Influence of Organic Selenium Application in Concentrate Mixtures on Selenium Content in Blood Plasma and Duck Feces

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ABSTRACT

The aim of this study was to investigate the effect of the addition of different amounts of organic selenium (ALKOSEL® R397) in concentrate mixtures on the selenium content in the blood plasma and feces of ducks.

The experiment was performed on 240 one-day-old ducklings (Cherry Valley) which were freely selected into 4 groups, one control K0 group, and three experimental K1, K2 and K3 groups. In two phases of feeding in fattening, ducklings were fed with two and nutritionally different concentrate feed mixtures: starter (from 1 to 14 days) and finisher (from 15 to 49 days of fattening). The control group of ducklings (K0) during feeding received food without added organic selenium in both phases of fattening. In both phases of fattening, the experimental group of ducks K1 was fed with food as well as the control group, but with the addition of 0.2 mg/kg of organic selenium, while the experimental group K2 used food with 0.4 mg/kg, and the experimental group K3 with 0.6 mg/kg of organic selenium. The addition of organic selenium to duck feed had the effect of increasing selenium content in both blood plasma and duck feces. The highest content of selenium in blood plasma and feces was determined in the group of ducks that received the highest amount of organic selenium through food during the entire experiment.

Keywords- Ducks, Organic Selenium, Blood Plasma, Feces.

I. INTRODUCTION

Today, it is a well-known fact in the world that animal nutrition can affect the nutritional value of meat, milk, eggs, and that it is one of the ways to obtain food with special properties known as functional food. Selenium-enriched foods can also be considered functional foods. Selenium performs its biological role in the body through the enzyme glutathione peroxidase (GPx), in the active site of which this element is located. Plasma GPx activity is a reliable indicator of selenium status in animals, but only at suboptimal and optimal selenium levels. However, high levels of selenium do not lead to a proportional increase in selenium enzymes. The dependence of GPx activity on the levels of supplemented selenium has been established in numerous studies in many animal species. In developed countries, ducks are mainly reared for fattening in closed intensive systems, without access to pasture and water, except for drinking water. Organic production is used less frequently. In Asia, most of the duck production is extensive and related to water surfaces (ponds), and even to rice fields (Adeola, 2006; Mahmutović Hava, 2014; Baeza, 1995). Intensive duck breeding systems require, in addition to appropriate housing conditions, that the animals be provided with all the necessary ingredients that enable the full use of their genetic potential. One of the most widespread breeds of ducks in the world is the Peking duck, and duck meat production is mainly based on commercial crosses of different Peking species (Anas platyrhynchos) (Pinigel, 1997; Zejidler, 1998). It originated in China, from where it spread to Europe and America. The most famous is the American and German strain, both white in color. Males reach a weight of about 4-6 kg, and females 3-4 kg. Nutrition is also an important factor influencing carcass yield. Poultry nutrition is based primarily on knowing the needs and providing adequate food in order to achieve optimal production results and obtain a satisfactory amount of high-value foods of animal origin for human consumption, as well as the appropriate choice of nutrients (Ševković et al., 1991; Hayes et al., 1979; He et al., 2003; Underwood and Suttle, 1999; US / NAS, 1980; NRC, 1980, 1994).

The concentration of selenium in soil varies significantly from 0.1 to 2 mg / kg and no direct correlation has been established between total selenium in soil and water-soluble fractions. The content of selenium in the soil is directly related to the content of selenium in plants. The content of selenium in food of plant origin, which is used in animal nutrition, varies depending on the area in which it is produced. If the soil is poor in selenium, the grain content of cereals can contain up to five times less selenium content than grain grains grown on soil rich in selenium. Cereal production, on land poor in selenium, can endanger the health of animals and humans both in the area and in the area rich in selenium if these grains are used in the diet. Plants absorb selenium...
from the soil in varying amounts, and then humans and animals eat those plants. Plants absorb and transport selenium better than selenite or the organic form of selenium (Terry et al., 2000). After the absorption of selenite or selenium from the soil, the plants synthesize organic forms of selenium. The organic form of selenium is selenomethionine (SeMet) which represents more than 50% of the total selenium in cereal grain (Olson and Palmer, 1976). In addition to selenomethionine, there are organic forms of selenium: Se-methyl-selenomethionine, selenocysteine (SeCys), gamma-glutamyl-Se-methylselenocysteine. Organic forms of selenium are known to be natural sources of selenium and are found in foods of plant and animal origin. The utilization of selenium from nutrients of plant origin is much better compared to nutrients of animal origin (Combs and Combs, 1986). Many factors affect digestion and resorption, metabolism and selenium excretion, and in order to accurately determine the needs, it must be known that the composition of food, the amount of food taken, the presence of sulfur, heavy metals, and especially the amount of vitamin E present. The amount of protein in food reduces the effectiveness of selenium in protecting broilers from exudative diathesis (Zhou and Combs, 1984) and reduces the concentration of selenium in tissue and GPx activity in rats, when the amount of selenium in food is small (Zhou et al., 1983). In poultry, the form of selenium, organically or inorganically bound, according to various authors, has no effect on GPx activity in the blood, as is the case in humans (Burk et al., 2006; Payne and Southern, 2005; Petrović et al., 2006). The effects of increased selenium in broiler feed mixtures are discussed in Stanley et al. (1996), Fairbrother and Flowes (1990), Heinz et al., 1987, Heinz et al., 1989, Heinz and Sanderson (1990). In 1974, the Food and Drug Administration (FDA) approved the addition of selenium to poultry and pig feed in the form of selenite or selenates. So far, the needs for selenium for all animals have not been fully defined and there is no general agreement on that. Selenium requirements primarily depend on the form of selenium ingested. Animals can use selenium, from its inorganic salts, and from organic forms. Selenium needs depend on the type of life and health status of the animal, production status and production levels, selenium sources, selenium status in the body, as well as the presence of interfering substances in the meal. In general, the needs of animals in growth and reproduction, as well as in production are significantly higher than the needs for life support.

| Animal species | Selenium | Animal species | Selenium |
|----------------|----------|----------------|----------|
| Cattle         | 0.20 – 0.30 | Pigs           | 0.10 – 0.30 |
| Sheep, goats   | 0.10 – 0.20 | Poultry        | 0.15 – 0.20 |
| Horses         | 0.10     | Dogs, cats     | 0.10     |

The degree of selenium resorption depends on the type of animal and the source of selenium. The digestibility of selenium is lower in ruminants than in non-ruminants due to the reduction of selenite to insoluble forms in the red by means of the microflora. The presence of sulfur and heavy metals and metalloids (Cu, Hg, As, Cd, Ag) reduces the digestibility of selenium from food. The average utilization of selenium from food in non-ruminants is around 65-85%, and in ruminants it is significantly lower at the level of about 30 to 35%. After resorption, in cells, selenium from organic carriers of selenocysteine and selenomethionine is converted back to the inorganic form and then incorporated as selenocysteine-tRNA and selenomethionine-tRNA, which have a regulatory role in selenium homeostasis in human tissue (Backović, 2005). When the selenium content in food satisfies the needs of the organism, the kidneys contain the highest concentration of selenium, followed by the liver and glandular tissues (spleen, pancreas), while in case of excess, selenium is deposited mainly in the liver and muscles. In addition, wool, hair and feathers can contain significant amounts, while nervous tissue contains minimal amounts of selenium. Selenium is excreted from the body through three main excretory pathways: the urinary tract, the digestive tract and the lungs. The amount and distribution of selenium excreted mostly depends on the amount of selenium that the organism ingests, the form in which it is ingested and the composition of the meal. Burk et al. (1978) using an injected labeled dose of 75-selenite in rats, found that fecal excretion was constant for 10 days with minimal variation and was about 10% of the given dose; urinary excretion was directly dependent on the amount of selenium given and ranged from 6% of the given dose in the basal diet with a low amount of selenium, to 67% in the diet containing 1 mg / kg of selenium. They came to the level of excretion through the lungs on the basis of a calculation, adding the percentage that the body does not retain and the percentage that was not eliminated through the previous two ways, and showed that only a few percent (with slight variations) are eliminated by exhaled air. This led to the conclusion that under normal conditions, the adjustment of rats to the amount of selenium in food depends primarily on urinary excretion. The degree of selenium excretion is proportional to the content of selenium in food, and inversely proportional to the status of selenium in the body, which can be a kind of homeostatic control. In addition, the intensity of excretion depends on the chemical form present in the food, as well as on the antagonist (Hg, S). Selenium excretion in non-
ruminants is performed mainly in urine (95%) with an increase in the content of selenium in food, its excretion in the feces increases (Drljačić, 2013).

II. MATERIAL AND METHOD OF WORK

The study of the influence of organic selenium on the production results of fattening ducks was conducted on a total of 240 one-day-old ducklings. The ducklings were randomly divided into 4 groups, namely the control K0 group, and the three experimental K1, K2 and K3 groups. There were 60 one-day-old ducklings in each group, and fattening was performed in three repetitions of 20 ducklings. Immediately on the day of moving in on the 1st day of fattening, in each experimental group the ducklings were marked by putting rings on their feet, with numbers from 1-240 according to the experimental groups (K0 = 1-60, K1 = 61-120, K2 = 121-180 and K3 = 181-240). At the beginning of fattening, the chemical composition of the concentrate mixtures as well as the selenium content were determined (to determine the total selenium content, i.e. basal derived from food and to add organic selenium). At the end of fattening, after the last weighing on the farm, the ducks were placed in marked plastic transport cages according to the experimental groups for each repetition. Fattened ducklings that did not reach the minimum body weight of 1800 g (scrap ducklings) were separated from the experiment. After marking the transport cages and loading into the vehicle, the fattened ducklings were transported to the private poultry slaughterhouse in Gračanica according to the dynamics of moving (repetitions).

Table 2: Inspection plan

| Experimental groups | K0 | K1 | K2 | K3 |
|---------------------|----|----|----|----|
| Number of ducklings |    |    |    |    |
| According to repetitions |    |    |    |    |
| I - V1             | 20 | 20 | 20 | 20 |
| II - V2            | 20 | 20 | 20 | 20 |
| III - V3           | 20 | 20 | 20 | 20 |
| Total              | 60 | 60 | 60 | 60 |

Ducklings are in two phases of feeding in fattening, fed with two and nutritionally different concentrate feed mixtures: starter (from 1 to 14 days) and finisher (from 15 to 49 days of fattening). The raw material compositions of the mixtures used in duck fattening (starter and finisher) are shown in Tables 2 and 3.

Table 3: Raw material composition of starter fattening concentrate mixture

| Raw material (%) | Starter concentrate mixture (1st to 15th day) |
|------------------|---------------------------------------------|
|                  | Experimental groups                        |
|                  | K0  | K1  | K2  | K3  |
| Corn             | 54,83 | 54,63 | 54,43 | 54,23 |
| Soybean semolina | 18,00 | 18,00 | 18,00 | 18,00 |
| Soybean meal     | 16,00 | 16,00 | 16,00 | 16,00 |
| Soy protein concentrate | 5,00 | 5,00 | 5,00 | 5,00 |
| Alcoholic yeast  | 2,50  | 2,50  | 2,50  | 2,50  |
| Mono-Ca-phosphate | 1,30  | 1,30  | 1,30  | 1,30  |
| Premix for fattening ducks | 1,00  | 1,00  | 1,00  | 1,00  |
| Livestock chalk  | 0,90  | 0,90  | 0,90  | 0,90  |
| Livestock salt   | 0,35  | 0,35  | 0,35  | 0,35  |
| DL-Methionine    | 0,12  | 0,12  | 0,12  | 0,12  |
| Organic selenium(Se) mg/kg | -  | 0,20  | 0,40  | 0,60  |
| Σ                 | 100,00 | 100,00 | 100,00 | 100,00 |
### III. RESULTS AND DISCUSSION

Determination of selenium content in samples of food, blood and feces of ducks was performed by atomic absorption spectrometry with a hybrid technique (HGGS). Samples of food, blood, feces of ducks were prepared for analysis and poured with HNO3 and H2O2, and then microwave digestion was performed on the device (MULTIWAVE 3000 ANTON PAAR). Determination of selenium by hydride atomic absorption spectrometry (HGAAS) after microwave digestion (digestion with 65% nitric acid and 30% hydrogen peroxide) (SRPS EN 16159: 2012).

Results and discussion of selenium content testing in duck feed mixtures

The average selenium content in concentrate mixtures (starter, finisher) of the examined groups of ducks is shown in Table 5.

| Table 4: Raw material composition of duck fattening finisher concentrate mixture |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Raw material (%)                | Finisher concentrate mixture (15th to 49th day) |
|                                 | Experimental groups             |
|                                 | K0                              | K1                              | K2                              | K3                              |
| Corn                            | 72,02                           | 71,82                           | 71,62                           | 71,42                           |
| Soybean meal                    | 11,00                           | 11,00                           | 11,00                           | 11,00                           |
| Soybean semolina                | 9,00                            | 9,00                            | 9,00                            | 9,00                            |
| Alcoholic yeast                 | 2,50                            | 2,50                            | 2,50                            | 2,50                            |
| Soy protein concentrate         | 2,00                            | 2,00                            | 2,00                            | 2,00                            |
| Premix for fattening ducks II   | 1,00                            | 1,00                            | 1,00                            | 1,00                            |
| Livestock chalk                 | 0,90                            | 0,90                            | 0,90                            | 0,90                            |
| Livestock salt                  | 0,30                            | 0,30                            | 0,30                            | 0,30                            |
| DL-Methionine                   | 0,08                            | 0,08                            | 0,08                            | 0,08                            |
| Organic selenium (Se) mg/kg     | -                               | 0,20                            | 0,40                            | 0,60                            |
| Σ                               | 100,00                          | 100,00                          | 100,00                          | 100,00                          |

#### Table 5. Average selenium content in concentrate mixtures for duck fattening during the experiment (µg / 100g)

| Group | (X ±Sd) |
|-------|---------|
|       | Starter | Finisher |
| K0    | 0,381[^ABC]±0,023 | 0,411[^ABC]±0,020 |
| K1    | 2,395[^ADA]±0,019 | 2,438[^ADB]±0,045 |
| K2    | 4,397[^FDR]±0,051 | 4,445[^RDF]±0,040 |
| K3    | 6,396[^FEC]±0,023 | 6,464[^CEF]±0,030 |

Legend: Same words: A, B, C, D, E, F (p<0,01)

Selenium was considered a toxic element until 1957. However, selenium as a micronutrient has been found to play an essential physiological role in all animal species (Schwarz and Foltz, 1957). Shortly thereafter, selenium was shown to prevent muscle degeneration in lambs (Schubert et al., 1961). It is now well known that selenium plays a significant role in aerobic respiration, and its importance is evidenced by the fact that the twenty-first amino acid named selenocysteine has been identified. This amino acid is important in all mammals. So far, over 35 selenoproteins have been identified in humans and animals and all of them contain selenocysteine (Kryukov et al., 2003; Raymond and Ralston, 2004). Adequate selenium levels are important for animal reproductive performance, bone metabolism, immune function, and iodine metabolism. Selenium is an element whose concentrations in the soil, and hence in plants, are not equally represented in all parts of the world, so certain regions, including the Balkans, are considered selenium deficient areas. In order to compensate for the lack of selenium in the diet of domestic animals, it is recommended to add it to animal feed mixtures.

The average selenium content in the feed starter of the control group of ducks (group fed without selenium supplementation) was 0.381 ± 0.023 µg / 100 g, and in the finisher for the same group of ducks the average selenium content was 0.411 ± 0.020 (g / 100 g. It was
determined that in the starter of the experimental group K1 (group to which 2.00 µg / 100 g of selenium was added) the selenium content was 2.395 ± 0.019 µg / 100 g, and in the finisher 2.438 ± 0.045 µg / 100 g. The average selenium content in the starter of the experimental group K2 (group to which 4.00 µg / 100 g of selenium was added) was 4.397 ± 0.051 µg / 100 g, and in the finisher 4.445 ± 0.040 µg / 100 g. In feed mixtures of ducks of the experimental group K3 (group to which 6.00 µg / 100 g of selenium was added), the average selenium content in the starter was 6.396 ± 0.023 µg / 100 g, and in the finisher 6.464 ± 0.030 µg / 100 g (Graph 1).

Graph 1: Selenium content in feed mixtures of ducks during the experiment

The addition of selenium to animal feed mixtures is recommended (NAS, 1980). These recommendations range from 0.1 to 0.3 mg/kg. Selenium toxicity tests were performed with different selenium content in duck feed mixtures or different selenium content in drinking water. The addition of selenomethionine in an amount of 3.5 mg/kg of the mixture still has no negative effect on the reproduction of ducks.

A negative effect is observed when the selenium content is 7 mg/kg and higher (Stanley et al., 1996). The effect on the immune system (suppression) is observed when 2.2 mg/L of selenomethionine is added to ducks in drinking water (Fairbrother and Fowles, 1990). Heinz et al. (1989) observed no adverse effects when administered 5 mg/kg to a selenium (sodium selenite) mixture for seventy-eight days. When using 1, 2 or 4 mg/kg of selenomethionine in the mixture for feeding ducks for one hundred days, no negative effects were observed, and mortality was recorded when the selenium content in the mixture was 40 mg / kg regardless of the form of selenium (inorganic, organic). Food consumption and reduction in growth were recorded when the selenium content was 20 mg/kg (Heinz et al., 1989). Heinz and Sanderson (1990) consider that the toxic effects of selenium are not observed if the selenium content is less than 10 mg / kg of the mixture.

Results and discussion of selenium content in blood plasma and duck feces

The average content of selenium in the blood plasma of ducks of the control and experimental groups during the experiment is shown in Table 6.

Table 6: Average selenium content in the blood plasma of ducks of control and experimental groups during the test (µg / 100 g)

| Group | Days of experiment (X ±Sd) | 1. | 14. | 49. |
|-------|--------------------------|----|-----|-----|
|       |                          | 17.28±1.38 | - | - |
| K0    |                          | 7.38<ABC ±0.84 | 14.17<ABC ±1.04 |
| K1    |                          | 24.76<ABC ±1.53 | 53.11<ABC±2.89 |
| K2    |                          | 33.72<ABC ±2.61 | 75.39<ABC ±1.04 |
| K3    |                          | 48.19<ABC ±3.15 | 93.31<ABC ±2.70 |

Legend: Same words<ABC,D,E,F (p<0.01)
Although the status of selenium in animals is often taken to be GPx activity, its determination in the blood and tissues of animals is most important for this status. Most often, selenium content tests are performed from blood plasma samples, although the plasma selenium content is lower than the selenium content in the kidneys, liver and muscles. The utilization of selenium in animal nutrition depends on the status of selenium in the organism and its chemical form. Selenium from organic sources is deposited more in tissues than inorganic selenium.

The average content of selenium in the blood plasma of ducks on the 1st day of the experiment was 17.28 ± 1.38 µg/100 g. In the blood plasma on the 14th day of the experiment, the selenium content was in the control K0 group 7.38 ± 0.84 µg/100 g, in the K1 group 24.76 ± 1.53 µg/100 g, K2 group 33.72 ± 2.61 µg/100g, and K3 groups 48.19 ± 3.15 µg/100g. A statistically significant difference (p<0.01) was found between the selenium content in the blood plasma of ducks on the 49th day of the experiment between all compared groups of ducks. The average selenium content in the blood plasma of ducks from day 1 to 14 decreased statistically significantly (p <0.01), and then increased until day 49, but on day 49 it was still statistically significantly lower (p<0.01) of the average selenium content in the blood plasma of ducks at the beginning of the experiment. In the experimental groups of ducks, the average content of selenium in blood plasma increased from the 1st to the 49th day and in all cases of comparison, a statistically significant difference (p<0.01) was found.

At the end of the experiment, the average content of selenium in the blood plasma of ducks of the control K0 group was 14.17 ± 1.04 µg / 100 g, K1 group 53.11 ± 2.89 µg/100 g, K2 group 75.39 ± 1.04 µg/100 g and K3 group 93.31 ± 2.70 µg/100 g. A statistically significant difference (p <0.01) was found between the blood selenium contents in the blood plasma of ducks on the 49th day of the experiment between all compared groups of ducks. The average selenium content in the blood plasma of ducks of the control group from day 1 to 14 decreased statistically significantly (p <0.01), and then increased until day 49, but on day 49 it was still statistically significantly lower (p<0.01) of the average selenium content in the blood plasma of ducks at the beginning of the experiment. In the experimental groups of ducks, the average content of selenium in blood plasma increased from the 1st to the 49th day and in all cases of comparison, a statistically significant difference (p<0.01) was found.

In experiments performed on broilers, it was determined that with the increase of selenium content in food, its content in blood plasma also increases (Marković, R; 2007; Mihaljev et al., 2007).

Both GPx activity and selenium content in blood plasma are characterized by the appearance of a "plateau" of GPx activity, ie selenium content, which means that after a certain time of fattening with mixtures with added selenium GPx activity, ie selenium content reaches its maximum, ie does not increase (plateau) (Joksimović-Todorović et al., 2006; Drljačić, 2013).

Table 7 shows the average content of selenium in the feces of the examined groups of ducks, on the 14th and 49th day of fattening.

| Group | Days of experiment (X ±SD) | 14 | 49 |
|-------|--------------------------|----|----|
| K0    | 5.98 ±0.52               | 30.70 ±2.97 |
| K1    | 40.13 ±2.36              | 126.70 ±3.34 |
| K2    | 80.61 ±4.52              | 209.70 ±1.44 |
| K3    | 176.50 ±3.91             | 258.70 ±5.88 |

Legend: Same words (p<0.01)

Graph 2: Selenium content in the blood plasma of control and experimental groups of ducks
It was determined that the average content of selenium in the feces of ducks of the control KO group on the 14th day of the experiment was 5.98 ± 0.52 µg / 100 g, K1 40.13 ± 2.36 µg / 100 g, K2 80.61 ± 4.52 µg / 100 g and K3 176.50 ± 3.91 µg / 100 g. In the faeces of ducks of the control K0 group on the 49th day of the experiment, the average selenium content was 30.70 ± 2.97 µg / 100 g, K1 126.70 ± 3.34 µg / 100 g, K2 209.70 ± 1.44 µg / 100 g, and K3 258.70 ± 5.88 µg / 100 g. A statistically significant difference (p <0.01) was found between the average selenium content of all examined groups of ducks on the 14th and 49th day of the study, respectively.

It was found that the average selenium content in duck feces in all groups was statistically significantly higher on day 49 compared to day 14 of the experiment, which can be seen in Graph 3.

The content of selenium in the feces of ducks on the 14th and 49th day increases with the increase of the content of selenium in food, which means that the degree of selenium excretion is proportional to its content in food. It is inversely proportional to the status of selenium in the body, which can be explained by homeostatic control. The selenium content in the feces of ducks of all groups was always higher on day 49 than on day 14 (Chart 3).

According to the results of Drljačić (2013), the content of selenium in the feces (broilers) on the 21st day of the experiment was higher than the content of selenium in the feces on the 42nd day of the experiment, i.e at the end of fattening.

**IV. CONCLUSIONS**

Concentrate mixtures of fattening ducks differed in selenium content. The basal selenium content (originating from certain nutrients) was lower in the starter (0.381 µg/100 g) than in the finisher (0.411 µg/100 g). The K0 group of ducks was fed with selenium-free concentrate mixtures. The content of selenium in concentrate mixtures for feeding K1 group (added to food 0.2 mg/kg selenium) was 2.395 µg/100 g (starter), or 2.438 µg/100 g (finisher), for feeding K2 group (added to food) 0.4 mg/kg selenium) was 4.397 µg/100 g (starter) and 4.445 µg/100 g (finisher), respectively, and the K3 group (0.6 mg/kg selenium added to food) was 6.396 µg/100 g (starter), or 6,464 µg/100 g (finisher).

The average selenium content in the blood plasma of ducks on the first day was 17.28 µg/100 g. The content of selenium in the group of ducks fed concentrate mixtures without the addition of selenium on day 14, the content of selenium in blood plasma was more than twice lower (7.38 µg/100 g), and on day 49 it was 14.17 (g /100 g. The selenium content in the blood plasma of other groups of ducks increased in proportion to the increase in the amount of selenium added to the food so that on day 14 it was from 24.76 µg/100 g (K1 group) to 48.19 µg/100 g (K3 group), and On day 49, from 53.11 µg/100 g (K1 group) to 93.31 µg/100 g (K3 group). In all cases of comparison, the content of selenium in the blood plasma was higher on the 49th day compared to the 14th day of fattening ducks.

The content of selenium in the feces of ducks on the 14th and 49th day increases with the increase of the content of selenium in the food, which means that the degree of selenium excretion is proportional to its content in the food. It is inversely proportional to the status of selenium in the body, which can be explained by homeostatic control. The selenium content in the feces of ducks of all groups was always higher on day 49 than on day 14.
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