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Biochemical Changes in Blood Serum after Selenium and Zinc Doses and their Effect on the Health and Environment of Kurdi Sheep in Kurdistan Region

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ABSTRACT

This study intended to know the effect of sodium selenite and zinc sulfate on the biochemical metabolites in the blood serum of sheep. The experiment was conducted on male sheep of the Kurdi ram and lamb breed in four treatments each as follows; Treatment 1: (control group) without addition, Treatment 2: addition of selenium at a level of 0.5 mg/kg feed, Treatment 3: addition of zinc at a level of 100 mg/kg feed, Treatment 4: addition of selenium with zinc at a level of 0.5 + 100 mg/kg feed for 90 days. The results were significant in the levels of glucose, LDL cholesterol, creatine, immunoglobulin M (IgM), and HDL cholesterol in the second treatment compared to the other treatments and there were no significant differences in the levels of cholesterol and triglycerides in the blood serum. The level of cholesterol increased in the fourth treatment compared to the other treatments in the blood serum, and significant differences were found in the level of albumin and immunoglobulin (IgM) in the fourth treatment compared to the rest of the treatments, and immunoglobulin level (IgG) lowered in all addition treatments compared to the control treatment in the blood serum. We conclude from these results that the addition of selenium and zinc mixture improves some biochemical and immunological properties in the blood serum of rams and lamb of the Kurdi sheep breed.

INTRODUCTION

Livestock intensification aims to significantly increase the production of meat, milk, and other animal products by enhancing livestock productivity, thereby achieving the highest output per consumed feed unit with minimal cost and labor. Basic the primary method for improving animal productivity is through proper nutrition, as minerals absorbed from feed and water play a vital role in animal health. Deficiencies in certain trace elements can lead to a decline in productivity and a reduction in the overall output from livestock. Moreover, mineral deficiencies can cause substantial economic losses for livestock operations, as they often result in diseases and metabolic disorders in animals (Zaslavskyi, 2024).

Selenium (Se) is an essential dietary mineral element in farm animal diets that acts as an essential component of at least 25 selenium chelates (SELs) and plays a vital role in antioxidant defense states. It has been confirmed that selenium supplementation supports livestock health, especially the digestive system, productive performance, antioxidant status, and reproductive and immune efficiency (Zheng et

al., 2022; Bolshakova & Aljaf, 2023). However, the mechanism of action of selenium in the heart and its association with it in the blood has not yet been precisely determined (Rose & Hoffmann, 2015) (Joseph, 2013).

To increase the productivity of farm animals, the farmer is trying to develop a new feed handling technique to improve the quality of feed production and economy (Sunarso, 2003). In this technology aspect regarding feed quality, farmers and livestock keepers are looking to reduce the use of mineral salts in the diet, and experiments and research indicate that the mineral salt requirements of ruminants are very small. Most Forage plants typically contain varying levels of both trace and essential minerals, which are crucial for maintaining mineral balance in livestock. To prevent deficiencies that could lead to various diseases and ensure optimal productivity, livestock are often supplemented with mineral salts (Solaiman et al., 2006). Among these minerals, selenium and zinc are particularly important for the raising of livestock and breeding.

The absorption of trace minerals by the animals is utilized in metabolic processes and contributes to improved performance, overall health, immune function, and reproductive efficiency. Although selenium was once regarded as a toxic substance in certain plants and animals, but recently been considered as an essential nutrient that plays a critical role in promoting growth and reproductive health in livestock (Engle., 2007). The primary function of selenium is to synthesize glutathione peroxidase, which helps inhibit oxygen radicals such as hydrogen peroxide and prevent them from generating cell harm (NRC. 2005). Zinc promotes cell proliferation and division, is an antioxidant, immune cells, sexual development, dark adaptation, taste, and consumption (Solomon., 1993). Selenium as well as zinc are rare important nutrients that play a major role in well-being. They are contributed by various enzymes and play a major role in improving metabolic processing and defending against oxidative destruction (Tinggi, 2008; Wang et al., 2017; Chung et al., 2019; Marreiro et al., 2017).

Many studies indicate that they are very necessary to maintain reproductive efficiency and the high performance of the endocrine gland and immune system (Tinggi, 2008; Wang et al., 2017). Zinc is the basis of metabolic reactive, as it acts as a multifactor for more than three hundred enzymes, in addition to acting as a powerful antioxidant (Chung et al., 2019). All scientific experiments that analyze the effect of selenium and zinc supplements in the blood on hypertension of arteries are inconsistent (Kuruppu et al., 2014). The only confirmed association is related to low selenium concentrations in the blood, leading to diseases in the body that disappear with selenium supplements (Sun et al., 2019). Other investigations are conflicting, as some indicated no relation between diseases and the concentration of zinc or selenium in the blood (Yao et al., 2018; Arnaud et al., 2007). while scientific experiments indicate the existence of such an association. Many studies have found that higher amounts of selenium and zinc in the blood lead to the presence of the disease (Vinceti et al., 2019) (Grotto et al., 2018) (Kunutsor and Laukkanen., 2016). While other scientific studies and research have found opposite results (Li et al., 2019; Hu et al., 2017).

Research has demonstrated that minerals play a critical role in various physiological processes, including the intracellular detoxification of free radicals, the biosynthesis of steroids, and the cellular metabolism of carbohydrates, proteins, and DNA. These minerals exert either beneficial or detrimental effects on the animal's physiological state, depending on their balance within the body. Both insufficient and excessive intake of dietary minerals can lead to a range of negative health outcomes. Notably, the effects of mineral imbalances often manifest in higher neural centers, particularly within the hypothalamus, which plays a central role in regulating various bodily functions. In this context, the present study aimed to investigate the effects of selenium and zinc either individually or in combination on key immunological and biochemical parameters in the serum of Kurdi sheep, specifically lambs and rams. This research seeks to enhance our understanding of how these minerals influence the health and physiological functions of livestock.

MATERIALS AND METHODS

The current scientific experiment was carried out in the summer season in Bakrajo, located in the city of Sulaymaniyah, Sulaymaniyah Governorate, Kurdistan Region of Iraq. 16 rams of Kurdi sheep

breed were used, aged between 16-18 months, and 16 Kurdi sheep breed lambs, between 3-4 months. The rams and lambs were allocated into 4 groups, with 4 lambs and 4 rams in every group.

The first group was considered as a control group and fed normally without any additions; the second group was fed selenium in the form of (sodium selenite) Na_2SeO_3 (0.5 mg per kg feed), the third group was fed zinc in the form of (zinc sulfate) $ZnSO_4$ at a (100 mg per kg acre), and the fourth group was fed (mixture of selenium and zinc) (0.5 + 100 mg per kg acre). Then, all animals were randomly divided and fed individually for sixty days, the diameter of the cages was 1x1.5 m^2.

The feed composition was barley (60%), soybeans (12%), and without any amount of selenium. The feed also contained wheat bran (26%), salt (1%), limestone (0.5%), and a mixture of vitamins and minerals (0.5%). Empty gelatin capsules were used for the current experiment to put selenium and zinc and give them to the sheep daily when the feed is provided in the morning meal. The amount of zinc and selenium is weighed using a sensitive balance and this amount is counted according to the daily feed consumed amount by animals. Selenium and zinc are mixed alone or mixed with corn flour and placed in an empty capsule. The capsule is given daily to the animals for ninety days before feeding in the morning time.

The experimental animals are cut off from feed and water for 12 hours before blood collection. Blood samples were collected via the jugular vein using a 5 ml sterile syringe. The blood was drained into gel tubes and centrifuged at 3000 rpm for 15 min to obtain the serum. The serum was placed in sterile plastic tubes, numbered, and stored at -20°C in the freezer until the experimental samples were analyzed.

The biochemical characteristics of the serum were analyzed using a special kit (CORMAY SA) in an automatic chemical analyzer (accent 200, Poland). The experimental design was a completely randomized factorial design (CRD) to estimate the impact of selenium and zinc on human lifespan. The analysis was performed using XLstat (2016) according to this equation:

$$Yijk = \mu + Ai + + Bj + AB\ (ij) + eijk$$

Where Yijk = dependent variable, μ = overall mean, Ai = effect of applying Se and Zn factor, Bj: effect of age factor, ABij = effect of interactions between two factors, eijk = standard error, comparison of mean according to Duncan (1955) within the program.

RESULTS AND DISCUSSION

The results of the current experiment, presented in Table 1, indicated that the addition of selenium led to the most significant increase in glucose levels compared to the other treatments. However, no significant differences were observed in the levels of cholesterol and triglycerides in the blood serum of lambs across the various treatments. In rams, the glucose levels also showed the highest increase with selenium supplementation, while the cholesterol levels were notably higher in the group receiving a mixture of selenium and zinc (fourth treatment) compared to all other treatments. These findings highlight the varying effects of selenium and selenium-zinc supplementation on different biochemical parameters in the blood serum of lambs and rams.

Table 1. Effect of selenium and zinc supplementation on glucose, cholesterol, and triglyceride levels in the serum of lambs and rams of Kurdi sheep

Treatments	Lamb			Ram		
	Glucose (mg/dl)	Cholesterol (mg/dl)	triglycerides (mg/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	triglycerides (mg/dl)
T1(Control)	66.000 b ± 0.58	36.000 a ± 3.21	11.333 a ± 1.45	71.667 b ±0.67	35.000 b ± 1.73	12.667 b ± 0.88
T2(Se)	72.667 a ± 3.53	48.333 a ± 7.42	17.667 a ± 4.26	75.667 a ±0.67	38.667 b ± 0.33	14.000 b ± 2.08
T3(Zn)	72.000 ab ± 0.58	38.333 a ± 1.2	21.000 a ± 6.81	73.000 b ± 1	38.000 b ± 1.53	25.333 a ± 0.33
T4(Se+Zn)	70.000 ab ± 1.15	45.333 a ± 1.45	14.667 a ± 1.45	71.333 b ±0.33	43.667 a ± 0.88	15.667 b ± 1.2

Means with different letters within each column differ significantly (P≤0.05) according to Duncan's test.

These results are in agreement with the results of Shahat and Abdel Monem (2011) and with the results of Ziaei (2015). where no significant differences appeared. This element plays an important and necessary role in many physiological processes in the body and other processes in goats (Ganabadi et al. 2010). The results were also in agreement with the study (Sushma et al. 2015) when adding selenium to some blood measurements of Indian Nellore male lambs, because the results were not significant in biochemical blood measurements, and the researchers explained that the reason may be due to the presence of selenium in the feeding system such as green fodder 0.009 ppm and dry bran 0.11 ppm or a concentrate mixture of 0.19 ppm and this satisfies sheep's selenium requirement of 0.3 ppm (NRC 2001). (Zarczynska et al. 2013) indicated that the selenium concentration in the blood serum varies from one region to another according to consumption and type of fed, and the result data cannot be adopted for another region.

Table 2. Effect of selenium and zinc supplementation on HDL, LDL and creatine levels in the serum of lambs and rams of Kurdi sheep

Treatments	Lamb			Ram		
	HDL (mg/dl)	LDL (mg/dl)	Creatine (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	creatine (mg/dl)
T1(Control)	20.333 a ± 2.03	16.333 b ±0.33	0.900 ab ± 0	24.667 a ± 0.67	21.667 c ± 0.33	0.800 a ± 0.35
T2(Se)	22.333 a ± 1.45	27.333 a ± 2.96	0.967 a ± 0.03	26.667 a ± 0.67	35.667 a ± 0.67	1.367 a ± 0.03
T3(Zn)	24.000 a ± 1.15	16.333 b ±0.33	0.800 c ± 0	26.333 a ± 3.18	11.667 d ± 0.33	1.067 a ± 0.09
T4(Se+Zn)	24.000 a ± 1.53	20.667 b ±0.33	0.833 bc ± 0.03	28.333 a ± 0.67	26.000 b ± 1	1.033 a ± 0.07

Averages with different letters within each column differ significantly (P≤0.05) according to Duncan's test.

The data presented in Table 2 indicate that there were no significant differences in the concentration of high-density lipoprotein (HDL) across all treatments when compared to the control group. Similarly, in lambs, the levels of low-density lipoprotein (LDL) and creatine were higher in the second treatment, which involved selenium supplementation, compared to the other treatments and the control group, although these differences were not statistically significant. In rams, no statistically significant differences were observed in HDL and creatine concentrations across the various treatments, including the selenium supplementation, when compared to the control group. However, the LDL levels were higher in the second treatment (selenium addition) compared to both the other treatments and the control group. These variations in serum biochemistry may be influenced by environmental factors, which can significantly affect nutrient absorption and metabolism (Bolshakova and Aljaf, 2022). Additionally, the observed differences may be attributed to the specific selenium and zinc supplementation levels, as well as the type and breed of the animals used in the study.

The results of the experiment from Table (3) showed that the albumin concentration decreased in the fourth treatment compared to the other treatments and the standard group, while the IgM concentration increased significantly in the fourth treatment compared to the other treatments and the control group, and the IgG concentration decreased in all addition treatments compared to the control group in the blood serum of lambs.

Table 3. Effect of selenium and zinc supplementation on Albumin, IgM, and IgG levels in the serum of lambs and rams of Kurdi sheep

Treatments	Lamb			Ram		
	Albumin (g/dl)	IgM (g/dl)	IgG (g/dl)	Albumin (g/dl)	IgM (g/dl)	IgG (g/dl)
T1(Control)	4.233 a ± 0.03	16.333 d ±0.88	1.233 a ± 0.03	4.633 b ± 0.13	14.667 c ± 0.67	1.133 a ± 0.03
T2(Se)	4.233 a ± 0.09	26.333 b ±0.33	0.967 b ± 0.07	5.900 a ± 0.25	24.333 a ± 0.33	0.833 b ± 0.03
T3(Zn)	4.233 a ± 0.09	21.667 c ± 0.67	1.133 ab ± 0.03	5.167 b ± 0.15	20.000 b ±0.58	0.900 b ± 0.1
T4(Se+Zn)	3.967 b ± 0.07	28.333 a ± 0.33	0.933 b ± 0.09	6.367 a ± 0.09	25.000 a ± 0.58	0.733 b ± 0.03

Means with different letters within each column differ significantly (P≤0.05) according to Duncan's test.

The results in the blood serum of rams showed that the concentration of albumin and IgM increased in the second treatment when selenium was added alone and the fourth treatment when a mixture of selenium and zinc was added compared to the third treatment when zinc was added alone and the standard group without any addition, and the concentration of IgG decreased in all addition treatments compared to the standard group.

Habib et al. (2013) show that blood plasma zinc concentration correlates with feed Zinc levels. (Yue et al. 2009) The reason may be that zinc plays the role of an antioxidant and a cofactor for many enzymes. It protects the cell membrane against oxidative damage. Selenium and zinc supplementation has been shown to significantly improve antioxidant status and some serum biochemical in Kurdi rams. (palani et al.,2018). (Bettger and ODeel, 1981) addition of selenium improves the state of oxidative stress in sheep (Moasaie et al, 2017) It is necessary to protect cells from free radical damage (Zurczynska et al., 2013) and acts as an influential antioxidant (Hassan et al, 2017).

We did not find in this experiment previous studies similar to the current experiment and with the same concentrations and sources to compare with our results. The reason may be the increase in blood serum when treated with selenium in the form of sodium selenite and zinc in the form of zinc sulfate due to their great role in improving the immune system because they contain effective antioxidants. Since sheep farming in Kurdistan depends on natural pastures from mountains and plains as a main source of nutrition, this may lead to a deficiency of rare mineral salts and limit the productivity of agricultural animals. Therefore, we recommend adding selenium and zinc to the feed. The results of the current experiment provide an overview of some biochemical properties and some immune properties in the blood serum of rams and lambs from the Kurdi sheep breed located in Sulaymaniyah Governorate in the Kurdistan Region of Iraq. Adding a mixture of selenium and zinc was the best performance to improve immunity. It is recommended to add additional supplements of these rare mineral salts. Additional factors that may affect the variation in the status of mineral salts are the season, maturity of feed types, and the biological availability of chemical forms of rare mineral salts. From the above, it is clear that the combined doses of selenium and zinc used in this study did not have any toxic effects on the Kurdi sheep breed.

CONCLUSION

The current research has shown the positive effect of selenium supplements in the form of sodium selenite and zinc in the form of zinc sulfate or their combination in strengthening and improving immunity in the blood serum. This provides better protection for sheep from oxidation states, as well as protecting the body from the accumulation of harmful organisms and giving it strong immunity against various diseases in the body. The dose of their combination of selenium and zinc at a concentration of 0.5 and 100 mg/kg feed has significantly and significantly improved the level of immunity in the blood serum of lambs and rams of the Kurdish sheep breed.

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