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THE EFFECTS OF FOODER SUPPLEMENTATION WITH ORGANIC SELENIUM ON HAEMATOLOGICAL AND BIOCHEMICAL MARKERS IN BROILER CHICKENS

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Abstract

Selenium is an essential trace mineral that prevents the formation of pro oxidative free radicals. The role the selenium plays in the protection of hemoglobin against peroxidation is very well documented and researched. In order to perform this study, a number of 20 3-weeks-old broiler chickens of the Cobb breed were analyzed. The individuals were distributed into two even lots. The batching of the individuals was realized within the bio base of the Faculty of Veterinary Medicine in Bucharest. The duration of the experiment was of 4 weeks. Both the experimental batch and the control batch were fed a 21/1 ratio feed diet for broiler chicken, finishing period. The experimental batch's diet was additionally supplemented with 0.5 ppm of selenium, with 0.25 ALKOSEL R397 g/kg mixed fodder.
At the end of the experimental period, blood samples were taken by venipuncture of the cubital vein for hematological and biochemical laboratory analysis. The results were tabled and bio statistically interpreted. The experimental lot has shown significant increases in the values of the hematocrit (7.68%) and of the blood levels of white blood cells (14.01%), aspartate aminotransferase (8.19%), calcium (10.59%) and selenium (42.56%). They also showed a significant decrease in the values of blood protein levels (8.33%). The biochemical parameters influenced by the organic selenium supplemented feed lead to the prevention of the oxidative stress and a higher efficiency of fodder conversion rate

Key words: *biochemistry, haematology, broiler, selenium.*

INTRODUCTION

Growth and immunity are negatively impacted by heat stress that induces physiological, hormonal and molecular alterations, as well as lipid peroxidation. (Donker et al., 1990). Baziz et al. (1996) observed that for each degree increase in temperature between 22°C and 32°C, broilers' feed intake and growth decrease with approximately 3.6 and 1.5%

Research has shown that supplementation of broilers' diets with antioxidants reduces the effects of heat stress on lipid peroxidation and metabolism (Cantor, 2003).

Selenium helps protect the hemoglobin against peroxidation by means of three enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (Curcă, 2008; Răduță 2016).

Low levels of selenium can be responsible for the peroxidation of cellular membranes that lead to an increased production of prostaglandins.

Membranes peroxidation leads to many molecules, including the DNA molecule, being structurally damaged, favouring the development of neoplastic diseases (Ghergariu 1980; Curcă, 2005)

A deficiency in selenium is directly linked to disorders such as anaemia and/or haemolysis most frequently observed in rats, dogs, primates, but also chickens. (Curcă, 2005).

Supplementation of the feed with selenium can prevent the apparition of such conditions as myopathy effusion, bleeding diathesis etc.

Selenium in poultry diets protects them from pancreatic fibrosis and exudative diathesis (Combs, 1986).

Based on these reports, it is established that selenium is an essential nutrient for poultry. Due to regional variation in selenium content of natural feeds, broilers' diets are commonly supplemented with selenium.

Dietary and supplemental selenium is needed to form part of the amino acid selenocysteine, the only amino acid to have a selenium component. Selenocysteine is incorporated into at least 25 selenoproteins that have catalytic and antioxidant functions in the body. The chemical structure of selenocysteine, which is the 21st amino acid, is almost identical with the

structure of the amino acid cysteine (Okunlola et. al., 2015). The basic difference is that selenium has taken the place of sulfur in the molecule Allan et. al., 2000, Zelenka, 2005).
Selenium administered in its organic form (selenomethionine) improves its bioavailability, increasing the retention of selenium by the organism due to the fact that these amino acids are not excreted in urine (Surai, 2006, Okinlola 2015).

MATERIALS AND METHODS

The experiment took place in the bio base of the Faculty of Veterinary Medicine of Bucharest and was conducted on two batches of broiler chickens of the Cobb 500 breed. Each lot contained 10 3-weeks-old individuals at the beginning of the experimental period (Figure 1).

Fig. 1. Broiler batch study group

Both the experimental group and the control group received the same feed regime: 21/1 ratio feed diet for broiler chicken, finisher stage. The fodder contained cereals, soybean meal, sunflower meal, corn gluten meal, calcium phosphate, calcium carbonate, salt, amino acids and vitamins and minerals premix.
The feed had the following nutritional values: crude protein – 17.20%, metabolizable energy – 3140 kcal/kg, methionine and cysteine – 0.65%, lysine 0 0.90%, calcium – 0.86%, phosphorus – 0.70%, choline – 0.004%, salt – 0.30%. It did not contain coccidiostats.
The experimental group had its feed supplemented with 0.25 g/kg M.F, 0.5 ppm organic selenium as ALKOSEL R397, obtained from Lallemand Animal Nutrition SA France. The control group received the normal feed.
After 30 days, at the end of the experimental period, blood was sampled by puncture of the cubital vein using EDTA anticoagulant 1-2 mg/ml of blood, for hematological analysis and for biochemical analysis were used clean dry tubes without anticoagulant (Figure 2).

Fig. 2. Blood sample collecting from the cubital vein for hematological and biochemical determinations

The RBC (red blood cells) count was performed using an automated Coulter Counter, CP-diff analyser ACT 5 Beckman. The WBC (white blood cells) count was performed within the laboratory of the department of Physiopathology of the Faculty of Veterinary Medicine of Bucharest, using the Burker hemocytometer and the Natt-Herrick dilution solution (Figure 3).

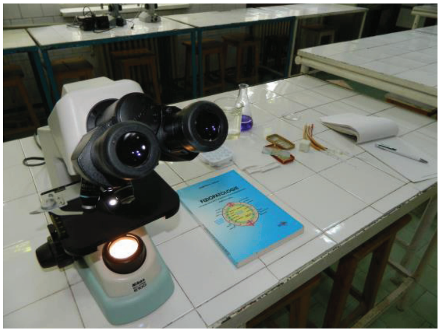

Fig. 3. The WBC count

The analysis of selenium, cholesterol, magnesium, calcium, ALT/GPT (alanine aminotransferase) and AST/GOT (aspartate aminotransferase) blood levels were performed by Bucharest's Institute of Animal Diagnostic and Health, using molecular absorption spectrometry. Glucose, proteins, ALKP/ALP (alkaline phosphatase), amylase and lipase blood levels were determined by the Laboratory of the Faculty of Veterinary Medicine of Bucharest, using specific kits for the aforementioned analysis. The kits were purchased

from S.C. Nova Group Investment S.R.L. The device used for these determinations was VeTTest, from Idexx Laboratory.
The results of these investigations were statistically calculated using ANOVA, a specialized statistics program. The data was processed by means of Microsoft Office 2010 software. The results were tabled, plotted and biostatistically interpreted (Table 1).

RESULTS AND DISCUSSIONS

The individuals in the experimental lot, whose diet was supplemented with organic selenium, showed statistically significant increases of the haematocrit and of the blood levels of white blood cells (Figure 4).
Other statistically relevant increases were registered by biochemical markers such as AST, calcium and selenium.
The blood levels of proteins have decreased significantly. Other changes of some parameters of the individuals in the experimental group have been observed, but with no statistical significance (Figure 4 and Figure 5).
Lipase, ALT and magnesium blood levels tend to increase while the MCHC and the blood levels of ALKP, cholesterol and glucose tend to decrease, but none statistically significant.
Supplementing the feed with selenium favours erythropoiesis and therefore that will lead to a better oxygenation of the tissues. (Mertz, 1987; Curcă, 2005 and 2007; Răduță, 2011).
ALKOSEL R397 is one of the most efficiently utilized concentrated sources of additional dietary organic Se, favouring a greater activity of glutathione peroxidase, an antioxidant enzyme that reduces peroxides and other free radicals that could compromise cellular membranes (Edens and Gowdy, 2005). Apsite (1993, 1994, 2004) discovered that selenium metabolites in the organism stimulate the activity of glutamine peroxides, leading to the elimination of lipid hydroxyl peroxides present in cellular structures, decreasing the risk of oxidative stress. Such results were later obtained by other researchers.
Results showed that the haemoglobin blood levels only increased by 5.44%, as a result of a larger population of young erythrocytes, thereby influencing the increase of the MCHC (mean corpuscular haemoglobin concentration). This is by 1.26% higher than the results of the same analysis performed on the individuals in the control batch and it confirms a better load of the erythrocyte with haemoglobin (Smith and Picciano, 1987).
The high intensity of erythropoiesis leads to a larger number of young erythrocytes being developed by the bone marrow and, as a consequence, to a higher haematocrit, that suggests there is an increase in the cellular mass, detrimental to the plasmatic mass (Surai, 2002, Payne, 2005).
An increasing trend was observed in the MCV (mean corpuscular volume) values as well, due to the large number of young erythrocytes that have a lower volume than that of mature red blood cells (Tayeb, 2012).
In this experiment, the results obtained from the experimental group individuals were by 2.58% larger than those obtained from the control group individuals (Aristide Popescu L. and N. Aristide Popescu, 1990; Răduţă et. al., 2015).
The decrease of the MCHC of 1.29% can be explained by the larger quantity of circulating haemoglobin and by the MCV increase. The blood levels of white blood cells show significant decrease.
From a biochemical point of view, ALT blood levels tend to increase with 2.99%, compared to the control group.
Lipase blood levels in individuals whose diet has been supplemented with organic selenium tend to increase to 181.16 U/L. This is 32.62% larger than the results obtained by the control group individuals.
Other researchers showed that the blood levels of lipase also tend to increase, demonstrating that a selenium deficiency can lead to a poor absorption and to a low hydrolysis of lipids in the digestive tract, which result in a significant decrease of vitamin E absorption, whose low levels can lead to necrotising dystrophy of the pancreas (Poll, 1968; Apsite et. al., 1993; Mahan, 1995; Aye et. al., 1999; Agate et. al., 2000).
The association between an intense activity of lipase and AST and a decrease in the ALKP levels, leads to a higher permeability of the cellular membranes, especially that of the sarcolemma.
Enzymes are released from the cytosol into the blood circulation, which leads to the instatement of muscle degeneration, not perceptible

by the anatomopathological examination (Bansal, M.P. şi Kaur, T. 2002, Cornell University College of Veterinary Medicine, 2011, Hassan, S. 1990, Oster, O. şi Prellwitz, W. 1990, Pappas, A.C.et. all., 2004).

Experimental group individuals' amylase blood levels results were 179.83 U/L, lower by 0.73% than those obtained by the control group individuals.

Cholesterol blood levels reached 100.6 mg/dL of serum, 17.54% lower than those of the control group, whose feed was not selenium supplemented. This state can be explained by the improved lipid metabolism, without intermediary metabolites.

Blood glucose levels of the experimental batch registered a large decrease, reaching 203.8 mg/dL blood, which is 2,16% lower than the results obtained for the control batch.

Calcium and magnesium blood levels were larger for the experimental group, with 10.59% for the former, and 6.93% for the latter.

This indicates there is a link between selenium and the mineral metabolism (Upton, 2009).

One of the world's problems in raising broiler chickens is the development of the musculoskeletal disorders as a consequence of the high growth rate of the birds. Because of these disorders the chickens can no longer move and prefer abnormal decubitus positions, favouring the apparition of chemical burns on their limbs and pectoral muscles.

Pain and lack of movement lead to the stop of the growth process and therefore to a decrease of the body mass and ultimately, due to various complications caused by bacterial infections, to death (Avanzo J. 2001).

Table 1. Statistically modified parameters in the experimental group

Parameters	Value average	Percentage average (%)	P value
Ht (%)	35.72	↑ 7.68	**.407**
WBC (x 10^3/µl)	27.33	↑ 14.01	**.0013**
GOT (U/L)	117.5	↑ 8.19	**.0236**
Calcium (µg /dl)	10.176	↑ 10.59	**.0044**
Selenium (µg /dl)	31.62	↑ 42.36	**<.0001**
Proteinemia (g/dl)	3.3	↓ 8.33	**.0213**

P<0,05 – significant differences

P<0,01 – differences significantly distinct

P<0,001 –differences very distinct significant

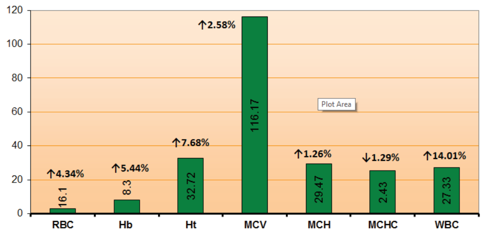

Fig. 4. Average values of haematological indices in the experimental group compared with the control group

Legend:

RBC – red blood cells (E x 10^6 / µl)

Hb – Hemoglobin (g/dl)

Ht – hematocrit (%)

MCV – mean corpuscular volume (μ^3)

MCH – mean corpuscular hemoglobin (pg Hb/Eritheremie)

MCHC – mean corpuscular hemoglobin concentration (g Hb/dl Erithremie)

WBC – white blood cells (x 10^3/µl)

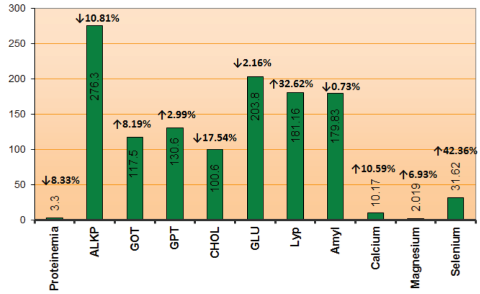

Fig. 5. Average values of biochemical indices in the experimental group compared with the control group

Legend:

Proteinemia – Proteinemia (g/dl)

ALKP – alkaline phosphatase (U/L)

GOT/AST – aspartate aminotransferase (U/L)

GPT/ALT – alanine aminotransferase (U/L)

Chol – cholesterol (mg/dl)

Glu – glucose (mg/dl)

Lyp – lipase (U/L)

Amyl – amylase (U/L)

Calcium – calcium (µg/dl)

Magnesium – magnesium (µg/dl)

Selenium – selenium (µg/dl)

CONCLUSIONS

1. The conducted experiment demonstrates the beneficial effects of feed supplementation with selenium, by improving of some haematological and biochemical parameters.

2. Selenium has a biologically active role in the activity of the hematopoietic bone marrow and

therefore in the formation of new, young, red blood cells, in the experimental group the RBC average was with 4.34% higher then in the control group.

3. The increased blood levels of calcium, 10,17% and selenium, 42.36% lead to the improvement of the mineral metabolism and a better growth rate of the individuals in the experimental batch.

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