

EFFECT OF SELENIUM AND VITAMIN E SUPPLEMENTATION DURING LATE PREGNANCY ON COLOSTRUM AND PLASMA Se, Cu, Zn AND Fe CONCENTRATIONS OF FAT TAIL SANJABI EWES AND THEIR LAMBS

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Effect of selenium and vitamin E supplementation during late pregnancy on colostrum and plasma Se, Cu, Zn and Fe concentrations of fat tail Sanjabi ewes and their lambs

Twenty seven fat tail ewes were randomly assigned to three treatment groups. Four and two weeks before expected lambing, ewes were injected intramuscularly 0 ml (C) 5 ml (T1), 10 ml (T2) selenium and vitamin E respectively. Blood samples were collected from ewes four weeks before expected lambing and on the day of lambing. Blood samples of their lambs were taken from the jugular vein at birth and 7 days of age. Selenium, copper, zinc and iron concentrations of plasma and colostrum were measured by inductively couple plasma (ICP-OES). The plasma Se concentration of lambs in T2 was significantly increased compared with control group at 7 days of age ($P < 0.05$). The colostrums Se and Cu concentrations were significantly increased in Se supplemented ewe and their lambs ($P < 0.05$). The plasma Zn concentration of ewes at lambing and colostrum were significantly decreased in T2 compared with controls. The plasma Zn concentration of lambs of ewes of T2 was significantly decreased compared with other groups at 7 days of age ($P < 0.05$). The serum concentration of Cu increased in supplemented ewes, which was concomitant with a reduction in Zn concentration in serum and colostrums. It seems that an increase in serum concentration of Cu at the end of pregnancy in ewes given Se supplement could disturb the Zn: Cu ratios which in turn lead to Zn reduction. There was a positive correlation between serum Se concentration with Cu and Fe levels and also a negative correlation between Se and Zn in treated ewes.

Key word: sheep / breeds / Sanjabi / animal nutrition / selenium / vitamin E / pregnancy / colostrum / blood / composition

Vpliv dodajanja selena in vitamina E med pozno brejstjo na koncentracijo selena, bakra, cinka in železa v kolostrumu in v plazmi pri debelorepih Sanjabi ovkah in njihovih jagnjetih

Sedemindvajset debelorepih Sanjabi ovac smo razdelili v tri poskusne skupine. Štiri in dva tedna pred pričakovano jagnitvijo smo ovcam intramuskularno vbrizgali 0 ml (C) 5 ml (T1), 10 ml (T2) selena in vitamina E. Vzorce krvi smo ovcam vzeli štiri tedne pred pričakovano jagnitvijo in na dan jagnitve. Jagnjetom smo kri vzeli iz jugularne vene ob rojstvu in po sedmih dnevih. Koncentracije selena, bakra, cinka in železa v plazmi in kolostrumu smo merili po ICP-OES metodi. Koncentracija selena v plazmi jagnjet je bila pri skupini T2 značilno višja kot pri kontrolni skupini po sedmih dnevih ($p < 0,05$). Koncentracija Se in Cu v kolostrumu in plazmi je bila značilno povišana pri ovkah, ki so dobole dodatek selena in pri njihovih jagnjetih ($p < 0,05$). Koncentracija Zn v plazmi ob porodu in v kolostrumu je bila značilno znižana pri skupini T2 v primerjavi s kontrolami. Koncentracija Zn pri jagnjetih ovac iz skupine T2 je bila značilno znižana v primerjavi z drugimi skupinami 7 dni po porodu ($p < 0,05$). Koncentracija Cu v serumu ovac, ki so bile tretirane s Se in vitaminom E, je bila povišana, kar je skladno z znižano koncentracijo Zn v serumu in kolostrumu. Povišana koncentracija Cu ob koncu brejosti pri ovkah, ki so prejele dodatek selena, lahko poruši razmerje med cinkom in bakrom, kar vodi do zmanjšanja koncentracije cinka. Korelacija med serumsko koncentracijo Se in koncentracijama Cu in Fe je bila pri ovkah, ki so bile tretirane s Se in vitaminom E, pozitivna, med koncentracijo Se in Zn pa negativna.

Ključne besede: ovce / pasme / Sanjabi / prehrana živali / selen / vitamin E / brejost / kolostrum / kri / sestava

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1 INTRODUCTION

Mineral elements, considered essential for sheep and goats, consist of seven major elements and twelve trace elements (Lee *et al.*, 2002; Meschy, 2000). The concentration of these elements must be maintained within narrow limits if the functional and structural activities of the tissues are to be safeguarded and the growth, health, and productivity of the animal are to remain unimpaired (Underwood *et al.*, 1999). Selenium is an important trace mineral, acting in synergism with vitamin E and other anti oxidative agents such as Cu and Zn which inhibit the oxidation of membrane fat polyunsaturated acids and DNA by oxygen radicals produced during aerobic metabolism (Florence, 1995). Se, Cu, Fe and Zn are micro-elements that rank among substances with a biological activity in intermediate metabolism. These trace minerals enter the organism mainly as components of animal diet and their trans-placental transfer is an important factor in the young animal. The level of absorption and retention of microelements is modulated by their actual levels in the tissue and their concentrations in the diet (Knowles *et al.*, 1999; Cao *et al.*, 2000).

Petrera *et al.* (2009) showed that even if goats are well supplemented with selenium, its concentration in milk can only be increased by adding organic selenium in the form of yeast. Burk *et al.* (2001) demonstrated that Se from Se-Met can be incorporated into proteins, presumably as Se-Met within the Met pool. Conversely, in goats supplemented with sodium selenite, the Se-Met content of serum increased only slightly because some animals have no efficient mechanism for Se-Met synthesis and are also unable to synthesize Se-Met (Schrauzer, 2003).

The occurrence of significant changes in Se, Cu and Zn concentrations in calves' colostrum and serum was probably due to vitamin E and Se supplementation given to pregnant heifers (Moeini *et al.* 2011). It seems that large doses of Se injected in pregnant heifers may inhibit Zn levels in their colostrum which, in turn, lead to Zn reduction in heifers and their newborn calves. Zn should be supplemented when more than 40 ml Se supplements were administered to pregnant heifers (Moeini *et al.* 2011).

Se supplementation to lambs on Cu deficient pastures increased Cu absorption and improved the growth rate (Cristaldi *et al.*, 2005; Feroci *et al.*, 2005). A high doses Se supplement could disturb the Zn: Cu and Zn: Fe ratios which can lead to Zn deficiency in young ruminant animals (Kojouri *et al.*, 2007). The beneficial effects of nutritional or parenteral administration of Se have not been universal among studies, and potentially confounding factors, such as Se status at the time of administration, duration and dose, and pre-existing incidence of

disease within a herd before administration can all affect the outcome of treatment.

Since the soils and feeds in many areas of Iran are deficient in Se, it is suggested mineral premix for animals should be supplemented with Se (Izadyar, 1987, Mohri *et al.*, 2005, Moeini *et al.* 2011b). Experimental studies in ruminants have demonstrated that injection of Se caused an increase of Cu concentration in the liver in animals receiving either normal (Hussein *et al.*, 1985) or Cu-deficient diets (Thomson & Lawson 1970) while Fe have minimal negative effects on Zn absorption and utilization (Alonso *et al.*, 2004, Zavoshti *et al.*, 2011). Neither vitamin E or Se supplementation caused changes of the serum concentrations of Fe, Zn, and Cu under thermo neutral conditions, whereas dietary supplemental Se at 1 mg/kg Se caused a significant reduction of the serum Cu concentrations of heat-stressed broilers (Ghazi *et al.*, 2012).

This study was preformed to investigate the effect of different levels of selenium and vitamin E supplementation during late pregnancy on colostrum and plasma Se, Cu, Fe and Zn status of fat tail Sanjabi ewes and their lambs.

2 MATERIALS AND METHODS

This experiment was performed at Mehregan research station in Kermanshah province in west of Iran. Twenty seven Sanjabi ewes were randomly assigned to three treatment groups, Animal were same for age, weight and lambed at the same time.

The ewes were fed alfalfa hay, concentrate and oat (*Sativa avena*) according NRC 1985. The nutrient content of dietary is presented in Table 1. The Se content of the ration was 0.06 mg/Kg DM. Four and two weeks before expected lambing, ewes were injected intramuscularly: no injection (C), 5 ml (T2), 10 ml (T3) Se and vitamin E respectively (each ml of the supplement containing of 0.5 mg Selenium as sodium selenite and 50 mg vitamin E as D, L-alpha-tocopheryl acetate).

Blood samples were collected from the jugular vein of ewes four weeks before the expected time of lambing (before administration supplement) and on the expected time of lambing. Blood samples of the lambs were drawn from their jugular vein at birth (before ingesting colostrum) and at 7 days of age. Colostrum samples were collected from ewes in 1, 10 & 18 hours after lambing. Blood samples were centrifuged for 15 min at 1000 rpm. The plasma & colostrum samples stored at -20 °C until analysis.

Se, Cu, Zn and Fe concentrations in plasma and colostrum were measured by working standards for in-

Table 1: Nutrient composition (DM basis) of alfalfa hay, concentrate and oat**Preglednica 1:** Vsebnost hrani (preračunano na suho snov) v lucerninem senu, koncentratu in ovsu

Nutrient	Alfalfa hay	Wheat straw	Oat	Concentrate
% DM	92.53	93.66	93.11	88.1
% CP	15.30	3.21	10.33	18
EE, kcal/kg	4472.08	4248.34	4235.91	5325
% CF	32.15	40.10	6.03	-
Ca %	1.87	0.43	0.18	0.33
Mg %	0.33	0.12	0.15	0.16
P %	0.20	0.09	0.31	0.29
Na %	0.12	0.03	0.04	0.26
Zn, mg/kg	29.13	14	34.5	33.02
Mn, mg/kg	37.16	40.3	20.24	46.9
Iron, mg/kg	354.36	617.61	216.61	-
Cu, mg/kg	13.49	5.1	7.94	-
Se, mg/kg	0.19	0	0.07	0.07

ductively coupled plasma optical emission spectrometry (ICP-OES) analysis were prepared from proper standard solutions containing 1000 PPM for each tested element obtained from Perkin Elmer (USA) according to the method described by Kachuee (2012). The means were separated and compared using Duncan's multiple range tests when ANOVA indicated significant at $P < 0.05$. The differences between treatment groups were estimated using the following model:

$$X_{ij} = \mu + T_j + \epsilon_{ij}$$

where X_{ij} is dependent variable, μ is the overall mean, T_j is the effect of treatment and ϵ_{ij} is the random error. The statistical analyses were performed using the SPSS package 18 (2009).

Table 2: Mean selenium (Se) concentrations of ewes and their lambs ($\mu\text{g/l}$)**Preglednica 2:** Povprečne koncentracije selena (Se) v serumu ovac in njihovih jagnjet ter v kolostrumu

	Time	Control	T1	T2
Ewes	Before supplementation	68 ± 3.6	69 ± 4.1	67 ± 3.5
	Lambing day	54 ± 3.2 ^{bc}	71 ± 2.5 ^{ab}	83 ± 3.8 ^a
	Colostrum	116 ± 6.5 ^b	140 ± 8.2 ^a	142 ± 7.5 ^a
Lambs	At birth	49 ± 1.7 ^b	62 ± 3.4 ^a	67 ± 2 ^a
	7 days of age	56 ± 4.6 ^{bc}	65 ± 5.1 ^{ab}	72 ± 4 ^a

($\mu\text{g} \pm \text{SE}$) = Mean ± standard error / povprečje ± standardna napaka; Means with different superscripts in the same row differ at $P < 0.05$ / Povprečja z različnimi oznakami v isti vrsti so statistično značilno različna ($p < 0.05$)

3 RESULTS AND DISCUSSION

The plasma and colostrum Se concentration of ewes and their lambs are shown in Table 2. The results indicated that the plasma Se concentrations of ewes before injection of Se and vitamin E supplement did not differ ($P > 0.05$) among all groups but at lambing day, the mean plasma Se concentrations increased in T2 compared with controls ($P < 0.05$).

Plasma Se concentrations of new born lambs at birth day was higher in lambs in T1 and T2 compared with control group ($P < 0.05$) as well as plasma Se concentrations of lambs at 7 days of age was higher in lambs in T2 compared with control group ($P < 0.05$). The mean values of colostrum Se concentrations were higher in T1 and T2 compared with control group ($P < 0.05$).

It was obvious that the plasma Se content was markedly increased in treated ewes and their lambs. This was in agreement with previous reports (Abdelrahman and Kincaid, 1995; Davis *et al.*, 2006). Moeini *et al.* (2011) reported that serum Se concentrations in heifers and their calves did not differ among all groups before injection of selenium and vitamin E supplement but, at calving, the serum Se concentration increased in the treatment groups.

Knowles *et al.* (1999) and Juniper *et al.* (2006) showed a similar result in dairy cows. In lactating cows, the intake of more than 6 mg of Se/day is suggested to maintain the level of serum to 70 μg

Table 3: Mean copper (Cu) concentrations of ewes and their lambs ($\mu\text{g/l}$)
Preglednica 3: Povprečne koncentracije bakra (Cu) v serumu ovac in jagnjet ter v kolostrumu

	Time	Control	T1	T2
Ewes	Before supplementation	665 ± 15.3	652 ± 16.7	670 ± 15
	Lambing day	660 ± 11.6 ^b	785 ± 13.6 ^a	805 ± 12.2 ^a
	Colostrum	490 ± 11	500 ± 12.8	525 ± 14.3
Lambs	At birth	385 ± 9.5	396 ± 8.8	400 ± 10.1
	7 days of age	599 ± 6.4 ^b	611 ± 7.9 ^b	643 ± 9 ^a

($\mu \pm \text{SE}$) = Mean ± standard error / povprečje ± standardna napaka; Means with different superscripts in the same row differ at $P < 0.05$ / Povprečja z različnimi oznakami v isti vrsti so statistično značilno različna ($p < 0.05$)

of Se/L (Gerloff, 1992). In our study lambs from treated ewes had higher plasma Se concentrations ($P < 0.05$).

The value of our results were more than nearly double the plasma Se concentrations suggested and plasma Se concentrations of control lambs were only slightly above the normal range. Close correlations exist between the concentration of Se in blood of calves and in dams at birth (Kincaid *et al.*, 1989).

Furthermore Kim and Mahan (2001) reported that elevated serum and tissue Se concentrations in neonate pigs when dietary Se levels of sows were increased. Concentrations of Se in the plasma of lambs were affected by dietary Se concentrations of their dams (Davis *et al.*, 2006). Placental and colostrum transfer of Se from ewe to lamb has been shown to occur even when the dam is deficient in selenium (Abd El-Ghany *et al.*, 2007, 2008, 2010). The efficiency of placental transfer is highly dependent on the levels and the chemical form of Se supplementation. Concentrations of Se in colostrum were significantly lower in control group (Table 2). The total Se content of colostrum in controls (Table 2) was in the range reported in other study (Benemariya *et al.*, 1993). Dietary Se supplementation of pregnant beef cows, markedly increased

concentrations of Se in colostrum and milk (Ammerman *et al.*, 1980).

The concentrations of Se in Colostrum was affected by Se supplementation of the ewe's diet and increased linearly as dietary Se increased (Davis *et al.*, 2006). The results of this trial are further supported by Kim and Mahan (2000) and Puls (1994), who demonstrated that colostrum Se was increased by increasing Se in pre partum and post-partum sow's diet. According to Taylor *et al.* (2009) ewes fed Se-enriched wheat grain had greater placental transfer of Se

to the fetus compared with ewes supplemented with sodium selenite.

The mean values of colostrum Se concentrations are shown in Table 2, these results confirm many other results obtained when dietary Se and vitamin E supplementation of pregnant beef cows and heifer, markedly increased concentrations of Se in both their colostrum and milk (Koller *et al.*, 1984, Moeini *et al.*, 2011).

The plasma and colostrum Cu concentration of ewes and their lambs are shown in Table 3. The plasma concentration of Cu in ewes at lambing day increased in supplemented groups ($P < 0.05$). The plasma Cu concentration of lambs in T2 was significantly increased compared with control group at 7 days of age ($P < 0.05$). The colostrum Cu concentration did not differ in ewes ($P > 0.05$).

The relatively important and rapid increase in serum Cu concentrations of newborn calves during the period of colostral nutrition may therefore be associated with increasing Cu colostrum concentration and improving the ability of the body to produce caeroloplasmin and, thus, distribute Cu from the liver (Pavlata *et al.*, 2001, 2004, 2005; Enjalbert *et al.*, 1999). These results were in agreement with the finding of another study in which supplementary administration of Se to sheep on

Cu deficient pastures increased Cu status (Cristaldi *et al.*, 2005). This finding is also in agreement with the result of Kojouri and Shirazi (2007). Kojouri and Shirazi (2007) and NRC 2001 indicated that the vitamin E and Se supplementation increases serum Cu concentration in lambs of treated ewes from birth to the fourth week of age (NRC 2001). This is, however, in contrast with the results of Gooneatne and Christensen (1989) and Muehlenbein *et al.*, (2001).

Table 4: Mean zinc (Zn) concentrations of ewes and their lambs ($\mu\text{g/l}$)

Preglednica 4: Povprečne koncentracije cinka (Zn) v serumu ovac in njihovih jagnjet ter v kolostrumu

	Time	Control	T1	T2
Ewes	Before supplementation	1142 ± 57	1128 ± 72	1150 ± 61
	Lambing day	1018 ± 32 ^a	946 ± 28 ^{ab}	912 ± 35 ^{bc}
	Colostrum	13280 ± 213 ^a	12760 ± 271 ^{ab}	12080 ± 381 ^{bc}
Lambs	At birth	887 ± 14 ^a	861 ± 17 ^{ab}	812 ± 16 ^{bc}
	7 days of age	1040 ± 31 ^a	985 ± 18 ^a	905 ± 10 ^b

($\mu \pm \text{SE}$) = Mean ± standard error / povprečje ± standardna napaka; Means with different superscripts in the same row differ at $P < 0.05$ / Povprečja z različnimi oznakami v isti vrsti so statistično značilno različna ($p < 0.05$)

Table 5: Mean iron (Fe) concentrations of ewes and their lambs ($\mu\text{g/l}$)

Preglednica 5: Povprečne koncentracije železa (Fe) v serumu ovac in njihovih jagnjet ter v kolostrumu

	Time	Control	T1	T2
Ewes	Before supplementation	955 ± 13	968 ± 17.5	948 ± 15
	Lambing day	815 ± 14.2	821 ± 16	826 ± 19
	Colostrum	1180 ± 28	1200 ± 31	1205 ± 19
Lambs	At birth	497 ± 10.1	501 ± 14.2	510 ± 11
	7 days of age	619 ± 15.1	624 ± 13	620 ± 12.2

($\mu\pm \text{SE}$) = Mean ± standard error / povprečje ± standardna napaka

Ahmed *et al.*, (2001) reported that the low plasma Cu level obtained in lactating animals to the small role of Cu in milk formation and to the fact that some Cu could be stored in the liver before being excreted into milk. Also they suggested that, the slight differences obtained between high and low yielders could be taken as indication of the low genetic makeup effect of Cu on lactation. These results are in agreement with the findings of Enjalbert *et al.* (2002), who also reported low plasma Cu concentrations in calves in week 1 post partum compared with those found in their dams, and with our previous findings (Pavlata *et al.*, 2004).

Also Moeini *et al.* (2011) reported that the supplemented heifers and their progenies had higher total Cu contents in serum at calving and birth day when compared to controls. Our results showed the positive effect of Se administration to the pregnant ewes on Cu status of their offspring. This is however contradicts with the results of Gooneatne and Christensen (1989) and Muehlenbein *et al.*, (2001).

The plasma and colostrum Zn concentration of ewes and their lambs are shown in Table 4. The Zn concentration of ewes at lambing day, lambs at birth and colostrum were significantly decreased in T2 compared with controls ($P < 0.05$). The plasma Zn concentration lambs of T2 was significantly decreased compared with other groups at 7 days of age ($P < 0.05$). It seems the large quantities of Zn are used up by the developing fetus but high concentration of Zn are present in colostrum and milk (Pavlata *et al.*, 2004, 2003).

The interaction between Cu and Zn assumes practical significance when more Cu is added to ration (Underwood and Suttle, 1999). The absorption of Zn is impaired by elements such as Cu and cadmium, through an increase the mucosal binding of Zn by metallothionein (Bremner, 1993). Se injection increased Cu absorption while Cu changes may have an indirect effect of Se on serum Zn concentrations.

Kojouri and Shirazi (2007) reported that changes in the Zn: Cu ratios at the end of the fourth week, in turn,

might have led to Zn reduction. The plasma Zn concentration slightly increased during the first week of life and then gradually decreased from the second week and declined significantly in the fourth week of life in lambs of the Se treated group. The Zn levels were insufficient for the late pregnancy and early lactation periods. This is possibly so because large quantities of Zn are used up by the developing fetus and because concentrations of Zn are present also in colostrum and milk (Muehlenbein *et al.*, 2001; Kracmar *et al.*, 2003; Pavlata *et al.*, 2004).

A remarkable feature of Se consists of its ability to oxidize thiols under reducing conditions. Thus, one mode of action recently suggested is the oxidation of thiol groups of metallothionein, thereby providing Zn for essential reactions. The results of studies indicate that Zn finger motifs are highly reactive towards oxidizing Se compounds. This could affect gene expression, DNA repair and, thus, genomic stability (Blessing *et al.*, 2004).

These results are in agreement with the findings of Moeini *et al.* (2011), who also reported that the serum Zn concentrations at parturition day decreased and tended to be lower for the Se treated heifers compared with controls. Also the Zn concentration of colostrum decreased in treated heifers. They stated that more Zn supplement should be administered, when more than 80 ml or (40 mg) Se supplements were given to pregnant heifers.

The plasma and colostrum Fe concentration of ewes and their lambs are shown in Table 5. The Fe concentration did not differ ($P > 0.05$) in plasma and colostrum of all treatments. Fe deficiencies except in young ruminants and milk-fed calves do not normally occur in ruminants (Mollerberg, 1975). Kojouri and Shirazi (2007) indicated that administration of vitamin E and Se affected the Fe concentrations in lambs and Fe concentrations of treated lambs were increased from birth to 4 weeks of life. But in other study the Fe concentrations in calves were similar and in the normal range and Se injection did not affected the Fe concentrations in calves of the treated heifers (Moeini *et al.*, 2011).

The significant antagonisms between most of these nutritional essential metals have been largely demonstrated in studies in animals exposed both at high or deficient mineral intakes (NRC 2001; Mertz 1986; Puls 1994).

4 CONCLUSION

The plasma Se and Cu concentrations increased in Se treated ewes compared with those in controls but no changes were seen in plasma Fe concentrations. The serum concentration of copper increased in supplemented ewes, which was concomitant with a reduction in Zn concentration in serum and colostrums. It seems that an increase in serum concentration of Se and Cu at the end of pregnancy in ewes given Se supplement could disturb the Zn: Cu ratios which in turn lead to Zn reduction in ewes and their newborn lambs. It was seen a positive correlation between plasma Se concentration with Cu and Fe levels and also a negative correlation between Se and Zn in treated ewes and their lambs.

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