THE INFLUENCE OF DIFFERENT LEVELS OF DIETARY SELENIUM ON ITS DISTRIBUTION IN THE ORGANS OF BROILER CHICKENS

ABSTRACT: The content of selenium in basic broiler diets was 50 ± 10, 100 ± 10, 150 ± 10 and 250 ± 10 µg kg⁻¹. At day 14, 28 and 42 tissue samples were collected. Dynamics and selenium deposits in blood, liver, muscle and heart were estimated in relation to the level of dietary selenium and age of broilers. When broilers were fed with diet containing 250 µg kg⁻¹ Se, at day 42 the highest concentration of Se was in liver (570.8 ± 44.1 µg kg⁻¹), blood (430.3 ± 46.5 µg L⁻¹) and heart (237.5 ± 42.8 µg kg⁻¹). At day 14, increase of dietary selenium content from 50 µg kg⁻¹ to 250 µg kg⁻¹ was followed by decrease of selenium deposits in heart: when broilers were fed with basic diet containing 50 µg kg⁻¹ Se measured content of Se in heart muscle was 49.8 ± 15.6 µg kg⁻¹ (99.6%) while in broilers fed with basic diet containing 250 µg kg⁻¹ Se measured content of Se in heart muscle was 147.2 ± 33.4 µg kg⁻¹ (58.9%), respectively. The point of saturation as well as maximal concentration of selenium in liver was reached in period between fourth (556.7 ± 40.6 µg kg⁻¹) and sixth (570.8 ± 44.1 µg kg⁻¹) week of age. Also, feeding with basic diet containing 250 µg kg⁻¹ Se the selenium blood level reached 162.8 ± 28.9 µg L⁻¹ already at day 14 that represent 65.1% of dietary selenium.

KEY WORDS: broilers, blood, heart muscle, liver, meat, selenium

INTRODUCTION

Selenium is one of the most significant essential microelement. It is present in all living systems and because of its high significance for the health of people and animals interest for selenium is recently rising. Many of the present knowledge is pointing out the significance of selenium such as: micro levels of selenium: in many tissues and organs which have high functional acti-
Selenium is showing stimulative effect on development and growth, influences on reproductive ability and is a part of anti-oxidative system (burst) of the organs of animals and people. Selenium also inhibits harmful effects of toxic elements (As, Cd, Hg, Pb).

Selenium is a cofactor in some enzyme's systems from which two are the most extensively studied: glutathione, where selenium is cofactor of glutathione peroxides acting as an antioxidant and destroying peroxide radicals which have harmful effect on cell membrane; and in the system of iodotireonin 5'-deiodinase which translates thyroxin in triiodtireonin and iodine is released (Jacques, 2001).

Research has shown that on the crops there are much more areas where ground does not contain enough selenium then there are the cases that it could be present at the toxic level (May and James, 1989). Results of measurements of the level of selenium in the ground from the different localities in Serbia (Maksimovic et al., 1992) show that the level of whole or in water soluble selenium in the samples that were investigated is extremely low. This has led to the conclusion that the home substrate on which the crops are established is very poor in selenium. Results provided when selenium content was measured in different regions in Serbia (Mihailovic et al., 1996) also show that plants in Serbia are poor in selenium.

Feed of plant origin and animal feed stuff in which there is low level of selenium are not suitable for successful agricultural production. Indirectly, over food chain, selenium reaches men so if there is insufficient supply of selenium that will reflects on plants or animals and also on humans.

Taking into consideration the importance of selenium in the metabolism of animals and it's insufficiency as an important factor of risk in the majority of human population it is indispensable that appropriate intake of the optimal level of selenium for animals and humans is provided. To realize this goal there is a need of giving selenium in order to achieve appropriate concentration in the granule and vegetative mass of plants because amount of selenium in food from plant or animal origin depends of the concentration of the selenium in the crop of certain quality (Giuseppe et al., 1984).

Although laboratory investigations have been done the role of selenium in the biochemical process is still not understood well. Also, needs of certain domestic animals for selenium is not defined clearly and there is no general agreement about this issue. Adding adequate level of selenium in the animal feed will lead to the better production results, and over "functional" food of animal origin, mostly meat or eggs there will be possible to enhance ingestion of selenium in humans. This will prevent various diseases among which carcinogen or cardiovascular diseases are the most significant (Wanger, 2003).

When it was discovered that selenium is integral part of the enzyme glutathione peroxides Rotruck et al. (1973) proved its role in cell antioxidative metabolism. Assumption was that selenium is an integral part of the 30—50 proteins in the organism (selenoprotein) (Koreh et al., 2000) that have a role in the function of triode hormones, immune system, in forming and viability of the sperm and the function of the prostate gland. As a consequence of the deficiency of selenium, in animals, there is a low immuno-
competence, high embryonic rate, lower fertility, and high rate of mortality in chickens in the first days of life (Suraí, 2002).

Intensive growth of animals is often connected with various stresses. The major cause of the stress in animals could be divided in three major categories: nutritive stress (high amount of unsaturated acids, deficiency in vitamin E, selenium zinc or manganese, higher amount of irons, hypervitaminosis A or presence of various toxic elements); ambient (higher temperature or humidity, hyperoxia or irradiation) inside stress causes (bacteria and viral infections and allergic reaction-activity of macrophages). Laying eggs is stress for hens. Prolonged keeping in the hatchery cabinet, transportation from the hatchery to the farm and vaccination all presents stress and causes free radicals to evolve (Suraí, 200).

Our goal was to investigate the influence of selenium the feed and its mass in tissue (whole blood, meat) and organs (liver and hurt) in broiler chickens.

MATERIAL AND METHODS

Experimental animals

Our experiments were performed in vivo on chickens of heavy hybrid Arbor Acres, which were treated from first to forty-two days of life. Feeding and water were given ad libitum. At the time when the experiments were starting chickens appear healthy and vital and they were raised according to the requirements for the hybrid.

Preparation of the feed for the chickens

In order to create low selenium intake through feed we tested the level of selenium in the plant feed from different regions (this included testing of corn, soybean meal and sunflower meal), (Mihaljev et al., 2003). During the mixing process we used only plant feed for which we discovered that selenium concentration was very low (5—10 ug Se/kg). As a source of selenium the specific amount of sodium selenite (Na2SeO3) was added. This way we achieved that feed mixture for the chickens have exactly the planned level of selenium, such as: < 10, 50 ± 10, 100 ± 10, 150 ± 10, 250 ± 10, fig Se/kg.

Experiment design

Five groups of chickens were formed in total. Each group consisted of 60 one-day old chickens of both sex (300 chickens). First group was fed with feed without additional selenium, second group was fed with the feed that was supplemented with 50 ug Se kg⁻¹, third group was fed with the feed containing 100 ug Se kg⁻¹, fourth group received feed with 150 ug Se kg⁻¹ and fifth group was fed (given) with the feed containing 250 ug Se kg⁻¹. After day 14, 28, and 42, chickens were measured and blood samples were taken.
Preparing of samples and measurement of selenium content

Samples were prepared for the measurement by the method of wet digestion with automatic regulation of the temperature in the aluminum thermo block AC 300. The destruction of the collected samples of blood, meat and liver was done with HNO₃ and H₂O₂ at the temperature of 20°C per hour. Since only Se (IV) ion forms hydrides, reduction of Se (VI) was done with 8M HCl at t = 120°C (Stoeppler, 1997 and Amparo, 1998). Hydrogen selenide is generated with sodium tetrahydroborate (0.6% in 0.5% NaOH) in the system VGA-76, and concentration of selenite is measured on atomic absorption spectrophotometer VARIAN SpectrAA-10. The conditions for the measurement were as follows (Lucinda Beach, 1992):

<table>
<thead>
<tr>
<th>Hollow cathode lamp</th>
<th>Slit = 1.0 nm</th>
<th>Acetylene = 3.5 flow units/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = 196.0 nm</td>
<td>Delay time = 60 sec</td>
<td>Air = 1.0 flow units/min</td>
</tr>
<tr>
<td>Lamp current = 10 mA</td>
<td>Measurement time = 2.0 sec</td>
<td>Inert gas = Nitrogen</td>
</tr>
<tr>
<td>Replicates = 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis

For liver samples (n = 12), whole blood (n = 12), meat (n = 12), and heart muscles (n = 12) the results are given as a mean value ± standard deviation. The statistical significance between experimental groups was done by analysis of variance and by Student t test.

RESULTS

Results given in Table 1 lead to the conclusion that among granular feed the highest level of selenium in native sample was for soybean (42.7 ug/kg). The medium level of selenium in corn was 26.6 ug/Kg. In protein feed the averaged measured level of selenium in native samples of soybean meal was 92.7 ug/kg, and in sunflower meal 77.2 ug/kg.

Tab. 1. — Content of selenium in feed of plant origin

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of feed</th>
<th>Se fug kg⁻¹</th>
<th>Range [ng kg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Corn (northern part — Vojvodina)</td>
<td>36.3 ± 5.4 n</td>
<td>25.5—40.5</td>
</tr>
<tr>
<td>2.</td>
<td>Corn (central part)</td>
<td>25.3 ± 3.8 n</td>
<td>20.8—31.1</td>
</tr>
<tr>
<td>3.</td>
<td>Corn (southern part)</td>
<td>18.1 ± 3.7 n</td>
<td>12.5—22.2</td>
</tr>
<tr>
<td>4.</td>
<td>Soybean</td>
<td>42.7 ± 8.5 n</td>
<td>30.3—50.1</td>
</tr>
<tr>
<td>5.</td>
<td>Soybean meal</td>
<td>92.7 ± 17.4 n</td>
<td>75.8—122.4</td>
</tr>
<tr>
<td>6.</td>
<td>Sunflower meal</td>
<td>77.2 ± 15.1 n</td>
<td>61.1—102.5</td>
</tr>
</tbody>
</table>

The values represent the mean ± SD
The values represent the mean ± SD.

**Fig. 1.** Measured amount of selenium in native samples of liver.
### Content of selenium [µg kg⁻¹]

<table>
<thead>
<tr>
<th>Dietary Se [µg/kg]</th>
<th>14 day</th>
<th>28 day</th>
<th>42 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (≤10)</td>
<td>8.5 ± 2.6</td>
<td>15.3 ± 4.0</td>
<td>15.8 ± 4.5</td>
</tr>
<tr>
<td>Basal + 50</td>
<td>38.5 ± 10.7</td>
<td>92.2 ± 18.0</td>
<td>117.0 ± 20.7</td>
</tr>
<tr>
<td>Basal + 100</td>
<td>64.9 ± 22.8</td>
<td>145.9 ± 30.5</td>
<td>181.9 ± 29.4</td>
</tr>
<tr>
<td>Basal + 150</td>
<td>81.9 ± 28.7</td>
<td>182.0 ± 31.5</td>
<td>224.8 ± 30.0</td>
</tr>
<tr>
<td>Basal + 250</td>
<td>152.8 ± 28.9</td>
<td>385.1 ± 39.5</td>
<td>430.3 ± 46.5</td>
</tr>
</tbody>
</table>

The values represent the mean ± SD.
The values represent the mean ± SD.

Fig. 3. — Selenium deposition in organs and tissue of broilers fed with the feed with basal level of selenium + 250 μg Se/Kg.
Inorganic selenium (sodium selenite) is still the most frequently added form of selenium into animal diets. After resorption in gut, sodium selenite gets in the liver where is transformed into biologically usable form of selenide (Se-²). In the presence of cystein, selenocystein is built through specific catalysed reactions. However the above mentioned mechanism becomes saturated when Se reached level of 300 mg kg⁻¹ Se in liver (Pehrson, 1993). Our experimental results lead to similar conclusion (Fig. 1). In broilers fed with diet containing 250 µg kg⁻¹ Se, at day 28 concentration of selenium in liver was 556.7 ± 40.6 µg kg⁻¹ and at day 42 only 570.8 ± 44.1 µg kg⁻¹, respectively. Since there was no significant difference between these two values, it can be concluded that the point of saturation as well as maximal concentration of selenium in the liver reached the maximum levels in a period between fourth and sixth week.

**DISCUSSION**

Inorganic selenium (sodium selenite) is still the most frequently added form of selenium into animal diets. After resorption in gut, sodium selenite gets in the liver where is transformed into biologically usable form of selenide (Se-²). In the presence of cystein, selenocystein is built through specific catalysed reactions. However the above mentioned mechanism becomes saturated when Se reached level of 300 mg kg⁻¹ Se in liver (Pehrson, 1993). Our experimental results lead to similar conclusion (Fig. 1). In broilers fed with diet containing 250 µg kg⁻¹ Se, at day 28 concentration of selenium in liver was 556.7 ± 40.6 µg kg⁻¹ and at day 42 only 570.8 ± 44.1 µg kg⁻¹, respectively. Since there was no significant difference between these two values, it can be concluded that the point of saturation as well as maximal concentration of selenium in the liver reached the maximum levels in a period between fourth and sixth week.

![Graph showing selenium content over time](image)

The values represent the mean ± SD

**Fig. 4.** Selenium level in native samples of heart muscle

<table>
<thead>
<tr>
<th>Dietary Se (µg/kg)</th>
<th>14 day</th>
<th>28 day</th>
<th>42 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bazal (&lt; 10)</td>
<td>9.2 ± 2.6</td>
<td>9.3 ± 3.1</td>
<td>9.7 ± 3.0</td>
</tr>
<tr>
<td>Bazal + 50</td>
<td>49.8 ± 15.6</td>
<td>62.7 ± 17.6</td>
<td>99.4 ± 20.4</td>
</tr>
<tr>
<td>Bazal + 100</td>
<td>84.1 ± 19.2</td>
<td>132.2 ± 27.9</td>
<td>178.5 ± 32.9</td>
</tr>
<tr>
<td>Bazal + 150</td>
<td>101.9 ± 24.1</td>
<td>152.3 ± 28.9</td>
<td>196.9 ± 28.9</td>
</tr>
<tr>
<td>Bazal + 250</td>
<td>147.2 ± 33.4</td>
<td>164.0 ± 30.1</td>
<td>237.5 ± 42.8</td>
</tr>
</tbody>
</table>

The values represent the mean ± SD.
The mechanism of biotransformation inorganic to organic form decelerates in time. Also, production of selenium deposits in different tissues is limited. A certain quantity of inorganic selenium that was not used in selenoprotein synthesis in liver is excreted in urine. In short time selenium is being incorporated in liver, the site of selenoprotein P synthesis, subsequently increasing the level of selenium in blood plasma that is good indicator of supply with this mineral (Zust et al., 1998). The effect is particularly prominent with content of dietary selenium of 250 ugkg⁻¹ (Fig. 2). According to our experimental results, already at day 14 the blood level of selenium reached 162.8 ± 28.9 ugL⁻¹ that represents 65.1% of dietary selenium.

The affinity for selenium as well as duration of its deposits varies among tissues. For example, feeding with diet containing 250 ugkg⁻¹ Se at day 42 the highest concentration of selenium of 570.8 ± 44.1 ugkg⁻¹ measured in liver (Fig. 3), than 430.3 ± 46.5 ugkg⁻¹ in whole blood, 237.5 ± 42.8 ugkg⁻¹ in heart muscle. The lowest concentration of selenium of 160.0 ± 35.8 ugkg⁻¹ was measured in meat. Tissue deposits of selenium in such order in chickens coincide with findings of Echevarria (1988).

Certain tissues bind less selenium with increase of dietary selenium content. Our results (Fig. 4) show that at day 14 after the increase of dietary selenium from 50 ugkg⁻¹ to 250 ugkg⁻¹ concentration of selenium in heart muscle subsequently decreased: when broilers were fed with basic diet containing 50 ugkg⁻¹, 100 ugkg⁻¹, 150 ugkg⁻¹ and 250 ugkg⁻¹ Se measured content of Se in heart muscle was 49.8 ± 15.6 ugkg⁻¹ (99.6%), 84.1 ± 19.2 ugkg⁻¹ (84.1%), 101.9 ± 24.1 ugkg⁻¹ (67.9%) and 147.2 ± 33.4 ugkg⁻¹ (58.9%), respectively.

Knowledge of speed of forming and quantity of deposits of selenium in meat is important for production of so-called "functional food". There is constantly increase of number of food products with improved nutritive value by adding selenium, like food and drink for sportsmen and children, eggs, meat and milk. Studies suggest that deposits of inorganic selenium in meat are significantly lower than in other tissues and organs (B eale et al., 1990). In our investigation similar results were obtained. The level of selenium in meat was significantly lower than in other evaluated tissues and organs (Fig. 3). We also observed that considerably higher deposits of selenium were determined at day 42 when diet with 250 ugkg⁻¹ Se was used.

The amount of selenium deposits in tissue and organs is in relation to quantity and form of selenium, age and animal species. Increase of intake of selenium increases its concentration in tissue and organs, but in nonlinear regression line. After assessing a certain determined level of dietary selenium, concentration of selenium in tissue reaches and remains at maximum and is no longer dependent on concentration of dietary selenium.

ACKNOWLEDGEMENTS

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УТИЦАЈ РАЗЛИЧИТIH НИВОA ДИЈЕТАРНОГ СЕЛЕНА НА ЊЕГОВУ ДИСТРИБУЦИЈУ У ОРГАНИЗМУ БРОЈЛЕРА

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Резиме

Потупне смеше за исхрану бројлера садржавале су 50 ± 10, 100 ± 10, 150 ± 10 и 250 ± 10 ng Se kg-1. Узорци су узимани 14-ог, 28-ог и 42-ог дана огледа. Праћене су динамика и количина накупљања селена у крви, јетри, месу и срчаном мишићу, у зависности од нивоа дијетарног селена и времена његовог уношења у организам. После 42 дана това смешом са 250 ug Se/kg највећи садржај селена измерен је у јетри (570.8 ± 44.1 ug/kg), затим у пуној крви (430.3 ± 46.5 Hg/l) и срчаном мишићу (237.5 ± 42.8 ng/kg). Најнижи садржај селена измерен је у месу (160.0 ± 35.5 ug/kg). Повећање садржаја дијетарног селена у храни од 50 до 250 ug Se/kg, после 14. дана това праћено је смањењем количине депонованог селена у срчаном мишићу: за смешу са 50 ug Se/kg количина селена у срчаном мишићу износила је 49,8 ± 15.6 ug/kg (99,6%), а за смешу са 250 ug Se/kg измерена количина селена у срчаном мишићу износила је 147,2 ± 33.4 ug/kg (58,9%). Између четврте (556.7 ± 40.6 ug Se/kg) и шесте недеље (570.8 ± 44.1 ug Se/kg) огледа дошло је до засићења и постигнут је плато концентрације селена у јетри. Такође се може закључити да са храном од 250 ug Se/kg, већ после 14 дана, ниво селена у пуној крви достиже вредност од 162,8 ± 28.9 ug/l, што представља 65,1% од количине дијетарног селена.