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Original Research Article

Correlations between sodium selenite and vitamin E with serum macro-minerals in suckling male lambs

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Abstract
The effect of selenium (Se) and vitamin E (VitE) injection was evaluated on the concentration of serum macro-elements and the relationships were also surveyed in 16 suckling Makuei male lambs in 90 days. Lambs were classified into 4 groups of 4 lambs including control, VitE, sodium selenite (NaSe) and NaSe/vitaminE (Se/E) groups. The 0.1 mg/kg body weight NaSe and 8 mg/kg VitE were injected. Mean blood Se was higher in NaSe than other groups ($P<0.01$). Blood Se in the Se groups was continuously increased, which was not noticeable in the NaSe and VitE. The least and upper blood Se were in VitE and NaSe groups, respectively. The mean concentrations of serum macro-minerals in the groups were within the normal range. Calcium was variable among the groups until day 30, then increased in control and VitE, it increased in Se groups until day 60 and then decreased not significantly to 90 days. Phosphorus was the opposite way of calcium. Magnesium decreased in the groups for 30 days and then increased insignificantly. The trend of calcium and magnesium in Se groups was to increase until day 60 but phosphorus was to decline. The lowest and highest calcium concentrations were in the control and NaSe, for magnesium in the VitE and control, and for phosphorus were in the control group, respectively. Mean comparison of minerals among groups and sampling times showed that Se had a significant increase from day 14 to 90, especially in NaSe ($P<0.01$). Selenium showed the most positive relationships with calcium and phosphorus in VitE and NaSe. In total groups, Se was correlated with all macro-minerals on day 90 and overall sampling times was correlated only with calcium. In conclusion, administration of Se with VitE increased Se, which was more pronounced in NaSe. NaSe alone or with VitE had no effect on the amount of macro-minerals. The least correlation was observed between Se and macro-minerals in Se/E administration. The correlation between macro-minerals was positive and significant. Finally, the effect of NaSe on treatment and prevention of Se deficiency was more effective than VitE and Se/E. VitE accelerates the process of increasing blood Se in a short time. Selenium was associated with calcium and their co-administration is recommended.

Keywords: Selenium, sodium selenite, vitamin E, calcium, lambs

Introduction

One of the most predominant antioxidants in animals is selenium (Se) and vitamin E (VitE), which minimize cell damage from membrane and intracellular peroxides, thus increasing the durability and efficiency of cells (Dhanasree et al. 2020). Selenium has a biological action close to VitE at the cellular level and is one of the basic constituents of glutathione peroxidase, which is an indicator of oxidative stress, in the removal of hydrogen peroxide and hydroperoxide from fats (Barcelos et al. 2022). Selenium components as organic and inorganic Se in the diet of lambs is effective in fortified the immune and nervous systems (Ataollahi, et al. 2020). Therefore, Se deficiency is associated with reproductive problems (Awawdeh et al. 2019), production deficiency (Ibrahim et al. 2017), reduced neonatal growth performance (Farghaly et al. 2017) and causes great economic losses. Meanwhile, animals need VitE based on their requirements to prevent oxidation of cell membranes that are very sensitive to peroxides and ultimately neutralize oxidative stress (Mohd Mutalip et al 2018, Tsiplakou et al. 2021). Therefore, the combination of VitE and Se protects body cells from any oxidative stress damages (Barwary et al. 2016). The importance of Se as a metalloenzyme in the health and production of livestock is vital (Shi et al. 2018) so its deficiency is related to immune, gastrointestinal, and respiratory disorders which characterized by signes of anorexia, emaciation, pica, anemia, decreased wool production, milk, and death (Morsy et al. 2019; Zhou et al. 2021). Selenium in soil and forage is variable, so organic and minerals supplements such as selenium methionine (Mousaie et al. 2017) and NaSe (Sherief et al. 2019), respectively in concentrate (Bampidis et al. 2019) or in newborns and growing domains will be associated with favorable results (Maraba et al. 2018).

The increasing role of macro-minerals in anatomical, physiological, enzymatic structure, homeostasis and acid-base balance of blood, interstitial fluids, osmolarity and finally, in growth, production and reproduction mechanisms is clear (Azarzar 2020). Calcium is the most predominant and abundant macro-minerals with various physiological functions in safety, energy, contraction and neuromuscular stimulation (Ataollahi et al 2018), animals' growth (Abdelrahman et al. 2021), metabolic procedures, production performance and carcass consistency (Cirne et al 2020). Magnesium is the third major mineral with many anatomical, physiological, enzymatic, immune, weight gain and reproductive functions in ruminants (Radostits et al. 2010; Ataollahi et al 2018). Phosphorus has also a vital role in coordination with calcium in skeletal formation, as a component of cell membranes, various physiological functions, and as a part of nucleic acids in energy storage (Radostits et al. 2010; Dittmer et al. 2017). These minerals' investigation in each animal give an idea of the healthy, growth, production and reproduction statues (Azarzar 2020).

Macro-minerals may be reduced directly due to their deficiency in food or indirectly in interactions with trace minerals and cause disturbances in animals' life (Kargin et al. 2004). One of the trace minerals is Se, which sometimes causes side effects such as competition and inhibition of absorption of the macro-minerals such as Ca, Mg or Pi (Abrahem et al. 2019). Researchers have shown low concentrations of macro-minerals with high Se in the diet (Kojouri and Shirazi, 2013) while others have not reported the same results (Bagnicka et al. 2017). Such descrepancies results in the use of Se, especially in lambs who are in the fast growing stage and have an urgent need for Se, Ca, Mg and Pi, need to be investigated and the interactions and ultimately the relationships among them. The objectives were: 1- To determine the level of Se in the blood of NaSe, VitE and Se/E groups 2- To identify the serum macro-minerals in the lambs' groups, and finally, 3- to present the relationships among minerals in groups and sampling periods.

Materials and methods

Animals and sampling

Sixteen Makui male lambs were selected. They were classified into 4 groups of 4 heads including control, VitE, NaSe and Se/E. Mean and standard deviation of weight were 21.7±1.13, 20.9±0.75, 25.2±1.5 and 23.4±0.75 kg, respectively. The overall mean±SE of lambs was 21.89±0.59 kg. The lambs were separated from the ewes and mixed in the morning and evening meals for breast feeding. Between milkings, the lambs were free to graze on the grains and legumes pasture. Before performing any tests and medications, the lambs were examined for vital and clinical signs such as temperature, respiration, and heart rate to ensure their health.

The lambs in control group were administered 0.1 ml/kg saline subcutaneously, VitE group were received 8 mg/kg intramuscular VitE, NaSe group have acquired 8 mg/kg NaSe subcutaneously and Se/E group were injected the VitE/Se according to the dose of medicine (Shi, et al. 2011). The study began on the first day with blood sampling and then medication. After calculating the required dose, it was prescribed based on body weight. Then, on days 7, 14, 30, 60, 90 (overall 6 times) the 5 ml of the jugular blood was taken with a long 18-gauge needle and collected in a test tube. Whole blood was administered for Se evaluation and serum for Ca, Mg and Pi assessment.

Samples evaluation

Blood Se concentration was measured by atomic absorption method using atomic absorption device (Technicon RA-1000, USA) made by Shimatzo and Se lamp. In this procedure, Se (Se+4) is combined with 2 and 3 diamino naphthalene and converted to a fluorescent form derived from 4 and 5 benzapiazel selenol. For this purpose, 0.25 ml of hemolyzed blood, 1 ml of a mixture of 15.8 mol/l nitric acid and 11.8 mol/l perchloric acid were added and heated at 150C° for 30 minutes. It was then stored for 2 hours at 190C° and 2 hours at 210C°. After cooling the mixture, 0.2 ml of 6 mol/l hydrochloric acid was added and stored at 150C° until nitric acid was no longer evaporated. Add one ml of solution containing 20 mmol of EDTA, 7 mol of ammonia solution and 10 mg of purple cresol bromide per liter to the mixture and heated at 140C° until the solution turns yellow. After this step, it was transferred to a cold medium and 1.5 ml of 66 mmol/l hydrochloric acid was added to adjust the pH between one and two. Fluorescence of solution obtained by spectrofluorometer (jasco, UK, London) was measured at wavelength at 366 nm (excitation nm; Emission at 544 nm). Calcium, magnesium and phosphorus were determined by autoanalyzer using commercial kits made by Pars Azmoun Iran.

Statistical analysis method

SPSS23 statistical program and case summaries, t-test, ANOVA and correlation tests were used for each of the parameters in the groups and sampling times by individually or for overalls. The results were drawn and interpreted as Tables and Figs. in groups and daily sampling.

Results

Mean blood Se concentration was the highest in NaSe group (F=19.4, df=3, P<0.01) (Table 1). Blood Se was continuously increased in the Se groups with time increasing, which was not visible in the control and VitE groups. The lambs of VitE group showed a slight increase up to day 14 which was not different from the control group (Fig. 1). The highest percentage of increase in Se was in NaSe (15.7%) and the lowest in the control group (7.1%), which was significantly high in the Se groups. The minimum and maximum blood Se concentrations were 180.4 and 262.8 nmol/l in the VitE and NaSe.

The overall mean blood Ca concentration did not show any difference between the groups (F=0.60, df=3, P=NS) and was within the standard range (Table 1). Calcium varied between groups for 30 days and showed a slight increase at the end, but decreased in Se groups (Fig.2). The lowest and highest Ca level were in the control group and NaSe groups ((3.96 and 10.5 mg/dl, respectively).

The overall mean serum Mg concentration in lambs was still not statistically different among groups (F=1.61, df =3, P=NS) and was in normal condition (Table 1). Magnesium decreased during the first 30 days in groups but then increased not significantly by day 90 in groups except NaSe and VitE. The increasing trend of Mg compared to the first day in the Se groups was not significantly less than the VitE and control groups, it decreased insignificantly at the end of the study. The minimum and maximum Mg level were in VitE and control groups (0.72-2.61 mg/dl), respectively.

The overall mean serum Pi concentration in the groups was not statistically different (F=0.17, df=3, P=NS) (Table 1) and no deficiency was expected. Phosphorus changes during the study were varried especially in the Se groups until day 60, then non-significantly increased by day 90 (Fig. 4). The minimum and maximum serum Pi level were both in the control group (3.84 and 8.86 mg/dl).

Mean comparison (ANOVA) of Se, Ca, Mg and Pi concentrations among 6 sampling times (Table 2) showed significant differences in Se on days 30, 60 and 90 (P<0.01) which were the highest increase in NaSe (Fig. 1). Phosphorus was significant on day 60 and all minerals were significant on day 90 (P<0.01). Mean comparison (ANOVA) of Se, Ca, Mg and Pi concentrations within groups (Table 3) showed significant differences in Se, Mg and Pi with the exception of Pi in the control group (P<0.01). Calcium did not differ within groups.

Pearson correlation analysis of Se with Ca, Mg and Pi based on groups and sampling frequency (Table 4) showed positive and significant relationships (P<0.05) between Se/Ca on day 7 in VitE, day 14 in Se/E and day 90 in all samples. Selenium showed a significant negative relationship with Mg on days 60 and 90 in all groups. Also, Se showed a significant positive relationship with Pi on day 7 in the control, days 60 and 90 in all groups (P<0.05). Therefore, the correlations between Se and all minerals were significant and positive on day 90 when groups were mixed and was positive and significant with Ca when sampling days were mixed (Table 4). The relationships between Ca/Mg (r=0.99), Ca/P (r=0.98) and P/Mg (r=0.89) were all positive and significant (P<0.05).

Table-1: Mean±SE lambs blood minerals in 4 groups and 6 sampling times in 90 days (n-24)

Groups/Minerals	Selenium[1]	Calcium[2]	Magnesium[2]	Phosphorus[2]
Control	200.5±1.13	8.82±0.33	1.58±0.16	6.72±0.25
Vitamin E	198.6±1.64	8.76±0.22	1.34±0.17	6.24±0.22
Sodium Selenite	226.9±4.82	8.54±0.29	1.20±0.18	6.45±0.20
Sodium Selenite/E	208.4±2.68	8.27±0.35	1.27±0.18	6.38±0.22
Overall	208.6±2.82	8.60±0.30	1.35±0.17	6.45±0.22

nmol/l = [1] ، mg/dl = [2]

Table-2: Mean comparison of lambs' blood minerals among sampling times in 90 days (n-24)

Days/Minerals	Selenium[1]	Calcium[2]	Magnesium[2]	Phosphorus[2]
Day 1	1.06	0.02	2.06	0.33
" " " 7	3.03†	1.13	0.195	0.556
" " " 14	9.75**	0.97	1.67	0.18

" " " 30	21.28**	0.26	0.51	0.87
" " " 60	48.45**	0.43	0.61	10.58**
" " " 90	17.21**	4.45*	6.51**	10.67**

nmol/l = 1، mg/dl = 2 P<0. 1>0.05=† P<0.05 *= P<0.01**=

Table-3: Mean comparison of lambs' blood minerals among sampling times in 4 groups (n=23)

Groups	Parameters	Sum Square	Mean Square	(F-values
Control	1	437.6	87.52	6.00**
	2	15.18	3.04	1.27
	3	8.09	1.62	4.28**
	4	8.68	1.74	1.25
Vitamin E	1	831.4	166.3	4.54**
	2	18.84	3.77	1.85
	3	9.50	1.90	6.07**
	4	11.54	2.31	2.85**
Sodium Selenite	1	11389.2	2277.9	28.20**
	2	8.03	1.60	0.76
	3	6.98	1.40	2.42**
	4	12.48	2.50	5.16**
Sodium Selenite/E	1	3311.94	662.4	18.49**
	2	18.20	3.64	1.37
	3	9.41	1.88	4.17**
	4	14.83	2.97	4.41**

1= Selenium, 2= Calcium, 3=Magnesium, 4= Phosphorus P<0.01**=

Table-4: Correlations between selenium and blood minerals in groups and sampling times (n=4)

Groups	Calcium2	Magnesium2	Phosphorus2
Selenium1 day 7 (Control)	0.84	0.13	0.95*
Selenium day 7 (VitE)	0.99**	0.70	0.86
Selenium day 14 (Se/E)	0.97**	-0.47	-0.40
Selenium day 7 (Overall)	0.11	-0.70**	0.58**
Selenium day 7 (Overall)	0.76**	-0.53**	0.74**

nmol/l = 1 ، mg/dl = 2 P<0.01**= P<0.05*=

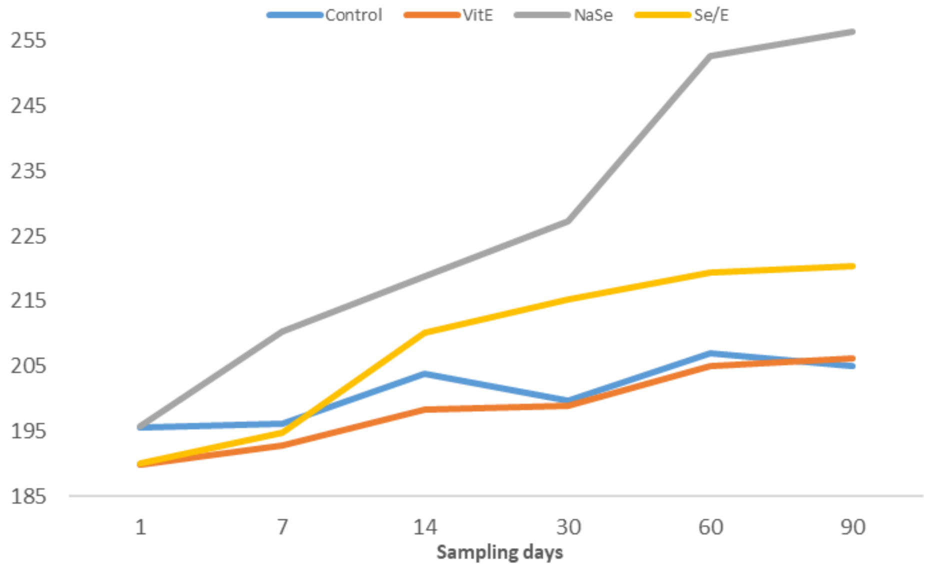

Fig.-1: Mean comparison of lambs' serum selenium (nmol/l) among groups in 90 days

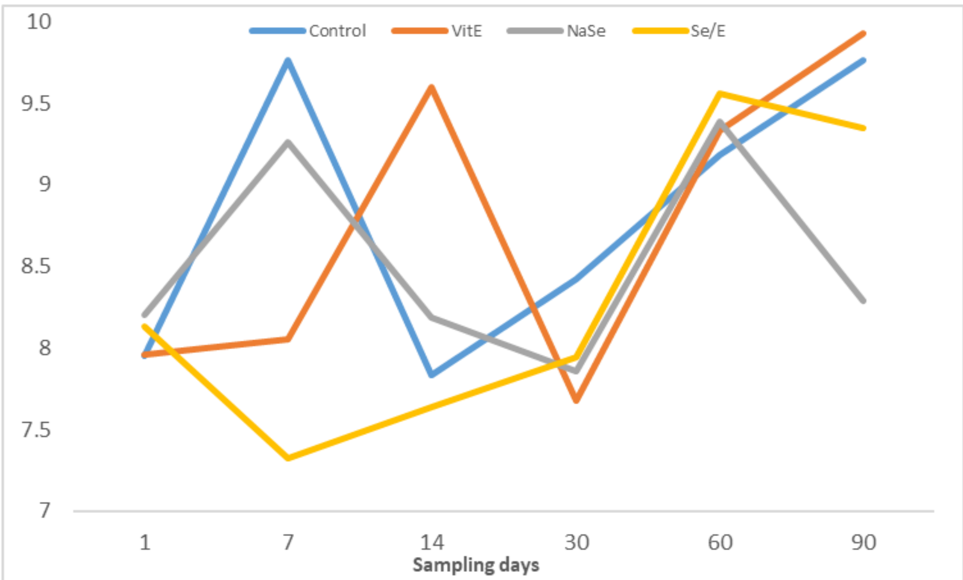

Fig.-2: Mean comparison of lambs' serum calcium (mg/dl) among groups in 90 days

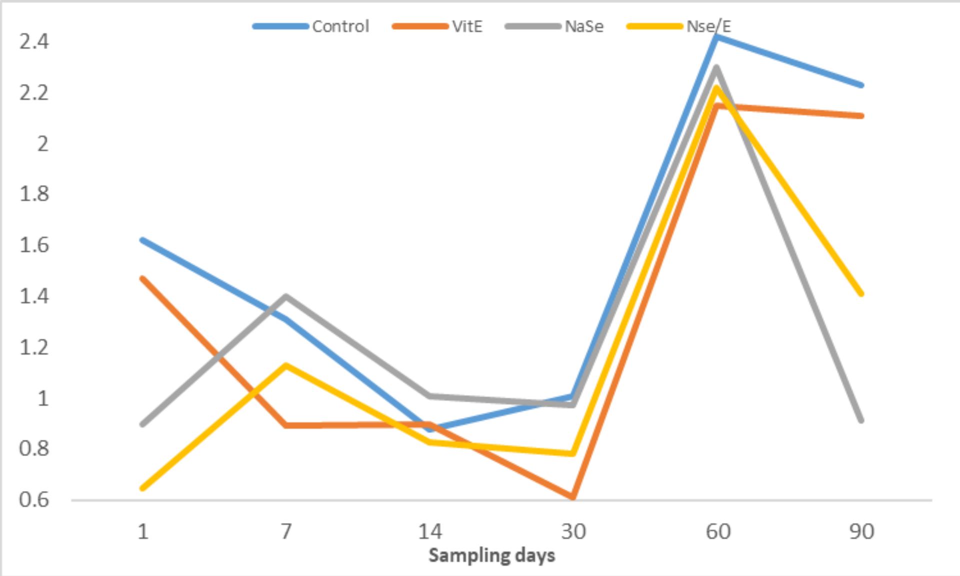

Fig.-3: Mean comparison of lambs' serum magnesium (mg/dl) among groups in 90 days

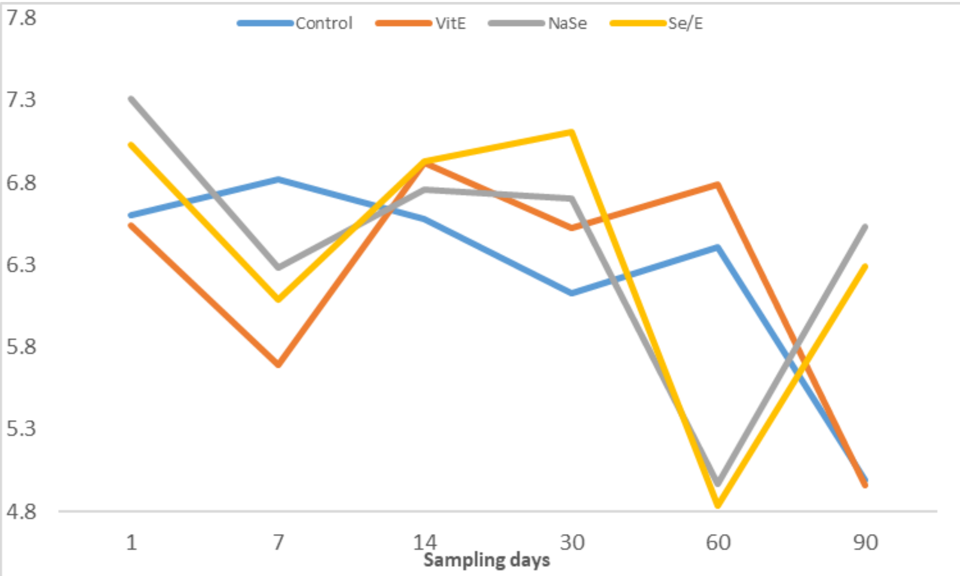

Fig.-4: Mean comparison of lambs' serum phosphorus (mg/dl) among groups in 90 days

Discussion

Selenium is considered as the prominent micronutrients in the prevention and elimination of oxidative stress in the cells of organisms (Ramadan et al. 2018). If the rang of Se in blood is considered around 70-100 ng/ml, the animal will be safe from reproductive disorders, losses resulting from heart myopathy and respiratory diseases (Mehdi and Dufrasne 2016). Selenium is involved in the metabolic processes of ruminants (Sayiner, et al. 2021), and its deficiency results weakens the immune system, increases the incidence of tumors (Shokrollahi et al. 2013), enhance thyroid hormone disorders (Novoselec et al. 2018) and muscular dystrophy (Kivanç, et al. 2021). For this reason, the assessment of Se in ruminants that need both Se and Ca, Mg and Pi as macroelements will increase its importance in terms of co-occurring or interacting effects.

The mean concentration of Se in Se groups was significantly higher than the other groups. Karren et al. (2014) showed an increase in blood Se after administration of Se supplements, which is consistent with the results of this study. Blood Se was continuously increased in the Se groups for 90 days, which was 2 times higher in NaSe than in Se/E group, while it was not significant in control and VitE groups. It means that VitE has no role in increasing blood Se, although it physiologically binds to each other to remove harmful peroxides at the cellular level (Dhanasree et al. 2020). Similar results have been reported by Shi et al. (2011) showing a significant increase in blood and tissue Se following dietary Se supplementation. The importance of Se as the main antioxidants is related in the elimination of oxidative stress, which prevents cell damage against peroxides, and finally, leads to increase durability and efficiency of body cells (Raisbeck 2020). The presence of Se components in food, in addition to the mentioned effects, will results fortification of the immune and nervous systems (Kumar et al. 2008), its deficiency accompanied by reproductive complications (Sayiner et al. 2021), production disorders (Cirne et al. 2020) and reduction in neonatal growth performance (Farghaly et al. 2017). Therefore, Se, especially with VitE, will protect cells from any oxidative stress damages (Dhanasree et al. 2020).

The physiological range for Ca, Mg and Pi concentrations reported as Azarzar (2019) in ewes were 8.4-11.2, 1.9-2.77 and 2.8-9.8 mg/dl, respectively which with the values of this study were at the lowest normal condition mainly for Ca and Mg, although those values were reported for adult ewes and our experimental animals were lambs, it means that no macro-mineral deficiencies were expected in these lambs. These results show that, the concentrations of lambs' macro-minerals were at its lowest level, which is impossible to be supplied through dams' milk, and because lambs probably do not receive concentrate, and despite milk is rich in Ca, so lambs will be at the risk of deficient for a period of time, and therefore, they need mineral supplements just for Ca and Mg while the situation for Pi was favorable and increased at the end of the study. According to current information, lambs under 2 months of age are also deficient in Se (Ibrahim et al. 2017), so they should be fed with Ca and Mg together (Ataollahi et al. 2020). These results show that oral administration of Se and VitE supplements separately and together did not affect the concentration of the macro-elements, but up to day 60 caused a slight increase in Ca and Mg. Therefore, the combination of Se and macro-minerals in the forms of injection or dietary supplements is useful (Farghaly et al. 2017).

Abrahem et al. (2019) emphasize that despite the information on the effect of Se and VitE on Ca level is limited and also controvertial. Juniper et al. (2006), Kumar et al. (2008), Shokrollahi et al. (2013) and Bagnicka et al. (2017) reported that Se supplements were not effective on Ca absorption in lambs. In another study, intramuscular injection of Se/E was not significant in serum Ca and Mg levels (Bednarek et al. 1996). Contrary to the mentioned findings, Mahmoud et al. (2013) found that administration of 5 mg NaSe and 450 mg VitE significantly increased serum Ca concentration in male lambs. Also, Bickhardt et al. (1998) showed that administration of Se and Ca are effective in the treatment of ketosis and hypocalcemia in cows. While Kessler et al. (1993), Garcia et al. (2011) and Mehdi and Dufrasne (2016) showed that high Ca nutrition has a negative effect on dietary Se absorption. There are no available sources about the effects of Se on Mg and Pi, or vice versa, and may indicate that these minerals are not sensitive to each other. Deficiency of macro-elements has been observed in metabolic and nutritional diseases in ewes (Kojouri and Shirazi 2013), which can also be observed in lambs that also need Se. The results of this study showed that their co-administration fortified each other and is suitable for growth and production.

In this study, the highest correlation was between Se/Ca, followed by Pi and Mg. In the combined groups, all macro-minerals were related on days 60 and 90 and in sampling times gathering only Ca was associated. These results show that co-administration of Se and Ca is appropriate and ideal, does not interfere with each other and acts independently and can even strengthen each other too. The results of this study was contradicted with the findings of Kessler et al. (1993), Garcia et al. (2011) Mehdi and Dufrasne (2016) Sayiner et al. (2021) who mentioned a negative relationship and also Bagnicka et al. (2017) which no relationship mentioned between Se and Ca. Nazıroğlu (2009) reported a link between Se and Ca-induced nerve waves and oxidative stress in human epilepsy, in which Se deficiency may exacerbate oxidative stress by decreasing GPX, so it causes nerve waves via a Ca factor, which may indicates an indirect relationship between Se and Ca. The relationships among macro-minrals in this study were in consistent with the findings of others (Azarzar 2020, Li et al. 2020, Fadlalla et al. 2020).

In conclusion, co-administration of Se with VitE increased blood Se, which was higher in NaSe than Se/E. NaSe and Se/E had no effect on the values of the macro-elements. There was no correlation between Se and macro-elements in NaSe and Se/E administration, but in overall groups, the correlation was positive and significant for all elements. There were positive and significant relationships among the macro-elements. Finally, the role of NaSe in Se deficiency is more favoable than Se/E. VitE injection is not effective in increasing blood Se. Selenium had the highest correlation with Ca, and co-administration is useful.

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