISE-Na	148	mEq/l	140	154
ISE-K	4.1	mEq/l	3.8	5.6
TOT PROT	5.9	g/dl	6.0	8.0
AMILASES BP	745	U/I	260	1500
TOT BILIRUB.	0.64	mg/dl	0	1.00
P	5.01	mg/dl	2.9	6.2

Histogram of hematological constants in 30 days within food consumption

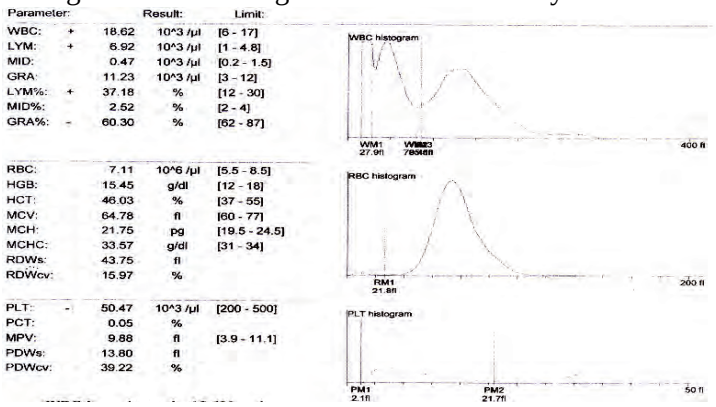


Hematological constants in 30 days within 18% protein content food consumption: bloodwork - PMN=43%; L=48%; M=7%; Eo=2%.

Table 4. Biochemical examination in 60 de days within consumption of food with 18% protein

TEST	RESULT	UNIT	MIN	MAX
CHOL	139	mg/dl	120	250
TRIGL	102	mg/dl	10	150
TOTAL CA	8.60	mg/dl	8,7	**11.9**
MG	1.74	mg/dl	**1.7**	2.7
ALP	620	U/l	10	150
GAMMA GT	11	U/l	0	9
UREA	29	mg/dl	10	30
GLYCEMIA	88	mg/dl	62	110
CREA	0.41	mgldl	0.5	1.3
GOT	48	U/l	10	50
GPT	55	U/l	0	55
ISE-Na	149	mEq/l	140	154
ISE-K	4.3	mEq/l	3.8	5.6
TOT PROT	6.3	g/dl	6.0	8.0
AMILASES EP	505	U/l	260	1500
BILIRUB.TOT	0.66	mg/dl	0	1.00
P	4.75	mgldl	2.9	6.2

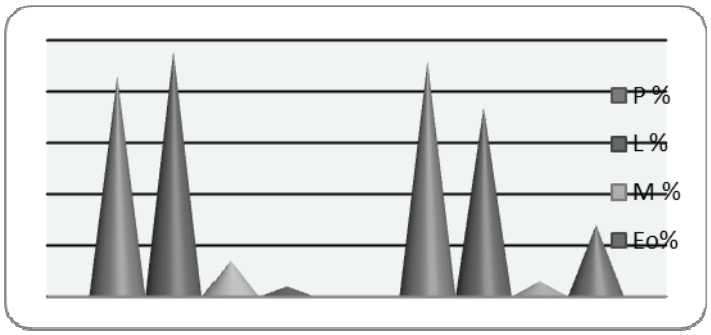
Histogram of hematological constants in 60 days from food consumption.



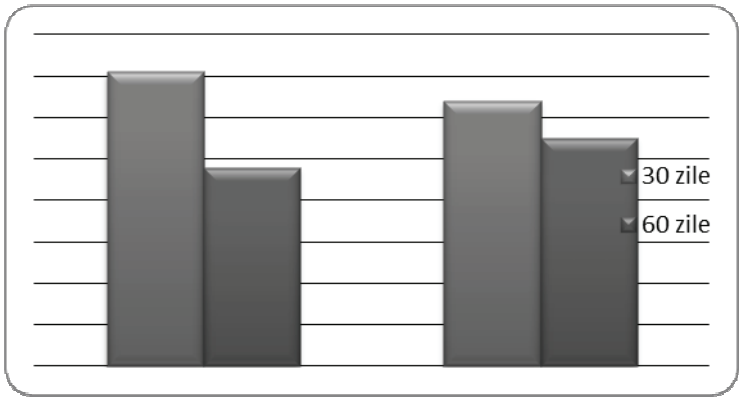
Hematological constants in 60 days from consumption of food with 18% protein content: bloodwork - PMN=46%; L=37%; M=3%; Eo=14%.

Within 30 days of consumption of dry food with 18% protein, low hemoglobin values are found, reduced number of red blood cells, hematocrit and platelets drop, indicating a microcytic anemia. Within 60 days they

return to normal blood constants. The picture shows blood neutropenia, monocytosis, lymphocytosis and thrombocytopenia in 30 days and in 60 days there is eosinophylia, lymphocytosis, neutropenia and thrombocytopenia. Biochemical values indicate increased GOT and GPT within 30 days of consumption, and in 60 days, the two values appear at the maximum (Graph 3).



Graph 2. Blood work in 30 and 60 days within food with 18% protein administration



Graph 3. Values of GOT and GPT in 30 and in 60 days from dry 18% protein granulated food

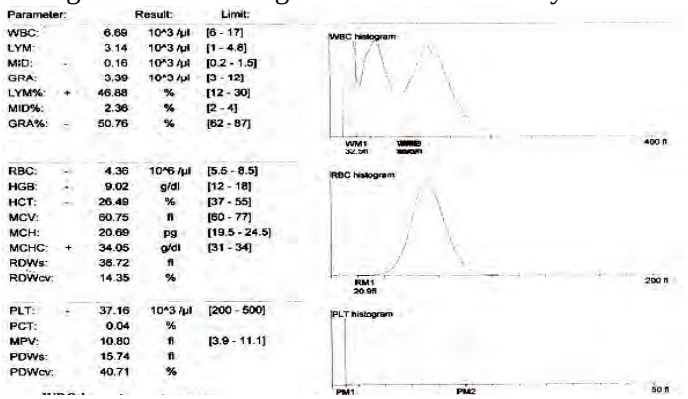
In the group fed with dry granulated food containing 30% protein, the values of biochemical examination within 30 days of consumption are included in Table 5, and blood values are presented as histograms. Interpretation of data obtained is presented in graphical form (Graph 4). The values of biochemical examination within 60 days of consumption are included in Table 6, and Blood constant values are presented as histograms.

Interpretation of data obtained is presented in graphical form (Graph 4).

Table 5. Biochemical examinatiun in 30 days within 30% protein food cosumption

TEST	RESULTS	UNIT	MIN	MAX
CHOL	110	mg/dl	120	250
TRIGL	68	mg/dl	10	150
TOTAL CA	8.69	mg/dl	8.7	11.8
MG	1.67	mg/dl	1.7	2.7
ALP	620	U/I	10	150
GAMMA GT	8	U/I	0	9
UREA	26	mg/dl	10	30
GLYCEMIA	69	mg/dl	62	110
CREA	0.17	ms/dl	0.5	1.3
GOT	69	U/I	10	50
GPT	72	U/I	0	55
ISE-Na	134	mEq/l	140	154
ISE-K	3.9	mEq/l	3.8	5.6
TOT PROT	5.8	g/dl	6.0	8.0
AMILASES EP	864	U/I	260	1500
TOT BILIRUB.	0.74	mg/dl	0	1.00
P	4.30	mg/dl	2.9	6.2

Histogram of hematological constants in 30 days within food administration

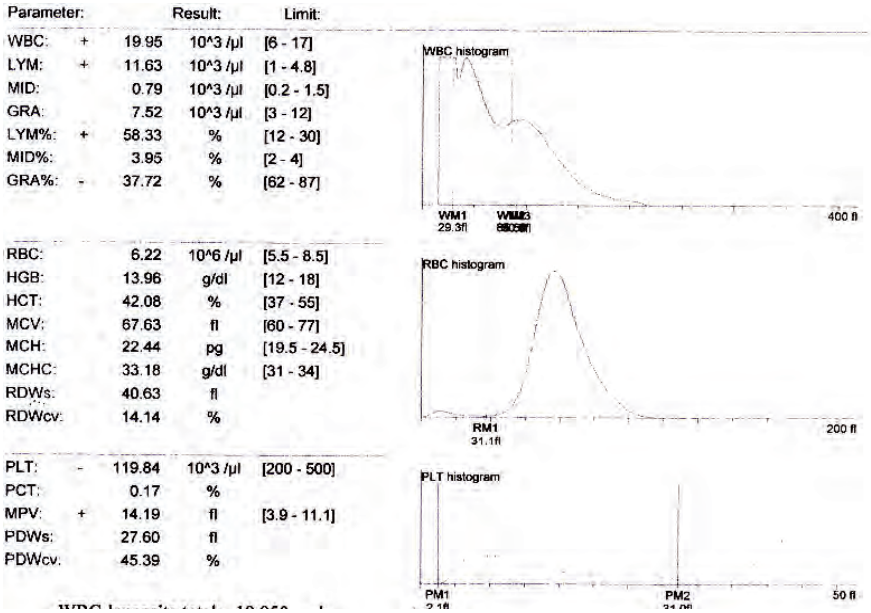


Hematological constants in 30 days within 30% protein food administration: bloodwork - PMN=50%; L=47%; M=2%; Eo=1%.

Table 6. Biochemical examination in 60 de zile within 30% protein food consumption

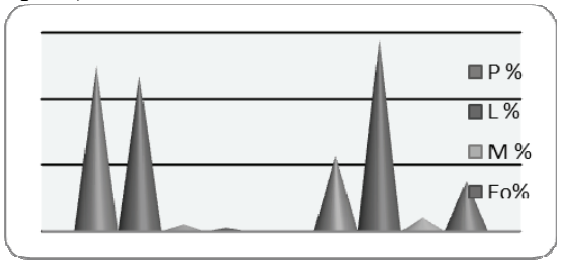
TEST	**RESULT**	**UNIT**	**MIN**	**MAX**
CHOL	180	mg/dl	120	250
TRIGL	76	mg/dl	10	150
TOTAL CA	8.64	mg/dl	8.7	11.8
MG	1.74	mg/dl	**1.7**	2.7
ALP	703	U/I	10	L50
GAMMA GT	12	U/I	0	9
UREA	22	mg/dl	l0	30
GLYCEMIA	79	mg/dl	62	110
CREA	0.73	mg/dl	0.5	1.3
GOT	84	u/l	10	50
GPT	76	ull	0	55
ISE-Na	15l	mEq/l	140	154
ISE-K	5.1	mEq/l	3.9	5.6
TOT PROT	6.3	g/dl	6.0	8.0
AMILASES EP	902	ull	260	1500
TOT BILIRUB	0.82	mg/dl	0	1.00
P	4.21	mg/dl	2.9	6.2

Histogram of hematological constants in 60 days within food administration.

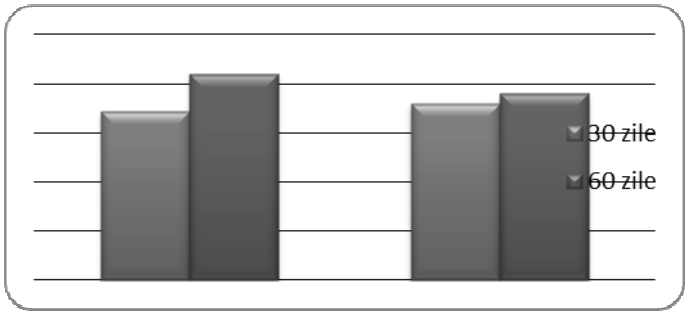


Hematological constants in 60 days within 30% protein food administration: bloodwork - PMN=23%; L=58%; M= 4%; Eo=15%.

At 30 days of consumption of 30% protein feed we found a decrease in red blood cells, hemoglobin, hematocrit and platelets, indicating a microcytic anemia; the bloodwork reveals neutropenia, lymphocytosis and thrombocytopenia. At 60 days of 30% protein food consumption only an increase of leukocytes is observed. Bloodwork in 60 days revealed leukocytosis, neutropenia, eosinophilia, lymphocytosis and moderate thrombocytopenia. Biochemical constants show an increase in GOT and GPT, which remain elevated even after 60 days of 30% protein food consumption (Graph 5).



Graph 4. Values of the blood work in 30 and 60 days within 30% protein food administration



Graphic 5. GOT and GPT values in 30 and in 60 days within 30% protein food adminstration

3. CONCLUSIONS

After the analysis we performed, we can conclude the following:

3.1 Mixed food consumption, especially food prepared at home, does not cause major changes in biochemical and hematological constants as long as the diet is associated with a corresponding water intake.

3.2 Elevated alkaline phosphatase is found only in lots that included puppies, the maximum value of the reference interval of alkaline phosphatase is 1200 U/l.

3.3 30 days of consumption of granulated dry food containing 18% protein causes microcytic anemia, neutropenia, monocitoză, lymphocytosis and thrombocytopenia, and after 60 days of consumption, eosinophilia, lymphocytosis, neutropenia and thrombocytopenia is found.

3.4 The consumption of granulated dry food with 18% protein content produces increased GOT and GPT, the value of the constants returns to maximum physiological levels after 60 days.

3.5 Consumption for 30 days of granulated dry food containing 30% protein causes a decrease in red blood cells, a decrease in hemoglobin, hematocrit and platelets, indicating a microcytic anemia. The picture shows neutropenia, lymphocytosis and thrombocytopenia.

3.6 Consumption for 60 days of granulated dry food containing 30% protein causes only a slight increase in leukocytes. Bloodwork revealed leukocytosis, neutropenia, eosinophilia, lymphocytosis and moderate thrombocytopenia.

3.7 Consumption for 60 days of granulated dry food containing 30% protein

causes a change in biochemical constants which reveals an increase of GOT and GPT, a phenomenon which remains increased after 60 days of administration

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THE INFLUENCE OF OXYTETRACYCLINE HYDROCHLORIDE ON THE HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF BROILER CHICKEN

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Key words: hematology, metabolic profile, oxytetracycline, doses

SUMMARY

The analysis of erythrocyte, leukocyte and biochemical parameters was the basis for estimation of oxytetracycline hydrochloride tolerance in Broiler chickens to facilitate the safety evaluation of a veterinary product *(Galiprotect comprimate).*

Investigations were conducted on a sample of clinically healthy Broilers (n = 90), divided into two age categories, each containing two experimental groups (n = 15), treated with oral doses of oxytetracycline hydrochloride (therapeutic and double) and 1 untreated control (n = 15).

During the experiments two sets of hematological and biochemical assessments (pre-and post-treatment) were performed, and the results were statistically interpreted and compared with reference values.

The mean recorded values of the erythrocyte parameters, showed no statistically significant alterations (p> 0.05). The oscillations observed were consistent with the reference data for the species. Thus the PCV and the number of erythrocytes were maintained within the physiological limits, a slight decrease being observed in hemoglobin concentration (8.00±0.88 to 7.03±1.07 g/dL). Mean erythrocyte constants showed minor variations from reference in case of MCH (25.18±4.65 to 35.11±7.26 pg), slightly increased MCV (136.80±27.29 to 177.92±21.61 fL) and slightly lowered (18.80±3.58 to 21.97±4.37 dl) MCHC.

Overall development of the leukocyte parameters outlined a profile of good defensive effectiveness, expressed as mean leukocyte levels situated within physiological limits. Leukocyte population was characterized by mean heterophile oscillations, slightly above normal, but statistically irrelevant (p> 0.05). Less important variations (from 36.14±13.20 to 45.86±15.27%) were seen in lymphocyte population. The proportion of monocytes was situated within reference intervals, ranging between 10.29±4.54% and 16.43±6.85%. Distribution of eosinophile subpopulations did not exceed physiological limits, the highest recorded mean value being 2.11 ± 1.38%.

The metabolic profile of the birds showed no significant statistical variations, slightly influenced by the experimental variables. Thus, mean protein levels presented variations within physiological range. The recorded albumin (1.73±0.33 to 2.7±0.86 g/L) and globulin (0.75±0.24 to 1.63±0.58 g/L) variations were without statistical significance. The same connotation was found in the other investigated biochemical parameters: glucose (202.20±16.50 to 278.20±25.43g/dL), aminotransferase (93.20±22.62 to 204.62±7.52U/L), creatine phosphokinase (737.82±206.95 to 1134.60±212.33U/L), uric acid (4.15± 0.94 to 7.02±1.04 mg/dL), calcium (4.46±1.42 to 9.58±0.73 mg/dL), phosphorus (4.46±1.42 to 7.58±1.25mg/dL), sodium (146.60±3.42 to 184.40±2.45mmol/L) and potassium (4.68±0.18 to 6.64±0.11mmol/L).

Information provided by hematological and biochemical investigations have proved relevant for establishing the health status of birds, seeing as their physiological characteristics differ from those of mammals (Clark and Raid, 2009). Hematological and metabolic investigations are also an essential component of protocols used in determining the degree of safety and tolerance of active substances (Ognean et al., 2011).

Research in the field of avian hematology highlights significant variations in erythrocyte and leukocyte parameters, influenced by various physiological factors such as species, gender or nutrition (Chubb and Rowell, 1959, Sturkie, 1965).

Metabolic profiling is an investigation commonly used in birds, knowing the fact that they manifest obvious clinical symptoms only in cases of severe disease; therefore require continuous monitoring of health status (Kendal, 2005).

Oxytetracycline hydrochloride is among the broad-spectrum antibiotics, commonly used in poultry, a synthetic tetracycline almost with an exclusive bacteriostatic action (Adams, 2001).

According to the aforementioned arguments the aim of this study was to assess the influences of orally administered oxytetracycline hydrochloride on the hematological and metabolic profile of Broiler chickens in the current context of veterinary medical product evaluation.

1. MATERIALS AND METHODS

The present research has been conducted on a population of Broilers (n = 90) derived from an intensive exploitation farm (Avicola Crăieşti-Mureş), and raised in similar conditions, maintaining the starter type combined feed. The birds were divided into six groups, corresponding to experimental variables: age, weight, duration of treatment and dose.

Groups 1A and 2A, consisting of 15 clinically healthy Broiler chickens each, of 9 to 10 days with a weight of approximately 0.5 kg, treated with the recommended and double dose (3 mg and 6 mg) oxytetracycline hydrochloride, 2 consecutive days.

Groups 1B and 2B, also consisting of 15 clinically healthy Broilers each, 32 to 38 days, weighing at least 1 kg, receiving, the recommended and double doses of oxytetracycline hydrochloride (12 mg and 24 mg) for 7 consecutive days.

Groups 3A and 3B, composed of 15 untreated controls each.

Pre-and post-treatment blood samples were collected on heparin, by puncture of the basilica vein, and subjected to hematological and biochemical investigations.

Among the determined hematological parameters were: total number of erythrocytes (Erit.) and leukocytes (Leuk.) (hemocytometric method), packed cell volume (PCV, microhematocrit method), hemoglobin concentration (Hb., spectrophotometric method), mean erythrocyte constants (MCV, MCH, and MCHC) and leukocyte subpopulations (on smears stained by panoptic method Dia Qiuck).

The metabolic profile indices were determined with *reptilian and avian profile* kits (Vetscan2 analyzer), which included: aspartate aminotransferase (AST), bile acids (BA), creatine phosphokinase (CK), uric acid (UA), glucose (GLU), calcium (CA), phosphorus (PHOS), total protein (TP), albumin (ALB), globulin (GLOB), potassium (K^+) and sodium (Na^+).

The recorded data was statistically analyzed with Graph Pad InStat V3.0 and Microsoft Excel applications, establishing the mean value, standard deviation and the index of probability "p" (Student test).

2. RESULTS AND DISCUSSIONS

The overall analysis of physiological parameters showed variable mean values mainly falling within physiological limits of the species and category, with few deviations from references. Thus, development of the erythrocyte parameters was characterized, according to data recorded in table 1, mean PCV values within physiological limits (34.00 to 43.00%) and hemoglobin concentration (Hb) slightly below the reference values (8.90 to 13.50 g/dL) (Gylstorff, 1983).

PCV oscillations (36.31 ± 4.98 to 40.50 ± 3.95%) and Hb (7.03 ± 1.07 to 8.00 ± 0.88 g/dL) were not influenced by age and could not be related to experimental variables. This apparent trend towards anemia was offset by developments of the mean values of total number of erythrocytes within the physiological range (2.20 to 3.30T/L) with group variations between a minimum of 2.29±0.31 T/L and a maximum 2.84±0.39 T/L.

The mean levels of the erythrocyte constants showed less important fluctuations compared to reference values for MCH (25.18±4.65 to 35.11±7.26 pg), slightly increased MCV (136.80±27.29 to 177.92±21.61 fL) and slightly decreased, both pre- (19.39±2.52 to 21.31±2.84 g/dL) and post-treatment (18.80±3.58 to 21.97±4.37g/dL) for CHEM. Comparative analysis of the data revealed a normal erythrocyte profile for the investigated categories, indicating a good tolerance of therapeutic and double doses of oxytetracycline hydrochloride and the lack of negative effects on fluid and electrolyte homeostasis, osmotic balance, erythropoiesis and other erythrocyte functions.

Table 1.

Mean erythrocyte parameters in Broilers treated with oxytetracycline hydrochloride (groups 1A and 2A -3 respectively 6 mg/bird/day for 2 days, lots 1B, 2B - 12 mg and 24 mg/bird/day, 7 consecutive days) and untreated controls (groups 3A and 3B)

Group	***9-10 days***			***32-38 days***		
	1A	***2A***	***3A***	***1B***	***2B***	***3B***
	Mean/ st.dev.	***Mean/ st.dev***	***Mean/ st.dev.***	***Mean/ st.dev***	***Mean/ st.dev***	***Mean/ st.dev***
Parameter	***Pre-treatment/initial***					
PCV(%)	40.00 ±3.49	37.25±2.72	36.31 ±4.98	40.25±3.25	39.13±3.16	36.88±4.62
Hb(g/dL)	8.00±0.88	7.86±0.70	7.10±0.93	7.73±0.53	7.55±0.76	7.03±1.07
Erit.(T/L)	2.32±0.19	2.35±0.20	2.76±0.41	2.37±0.12	2.50±0.19	2.72±0.36
MCV(fL)	173.43±17.29	159.69±19.61	138.19±31.90	170.23±11.96	157.01±15.62	136.80±27.29
MCH(pg)	34.91±5.21	33.73±4.77	27.09±5.57	32.78±3.47	30.36±3.95	26.18±5.73
MCHC(g/dL)	20.27±3.16	21.31±2.84	19.94±3.83	19.39± 2.52	19.44±2.30	19.45±3.97
Post-treatment/final						
PCV(%)	40.50±3.95	36.88±5.31	38.25±3.72	40.19±3.53	39.75±2.81	37.94±4.74
Hb(g/dL)	7.95±1.30	7.81±0.58	7.10±1.04	7.66±0.60	7.93±0.62	7.08±1.03
Erit.(T/L)	2.29±0.31	2.49±0.21	2.84±0.39	2.37±0.34	2.58±0.19	2.79±0.38

MCV(fL)		177.92±21.61	148.05±19.39	136.65±26.84	170.89±24.25	154.67±15.09	138.60±31.15
MCH(pg)		35.11±7.26	31.55±2.95	25.18±4.65	32.76±5.45	30.83±2.79	25.89±5.77
MCHC(g/dL)		19.90±5.28	21.97±4.37	18.80±3.58	19.28±2.75	19.99±1.41	18.94±3.62
Ref	**Source**	**PCV (%)**	**Hb (g/dL)**	**Erit.(T/L)**	**MCV(fL)**	**MCH(pg)**	**MCHC(g/dL)**
	R[1]	*27.40*	*8.23*	*2.07*	*134.93*	*24.99*	*34.17*
	R[2]	*34.00-43.00*	*8.90-13.50*	*2.20-3.30*	-	-	-

R[1] Ghergariu et all., 1999. Manual de laborator clinic veterinar; **R[2]** Gylstorff I, 1983. Handbuch der Geflügelphysiologie (Gylstorff I, Grimm F, 1987 Vogelkrankheiten).

Developments in the leukocyte parameters, revealed by the data presented in table 2, showed wide variations in mean levels of total leukocytes, from 15.17±6.12 to 24.11±7.38 G/L, but within the normal limits.

The analysis of mean leukocyte subpopulations values show that the percentage heterophiles exceeded physiological limits (from 19.80 to 32.60%), both pre- (38.00±23.04 to 43.00±3.45%) and post-treatment (36.14±13.20 to 41.73±17.08%), but these deviations were statistically insignificant (p> 0.05).

Tabel 2

Mean values of leukocyte parameters in Broilers with oxytetracycline hydrochloride (groups 1A and 2A -3 respectively 6 mg/bird/day for 2 days, lots 1B, 2B - 12 mg and 24 mg/bird/day, 7 consecutive days) and untreated controls (groups 3A and 3B)

Group		***9-10 days***			***32-38 days***		
		1A	***2A***	***3A***	***1B***	***2B***	***3B***
		Mean/st.dev.	***Mean/st.dev.***	***Mean/st.dev.***	***Mean/st.dev.***	***Mean/st.dev.***	***Mean/st.dev.***
Parameter		***Pre-treatment/initial***					
Leuk.(G/l)		17.69±3.61	16.11±8.08	19.35±6.12	20.82±3.11	22.31±12.23	19.32±8.61
Heter.(%)		43.00±3.45	41.55±22.12	43.18±18.12	39.57±4.06	38.00±23.04	38.29±15.75
Eos.(%)		1.36±1.00	0.91±0.84	1.54±0.92	2.11±1.38	1.59±0.99	1.00±0.05
Bas.(%)		0.82±0.40	1.82±1.29	1.37±1.23	1.75±1.18	1.84±1.55	1.14±1.13
Limph.(%)		43.18±3.95	43.64±22.92	41.36±18.21	46.12±3.15	43.00±29.84	43.14±18.63
Mono.(%)		11.64±4.20	12.09±5.81	12.55±5.48	10.29±4.54	15.57±7.54	16.43±6.85
Post-treatment/final							
Leuk.(G/l)		16.95±6.62	15.17±6.12	18.21±6.63	22.53±15.71	24.11±7.38	20.56±8.78
Heter.(%)		41.73±17.08	38.91±18.12	39.36±16.25	41.26±14.21	37.43±15.00	36.14±13.20
Eos.(%)		1.75±1.48	1.72±0.91	1.36±0.79	1.29±0.78	1.62±0.43	2.02±0.64
Bas.(%)		1.18±1.18	0.64±1.23	1.27±0.96	1.71±1.52	1.91±1.11	1.26±0.79
Limph.(%)		42.82±17.87	45.45±18.21	44.64±15.35	42.43±28.00	44.17±7.46	45.86±15.27
Mono.(%)		12.51±5.220	13.27±5.48	13.36±5.54	12.71±7.55	13.63±5.02	14.52±6.50
Ref.	**Source**	**Leuk.(G/L)**	**Heter.(%)**	**Eos.(%)**	**Bas.(%)**	**Limph.(%)**	**Mono.(%)**
	R[1]	*19.80-32.60*	*7.90-24.20*	*1.50-2.70*	*1.70-4.30*	*45.00-75.00*	*8.1-16.50*
	R[2]	*20.00-30.00*	*14.00-49.00*	*2.00-14.00 (7.0)*	*1.00-17.00 (9.00)*	*31.00-72.00*	*1.00-4.00 (2.00)*

R[1] Gylstorff I, 1983. Handbuch der Geflügelphysiologie (Gylstorff I, Grimm F, 1987 Vogelkrankheiten).; Gylstorff I: Handbuch der Geflügelphysiologie, 1983;**R**[2] Hoffmann G., 1961 „Abriss der LaboratoriumstierKunde", Veb Gustav Fisher Verlag, Jena.

Distributions included within physiological limits were observed in the development of eosinophil subpopulation, which did not exceed the upper reference limit (2.70%), the highest mean recorded value was (2.11±1.38%). Lymphocyte population development was characterized by minor variations, from 41.36±18.21 to 46.12±3.15%, falling within physiological limits (45.00 to 75.00%). The proportion of monocytes was situated within the reference intervals (8.10 to 16.50%) both pre-and post-treatment, ranging between 10.29±4.54% and 16.43±6.85%. Quantification of the total number of leukocytes and leukocyte subpopulations proportions confirmed tolerance to therapeutic and double doses of oxytetracycline hydrochloride, expressed by the lack of sensitization or adverse effects on leucopoiesis and main leukocyte function.

Developments in the determined metabolic parameters revealed, according to data presented in table 3, a characteristic profile of Broilers, with more or less important deviations.

Table 3

Mean values of the metabolic parameters in Broilers with oxytetracycline hydrochloride (groups 1A and 2A -3 respectively 6 mg/bird/day for 2 days, lots 1B, 2B - 12 mg and 24 mg/bird/day, 7consecutive days) and untreated controls (groups 3A and 3B).

Group	***9-10 days***			***32-38 days***		
	1A	***2A***	***3A***	***1B***	***2B***	***3B***
	Mean/st.dev.	***Mean/st.dev.***	***Mean/st.dev.***	***Mean/st.dev.***	***Mean/st.dev.***	***Mean/st.dev.***
Parameter	***Pre-treatment/initial***					
TP(g/dL)	3.41 ± 0.23	3.35±0.30	3.28 ±0.52	3.52 ±0.15	3.54±0.16	3.48±0.17
ALB(g/dL)	1.75±0.35	1.73±0.33	1.74±0.36	1.92±0.54	1.96±0.15	1.75±0.27
GLOB(g/dL)	1.11±0.55	1.12±0.24	0.75±0.24	1.04±0.40	1.63±0.58	0.95±0.15
GLU(mg/dL)	212.84±35.64	230.42±15.75	225.40±35.35	254.60±33.32	261.80±16.80	215.60±15.63
AST(U/L)	155.22±28.24	183.20±22.32	204.62±7.52	185.80±24.23	192.80±31.62	185.40±20.12
CK(U/L)	1005.80±201.21	1050.20±72.83	1134.60±212.33	925.40±64.21	836.80±86.32	954.40±62.73
BA(µmol/L)	< 35	< 35	< 35	< 35	< 35	< 35
UA(mg/dL)	4.66±0.75	5.38±/0.65	6.15±0.72	7.02±1.04	6.26±1.48	6.18±0.62
CA(mg/dL)	8.64±0.96	7.35±1.86	7.96±1.15	8.26±1.23	9.58±0.73	8.22±1.50
PHOS(mg/dL)	6.80±0.42	6.80±1.00	7.58±1.25	7.56±0.74	6.35±0.94	7.55±0.52
NA^+(mmol/L)	175.2±03.53	184.40±2.45	175.40±9.92	168.80±4.22	155.00±1.56	146.60±3.42
K^+(mmol/L)	6.64±0.11	5.54±0.23	4.88±0.15	4.78±0.15	4.68±0.18	5.55±0.28
Post-treatment/final						
TP(g/dL)	3.40±0.32	3.50±0.28	3.26±0.65	3.68± 0.38	3.56±0.41	3.36±0.42
ALB(g/dL)	1.85±0.40	1.93±0.24	1.90±0.34	2.64±0.45	2.72±0.86	2.28±0.45
GLOB(g/dL)	1.48±0.65	1.58±0.28	1.05±0.45	0.95±0.23	0.78±0.52	1.12±0.21
GLU(mg/dL)	228.60±30.0	206.00±13.27	202.20±16.50	278.20±25.43	252.80±58.15	277.20±54.65
AST(U/L)	170.45±11.82	167.20±22.45	178.40±14.45	93.20±22.62	128.60±51.14	142.60±38.01

CK(U/L)		981.24±172.65	867.00±196.83	1063.80±352.49	737.82±206.95	757.40±452.31	953.20±82.12
BA(µmol/L)		< 35	< 35	< 35	< 35	< 35	< 35
UA(mg/dL)		4.32±1.13	4.15±0.94	4.54±1.45	4.75±1.35	4.76±1.75	4.82±1.05
CA(mg/dL)		8.62±1.47	8.76±1.32	8.22±1.62	9.45±0.32	8.94±1.35	9.57±0.51
PHOS(mg/dL)		6.42±1.03	6.24±0.75	5.25±0.62	5.52±1.18	4.46±1.42	4.60±0.86
NA^+(mmol/L)		155.60±9.43	152.20±7.62	152.20±7.22	157.20±5.72	157.40±8.96	156.60±6.58
K^+(mmoL/L)		5.52±1.51	4.32±0.56	4.70±1.02	4.82±0.85	4.95±1.26	4.71±0.82
Ref.	**TP(g/dL)**	**ALB(g/dL)**	**GLU(mg/dL)**	**UA(mg/dL)**	**CA(mg/dL)**	**PHOS(mg/dL)**	**NA^+(mmol/L)**
	*3.00 - 3.70**	*1.50-1.80**	*130.00-270.00 **	*5.80-8.30**	*7.70-10.00**	*6.00-7.60**	*151.00-161.00***

* Ghergariu et al., 2000. Manual de laborator clinic veterinar;** Reece. 1996. Physiology of domestic animals.

Thus, the development in protein levels was characterized by a relatively constant mean level, group oscillations ranging from 3.26±0.65 to 3.68±0.38g/L, physiological ranges for this parameter being 3.00 to 3.70 g/L (Ghergariu et al., 2000). Albumin (1.73±1.33 to 2.72±0.86g/L) and globulin values (0.75±0.24-1.63±0.58g/L) also showed slight deviations from the physiological limits both pre-and post-treatment. Within the reference limits (130.00 to 270.00 mg/dL) established by Reece (1996) was situated the blood glucose (202.20±16.50 to 278.20±25.43g/dL).

In the same context could be framed the minor and statistically insignificant deviations reported for aspartate aminotransferase (93.20±22.62 to 204.62±7.52U/L), creatine phosphokinase (737.82±206.95 to 1134.60±212.33U/L), uric acid (4.15±0.94 to 7.02±1.04 mg/dL), calcium (4.46±1.42 to 9.58±0.73 mg/dL), phosphorus (4.46±1.42 to 7.58±1.25 mg/dL), sodium (146.60±3.42 to 184.40±2.45 mmol/L) and potassium (4.68±0.18 to 6.64±0.11 mmol/L).

The mean values reported for protein, carbohydrate, enzyme and mineral metabolic indices did not highlight any toxicity or induced metabolic syndromes by therapeutic or double doses of oxytetracycline hydrochloride.

During the course experiment ethic and welfare standards were maintained which increased the relevance of data and the interpretation in the general context of medical and veterinary practice (Mitchell. 1998).

3. CONCLUSIONS

3.1. Mean PCV values remained within physiological (34.00 to 43.00%) while Hb concentration fell slightly below the lower reference values (8.90 to 13.50 g/dL). Hb decreasing trend was not correlated with the total number of erythrocytes, which was within the physiological range.

3.2. The total number of leukocytes showed wide variations (15.17±6.12 to 24.11±7.38 G/L), but which did not exceed normal values.

3.3. Leukocyte population showed a slight heterophilia (36.14±13.20 to 43.18±18.12%) and physiological variations within the normal limits for lymphocyte (41.36±18.21 to 46.12±3.15%), monocite (10.29±4.54% to 16.43±6.85%) and eosinophile (0.91±0.84 to 2.11 ±1.38%) subpopulations.

3.4. Biochemical analysis revealed deviations more or less important of the mean values for protein, carbohydrate, enzyme and mineral profile, which were not influenced by the

experimental variables pre-and post-treatment.

3.5. The health monitoring through hematological and metabolic profile indices in the tested Broilers did not identified risks associated with doubling the dose of orally administered oxytetracycline hydrochloride, showing a good level of tolerance and lack of immediate or delayed adverse reactions.

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OLFACTORY, AUDITIVE, TACTILE, GUSTATIVE AND VISUAL ACCURACY IN HENS IN SMALL PROPERTY

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SUMMARY

To investigate modeling adaptive behavior and perception of smell, hearing, touch, taste, sight, in fowls in small property, one requires the development of a specific experimental design for each analysis. This study intended following feeding behavior and adaptation methods to natural growth conditions of chickens found in private households, reported to the type of perception.

In the current economic situation, farming chicken in small households has its own utility by turning food waste from households and producing eggs as to meet the own requirements. Often, economic contribution is increased by increasing the effective, ensuring stable food sources, housing according to the size of the lot, so as to increase the production of eggs, meat or even selling them to obtain a material benefit. The small community tends to uniformity of specimens that compose it on breed, age, color [5,6,7].

Chicken farming requires knowledge of anatomy, physiology, ethology, nutrition and diet due to the following: the large number of breeds with biological peculiarities of growth and maintenance very different from each other, the complexity of production and especially the production of eggs, the very intense metabolism that makes birds react very quickly to the slightest change in living conditions, the characteristic breeding behavior and adaptive behavior modeled according to the influence of natural environmental conditions [1,2,3,4].

1. MATERIAL AND METHOD

Experimental investigations on modeling adaptive behavior and perception of smell, hearing, touch, taste, sight, in fowls in small property were conducted on a total of nine hens and a rooster based on a specific experimental design.

2. RESULTS AND DISCUSSIONS

The results and their interpretation were based on an experimental design for each sensory channel studied.

Olfactory perception. To study olfactory perception, the cephalic extremity of the chicken was covered with a block to exclude identification of feed within sight. Then the chicken was placed in the middle of feed that enters the normal ration of the species after a day of fasting. A state of immobility, rigidity and inability to identify objects around the chicken followed. Not even lifting up the feed to its beak improved the situation. In a later stage the block we placed was removed and the corn was soaked in acetic acid, a strong olfactory stimulus, and placed in Petri plates. The hens consumed the feed. Normally, heavy smell of acetic acid eliminates any mammal or bird that use smell to identify the feed. This means that identification of feed in chicken is performed by sight and not by smell.
The data obtained from the morphological examination of the chickens revealed that they are free of olfactory lobes, which excludes the reception and analysis of odors in the environment and especially in the feed (Fig. 1).

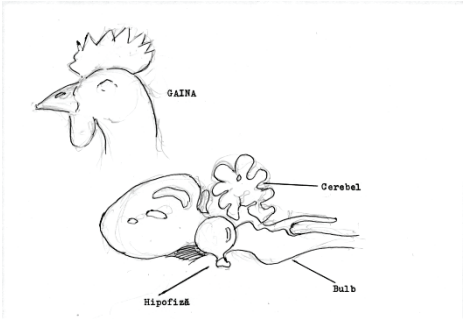

Fig. 1. One remarks the lack of the olfactory lobe in the chicken (original)

Auditory perception. At the end of hatching, when chicken are about to leave the egg, they have already communicated with the hen. Sound Communication is essential. After hatching, recognition between chicks and hen is not through sight but by sound. Communication sounds for social integration are almost constant, especially in chickens with different modulations. Rooster needs to impose,

dominate, conquer, protect, get known by his sounds, his presence means stability. Sounds of moderation, of quietness on the perch, seem like the ones found in hen houses when they soothe the young. Auditory perception is satisfactory and fulfills the role of communication and cohesion in the whole group.

Taste perception requires a slight dissolution of the feed, achieved with the help of salivary glands, which requires a certain stagnation of the feed in the mouth. They remember the appearance of the feed so they no longer require a new tasting so that food is accepted or rejected. The conditions offered by growing under the hen, she identifies what is good for the chicken, calling and learning follow. The taste image combined with the image it gives is permanent. The priority in eating is represented by insects, earthworms, etc. which move. Insects mimic death by transient immobilization often escaping observation. Some insects produce bad-smelling secretions, which impresses insectivorous mammals, in birds there is more of a taste repellent effect. Black ants are eaten by chicken, whereas the red ones are not.

Visual perception. In birds, the eye is the main sense organ involved in facilitating all adaptation activities to the life environment, orientation in space and balance. Visual quality go beyond the visual possibilities of any other species. A falcon or hawk identifies a mouse in the grass from a height of 400 m, a chicken picks a grain of poppy from the ground on the ground at the first attempt, which are unprecedented performances in mammals. For identification of color perception we used the principle of colored vessels. We used nine vessels stained with the four basic colors and five complementary colors, located at distances of 3 m apart. Getting used to a color lasts 5 days, afterwards feed is moved to another vessel after a break of one week. This experiment takes time and requires patience. When the chicken recognizes a color, it becomes familiar with it. Problems arise when they confuse a color with one or two other colors, green with blue, orange with yellow or ocher, but they are colors of the same range of radiation

The arrows indicate the recognized colors. Where there are two or three arrows, it means that they are confused and their interpretation is the same: green with blue, orange with yellow and ocher (Fig. 2).

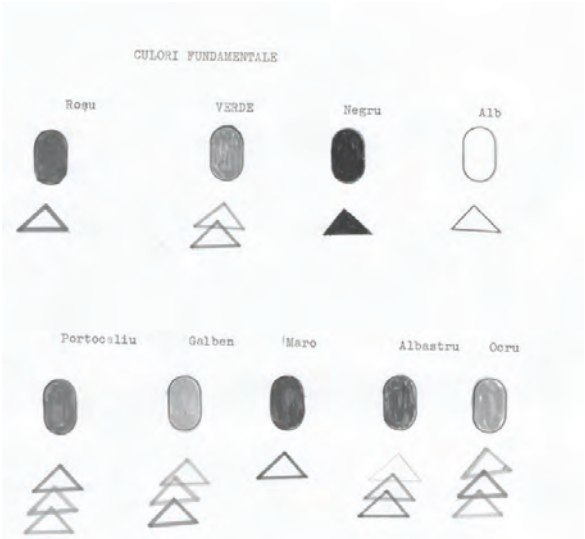

Fig. 2. Identification of color perception in chicken.

To identify and memorize shapes in chicken, we used the following experimental design: four boxes were made with a 20 cm side painted white and on each figure 1-4 was printed in black. Feed was introduced only in box no. 2 for 5 days. We used four hens and a rooster, feed being regularly administered to the box no. 2. After five days the hens go directly to box no. 2 without exceptions. On the 6th day box no. two is changes with box no. 4, chickens finding o feed, they go straight to box no. 2. Next day, they remain within the same box (Fig. 3).

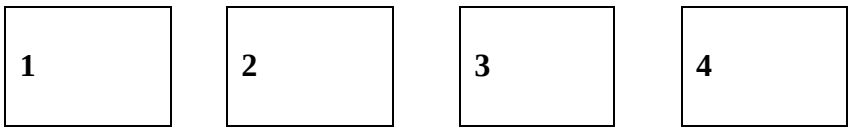

Fig. 3. Shape identification and memorization.

Following the experiment performed we can conclude the following: laying hens remember the topography of the feed box, they remember box no. 2 by shape. Memorization required 5 days. We took into account wind direction; although we already knew chickens are free of odor sense. The process of memory leads to remembering shape and topographic position.

Establishing the visual field. Mobility of the eye is particular, being aided by nine muscles and the anatomical position facilitates a field view of 350 ° allowing control of the beak to the cephalic extremity, so the chicken can control the exact collection of a grain of poppy at the first attempt. Cervical mobility contributes to turning the head easily so as to receive the secretion of the uropigeal glands. Comparing the brightness of the eye with the pupil

at maximum opening though, the brightness is reduced to about 6.3, which is little. Reporting from our research in the number of rod cells compared to cone cells for color perception, the ratio would be 1/3 (Fig. 4).

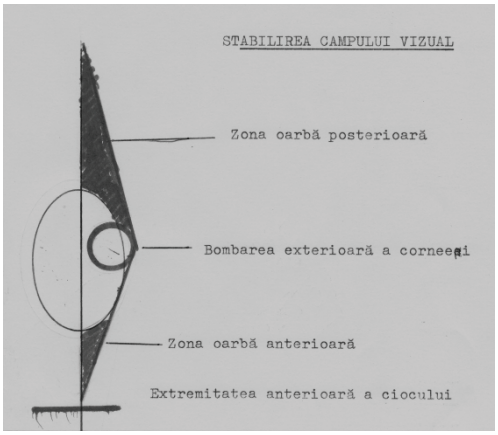

Fig. 4. Stability of visual field

This explains the low visibility of chicken in twilight and unfortunately the reduced brightness of the eye, the cone cells for color perception require an intense light. We consider the growth of chicken I blind halls as a contravention of health technology, regardless of the arguments in its favor. Even though the lens in the chicken is equivalent to a wide angle one, low brightness reduces its value.

3. CONCLUSIONS

3.1. Experiments have shown the lack of sense of smell, hens are anosmatic. They lack the presence of the olfactory lobe.

3.2. Chickens have a language of communication, social integration and cohesion of the group. It starts with the first sounds from the period before hatching and continues with a sound gender differentiation, the sounds fit into a complex language of social signals.

3.3. Tactile perception and taste are associated predominantly with tactile identification of food in the mouth.

3.4. The ratio of cones and sticks present of the retina is 3 / 1 in favor of cones, so a fotopic view, colored, which requires a satisfactory luminance. Chicken clearly distinguish red, black, white, brown, but green is confused with blue, orange and yellow with ocher. The small number of rod cells, the scotopic view explains nyctalopia. In the experiment we used the colored vessels principle.

3.5. The experiment demonstrates the ability to identification and memory object size.

3.6. The view field in chicken is 350 °.

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PHENOTYPIC CHARACTERIZATION OF STAPHYLOCOCCI STRAINS ISOLATED FROM ANIMALS

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Key words: staphilococi, methicillin-resistance, animals

SUMMARY

In routine diagnosis, staphylococcal infections, it is necessary to use a simplified working scheme to differentiate pathogens such as *Staphylococcus* or other nonpathogenic and hull, which is based on some characteristic phenotypic characters.
Methicillin-resistant staphylococcus bacteria are considered with zoonotic risk, causing nosocomial infections in humans. Methicillin-resistance is present in several species coagulase positive or negative, in laboratories, its presence being compulsorily tested.
In this study have been identified 27 of methicillin-resistant strains, from all animal species, is shown as epidemiological cycle of intra-and interspecific these strains.
Bacterial performed according to the methodology used allowed the isolation and identification of three species of staphylococci (*S. aureus* subps. *aureus*, *S. hycus*, *S. xylosus*) from animals of rent and the Group *intermedius* from dogs and cats.

Staphylococcal infections in animals are quite common, have a variable clinical course, being produced by several species of staphylococci, with an intra-and interspecific epidemiological circuit that includes people (3).
In recent years methicillin-resistant *Staphylococcus* bacteria are considered particularly zoonotic risk, causing nosocomial infections in humans. Methicillin-resistance is present in several species coagulase positive or negative, in laboratories, its presence being compulsorily tested (1, 2, 4, 5).
In routine diagnosis, staphylococcal infections, it is necessary to use a simplified working scheme to differentiate pathogens such as *Staphylococcus* or other nonpathogenic and hull, which is based on some characteristic phenotypic characters (3).
In this context research covered by this paper were performed in order:
-to characterize the phenotypic strains of staphylococci isolated from

animals, based on a simplified scheme;
-to identify staphylococci coagulase positive and negative rapid test;
-to identify methicillin-resistant strains of staphylococci present in animals.

1. MATERIALS AND METHODS

Pathological material samples were taken from animals (sheep, cattle, horses, pigs, dogs and cats), with different clinical disease or healthy. Pathological samples were taken from a number of rent and 50 animals from 55 different pets with skin diseases or healthy (Table 1).
Of pathological material were made early sowing on agar sheep blood defibrinated 5%, the plates were incubated at 37˚C in normal atmosphere for 18 -20 hours. Early sowings were made in this way to obtain isolated colonies, in order to assess cultural character. Strains of staphylococci were isolated and purified on biochemical tests and pathogenicity of characters.
Controlled biochemical properties were manita the environment Chapmann fermentation and fermentation sugars present in several API Staph system. Control pathogenicity factors were the presence of coagulase-related (clumping factor) and hemolisins. Haemolytic activity was assessed on sheep blood defibrinated agar 5%.
Highlighting related coagulase was highlighted Staph Latex Kit KIT PROLEX quickly. Related strains possessing coagulase (clumping factor) or protein A, placed in contact with latex particles sensitised with fibrinogen and IgG, producing mixed agglutination. Indicates the emergence of coagulase-related small clot.
For susceptibility testing of staphylococci and novobiocine, strains of staphylococci isolated was used Kirby-Bauer difusimetric method were used the following ingredients: Mueller-Hinton broth and agar, Petri plates and impregnated biodiscs meticilin-novobiocine and Oxoid products.

2. RESULTS AND DISCUSSION

Bacteriological examination of samples submitted were isolated 105 strains of staphylococci, in October 2010 - May 2011, in the Bacterial Infectious Diseases Research Laboratory - Faculty of Veterinary Medicine Timisoara.
The 105 strains studied, the Gram stain were assigned to Gram-positive group and were presented in the form of cocci, with size between 0.8-1.5 mm in diameter, and how was the predominant group cluster and

occasionally in pairs or solitary bacterial cells.
Both in primary cultures and subcultures isolates produced yellow pigment is either white pigment beta haemolysis type, more commonly, as well as alpha (incomplete).

Table 1

The result of bacteriological examination

Crt. No.	Species	Samples examinated	
		Pozitive	Sterile
1.	Cattle	8	-
2.	Sheep	7	-
3.	Swine	15	-
4.	Goats	10	-
5.	Horses	10	-
6.	Dogs	40	-
7.	Cats	15	-
Total		105	-

Strains of staphylococci isolated from animals have variable biochemical behavior, and the tests used could make a difference summary, the results of these investigations are presented in Table 2. All isolates were susceptible to novobiocină, allowing their classification in the genus *Staphylococcus*.
The Chapman agar is a selective medium for isolation and presumptive identification of staphylococci manita-positive and fermented rose manita a total of 29 strains, all isolated only from animals of rent.

Table 2

Biochemical test results

Crt. No.	Species	Laboratory test results		API Staph test results			
		Chapmann agar	Pastorex Staph Latex Kit	*S. aureus*	*S. xylosus*	*S. hycus*	*Intermedius* group
1.	Cattle	1	2	1	5	2	0
2.	Sheeps	2	2	4	3	-	0
3.	Swine	7	10	5	-	5	0
4.	Goats	9	2	8	2	-	0
5.	Horses	10	10	7	2	1	0
6.	Dogs	11	18	11	0	0	21
7.	Cats	9	2	9	0	0	4

Bound coagulase was present in 46 of the 105 isolated strains. API Staph system was tested in all isolates, interpretation of results is made with a program softwer, and the results are shown in Table 2. This system allowed the staphylococci isolated from dogs and cats to be involved in the group without being differentiated intermedius species of this group.

The phenomenon of methicillin-resistant strains of staphylococci characterizing, zoonotic risk, was present in 16 strains isolated from animals of rent and 11 strains isolated from pets. At present this phenomenon governed by factors plasmid (R) and chromosomes (gene *mec*) is widely studied in both animals and humans because it is associated with other resistotypes and methicillin-resistant strains have a complex epidemiological circuit (5, 6, 7).

3. CONCLUSIONS

3.1. Bacterial performed according to the methodology used allowed the isolation and identification of three species of staphylococci (*S. aureus* subps. *aureus, S. hycus, S. xylosus*) from animals of rent and the Group *intermedius* from dogs and cats.

3.2. Use novobiocine strains allowed the classification of the genus *Staphylococcus* and pigmentogenesis, hemolysis, the presence of related coagulase, fermentation manita and API Staph system allowed the classification of strains in the three species and intermedius group.

3.3. Have been identified methicillin-resistant strains 27, from all animal species, is shown as epidemiological cycle of intra-and interspecific these strains.

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NATIONAL SYSTEMS OF INNOVATION - A COMPLEX ENVIRONMENT FOR SCIENTIFIC RESEARCH EXPLOITATION POLICIES, COMPARATIVE ANALYSIS

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Key words: innovation, scientific research, technological transfer

SUMMARY

Investments in research - development of each country and the human skills training for the knowledge industries are vectors of its socio-economic development. A fundamental aspect of the knowledge industry, already made a reality in the economies of advanced countries is the creation of national innovation systems, to achieve a balanced interaction between universities, companies, research laboratories and corporations, in order to capitalize on the best scientific research results.

1. GENERAL FRAMEWORK - CONCEPTUAL ELEMENTS

In carrying out the building and development of knowledge society process, knowledge industry became a reality. This creative dimension that produces knowledge consists of two main areas:

1. The area of organizations that produce new knowledge as such (research, information production and processing, innovation, dissemination of information in the global society system;
2. The area of the organizations based on intensive knowledge related to dynamic innovation networks (this may attract an increased volume of capital invested in research -development and can provide rapid transfer of research - development results in economic - social system).

The specialized literature distinguishes between knowledge-based industries (science) and industry created by human intelligence as follows:

a. A science-based industry has emerged in the twentieth century and propelled the deliberate creation (invention) of new products. In Europe, the chemical engineering industry in Germany is considered the first industry of this kind;

b. Industry created by human intelligence emerged in the '90s and recorded the fastest growth rate, considered as the industry of the next century. Here

are some of its components: biotechnology, microelectronics, telecommunications industry, the production of civil aircraft, tools machine, robots, computers, equipment, programs, and new materials industry (Thurow, 1996).

The development of these components of the industry created by human intelligence does not depend on natural resources that a country is endowed or not with, but on the size of each country's investment in research - development and the human skills training for these industries. Thus, if a country has not developed a base of human expertise in immunology, virology and / or microbiology, for example, by configuring the required number of specialists, doctors in these areas, it cannot sustain a biotechnology industry.

According to Michael Gibbons, the core of the process of transition to knowledge industry comprises this new way of production and dissemination of knowledge based on research —development - innovation. The beginning of this process is placed at the end of the twentieth century and was determined by two factors:

- Development of higher education at high rates;
- The emergence and development of companies providing specialized services at demand, which have reduced the corporations' monopoly —those that provide production for mass consumption.

Regarding the mass-scale development of higher education, this has led to recognition by more and more countries of the fact that universities can generate new knowledge. This framework recognized in most universities around the world led to the establishment of scientific research as a function as such, along with the training function. In this respect, Michael Gibbons believes that "*... the dissemination of higher education inside the society had the effect of continuous offerings of workforce educated for the industrial system. Research has become a core function for universities, first of the elite, then the other. This process increased the level of familiarity with science and technology, with scientific methods and procedures and has increased the number of sites where research is conducted as a recognized occupation...* " (Gibbons, 1998).

Within the knowledge industry, data, information, knowledge are the main range of goods that are produced and marketed. Today, in the knowledge industry, the factories are: systems of science and technology, innovation

systems, companies specialized in production of knowledge on demand and they are the equivalent for what traditional factories were within industrial society. The main purpose of scientific research regardless of where it is conducted is to produce new knowledge that can lead, of course, on different levels of use, to a practical purpose (Purdoiu, 2010).

Higher education s as components of national innovation systems have a well defined and extremely important role in knowledge industry through scientific research process that they develop and through comprehension of research results in the socio-economic area.

Today, one of the most important aspects of the knowledge industry is the creation of ***national innovation systems***, the interaction between universities, corporations, research laboratories and companies.

The concept *of national innovation system*[1] was first introduced in the literature by Christopher Freeman in his *Technology, Policy and Economic Performance: Lessons from Japan* (Freeman, 1987). The author distinguishes a number of factors that characterize national systems of innovation, which actually determine the specificity of each system:

- Institutional factors related to the structure and quality of educational institutions, the forms of their financial institutions, research - development institutions, juridical institutions;
- Factors related to public policies of a country and expressed through the objectives of this policies relating to areas where innovation is stimulated, the type of institutions encouraged to innovate and forms of financing;
- Innovation incentives such as: local demand, local production prices, natural resources, public and private investment activities;
- Other possible elements of national innovation systems, such as: level of education and workforce training, companies' attitude to innovation, their management culture, etc.

[1] After 1987 around the concept of "*national innovation system*" has been created an entire literature, of which we mention the works: LUNDVALL, B.A. *National Systems of Innovation: Towards a Theory of Innovation and Interactive Learning.* London: Frances Pinter, 1992; NELSON, R. R. *National Systems of Innovation: A Comparative Analysis.* Oxford: Oxford University Press, 1993.

2. COMPARATIVE ANALYSIS OF SOME INNOVATION SYSTEMS IN THE WORLD

Currently, the most diversified national innovation system exists in the U.S. and was established in the early 1950s, the political decision of the National Ministry of Science. That was established by law, the National Science Foundation, which operated under the auspices of the National Ministry of Science. Its first assignment was collection and analysis of quantitative information on the research - development in the U.S., as a basis for taking political decisions in the field. National Ministry of Science has allocated large sums from its budget for the conduct by the National Science Foundation of studies and analysis on the research - development (R-D), on R-D funding by Federal Government funds, R-D financing in industry, colleges and universities. If we refer only to the year 1952 we see the deep involvement of the U.S. in R —D; at that time the National Science Ministry has allocated one million dollars from its budget, at that time about 13.5 million U.S. $, for the development of a large study in R-D area conducted by the National Foundation for Science. Achieving these studies and analysis regarding R-D created the ground for a R-D national policy, a policy to promote basic research and scientific education.

The first law for R-D domain in the U.S., developed in 1950, still applies today, with some amendments, and formulated the requirement for the National Ministry of Science to submit to the President each leap year a report on indicators for science and engineering, to be presented in plenary Congress. This National Ministry of Science obligation is valid even today. We refer to the National Ministry of Science report of 1998 called *Science and Engineering Indicators 1998*, which presents major changes in American science and technology industry reported in the transition process to 21st century and to a knowledge-based economy.

There are presented major trends in research - development, technology and science development:

- *Extension of globalization in science, technology and economy* - in this regard the report emphasizes that investments in financial and human resources for science and technology are essential for social and economic welfare in the global economy;
- *Increased emphasis on education and professional formation for science and engineering* - in this regard the report shows that the

diverse workforce must have a minimum of technical knowledge and citizens must acquire a sufficient amount of knowledge on science and technology to meet appropriately the needs of a new century;

- *Priorities and structural changes in science and engineering* - on these points in the report it is highlighted the role of the Federal Government, which remains essential in funding basic research conducted in universities and in funding workforce education for science and engineering. Also it has been showed an increased role in funding research - development industry and the growing importance of R-D in the service sector;
- *Increased impact of science and technology on everyday life* - in this respect in the report is highlighted the profound impact of science and technology on everyday life due to changes produced by information and communication technology in the workplace, at schools and homes.

Regarding the national research and development system of Japan, although The World War II had a devastating effect on this country, the Japan revived and transformed in the last 40, 50 years in a national complex innovation system, characterized by functionality, efficiency, creativity, becoming the main opponent of the U.S. innovation system. Japanese innovation system development was supported by: the massive import of technology, adaptation and renewal of permanent technical knowledge in industrial firms, permanent learning and technological innovation systems. Unlike the U.S., in Japan a major role in financing research - development have private companies (at a rate of over 60%), the Japanese government was involved in coordinating and funding R-D in high education system, industry and military.

When referring to the innovation systems of the EU Member States, we see that they are characterized by a wide diversity derived from traditions, specific economic and research - development policies. By reference to the first four EU countries which currently allocate the highest percentage of gross domestic product (GDP) in research and development funding, we see that France and Germany are on the first place with a rate of 2.3% of GDP, followed by UK 2.1% of GDP and Italy 1% of GDP (Eurostat source). Even though this is not the only financial indicator that characterizes the national innovation systems, it provides a clear criterion for positioning in the

ranking of European States in the R-D. Regarding Romania, in 2007 research - development expenditure represented 0.53% of GDP and for 2008 was 0.58% of GDP (Statistical Yearbook of Romania, 2008-2010).
If the French system of innovation is based mostly on government sector (50%), in Germany research is funded at the rate of over 61% of firms and is applicative-oriented. Currently the UK and Germany are countries with the largest number of researchers for private companies. Research - development sectors of four other EU member states (Greece, Ireland, Portugal, Spain) have a fast growth rate. The research - development system of Ireland has 69% support from the private sector, a percentage exceeded only by Sweden at present (72%). Ireland also promotes the presence of foreign multinationals that finance R-D, as well as Belgium and Sweden.
This diversity of European innovation systems is considered by authors as Dodgson and Bessant as an important advantage for the European Union countries. In their meaning, "*Europe can and must learn from its diversity and enhance the supra-national systemic force. This implies the need for policies coordination and to ensure their subsidiarity, specialization of individual Member States, but also to encourage complementarities and learning through cooperation and R-D networking, as facilitating streaming distribution of technologies* "(Dogson and Bessant, 1996).

3. CONCLUSIONS

3.1 Even if national systems of innovation, both of the EU Member States, as well as of the states on other continents are characterized by diversity and even disparities, we can shape some trends of research-development-innovation policies, which occurred due to several factors of change:

- budgetary pressures faced by most Member States which have induced a reduction in government allocations for research-development-innovation, as a percentage of GDP;
- expectations from R-D policies are increasingly linked to solving concrete problems such as health, quality of life, unemployment;
- evolutionary phenomenon of globalization and interdependence of national innovation systems and economies.

3.2 This framework of change led developing new R-D policy objectives in European and international zone, such as:

- Promoting research-development to meet the expectations of society;
- Creation of jobs;
- Creating a closer link between the public, private and high education system;
- Promoting innovation and technology transfer;
- Creating a favourable environment for investment in research-development;
- Promoting technology dissemination in European area.

3.3 When referring to the appearance of a common EU-wide research — development policy, the first milestone in this respect was *the first Framework Programme for Research and Technological Development* (R-T-D) developed in 1984. So far were carried out in succession, several EU framework programs, we make a brief reference to the Fifth Framework Programme (1999-2002) which has set as priority axes in research - development the following: unlocking living resources and the ecosystem, confirming the international role of European research, promoting sustainable competitive growth, creating an information society accessible to users, improving human potential. The following Framework Programmes conducted in the area are the Sixth Framework Programme (2003-2006) and Seventh Framework Programme which is ongoing (2007-2013). These last two programs in research - development field support and develop the axes set in the fifth program, with particular emphasis on development of information society in Europe as a knowledge society sustainable in the future.

3.4 The overall conclusion of this paper refers to the fact that researchers, scientists, academic institutions, companies and corporations must be supported through strong national innovation systems to benefit from growing international nature of science and technology, to a maximum capitalization of scientific research results. Important aspects in this respect are: increasing job mobility in science and technology, increasing the number of publications featuring joint/ collaborative papers, developing international coalitions for technology transfer, fluidization of global know-how flow.

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CHARACTERISTICS OF MESENCHYMAL STEM CELLS FROM BONE MARROW, UMBILICAL CORD, PLACENTA AND AMNIOTIC FLUID

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Key words: mesenchymal stem cells, markers, imunomodulation;

SUMMARY

Mesenchymal stem cells (MSCs) are considered to be adherent, fibroblast-like, pluripotent, nonhematopoietic progenitor cells and, nowadays, the most important source of stem cells for regenerative medicine. MSCs of human and nonhuman mammalian species are often studied for various applications in regenerative medicine research. These cells have been isolated from many adult human and animal tissues, such as bone marrow, placenta, adipose tissue, scalp tissue, various foetal tissues and teeth. They can be identified according to their ability to form fibroblast-like colony forming units that develop into stromal like cells when expanded in culture. Their multilineage differentiation potential is very large, being able to differentiate into various tissues of mesenchymal and nonmesenchymal origin. The present paper is a review of mesenchymal stem cells characteristics, cells obtained from bone marrow, umbilical cord, placenta and amniotic fluid regarding their transforming potential including the immunomodulatory capacities and the possibilities of using these cells in allotransplantations or xenotransplantations.

The presence of MSC of bone marrow origin was formally demonstrated in the second half of the 1970s, by seeding whole bone marrow samples in culture plastic disks and removing non-adherent cells after some hours. The few adherent "fibroblastic-like" cells formed small cell clusters, defined fibroblast-colony forming units (CFU-F). After several culture passages, surviving cells became homogeneous and retained their ability to replicate and form cartilage and bone cells (Mauro Krampera et al., 2007). MSCs can diferentiate one or more of the cell types resident in the injured tissues, reduce local inflammation and repair injured tissues by secreting cytokines and growth factors that can restore tissue homeostasis (Bianco P et al., 2001; Spees JL et al. 2003).

BONE MARROW MESENCHYMAL STEM CELLS

The most important source, so far, regarding the mesenchymal stem cell biotechnologies is the bone marrow. The microenvironment of mammalian bone marrow is composed of several different elements that support haematopoiesis and bone homeostasis. It includes a heterogeneous population of cells: macrophages, fibroblasts, adipocytes, osteoprogenitors, endothelial cells and reticular cells. Among these, there are also non-haematopoietic stem cells that possess a multilineage potential. These stem cells are commonly described as marrow stromal stem cells or mesenchymal stem cells (MSCs). Mesenchymal cells are primordial cells of mesodermal origin, able to give rise to skeletal muscle cells, vascular and urogenital systems and to connective tissues throughout the body. MSCs are of interest because they can easily be isolated from a small aspirate of bone marrow and expanded through as many as 50 population doublings in about 10 wk (Galderisi U et al. 2010).

MSCs isolated from human and other mammalian species including baboon, dog and rodents do not elicit a proliferative response from allogeneic lymphocytes. They suppress the immune response to alloantigen and modify the proliferation of T cells. $CD4^{+}CD25^{+}$ regulatory T cells have strong immunomodulatory potential. One of the most intriguing features of MSCs is that they can escape immune recognition and inhibit immune responses. However, the exact molecular mechanism mediating the immunosuppressive effect of MSCs is not completely understood. Although the investigation of specific MSC markers is still ongoing, CD54- and integrin β1 $(CD29)^{-}$ positivity, $CD14^{-}$ and $CD45^{-}$ negativity are reportedly expressed in MSCs. MSCs have a profound inhibitory effect on the activation of T cells by their cognate peptides *in vitro*; both naive and memory cells are subject to MSC mediated suppression.

MSCs modulate the immune function of the major cell populations involved in alloantigen recognition and elimination, including antigen presenting cells, T cells and natural killer cells. Considerable data indicate that MSCs inhibit lymphocyte proliferation in mixed lymphocyte culture. The inhibitory effect of rMSCs on T cell proliferation is triggered by mitogenic stimuli. When CD3+ T cells co-cultured with rMSCs were induced by PHA (10 μg/ml), the proliferation of CD3+ T cells was markedly inhibited. The degree of immune suppression was dependent on the concentration of

rMSCs in MLR, but the inhibitory effect was not evident at very low concentrations. MSCs suppress proliferation of allogeneic T cells in an MHC-independent manner. $CD4^+$ T cells subset expressing the CD25 molecule, which appears to actively suppress T cell activation. $CD4^+CD25^+$ regulatory T cells significantly increased in the presence of rMSCs, the level being dependent on the concentration of rMSCs. MSC-induced inhibition of mitogen-stimulated T cells reduces the expression of CD25, as well as CD38 and CD69.

Rat MSCs induced the generation of $CD4^+CD25^+$ Treg in MLR and the immunosuppressive activity of rMSCs was related to the percentage of $CD4^+CD25^+$ Tregs. The secretion of pro-inflammatory TNF-α decreased while the anti-inflammatory TGF-β and IL-10 increased in the supernatant of the MLR, revealing that rMSC-mediated suppressive activity *in vitro* was partly attributed to soluble mediators. rMSCs induced a more anti-inflammatory or tolerant phenotype and caused an increase in the proportion of $CD4^+CD25^+$ T cells (Tregs) when $CD3^+$ T cells were co-cultured with rMSCs in MLR. MSCs hold potential not only for the suppression on the proliferation of immunocytes *in vitro* but also for the development of applications that could assist in transplantation through inducing regulatory T cell proliferation.

Primarily, two populations of these inducible Tregs are important for transplantation tolerance: Th3 cells and T regulatory cells 1 (Tr1). Th3 cells were first identified because of their role, the secretion of TGF-β, in the development of immune tolerance following the ingestion of antigens (termed oral tolerance). Tr1 cells are similar to Th3 cells, but they secrete large amounts of IL-10 and were first characterized on the basis of their role in preventing autoimmune colitis. Importantly, $CD4^+CD25^+$ Tregs which were found inside the tolerated graft have indirect allospecificity for donor antigens. This raises the possibility that $CD4^+CD25^+$ Tregs with indirect anti-donor allospecificity could be potential reagents to promote clinical transplantation tolerance. rMSCs have a tolerogenic profile *in vitro* in the rat allogeneic system. (Zhou Ye et al. 2008)

UMBILICAL CORD MESENCHYMAL STEM CELLS AND THEIR POTENTIAL

Although bone marrow (BM) has been regarded as a major source of MSC, umbilical cord blood has recently been regarded as an alternative source for isolation of MSC. Human and animal umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) have a capacity similar to that of BM-MSCs for multi-lineage differentiation. In addition, hUCB-MSCs are also proved to possess activities for immune modulation, tumor tropism and nursing effect (Bieback and Klüter H, 2007; Lee DH et al. 2009; Ju-Yeon Kim et al. 2010).

Immune rejection in recipient patients is the primary issue associated with use of MSCs as an allogeneic cell source for cell based therapy involving transplantation. In fact, previous studies of the properties of immune-privilege have been carried out primarily in BM-MSCs where the surface immunogenic markers were hypo-immunogenic which may prevent proliferation of allogeneic lymphocytes. In the same fashion as BM-MSCs, major histocompatibility complex-II class molecules and costimulatory molecules, such as CD40, CD40 ligand, CD80 and CD86 which are involved in T cell activation response for transplant rejection, are not expressed in hUCB-MSCs even when mitogenic or allogeneic stimulated signals are delivered. In addition, differentiation of hUCB-MSCs into chondrocyte or neuron-like cells did not elicit expression of these immunogenic surface molecules and could not provoke allocative lymphocyte proliferation in mixed lymphocyte reactions (MLR) *in vitro* Compared with BM-MSCs, hUCB-MSCs showed lower immunogenicity than BM-MSCs because of primitive characters originating from UCB. Indeed, undifferentiated or differentiated hUCB-MSCs can be successfully transplanted for cell based therapy MSCs are known to have immune suppressive action on lymphocyte proliferation in MLR by alloantigen and mitogens such as phytohemagglutinin and to reduce the level of proinflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α).

Recent evidence has demonstrated that hUCB-MSCs can suppress not only the function of mature dendritic cells but also increase the portion of regulatory T cells related to immune regulation. At present, several soluble factors involved in immune suppression have been reported including

transforming growth factor-β (TGF-β). However, induction of these cytokines was not observed under conditions of immune suppression by hUCB-MSCs and study of contact dependent inhibition by hUCB-MSCs is in progress. hUCB-MSCs elevated the level of prostaglandin E2 and induced indoleamine 2, 3-dioxygenase (IDO). In addition, the surface molecule HLA-G which is involved in immune tolerance in pregnancy was detected in hUCB-MSCs by fluorescence activated cell sorter analysis. (Ju-Yeon Kim et al. 2010) Recent comparative studies on human mesenchymal stem cells show that there are still several differences between hUCMSCs and BM-MSCs.

Firstly, the fibroblast colony-forming units (CFUF) frequency was significantly higher in UC derived nucleated cells than in BM derived nucleated cells. Since CFUF represents the mesenchymal progenitor cell, this suggested a higher frequency of MSCs in the nucleated cells of UC than in those of BM. Secondly, the proliferation analysis revealed that hUCMSCs have a faster population doubling time, that did not change after 30 passages. In contrast, BMMSC showed significantly slower population doubling time which became even longer after Passage 6. hUCMSCs had a higher proliferative capacity in comparison with BM-MSCs indicating that UCMSCs may be a novel alternative source MSCs for clinical application. In addition, hUCMSCs showed lower expression of CD106 and HLA-DR in comparison with hBMMSC.

The different expression of CD106 in hUCMSCs and BMMSCs may represent a specific indicator for identifying peripheral MSCs from BMMSCs because low expression of CD106 has also been identified in adipocyte derived MSCs. (Arianna Malgieri et al. 2010)

PLACENTA AND AMNIOTIC FLUID DERIVED STEM CELLS

An alternate source of stem cells is the amniotic fluid and placenta. Amniotic fluid and the placenta are known to contain multiple partially differentiated cell types derived from the developing foetus. Stem cell populations from these sources, called amniotic fluid and placental stem cells (AFPSC), express embryonic and adult stem cell markers. The undifferentiated stem cells expand extensively without feeders and double every 36 hours. Unlike hES cells, the AFPSC do not form tumors *in vivo*. Lines maintained for over 250 population doublings retained long telomeres

and a normal karyotype. AFS cells are broadly multipotent. Clonal human lines verified by retroviral marking can be induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages. In this respect, they meet a commonly accepted criterion for pluripotent stem cells, without implying that they can generate every adult tissue.

Examples of differentiated cells derived from AFS cells and displaying specialized functions include neuronal lineage cells secreting the neurotransmitter L-glutamate or expressing G-proteingated inwardly rectifying potassium (GIRK) channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue engineered bone. The cells could be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth. The cells could be preserved for self-use and used without rejection, or they could be banked. (Jennifer L. et al. 2011)

Zhang et al (2004) described that the mesenchymal stem cells in human placenta produce fibronectin, laminin, and vimentin, have the potential to differentiate into osteogenic, adipogenic, and chonrogenic lineages and are able to suppress T-cell proliferation induced by cellular stimuli.

Yen et al. (2005) showed that placenta-derived stem cells have the same surface markers of embryonic stem cells such as SSEA-4 differentiate into mesodermal-lineage cells of osteoblasts and adipocytes, as well as ectodermal, neuron-like cells. Human placental mesenchymal engrafted stem cells may undergo differentiation into various phenotypes after engraftment. Cell tracking, FISH and real-time PCR demonstrated that hPMCs are capable of engrafting in multiple foetal tissues (brain, heart, lung, liver and spleen) and can survive post-natally for at least 12 post-natal weeks after in utero transplantation.

These cells did not express hematopoietic markers (CD34, CD45) but they did express a number of adhesion molecules and mesenchymal markers, including CD29, CD44, CD73, CD105 and CD166. Cells were able to proliferate in vitro, maintaining a homogeneous morphology, and, under appropriate stimulation, differentiated into multiple cell lineages including osteocytes, adipocytes, hepatocytes and endothelial cells. The endothelial cells could also be induced to undergo tube formation in vitro. hPMCs

express many of the genes derived from mesoderm, ectoderm and endoderm.

Engraftment involves many complex steps to allow transiently circulating hPMCs to become resident within the tissue of various organs. hPMCs express human leukocyte antigen class I (HLA-ABC) but not class II antigens (HLA-DR), which may limit the immune response and antigen recognition by the host. The major MHC appears to have little adverse influence on engraftment at an early gestational age. High rates (50%) of long-term engraftment are thus theoretically achievable, although the degree of engraftment may be very low (0.0001–1%). Even when donor cells are fewer than 0.01% in recipient blood or tissues, they appear to be sufficient to induce and maintain tolerance. Mesenchymal progenitors isolated from amnion and chorion did not induce allogeneic or xenogeneic lymphocyte proliferation responses.

Culture-expanded hPMCs were observed to inhibit proliferation of cord blood lymphocytes triggered by allogeneic peripheral blood lymphocytes or phytohemagglutinin in a dose-dependent manner. A similar effect was shown on allogeneic lymphocyte proliferation too (Chie-Pein Chen et al. 2009; Bailo M et al. 2004; Le Blanc K et al. 2005).

CONCLUSIONS

MSCs therapy in regenerative medicine studies on humans and animals show a vast potential. This stem cell therapeutic approach is proved to be one of the most effective, considering the many advantages of mesenchymal stem cells, like availability, transplantability, expandability or immune regulation. Even though many questions about these stem cells are still pending, they are considered in this moment the best candidates for allotransplantation and xenotransplantation, due to their capacities of avoiding rejections.

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CONTRIBUTIONS OF THE GHEORGHE IONESCU-BRAILA (1879-1947) TO THE ADVANCEMENT OF THE VETERINARY MEDICAL IN ROMANIA

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Key words: Gheorghe Ionescu-Brăila, advancement, veterinary medicine, Romania.

SUMMARY

Ionescu Gheorghe-Brăila attended the Higher School of Veterinary Medicine from Bucharest, in December 1900 he was promoted as a diplomate, and in February 1901 he joined the public service, as a provisional veterinary surgeon in the CFR Galati Circuit.
During 1908-1909 he benefited from a grant abroad at the Pasteur Institute and the Veterinary National School from Alfort. He studied the organisation of the estate veterinary services from The Agricultural Ministery in France, and the new sanitary veterinary laws in order to eradicate epizooties.
After 1909 he was head of the Technical Department and from 1919, at the age of 40, he was promoted as general inspector of the Veterinary Service from Romania. From 1922 till 1939 he was general manager of the Zootechnical and Sanitary Veterinary Department from the Agricultural and Estate Ministery.
In the same year, the first National Zootechnical and Veterinary Hygiene Congress was organised, during 12-14 September 1924, in Cluj, under the initiative of the General Association of the Veterinary Surgeons from Romania, whose president he was. In the same time there took place the Zootechnical Exhibiton of Ardeal and Banat.
He took part in the European Congresses for rural economy, obtaining favourable riders for the Romanian exports of animals and animal products, according to the comercial conventions with different countries such as: Italy, Germany, France and Austria.

Gh. Ionescu-Brăila (Fig.1.) was born on August 19, 1879, in Brăila. After graduating the primary and secondary school from his native town, he attended the High School of Veterinary Medicine from Bucharest.
In December 1900 he was promoted as a diplomate, and in February 1901 he joined the public service, as a provisional veterinary surgeon in the CFR Galati Circuit. From May 1902 he was in charge with the veterinary service from Oltenia (3, 5, 6, 7).

During 1908-1909 he benefited from a grant abroad at the Pasteur Institute and the Veterinary National School from Alfort. He studied the organisation of the estate veterinary services from The Agricultural Ministery in France, and the new sanitary veterinary laws in order to eradicate epizooties.

After 1909 he was head of the Technical Department, and from 1919 at the age of 40, he was promoted as general inspector of the Veterinary Service from Romania. From 1922 till 1939 he was general manager of the Zootechnical and Sanitary Veterinary Department from the Agricultural and Estate Ministery (10).

Fig.1. Gheorghe Ionescu-Brăila (1879 —1947)

In order to solve the difficult tasks for the zootechnical and sanitary veterinary fields, Gh. Ionescu-Brăila kept a permanent contact with the distinguished professors: I. Athanasiu, C. Motaş, P. Riegler, I. Poenaru, I. Ciurea, Gh. Udriski, Al. Ciucă etc.

In 1922, in order to develop the animal breeding, he founded the regional Zootechnical Committees, a lot of progress being made concerning the breading and improvement of animal breeds, organising a lot of areal, regional or national zootechnical exhibitions, for example in 1923 in Iaşi, 1924 in Chişinău and Cluj, in 1926 in Timişoara, The Agricultural Chambers were founded in1926.

The first regional bacteriological laboratories were founded in 1924 in order to supervise and diagnose the epizootic disease in animals, in: Iaşi, Chişinău, Cluj, Constanța, Craiova. In the same year, the first National Zootechnical and Veterinary Hygiene Congress was organised, during 12-14 September 1924, in Cluj, under the initiative of the General Association of the Veterinary Surgeons from Romania, whose president he was. In the same time the Zootechnical Exhibiton of Ardeal and Banat took place.

The Law concerning the breeding, improvement and animal welfare was elaborated and headed to the Parliament to be aproved in 1925, and in 1926, the Law concerning the veterinary group organisation, as well. The Law for sanitary veterinary police, concerning the increasing of the compensations

for slaughters due to epizooties, was modified, and also the procedure of judging the sanitary veterinary minor offences in the first instance by the primary veterinary surgeons from the counties, was established (1, 2, 10).
The Balkan Conference was organised in Bucharest in 1929, under the participation of: Bulgary, Greece, Jugoslavija, Romania and Turkey, concerning the uniformity of the epizooties prevention and controlling measures in Balkans. Ten zootechnical and sanitary veterinary regional inspectorates were refounded in 1931, for a better zootechnical and sanitary veterinary country organisation. He was interested especially in public hygiene, food control in slaughter houses and markets.

He insisted on the need to increase the pastures for the farms, in order to stimulate the animal breeding. He took part in the European Congresses for rural economy, obtaining favorable provisions for the Romanian exports of animals and animal products, according to the commercial conventions with different countries such as: Italy, Germany, France and Austria (6, 8, 9).

He contributed to animal breeding conditions improvement by organisatorical measures, by founding Zootechnical Stations in every country region, great zootechnial valuable sires being provided. The Zootechnical Station Florica-Muscel was founded in 1928 and the Arabian Horses Stud from Mangalia, in 1929, offering his whole support in order to develop the trot horse.
As a General Manager of the Zootechnical and Sanitary Veterinary Department, Gh. Ionescu-Brăila offered material and moral support in order to organize and modernize the veterinary education, up-to-dating and completing the material basis for departments such as: Animal Breeding, Food, Hygiene, Fish Breeding etc. from the recent founded National Zootechnical Institute (1926). He contributed to the equipment of the Catching Disease Clinic, Microbiology, General Pathology etc., he offered in 1924 a 68 ha area from Giuleşti village, nowadays part of Bucharest, as an enclosing part of the Pasteur Institute, which was administrated by and considered a technical part of the Faculty of Veterinary Medicine. On this area there were built sheds for the animals, in order to produce serums and vaccines, developing mainly the anti-plague serum, and the labs where animal viroses were studied (1, 2,10).

Gh. Ionescu-Brşila's publishing activity was represented by a great

number of scientifical papers from Animal Breeding (Fig. 2.), Zooeconomy etc., papers that were presented in national and international congresses of Veterinary Medicine and in the International Office of Epizooties from Paris, as well (4).

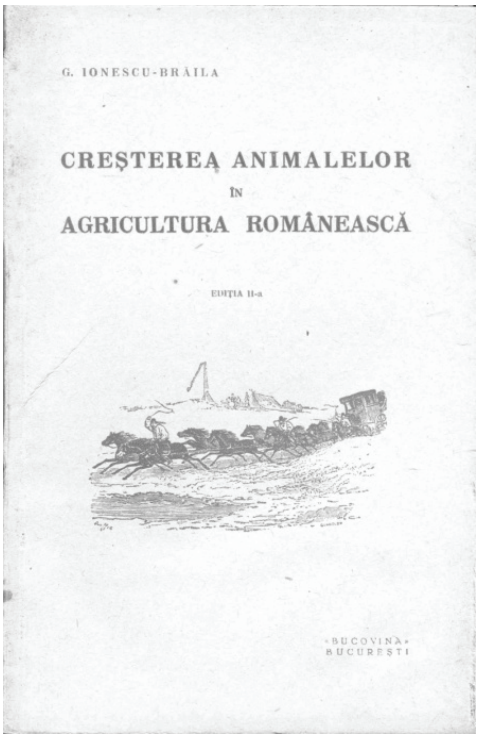

Fig.2. Book of Animal Breeding in Romania, by Gh. Ionescu-Brăila

Fig. 3. Building of the House of the House of the General Association of the Veterinary Surgeons from Romania, in 1929-1933

His professional love was demonstrated by his militant character, in order to join together in responsibility the veterinary surgeons group, which already had had a statute and had been grouped in a Professional Association that had relied on juridical basis, materials and by his harmonious activity could serve as a model for every other profession. His true wish was as the veterinary surgeon, by a dignifying and honest behavior, to win each and every person's trust, even as a human being or a professionist. Gh. Ionescu-Brăila contributed to the building of the House of the General Association

of the Veterinary Surgeons from Romania (Fig.3.), in 1929-1933 the necessary funds being obtained by voluntary contribution of every veterinary surgeon.

The inauguration festivity took place on February 26th, 1933 (Fig. 4). After 1947, this beautiful building from Bucharest downtown belonged to the Justice Ministry, as a tenant, and never as an owner. The Justice Ministry owns this building abusively even nowadays, after 21 years of democracy (Fig. 5).

Fig. 4. The inauguration festivity of the « **Our house** » of the Veterinary Physician' Association Palace in February 26th, 1933, in the presence of King Carol the 2nd (**Universul** newspaper, February 27th ,1933)

Fig. 5. Medalions on the front door in the Veterinary Physician' Association Palace, designed by Iosif Fekete.

Gh. Ionescu-Brăila owned numerous titles, as a result of his prodigious activity: member of Veterinary Medicine Society, member of the Medicine Academy of Romania, elected associated member of the Veterinary Professoral College of Great Britain, member in the International Office Committee for Epizooties from Paris, member of the International Commission for Agriculture from Paris, member of the Preparing Committee for International Congresses from Hague, president of the Veterinary Medicine Society from Romania, president of the General Association of the Veterinary Surgeons from Romania (GAVSR), dean of

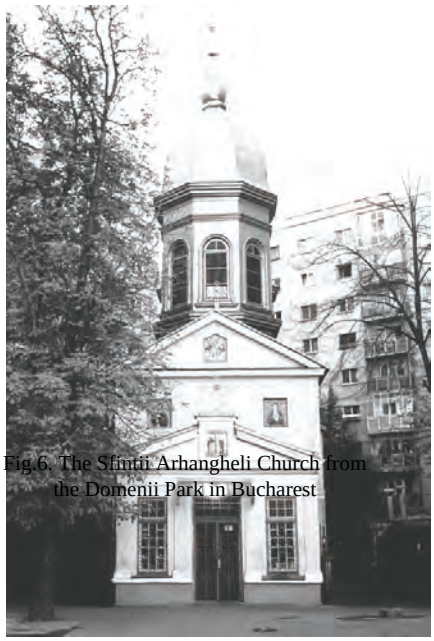

Fig.6. The Sfinții Arhangheli Church from the Domenii Park in Bucharest

the Veterinary Surgeons College, and, at the proposal of the GAVSR members, elected senator, in 1938.

A giving nature from all the points of view, he gave meaningful donations in money, created an award fund for the best scientifical papers with practical use, selected from those presented in the Veterinary Medicine Society meetings.

He also founded some churches, such as Casin Monastery, situated in the northern part of Bucharest. Like many other churches in Romania, this one in a mixed style between the Brancovian and Byzantine styles.

Like any other Brancovian monuments at the entrance we can see some beautiful columns characteristic for this style. Because it has few characteristics from a Byzantine Basilica, the Casin Monastery is a very tall and very roomy church. It was built in 1937 nearby the Triumphal Arch.

It is a great monument which combines harmoniously the traditional architectural elements with the modern ones.

Its mural decoration is made of multi-colored mosaic and marble. Its rood screen and apse are also made of marble with icons made of mosaic and enamel. Nadia Comaneci (one of the world's best gymnasts) and Bart Conner got married here on April 27, 1996, in a lavish church ceremony. Their godparents were Adrian Năstase (Prime Minister at that time) and his wife Dana Năstase.

He also founded the Sfinții Arhangheli Church (Fig.6.) from the Domenii Park, where his last home was, since February 26, 1947 (10).

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STUDY OF SOME BLOOD MINERAL LEVELS IN DIFFERENT SEASONS IN CATTLE AND SHEEP

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Key words: mineral elements, cattle, sheep.

SUMMARY

The main goal of this study was to determine Cu, Zn, Ca, Mg, K, and P levels in different period of the year in cattle and sheep and to determine the relationships between seasons. For this purpose, blood samples of 15 healthy, gestating BNR cows fed under normal condition and 40 gestating Turcana ewes were used. Blood samples were analyzed using an ICP spectrometer. There were significant differences between Zn and K blood level in the autumn season compared to other periods, in cattle. In cattle, P blood levels were significantly low during summer season, compared to its blood levels in sheep, which were significantly low during autumn and summer seasons. In both cattle and sheep, during all seasons, Ca blood levels were normal.

The mineral requirements in animals vary by dietary mineral concentrations, but also, are dependent of the growth, health, fertility, or other relevant criteria. Metabolic processes sensitivity to the lack of an essential element vary dependent to the species and within species, to the age and sex of the animal and to the rapidity of deficiency developing.

For example in sheep, the pigmentation and keratinization of wool appear to be the first to be affected by a low-copper status, so that at certain levels of copper intake, no other function involving copper is impaired. In this species, it has also been shown that the zinc requirements for testicular growth and development and for normal spermatogenesis are significantly higher than those needed for the support of normal growth and appetite. Trace minerals participate in many important catalytic, enzymatic and structural functions of vertebrates and their concentrations in mammals depend on several environmental and biological conditions (4,11).

Trace elements are required for numerous metabolic functions in livestock, and optimal production and performance require adequate intake of

balanced trace minerals (10). As trace mineral status of the animal declines from adequate to marginal, immunity and enzyme function are compromised followed by the loss of performance and reproduction, but also, high dietary levels of the same elements or interaction between them can induce poisonings.
The primary objectives of this study was to determine Cu, Zn, Ca, Mg, K, and P levels in different period of the year in cattle and sheep and to determine the relationships between seasons.

1. MATERIALS AND METHODS

In this study, blood samples of 15 healthy, gestating BNR cows (5–8 yr) fed under normal conditions and 40 gestating Turcana ewes (3–5 yr) were used. The serum was separated by centrifugation and freeze (–20°C) until analysis. The samples were analyzed for Cu, Zn, Ca, Mg, K, and P levels using a Thermo XS series 2 ICP-MS.
During summer and autumn season the studied sheep were in their lactation period and during winter they were gestating. In cattle, during summer and winter season they were in their lactation period and during autumn the cows were gestating.

2. RESULT AND DISCUSSION

Table 1 presents the results of the analysis for blood Cu, Zn, Ca, Mg, K, and P levels in cattle and table 2 the results of the analysis for serum Cu, Zn, Ca, Mg, K, and P levels in sheep.

Table 1
Some blood minerals levels in cattle in different seasons

Minerals	**June 2010**	**October 2010**	**February 2011**
Cu (µg/dl)	274.10	320,87	252.77
Zn (µg/dl)	249.78	161.21	235.93
Ca (mg/dl)	8.73	10.37	9.28
Mg (mg/dl)	3.72	3.79	3.57
K (mg/dl)	22.59	24.57	23.63
P (mg/dl)	1.72	5.16	4.73

As shown in Table 1, there were significant differences between summer and autumn, and autumn and winter period for Zn and K blood levels in cattle.
As shown in Table 2, there were significant differences between autumn and summer period for Cu blood levels, and autumn and winter for K blood levels, and also, between autumn and summer period for Mg blood levels in sheep.

Table 2
Some blood minerals levels in sheep in different seasons

Minerals	**August 2010**	**January 2011**	**June 2011**
Cu (µg/dl)	465.08	346.99	320.62
Zn (µg/dl)	245.84	249.08	223.89
Ca (mg/dl)	8.77	8.69	9.26
Mg (mg/dl)	3.51	4.11	4.52
K (mg/dl)	24,43	26.31	26.84
P (mg/dl)	1.56	3.65	1.95

Macronutrient minerals and trace elements play an important role in metabolism, growing, and especially, the functions of reproduction system in animals (11).
The normal sanitary status of animals, and also, developing of both fetus and new-born and being healthy after birth are very important, and this is determined by the balance of all nourishment and mineral substances taken by the mother (2).
Animals mineral elements necessary are different dependent to the physiological status and their use can increase because both the new-born and especially mother need them during gestation and lactation.

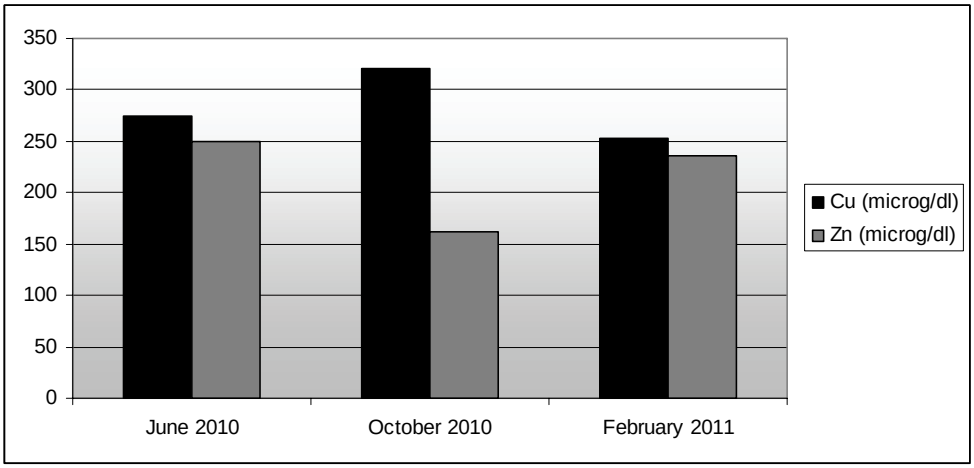

Fig. 1. Cu and Zn levels in cattle in different seasons

In this study, the Zn blood level was low and the K blood level was high in the autumn season compared to other periods; no difference was found in Cu and Mg levels between seasons in cows (Fig. 1).

Zn blood concentration was decreased during autumn season. This can be dependent to increases Zn necessary during gestation (11).

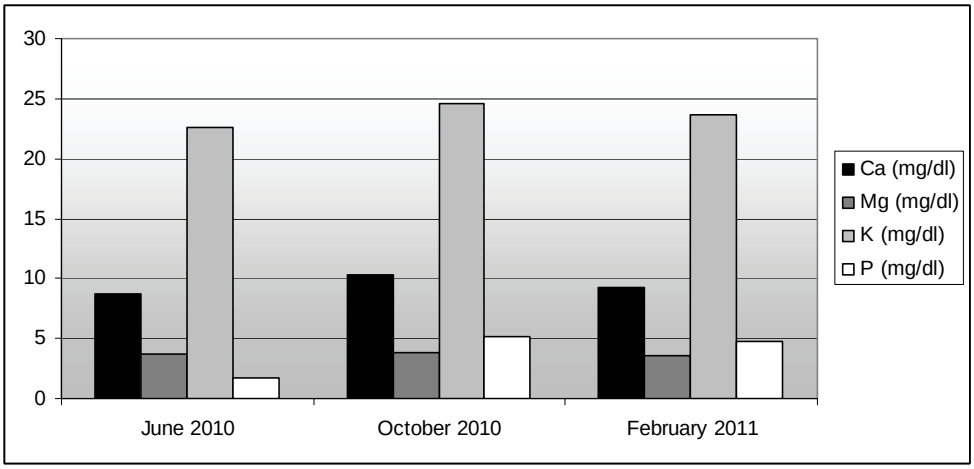

Fig. 2. Ca, Mg, K and P levels in cattle in different seasons

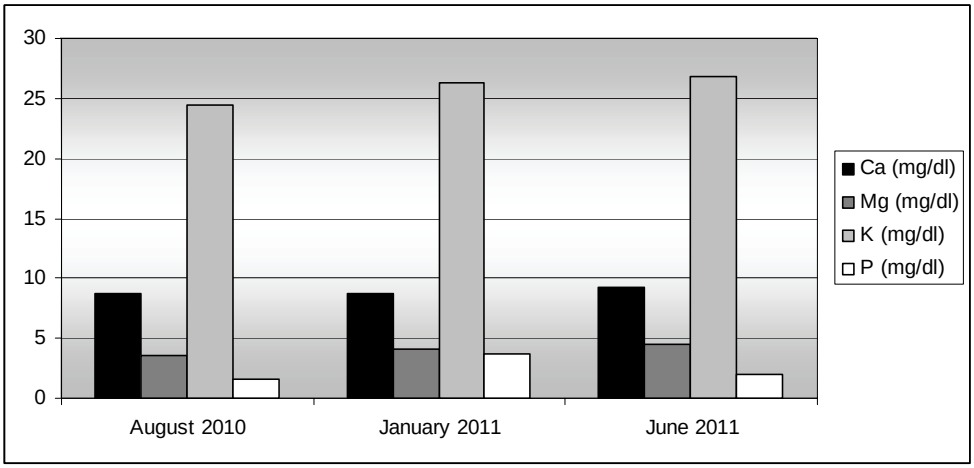

Fig. 3. Ca, Mg, K and P levels in sheep in different seasons

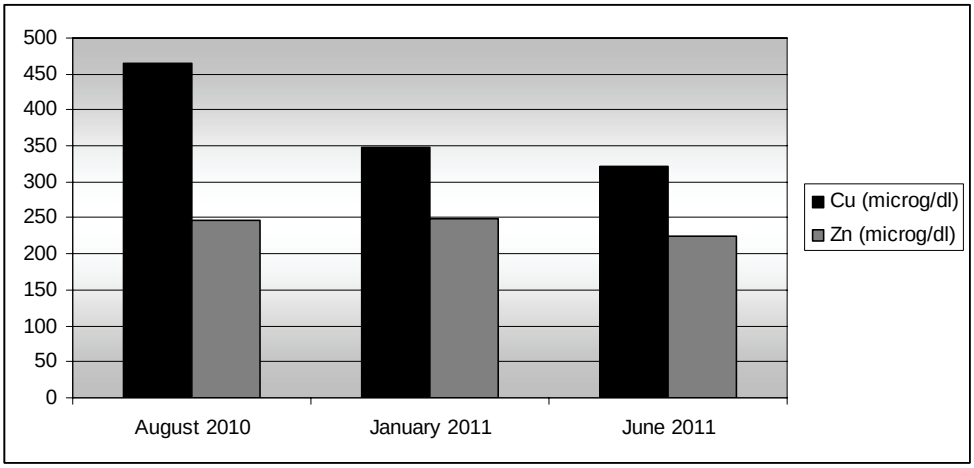

Fig. 4. Cu and Zn levels in sheep in different seasons

In this study, the Cu blood levels in the autumn season were higher than in the summer season (Fig. 4); the Mg blood concentrations in autumn season were lower than its levels in the summer season in sheep (Fig. 3).

The Cu blood levels during gestation were higher than in the lactation period (9). Also, Cu blood concentrations was registered the highest level at the beginning of lactation and the lowest level during the fourth month of lactation (5), and blood Cu levels were not significant during the gestation (8).

In this study, K blood levels during winter season were higher than in the autumn season in sheep (Fig. 3). Also, in other studies (2,6), it was observed that the K blood levels increased during gestation.
In this study, in both cattle and sheep, during all seasons, Ca blood levels were in normal range for these species. P blood levels were significantly low during autumn and summer seasons in sheep, and in cattle, during summer season, my be due to increasing needs for P in animals related to physiological status and also, to the interaction to its antagonists.

3. CONCLUSIONS

3.1. In cattle, Zn blood level was low and the K blood level was high in the autumn season compared to other periods.
3.2. In cattle, P blood levels were significantly low during summer season.
3.3. In sheep, Cu blood levels in the autumn season were higher than in the summer season.
3.4. In sheep, Mg blood concentrations in the summer season were higher compared to those in the autumn season.
3.5. In sheep, P blood levels were significantly low during autumn and summer seasons.
3.6. In both cattle and sheep, during all seasons, Ca blood levels were normal.

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THE EVOLUTION OF SOME METABOLISMS IN THE MECHANISM OF HORMONALLY INDUCED FORCED MOLTING IN LAYING HENS

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Key words: laying hens, molting, thyroxin, metabolism

SUMMARY

In this paper it was determined the evolution of some nutrient metabolism in laying hens at the end of the laying cycle, following the administration of thyroxine (levothyroxine sodium 100 mg) to induce molting process. Research has been conducted on Hy Line Brown hens, 83 weeks of age, to whom it was administered 100 mg of thyroxine, for 10 days, during which molting was induced. The results demonstrate that forced molting was induced by administration of thyroid hormones. Simultaneously there are significant effects on the evolution of the main metabolisms, such as lipid, protein, carbohydrate, cholesterol and calcium and blood plasma can reflect changing of their parameters. Administration of thyroxine to reproduce forced molting reveals indirect involvement of this hormone in reproductive development, as the experimental treatment caused stoping the process of laying.

Thyroid hormones are important for development processes like hatching, molting and laying at birds. Molting is due in restoration of the reproductive system of old hen, preparing them for a new laying cycle. Increased concentration of thyroid hormones in the period before brooding stimulates some metabolic processes which are necessary for a successful laying cycle.

The aim of the research was to reveal the possibility of using thyroxin to induce molting in adult hens, as an altenative of methods, which involve severe starvation of food and water, and reveal the metabolic changes that accompany the biological process.

Research conducted untill present in wild and domestic birds indicating a close relationship between thyroxine and natural molting process.

1. MATERIAL AND METHODS, 9pt Time New Roman

Laying hens were the biological material, hybrids Hy Line Brown clinically healthy, aged 83 weeks (at the end of the first laying cycle) divided in two groups as follows:

- A control group (CG) consisting in ten laying hens, which were fed with the receipt of complete feed (for laying hen production) containing 17.3% protein, technical fat 6-7%, ash 11%;
- An experimental group (TG) consisting in ten laying hens, which were fed with the same receipt of complete feed, receiving daily per os Euthyrox 100 mg;

Euthyrox is a pharmaceutical preparation containing levothyroxine sodium 100 mg and excipients.

Hens were fed *ad libitum* for a period of 55 experimental days, at the end of this 49% of them returned to laying. Blood samples were taken from the axial vein (6 ml per sample) on the first day. At the beginning of the experimental treatment and on the fifth and tenth day of treatment, blood samples were collected from all laying hens of each group. To characterize the effect of thyroxine on different metabolisms, it was considered appropriate to determine the evolution of the following biochemical parameters: glucose, cholesterol, triglycerides, total protein and total serum calcium.

The data were statistically processed determining the mean and the standard error of mean, and the differences between the groups were assessed based on t test (Student's t test).

2. RESULTS AND DISCUSSION

In clinically terms, by the end of the ten days of treatment with thyroxine, at the experimental group were found fallen feathers and the percentage of laying was reduced. In the next period it was also found that hens have reduced feed consumption. These demonstrated that the administration of thyroxine was able to induce moulting phenomenon after five days of experimental treatment.

About the evolution of plasma parameters, investigated in this study, it was observed in the laying hens from TG group a slight decrease of protein values in the plasma compared with the values recorded in CG. **A** decline of values during the monitoring was observed in CG. Starting from a value of 7.1 g / dL, after a five day period it reached the value of 5.2 g / dL, reaching a minimum level of 4.9 g / dL of blood after ten days of treatment. The TG had an initial level of 7.1 g / dL that decreased down to 4.9 g / dL after five days, maintaining its value at 4.9 g / dL after ten days of treatment (Fig. 1).

Statistical analyses reveal no significance differences regarding the level of plasma proteins between the two groups after the ten days of monitorization and experimental treatment ($P>0.05$).

Müller and Seitz (2004) found that serum protein is slightly reduced or unchanged in hyperthyroidism. Increased turnover induced by thyroid hormones on the total protein from the body is part of the increased metabolism seen in hyperthyroidism. The reduced effect of thyroxine on the plasma protein concetration can be explained by keeping a balance between anabolic processes and protein catabolized processes.

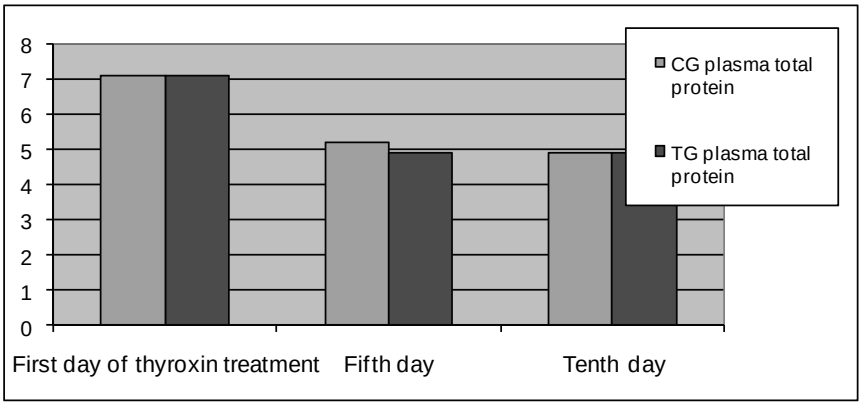

Fig.1. The evolution of the plasma protein values (in mg/dL of blood) in laying hens for a 10 days period of thyroxin (Levothyroxine) administration to induce a moulting

In the evolution of blood glucose concentration it was found an increase in glucose in the first five days of thyroxine administration, with a slight decrease in the coming days, but with similar values found in the control group values (Fig. 2).

It is known that thyroid hormones increase intestinal absorption of glucose and facilitates passage of glucose in the muscle and adipose tissue. This would mean a faster turn-over, which might reflect the decrease or increase in blood glucose concentration.

Also, just as it was shown, thyroid hormones facilitate taking over insulin-mediation of glucose by the cells. High doses of thyroid hormones stimulate the hepatic gluconeogenesis and glycogenolysis. Elevated T3 values in hyperthyroidism exacerbates diabetes symptoms.

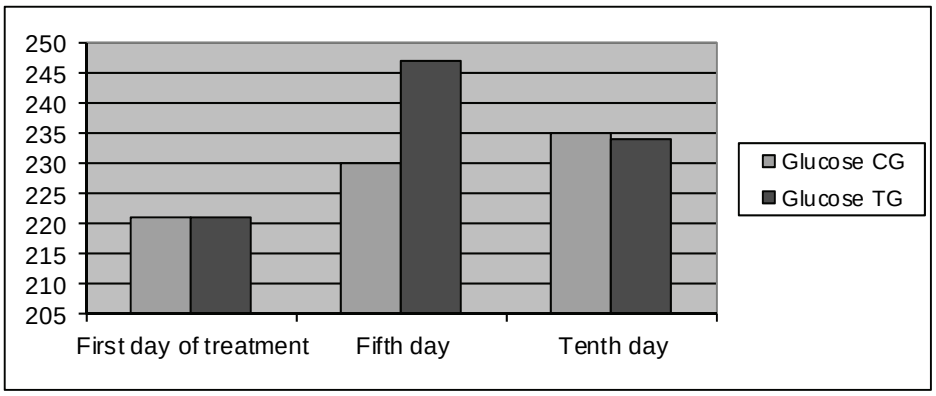

Fig.2. Evolution of blood glucose concentration values (in mg / dL) in laying hens experimentally treated with thyroxin (Levothyroxine) to induce molting

During the monitoring of blood glucose concentration, the higher difference was found between groups after five days of treatment. After ten days, the differences between groups were eliminated. Regarding the evolution of this parameter in hens, Brake and Thaxton (1979) found that plasma glucose levels is growing in hens under program with restriction of food and water. Edwards *et al.* (1999) reported a slight decrease in muscle glycogen for the first 24 hours of food restriction in 6-week-old birds, with a return to normal glycogen afterwards.

Regarding the evolution of plasma triglycerides and cholesterol over a moulting, decreased triglyceride levels and increased cholesterol levels were found when compared to values of hens that are subject to production and they are not under moulting programs. This is because while working in the reproductive period, triglyceride levels are increased due to their migration from the liver to the developing follicles (Tanaka *et al.*, 1996). Because follicular development is stopped during the periodicity of moulting due to significant decrease in circulating gonadotropins, follicular atresia will occur. As a result it will produce an absorption of lipoproteins in the follicular blood flow, that leads to a higher level of cholesterol. Increased cholesterol and decreased triglycerides values can be interpreted as a sign of reproductive involution and cessation of follicular development, whose result is a successful moulting. From the research conducted in laying hens a marked

decrease in triglycerides was found, from 1456.43 mg/dL to 247.03 mg/dL to undergo moulting hens (Lien *et al*., 1993). In our study, after administration of levothyroxine 100 for ten days a significant decrease in triglyceride values was found, from 873 mg/dl to 681 mg/dl, with a slight increase in value toward the end but it remained low in comparison with the control group values (Fig. 3).

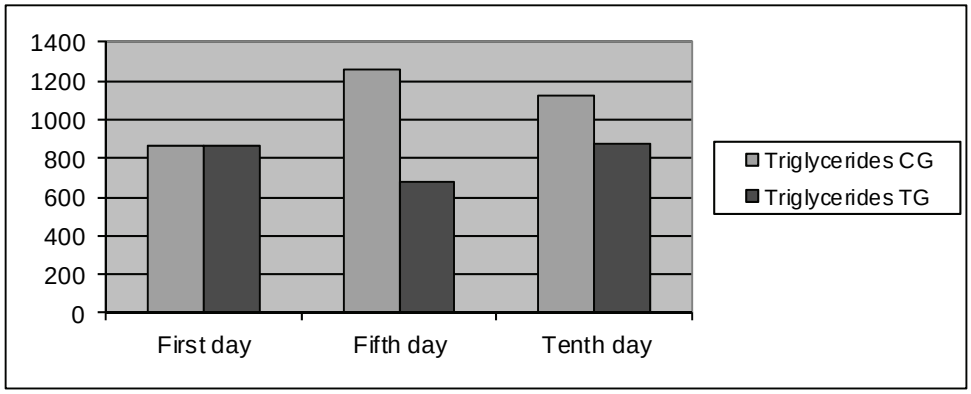

Fig.3. Evolution of plasma triglycerides (mg / dL of plasma) in laying hens for a period of 10 days of treatment with thyroxin (Levothyroxine) to induce forced molting

Plasma cholesterol of TG recorded a noticeable decrease compared with the values obtained in the CG after the first five days of taking levothyroxine. A slight increase of its value can be observed after the tenth day but they are still close to the values obtained in the CG (Fig. 4).

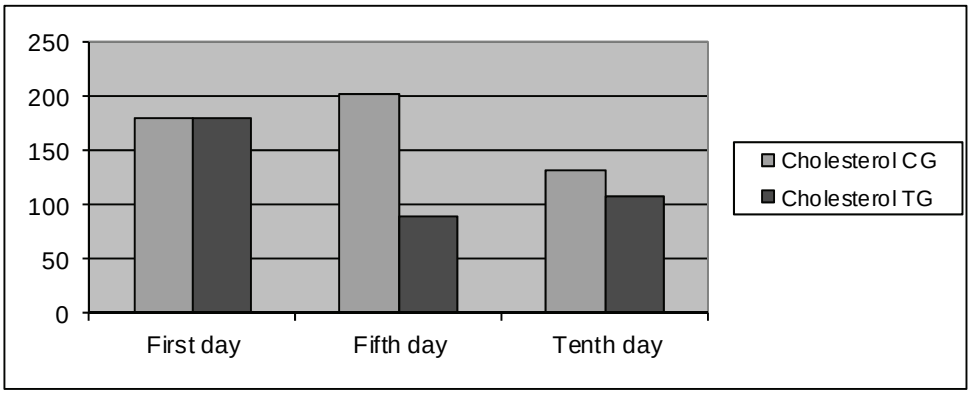

Fig.4. Evolution of plasma cholesterol (mg / dL of plasma) in laying hens for a period of 10 days of administration of levothyroxine to induce forced molting

Calcium is an important element for maintaining the production of eggs, not only because of the very large amount that is needed for the shell formation but also because of the role it plays in gonadotropic hormone secretion. The level of calcium in the body of

birds greatly influences the pituitary gland activity, calcium being more important in terms of the number of eggs, then in terms of the shell quality. After the administration of thyroxine a very pronounced decrease in total serum calcium in the first five days occured, at which there was a drop in egg production, with a slight increase in the tenth day (Fig.5) .

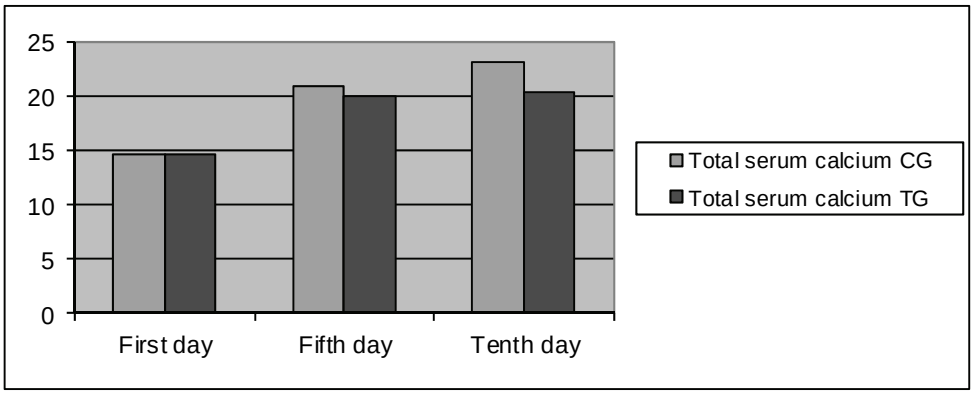

Fig.5. Evolution of total calcium in serum (mg / dL of plasma) at laying hens over a period of 10 days of administration of levothyroxine to induce forced moulting

3. CONCLUSIONS

Research conducted on laying hens at the end of the first laying cycle demonstrates that molting could be induced by the use of thyroxine, without affecting the welfare of hens.

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ANTIBIOTICS SUSCEPTIBILITY OF E.COLI STRAINS ISOLATED FROM CHICKENS WITH COLISEPTICEMIA IN VEST OF ROMANIA BETWEEN 2010-2011

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Key words: *E. coli*, colisepticemie, antibiotics, broilers

SUMMARY

In this study are presented isolation and identification a some outbreaks with colisepticemie. From corpses with colisepticemie were isolated 54 strains of E. coli. Antibiotic susceptibility testing was performed by the single disc-diffusion method, using a set of standard antibiotic discs. Multiple resistances to antibiotics were observed in all isolates, 81-96% of the strains were resistant to neomycina, lincomycin, tetracycline, doxyciclin, erythromycin, 76% were resistant to enrofloxacin, 55% to spectinomycin and 33% to florfenicol. High rate of resistance is due to incorrect therapy performed in broilers.

Colibacilosis is the primary cause of morbidity, mortality and condemnation of carcasses in the poultry industry worldwide.
Colisepticemia is the most common form of colibacillosis and is responsible for significant economic losses in aviculture (Ewers et all. 2003).
Escherichia coli is the most common agent causing secondary bacterial infection in poultry and may also be a primary pathogen.
E. coli may be sensitive to many antibiotics, but isolates of *E.coli* from poultry are frecvently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over a long period. Resistance to two or more classes of antibiotics is now commonplace in both veterinary and human medicine (Wasyl et all., 2010).

1. MATERIAL AND METHOD

E. coli strains studied were from broiler poultry in a farm located in western Romania.
Isolation and identification of *E.coli* were performed by standard bacteriological methods.

Bacteriological examination samples were taken from corpses that had specific colisepticemie lesions, there were sampled long bone and liver, from which were made primary isolations by common methodology in broth and on agar medium. Specimens were cultured on McConkey and EMB agar and the colonies suspected to be *E.coli* were identified by standard methods.

From corpses with colisepticemie were isolated in 54 strains of E. coli, of which a total of 30 strains were tested on API 20E system.

All strains were isolated from broiler chickens that had died from colisepticemia and the chickens were between 1 to 42 days of age.

Antibiotic susceptibility testing was performed by the single disc-diffusion method, using a set of standard antibiotic discs. The following antibiotics discs on Mueller Hinton agar were applied: florfenicol (FFc/30 μg), gentamicin (CN/10μg), colistin sulphate (CS/10 μg), neomycin (N/10 μg), tetracycline (TE/30μg), erythromycin (E/15 μg), lincomycin (L/10 μg), doxycycline (Do/30 μg), enrofloxacin (ENF/5 μg), spectinomycin (SPT/10μg), streptomycin (S/10 μg), amoxicillin / clavulanic acid (AMC 2 / 1 μg).

The diameters of inhibition zones were interpreted using EUCAST (European Committee on Antimicrobial Susceptibility Testing) disk diffusion methodology (EUCAST 2011).

2. RESULTS AND DISCUSSIONS

Cultural, all strains of *E. coli* isolates grew on Mac Conkey and EMB environment, most strains developing similar and characteristic colonies.

Biochemistry, the TSI medium, all strains tested fermented glucose, lactose and sucrose, produced gas and all strains did not produce H_2S. The MIU medium, all isolates showed mobility, produced indole and urease.

In this study, multiple antibiotic resistance was observed in all of the examined strains.

The isolated strains were subjected to an antibiotic sensitivity test and the percentages of susceptible, intermediate or resistant strains to each antibiotic are present in table 1.

Table 1

Percentages of antibiotic susceptibility of isolated E.coli from broiler

Antibiotic Biodisc	**Disc Content (µg)**	**Diffusion Zone Breakpoint (mm)**	**Sensitive (%)**	**Intermediary (%)**	**Resistant (%)**
Amoxicilin/acid clavulanic	1/2	≤ 14	6	33	41
Colistin sulfat	10	8	5	56	39
Doxycycline	30	≤12	8	6	86
Enrofloxacin	5	≤ 16	2	22	76
Erytromicyn	15	≤13	0	4	96
Florfenicol	30	≤19	62	5	33
Gentamicin	10	≤12	40	54	6
Lincomycin	10	≤14	0	9	91
Neomycin	10	≤12	11	8	81
Spectinomycin	10	≤14	36	9	55
Streptomycin	10	≤11	0	33	67
Tetracycline	30	≤14	2	7	91

In table 1 can be observed that 81-96% of the strains were resistant to neomycina, lincomycin, tetracycline, doxyciclin, erythromycin, 76% were resistant to enrofloxacin, 55% to spectinomycin and 33% to florfenicol.

In this study, multiple antibiotic resistances was observed in all strains examined similar to the findings obtained by other researchers (Salehi et all. 2006, Wasyl et all. 2010) in other countries.

Resistant faecal *E. coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora (Barnes et all. 2003).

Almost all the *E. coli* isolated showed high percentage of resistance to the antibiotics.

Gentamicin is injectable solution and they aren't used as mass medication in broiler chickens.

The incidence of antimicrobial resistance in the *E. coli* strains is most probably due to increased use of antibiotics as feed additives for prevention of diseases, for treatment of diseases, resistance transfer among different

bacteria and possible cross resistance between antibiotic used in broiler.
High levels of resistance were against erythromycin 96% and low levels of resistance were against florfenicol 33% recent introduced in poultry therapy

3. CONCLUSIONS

3.1. Isolated *E. coli* strains are sensitive to florfenicol (62%) and gentamicin (40%).
3.2. The strains were resistant to neomycina, lincomycin, tetracycline, doxyciclin, erythromycin 81-96%, to enrofloxacin 76%, to spectinomycin 55% and to florfenicol 33%
3.3. Highest rate of resistance is due to incorrect therapy performed in broilers.

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*** EUCAST European Committee on Antimicrobial Susceptibility Testing www.eucast.org. 2011

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STRESS INDUCED ALTERATIONS IN THE MAIN BIOCHEMICAL PARAMETERS OF THE HEMOLIMPH OF APIS MELIFERA CARPATHICA IN ROMANIA

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Keywords: haemolymph, honey bee, stress, biochemical parameters

SUMMARY

The experimental study presents changes in biochemical parameters profile of the stressed honey bee *Apis mellifera carpathica* hemolymph compared with values observed in hemolymph of natural living bees. Investigations were made in 2009 on a total of 10 colonies under stress factors and 26 colonies of healthy bees. Harvesting and processing of hemolymph fresh samples from both groups were followed by analysis via classical biochemistry. In stressed bees hemolymph were found significant increases in alkaline phosphatase (ALP), aspartataminotransferaze (AST), gammaglutamiltranspeptidaze (GGT), creatinine (Cre), total calcium (total Ca), triglycerides (TG) and significant reduction of magnesium (Mg), compared with values in natural living bees. The others biochemical analyzed parameters (GPT/ALT, CPK, GLU, T-Pro, UA and Urea) showed no semnificative differences between stressed bees and natural living bees. Monitoring the metabolic health of bees in stress conditions requires evaluation only of a few biochemical parameters (ALP, AST, GGT, total Ca, Mg, Cre and TG). The results showed variations in some cells types from haemolymph between the 2 groups of natural living honey bees and the 2 groups of honey bees keeped in stressful conditions.

The haemolymph is transparent, uncoloured (or light yellowish) and represents about 25-30% from the total weight of the bee at the hatching stage and decreases as the bee becomes older. The haemolymph contains proteins, amino acids, sugars, hormones, enzymes and many other substances in different concentration as compared with vertebrate blood. (Milani, 1988)

In specific literature the most part of researches in biochemical filed has focused on sugars (fructose, trehalose and glucose), proteins (vitellogen), amino acids (proline), hormones (juvenile hormone) contents and the activity of some enzymes. For example, a series of data shows that the total sugars level from honeybees' haemolymph is the highest from all the insect species, being used as the main energetic supply in the flight activity which requires a very high energetic consumption (Bozic and Woodring, 1997; Leta *et al.*, 1996). This paper continues our research in biochemistry of haemolymph (Şapcaliu *et al.*, 2008; Şapcaliu, 2010).

The main stress factors found in natural conditions to *Apis mellifera carpathica* bee causing serious losses to beekeepers and country economy are traveling for long distances, tiring, in poor conditions, at high temperature and poor ventilation, low humidity and insufficient food, correlated with poor access to water supply (Șapcaliu, 2010).
The goal of this experimental study was to determine the variability of the main biochemical parameters types profile from natural living honeybees, compared to stressed bees (exposed to high temperature, over 30°C for about 36 hours, poor ventilation)

1. MATERIALS AND METHODS

The study was performed in October 2009 and February 2010 on two distinct categories of subjects: healthy natural living honey bees and healthy honey bees affected by stress belonging to colonies of Beekeeping Research and Development Institute Bucharest, sanitary-veterinary surveyed.
Stress conditions were achieved by maintaining bee families in Apiclimatron up to 24 hours, in high temperature (over 30°C), low humidity, poor ventilation in the internal tube, compared to healthy bees that were kept in hives in normal microclimate conditions in the external tube.
Measurements were performed on samples collected in inactive season - October 2009 and February 2010, for both natural living and stressed bees. For biochemical tests each sample contains haemolymph randomly harvested from about 50 bees.
Collection of the required quantity of fresh haemolymph (300 µl/determination) was made by means of a glass capillary introduced between 3 and 4 tergites, in the sinus dorsalis of the bee's circulatory system, and collecting of the haemolymph from capillary in a sterile syringe. The samples were analyzed by wet chemistry using Alfawassermann device aiming at assessing ALP (IU/L), GPT/ALT (IU/L), GOT/AST (IU/L), Ca total (mg/dl), CPK (IU/L), Cre (mg/dl), GGT (IU/L), GLU (mg/dl), Mg (mg/dl), T-Pro (g/dl), TG (mg/dl), UA (mg/dl) and Urea (mg/dl).

2. **RESULTS AND DISCUSSION**

The biochemical parameters results obtained during the study are presented in Tables 1, 2, 3, and 4.

Table 1

The main biochemical parameters of haemolymph in natural living honey bees
October 2009

No.	**Parameter (UM)**	**Value**
1	ALP (UI/l) —alkaline phosphatase	70
2	GPT/ALT (UI/l) —alanine amino transferase	368
3	GOT/ AST (UI/l) —aspartate amino transferase	336
4	Total Ca (mg/dl) —calcium	3.1
5	CPK (UI/l) —creatininphosphokinase	506
6	Cre (mg/dl) —creatinine	6.1
7	GGT (UI/l) —gamma glutamyl transpeptidase	3.33
8	GLU (mg/dl) —glucose	377
9	Mg (mg/dl) —magnesium	3.4
10	T-Pro (g/dl) —total protein	2.4
11	TG (mg/dl) —triglycerides	34.9
12	UA (mg/dl) —uric acid	2.62
13	BUN —urea (urea nitrogen)	41.2

Table 2

The main biochemical parameters of haemolymph in natural living honey bees
February 2010

No.	**Parameter (UM)**	**Value**
1	ALP (UI/l) —alkaline phosphatase	60
2	GPT/ALT (UI/l) —alanine amino transferase	311
3	GOT/ AST (UI/l) —aspartate amino transferase	393
4	Total Ca (mg/dl) —calcium	3.28
5	CPK (UI/l) —creatininphosphokinase	498
6	Cre (mg/dl) —creatinine	6.90
7	GGT (UI/l) —gamma glutamyl transpeptidase	2.56
8	GLU (mg/dl) —glucose	340.5
9	Mg (mg/dl) —magnesium	3.99
10	T-Pro (g/dl) —total protein	2.51
11	TG (mg/dl) —triglycerides	36.7
12	UA (mg/dl) —uric acid	2.88
13	BUN —urea (urea nitrogen)	42.63

Table 3

The main biochemical parameters of haemolymph in stressed honey bees
October 2009

No.	Parameter (UM)	Value
1	ALP (UI/l) –alkaline phosphatase	158.5
2	GPT/ALT (UI/l) –alanine amino transferase	372.5
3	GOT/ AST (UI/l) –aspartate amino transferase	521
4	Total Ca (mg/dl) –calcium	6.19
5	CPK (UI/l) –creatininphosphokinase	535.5
6	Cre (mg/dl) –creatinine	10.79
7	GGT (UI/l) –gamma glutamyl transpeptidase	241.67
8	GLU (mg/dl) –glucose	337
9	Mg (mg/dl) –magnesium	0.42
10	T-Pro (g/dl) –total protein	2.14
11	TG (mg/dl) –triglycerides	66.35
12	UA (mg/dl) –uric acid	4.03
13	BUN –urea (urea nitrogen)	41.78

Table 4

The main biochemical parameters of haemolymph in stressed honey bees
February 2010

No.	Parameter (UM)	Value
1	ALP (UI/l) –alkaline phosphatase	125
2	GPT/ALT (UI/l) –alanine amino transferase	337
3	GOT/ AST (UI/l) –aspartate amino transferase	458.5
4	Total Ca (mg/dl) –calcium	5.25
5	CPK (UI/l) –creatininphosphokinase	561
6	Cre (mg/dl) –creatinine	8.85
7	GGT (UI/l) –gamma glutamyl transpeptidase	32.15
8	GLU (mg/dl) –glucose	327.25
9	Mg (mg/dl) –magnesium	0.48
10	T-Pro (g/dl) –total protein	2.96
11	TG (mg/dl) –triglycerides	62.03
12	UA (mg/dl) –uric acid	2.33
13	BUN –urea (urea nitrogen)	46.2

The results analysis summarized in table 1-4 shows the existence of a significant increasing of biochemical parameters values characterizing enzyme activity (ALP, AST, GGT), energy metabolism (CRE) and total

calcium levels in haemolymph. It was found that alkaline phosphatase activity increases under stress, very significantly, from 60-70 IU/L to 125-158.5 UI/L, AST enzyme activity significantly increased from 336-393 IU/L to 458.5-521 IU/L.

Under stress GGT enzyme activity increased very significantly, from 2.56-3.33 IU/L to 32.15-241.67 IU/L. Creatinine values increased significantly from 6.1-6.9 IU/L to 8.85-10.79 IU/L, values are 5-6 times higher than the mammal blood. In comparison, total calcium values increased significantly from 3.1-3.28 mg/dl (bee sound) to 5.25-6.19 mg/dl (in terms of stress). Triglycerides increased very significantly from 34.9-36.7 mg/dl to values between 62.03-66.35 mg/dl in terms of exposure to stressful factors.

It is noted that for monitoring the bees health in metabolic stress conditions, is necessary to evaluate a small number of biochemical parameters: ALP, AST, GGT enzymes, creatinine, total calcium, magnesium and total triglycerides, other biochemical parameters of moving insignificant hemolymph.

Compared with previous research conducted on healthy bees (Șapcaliu *et al.*, 2008) which were followed seasonal variations of biochemical parameters of honey bees hemolymph, we found low variability in most biochemical parameters studied in natural living healthy bees in the two years of the experiment. Glucose and total protein values were comparable to those reported in literature, and activity levels of enzymes AST, ALT, GGT, CPK, and total urea showed higher values compared with those found in the blood of mammals, while calcium, magnesium, uric acid and triglycerides had values within the range in blood plasma of mammals (Rădoi, 2001).

This experimental study continues our previous researches in biochemistry of haemolymph, but this time we investigate the biochemical changes in stress conditions. We propose to identify some changes that are manifesting in the bee organism in stressful conditions, in order to monitor the bee health status. Biochemical investigations reflect the transformations that occur under the action of stressors in bee haemolymph. Metabolic investigations may have important preventive value by highlighting some fine changes occurring before the clinical manifestation. Under stress conditions in the bee body occur important changes revealed by the existence of significant changes in biochemistry of haemolymph.

3. **CONCLUSIONS**

3.1. The wet chemistry results performed on the two groups of honey bees kept in natural like conditions and the two groups of honey bees kept under the stress influence showed significant changes in biochemical parameters concentration of hemolymph**.**

3.2. Metabolic profile investigation of bee colonies under stress conditions compared with bees colonies unstressed in Apiclimatron terms, based on the assessment of major changes in biochemical parameters in haemolymph, shows increased enzyme activity, the amount of total calcium, creatinine and triglycerides, while decreasing total magnesium under conditions of stress.

3.3. Assessment of metabolic profile of bees in stressful conditions shows the importance of monitoring enzyme activity (alkaline phosphatase, aspartataminotransferaze, gammaglutamil-transpeptidaze) and calcium and magnesium total concentration, creatinine and triglycerides, reducing the number of investigated biochemical parameters.

3.4. Biochemical investigations more accurately reflect the transformations that occur under the action of stressors in bee haemolymph.

3.5. Metabolic investigations may have important preventive value by highlighting some fine changes occurring before the clinical manifestation.

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ANATOMICAL AND RADIOLOGICAL PARTICULARITIES OF THE PELVIC AUTOPODIUM AT THE DOMESTIC SOLIPEDES

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Key words: radiological particularities, autopodium, horse, donkey..

SUMMARY

The investigations were performed on 10 specimens of each species Equus caballus and Equus asinus. Taken together, the anatomical region of the donkey's tarsal bone resembles that of the horse but there are some obvious features. It is noted that talus has relationships with scaphoid bone by means of a joint surface which is uniformly convex on plantar branch from side to side, while the horse form two connected flat angle. The scaphoid conformation is characterized by a reverse. Compared with the horse, donkey has a finer primary metatarsus. Also, the tuberosity from proximal extremity of metatarsus which providing lateral support is more developed than the horse. The distal extremity of donkey's condyles are more unequal than the horse.

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

1. MATERIALS AND METHODS

The study material was represented by ten horse pelvic autopodes and ten donkey pelvic autopodes. Were used animals destined for dissection, demonstration and reasearching in the Domestic Animal Anatomy Laboratory from The Faculty of Veterinary Medicine. After the achievement of dorso-palmar and latero-lateral radiographs, for the bone study, the autopodes were macerated in differente pots and labeled.

2. RESULTS AND DISCUSSIONS

Taken together, the tarsal bones forming a bone complex, with four sides, apex and base.

The front have a upper part which is correspond with the edge of the calcaneus. The middle part is formed by trochleea of the talus. The lower part contains digital fossa of the talus, the front sides of the cuboid, scaphoid and cuneiform bones. Ventral from the lateral lip of trochleea of the talus is observed the entry in the tarsal conduct.

External face is represented by an upper part formed by the external side of calcaneus. The middle part is represented through a area of insertion of ligament, placed on the lateral side of the talus. The lower part is formed by the external side of the cuboid and the adjacent extremity of great cuneiform and scaphoid bones.

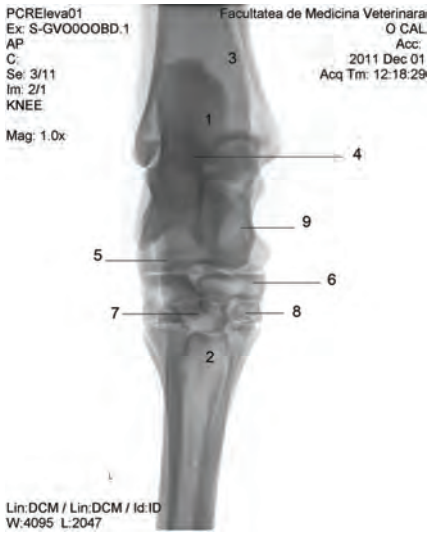

Fig. 1 Radiography of the tarsal region, left leg, horse, plantar aspect
1-tuberosity of calcaneus, 2-metacarpal III bone; 3-tibia; 4-trochleea of the talus; 5-cuboid bone; 6-scaphoid bone; 7-great cuneiform; 8- little cuneiform; 9-sustentaculum tali.

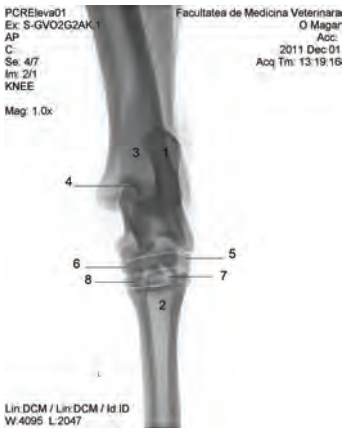

Fig. 2 Radiography of the tarsal region, left leg, donkey, dorsal aspect
1-tuberosity of calcaneus, 2-metacarpal III bone; 3-tibia; 4-trochleea of the talus; 5-cuboid bone; 6-scaphoid bone; 7-great cuneiform; 8- little cuneiform;

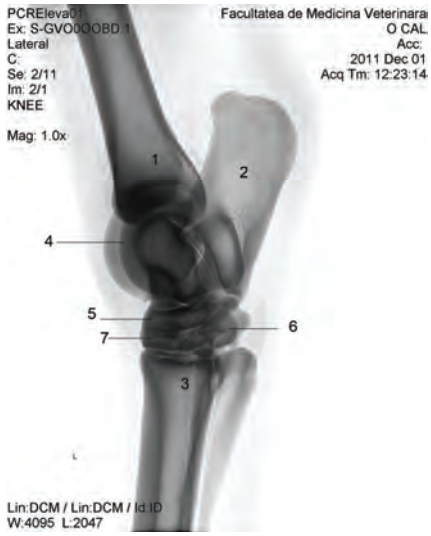

Fig. 3 Radiography of the tarsal region, left leg, horse, lateral aspect
1- tibia, 2- tuberosity of calcaneus; 3-metacarpal III bone; 4-trochleea of the talus; 5-scaphoid bone; 6- little cuneiform; 7-great cuneiform.

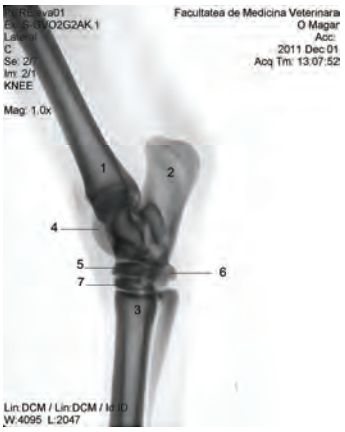

Fig. 4 Radiography of the tarsal region, left leg, donkey, lateral aspect
1- tibia, 2- tuberosity of calcaneus; 3-metacarpal III bone; 4-trochleea of the talus; 5-scaphoid bone; 6- little cuneiform; 7-great cuneiform.

At the top, the internal face is represented by the internal, concave face of the calcaneus. In the middle it is the tendinous groove of talus. In the bottom is the external face of cuboid and adjacent extremity of two cuneiform bones (Fig. 1,2).

The plantar edge is formed by the calcaneus. In the middle part is the tendinous groove of sustentaculum tali. Lower portion is anfractuous and irregular.

The apex of osseous complex correspond with proximal extremity of the calcaneus.

The base or metatarsal side is represented by plantar faces of the cuboid, great cuneiform and small cuneiform (Fig. 3,4).

The most important differences in donkey are: talus has relationships with scaphoid bone by means of a joint surface which is uniformly convex on plantar branch from side to side, while the horse form two connected flat angle. The scaphoid conformation is characterized by a reverse. Compared with the horse, donkey has a finer primary metatarsus. Also, the tuberosity from proximal extremity of metatarsus which providing lateral support is more developed than the horse. In the donkey the condyles of the distal extremity are more unequal than the horse.

3.CONCLUSIONS

3.1. In donkey talus has relationships with scaphoid bone by means of a joint surface which is uniformly convex on plantar branch from side to side, while the horse form two connected flat angle..

3.2. Compared with the horse, donkey has a finer primary metatarsus. The large metacarpal bone, at donkeys, is thicker, less flettened craniocaudal.

3.3. The tuberosity from proximal extremity of metatarsus which providing lateral support is more developed than the horse.The first and second phalanx are relatively longer at donkeys and the third phalanx has less proeminent angles.

3.4. In the donkey the condyles of the distal extremity are more unequal than the horse.

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COMPARATIVE ASPECTS REGARDING THE MORPHOLOGICAL BIODIVERSITY OF CERVICAL VERTEBRAE IN AFRICAN OSTRICH (STRUTHIO CAMELUS) AND RHEAS (RHEA AMERICANA)

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Key words: ostrich, vertebrae, cervical region

SUMMARY

Research conducted on bones from four specimens of Struthio and four specimens of Rhea have shown that there are many features on which we can identify the species from which the bones belong. We observed that the ratites cervical region is very long, consisting of 17 vertebrae in Struthio and 15 vertebrae in Rhea. Except the first two cervical vertebrae, the rest are long and meet the characters common to all species of birds. Except atlas and axis, cervical vertebrae in ratites have long body, the longest being those of the middle series (VII, VIII, IX) (average from 5 to 5.5 cm and 7-8 cm in Struthio Rhea). The last two or three vertebrae are shorter.\

Compared with data from the literature, quite detailed, regarding the morphology of the cervical vertebrae in poultry (and web-footed fowls) (1,2,5,7), in the ratites the peculiarities were briefly described (3, 4,6,8,9). For this reason we approached this study with the aim of presenting detailed morphology of the cervical vertebrae and the differences that arise between the two species.

1. MATERIAL AND METHODS

The researches has been made on 8 birds of different sexes and different ages. After the boning, the material was prepared from maceration,

then the bones were degrease and whitened with perhidrol 3%. We used Nomina Anatomica Avium —1993.

2. RESULTS AND DISCUSSION

Cervical region in ratites is very long consisting of 17 vertebrae in Struthio and 15 vertebrae in Rhea. Except the first two cervical vertebrae, the rest are long and meet the characters common to all species of birds. There are also some features that characterize the group of ratites and on which it can be quite accurate their membership.

Atlas is the lowest cervical vertebra, the dorsal arch is delicate (Fig. 1). From both sides of it is detaching one caudal articular process (Postzygaphophysis), oriented caudodorsal, with a length of approximately 2.5 mm. In Struthio each process supports in dorsal side a small tuber (Anapophysis). Transverse holes in both species are transformed into notch. On the cranial atlas presents a joint cavity, deep, with a diameter of 7-8 mm in Struthio and 5 mm in Rhea, for articulation with the only one condyle of occipital bone.

Above the joint cavity, there is a distinct notch, where entering the odontoid process of axis.

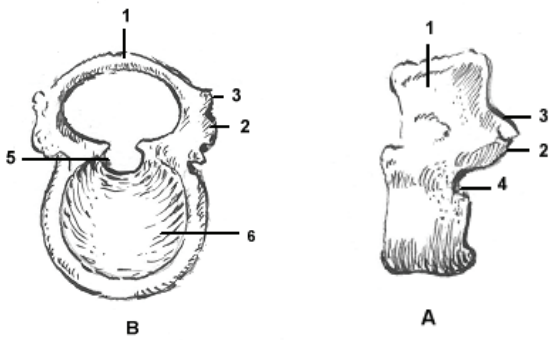

Fig. 1 Atlas in Struthio (original)
A-lateral view; B-caudal view
1-dorsal arch; 2-caudal articular processes (postyzgapophysis); 3-anapophysis; 4-transversal notch; 5-the notch for odontoid process; 6-caudal terminal face.

In both species the odontoid process of axis is relatively short, widened, developed on behalf of a own nucleus of ossification.

Costotransversar processes are small (2-3 mm), oriented dorsal (Fig. 2). At the base of them it can see a area provided with numerous vascular openings. Cranial articular processes have approximately the same size like costotransversar processes and its are joined by a sharp ridge with caudal articular processes. Spinous process, high and tuberous, tends to divide in the caudal side. This aspect is more accentuated in Rhea. Caudal articular processes (Postzygapophysis) are provided with rough anapophysis, about 2 mm high. Terminal caudal face, is convex from side to side and concave dorsoventral, and has a height greater than width.

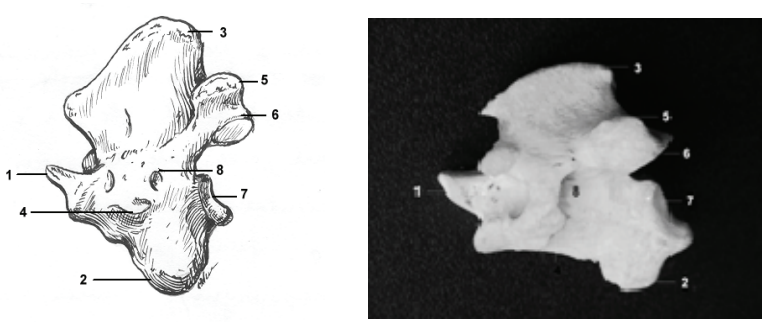

Fig. 2 Axis in Strurhio (lateral view)(original)
1-odontoid process; 2- vertebral ventral ridge(hypapophysis); 3- spinous process;
4-costotransversar process; 5-anapophysis;
6-articular caudal processes(postzygapophysis); 7-caudal terminală faces;8-transvers conduit.

Cervical vertebrae III-XIV (Rhea) and III-XVIII (Struthio) Except atlas and axis, other cervical vertebrae in ratites have long body, the longest being those of the middle series (VII, VIII, IX) (average from 5 to 5.5 cm in Rhea and 7-8 cm in Struthio). The last two or three vertebrae are shorter (Fig. 3,4).

Vertebral body (Corpus vertebrae) increases in thickness from first to last, and it delimits the terminal faces (Facies articularis cranialis et Facies articularis caudalis) will become more broad transversary. Lateral grooves (Sulcus lateralis), for ascending vertebral arteries and veins are not well defined. On the front side (Facies lateralis) we can identified the costal processes, oriented caudally (Proc. costalis, Pleurapophysis sin), gradually

thickened towards the end of the series. On the ventral side of the body (Facies ventralis) in the cranial region can be seen carotid processes (Proc caroticus, sin. Proc. Hemalis) that delimit carotid groove (Sulcus caroticus). These are very small and spaced at the beginning of the series, then increase in size, gradually approaching, so they tend to last vertebrae form a bony canal.

Transverse processes (Proc. transversus, sin. Diapophysis) are much lower compared with those of thoracic vertebrae, directed ventral from the base of cranial articular processes (Proc. articularis cranialis, sin. Zygapophysis cranialis). With coastal processes its delimits a transverse hole increasingly wider (Foramen transversarium).

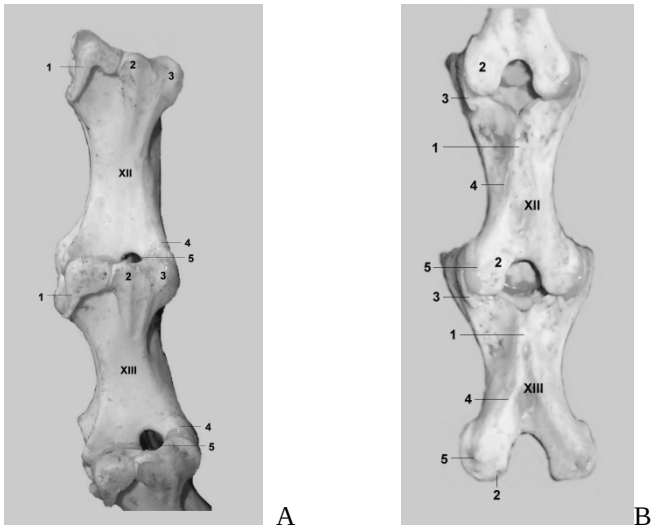

Fig. 3 Cervical vertebrae XII and XIII in Rhea americana în lateral view (A) and dorsal view (B) (original)

A-1-coastal processes; 2- transvers processes; 3- cranial articular process; 4- caudal articular process; 5-intervertebral holes;

B-1- spinous process; 2- caudal articular process; 3- cranial articular process; 4-transverse-oblique ridges; 5-hiperapophysis.

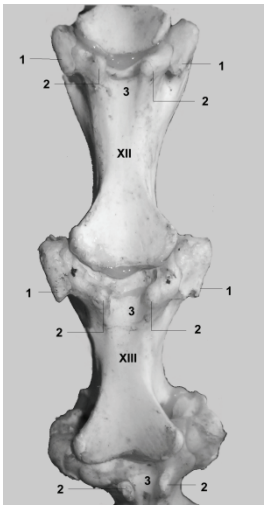

Fig. 4 Cervical vertebrae XII-XIII in Rhea americana,ventral aspect (original)
1- costal process; 2-procesele carotic processes; 3-carotic grove.

The articular faces (Facies articularis) of articular processes are closer at the first vertebra, becoming more distant in caudal region. Transverso-oblique ridges (Crista transverso-obliqua) are more evident for the last vertebrae. Spinous process (Proc. dorsalis, sin. Proc. Spinosus), represented by two tubers, located in caudal half of the vertebral arch of the III-IV cervical vertebrae, is reduced in volume in other vertebrae, appearing as an elongated ridge located in the cranial half of the arch. In the last three vertebrae, the crest is highlighted, thickens and divides, continue with ridges transverso-oblique.

3. CONCLUSIONS

3.1. Cervical region in ratites is very long consisting of 17 vertebrae in Struthio and 15 vertebrae in Rhea.
3.2. Atlas has a delicate dorsal arch, from this are detaching, on the both side, a caudal articular process (Postzygaphophysis). This has a caudodorsal direction with a length of approximately 2.5 mm. In Struthio each process in the dorsolateral parts, supports a small tuber (Anapophysis).

3.3. In both species the odontoid process of axis is relatively short, widened, developed on behalf of own nucleus of ossification. Spinous process, high and tuberous, tends to divide in the caudal side.

This aspect is more accentuated in Rhea.

3.4. Except atlas and axis, cervical vertebrae in other ratites have long body, the longest being those of the middle series (VII, VIII, IX) (average from 5 to 5.5 cm in Rhea and 7-8 cm in Struthio). The last two or three vertebrae are shorter.

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ASPECTS REGARDING THE MORPHOLOGICAL BIODIVERSITY OF HIP JOINT IN RHEAS (RHEA AMERICANA)

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Key words: rhea, hip joint, femur

SUMMARY

Research conducted on four adult specimens have shown that American Rhea 's coxofemural hinge joint is strengthened especially by the strong adjacent muscles. The means of connection are the joint capsule inserted on the acetabular cavity and on the articular surface of the femoral head and the great trochanter . The capsule appears condensed in the traction force exerted anterodorsal during the hip extension. In the caudoventral region the capsule weaves on the outside with the internal obturator muscle tendon. Intracavitary there is the femoral head's own ligament.

In the lower limb joints in ratite study, the literature provides data in particular on the tibio-patellar-femur (1,4,5), tibio-tarsometatarsian (2) and toes (3). The coxofemural joint was described in less detail. Research conducted on pieces from four copies of rhea allowed the description of morphological aspects of this articulation of the species above.

1. MATERIALS AND METODES

The research was conducted on four copies of rheas from the Bucharest zoo. Dissection was carried out by the usual technique using SMZ 2 T Nikon stereomicroscope and camera with which images were taken. Description of formations was made according to Nominal Anatomica Avium - 1993.

2. RESULTS AND DISCUSSIONS

In Rhea, the coxofemural joint (Articulatio coxae) is a particularly strong hinge reinforced by adjacent muscles.

The articular femoral head has an articular surface that is extended on the back of the neck, joining the articular surface of the great trochanter (facies articularis antitrochanterica). Fissures joint is wide, placed in the back of the femoral head.

The hip Offers for articulation the acetabular hole, completată completed in depth by a fibrocartilaginos membrane. The edge of the acetabular hole (acetabular labrum) protrudes craniodorsal where it appears thin and sharp, the dorsal and ventral area is bounded by one depression, where the edge appears bevelled.

The means of connection are the joint capsule inserted on the edge of the acetablare cavity offered by the femoral head and great trochanterul joint. The capsule appears condensed in the anterodorsal due to the traction force exerted during extension trough. In the caudoventral side the capsule weaves on the outside with the internal obturator muscle tendon. The latter after comeing out through the obturated hole in the abdominal cavity has craniodorsal oblique trajectory and inserts to the external face of the high trochanter, limiting its movement in anteromedial direction (Fig. 1, 2).

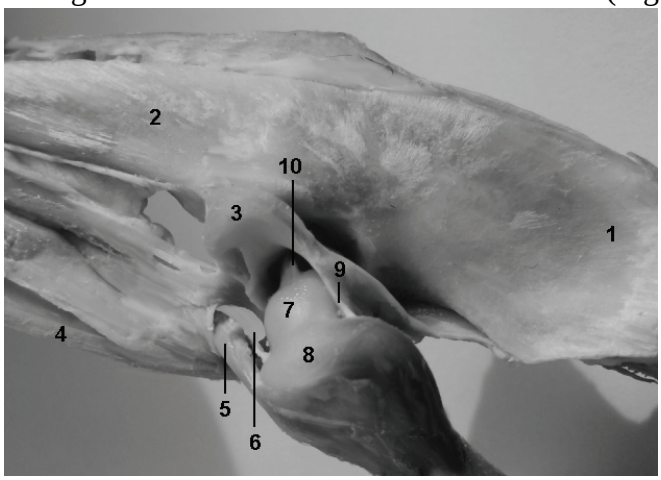

Fig.1 A joint coxofemural from American Rhea (dorsolateral view) (original)
1-preacetabular part of the ilium; 2-postacetabular portion of Ilium, 3-antitrochanter, 4-pubis; 5-the internal obturator muscle tendon; 6-the obturated hole; 7-femoral head, 8-femoral trochanterul 9-joint capsule.

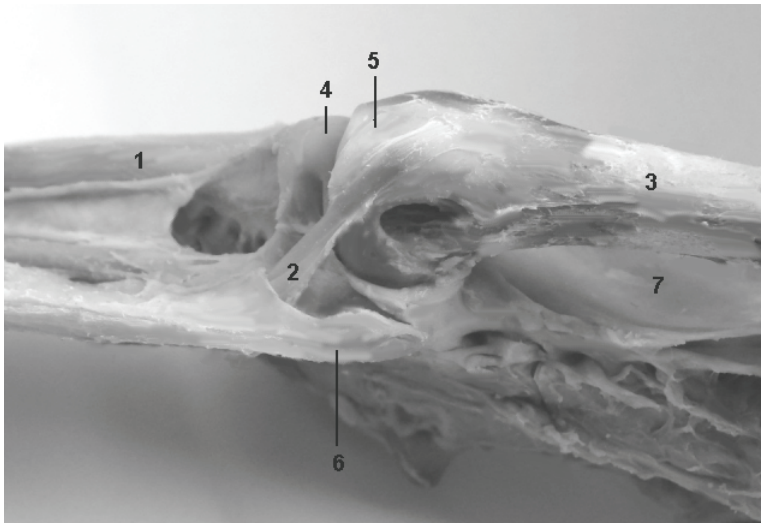

Fig. 2 joint coxofemural from American Rhea (view caudoventral) (original)
1 the postacetabular portion of the ilium; 2 the internal obturator m. tendon; 3 femur; 4-antitrochanter; 5-the femoral trochanter; 6 pubis; 7 preacetabular part of the Ilium.

Intracavitary exists the ligament appanage of the femoral head (Lig. capitis femoris). It is short and strong and insert on one side of the femoral head ligament in the fissures and on the other hand, it weaves with the fibrocartilaginos membrane from the depth of the acetabular cavity. In the ligament insertion area, the fibrocartilaginos membrane appears more condensed and inserted from the lateral edge of the sinsacrum.

3. CONCLUSIONS

3.1. The means of connection are represented by articular capsule inserted on the edge of the acetabular cavity and on the edge of the articulation surface offered by the femoral head and great trochanter. Intracavitary exists appanage the ligament of the femoral head (Lig. capitis femoris). It is short and strong.

3.2. In the caudoventral side the capsule weaves on the outside with the internal obturator muscle tendon.

3.3. The internal obturator tendon as well as the strong musculature in the area are additional means to strengthen this articulation

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THE ROLE OF ADVANCED GLYCATION END PRODUCTS IN THE DEVELOPMENT OF DIABETES COMPLICATIONS

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Key words: glycation, animal study model, collagen, AGEs, diabetes

SUMMARY

To confirme the potential pathogenic role of advanced glycation end products (AGEs) in the development of diabetes complications such glomerulosclerosis and in order to shunt the metabolic pathways activated by hyperglycemia, this study used as an animal study model, rats treated i.v. with glycated rat albumin (AGE-RSA), which simulate the development of circulating glycation end products. The efficiency of this particular study model was investigated in the presence of aminoguanidine, an inhibitor of AGEs formation. The amount of AGEs accumulated within the kidneys was done by competitive ELISA, and the effect of AGEs on the protein expression of RAGE receptors and type IV collagen at this level was perform by Western blot. Moreover, we tried to correlate the levels of HbA1c and AGE products accumulation with diabetes complications such as an increase of RAGE and collagen IV protein expression and albuminuria. This study demonstrates that AGEs are implicated in the development of diabetes complications because the coadministration of aminoguanidine with AGE-RSA reduced albuminuria, AGE products level and the protein expression of RAGE and collagen IV.

Elevated levels of blood glucose give birth to a vicious cycle of metabolic disturbances within the intracellular and extracellular environment and lead to a broad array of microvascular and macrovascular complications of diabetes such retinopathy, neuropathy, nephropathy and atherosclerosis (Yan, Yan et al. 2006). A number of pathways are activated in the hyperglycemic milieu, such as the aldose reductase pathway (AR), activation of protein kinase C (PKC), and the generation of advanced glycation endproducts (AGE) (Wendt, Tanji et al. 2003)

The non-enzymatic glycosylation of proteins or glycation reaction is a consequence of elevated levels of glucose. This process occurs in normal aging and it is accelerated in diabetes. The glycation reaction is beginning when the amino groups of a protein react non-enzymatically with glucose to form a Schiff base which is temporarily stabilized through Amadori rearrangement and represent an early step in the glycation process

(Thornalley 2005). The Amadori products best known is HbA1c and fructosamine (fructoselysine)(Jakus, Bauerova et al. 2001). The AGE formation from fructoselysine involves booth oxidative and non-oxidative mechanisms to form new reactive intermediates that again modify proteins to form AGEs of various chemical structures for examples: N^{ε}-carboxymethyllysine (CML) (Iacovella, Alderson et al. 2004), pentosidine (Biemel, Reihl et al. 2001), glucosepan (Sell, Biemel et al. 2005), crossline (Biemel, Conrad et al. 2002), etc. Recent studies have suggested that AGEs are not only produced from sugars such as glucose, but also from α-hydroxyaldehyde (glyceraldehyde and glycolaldehyde), and dicarbonyl compounds (glyoxal, methylglyoxal and 3-deoxyglucosone) that could derived from the glycation process, autoxidation of sugars (Wolff pathway) or fragmentation of Schiff base (Namiki pathway) and other metabolic pathways (Thornalley 2005).

AGEs can cause cellular dysfunction by multiple mechanisms, including receptor-independent and receptor-dependent processes. In the former setting, AGEs may directly influence the structural integrity of the vessel wall and underlying basement membranes through excessive deposit, cross-linking of matrix molecules such as collagen and disruption of matrix-matrix and matrix-cell interactions. Also, diabetes is accompanied by aortic and glomerural accumulation of total collagen, specifically collagens I, III, and IV, as well as increases in the profibrotic cytokines transforming growth factor- β (TGB-β) and connective tissue growth factor (CTGF) (Iglesias de la Cruz, Isono et al. 2002; Mentink, Hendriks et al. 2002; Twigg, Joly et al. 2002; Twigg, Cao et al. 2002; Yang, Litchfield et al. 2003). On the basis of these findings and other evidence suggesting impact of AGEs in the pathogenesis of diabetic complications, pharmacologic inhibitors of AGE formation were developed to test the concept that reduced AGEs formation would lead to decreased complications. The best example of these is aminoguanidine (AG), an agent of which the major biologic target is AGE formation.

The aim of the present study was to test a new animal study model (rats infused with AGE compounds) significantly cheaper and easier to work with in order for researchers to comprehend and discover the pathways involved in the development of diabetes complications and prevent them from happening.

1. MATERIAL AND METHODS

1.1. Preparation of AGE-RSA and evaluation the glycation process. Soluble glycated rat serum albumin (AGE-RSA) was synthesized in *vitro*, according to the methods previously described by (Serban, Condac et al. 2009), but the incubation was prolongeted to 3 months. The glycation of RSA was performed by SDS-PAGE, gel filtration chromatography and fluorescence assay.

1.2. Animal studies. Male rats, Rattus norvegicus species, Winstar line, aged 10 months were divided into 3 study groups of 5 rats each. Each rat was placed into an individual cage and was given standard food (Labdiet 3001) and water. After a two week acclimatization period, the rats were treated for 3 months as follows: 1st group (control): i.v. shot in the tail with 50 mg/kg/day RSA, 2nd group: i.v. shot in the tail with 50 mg/kg/day AGE-RSA and 3rd group: i.v. shot in the tail with 50 mg/kg/day AGE-RSA + 200 mg/kg/day AG.

1.3. Biochemical assays. The rats were monitored during the 3 months of treatment by analysing the blood glucose, and HbA1c levels. The glucose level was assessed using the Cayman Kit and HbA1c was determinate using Nycocard Reader II device (sensibility 3-18 % HbA1c). Twenty-four-hour urine samples were obtained for three consecutive days every month for total protein (Bradford 1976), and total albumin determinations using Nycocard Reader II device.

1.4. Immunochemical assays

1.4.1. Evaluation of CML level through competitive ELISA: 96-well plates are coated with specific CML-antigen. During the analysis, the sample is added together with the specific primary anti-CML antibody. If the sample contains a certain amount of CML-antigen, it will be in competition for linking to the primary antibody to the CML-antigen that the 96-well plates were coated with. TGFβ Coat mAb, which binds soluble TGFβ1. After washing, the amount of the captured primary antibody anti-CML is detected using a second specific polyclonal antibody conjugated to horseradish peroxidase. The unbound conjugate is removed by washing, and following an incubation with a chromogenic substrate, the color change is measured. The amount of CML-antigen in the sample is reverse proportional to the color generated in the oxidation-reduction reaction.

1.4.2. Western blot assays. The col IV and RAGE protein expression were investigated by Western blot analysis using anti-col IV antibody and anti-RAGE antibody Protein samples are separated in a 10% SDS-PAGE and then electro-blotted onto PVDF membranes. Specific mouse monoclonal IgG1 antibodies (Santa Cruz) anti-target proteins and Chromogenic Western Blot Immunodetection kit which contains secondary antibodies anti-mouse IgG1 conjugated with alkaline phosphatase (Invitrogen) are used.

2. RESULTS AND DISCUSSION

During 3 months treatment period all animals gained weight normally. The glucose and Hb1c levels were determinated from blood samples and from urine samples we determined the total protein levels and albuminuria. After 3 months, the rats were sacrificed and CML levels were determined from serum and kidneys extracts and the protein expressions of collagen IV and RAGE from the kidneys extracts, too.

Mean blood glucose values among the groups treated with RSA and AGE-RSA were not significantly different during the 3 months of treatment (155 mg/dl±5), demonstrated that circulating AGE compounds had no influence on the glucose level. The slight decrease in glucose levels following AG treatment reflects this inhibitor's potential to induce hypoglycemia (Fig. 1).

In the presence of circulating AGE compounds which can attack free amino group on proteins, an increase in HbA1c takes place, reaching after 3 months of treatment a two-fold increase, compared to control. It is noticed that AG has the capacity to maintain HbA1c levels within the physiological limits (Fig. 2).

AGE-RSA treatment was associated with 3.2-fold increase in total urinary protein excretion/24 hours, compared to the control (unmodified-RSA-treated group). The cotreatment with aminoguanidine was associated with a significant, although not complete inhibition of this proteinuria (Fig. 3). After 3 months of treatmenr, urinary albumin excretion in the AGE-RSA-treated rats was 2.2-fold greater than in the RSA-treated rats; in contrast, the AGE-RSA+AG-treated rats had near normal urinary albumin levels (Fig. 4).

The results demonstrate that AGEs are implicated in development of kidney disease and confirm the capacity of AG to neutralize dicarbonyl intermediates compounds and to interrupt the glycation reaction.

CML levels from serum and total kidney extracts were determined through competitive ELISA after sacrificing the rats. Circulating CML-protein levels in AGE-RSA-treated rats were 2.5-fold higher than in control group, whereas in the AGE-RSA+AG-treated group, serum CML values fell between the AGE-RSA and control group values (Fig. 5). The daily treatment of rats with AGE-RSA over a period of 3 months resulted in renal-tissue CML accumulation, compared to RSA-treated group (Fig. 6). In animals cotreated with the AGE inhibitor aminoguanidine, protein-associated CML levels increase sligtly above normal. These results reveled that AG was able to annihilate CML development on glyoxal pathway, stopping therefore the accumulation of AGEs in the kidneys.

The protein expression of RAGE and collagen IV assessed by western blot from kidneys extracts of AGE-RSA-treated rats, showed a 2.7-fold and respectively 3.1-fold relative increase, compared to control. The cotreatment with AG induced a decrease of protein expression of both RAGE and collagen IV (Fig. 7). The perfect correlation between RAGE protein expression and collagen IV protein expression demonstrates that the receptor dependent signaling pathway was activated and also it’s potential involvement in development of glomerulosclerosis.

3. CONCLUSIONS

3.1. *In vivo* study of the effects of AGE compounds on normal rats infused for 3 months with AGE-RSA, in the absence of hyperglycemia revealed the significant role that the advanced glycation end-products have in the development of diabetes complications, affecting especially the renal system.

3.2. The increase in HbA1c level in AGE-RSA-group demonstrates that the glycation reaction takes place in the absence of hyperglycemia but in the presence of Schiff base and Amadori products.

3.3. The CML levels and albuminuria are in perfect correlation with the protein expression levels of RAGE, CML having therefore epitops

recognized by RAGE with great implications in the development of kidney disease.

3.4. With regard to the renal system, AGE compounds are able to induce glomerulosclerosis either by performing cross-links between AGE compounds and extracellular matrix proteins or by activating certain signaling pathways, dependent on the RAGE receptors which finally lead to an increase in extracellular matrix proteins such as type IV collagen.

3.5. AG determined a decrease only in CML levels developed from the glyoxal pathway, demonstrating that this inhibitor has great efficiency in neutralizing dicarbonyl AGEs intermediates compounds.

3.6. The animal study model described in this research may be used successfully in testing several inhibitors of the glycation reaction as well as in determining the diabetes complications induced by AGEs.

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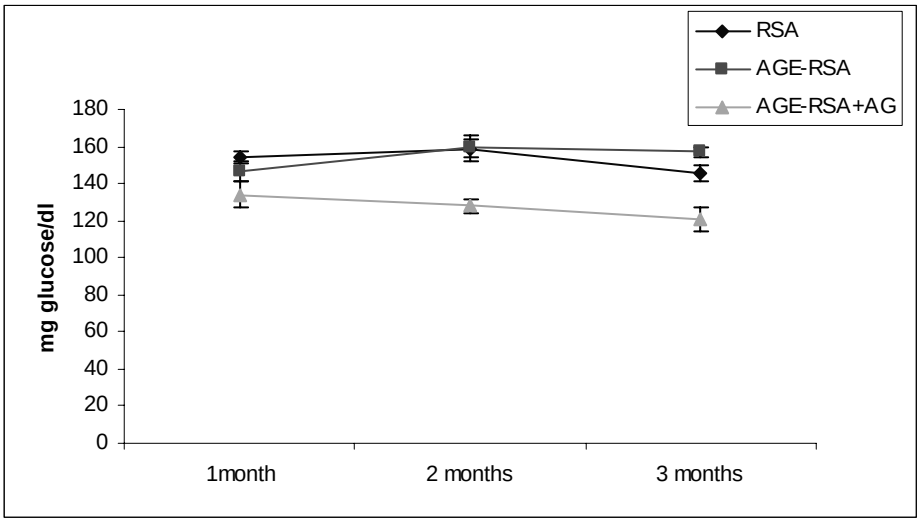

Fig. 1. The variation of glucose level during 3 months of treatment

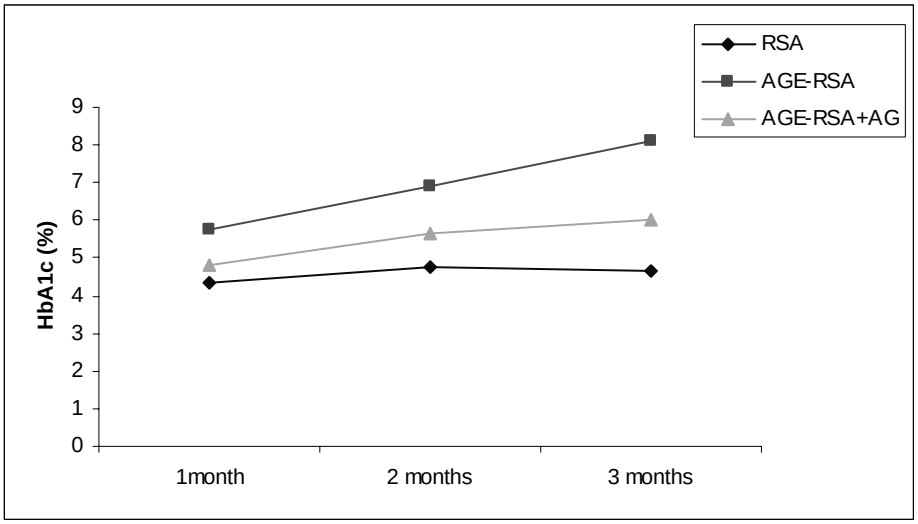

Fig. 2. The variation of HbA1c level during 3 months of treatment

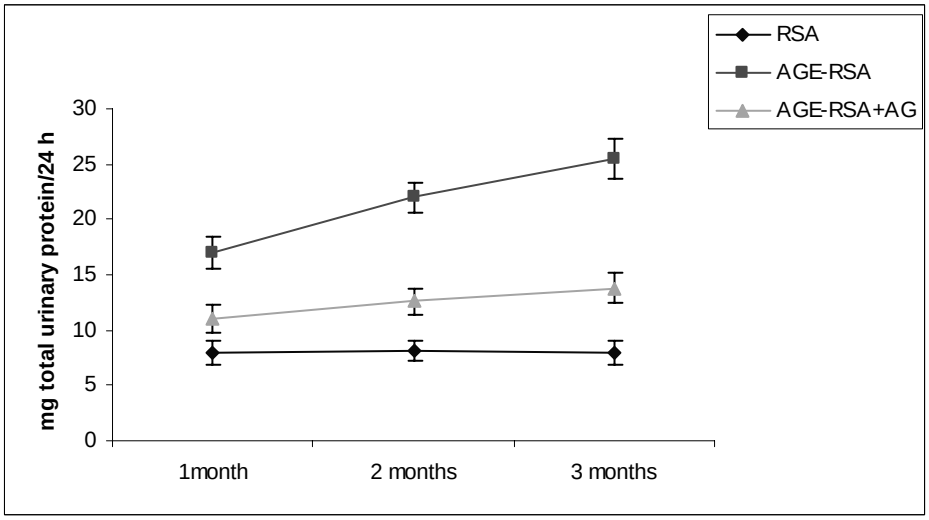

Fig. 3. The variation of total urinary protein/24 hours level during 3 months of treatment

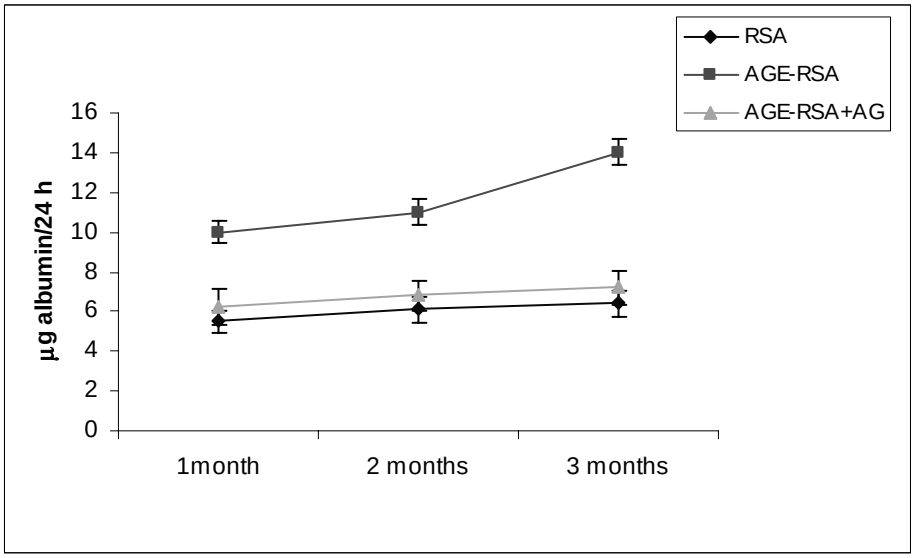

Fig. 4. The variation of urinary albumin/24 hours level during 3 months of treatment

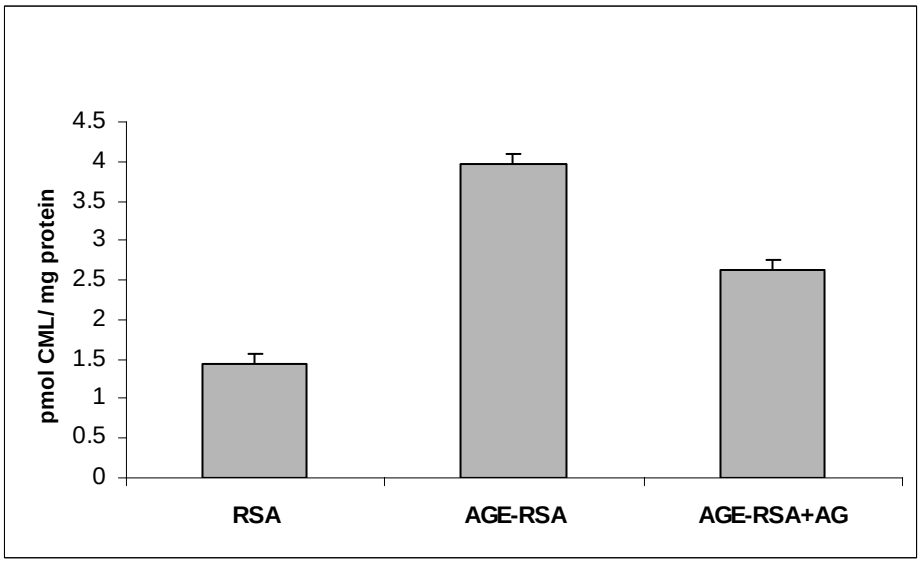

Fig. 5. Circulating CML-protein level after 3 months of treatment

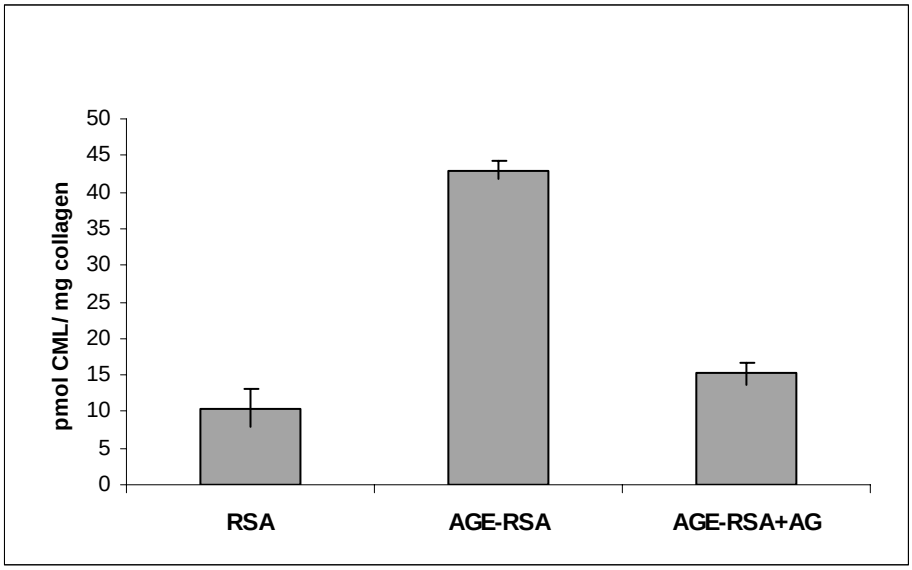

Fig. 6. Renal-tissue CML accumulation after 3 months of treatment

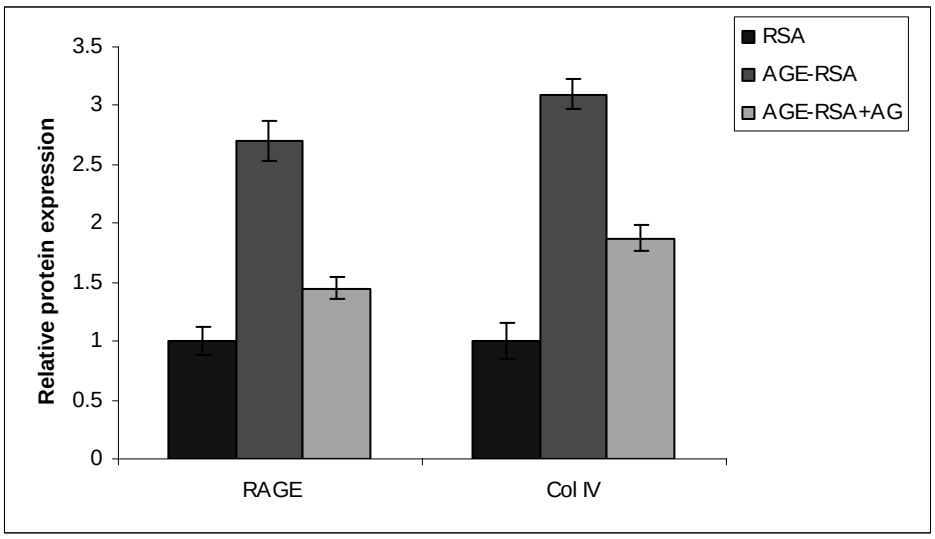

Fig. 7. The relative protein expression levels of RAGE and collagen IV in renal-tissue after 3 months of treatment

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THE HISTOLOGICAL RESEARCH FOR SWINE TESTICLE MONOCLONAL ANTIBODY

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***Key words**: seminiferous tubules, gonocytes, Sertoli cells, Interstitial gland*

SUMMARY

In sections made in the testicular parenchyma is observed that the lumen of seminiferous tubule germinal epithelium is delimited by a basal membrane located on the outlines of seminiferous tubules. Basal lamina or internal layer called is linked collagen and elastic fibers to cells that form a blanket peritubulare consists of 2-5layers. Peritubulare cells the characteristics of smooth muscle cells and are involved in providing tubulare.In contractions seminiferous tubules sinuous structure meet 4-5 generations of cells. In prepubertal testis cells presents a core support of small andlarge in size gonocitele globular nuclei. Sertoli cells are active in terms of mitotic and contain large amounts of rough endoplasmic reticulum

1.MATERIAL AND METHODS

For immunohistochemical highlighting of tissular antigene was used the indirect two-staged method based on polymerized dextran (DAKO ENVISION). The sections were displayed on spangles treated with Poly-l-lysin.Next stage was bounding of nonspecific situses of Fc type by adding normal serum for 20 minutes.Second day ,after washing for 20 minutes in PBS, we add the secondary biotinilat antybody (with a bridge role) with whom the sections are incubatedfor 30 minutes at room temperature. After that , a 45 minutes incubation follows ,using the Streptavidina complex(Kit Dako En Vision). After washing in normal water we proceed with a development in a dediaminobensidine solution (10mg DAB în 88ml PBS) with 0,0025 peroxide water.The diaminobenzidine splited of free peroxidase from the Streptavidina-biotina complex, creates a brown precipitate locating with precision the searched antigen, be it in cytoplasm , be it in nucleum.

2. RESULTS AND DISCUSSIONS

The differentiation of the sustaining cells takes place during puberty, when the multiplication capacity is reduced. The basal compartment contains spermatogonium and it is placed peripherally and it is the place where the spermatogonium multiplication and the stem cells renewal take place.

These cells are pyramidal, tall, have an irregular shape and they are placed on a single row, having the base on the membrane bazala and the apical pole to the tubes lumen.

On a cross section, in the semniferous tube it can be noticed that these cells have basal nucleus with a large nucleolus, multihued, often triangular and with multiple indentations.

The spermatogonium line cells are connected to the apical folds of the Sertoli cells cytoplasmic membrane. This net made out of cells, represents the physical support for the ramifications of Sertoli cells. The A spermatogonium has an ovoid nucleus with prominent nucleoli and has a large contact area with the basal lamina. The B spermatogonium is a spherical cell with less prominent nucleoli. From the mitotic division of the B spermatogonium are formed the primary spermatocite .These are the largest cells of the seminal line placed in an intermediary place between the spermatogonium and the sperm cells, their nuclei are large and round, with prominent nucleoli. They gradually lose contact with the basal lamina and they move in the adluminal compartiment through the intercelular joining of the Sertolli cells.

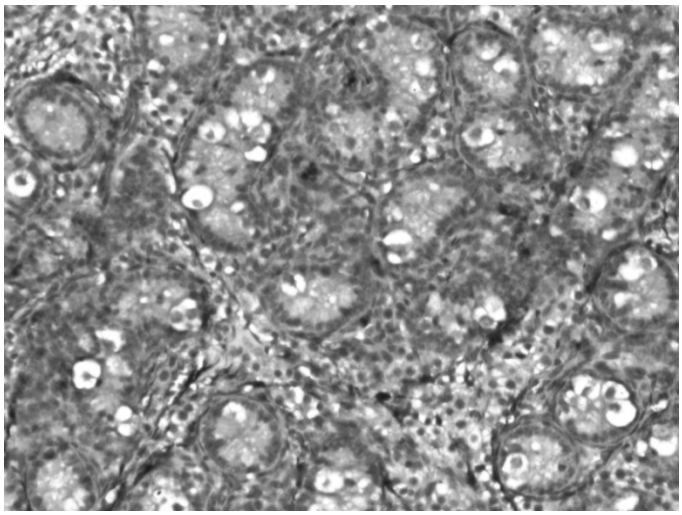

Fig. 1. Swine testicle at birth –Leu 7. Ob 20. Positive reaction at basal layer level

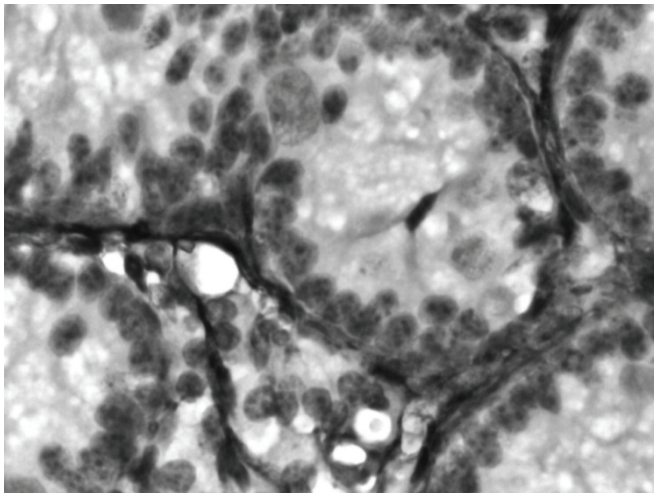

Fig. 2 Grown-up swine testicle –Leu 7 Ob 40x .Positive reaction at basal layer level and positive reaction at interstitial cells.

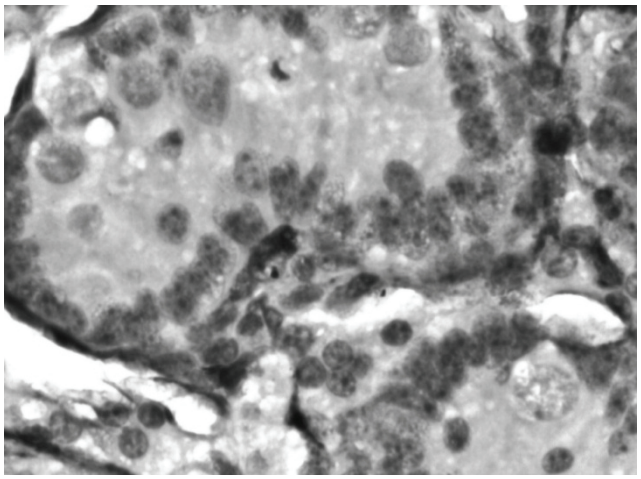

Fig.3.Swine testicle at weaning-Leu 7 Ob40X.Positive reaction.At basis layer level and la interstit cells level

Through the spermiogenesis process, the new formed spermatide become sperm(differentiate into sperm). The excess material of the spermatide (cytoplasm, water, organelles) that is not needed by the sperm formed after the morphological transformation during the spermatogenesis (after the formation of the acrosome, the condensation of nuclear chromatin) is phagocitized by the Sertoli cells.

The interstitial endocrinocite are numerous in the adult pig and they take 20-30% of the

testicular tissue. The connective tissue between the tubes contains many fibroblasts, macrophages, mastocytes, blood and lymph capillaries. During puberty, the connective cells, round or polygonal , have a central nucleus, eosinophillic cytoplasm, and with lipid vesicles. The form of the endocrinocite is irregularly polyhedral spheroidal nuclei and peripheral chromatin, nucleoli are distinct. The cytoplasm is a characteristic of the steroid secreting cells, having a smooth endoplasmic reticulum, abundant at puberty (with function in steroid genesis), lipid, protein, pigment, secretory granules inclusions.

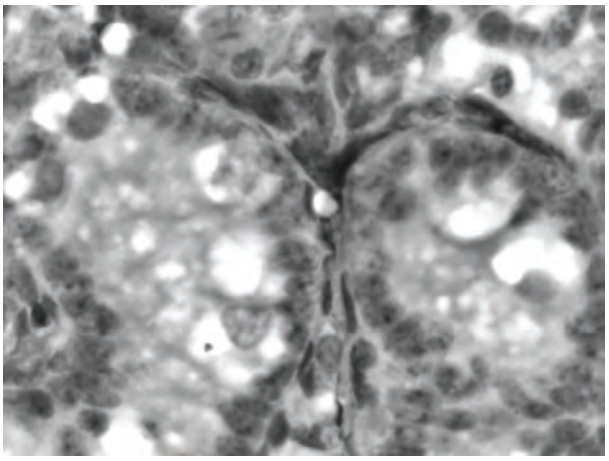

Fig 4.Swine testicle at birth –Leu 7 Ob 40. Positive reaction at basal layer level

EXTERIOR DIAMETER OF SEMINIFER TUBES, LUMENE DIAM AND THE HEIGHT OF EPITHELYUM AT MATURITY

Seminifer tubes	Ext.(µm) Diam	Lumen(µm) Diam	*Height of* ephitelium(µm)
1	352,279	185,121	92,173
2	298,736	176,198	98,231
3	387,836	189,213	89,123
4	352,243	192,162	99,112
5	375,127	199,192	89,178
6	299,374	189,278	88,987
7	324,376	188,176	97,243
8	312,126	199,298	96,876
9	296,989	189,989	90,345
10	311,121	209,222	97,278

During puberty sustentaculare cell differentiation is accompanied by a morphological transformation and loss of mitotic capacity (adults Sertoli cells no longer divide). Sustentaculare cells are pyramidal with irregular contour, contour Sertoli cell is not visible due to the presence of numerous germ cells were seen in the sustentaculare cells emit ramifications extended around and between germ cells making an intimate contact with thems.Rennewal of and multiplication is performed in the basal compartment in the intertubular tissue fluid has a relatively free access. Hemo-testicular barrier prevents many substances selectively into adluminal compartment where meiosis takes place and vital processes of a controled microenvironment.Spermatocytes intercellular junctions early pass through the barrier without interrupting the physiological hemo-testicular. An effective barrier could be observed in tubules that contained spermatydes early primary spermatocytes and the phase of leptoten were still inside the barrier deschis.This way, training was correlated with haployde germ cells. Complete compartimentation leaving only seminiferous tubules in inside the open, was performed in tubules that contained elongated spermatydes maturation.In phase during the final stages of sperm maturation in the cytoplasm spermatydes organelles are removed and the residual body of cytoplasm attached to the caudal portion of cytoplasmic debris spermatyd.These are called the residual and are phagocytosed by Sertoli cells and degraded in lysosomes cell.

3.CONCLUSIONS

1. During puberty sustentaculare cell differentiation is accompanied by a morphological transformation and loss of mitotic capacity (in adults, Sertoli cells no longer divide).
2. Spermatogenetic stem cell renewal and multiplication is performed in the basal compartment, where the intertubular tissue fluid has a relatively free access.
3. The hemo-testicular barrier selectively prevents many substances from entering the adluminal compartment, where the vital processes of meiosis and spermiogenesis take place in a controlled microenvironment .Early spermatocite pass through these intercellular junctions without interrupting the physiological hemo-testicular barrier.
4. It has been observed in the seminiferous tubules , the frequent phenomenon of apoptosis in the spermatocytes of grade I and grade II ,and their position in the lumen of the seminiferous tubules.
5. During the spermatogenesis process, it has been observed the formation of second degree spermatocytes, as a result of the second mitotic division, and their arrangements to the lumen of the seminiferous tubules on two or three rows. The shape is sometimes polygonal with rounded edges and the nucleus is central or eccentric nucleus , vesicular, with one or more nucleoli.
6. Androgen producing mature Leydig cells, appear in pig at 5-6 months old. They appear in full secretion process , with crystalloid inclusions. Some Leydig cells have prominent nucleoli, and intracytoplasmic lipofuscin granules can be seen.
7. Through the immunohistochemical examination it has been established a profile of imunomarcarii with monoclonal antibodies of the seminisferous tubule cells.

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STUDIES ON THE INFLUENCE OF HORMONES CELL ULTURES PTAINED FROM THE TESTICULAR PARENCHYMA FROM PIGS

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***Key words**: gonocytes, Germ cells, Sertoli cells, cell cultures*

SUMMARY

The changes cytology seminiferous epithelium cellular elementsheld in a regular in shape and synchronize reaching associationshistologically different features picture tube timing seminifer.The syncronising factors ensure a new mitosis of the spermatogonites. Coordination factors have a significant influence on the cell population which causes coexistence of testicle cell populations at different stages of development.Testicular synchronising cell association and coordination occurs through Sertoli cellcytoplasmic intercellular bridges. This mode of conduct of the division intratesticular is a characteristic of cells that can be found in cytoplasmic bridges association closely through synchronous and have a successful outcome cells to separate addresses where such a development takes place.

1. MATERIAL AND METHODS

To perform this study there have been collected testicles from baby boars at birth and adulthood, the albuginea was removed, and the testicular parenchyma has been cut and left to incubate for 30 minutes at 32 C, without PBS.

The testicular fragments were also mechanically disrupted at 15 and 35 minutes of repeated pipetting . they were diluted with 30 ml PBS and the cells were collected by centrifugation for 20 minutes at 100 x g. the seminiferous tubules cells and interstitial cells were collected in supernatant (32). The supernatant was centrifuged for 20 minutes , and the resulting extract was concentrated pellet in the culture medium

The cells were counted in a hemocytometer, and less than 10% were associated with clusters of more than 5 cells. Cell viability was tested with blue Trypan (0.04%) exclusion, and more than 90% of the cells were viable. The cells were placed in culture vessels (diameter, 6.4 mm) at 0.7 × 10 5 -10 5 cells/100 µ in a humidified atmosphere of 5% CO 2 in air to allow cells to attach .

Cultures were monitored under the microscope to watch cell morphology until the culture can be seen in the monolayer. Cultured cells were pretreated with administered

testosterone of various dilutions and an herbal -based hormone substitute. We distribute equal volumes of cells in culture flasks.

Primary cultures made from normal adult cells can be under cultivated several times (4-6). After that, the cells die (apoptosis). It will be considered monitoring the cytopathic effects (cell detachment from the wall surface, and detecting changes. After 48 hours cell cultures obtained are colored and then examined under the microscope.

2.RESULTS AND DISCUSSIONS

The cells were cultivated in the first 24 hours, without adding any serum. Meanwhile, Sertoli cells were attached to the culture plate and began to flatten. Germ cells remained floating freely in the environment. The cells were then examined under a microscope after 24 hours and in the first day it was noticed that most germ cells were removed by washing with PBS.

In the fresh culture environment were added in increasing concentrations of testosterone. In some experiments, the testosterone was added immediately after the isolation procedure performed on the first day. After 24 hours the cultures were frozen at -20 ° C . In the first day all the Sertoli cells were examined in the absence of the hormone for the control group. The plate was washed with PBS to remove floating germ cells. The Sertoli cells attached to the plate and the germs have been partially removed. Remaining germ cells were removed with trypsin-EDTA solution (01% (w / v) trypsin and it was then supplemented with culture environment. Only the Sertoli cells remained attached to the culture plate. These cells have irregular shape and it has been observed the presence of nuclear vesicles.

The plasminogen test was later utilized by using fibrinogen labeled fibrinogen that was diluted with unlabeled fibrinogen and were then left to work on culture wells. Over a part of the culture was then added 10% of fetal bovine serum plasminogen for 2 hours at 37 ° C. The environment was removed and replaced with 100 Sertoli cells in culture. Other cultures were treated with fetal sheep serum as a source of plasminogen .

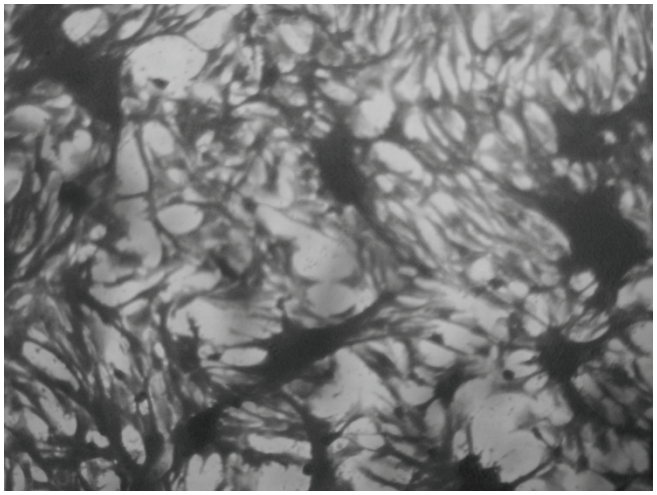

Fig.1.Primary cell culture. Control testis

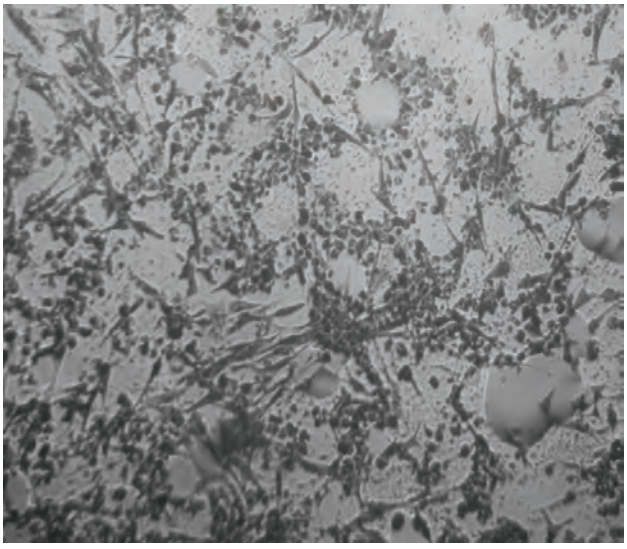

Fig.2.Primary cell culture. Swine testis at birth treated with testosteron

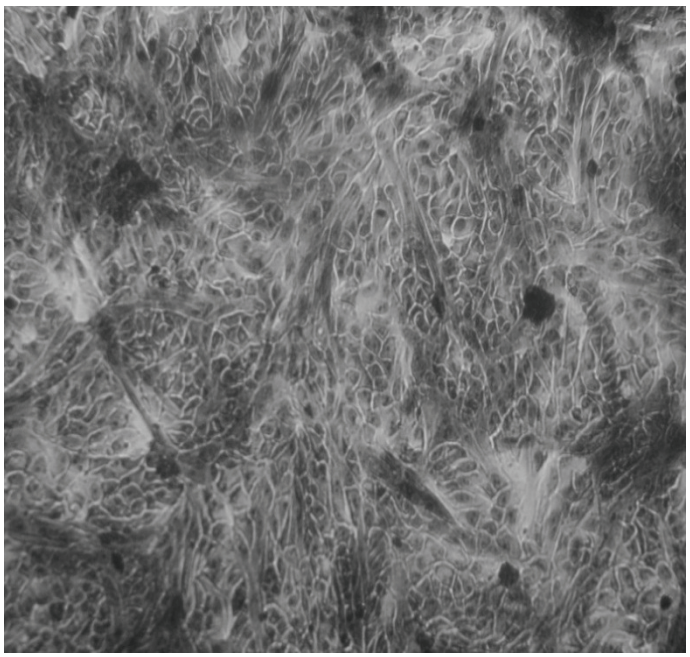

Fig.3.Primary cell culture. Control testis .

Under microscopic check it has been observed that under the influence of fetal sheep serum the cultures were more sensitive, having a more cytotoxic effect than when we used fetal bovine serum. Cell cultures were then incubated at 37 ° C for 24 hours. In the first day of culture, after 3 washes with PBS can be seen many Sertoli cells and their cytoplasmic extensions , and fewer germ cells. In the environments where the plasminogen was added it was seen that the number of Sertoli cells decreased by eliminating fibrinogen from the culture environment .

In the laboratory was then prepared a culture medium from Sertoli cells coming from boars from birth to adulthood. The cells were then cultured and have received increasing doses of testosterone in different dilutions. This effect was tested in cultures of Sertoli cells in boars at birth and boars at adulthood. In the plates from boars at birth, Sertoli cells become less sensitive to testosteron. Therefore, it seems like the use of trypsin for isolating the testicular cells of roars cannot destroy the Sertoli cells testosterone receptors. Each set forms an area containing a group of cells that adhere closely.

The fibroblasts are freely spread, forming homogeneous layers over the entire substrate. Rarely they get very close to each other. They sometimes intersect, forming multiple layers, never in association with germ cells. They are cylindrical, triangular, star shaped to polyhedral . Endothelial cells can form small colonies consisting of polygonal areas containing groups of associated cells. The shape can vary from round to polygonal with flat periphery and prominent centers.

Sertoli cells are scattered and juxtaposed in a single layer, the cells edges are not always definitive. Residual germ cells may remain attached to some free surfaces during the initial culture.

There have been performed cell cultures at boars in adulthood and it has not been conducted any specific treatment to remove germ cells .These cells were treated with an enriched special enviornment to enable particularly an increase in Sertoli cells density in cultures.

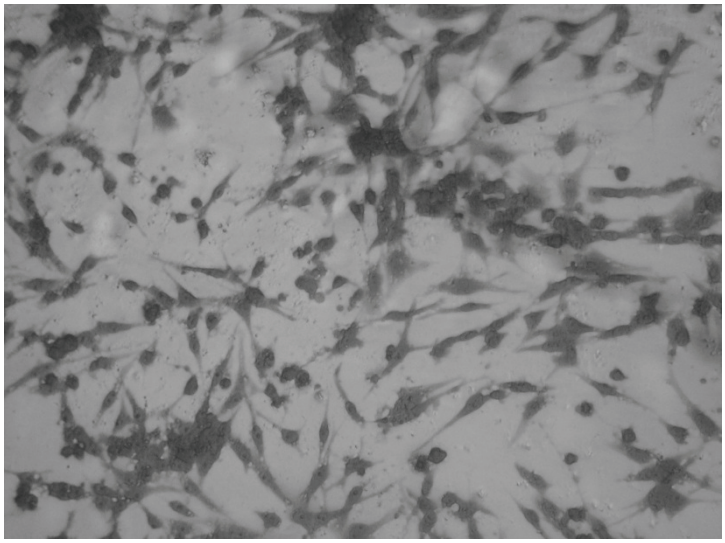

Fig.4.Primary cell culture. Swine testis treated with testosterone.

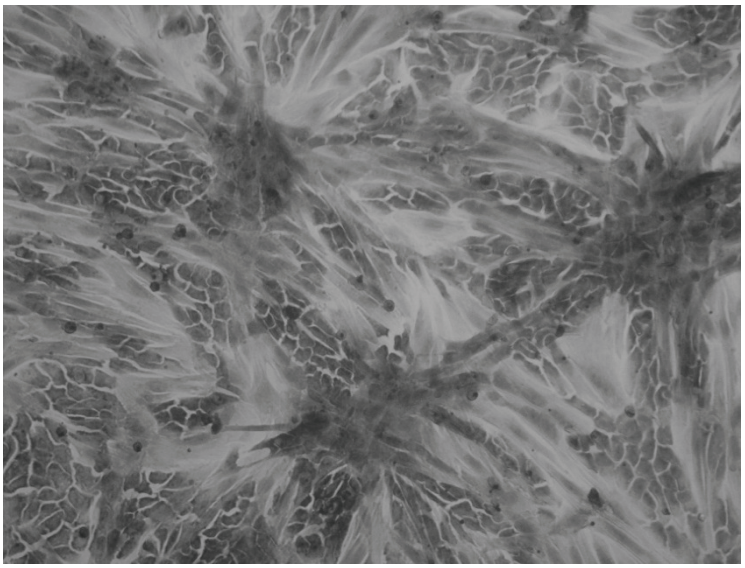

Fig.5.Primary cell culture, Swine testis treated with Manyphit.

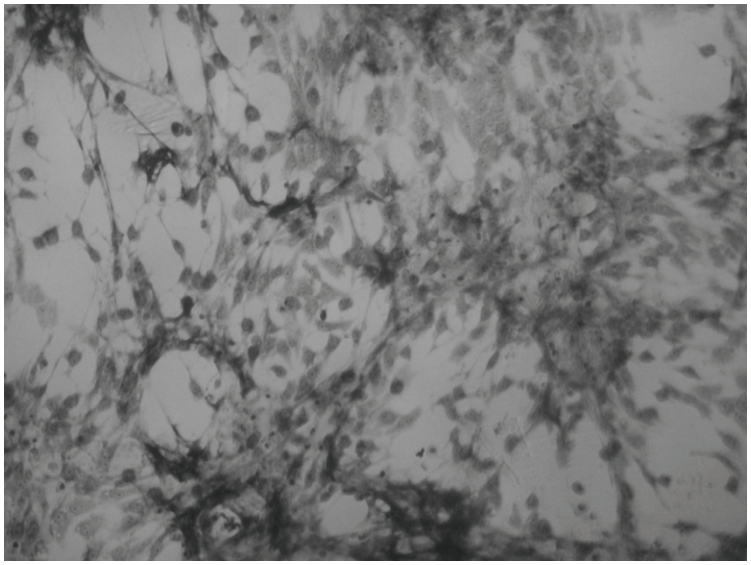

Fig.6.Primary cell culture. Adult swine testis treated with testosterone.

However, even in these conditions, Sertoli cells density declined sharply in this age group. It seems that these changes in the sensitivity of Sertoli cells were directly linked to changes in the physiological status of the boars' testis age and indirectly through changes in Sertoli cells density.

Sertoli cells could not be cultivated when isolated from the testes of 6 months old boars in early meiosis which is accompanied by their morphological differentiation their obvious metaphases. This physiological state of the Sertoli cells make them less resistant to mechanical disruptions during the isolation procedure. Sertoli cells from roars showed a higher sensitivity for testosterone from the testes coming from adult boars. At boars testosterone secretion has stimulated cell secretion at birth but during maturity these cells could not be grown under the influence of testosterone.

Furthermore, in the testicular cells treated with Manifyt (hormonal substitute made of plant extract) it was seen in that this product had a cytotoxic effect on these cells and the cells could not be further grown. The assumption that a similar testosterone concentration can be used to target androgen receptors present in the cells was based on previous results that have demonstrated the presence of only one type of androgen receptors in testicular plates obtained from the considered boars.

3. CONCLUSIONS

1.We can conclude that in the first day of culture aggregates of polygonal cells were observed which can be dispersed or isolated cells.

2.The fibroblasts were observed in the monolayer. These are free to form homogeneous layers spread over the entire substrate. They rarely get very close to each other. They sometimes intersect, forming multiple layers, never in association with germ cells. They are cylindrical, triangular, star shaped to polyhedral. They were also observed in monolayered cells peritubulare mioide

3.Sertoli cells were also observed in aggregates. Sertoli cells are scattered and juxtaposed in a single layer, their edges are not always definitive . These cells had an increased number of intracytoplasmic inclusions. The intracytoplasmic inclusions were scattered here and there only in the cytoplasm, and in larger quantities in the perinuclear cytoplasm. Inclusions have different sizes, round or oval .

4.The testosterone has stimulated the cell secretion at birth but during maturity these cells could not be grown under the influence of testosterone.

5. In cell cultures from adult boars it is observed that the Sertoli cell density declined sharply in this age group. It seems that these changes in sensitivity of Sertoli cells were directly linked to changes in the physiological status of the boars testis age and indirectly through changes in Sertoli cells density.The testes were also disrupted mechanically at 15 and 25 min by repeated pipetting.

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THE CYTOLOGY OF THE PROXIMAL CONVOLUTED TUBULE OF THE NEPHRON IN SPECIMENS OF GENUS PHASIANUS

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Key words: proximal convoluted tubule, epithelial cells, mitochondria, pheasant.

SUMMARY

By analyzing the transmission electron microscopy images, we could extend the study to the aspect of the cytoplasm of the epithelial cells. In this context, the intensity of the cytoplasm color could be correlated with the distribution of mitochondria, making possible for us to mention light cells, normochromic cells and dark cells. Therefore, the cells having evenly distributed mitochondria will appear in medium shades in light microscopy, when using usual staining methods but also some special ones. They are predominant in all the analyzed histological sections. Between them, grouped or dispersed, dense dark cells appear. Sometimes, the intensely colored cells will line large areas of the proximal convoluted tubule, occupying all the tubular territory in some slides.

In what concerns the proximal convoluted tubule, the dark aspect of the cytoplasm may be due to an excessive agglomeration of mitochondria. They appear in a great variety of shapes and sizes and are the predominant organelles observed even in the perinuclear space.

The clear cells have a shriveled nucleus. Even if the nucleoli maintain their structure, there can be observed some changes in the nuclear membrane. The nuclear membrane becomes discreetly wavy on its whole distribution. The nucleolemma layers will become distanced and a cysternal space will appear. The mitochondria will become swollen and will lose their ordered, lamelary aspect of the cristae, observing between them numerous vacuoles of different sizes.

The idea for the cytological study began from a series of histostructural studies on proximal convoluted tubules that indicated their different tinctorial affinity from that of the epithelial cells. Consulting the speciality literature, we learned that the histostructural and ultrastructural data concerning the renal parenchima referred mainly to mammals (Ohno, S. *et al*., 1992). Researches that would tackle the avian segment are moderate and mostly target species *Gallus domesticus* (Casotti, G. *et al.*, 2000). Even in this context, there are voids regarding the nephron ultrastructure correlated with the age of the subjects, with their hydric and food regimes,

and in other granivorous species, there is an important lack of data about their microscopic morphology (Nabipour, A *et al.*, 2009).

The study of the proximal convoluted tubule epithelium in the Caucasus Pheasant (*Phasianus colchicus colchicus*), in the Mongolian Ringneck Pheasant (*Phasianus colchicus mongolicus*) and in the Golden Pheasant (*Chrysolophus pictus*), creates the possibility of establishing some characteristics of subspecies.

The general transmission electron microscopy image shows the particular aspect of the proximal convoluted tubule epithelium. The lumen appears extremely reduced, difficult to notice because of the numerous microvilli of the epithelial cells. The epithelial cells are approximately pyramidal and are adherent to the basal membrane. The cytoplasm of these cells contains numerous mitochondria of different shapes and sizes, which are distributed in all the cellular territory except the perinuclear area (Beuchat, C.A. *et al.*, 1999; Wideman, R.F. *et al.*, 1981).

1. MATERIAL AND METHODS

For this study three 10-month-old male birds belonging to *Galliformes* order, *Phasianidae* family, *Phasianus* genus have been used, one from *Phasianus colchicus colchicus*, one from *Phasianus colchicus mongolicus* and one from *Chrysolophus pictus* species.

The biological material destined for the ultrastructural study, using TEM (*Transmission Electron Microscopy)* technique, needed laborious preparation requiring more stages, represented by: fixation, washing, dehydration, epoxy inclusion, sectioning, contrasting, microscope examination and photography. The specificity of the method is given by the reactives used and the complexity of each stage. It is worth mentioning that the first sectioning stage of the blocks is made with an ultratome, creating with 1 μm thick sections. These sections would be later on stained with Toluidine blue.

The semifine sections are examined with the light microscope and the areas of interest are chosen for photography. Then, then semifine sections are cut using the ultramicrotome, the thickness of the sections ranging between 60 and 80 nm. The technique of seriated sections allows the three-dimensional reconstruction of the structure of the tissues.

In order to visualize and capture the images of interest, the TEM PHILIPS EM 208S equipped with image acquiring and processing system, together with *Veleta* video camera and *iTEM Olympus Soft Imaging System* were used for acquiring and processing images.

2. RESULTS AND DISCUSSION

By analyzing the transmission electron microscopy images, we could extent the discussions concerning the aspect of the epithelial cell cytoplasm. In this context, we can observe that the intensity of the cytoplasmatic color may be correlated with the mitochondria distribution pattern. Thus, the cells having evenly distributed mitochondria will have a medium shade in light microscopy, when using the usual and some special staining methods. These cells are predominant in all the analyzed histological slides. Among them, grouped or dispersed, dense, dark cytoplasm cells appear. Sometimes the intensely chromophyle cells may line large areas of the proximal convoluted tubule, occupying all the tubular territory in some sections. The nucleus of these cells is spherical, vesiculos and intensly euchromatic.

The dark aspect of the cytoplasm is due, in the case of the proximal convoluted tubule cells to an excessive agglomeration of mitochondria. They are the predominant organelles and penetrate even in the perinuclear area. The mitochondria are characterized by a great shape and size diversity. The large ones, considerable elongated, have the tendency to be curve, becoming crescent-shaped. The medium ones are typical, oval, and the ones smaller in size are circular. Concerning the cristae arrangement, it may be observed that, not matter the shape and sizes of the mitochondria, the cristae are lamellated and intensely pleated.

Particular elements may be observed in the basal pole of the dark cells, too. The pleats of the basal plasmalemma become slightly distanced, thus contouring a basal intermembranary space.

The mitochondria reach right to this area, but do not get thru it. The pleats of the basal plasmalemma have a particular aspect. They do not appear to be ordered, with a visible parallelism, but narrow themselves towards the basal membrane. The narrowed area will expand in the vicinity of the basal membrane, thus enlarging the contact surface between the plasmalemma of the celullular basal pole and the basal membrane. In this

context, the pleats of the basal plasmalemma will have the aspect of some short and branched elongations, intimately attached to the basal membrane surface. In the spaces formed between the pleats, small exocytosis vesicles are identifiable, but also in the interstitial space or even while crossing the basal membrane. Also, numerous vesicles are seen in the cytoplasm of the pleats, from their forming place to near the plasmalemma, along with poliribosomes. Towards the base of the pleats, numerous fragments of the rough endoplasmic reticulum and microtubules appear (Figure 2.1).

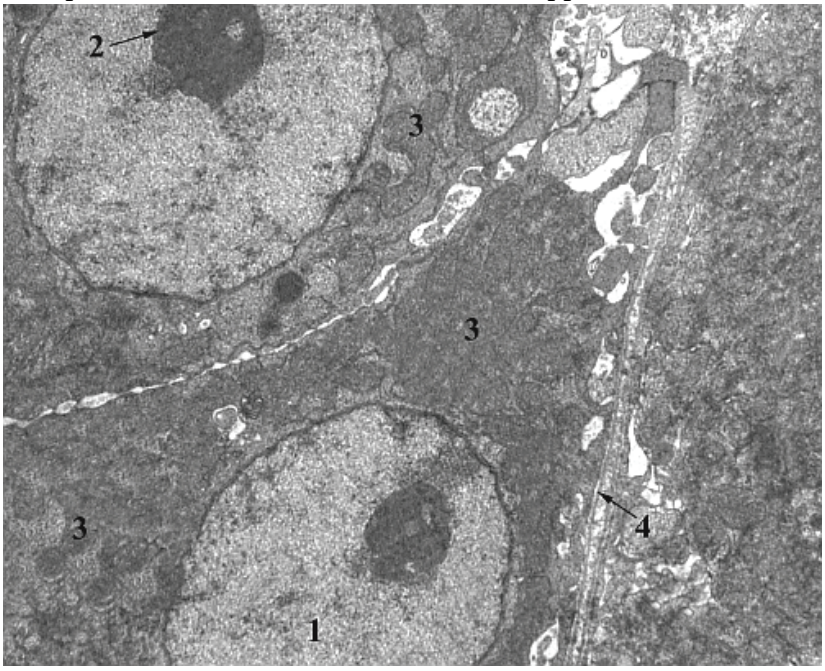

Figure 2.1. Ultrastructure of the dark cells in 10-month-old pheasant / TEM, 7 100x (original)
1. Nucleus; 2. Nucleolus; 3.Mitochondria; 4. Basal membrane.

The medium chromophyl cytoplasm cells have their mitochondria evenly distributed in all their surface, along with the other organelles. There is an exception in the perinuclear area, that is occupied by the components of the Golgi complex and that of the rough endoplasmic reticulum. The vesicles elaborated by the Golgy complex may be observed everywhere between the mitochondria, along side with heterolisosomes.

The nucleus will maintain the particularities observed in the dark cells too, thus will be large, spherical, euchromatic, with obvious nucleoli.

The nucleolus will have granular and fibrilar components, organized around the filamentous core, which will have a fragmented, clear-electron aspect.

The cellular apical pole appears to be occupied by very closed microvilli, uneven in height and diameter, which will form a tall, thick brush border. In the cytoplasm, near the base of the microvilli, numerous grouping of agglomerated microfilament may be observed.

In all the cytoplasm of the cellular apical pole, fragments of the rough endoplasmic reticulum, poliribosomes, herolisosomes, mitochondria, microvesicles and microfilament may be seen. The intercellular space is tight and the cells are stabilized with jonctional complexes.

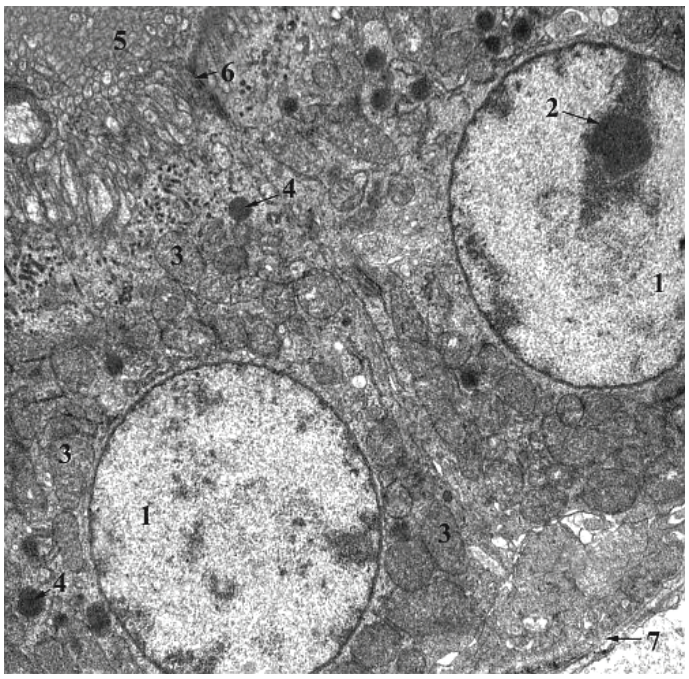

Figure 2.2. Ultrastructure of intermediary cells in proximal convoluted tubule in 10-month-old pheasant / TEM, 7 100x (original)

1. Nucleus; 2. Nucleolus; 3.Mitochondria; 4. Heterolisosome; 5. Microvilli; 6. Jonctional complex; 7. Basal membrane.

Numerous lamellated mitochondria with different shapes and sizes, rare heterolisosomes and rough endoplasmic reticulum fragments may be seen in the cellular basal pole. The cytoplasmatic region closer to the basal plasmalemma has poliribosomes and microvesicles, but it does not contain

mitochondria. Intensely interdigitated pleats, separated by discreet spaces appear. A extracellular space can also be seen between the basal plasmalemma and the basal membrane. In some areas, the basal membrane forms fine pleats, forming a larger contact surface with the basal plasmalemma. In the vicinity of the basal membrane of the proximal convoluted tubule epithelium, the basal membrane of a fenestrated capillary and the cytoplasmatic pellicle of an endothelial cell can be seen (Figure 2.2).

The clear cytoplasm cells present a slightly shriveled nucleus. Even if the nucleoli maintain their aspect, a series of alterations may be seen in the nuclear membrane. The nuclear membrane becomes discreetly wavy on its whole surface. The layers of the nucleolemma will distance themselves, thus appearing a dilatated cysternal space.

The mitochondria will become large and will lose their neat, lamelated aspect of the cristae. Numerous vaculoles with different sizes, microvesicles and poliribosomes are seen among mitochondria.

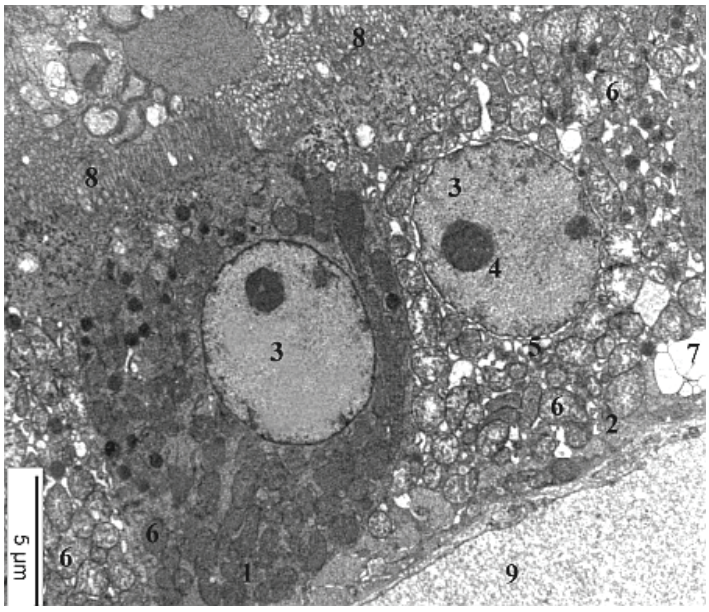

Figure 2.3. TEM image of clear and dark cytoplasm cells in proximal convoluted tubule epithelium in 10-month-old pheasant / TEM, 2 200x (original)

1. Dense cytoplasm cell; 2. Clear cytoplasm cell; 3.Nucleus; 4. Nucleolus; 5. Dilatated perinuclear cystern; 6. Mitochondria; 7. Vacuole; 8. Microvilli; 9. Capillary.

In the celullar apical pole microfilaments abound as consistent groups (Figure 2.3).

3. CONCLUSIONS

3.1. Depending on the density, the arrangement and the structure of the mitochondria, in the epithelium of the proximal convoluted tubules can be distinguished: dense cytoplasm cells, clear cytoplasm cells and intermediary cytoplasm cells.
3.2. All the cells of the proximal convoluted tubule epithelium have mitochondria of different shapes and sizes.
3.3. The dense cytoplasm cells have short distanced, branched pleats of the basal plasmalemma, and have no mitochondria in the areas where they form numerous circular or ovoidal spaces.
3.4. All the cells of the proximal convoluted tubules epithelium have spherical, euchromatic nuclei with evident globular nucleoli.
3.5. The transmission electron microscope image reveals that all the three categories of cells may be considered as a single cellular type of cells, in different functional stages.

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ULTRASTRUCTURAL ASPECTS CONCERNING THE CAPILLARIES OF THE RENAL PARENCHYMA IN *PHASIANUS COLCHICUS*

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SUMMARY

The renal parenchyma is intensely vascularized in *Phasianidae*. While the capillaries form a structured network packed in the renal corpuscles, they are following the trajectory of the uriniferous tubules in the rest of the parenchima.

There are many fenestrations in the vascular endothelium in the renal corpuscle. In the vicinity of the pores, numerous pleats are observable, pleats that make the dorsal and the ventral plasmalemmas of the endothelial cells noticeable and that frame a fine cytoplasm pellicle.

Lamina densa of the basal membrane is compact. Lamina rara externa appears visible near the podocytic processes, while lamina rara interna is difficult to observe.

Along erythrocytes, lymphocytes B and T are seen in the lumen.

The capillaries that accompany the uriniferous tubules have an almost continuous vascular endothelium. The fenestrations appear rare and narrow, unnoticeable. Lamina densa is thin and distanced from the interstitial space by the well-structured lamina densa, that belongs to some segments of the uriniferous tubules. In the interstitial space numerous vesicles are noticed.

Near the vascular endothelium, lymphocytes B and T were observed, exactly in the moment of adhering to the capillary wall, in the process of crossing the basal membrane, by which sometimes macrophages are visible.

Consulting the speciality literature, we learned that the histostructural and ultrastructural data concerning the renal parenchima referred mainly to mammals (Ohno, S. *et al*., 1992). Researches that would tackle the avian segment are moderate and mostly target species *Gallus domesticus* (Beuchat, C.A. *et al.*, 1999; Casotti, G. *et al.*, 2000). Even in this context, there are voids regarding the nephron ultrastructure correlated with the age of the subjects, with their hydric and food regimes, and in other granivorous species, there is an important lack of data about their microscopic morphology (Wideman, R.F. *et al.*, 1981).

The major aim of this study was to examine the ultrastructural

images of the renal corpuscles capillaries and to compare them with urinary tubes capillaries.

All the ultrastructural information is of practical interest in the renal pathology, especially in the behavior of this segment in different intoxications (Latshaw, J.D. *et al.*, 2004).

1. MATERIAL AND METHODS

The biological material destined to the ultrastructural study that used TEM (*Transmission Electron Microscopy)* technique, was taken from three 10-month-old males belonging to the *Phasianus colchicus* species, and required laborious preparation consisting of several stages: fixation, washing, dehydration, epoxy inclusion, sectioning, contrasting, microscope examination and photography. The specificity of the method is given by the reactives used and the complexity of each stage. It is worth mentioning that the first sectioning stage of the blocks is made with an ultratome, creating with 1 μm thick sections. These sections would be later on stained with Toluidine blue.

The semifine sections are examined with the light microscope and the areas of interest are chosen for photography. Then, then semifine sections are cut using the ultramicrotome, the thickness of the sections ranging between 60 and 80 nm. The technique of seriated sections allows the three-dimensional reconstruction of the structure of the tissues.

In order to visualize and capture the images of interest, the TEM PHILIPS EM 208S equipped with image acquiring and processing system, together with *Veleta* video camera and *iTEM Olympus Soft Imaging System* were used for acquiring and processing images.

2. RESULTS AND DISCUSSION

Glomerular and intertubular capillaries can be taken into discussion concerning the renal parenchyma.

The glomerular capillaries are covered on their whole surface with podocyte processes. The ones found toward the surface of the renal glomerulus present a larger lumen and are closer to the simple squamous epithelium that lines the Bowman's capsule parietal layer. The capillaries found deeper in the glomerulus have a perceivable labyrinthical aspect, thus the lumen becoming narrow and anfractuous. In the mesangial territory, the

capillaries are contoured as a fragmented and dense web, well represented between the connective elements. In general, many clustered erythrocytes can be noticed in the lumen.

The wall of the glomerular capillaries in contact with the podocite processes takes part in the filtration barrier. On one side, the podocytes and their long processes and pedicles, together with the filtration slit are observed, and on the other side the vascular endothelium is noticed.

The cross-section in an endothelial cell shows a fine cytoplasmic pellicle framed by dorsal and ventral plasmalemmas.

The dorsal plasmalemma is not smooth and it forms pleats. A part of the pleats become real extensions implicated in a real endocytotic process. The increase of compound transport from the capillary lumen in the podocyte territory, and from here to the filtration slit is made with the help of the numerous pores that fenestrate the vascular endothelium. The pores are large, easy to observe and have a variable diameter.

Figure 2.1. Glomerular capillary ultrastructure in the filtration barrier in 10-month-old pheasant/TEM, 11 100x (original)

1. Capillary lumen; 2. Podocyte nucleus; 3. Long processes; 4. Secondary podocytes processes; 5. Filtration slit; 6. Endothelial cell; 7. Fenestrations; 8. *Lamina densa*; 9. *Lamina rara externa*; 10. *Lamina rara interna*; 11. Lymfocyte.

The basal membrane of the capillary is formed by a thick, compact *lamina densa*, separated from the ventral endothelial plasmalemma by a discreet *lamina rara interna*. *Lamina rara externa* on which the podocyte pedicles are fitted, is visible. The pedicles are short and alternatively distributed with the endothelial fenestrations (Figure 2.1).

In cross-section, the central area of the endothelial cells that is occupied by the nucleus and the perinuclear components, presents different aspects correlated with the metabolic activity. Frequently, the nucleus of the endothelial cell appears oval, flattened, oblate, normochromatic. The heterochromatin predominates in the periphery, circling an euchromatic core. In the perinuclear zone, fragments of the rough endoplasmic reticulum and numerous poliribosomes are noticed.

Some mithocondria and actin microfilaments, together with elements of the rough and smooth endoplasmic reticulum appear in the cytoplasm. The poliribosomes are spread on the territory.

The whole cellular surface is crossed by numerous vesicles, of which constitutive endocytosis can be observed in the dorsal plasmalemma. They cross the cytoplasm of the endothelial cell by transcytosis and reach the ventral plasmalemma, where they are exocytosis in the *lamina rara interna* space. From here, they perambulate the *lamina densa* and *lamina rara externa*, reach the urinary space, if the basal membrane of the capillaries is covered by the podocyte processes. The vesicles may cross the *lamina densa, lamina rara externa* and *lamina fibro-reticularis*, coming in the interstitial space in the case of intertubulary capillaries.

The nucleus of the endothelial cell may sometimes be bulged, prominent in the vascular lumen. This normochromatic nucleus has a visible nucleolus. The plasmalemma forms evident pleats, which interest mostly the dorsal surface, hurrying the forming of pinocytosis vesicles.

Sometimes, the endothelial cells are especially active and incorporate significant quantities of fluid, enhancing its pinocytosis capacity. The cells observed in such a moment present dense processes derived from the dorsal plasmalemma both in the perinuclear area and near it, too. A well represented rough endoplasmic reticulum, poliribosomes, several mitochondria, alongside vesicles in transcytosis process, can be seen in the cytoplasm.

In the lumen of the glomerular capillaries, but also near the capillary

wall, different lymphoid cells can be noticed. Thus, in the lumen, alongside erythrocytes, lymphocytes appear. Some of them are perfectly spherical, have a heterochromatic nucleus, very little cytoplasm and present rare elongated pseudopods, while others are slightly oval. The oval ones have a normochromatic nucleus and have some mitochondria, lisosomes, poliribosomes and a represented rough endoplasmic reticulum in their cytoplasm.

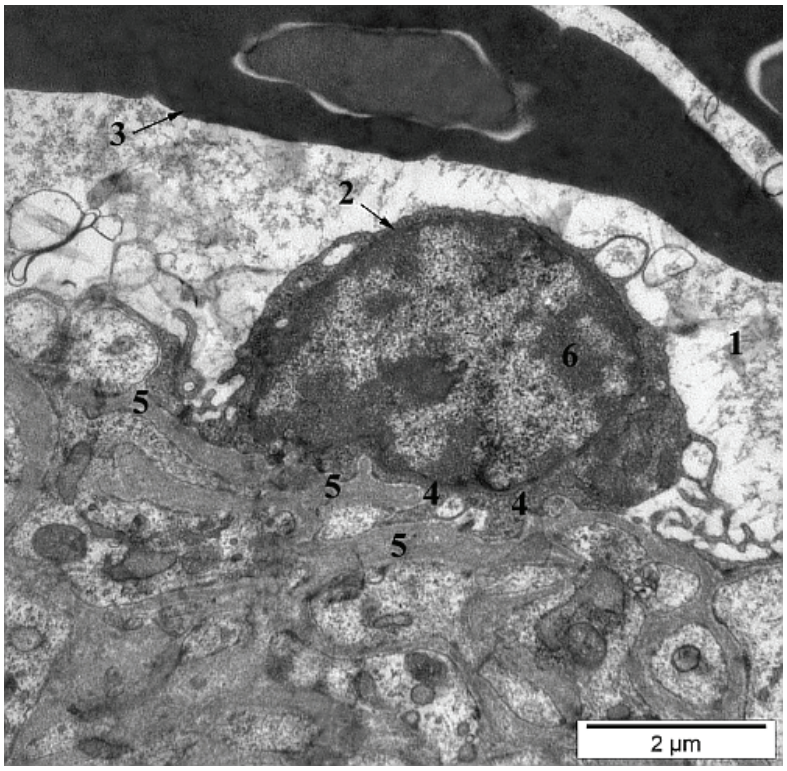

Figure 2.2. Lymphocytary diapedesis in the glomerulary capillary in 10-month-old pheasant /TEM, 11 100x (original)
1. Capillary lumen; 2. Lymphocyte; 3. Erythrocyte; 4. Pseudopods; 5. Basal membrane; 6. Heterochromatin.

The lymphocytes are observed also in the moment of diapedesis. They enlarge the fenestrations of the endothelial cells, cross the plasmalemma pores, reach the basal membrane of the capillary and then, with the enzyme equipment, fragment it in some areas, firstly sending their pseudopods in the interstitial space. These cells present a normochromatic

oval nucleus, with a smooth dorsal area and a pleated ventral area, more discreet than the cytoplasmic and plasmalemmal territories that touch the basal membrane of the capillary. In some cells that start diapedesis process, the heterochromatin has the tendency of being organized as radiary strips that alternate with the euchromatin. By adding the element of predominance of rough endoplasmic reticulum and that of the poliribosomes in the cytoplasm, but also of the plasmalemmal elongations, we could sum up that we are dealing with an active cell, in full process of transformation while crossing the capillary lumen in the mesangial adjacent connective tissue. It may be the case of the transformation of a lymphocyte B into a plasmocyte (Figure 2.2).

Also near the capillary basal membrane, adhering to the *lamina rara externa*, a machrophage is observed.

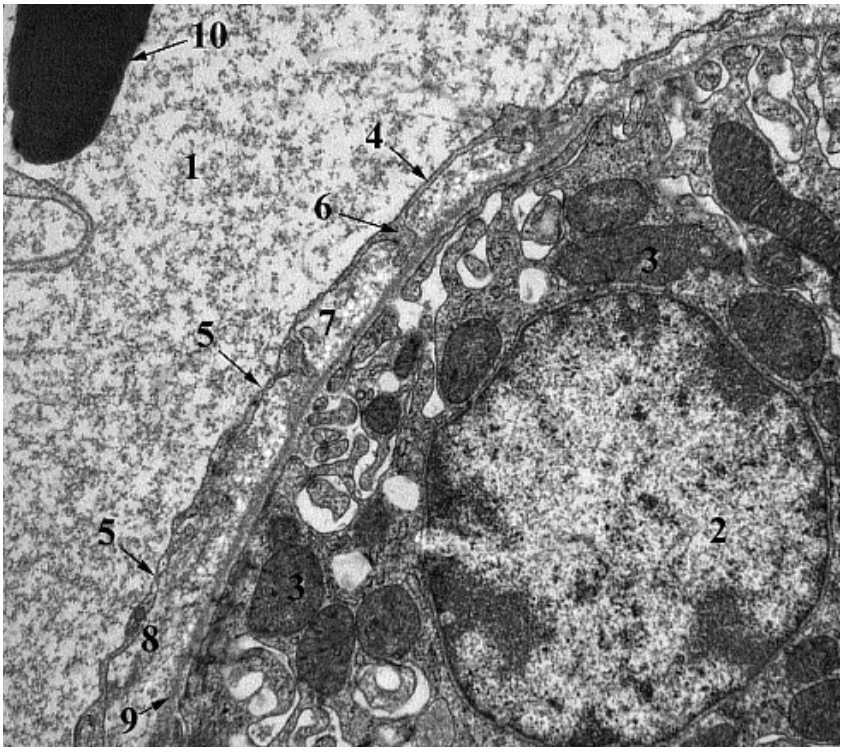

Figure 2.3. The ultrastructural image of the intertubular capillary wall in 10-month-old pheasant /TEM, 11 100x (original)

1. Capillary; 2. Nucleus of a cell from the uriniferous tubule; 3.Mitochondria; 4. Endothelial cell plasmalemma; 5. Endothelial narrowing; 6. Endothelial interstitial elongations; 7. Vesicles; 8. Capillary basal membrane; 9. Tubular basal membrane; 10. Erythocyte.

The capillaries that accompany the uriniferous tubules present an almost continuous vascular endothelium. The rare and narrow fenestrations, almost insesizable are noticed. Most of the times, only a slight narrowing given by the exaggerated closeness, in more or less closed points, of the dorsal and ventral plasmalemmas of the endothelial cells. The *lamina densa* appears thin and distanced from the structured *lamina densa* by an interstitial space that belong to segments of the uriniferous tubules. Numerous vesicles are seen in the interstitial space. Here and there, *lamina densa* becomes discontinuous and the endothelial cells form short elongations that penetrate in the interstitial space. While forming these elongations, only the ventral plasmalemma is implied, and the dorsal one remains smooth (Figure 2.3).

The intertubular capillary network is vast. These capillaries practically mold themselves onto the basal membrane of different segments of the uriniferous tubules that accompany them. In the intertubulary territories, they touch with the interstitial connective tissue, noticing an active exchange between these and the capillaries, by identifying some vesicles. Fibroblasts can be identified near the vessel.

3. CONCLUSIONS

3.1. The ultrastructure of the endothelial cells vary in some ranges, according to the position of the capillary in the renal parenchyma and the intensity of activity caught in the moment of preparation of the samples.

3.2. The fenestrated aspect of the intertubular endothelial cells is not constant, most of the time observing only a slight narrowing, by the exaggerated closeness, in less or more closed points, of the dorsal and ventral plasmalemma.

3.3. The basal membrane of the vascular endothelium has a different structure, depending of the location of the capillary.

3.4. The identification of the lymphoid cells in the lumen, in the proximity and while crossing the capillary wall, certifies its implication in immune-reactive processes.

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