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THE EFFECT OF DIFFERENT FORMS OF SELENIUM IN DIET ON LIVER FUNCTION AND BODY WEIGHT OF BROILER CHICKENS

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Abstract - The main biological role of selenium is performed via enzyme glutathione peroxidase, and includes the participation in the defense mechanism of the cell membrane from the peroxide damage. The aim of this study was to determine the effect of added selenium in foods in varying amounts and from various sources (organic and inorganic selenium). The experiment was conducted on the broilers, and the following parameters were monitored: body weight, total serum protein and the activity of the liver enzymes AST and ALT.

The study demonstrated that organically-bound selenium had the most pronounced protective property, while both forms of selenium did not significantly affect the growth and concentration of the total serum protein. Therefore, this research suggests economic benefits of organic selenium in the diet of broiler chickens.

Key words: selenium, body weight, liver function, broiler chickens

Introduction

One of the most important discoveries in nutrition in the last 50 years was the awareness of the importance of selenium for living organisms, and the explanation of its biochemical basis of activity and nutritional association with vitamin E and other factors (17).

The biological role of selenium is performed via enzyme glutathione peroxidase (GSH-Px). This enzyme, which in its active site contains selenium together with catalase, superoxide dismutase and vitamin E, participates in the defense mechanism of cell membrane from peroxide damages (17).
Diseases caused by deficiency of selenium and vitamin E have been diagnosed and described in 60 species of domestic, wild and laboratory animals. All of these diseases have their own clinical presentation and pathology: dystrophy of skeletal muscle and cardiac muscle, dietetic hepatoses, exudative diathesis, pancreatic necrosis, impaired reproduction and others. Selenium has strong anticancer effect on the development of many forms of cancer, which are caused by various chemical carcinogens (17).

Insufficient selenium in plant foods has sparked interest and directed it towards the introduction of measures that would increase the amount of selenium in food for animals.

Selenium performs its biological function only when it is incorporated into the variety of selenoproteins. First discovered selenoprotein was the enzyme GSH-Px, which has selenium in its active site (13).

Two forms of selenium are used as food supplements. The first is inorganic selenium, usually in the forms of selenite or selenate. They have a long history of nearly 60 years in animal nutrition (5). Part of that history includes cases of accidental poisoning of animals and humans with high-toxic inorganic forms (17). Natural feed for animals is supplemented with selenized yeast, which contains organic form of selenium (another form of selenium), mainly selenomethionine (23). However, all relevant animal selenoproteins contain selenocysteine as physiologically active component. Thus, in recent years the interest in the use of selenized yeast as an effective source of selenomethionine in animal feed has been raised (9). Selenomethionine can be deposited in the muscles and subsequently converted into selenocysteine, and incorporated into selenoproteins (10, 15).

Studies in recent years have shown that organic selenium is increasingly replacing the use of inorganic (22). Metabolic pathways of organic and inorganic selenium are different. Organic selenium is present in grains, feed and certain food ingredients, mainly in the form of selenomethionine. That’s why it has the same metabolic pathway as methionine (active transport through the intestinal membrane and active accumulation in the liver and muscle tissue) (21). Recent studies suggest that selenomethionine cannot be synthesized in the animal and human body, but also that it comes from the plant sources. Inorganic selenium remains in the tissues for a short period of time. A small amount incorporates into selenoproteins and the rest is being excreted in the urine. The animal organism is adapted to organic selenium, which is the main food ingredient, while inorganic selenium doesn’t represent a natural source. Therefore, it can be occasionally used as an antioxidant (22). Organic selenium effectively provides selenium-tissue reserves compared to non-organic (15).

In poultry production, selenium is added to food primarily to prevent certain diseases due to its positive effect on the immune system, and to increase production characteristics, particularly body weight (4, 24, 18, 14).

Selenium in the form of sodium selenite is usually added to the poultry feed in the dose of 0.15 mg of Se/kg (8, 16, 19). However, the current recommendations are
much higher ranging from 0.3 to 0.5 mg/kg, and selenium is usually added in organic form (selenized yeast) (15).

Alanine aminotransferase (ALT) is used as an indicator of hepatopathy, but is not considered an organ-specific enzyme. Increased activity of this enzyme may be an indicator of myopathy. For this reason, the increase of ALT is usually followed by the increase in activity of some other serum enzymes. Increased serum activity at first indicates increased release of enzymes from the cells which normally contain them, primarily due to the cell membrane damage caused by degenerative or necrotic processes. Cell membrane damage is always advanced to a certain degree. However, in the early, mild or chronic cases, serum activities can be increased moderately, without exceeding the marginal value (11).

Tissue damages with the increased activities of serum ALT as a consequence, may be the result of the effects of oxidative agents, which associates them with protective, antioxidant effect of selenium.

Aspartate aminotransferase (AST) is present in many tissues and therefore is not an organ specific enzyme, but is a good indicator of soft tissue damage. Generally, among birds, increased activity of serum AST may be a sign of hepatopathy or myopathy. The increase in serum AST activity may be the result of tissue damage under the influence of oxidative agents produced in conditions of hypoxia, hypercapnia and respiratory acidosis, which puts them in touch with protective, antioxidant action of selenium. Several papers indicate the impact of selenium deficit on increased serum AST. Bartholomew et al. (1) are showing the increased activity of some plasma enzymes such as aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase (CK) among chickens suffering from exudative diathesis. Hull and Scott (12) found elevated plasma aspartate aminotransferase activity among chickens suffering from nutritional muscular dystrophy. In addition, Campbell (3) shows that the muscle damages of different etiology among birds are accompanied by increased activity of aspartate aminotransferase, creatine phosphokinase, and lactate dehydrogenase (LDH).

Based on the aforementioned, the aim of this study was to investigate the effect of various sources of selenium (organic and inorganic) in different amounts on body weight and some biochemical parameters of blood serum.

**Material and Methods**

Broiler chickens "Cobb 500" of both sexes used in the experiment were divided in four experimental groups (A, B, C and D):

- Group A was treated with selenium only in the form of sodium selenite at the rate of 0.3 ppm;
- Group B was treated with selenium in the form of sodium selenite at the rate of 0.2 ppm, and supplemented by mixture of selenoaminoacids at the rate of 0.1 ppm;

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Group C was treated with selenium in the form of sodium selenite at the rate of 0.1 ppm, and supplemented by mixture of selenoaminoacids at the rate of 0.2 ppm;

Group D was treated with selenium in the form of additives represented by mixture of selenoaminoacids in the amount of 0.3 ppm.

Selenoaminoacids in the additive were selenomethionine, selenocistin, selenocysteine, and selenocistation metilselenocistein in the ratio shown in Table 1.

Chickens were fed ad libitum. For this trial, we used 10 chickens from each group (5 males and 5 females) at the age of 42 days. Body weight was measured individually before taking blood for analysis. Blood samples were taken by cardiac puncture (3 to 5 ml). Then the blood serum was separated from blood for biochemical tests.

Table 1. The composition of additive

<table>
<thead>
<tr>
<th>Selenoaminoacid</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenomethionine</td>
<td>50</td>
</tr>
<tr>
<td>Selenocistin</td>
<td>15</td>
</tr>
<tr>
<td>Selenocysteine</td>
<td>15</td>
</tr>
<tr>
<td>Selenocistation</td>
<td>10</td>
</tr>
<tr>
<td>Metilselenocistein</td>
<td>10</td>
</tr>
</tbody>
</table>

Blood serum was analysed for the enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and the concentration (content) of protein in the blood plasma. Biochemical parameters were measured by quantitative spectrophotometric method using photometric analyzer “CLIMA MC-15”. In the trial, we used test kits "BIOANALITICA" for the appropriate biochemical parameters, which were used according to the manufacturer's instructions.

The activities of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were determined (IFCC) kinetically, using the UV method. The activity of serum aspartate aminotransferase (AST) was determined (IFCC) kinetically, using the UV method. The concentration of total serum protein was determined by Biuret colorimetric method with the standard.

Statistical significance of differences between groups was determined using analysis of variance, and occasionally, Student's t-test. The calculation of averages and standard deviations and tests of significance of differences were all performed using the PC and software Microsoft Excel XP.

Results

Table 2 shows the results of measuring of the body weight, total serum protein concentration and liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST).
The effect of different forms of selenium in diet on liver function and body weight

Table 2. The body weight, total protein concentration and ALT and AST activity

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Average body weight (g)</th>
<th>Total protein (U/l)</th>
<th>ALT (U/l)</th>
<th>AST (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2183 ± 135,2</td>
<td>32,8 ± 3,5</td>
<td>11,9 ± 5,1</td>
<td>448,5 ± 25,2</td>
</tr>
<tr>
<td>B</td>
<td>2150 ± 170,2</td>
<td>34 ± 5,9</td>
<td>10,6 ± 4,8</td>
<td>384,5 ± 55,7</td>
</tr>
<tr>
<td>C</td>
<td>2139 ± 137,1</td>
<td>34,5 ± 4,7</td>
<td>8,2 ± 2,7</td>
<td>261 ± 70,8</td>
</tr>
<tr>
<td>D</td>
<td>2157 ± 114,3</td>
<td>33,5 ± 3,7</td>
<td>6 ± 2,5</td>
<td>354,5 ± 193,3</td>
</tr>
</tbody>
</table>

The differences between a and b are significant at the level (P<0,05) and between a and c at level (P<0,01)

Based on the data analysis from Table 2, we see there were no statistical differences in the weight gain and the amount of total serum protein between the groups. The lowest average weight of all experimental groups was in the group C, while the biggest average weight was in the experimental group A. The lowest concentration of total serum protein was found in the experimental group A, while the highest concentration was detected in the experimental group C. There was statistical differences between some experimental groups in serum AST and ALT activity.

Discussion

The results showed significantly lower ALT activity (P<0,05) in the group D compared to group A. The differences between another experimental groups in the average activity of alanine aminotransferase were not statistically significant. The highest activity of this enzyme was in the group A with an average value of 11.9 U/l. Normal serum activity of this enzyme among birds is less than 50 U/l (3). Basmacioglu et al. (2) describe the average serum ALT activity of 7.8 U/l in chickens at the age of 21 days with the addition of inorganic selenium only in the amount of 0.15 ppm

Serum AST activity among birds is normally less than 275 U/L (3). There was a statistically significant difference in the activity of aspartate aminotransferase. Experimental group A, which was given only inorganic selenium, had statistically higher (P<0,01) activity of serum AST compared to experimental groups B and C, who consumed a mixture of organic and inorganic forms of selenium. Experimental group A had statistically higher (P<0,05) activity of serum AST compared to group D (chickens who consumed pure organic selenium). Among other groups there weren’t any statistically significant differences noticed.

The lowest ALT activity was found in the experimental group D where pure organic selenium was used, while the highest value was found in the group where only inorganic selenium was used (group A). The highest AST activity was also found in the...
experimental group A, while the lowest activity of this enzyme was observed in the group C where it was used in 1/3 inorganic and 2/3 organic selenium diet. Broilers, genetically predisposed to rapid growth, have more dynamic oxidation processes that require high levels of oxygen. Proportionally smaller lung capacity compared to body mass, results in hypoxia, hypercapnia and respiratory acidosis development (20).

During hypoxia, the production of free radicals increases, including fatty peroxide, hydrogen peroxide and superoxide. Secondary, hypoxic tissue damages attract leukocytes, which also release free radicals, and in that way, the circle of tissue damages is closing (20).

Acidosis also directly affects the integrity of the cell membrane, and points on the effects of free radicals (20).

Based on the results obtained from using different forms of selenium, organic form of selenium (group D) showed the most protective effect on the liver. A combination of 1/3 inorganic and 2/3 organic form of selenium showed slightly less protective effect, which can be explained by the fact that inorganic selenium is immediately being used for a metabolic purpose, while organic deposits for future utilization.

**Conclusion**

Based on the results obtained, we can conclude:

1) The most protective effect was showed by organic selenium (experimental group D), and almost the same effect was showed by the combination of 1/3 inorganic and 2/3 organic selenium (experimental group C);
2) Less protective effect was exhibited in the experimental group A where only inorganic selenium was used, and experimental group B, which contained 1/3 organic and 2/3 of the inorganic forms of selenium;
3) Different forms of selenium had no significant effect on the amount of total serum protein;
4) These results justified the use of organic selenium in the diet of this product category.

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