



# The Effect of Maternal Supplementation of Zinc, Selenium, and Cobalt as Slow-Release Ruminant Bolus in Late Pregnancy on Some Blood Metabolites and Performance of Ewes and Their Lambs

Hassan Aliarabi<sup>1</sup> · Amir Fadayifar<sup>2</sup> · Reza Alimohamady<sup>1</sup> · Amir Hossein Dezfoulian<sup>1</sup>

Received: 3 April 2018 / Accepted: 6 June 2018 / Published online: 15 June 2018  
© Springer Science+Business Media, LLC, part of Springer Nature 2018

## Abstract

The aim of this study was to determine the effect of the supplementation of a slow-release bolus of zinc (Zn), selenium (Se), and cobalt (Co) at late gestation (6 week prepartum) on performance and some blood metabolites of Mehraban ewes and their lambs until weaning. Seventy pregnant ewes, 6 weeks prior to expected lambing, were randomly divided into two groups (35 heads each) including (1) control group and (2) slow-release bolus group. Blood samples of ewes were obtained on day 10 prepartum and 45 and 90 days postpartum, and milk samples were collected on day 45. Blood samples of lambs were collected on days 10, 45, and 90. Body weight at birth and weaning and average daily gain were higher and percentage of mortality and white muscle disease rate were lower in lambs whose mothers were given a bolus ( $P < 0.05$ ). Slow-release bolus administration increased serum alkaline phosphatase and whole blood glutathione peroxidase activity, plasma concentrations of Zn, Se, and vitamin B<sub>12</sub> in ewes and their lambs ( $P < 0.05$ ). In addition, serum creatine phosphokinase activity of lambs whose mothers were given bolus was lower ( $P < 0.05$ ). Serum concentration of T<sub>3</sub> in bolus given ewes and their lambs was higher ( $P < 0.05$ ) and serum T<sub>4</sub> concentration was lower ( $P < 0.05$ ). Zinc, Se, and vitamin B<sub>12</sub> concentrations in milk were significantly higher in treated ewes ( $P < 0.05$ ). Obtained results showed that maternal supplementation of zinc, selenium, and cobalt as slow-release ruminant bolus in late pregnancy improved some mineral status of ewes and their lambs until weaning and led to higher body weights of lambs at weaning.

**Keywords** Sheep · Trace minerals · Controlled release bolus · Vitamin B<sub>12</sub> · Serum enzymes

## Introduction

Nutrient provision during gestation not only has an effect on maternal status and reproductive performance [1] but also affects prenatal and postnatal litter growth and health [2]. Although trace elements are needed by the body in small amounts, they are essential nutrients for several metabolic functions such as growth, development, reproduction, and immunity [3]. Furthermore, newborn animals are dependent upon their dams for transfer of these nutrients via the placenta and the mammary gland [4].

It has been reported that supplementation of zinc (Zn) [5], selenium (Se) [4, 6], and cobalt (Co) [7] improved indices of reproductive performance, lamb production, and health. Soils and feeds in many regions of Iran are deficient in Zn [8, 9], Se [10, 11], and Co [12, 13], and deficiency symptoms have been observed in several flocks. In extensive production systems, supplementing animals with trace elements can be difficult. Using supplemental feed as a trace element carrier incurs the costs of both feed and labor, if additional feed is not required [14]. Free access minerals, mineral licks and blocks are subject to variable intakes with animals consuming between nothing and many times the required intake [15]. Oral dosing with trace element drenches is another possible alternative. Although this ensures that each animal receives a dose, it may need regular handling, storage mechanisms for the element and/or a high animal tolerance to the levels of element given for long-term administration [14]. Previous work has shown oral drenches to be effective for only short periods in the correction of clinical copper deficiency (1–2 weeks) [16]. The controlled release bolus route should provide each animal

✉ Hassan Aliarabi  
h\_aliarabi@yahoo.com

<sup>1</sup> Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Islamic Republic of Iran

<sup>2</sup> Department of Animal science, Faculty of Agriculture, Lorestan University, Khorramabad, Islamic Republic of Iran

with a consistent dose in line with its requirements sustained over a long period of time, such that one treatment of the animals should ensure adequate trace element cover for a number of months [14, 16–18]. Abdelrahman et al. [19] indicated that trace minerals slow-release bolus supplementation in Najdi ewes, raised under intensive system, improved Se, Zn, Cu, and Co status at parturition and Co, Zn, Cu, P, and Se in their newborns at birth. Additionally, the higher colostrum yield and the body weight of the newborns show positive effects of the trace mineral supplementation. However, to our knowledge, there are no studies on the mother's supplementation with slow-release trace elements ruminal bolus at late gestation and its effect on the status of the elements in the mother and the lamb until weaning. Furthermore, given that slow-release trace elements ruminal bolus should not be used for newborn lambs until weaning, the aim of this study was to determine the effect of the supplementation of slow-release bolus of Zn, Se, and Co at late gestation (6 weeks prepartum) on performance and status of mineral profile in milk and plasma of Mehraban ewes and their lambs until weaning.

## Materials and Methods

Seventy pregnant Mehraban ewes ( $58 \pm 4$  kg) were randomly assigned into two groups. In the first group, 35 ewes, 6 weeks prior to the expected lambing date, were given glass boluses containing Zn, Co, and Se via a bolus gun, while animals in another group (control), were not given boluses. The boluses used in this experiment had an average weight of 18.82 g containing 20% zinc, 0.50% cobalt, and 0.23% selenium. Their average release rate in the rumen was 103.55 mg per day, and the daily supply was 23.01 mg zinc, 0.535 mg cobalt, and 0.258 mg selenium. The ewes were kept indoors as a single flock from 42 days prepartum to 30 days postpartum and fed with the same base diet (Table 1). Thirty days after parturition ewes and their lambs were turned out to medium-quality pasture. Lambs were kept together with their mothers from birth until weaning at 120 days after birth. They were weighed to determine average birth and weaning weights. Lamb mortality and white muscle disease symptoms were also recorded.

## Sample Collection

Blood samples were collected from ewes (10 days prepartum, 45 and 90 days postpartum) and lambs (10, 45, and 90 days old). Blood samples were collected in two tubes in the morning (8:00 am) via the jugular vein, one containing heparin to obtain plasma and the other without heparin to obtain serum by centrifuging at 3000 rpm for 15 min. In addition, 5 mL of blood was collected into a heparinized vacutainer tube to estimate glutathione peroxidase (GSH-Px) activity. All samples

were stored at  $-80$  °C until further analysis. Plasma samples were used to determine concentrations of Zn, Se, and vitamin B<sub>12</sub> and serum samples to determine activity of alkaline phosphatase (ALP), tetraiodothyronine (T<sub>4</sub>), and triiodothyronine (T<sub>3</sub>) concentrations in ewes and their lambs. Also, serum creatine phosphokinase (CPK) activity was determined in lambs. Milk samples were collected from ewes on day 45 postpartum, stored at  $-20$  °C and finally used to determine milk Zn, Se, and vitamin B<sub>12</sub> concentrations.

## Minerals and Vitamin B<sub>12</sub> Determination

The concentrations of Zn and Se in plasma, milk, and feeds were determined in an air-acetylene flame on an atomic absorption spectrophotometer (Varian SpectrAA220, Australia). Cobalt concentration in feeds was determined using an atomic absorption spectrophotometer (PHILIPS Model PU9100, single beam) according to the procedures of [20]. Plasma and milk concentrations of vitamin B<sub>12</sub> were analyzed using a competitive binding radioimmunoassay kit, in which the non-specific vitamin B-binding R-protein was removed by affinity chromatography (ICN, Costa Mesa, CA, USA).

## Enzymes and Thyroid Hormone Determination

Serum ALP and CPK activity were estimated according to the recommendations of German Society of Clinical Chemistry by available commercial kits (Pars Azmon, Tehran, Iran) and GSH-Px was measured by the method [21] of using the Ransel kit (cat. no. RS 504, Randox Laboratories Ltd., UK) by a spectrophotometer (Varian SpectrAA220, Australia). Total amounts of T<sub>3</sub> and T<sub>4</sub> were determined by enzyme-linked immunosorbent assay (ELISA) methods as explained by the commercial kit (PactanGostar, Tehran, Iran) using an ELISA reader (ELX808, Bio-Tek, USA). Sensitivity and intra-assay coefficients of variation of the T<sub>3</sub> assay were 0.3 nmol/L and 7.4%, respectively. Also, sensitivity and intra-assay coefficients of variation of the T<sub>4</sub> assay were 12 nmol/L and 5.4%, respectively.

## Statistical Analysis

The GLM procedure of SAS [22] was used for analysis of data. Birth weights, weaning weights, and average daily gain of lambs were analyzed with least squares means (LSM) using *t* test. Because the interaction between gender (male or female) and type of birth (single or twin) was not significant, their interaction was excluded from the model:

$$Y_{ijkl} = \mu + T_i + A_j + B_k + e_{ijkl}$$

where  $\mu$  is the overall mean,  $T_i$  is the effect of treatment,  $A_j$  is the effect of sex (male or female),  $B_k$  is the effect of birth (single or twin), and  $e_{ijkl}$  is the effect of error.

**Table 1** Ingredients and nutrient composition of the basal diet

Item	Feedstuff				Basal diet
	Alfalfa hay (35%) <sup>a</sup>	Corn silage (10%)	Wheat straw (23%)	Barley grain (32%)	
Dry matter (%)	92	33	94	92.5	86.72
Organic matter (%DM)	91.6	96	92.4	97.1	93.98
Crude protein (%DM)	15.8	8.5	3	12	10.91
Ether extract (%DM)	3	3	2	2	2.45
Neutral detergent fiber (%DM)	46	46	85	28	49.21
Acid detergent fiber (%DM)	35.78	28	49.4	9.69	29.78
Ash (%DM)	8.4	4	7.6	2.9	8.18
Metabolizable energy (Mcal/kg)	2.2	2.6	1.5	3.1	2.36
Calcium (%DM)	1.2	0.28	0.31	0.05	0.53
Phosphorus (%DM)	0.24	0.25	0.10	0.34	0.24
Zinc (mg/kg DM)	16.04	18.12	12.02	22.12	17.26
Selenium (mg/kg DM)	0.11	0.015	0.02	0.29	0.053
Cobalt (mg/kg DM)	0.17	0.012	0.011	0.12	0.101

<sup>a</sup> Percentage of ingredients in the basal diet (DM)

All factors in milk and blood were analyzed according to a completely randomized design. The model used for analysis was as follows:

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

where  $\mu$  is overall mean,  $c_i$  is the effect of treatments, and  $e_{ij}$  is the residual effects.

Duncan’s multiple range tests was used for comparison of means, considering  $P \leq 0.05$  as the significant level.

Number and percentage of healthy, with white muscle disease (WMD) signs and lambs alive at weaning were compared by chi-square test.

## Results and Discussion

Number and percentage of lambs with WMD signs until weaning were significantly lower ( $P < 0.05$ ), and lambs alive at weaning were significantly higher ( $P < 0.05$ ) in lambs whose mothers were given bolus compared to animals in

**Table 2** Number and percentage of WMD sign and dead until weaning in lambs

	Control No. (%)	Bolus No. (%)	$\chi^2$	<i>P</i> value
Pregnant ewes	35 (100)	35 (100)	0.00	1.00
Non-pregnant ewes	0.0 (0.0)	0.0(0.0)	0.00	1.00
Lambs born alive	39 (100)	38 (97.44)	2.01	0.364
Lambs with WMD signs	7 (17.95) <sup>a</sup>	0.0 (0.0) <sup>b</sup>	7.50	0.006
Lambs alive at weaning	35 (89.74) <sup>b</sup>	38 (100) <sup>a</sup>	4.11	0.042

Different superscript letters in rows represent statistical differences

control group (Table 2). There were 4 dead lambs and 7 lambs with clinical signs of WMD in control group while no mortality and WMD incidence was observed in the treated group. Nutritional myopathy commonly known as “white muscle disease” is the most understood Se-responsive disease [3]. The present study showed that the boluses, by releasing selenium, ensured a substantial supply of selenium for the lambs. Similarly, Zervas [18] reported that boluses containing selenium, copper, and cobalt before pregnancy significantly decreased WMD incidence in newborn lambs.

Body weight at birth and weaning and average daily gain were significantly higher ( $P < 0.01$ ) in lambs whose mothers were given bolus compared to animals in control group (Table 3). Kendall et al. [17] reported that growing lambs receiving slow-release bolus containing Zn, Se, and Co had higher daily gains. In the present experiment, three trace elements were supplemented simultaneously which may cause difficulty in determining which element is responsible for growth response. However, individual supplementation of Zn [5, 23], Se [24], and Co [12] have been reported to improve performance.

Plasma Zn concentrations in bolus given animals and their lambs were higher ( $P < 0.01$ ) at all sampling times compared

**Table 3** Birth weight, weaning weight, and average daily gain in lambs

	Control	Bolus	SEM	<i>P</i> value
Birth weight (kg)	3.32 <sup>b</sup>	4.63 <sup>a</sup>	0.032	>0.0001
Weaning weight (kg)	27.49 <sup>b</sup>	33.87 <sup>a</sup>	0.353	>0.0001
Average daily gain (kg)	0.201 <sup>b</sup>	0.243 <sup>a</sup>	0.037	>0.0001

Different superscript letters in rows represent statistical differences. Least squares means (LSM) corrected on the basis of gender (male or female) and type of birth (single or twin)

**Table 4** Zinc concentration (mg/L) in plasma and milk of ewes and their lambs

	Time	Control	Bolus	SEM	<i>P</i> value
Ewe	Plasma				
	10 days before lambing	0.983 <sup>b</sup>	1.394 <sup>a</sup>	0.037	0.0005
	45 days after lambing	0.909 <sup>b</sup>	1.425 <sup>a</sup>	0.034	0.0001
	90 days after lambing	0.971 <sup>b</sup>	1.402 <sup>a</sup>	0.026	0.0001
	Milk				
	45 days after lambing	4.533 <sup>b</sup>	6.566 <sup>a</sup>	0.133	0.0001
Lamb	Plasma				
	10 days old	1.053 <sup>b</sup>	1.536 <sup>a</sup>	0.038	0.0003
	45 days old	0.957 <sup>b</sup>	1.444 <sup>a</sup>	0.033	0.0001
	90 days old	0.840 <sup>b</sup>	1.427 <sup>a</sup>	0.072	0.0022

Different superscript letters in rows represent statistical differences

to control group (Table 4). Considering plasma zinc concentration in a quoted normal range from 0.8 to 1.4 mg/L [3], all ewes and their lambs had normal plasma zinc concentration; however, zinc supplementation as slow-release bolus could increase plasma zinc concentration. Similar to our findings, Kendall et al. [17, 25] reported that administration of a slow-release bolus (Zn, Co, and Se) to sheep increased plasma Zn level in animals receiving bolus compared to control group. Similar to our findings, Abdelrahman et al. [19] reported that administration of a slow-release bolus of selenium (Se), copper, zinc (Zn), cobalt (Co), phosphorous (P), manganese [26], and iodine (I) at late gestation (60 days prepartum) to ewes increased serum Zn level in newborn lambs that were born from animals receiving bolus compared to control group.

Significant correlations were reported for plasma and milk Zn concentration [27]. Hence, the higher level of plasma Zn of lambs seen in the present study (Table 4) may be a reflection of higher Zn concentration of milk in the bolus-treated group. Our result is consistent with White et al. [28] and Zali and Ganjkanlou [29] who indicated that Zn supplementation to ewes increased milk and plasma Zn concentrations.

**Table 5** Serum alkaline phosphatase activity of ewes and their lambs (U/L)

	Control	Bolus	SEM	<i>P</i> value	
Ewe	10 days before lambing	166.33 <sup>b</sup>	184.23 <sup>a</sup>	2.916	0.0074
	45 days after lambing	169.52 <sup>b</sup>	210.47 <sup>a</sup>	2.973	>0.0001
Lamb	10 days old	201.34 <sup>b</sup>	246.18 <sup>a</sup>	3.097	0.0002
	45 days old	192.56 <sup>b</sup>	245.82 <sup>a</sup>	2.973	>0.0001
	90 days old	169.95 <sup>b</sup>	231.87 <sup>a</sup>	2.897	>0.0001

Different superscript letters in rows represent statistical differences

**Table 6** Selenium concentration in milk and plasma of ewes and their lambs (μg/L)

		Control	Bolus	SEM	<i>P</i> value
Ewe	Plasma				
	10 days before lambing	80 <sup>b</sup>	163 <sup>a</sup>	2.28	>0.0001
	45 days after lambing	91 <sup>b</sup>	171 <sup>a</sup>	1.82	>0.0001
	90 days after lambing	97 <sup>b</sup>	169 <sup>a</sup>	2.33	>0.0001
	Milk				
	45 days after lambing	21 <sup>b</sup>	45 <sup>a</sup>	1.87	>0.0001
Lamb	Plasma				
	10 days old	55 <sup>b</sup>	115 <sup>a</sup>	1.43	>0.0001
	45 days old	66 <sup>b</sup>	121 <sup>a</sup>	1.23	>0.0001
	90 days old	73 <sup>b</sup>	102 <sup>a</sup>	1.71	>0.0001

Different superscript letters in rows represent statistical differences

Alkaline phosphatase activity was higher in bolus-treated ewes, and their lambs at all sampling times as compared to animals in control group (Table 5). The activities of enzymes which have Zn as a cofactor are indicators of Zn status in the body [3] and changes in the rates of plasma ALP may reflect changes in concentration of Zn in plasma among animals [30]. Several reports indicate that ALP activity increases at higher levels of Zn supplementation [9, 31]. Probably level of Zn in the basal diet (17.26 mg/kg DM) was not sufficient for an adequate ALP activity in ewes and their lambs, so that the administration of a slow release bolus, containing Zn, can improve the ALP activity.

Selenium concentrations in plasma and milk of bolus given ewes during the course of the study were significantly higher than those of control animals (Table 6). Our results are consistent with findings of Zervas [18], who reported that administration of slow-release bolus (Se, Cu, and Co) enhanced plasma Se level of pregnant ewes at 3 months prepartum until 3 months postpartum. Abdelrahman et al. [19] also reported that administration of a slow-release bolus of Se, Cu, Zn, Co, P, Mn, and I at late gestation (60 days prepartum) to ewes

**Table 7** Whole blood glutathione peroxidase activity of ewes and their lambs (μkat/L)

	Control	Bolus	SEM	<i>P</i> value	
Ewe	10 days before lambing	217.3 <sup>b</sup>	960.5 <sup>a</sup>	56.18	0.0002
	45 days after lambing	205.0 <sup>b</sup>	1043.1 <sup>a</sup>	41.98	>0.0001
Lamb	10 days old	221.8 <sup>b</sup>	1033.8 <sup>a</sup>	36.72	>0.0001
	45 days old	265.5 <sup>b</sup>	1047.1 <sup>a</sup>	58.30	0.0002
	90 days old	294.5 <sup>b</sup>	997.5 <sup>a</sup>	59.71	0.0004

Different superscript letters in rows represent statistical differences

**Table 8** Serum T<sub>3</sub> concentration of ewes and their lambs (nmol/L)

		Control	Bolus	SEM	<i>P</i> value
Ewe	T <sub>3</sub>				
	10 days before lambing	1.61 <sup>b</sup>	1.86 <sup>a</sup>	0.037	0.0002
	45 days after lambing	1.41 <sup>b</sup>	1.84 <sup>a</sup>	0.047	>0.0001
Lamb	T <sub>3</sub>				
	10 days old	1.57 <sup>b</sup>	1.86 <sup>a</sup>	0.044	0.0057
	45 days old	1.49 <sup>b</sup>	1.81 <sup>a</sup>	0.029	0.0006
	90 days old	1.52 <sup>b</sup>	1.85 <sup>a</sup>	0.040	0.0021

Different superscript letters in rows represent statistical differences

increased serum Se level in newborn lambs. Furthermore, addition of Se to the maternal diet increased Se concentration in serum of newborn calves [32], kids [33], and lambs [34].

Concentration of Se in milk depends on the selenium status of sheep [35] and increase in plasma Se concentration can increase its concentration in milk. The concentrations of selenium in colostrum and main milk can both be raised by inorganic selenium supplements in sheep [4] and goats [36], but selenomethionine is by far the more effective [37–39].

Glutathione peroxidase activity of lambs born from Se-treated ewes (Table 7) was higher than those born from the control ewes, and these differences were sustained to weaning ( $P > 0.05$ ). The glutathione peroxidase has been used as an indicator of Se status in animals. Misurova et al. [40] reported a significant correlation between activity of GSH-Px and blood selenium concentrations in newborn kids. Increased activity of GSH-Px as a result of Se supplementation in the present, is in agreement with the results of Lacetera et al. [41] who reported that injections of selenium at 30 days before parturition increased the activity of glutathione peroxidase before and after parturition in pregnant ewes. Also, Zervas [18] indicated that administration of a slow release bolus (Cu, Co, and Se), 3 months prepartum to ewes, increased the glutathione peroxidase activity until 3 months postpartum. The antioxidant system of living organisms includes enzymes such as superoxide dismutase, glutathione peroxidase, and catalase. Cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen, than any single

**Table 9** Serum T<sub>4</sub> concentration of ewes and their lambs (nmol/L)

		Control	Bolus	SEM	<i>P</i> value
Ewe	T <sub>4</sub>				
	10 days before lambing	85.73 <sup>b</sup>	72.30 <sup>a</sup>	0.451	>0.0001
	45 days after lambing	85.45 <sup>b</sup>	72.33 <sup>a</sup>	0.608	>0.0001
Lamb	T <sub>4</sub>				
	10 days old	77.99 <sup>b</sup>	64.01 <sup>a</sup>	1.425	0.0010
	45 days old	81.23 <sup>b</sup>	68.36 <sup>a</sup>	1.940	0.0054
	90 days old	80.78 <sup>b</sup>	68.61 <sup>a</sup>	0.678	>0.0001

Different superscript letters in rows represent statistical differences

**Table 10** Serum creatine phosphokinase activity of lambs (U/dL)

		Control	Bolus	SEM	<i>P</i> value
Lamb					
	10 days old	284.57 <sup>b</sup>	77.98 <sup>a</sup>	39.04	0.0134
	45 days old	488.32 <sup>b</sup>	78.01 <sup>a</sup>	29.21	0.0002
	90 days old	279.02 <sup>b</sup>	77.36 <sup>a</sup>	28.02	0.0038

Different superscript letters in rows represent statistical differences

compound alone. In earlier research, the greater SOD and GSH-Px activity observed in Zn supplemented lambs and ewes [31] indicated that at least 15 mg Zn/kg DM supplementation was required for obtaining higher antioxidant enzyme activities.

Serum T<sub>3</sub> concentration (Table 8) was significantly higher and serum T<sub>4</sub> concentration (Table 9) was significantly lower in bolus given ewes and their lambs as compared to animals in control group ( $P > 0.01$ ). Similar to our findings, the increased serum T<sub>3</sub> and the decreased serum T<sub>4</sub> has been reported in growing male lambs supplemented with 0.2 mg Se/kg DM [11]. Normal thyroid status is dependent on the presence of some trace elements (I, Se, Zn, and Fe) for both the synthesis and metabolism of thyroid hormones [42]. Selenium has a critical role in the synthesis and homeostatic control of the thyroid hormones. About 80% of T<sub>3</sub> in serum is produced in the liver, kidney, and muscle, and all these tissues contain the selenium dependent enzyme deiodinases that convert T<sub>4</sub> to T<sub>3</sub> [43]. Positive correlations were also reported between plasma selenium level of ewes and T<sub>3</sub> level of their lambs ( $r = 0.72$ ) and between milk Se concentration and lamb plasma Se concentration ( $r = 0.84$ ) [35]. Probably, consumption of milk containing higher concentration of selenium by lambs born from ewes which received bolus is one of the reasons for the results obtained in the present study.

This result was in agreement with the finding of Abou-Zeina et al. [44] who reported that zinc supplementation increased total T<sub>3</sub>. In addition to its participation in protein synthesis, Zn is essential for proper thyroid function. It is involved in T<sub>3</sub> binding to its nuclear receptor [45]. Also, Zn participates in synthesis and action of thyrotropin-releasing hormone (TRH). Pekary et al. [46] reported that the processing of prepro-TRH to form TRH is Zn dependant via post-translational processing enzymes such as carboxypeptidase. El-Tohamy [47] indicated that Zn alone or combined with Se deficiencies resulted in a decrease in thyroid function. Zinc deficiency can also indirectly affect thyroid hormone status by decreasing energy intake [48].

Serum CPK in lambs, whose mothers received bolus, was significantly lower at all sampling times as compared to lambs born in control group (Table 10). Creatine phosphokinase is a muscle enzyme that is strictly related to muscular damage. So, serum activities of CPK were measured as an indicator of muscle injury [49]. Due to selenium deficiency in plants grown in several parts of Iran and low level of selenium in

**Table 11** Vitamin B<sub>12</sub> concentration in milk and plasma of ewes and their lambs

	Time	Control	Bolus	SEM	P value
Ewe					
Plasma (nmol/L)					
	10 days before lambing	432.53 <sup>b</sup>	737.47 <sup>a</sup>	14.34	>0.0001
	45 days after lambing	361.39 <sup>b</sup>	839.35 <sup>a</sup>	12.88	>0.0001
Milk (mg/L)					
	45 days after lambing	2325 <sup>b</sup>	3640 <sup>a</sup>	44.68	>0.0001
Lamb					
Plasma (nmol/L)					
	10 days old	175.03 <sup>b</sup>	305.14 <sup>a</sup>	12.81	0.0008
	45 days old	203.88 <sup>b</sup>	349.75 <sup>a</sup>	13.64	0.0006
	90 days old	303.89 <sup>b</sup>	561.78 <sup>a</sup>	40.29	0.0069

Different superscript letters in rows represent statistical differences

the basal diet, increased activity of CPK in the lambs born in the control group was expected. This could also be correlated with the higher level of WMD seen in this group. Similar to our findings, there are some reports on effect of Se supplementation [50] and Se injection [10] on CPK activity in sheep fed a basal diet deficient in Se. In contrast, there was no significant difference for CPK activity in fattening lambs fed a basal diet containing 0.06 mg Se/kg DM (control group) or basal diet + 0.2 mg Se/kg DM [11]. This difference may be related to the type of diet used, since it has been demonstrated that selenium absorption in forage based diets (present study) is less than diets with high levels of concentrate [51].

Plasma concentration of vitamin B<sub>12</sub> in bolus-treated ewes and their lambs was significantly higher as compared to control (Table 11). In addition, milk concentration of vitamin B<sub>12</sub> in treated ewes was significantly higher than that of animals in the control group. The only known animal requirement for cobalt is as a constituent of vitamin B<sub>12</sub>, which has about 4% cobalt in its chemical structure. This means that a cobalt deficiency is really a vitamin B<sub>12</sub> deficiency [52]. Microorganisms in the rumen are able to synthesize vitamin B<sub>12</sub> needs of ruminants, if the diet is adequate in cobalt. In lambs, until the rumen becomes functional, the only vitamin B<sub>12</sub> supply is their mother's milk. However, at birth, the rumen is not yet functional and becomes functional when the lambs are older. The lambs start to produce their own vitamin B<sub>12</sub> from about 1 month of age, when the rumen becomes functional. It is believed that the concentration of vitamin B<sub>12</sub> in the milk is a reliable index of cobalt sufficiency in ruminants [53]. Higher concentrations of vitamin B<sub>12</sub> in blood of the lambs born from ewes receiving bolus on day 10 may be associated with higher vitamin B<sub>12</sub> levels in milk. Considering increasing trend in both groups from d 10 to d 90 in plasma vitamin B<sub>12</sub> which should be due to cobalt supplied from the feed to the rumen microorganisms, the significant difference between the two groups may be related to the higher vitamin B<sub>12</sub> content of the bolus group. Similarly, Zervas [18]

indicated that administration of a slow-release bolus (Cu, Co, and Se) at 3 months prepartum to ewes increased the plasma concentrations of vitamin B<sub>12</sub> until 3 months postpartum. Furthermore, Andrews and Stephenson [54] reported that cobalt supplementation of pregnant ewes enhanced vitamin B<sub>12</sub> status of their lambs due to increases in the fetal reserves of the vitamin and supply of vitamin B<sub>12</sub> from milk.

## Conclusion

Obtained results showed that maternal supplementation of Zn, Se, and Co as slow-release ruminal bolus in late pregnancy improved some mineral status of ewes and their lambs until weaning and led to higher body weights of lambs at weaning.

**Acknowledgments** All funds were provided by Bu-Ali Sina University, Hamedan, Iran, as a Ph.D. dissertation program funding.

## References

1. Wettemann R, Lents C, Ciccioli N, White F, Rubio I (2003) Nutritional- and suckling-mediated anovulation in beef cows. *J Anim Sci* 81(14\_suppl\_2):E48–E59
2. Wu G, Bazer F, Wallace J, Spencer T (2006) Board-invited review: intrauterine growth retardation: implications for the animal sciences. *J Anim Sci* 84(9):2316–2337
3. Suttle NF (2010) Mineral nutrition of livestock. Cabi
4. Rock M, Kincaid R, Carstens G (2001) Effects of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. *Small Rumin Res* 40(2):129–138
5. Hatfield P, Snowden G, Head W, Glimp H, Stobart R, Besser T (1995) Production by ewes rearing single or twin lambs: effects of dietary crude protein percentage and supplemental zinc methionine. *J Anim Sci* 73(5):1227–1238
6. Monem UA, El-Shahat K (2011) Effect of different dietary levels of inorganic zinc oxide on ovarian activities, reproductive performance of Egyptian Baladi ewes and growth of their lambs. *Bul J Vet Med* 14(2):116–123

7. Quirk M, Norton B (1987) The relationship between the cobalt nutrition of ewes and the vitamin B12 status of ewes and their lambs. *Crop Pasture Sci* 38(6):1071–1082
8. Alloway BJ (2004) Zinc in soils and crop nutrition. International Zinc Association Brussels, Belgium
9. Fadayifar A, Aliarabi H, Tabatabaei MM, Zamani P, Bahari A, Malecki M, Dezfoulian AH (2012) Improvement in lamb performance on barley based diet supplemented with zinc. *Livest Sci* 144(3):285–289
10. Mohri M, Ehsani A, Norouzian M, Bami MH, Seifi HA (2011) Parenteral selenium and vitamin E supplementation to lambs: hematology, serum biochemistry, performance, and relationship with other trace elements. *Biol Trace Elem Res* 139(3):308–316
11. Alimohamady R, Aliarabi H, Bahari A, Dezfoulian AH (2013) Influence of different amounts and sources of selenium supplementation on performance, some blood parameters, and nutrient digestibility in lambs. *Biol Trace Elem Res* 154(1):45–54
12. Bishehsari S, Tabatabaei MM, Aliarabi H, Alipour D, Zamani P, Ahmadi A (2010) Effect of dietary cobalt supplementation on plasma and rumen metabolites in Mehraban lambs. *Small Rumin Res* 90(1):170–173
13. Kojouri G, Shirazi A (2007) Serum concentrations of Cu, Zn, Fe, Mo and Co in newborn lambs following systemic administration of vitamin E and selenium to the pregnant ewes. *Small Rumin Res* 70(2):136–139
14. Kendall N, Mackenzie A, Telfer S (2001) Effect of a copper, cobalt and selenium soluble glass bolus given to grazing sheep. *Livest Sci* 68(1):31–39
15. McDowell LR (1992) Minerals in animal and human nutrition. Academic Press Inc.
16. Kendall N, McMullen S, Green A, Rodway R (2000) The effect of a zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. *Anim Reprod Sci* 62(4):277–283
17. Kendall N, Mackenzie A, Telfer S (2012) The trace element and humoral immune response of lambs administered a zinc, cobalt and selenium soluble glass bolus. *Livest Sci* 148(1):81–86
18. Zervas G (1988) Treatment of dairy sheep with soluble glass boluses containing copper, cobalt and selenium. *Anim Feed Sci Technol* 19(1):79–83
19. Abdelrahman MM, Aljumaah RS, Khan RU (2017) Effects of prepartum sustained-release trace elements ruminal bolus on performance, colostrum composition and blood metabolites in Najdi ewes. *Environ Sci Pollut Res* 24(10):9675–9680
20. Horwitz W, Horwitz W (2000) Official methods of analysis of AOAC International. vol C/630.240 O3/2000
21. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70(1):158–169
22. SAS S (2004) STAT 9.1 user's guide. SAS Institute Inc, Cary, pp 1291–1320
23. Hatfield P, Snowden G, Glimp H (1992) The effects of chelated zinc methionine on feedlot lamb performance, cost of gain, and carcass characteristics. *Sheep research journal (USA)*
24. Gabryszuk M, Klewicz J (2002) Effect of injecting 2- and 3-year-old ewes with selenium and selenium–vitamin E on reproduction and rearing of lambs. *Small Rumin Res* 43(2):127–132
25. Kendall N, Mackenzie A, Telfer S (1997) Effect of a soluble cobalt, selenium and zinc glass bolus on humoral immune response and trace element status in lambs. In: *Trace elements in man and animals-9: Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals*. NRC Research Press, Ottawa, pp 442–443
26. Awati M, Awati AM (2014) Sedation in ICUs (384)
27. Van Niekerk F, Van Niekerk C, Heine E, Coetzee J (1990) Concentrations of plasma copper and zinc and blood selenium in ewes and lambs of Merino, Dohne Merino and SA Mutton Merino sheep. *S Afr J Anim Sci* 20(1):21–26
28. White C, Chandler B, Peter D (1991) Zinc supplementation of lactating ewes and weaned lambs grazing improved mediterranean pastures. *Anim Prod Sci* 31(2):183–189
29. Zali A, Ganjkanlou M (2009) Effect of zinc from zinc sulfate on trace mineral concentrations of milk in Varamini ewes. *Afr J Biotechnol* 8(22)
30. Vergnes H, Courdouhji M, Guelfi J, Grozdea J, Lamand M (1990) Effect of zinc deficiency in lambs on plasma and neutrophil alkaline phosphatase. *Small Rumin Res* 3(2):167–177
31. Nagalakshmi D, Dhanalakshmi K, Himabindu D (2009) Effect of dose and source of supplemental zinc on immune response and oxidative enzymes in lambs. *Vet Res Commun* 33(7):631–644
32. Phipps RH, Grandison AS, Jones AK, Juniper DT, Ramos-Morales E, Bertin G (2008) Selenium supplementation of lactating dairy cows: effects on milk production and total selenium content and speciation in blood, milk and cheese. *Animal* 2(11):1610–1618
33. Kachuee R, Moeini M, Souiri M (2014) Effects of organic and inorganic selenium supplementation during late pregnancy on colostrum and serum Se status, performance and passive immunity in Merghoz goats. *Anim Prod Sci* 54(8):1016–1022
34. Erdoğan S, Karadaş F, Yılmaz A, Karaca S (2017) The effect of organic selenium in feeding of ewes in late pregnancy on selenium transfer to progeny. *Rev Bras Zootec* 46(2):147–155
35. Hefhawey AE, Youssef S, Aguilera PV, Rodríguez CV, Pérez J (2014) The relationship between selenium and T3 in selenium supplemented and nonsupplemented ewes and their lambs. *Vet Med Int* 2014
36. Zachara B, Wardak C, Didkowski W, Maciag A, Marchaluk E (1993) Changes in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. *Gynecol Obstet Investig* 35(1):12–17
37. Pechova A, Misurova L, Pavlata L, Dvorak R (2008) Monitoring of changes in selenium concentration in goat milk during short-term supplementation of various forms of selenium. *Biol Trace Elem Res* 121(2):180–191. <https://doi.org/10.1007/s12011-007-8033-3>
38. Pechova A, Sevcikova L, Pavlata L, Dvorak R (2012) The effect of various forms of selenium supplied to pregnant goats on selected blood parameters and on the concentration of Se in urine and blood of kids at the time of weaning. *Vet Med* 57(8):394–403
39. Petrerá F, Calamari L, Bertin G (2009) Effect of either sodium selenite or Se–yeast supplementation on selenium status and milk characteristics in dairy goats. *Small Rumin Res* 82(2):130–138
40. Misurova L, Pavlata L, Pechova A, Dvorak R (2009) Selenium metabolism in goats—maternal transfer of selenium to newborn kids. *Vet Med* 54(3):125–130
41. Lacetera N, Bernabucci U, Ronchi B, Nardone A (1999) The effects of injectable sodium selenite on immune function and milk production in Sardinian sheep receiving adequate dietary selenium. *Vet Res* 30(4):363–370
42. Arthur JR, Beckett GJ, Mitchell JH (1999) The interactions between selenium and iodine deficiencies in man and animals. *Nutr Res Rev* 12(01):55–73
43. Köhrle J (2000) The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. *Cell Mol Life Sci* 57(13–14):1853–1863
44. Abou-Zeina H, Hassan S, Sabra H, Hamam A (2009) Trials for elevating adverse effect of heat stress in buffaloes with emphasis on metabolic status and fertility. *Glob Vet* 3(1):51–62
45. Liu N, Liu P, Xu Q, Zhu L, Zhao Z, Wang Z, Li Y, Feng W, Zhu L (2001) Elements in erythrocytes of population with different thyroid hormone status. *Biol Trace Elem Res* 84(1–3):37–43
46. Pekary AE, Lukaski HC, Mena I, Hershman JM (1991) Processing of TRH precursor peptides in rat brain and pituitary is zinc dependent. *Peptides* 12(5):1025–1032

47. El-Tohamy M (2002) Effect of single and combined zinc-selenium deficiencies of rams. In: XXII World Buiatrics Congress, Hannover
48. Ruz M, Codoceo J, Galgani J, Muñoz L, Gras N, Muzzo S, Leiva L, Bosco C (1999) Single and multiple selenium-zinc-iodine deficiencies affect rat thyroid metabolism and ultrastructure. *J Nutr* 129(1): 174–180
49. Radostits OM, Gay C, Hinchcliff KW, Constable PD (2007) A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Vet Med* 10:2045–2050
50. Faixova Z, Faix Š, Leng E, Vaczi P, Makova Z, Szaboova R (2007) Haematological, blood and rumen chemistry changes in lambs following supplementation with Se-yeast. *Acta Vet Bmo* 76(1):3–8
51. Del Razo-Rodriguez O, Ramirez-Briebesca J, Lopez-Arellano R, Revilla-Vazquez A, Gonzalez-Munoz S, Cobos-Peralta M, Hernandez-Calva L, McDowell L (2013) Effects of dietary level of selenium and grain on digestive metabolism in lambs. *Czech J Anim Sci* 58(6):253–261
52. Berger LL, Cunha TJ (1993) Salt and trace minerals for livestock, poultry and other animals. Salt Institute Alexandria, VA, USA
53. Ramos J, Saez T, Bueso J, Sanz M, Fernandez A (1994) Vitamin B12 levels in ewe colostrum and milk and in lamb serum. *Vet Res* 25(4):405–409
54. Andrews E, Stephenson B (1966) Vitamin B12, in the blood of grazing cobalt-deficient sheep. *N Z J Agric Res* 9(3):491–507