Cooperative and antagonistic reactions of heavy metallic elements and its impact on health that are supplemented with selenium and zinc in Kurdi sheep

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Abstract---The purpose of present study was to determine the variations in the levels of Selenium, Zinc, Iron, Copper, Molybdenum and Manganese in the serum of Kurdi sheep and to compare the age difference in the levels of minerals between lambs and rams that are supplemented with Selenium and Zinc individually or their combination. It was on two experiences. The males of the Kurdi sheep from lambs and rams for four coefficients each as follows; First Treatment: (control) without adding, Second Treatment: added Selenium with a dose of 0.5 mg/kg fed, Third Treatment: added Zinc with a dose of 100 mg/kg fed, Fourth Treatment: added Selenium with Zinc with a dose of 0.5 + 100 mg/kg fed, then they were given gelatinous capsules daily for 60 days. Selenium and Zinc supplementation and their combination led to significant increase in the Selenium and Zinc concentration in the blood serum of lambs and Rams. Selenium, Zinc and their combinations significantly contributed to changes in the analysed minerals (Selenium, Zinc, Iron, Copper Molybdenum and Manganese). The results of this experiment suggest that Selenium and Zinc supplementation might lead to a decrease in
levels of other health-promoting elements such as Copper, and an increase in Selenium, Zinc, Iron, Molybdenum, and Manganese in the blood serum of lambs and Rams of Kurdi sheep. A significant decrease in the Copper content may induce disease symptoms of the deficiency of these trace elements in animals.

Keywords---iron, copper, molybdenum, manganese.

Introduction

Sheep operations in Kurdistan rely on rangelands as their primary feed source, which could lead to clinical or subclinical trace mineral deficiencies and limit animal productivity. Minerals are inorganic substances found in all tissues of the body and fluids, and their presence is necessary to preserve some of the physical and chemical processes that are essential to life. Although minerals do not produce energy, they play important roles in many biological activities in the body (Malhotra, 1998). Maintaining good animal health, growth, and proper performance depends on the appropriate concentrations of trace elements and their correct balance in tissues and organs. The animal supply required with the partial and large elements is subject to animal species, physiological condition, age, and production type (Hylland et al. 2009). Small ruminant production is the main economic output, as it contributes to farmers' incomes in many parts of Asia and Africa (BenSalem and Smith 2008). The deficiency of trace minerals can affect the production and performance of sheep and goats (Lengarite et al., 2012) (Xin et al., 2011). Scarcity of mineral deficiency is more common in small ruminants raised under the traditional system and/or pasture of poor quality pastures (Kawas et al., 2010) (Sowande et al., 2008). Mineral deficiency is not easily recognized, especially in sheep and goats because the deficiency is present in flocks in a semi-clinical form and affects fertility, wool yield, and growth rate (Vázquez - Armijo et al., 2011). Also, mineral deficiency causes displacement in miscarriage, retention of placenta, death of lambs, poor savings, reduced immunity, increased susceptibility to bacterial and parasitic infections, and affects animal welfare (Suttle, 2010). Deficiencies in trace elements such as cobalt, copper, iron, zinc, and selenium have been reported in all climatic and geographic regions of the world (Underwood and Suttle, 2001). Selenium and zinc are among the rare elements that influence metabolism and health, and their deficiency leads to health disorders. Moreover, it is a very specific factor in the early stages of growth, especially with the decrease in fed protein, which is required for the synthesis of protein (Pavlata et al., 2009). Zinc is essential for growth hormones, as it plays an important role in metabolism, and is necessary for growth and feed conversion efficiency (Baltaci et al., 2004). It is necessary to add and provide rare mineral elements in the fed of sheep, as its deficiency causes changes in animal behavior and blood components (Ebrahim et al., 2016). Selenium and Zinc are among the most deficient minerals in animal feed and have great importance through their major roles in the growth and fertility of animals. (Page et al., 2016). Selenium and Zinc are important nutrients for animal health, hormone regulator, and some biochemical indicators in blood serum (Palani et al 2018a). Shown the beneficial effect of supplementation of selenium and zinc on Glutathione activity in Liver and kidney tissues which may provide better protection to from oxidative damage.
Which may play a role in efficiency of Kurdi lambs (Palani et al., 2020). The low level of Selenium and Zinc in the blood of Kurdi sheep is due to the low level in plants, and to the low concentration in the soil of Sulaimaniyah Governorate located in the Kurdistan Region of Iraq. The addition of selenium and zinc to the diets of lambs and rams raises their level in the blood of Kurdi sheep (Palani, 2019). Zinc significantly improves the efficiency of the growth of Kurdi lambs (Palani et al. 2018 b). That Selenium supplementation with a dose of 0.5 mg/kg of feed improves animal health and has no detrimental effect on the environment (Palani et al., 2019). No previous study has quantified trace mineral status in Kurdistan breeding sheep populations. Therefore, the aim of this study was to detect the increase and decrease of some trace elements by analysing blood in lambs and rams of the Kurdi sheep breed. In addition, the effectiveness of complementary therapy to correct the deficiency was another target.

**Materials and Methods**

The present study was conducted during summer season in Sulaymaniyah governorate in Kurdistan Region of Iraq. 12 Kurdi rams that aged between 16-18 months, then the rams were divided into four groups with three rams in each group. The first group control received normal fed without adding, the second group added Selenium (Sodium Selenite) Na2Seo3 with a dose of 0.5 mg/kg of fed, the third group added Zinc (Zinc sulfate) ZnSO4 with a dose of 100 mg / kg fed, the fourth group added (Selenium with Zinc) with a dose of 0.5 + 100 mg / kg fed. Moreover, 12 male Kurdi lambs were taken from 3-4 months aged, The lambs were divided into four groups with 3 lambs in each group. The first group control received normal fed without addition, the second one applied Selenium (Na2SeO3) with a dose of 0.5 mg/kg of fed, the third group added Zinc (Zinc sulfate) ZnSO4 with a dose of 100 mg / kg fed, the fourth group added (Selenium with Zinc) with a dose of 0.5 + 100 mg / kg fed. After that, all the rams and lambs were randomly distributed and each was placed in one cage with an area of 1 * 1.5 m² for 60 days individual feeding. The animal fed was consisted of 60% Barley, 12% Soybean which did not contain any amount of Selenium; besides that, the fed contained 26% Wheat bran, 1% salt, 0.5% limestone, and 0.5% mixed of minerals and vitamins. Capsule used to apply Selenium and Zinc for animals, in which amount of Se and Zn has been taken and weighted by sensitive balance, and these were according to amount of consumed pasture for each animal. Where the Selenium and Zinc mixed with corn powder and put in empty capsule. Then the capsule was daily given to the animals through 60 days in the morning with their fed. Where the fed and water were cut off from the experiment animals for 12 hours before the blood was withdrawn. The blood was collected through the jugular vein by a 5 ml medical syringe and the blood was emptied into sterile laboratory plastic tubes. To the laboratory and placed in the centrifuge 3000rpm/min for 15 minutes to separate the blood serum from the rest of the blood components and then put the blood serum in sterile plastic tubes and sealed and stored under the temperature of -20 ° C in the freezer until the analysis of the experiment, and it was determined by ICPE-9000 from Shimadzu Japanese made, used to evaluate the minerals content in blood serum after dilution of 1: 3 nitric acid, per chloric digested for 2 hours at a temperature of 120 C to completing deionized water 10 ml. The design of experiments was a Factorial Complete Randomized Design (CRD) to determine the effects of Selenium and Zinc on age.
The analysis is conducted by using XLstat (2016) according to this equation: \( Y_{ijk} = \mu + A_i + B_j + AB(ij) + e_{ijk} \) where \( Y_{ijk} \) is the dependent variable, \( \mu \) = overall mean, \( A_i \) = effect of apply Se and Zn factor, \( B_j \): effect age factor, \( AB(ij) \) = effect of interactions between two factors, \( e_{ijk} \) = standard error, means compared according to Duncan (1955) within the program.

Results and Discussion

The table (1) showed significant differences (\( P \leq 0.05 \)), as the level of Selenium (Se) increased in the second treatment and the fourth treatment when compared to the control group, and the highest increase in the level of Selenium in the fourth treatment compared to other treatments. It was found that the level of Zinc (Zn) increased in the third treatment, the differences were significant (\( P \leq 0.05 \)), addition of Zinc and the fourth treatment compared to the second treatment and the control group. No significant differences (\( P \geq 0.05 \)) appeared in the second treatment compared to other treatments and the control group. The Iron (Fe) level showed an increase in serum and the differences were significant (\( P \leq 0.05 \)) in all treatments compared to the control group. The highest increase in the level of Selenium in the fourth treatment compared to other treatments. While the serum Copper (Cu) level decreased, the differences were significant (\( P \leq 0.05 \)) in all addition treatments compared to the control group, and the highest decrease in the fourth treatment compared to other treatments. Significant differences (\( P \leq 0.05 \)) in serum Molybdenum (Mo) level showed an increase in the addition treatments compared to the control group. It showed significant differences (\( P \leq 0.05 \)) in the level of Manganese (Mn). In addition, treatments compared to the control group, and the highest increase in the third treatment compared to other treatments and the control group.

Table (1): Influence of Selenium and Zinc Supplemental on Serum Mineral Profile in Kurdi Lambs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Se ppb</th>
<th>Zn ppm</th>
<th>Fe ppm</th>
<th>Cu ppm</th>
<th>Mo ppb</th>
<th>Mn ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Control)</td>
<td>19.2±1.1 c</td>
<td>0.526±0.1 b</td>
<td>0.485±0.1 d</td>
<td>28.1±0.6a</td>
<td>128.3±3.1 c</td>
<td>0.711±0.1 c</td>
</tr>
<tr>
<td>T2 (Se)</td>
<td>22.6±0.3 b</td>
<td>0.507±0.1 b</td>
<td>0.727±0.1 c</td>
<td>22.3±0.8c</td>
<td>154.3±1.8 a</td>
<td>0.799±0.1 b</td>
</tr>
<tr>
<td>T3 (Zn)</td>
<td>19.8±1.1 c</td>
<td>0.784±0.1 a</td>
<td>0.781±0.1 b</td>
<td>24.6±0.4b</td>
<td>142.3±2.7 b</td>
<td>0.931±0.1 a</td>
</tr>
<tr>
<td>T4 (Se+Zn)</td>
<td>27.1±1.1 a</td>
<td>0.793±0.1 a</td>
<td>0.859±0.1 a</td>
<td>19.3±0.8d</td>
<td>161.1±2.0 a</td>
<td>0.785±0.1 b</td>
</tr>
</tbody>
</table>

Means with different letters within each column differ significantly (\( P \leq 0.05 \)) according to Duncan’s test.

Table (2) showed significant differences (\( P \leq 0.05 \)), as the Selenium (Se) level in the second treatment and the fourth treatment increased compared to the third treatment and the control group. It was found that the level of Zinc (Zn) increased in the third and fourth treatment, and the differences were significant (\( P \leq 0.05 \)) compared to the second treatment and the control group. The Iron level showed an increase in serum and the differences were significant (\( P \leq 0.05 \)) in all
treatments compared to the control group. While the serum Copper (Cu) level decreased, the differences were significant (P ≤ 0.05) in all addition treatments compared to the control group, and the highest decrease in the fourth treatment was the addition of Selenium with Zinc compared to other treatments. Significant differences (P ≤ 0.05) in serum Molybdenum (Mo) level showed an increase in the second and fourth treatments compared to the third treatment and the control group. It showed significant differences (P ≤ 0.05) in the level of Manganese (Mn) in the third and fourth treatments compared to the second treatment and the control group.

Table (2): Influence of Selenium and Zinc Supplement on Serum Mineral Profile in Kurdi Rams

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Se ppb</th>
<th>Zn ppm</th>
<th>Fe ppm</th>
<th>Cu ppm</th>
<th>Mo ppb</th>
<th>Mn ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Control)</td>
<td>21.3±0.3 b</td>
<td>0.324±0.1 b</td>
<td>0.362±0.1 c</td>
<td>41.1±1.2 a</td>
<td>98.3±1.5 b</td>
<td>0.548±0.1 b</td>
</tr>
<tr>
<td>T2 (Se)</td>
<td>26.3±0.8 a</td>
<td>0.344±0.1 b</td>
<td>0.551±0.1 b</td>
<td>33.6±1.4 b</td>
<td>131.3±2.9a</td>
<td>0.588±0.1 b</td>
</tr>
<tr>
<td>T3 (Zn)</td>
<td>19.6±0.8 b</td>
<td>0.474±0.1 a</td>
<td>0.608±0.1 b</td>
<td>35.3±0.3 b</td>
<td>97.3±3.3 b</td>
<td>0.685±0.1 a</td>
</tr>
<tr>
<td>T4 (Se+Zn)</td>
<td>27.1±0.5 a</td>
<td>0.505±0.1 a</td>
<td>0.739±0.1 a</td>
<td>29.1±1.1 c</td>
<td>134.1±5.9a</td>
<td>0.655±0.1 a</td>
</tr>
</tbody>
</table>

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

Table (3) showed that the Selenium (Se) level increased and the differences were significant (P ≤ 0.05) in the second treatment in rams compared to the second treatment in lambs. The differences were significant (P ≤ 0.05) in the level of Zinc (Zn), Iron (Fe), Molybdenum (Mo) and Manganese (Mn) in all lamb treatments, compared to the rams treatments. The Copper (Cu) level increased and the differences were significant (P ≤ 0.05) in all ram treatments compared to lamb treatments.

Table (3): Influence of Selenium and Zinc Supplemental on Serum Mineral Profile in Kurdi Lambs and Rams

<table>
<thead>
<tr>
<th>Kind</th>
<th>Treatment</th>
<th>Se ppb</th>
<th>Zn ppm</th>
<th>Fe ppm</th>
<th>Cu ppm</th>
<th>Mo ppb</th>
<th>Mn ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb</td>
<td>T1 (Control)</td>
<td>19.1±1.1 c</td>
<td>0.526±0.1 b</td>
<td>0.485±0.1 d</td>
<td>28.1±0.6c</td>
<td>128.3±3.1c</td>
<td>0.711±0.1 c</td>
</tr>
<tr>
<td></td>
<td>T2 (Se)</td>
<td>22.6±0.3 b</td>
<td>0.507±0.1 b</td>
<td>0.727±0.1 b</td>
<td>22.3±0.8d</td>
<td>154.3±1.8a</td>
<td>0.799±0.1 b</td>
</tr>
<tr>
<td></td>
<td>T3 (Zn)</td>
<td>19.1±1.1 c</td>
<td>0.784±0.1 a</td>
<td>0.781±0.1 b</td>
<td>24.6±0.4d</td>
<td>142.3±2.7b</td>
<td>0.931±0.1 a</td>
</tr>
<tr>
<td></td>
<td>T4 (Se+Zn)</td>
<td>27.2±1.2 a</td>
<td>0.793±0.1 a</td>
<td>0.859±0.1 a</td>
<td>19.3±0.8e</td>
<td>161.1±2.0a</td>
<td>0.785±0.1 b</td>
</tr>
<tr>
<td>Ram</td>
<td>T1 (Control)</td>
<td>21.3±0.3bc</td>
<td>0.324±0.1 c</td>
<td>0.362±0.1 e</td>
<td>41.3±1.2a</td>
<td>98.3±1.5 d</td>
<td>0.548±0.1 e</td>
</tr>
</tbody>
</table>
Means with different letters within each column differ significantly (P≤0.05) according to Duncan’s test.

This study was recent as it was conducted to find the difference between the effect of the addition of selenium and zinc each individually or mixture of them, and on the other hand to find the difference between the age for the reactions of minerals in the serum of Kurdi sheep. So only few reports are there in literature for comparison with the present study. Trace minerals in animal feedstuffs are the most highly variable nutrient due to factors of plant species, soil stage of maturity and climate conditions (Underwood, 1981; Berger, 1996). Mineral elements such as zinc (Zn), copper (Cu), cadmium (Mo) and selenium (Se) are necessary to animal health, production and survival as they are part of structural, physiological, catalytic and regulatory organism roles (Ries et al., 2010). In addition, farm animals are greatly relying on their nutritional status for their performance. Intracellular detoxification of Free radicals (Smith and Akinbamijo, 2000). Groups showed decrease in concentration of Copper in blood. Decrease of Copper concentration was more marked in lambs and rams of the Kurdi sheep breed that fed selenium and Zinc in which negative correlation between Selenium, Zinc and Copper in blood serum concentrations was seen. This indicates possible negative interaction between copper, selenium and Zinc. On the contrary, addition doses of selenium (Sodium Selenite) and Zinc (Zinc sulfate) in the feeding ration led to a rise in the levels of Selenium, Zinc, Iron, Molybdenum and Manganese in the blood of experimental sheep. Moreover, Soetan et al., (2010) illustrated that Copper, Magnesium, Selenium, Iron, Zinc, Molybdenum and Manganese are necessary co-factors found in some enzyme structure and are necessary in abundant biochemical pathways. Nevertheless, Selenium has a hostile relationship with some useful and toxic elements, such as: Zinc, Copper, Molybdenum, Manganese, Cobalt, Arsenic, Cadmium, Nickel, Tin, Gold, Bismuth, Silver, Lead and Mercury (Hosnedlova et al., 2017). Levels of toxic elements in this study were lower than in previous studies and were also lower than recommended levels in sources and organizations of fed, environment and health from EFSA, ANZFA and Institute of Medicine (US). Overall, this study found that all rams and lambs look healthy when Selenium used at dose 0.5 mg\kg fed and Zinc at dose 100 mg\kg fed, and their mixture. Palani (2019) pointed out that the low level of selenium in the blood of Kurdi sheep is due to its low level in plants, and to its low concentration in the soil of Sulaimani governorate which is in Iraq Kurdistan Region. In contrast to the research that’s done by Kyle and Allen (1990) who discovered that the rams (which all survived) received dose around 0.38 mg/kg selenium, the ewes (9 survived) received dose about 0.50 mg/kg, while the lambs (all 5 died) received dose 0.45 mg/kg Selenium of body weight. Furthermore, Tiwary et al., (2006) established that using selenium (Sodium Selenite) at dose of 2, 3, and 4 mg/kg resulted in Shortness of breath and/or tachypnea. In contrast to the research that’s done by (Mazurek and Wałkuska, 2014) when adding selenium led to a decrease in the levels of Zinc and Iron and were consistent with
a decrease in the level of Copper and an increase in the level of Manganese. The researches of other authors have confirmed the Zinc-Selenium and Copper-Selenium antagonism (Jensen 1975, Hause, Welch 1989). Iron (Fe) and Manganese (Mn) have also been shown to be Selenium antagonists (Martin et al. 1989). The present research shows that dietary supplementation with Selenium and Zinc affected the content of Selenium, Zinc, Iron, Copper, Molybdenum and Manganese in the blood serum of lambs. The influence of Metallothionein MT, a low molecular mass protein likely to participate in the uptake, transport and regulation of Zinc in biological systems; it is formed through the synthesis stimulated in mucous cells by competitive Zinc and Copper atoms (Eck, Pall auf 1999, Oh et al.1981). Metallothionein MT also acts as a Zinc and Copper store in the cell and has been documented to be a potent scavenger of free radicals (Park et al. 1985). Copper proves to be an essential component of different proteins and metalloenzymes. This trace element contributes to Iron metabolism, hemoglobin synthesis and erythrocyte production. The most pronounced changes were noted in the Copper and Iron concentration in blood serum. The observed behavior of the mineral elements indicates a strong antagonistic interaction in their deposit and metabolism in the liver. The current results agree with those reported by other authors (Bik, Bednarek 1997), who noted a significant decrease in the Copper level in sheep’s blood serum after a subcutaneous injection of sodium selenite aqueous solution. The experiment performed by South et al. (2000) on mice showed that Selenium deficiency played a role in iron accumulation in the plasma. Iron is essential to virtually all living organisms, it is a key component of cytochromes, an important electron carrier and plays a critical role in cellular respiration. Antagonism between Copper and Selenium has been documented in animal studies (Hill, 1974) some studies demonstrated that copper can inhibit selenite-induced cytotoxicity and apoptosis in HT-29 cells. Zeng and Botnen (2004) described that selenite and selenocysteine can cause cell cycle arrest through distinct mechanisms, and suggest that Copper may interact with selenite extracellularly, which represents the basis on antagonism between Copper and selenite. Wang et al. (2010) described interaction between selenomethionine and Copper ions. The copper coordinated with selenomethionine by the fashioning of Cu-Se and Cu-OCO bonds or by the formation of Cu-N and Cu-OCO bonds. Selenium interactions with other elements rate among the factors influence on Selenium metabolism in an organism. There are descriptions of interactions of Selenium, Copper, Manganese, Iron, and others (Shamberger, 1983). However, a dearth of data regarding serum Mo concentrations currently exists, and data contained herein will aid in guiding future reference ranges for sheep. Molybdenum is an essential trace mineral because of its role in the reduction of nitrate to nitrite in bacteria (Williams and Da Silva, 2002), though essential requirements are low and clear signs of deficiencies have been reported in few species (McDowell, 2017). Toxicity of Mo in ruminants varies by species, chemical form, type of diet (NRC, 2007). Selenium and Zinc supplementation has elevated the Mn concentration in both lambs and Rams groups with administered dietary Selenium and Zinc compounds. Selenium and Zinc supplementation of lambs and Rams seems to be beneficial with respect to the Magnesium function as an oxidant contained in superoxide dismutase, whose activity is conditioned by the presence of selenium, Zinc and Manganese. Beside Copper and Iron, Magnesium proves to be an essential trace element for hemoglobin synthesis.
In this study, there was a decrease in the level of Selenium and Zinc in the blood of Kurdi Sheep. Because Sheep operations in Kurdistan rely on rangelands as their primary fed source, which could lead to clinical or subclinical trace mineral deficiencies and limit animal productivity, so we recommend adding Selenium and Zinc in the fed of Sheep. Optimal concentrations of fed Selenium and Zinc for sheep are not well understood. However, The European Food Safety Authority (EFSA, 2014) has decided to allow the addition of Selenium to the fed of about 0.2 to 0.5 mg / kg dry matter to avoid toxins and environmental pollution, the high tolerance of Sheep to dietary Zinc (300 mg of Zn/kg of diet DM; NRC, 2005). Results from the current study provide insight on serum trace mineral concentrations in ram and lambs. On average, serum Selenium concentrations were lower in animals located in Sulaimani governorate which is in Iraq Kurdistan Region, which was likely due to soil and forage Se deficiencies. Selenium and Zn were the 2 most deficient and marginally deficient minerals across rams and lambs, and additional supplementation of these trace minerals is recommended. Additional factors that may influence mineral status variation include individual intake, season, forage species maturity, bioavailability of trace mineral chemical form and mineral antagonists. From the above-given, it follows that doses of Selenium and Zinc individually or their combination that were used in the present study did not have any toxic effect on Kurdi sheep.

**Conclusions**

Selenium and Zinc supplementation significantly has led to improvement in some important elements from the minerals: Selenium, Zinc, Iron, Molybdenum and Manganese. This is considered as an adequate for using it as nutritional supplements and which has no harmful effects on sheep and their levels within the limits allowed, Copper concentration decrease was reported in lambs and rams of males Kurdi sheep, that fed with dose of Selenium and Zinc individually or their combination. Moreover, we recommend adding Selenium and Zinc in the fed of Sheep, there are no adverse effects of metal elements in blood, which is important for animal health.

**References**

ANZFA (Australia Hew Zealand, Food Authority) Wellington, New Zealand.


