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by Mohammad Rudiansyah

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Original Article

The Role of Selenium on the Status of Mineral Elements and Some Blood Parameters of Blood Serum of Lambs

Jamal Rajab, W[1] *, Rudiansyah, M[2], Kadhim, M. M[3,4], Tolmasovich Shamsiev, A[5], Prakaash, A. S[6], Hadi Lafta, M[7], Aravindhan, S[8], Fakri Mustafa, Y[8]

1. Medical Laboratory Techniques Department, Al-Maarif University College, Ramadi, Iraq
2. Division of Nephrology and Hypertension, Department of Internal Medicine, Faculty of Medicine, Universitas Lambung Mangkurat, Ulin Hospital, Banjarmasin, Indonesia
3. Department of Dentistry, Kut University College, Kut, Wasit, 52001, Iraq
4. College of Technical Engineering, The Islamic University, Najaf, Iraq
5. Teaching Assistant, MD, Department of No.3 Therapeutic Disciplines, Tashkent State Dental Institute, Makhtumkuli 103, Tashkent, Uzbekistan
6. Department of Mathematics, Panimalar Institute of Technology Poonamallee Chennai, Chennai Tamilnadu, India
7. Iraqi Ministry of Education, Baghdad, Iraq
8. Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India

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Corresponding Author: islamov.2t@yahoo.com

Abstract

Selenium is one of the compounds belonging to the trace minerals group, which needs less than 100 mg/day. This element is one of the main constituents of selenoproteins, and the function of selenoproteins is to help make DNA and protect cells from damage and infection. This experiment aimed to evaluate the effect of different sources of selenium on some mineral elements in the blood serum of lambs. This experiment was conducted using twenty 4-month-old lambs with an average weight of 37±2.2 kg, 4 treatments, and 5 replications in a completely randomized design (CRD). The treatments tested included control, sodium selenite, nano selenium, and VitEsel. The experiment duration was 30 days, and blood sampling of lambs was performed at the beginning of the experiment (zero), 15, and 30 days. Different sources of selenium significantly affected the concentrations of iron, copper, and zinc (P<0.05). Different sources of selenium in this experiment decreased the concentration of iron and copper and increased the concentration of zinc and plasma selenium in different periods (P<0.05). Using different sources of selenium changed the concentration of the studied elements and showed the difference in their bioavailability.

Keywords: Selenium, Iron, Copper, Zink, Lamb

1. Introduction

Given that 75% of the cost of livestock is spent on the preparation and purchase of animal feed, recognizing food and identifying the nutritional needs of livestock is of particular importance (1, 2). Most foods used in animal feed are poor in some nutrients and require nutritional supplements (3). Among dietary supplements, high- and low-consumption minerals and vitamins are special importance (4). Selenium is one of the traces and essential mineral elements that is essential for the health (5), safety (6), and reproductive function of animals (7). Selenium was initially thought

to be a toxic element until Schwarz and Foltz proved the role of selenium as a nutrient (8). Selenium plays various roles in animals by expressing a wide range of selenoproteins (9). One of the most critical selenium-dependent enzymes in the body is the enzyme Triiodothyronine, which plays a vital role in converting the inactive form of thyroid hormones (thyroxin) to the active form (Triiodothyronine), and given that thyroid hormones are directly related to the body's general metabolism, thus increasing their concentration and activity can affect growth (10).

The role of selenium in thyroid peroxidase activity has also been identified and reported as a selenium enzyme that acts as iodine globulin and prevents degradation of the thyroid epithelial membrane (11). Because thyroid hormones affect lipid metabolism in the liver and tissues (12), the addition of selenium can affect the concentration of lipid parameters in the blood, including triglycerides, cholesterol, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (13).

The deficiency of this factor causes tissue damage, which leads to an increase in the enzymes aspartate aminotransferase (AST) and creatine phosphokinase (CPK) (14, 15). It usually occurs as white muscle disease in lambs born to selenium-deficient ewes (16). To know the status of selenium in sheep, its amount can be measured in serum, plasma, or red blood cells (17). Measurement of total blood selenium indicates the amount received through food (18) and normal blood selenium levels are 0.1 to 0.5 mg/L in sheep (19). The amount of this element in plasma shows the current state and its amount in erythrocytes in the last 3 months (20). Because selenium in ruminants is insoluble in the rumen by existing microbes (21). Selenium absorption is reported to be about 35% in ruminants and 85% in non-ruminants (22). From various sources of selenium supply, selenium methionine is absorbed in the small intestine through the methionine transport system, and sodium selenate and selenite enter the body as a passive (23). Selenium of any kind that is absorbed must be converted to selenide, which combines with selenocysteine and enters the structure of selenoproteins (24). Four factors such as grazing in sandy soils (25), annual rainfall above 450 mm (26), grazing in pastures with high clover content (27), and long-term use of sulfur and superphosphate fertilizers (28), cause selenium deficiency in pasture sheep. Deficiency of this element occurs when soil selenium is less than 0.5 mg selenium per kg of soil and less than 0.1 mg/kg forage (29).

Selenium is transmitted through the placenta to the fetus and milk and colostrum to the newborn, resulting in increased immunity and growth in the offspring (30). The sheep fed adequate selenium were reported to have higher concentrations of selenium in allantoic fluid, milk, and colostrum. Their lambs gained better weight during the two weeks of life (31). Soliman, Abd (32) stated that injecting 1 ml of vitamin E and selenium solution (containing 150 mg of vitamin E and 1.67 mg of selenium) into pregnant ewes increased the amount of T_3 hormone in ewes.

Abood, H. Judi (33) also showed an increase in CPK and AST activity in the blood of selenium and vitamin E deficient sheep, so there is a relationship between selenium and vitamin E levels and the concentration and activity of CPK and AST in the blood. However, in some other studies, no significant difference has been observed, possibly due to the breeding conditions and the amount of selenium in the basal diet (34, 35). Because the soil in many parts of the world is deficient in selenium, the symptoms of deficiency are common in livestock. This study was designed and conducted to investigate the effect of different sources of selenium on some mineral elements and blood parameters in lambs.

2. Materials and Methods

2.1. Animal Care and Experimental Treatments

In this experiment, twenty 4-month-old lambs with an average weight of 37.0±2.2 kg were used. The lambs used were first labeled, and then their weights were recorded. The selected animals were clinically examined at the beginning of this study, and their

health was ensured. Treatments included sodium selenite and nano-selenium orally at the rate of 0.15 mg/kg body weight for ten days, VitEsel (injected at the rate of 0.004 mg/kg body weight), and control (without selenium). Access to water and salt was *ad libitum*. The test was performed for 30 days, and blood samples were taken from lambs on the experiment days (zero), 15, and 30 days. The essential diet was adjusted based on racial needs (Table 1).

2.2. Preparation of Selenium Nanoparticles

The selenium oxide reduction method was used using ascorbic acid to prepare nano-selenium particles. The compounds used were selenium oxide and ascorbic acid produced by the German company Merck (36). There are several chemical and biological methods for preparing red selenium nanoparticles. This study used a chemical method based on the solution phase. To start the reaction, by adding a drop of ascorbic acid to the selenium dioxide solution, red nano-selenium particles begin to form, changing the solution's color from colorless to red. The resulting solution was placed in a quiet place for 48-72 hours to separate the nanoparticles without moving. In this method, the presence of water-soluble polymers was used as effective stabilizers and emulsifiers in selenium colloidal synthesis.

2.3. Blood Sampling and Sample Preparation

In this experiment, on days 0, 15, and 30 days, blood samples were taken from all lambs through the jugular vein, and blood samples were transferred to two separate tubes containing heparin and the other without heparin. After transferring the samples to the laboratory, they were centrifuged (at a speed of 3500 rpm), and their plasma or serum was separated for 20 minutes. Serum and plasma samples were stored at -20 °C until measurement. A standard kit measured plasma zinc and selenium concentrations (34) by atomic absorption spectrometer (VarianSpectrAA220) and serum iron. The optical density of the samples was read at 562 nm, and chromatography and electrophoresis instruments were used to measure serum ferritin. The activity of glutathione peroxidase and superoxide dismutase enzymes in whole blood was measured using a redox kit (made in England) according to the kit manufacturer's instructions and by spectrophotometer at wavelengths of 340 and 540 nm, respectively.

2.4. Statistical Analysis

The design was completely random. The statistical model of the design is as follows:

$Y_{ij}=\mu+T_i+e_{ij}$

Y_{ij}: The observed value of the i treatment in the j repeat

μ: Average effect

T_i: effect of i treatment

e_{ij}: The effect of experimental error related to i's treatment on j^{th} iteration

All collected data were edited using Excel software, and SAS statistical software (version 9.1) was used for statistical analysis with glmprocedure. Duncan's multivariate test at the significance level of 0.05 was used to compare the means.

Table 1. Chemical composition and ingredients of experimental diet

Feed ingredients	%	Chemical composition	%
Alfalfa hay	13.5	Metabolizable energy (Mcal/Kg)	2.78
Wheat straw	6.5	Crude protein	12.8
Beet pulp	10	Dry matter	60.7
Corn grain	32	Organic matter	93.5
Barley grain	32	Ether extract	3.1
Soybean meal	4.5	Ash	6.4
Carbonate calcium	0.6	Neutral detergent fiber	27.7
White salt	0.3	Non fibrous carbohydrate	54.1
Ammonium chloride	0.4	Calcium	0.6
Mineral vitamin premix*	0.2	Phosphorous	0.3

Non-fibrous carbohydrates = (ash + ether extract + Neutral detergent fibers + crude protein) -100

*Per kilogram of premix: 1,300,000 international units of vitamin A, 360,000 units of vitamin D, 1,200 units of vitamin E, 16 grams of zinc, 10 grams of manganese, 0.8 grams of iron, 0.12 grams of cobalt, 0.15 grams of iodine, and 0.08 grams of selenium

3. Results

The effect of different sources of selenium on iron concentration in the first period was significant (P<0.05). According to figure 1, using different sources of selenium reduced the plasma iron concentration compared to the control group, so using nano selenium compared to other sources of selenium reduced the plasma iron content to a lesser extent. With an increasing test period, injectable VitEsel increased the plasma iron concentration compared to the control group. While plasma iron concentration decreased significantly compared to the control group using oral sodium selenite. Analysis of day 30 samples showed that oral sodium selenite increased the plasma iron concentration compared to the control group, while the plasma iron concentration decreased significantly compared to the control group when using injected VitEsel (P<0.05). The highest plasma iron concentration in the whole period was related to the control group. The use of nano selenium reduced plasma iron concentrations to a lesser extent than oral sodium selenite and injectable VitEsel.

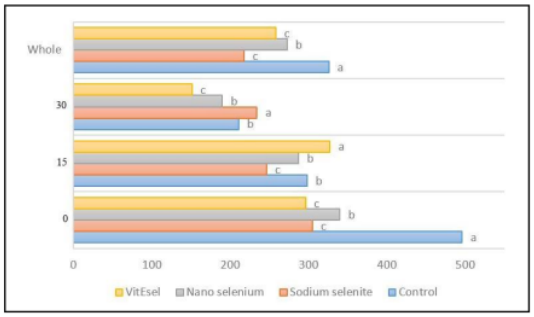

Figure 1. Comparison of average Fe (μg/dl) due to the use of different treatments in different periods
Dissimilar letters in each column indicate a significant difference (P<0.05)

According to figure 2, the effect of different sources of selenium on copper concentration in the first period was not significant (P>0.05), but in the second period, at a level of 5%, showed a significant difference (P<0.05). So that the concentration of copper at the time of using nano selenium had the highest decrease compared to the control group and other selenium sources. VitEsel injected selenium reduced plasma copper concentrations to a lesser extent than other sources of selenium. Analysis of variance of blood samples of the third period also showed that the use of VitEsel injected selenium reduced the amount of plasma copper compared to the control group and other sources of selenium (sodium selenite and oral nano selenium) (P<0.05). However, the highest plasma copper concentration in the whole period was related to the control group. In general, using VitEsel injected selenium significantly reduced plasma copper concentrations relative to oral sodium selenite and oral nano selenium.

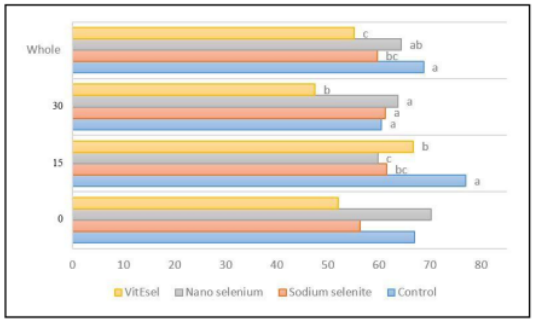

Figure 2. Comparison of average Cu (μg/dl) due to the use of different treatments in different periods
Dissimilar letters in each column indicate a significant difference (P<0.05)

According to the mean comparison in figure 3, using different sources of selenium reduced the element zinc compared to the control group in the first period. Among the sources of selenium used, oral sodium selenite had the most significant decrease in plasma zinc concentrations, while plasma zinc concentrations decreased the least when other selenium sources were used. The use of oral sodium selenite and injectable VitEselincreased the plasma zinc concentration compared to the control group in the second experimental period. While plasma zinc concentration decreased significantly compared to the control group using oral nano selenium (P<0.01). In the third period of the experiment, oral nano selenium increased the plasma zinc concentration compared to other treatments. Plasma zinc concentration showed a significant decrease

compared to the control group when injecting VitEsel (P<0.05). According to figure 3, the effect of different sources of selenium on plasma zinc concentration throughout the period was insignificant (P<0.05).

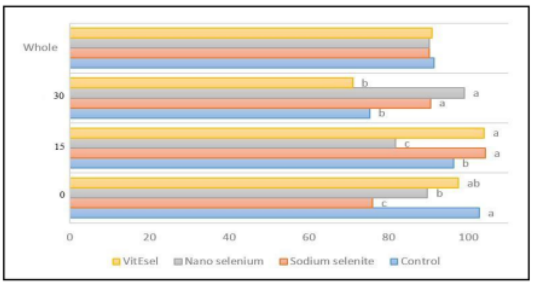

Figure 3. Comparison of average Zn (μg/dl) due to the use of different treatments in different periods
Dissimilar letters in each column indicate a significant difference (P<0.05)

The highest blood selenium in lambs was observed in oral sodium selenite treatment (P<0.01), and the control group had the lowest blood selenium (figure 4). The same effect was observed during the sampling period. The use of oral sodium selenite increased the selenium concentration in lambs. The effect of different selenium sources on blood selenium concentration in the third period was insignificant (P>0.05). However, in the analysis of variance of the whole period, he repeated the trend of the first and second periods. Blood selenium concentration increased significantly during the whole period when using oral sodium selenite. The use of oral nano selenium and VitEselalso increased the concentration of selenium in the blood compared to the control group.

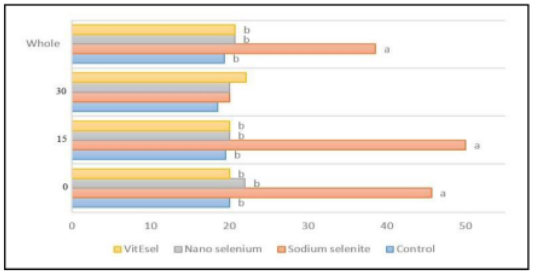

Figure 4. Comparison of average Se (μg/dl) due to the use of different treatments in different periods
Dissimilar letters in each column indicate a significant difference (P<0.05)

4. Discussion

The present study showed that using different sources of selenium in lambs reduced the concentration of iron in the blood plasma of these animals. Consistent with the results of this study, Aliarabi, Fadayifar (37) stated that adding selenium to the diet of fattening lambs significantly decreased plasma iron concentration. Kojouri, Jahanabadi (38) also showed that the use of selenium supplementation as sodium selenite reduced serum iron concentration. The researchers found that selenium supplementation in sheep reduced serum iron levels after 20 days and increased transferrin receptor gene expression at the level of bone marrow cells. Selenium may increase the transfer of transferrin into the cell by receptor-mediated endocytosis by increasing the expression of transferrin receptors on the cell surface of tissues, which reduces the serum iron concentration. However, when the cell is saturated with iron, the transferrin receptors decrease, and iron entry into the cell decreases. However, Jalilian, Moeini (39) did not observe a significant difference in plasma iron concentration in ewes receiving selenium and vitamin E supplementation. Injection of selenium into pregnant heifers also did not affect their iron concentration (40). Some studies have suggested that selenium is involved in regulating iron metabolism. It was also shown that selenium and glutathione peroxidase activity in their heart tissues decreased in mice that received high concentrations of iron for a long time, and by adding sodium selenate to their diet, the concentration of iron in heart tissue and oxidative stress decreased (38).

The results of this study showed that the use of different sources of selenium had a decreasing effect on plasma copper concentration. These findings contrast with the results of Cristaldi, McDowell (41), who stated that plasma copper concentrations in sheep receiving selenium and vitamin E were significantly increased. According to researchers (39), the copper concentration of selenium-receiving sheep in copper-deficient pastures also increased. The findings of Pechova, Misurova (42) also indicate an increase in the

concentration of copper in the serum of selenium-receiving goats. At the same time, Mohri, Ehsani (43) did not observe any change in plasma copper concentration by injecting selenium into newborn lambs. Also, Ali Arabi, Zand (44) did not observe any effect on copper concentration in male and female lambs by taking slow-release tablets of zinc, selenium, and cobalt. Administration of selenium to pregnant heifers did not affect their serum copper concentration (40).

Few studies have investigated the interaction of selenium and copper in ruminants. Most reports have been in the form of selenium injections, which have eliminated the rumen environment's possible effects of the rumen environment (43). Plasma copper concentrations in ruminants range from 0.55 to 0.95 mg/L (45). Due to the antagonistic relationship between zinc and copper, it has been reported that serum copper concentrations in zinc-receiving buffalo calves decreased by 250 and 1000 mg/kg of dry matter (46). High concentrations in the diet stimulate the synthesis of metallothionein, which inhibits the uptake of zinc and copper in intestinal enterocytes due to its strong affinity for zinc and copper. No significant difference in plasma copper concentration was reported at times receiving different levels of zinc per day in the form of zinc sulfate and methionine (47). On the other hand, no significant difference was observed in plasma copper concentration in calves supplemented with 35 mg/kg zinc as zinc sulfate and zinc propionate (48).

Oral sodium selenite, oral nano-selenium, and injectable vitamin E selenium increased the plasma zinc concentration compared to the control group. Plasma zinc concentrations in ruminants range from 0.8 to 1.4 mg/L (45). Consistent with the results of this study, it was reported that taking slow-release tablets containing zinc, cobalt, and selenium in sheep increased plasma zinc concentrations compared to the control group (49). Subsequent injections of two doses of selenium and vitamin E into pregnant heifers did not significantly change the plasma concentrations of heifers before and after calving (40). In a similar study, the addition of zinc to the diets of fattening male lambs increased plasma zinc concentrations (50). Due to the homeostasis of zinc uptake and metabolism (51), increasing plasma zinc concentrations by adding zinc to diets is very difficult except for zinc-deficient animals (52). However, considering that the storage capacity of zinc in the body is weak, therefore, this element needs to be supplied continuously through the diet. Researchers stated that the zinc concentration in selenium-receiving ewes was significantly reduced compared to the control group (39). Serum zinc concentrations also decreased in selenium-receiving heifers (53). They said that more zinc supplements should be given to pregnant heifers when more than 80 milliliters or 40 milligrams of selenium supplements. However, Kojouri and Shirazi (54) administered selenium and vitamin E to pregnant ewes and reported that their serum concentrations did not differ significantly, probably due to the level of zinc and selenium in the diet. Copper changes may have an indirect effect on serum concentrations. The absorption of zinc at the intestinal surface by copper and cadmium is impaired by increasing the synthesis of metallothionein (47). Metallothionein is a form of storage of divalent elements such as copper and zinc (except iron). As the synthesis of metallothionein increases, the excretion of zinc increases, and in the conditions of selenium deficiency, the thiolmetallothionein group is oxidized. As a result, urinary excretion of zinc is reduced, and this element is used for essential reactions (39). Therefore, the increase in lambs' plasma concentration can be considered a decrease in copper concentration.

Using different sources of selenium increased the concentration of selenium in cattle blood throughout the experiment. The comparison table shows that blood selenium concentration increased significantly during the whole period when using oral sodium selenite. The use of oral nano selenium and vitamin E selenium increased the concentration of selenium in the blood compared to the control group. Consistent with the results of the present study, ewes receiving slow-

release tablets of copper, cobalt, and selenium had higher blood selenium concentrations compared to the control group (55). In cows fed selenium, a higher concentration of plasma selenium was recorded in the control group. Also, adding different sources of selenium to the diets of pregnant ewes increased the serum selenium concentration before and after calving (56). Consecutive selenium injections at 4 and 8 weeks before calving and 1 week after calving to pregnant ewes increased the plasma selenium concentration compared to the control group before calving. These ewes had higher blood selenium concentrations than the control group up to 5 weeks after calving (54). Sheep with plasma concentrations of 25 to 53 selenium per liter were severely deficient in selenium, but plasma concentrations of 160 to 170 micrograms per liter indicated an excellent selenium concentration in the basal diet (57). Therefore, this study's results show that using different sources of selenium caused a continuous supply of selenium and reached the desired level of plasma selenium concentration.

5. Conclusion

Using different sources of selenium changed the concentration of the studied elements and showed the difference in their bioavailability.

Authors' Contribution

Study concept and design: W. J. R. and M. M. K.
Acquisition of data: M. R. and A. S. T.
Analysis and interpretation of data: A. S. P.
Drafting of the manuscript: M. H. L.
Critical revision of the manuscript for important intellectual content: W. J. R. and M. R.
Statistical analysis: Y. F. M.
Administrative, technical, and material support: S. A.

Ethics

The study was approved by the ethics committee of the Al-Maarif University College, Ramadi, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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