

## The Effect of Selenium Administration on the Selenium, Copper, Iron and Zinc Status of Pregnant Heifers and Their Newborn Calves

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### ABSTRACT

This study was performed to determine the effect of injecting selenium into pregnant heifers at the last stage of gestation on the serum Se, Cu, Zn and Fe status of the heifers and their calves. Fifty Holstein heifers were randomly assigned to one of five treatments. Four and two weeks before the expected time of calving, the heifers were injected intramuscularly 10 ml (T1), 20 ml (T2), 30 ml (T3), 40 ml (T4) of selenium and vitamin E, respectively. The control (C) group received no supplement. Each ml of the supplement® (Vet. Anim. Health BV) contained 0.5 mg Se as sodium selenite and 40 IU of *D-L* alpha-tocopheryl acetate. Blood samples were collected from heifers two weeks before the expected time of calving and on calving day. Blood samples of newborn calves also were taken from the jugular vein at birth and 7 days of age to measure the Se, Cu, Zn and Fe concentrations. The results indicated that the serum concentrations of Se increased in treated heifers compared with the controls. The selenium concentrations were significantly increased in the colostrum of treated heifers ( $P < 0.05$ ). Zn concentration of both serum and colostrum decreased in the treated group compared with controls but it was not significantly different. Serum Se, Cu concentrations of calves of treated heifers increased during the first week of age but the serum concentration of Zn decreased in newborn calves at 7 days of age ( $P < 0.05$ ). It seems that a high Se injection (T4) in pregnant heifers could increase the Cu and decrease the Zn concentrations and, thus, might disturb the Zn:Cu ratio which, in turn, leads to zinc reduction in heifers and their newborn calves. It can be concluded that a higher amount of Zn should be supplemented when more than 40 ml Se supplements are administered to pregnant heifers.

**Keywords:** Calves, Cu, Heifers, Interaction, Se, Zn.

### INTRODUCTION

Micro elements have a number of structural, catalytic and regulatory functions in organisms and play a major role in the functioning of the immune system (Underwood and Suttle, 1999).

Selenium (Se) is an important trace mineral, acting in synergism with vitamin E and other anti oxidative agents such as Zn and Cu which inhibit the oxidation of

membrane fat polyunsaturated acids and DNA by oxygen radicals produced during aerobic metabolism (Florence, 1995). Selenium, copper and zinc are microelements 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

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levels in the tissue and their concentrations in the diet (Knowles *et al.*, 1999; Cao *et al.*, 2000). Some reports indicated the inhibition of zinc absorption from a diet low in zinc when selenium was high in seleniferous regions (Underwood and Suttle, 1999). Recent research has demonstrated that sub-clinical or marginal deficiencies in key trace minerals like Zn, Cu and Se, as well as vitamin E and vitamin A are linked to the suppression of disease resistance and lower reproductive efficiencies in ruminant animals. Administration of Se supplementation to sheep grazing on copper deficient pastures increased copper absorption and improved the growth rate (Cristaldi *et al.*, 2005). However, a high Se supplement could disturb the Zn: Cu and Zn: Fe ratios which can lead to Zn deficiency in young ruminant animals (Kojouri and Shirazi, 2007). The beneficial effects of nutritional or parenteral administration of selenium 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 (Rodostits, O. *et al.*, 1994).

Since the soils and feeds in many areas of Iran are deficient in Se, all mineral premix should be supplemented with Se in animal nutrition (Izadyar, 1987; Mohri *et al.*, 2005). Owing due to the lack of reports about micro element interactions and induction or prevention of neonatal diseases, therefore this study was designed to determine the effect of different levels of Se and vitamin E supplement given to pregnant heifers on their serum Se, Cu, Zn and Fe status of heifers and that of their calves.

## MATERIALS AND METHODS

This experiment was conducted on a large commercial dairy farm in Kermanshah Province in western Iran. Fifty heifers were randomly divided into five treatments; they were homogeneous for age, weight and time of calving. The heifers were fed alfalfa hay, concentrate and corn silage according NRC 2001 (Table 1). The Se content of the rations without mineral supplements was marginal ( $0.16 \pm 0.35$  mg kg<sup>-1</sup>).

Heifers were transferred to a separate barn two months before the expected time of parturition. Four and two weeks before the due time of calving, the heifers were injected with 10 ml (T1), 20 ml (T2), 30 ml (T3) and 40 ml (T4) Se and vitamin E supplements, respectively (each ml of the supplement containing of 0.5 mg Se as sodium selenite and 40 IU of D, L-alpha-tocopheryl acetate, Vet.Anim.HealthBV®). The remaining 10 heifers were not treated and were assigned as a control group. Blood samples were collected from the jugular vein of heifers four weeks before the expected time of calving and on calving day. Immediately after parturition, newborn calves were taken away from the heifers. Blood samples of the calves were drawn from their jugular vein at birth and at 7 days of age. From birth to 80 days of age, calves were housed in individual pens. For the first 36 hours of life, at 8-10 hour intervals, calves of all groups were fed, 2 L of colostrum from their respective dams. From 3 through 6 days after birth, calves were allowed to consume 5 L of milk from their respective dams. Eight and 12 hours after calving, the heifers were milked and colostrum samples stored at -25°C until analysis. Selenium concentrations in the serum and colostrum were measured by hydride generation atomic absorption

**Table1.** Ingredient composition of concentrate mix fed to heifers (%DM).

Ingredient:	Barley	Corn	Beet pulp	Rap meal	Wheat	Cation-Anion
	34	6	8	14	36	2

spectrophotometer (AAS), according to the method described by Tiran *et al.* (1992). Serum and colostrum concentrations of Zn, Cu and Fe were determined by flame AAS. The experimental design was completely randomized with 10 replication and five treatments. Data for serum samples were analyzed by use of a repeated measure procedure using SPSS package 11.5 statistical software (2002). The comparative mean test was carried out by using Duncan method. The differences between injected groups of heifers were estimated using the following model:

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

where  $\mu$  is the overall mean,  $T_j$  the effect of  $j$ th injection Se and Vit E,  $\varepsilon_{ij}$  the random error.

## RESULTS AND DISCUSSION

Serum Se concentrations in heifers and their calves are shown in Table 2. These did not differ among all groups before injection of Se and vitamin E supplement but, at calving, the serum Se concentration decreased in the control group and increased in the treatment groups and were higher in the T3 and T4 groups ( $P < 0.05$ ). Abdelrahman and Kincaid (1995) reported that Se concentration decreased during the final 60 days of gestation, emphasizing the importance of Se supplementation during late gestation. The concentration of Se in colostrum samples has been shown in Table 2.

The selenium concentrations were significantly increased in the colostrum of treated heifers compared with the control ( $P < 0.05$ ). Dietary Se supplementation of pregnant beef cows, markedly increased concentrations of Se in both their colostrum and milk (Koller *et al.*, 1984).

The heifers given Se and Vit. E also produced more colostrum, and overall production of colostrum was higher in the treated heifers compared with the control. It should be mentioned that dry matter intake did not differ between treatments throughout the experiment.

The serum Cu concentration in heifers and their progenies is shown in Table 3. Treatments did not affect serum Cu concentrations in heifers at parturition day. The copper concentrations were significantly increased in the colostrum of treated heifers (T4) compared with the control group ( $P < 0.05$ ).

Serum Cu concentrations in calves of treated heifers were increased in the first week of age ( $P < 0.05$ ). During gestation, the copper concentrations increases progressively in the ovine and bovine fetal liver and decreases in maternal liver (Radostits, 2000). Blood Cu concentrations of calves rapidly increased quite during colostrum feeding. The lower blood Cu concentrations of calves compared with those of their mothers may be due to the fact that, in newborn calves, caeruloplasmin which is the main form of Cu transport in blood, is not produced to a sufficient extent

**Table 2.** Selenium concentrations of heifers and their calves ( $\mu\text{g l}^{-1}$ ).

	Control	T1	T2	T3	T4
Before calving	73±17	68±21	67±12	70±9	67±13
After calving	56±12 <sup>a</sup>	73±23 <sup>ab</sup>	85±15 <sup>bc</sup>	92±13 <sup>c</sup>	95±18 <sup>c</sup>
Colostrum	111±18 <sup>a</sup>	135±25 <sup>b</sup>	138±12 <sup>bc</sup>	145±13 <sup>bc</sup>	156±20 <sup>c</sup>
Calves at birth	51±10	69±10	76±6	82±12	85±12
Calves at 7 age in days	50±13 <sup>a</sup>	74±10 <sup>b</sup>	83±5 <sup>c</sup>	87±11 <sup>c</sup>	92±11 <sup>c</sup>
Sc2/Sc1	0.98±0.07 <sup>a</sup>	1.07±0.07 <sup>ab</sup>	1.09±0.04 <sup>ab</sup>	1.06±0.16 <sup>ab</sup>	1.08±0.04 <sup>b</sup>
Sc1/Sh2	0.91±0.17	0.94±0.28	0.89±0.15	0.89±0.10	0.90±0.14

Different letters within rows relate to significant (\* =  $P < 0.05$ )

Sc2/Sc1: Ratio of serum Se concentration in calves in 7 days/serum Se concentration in calves at birth.

Sc1/Sh2: Serum Se concentration in calves at birth/serum Se concentration in heifers at calving day.

**Table 3.** Copper concentrations in colostrums of heifers and their calves ( $\mu\text{g l}^{-1}$ ).

Heifers	Control	T1	T2	T3	T4
Before calving	690 $\pm$ 45	765 $\pm$ 54	712 $\pm$ 67	652 $\pm$ 59	728 $\pm$ 59
After calving	779 $\pm$ 38	790 $\pm$ 43	825 $\pm$ 64	817 $\pm$ 71	823 $\pm$ 74
Colostrum	500 $\pm$ 54 <sup>b</sup>	488 $\pm$ 54 <sup>b</sup>	528 $\pm$ 56 <sup>b</sup>	502 $\pm$ 54 <sup>b</sup>	595 $\pm$ 50 <sup>a</sup>
Calves at birth	420 $\pm$ 65	338 $\pm$ 33	410 $\pm$ 47	418 $\pm$ 39	461 $\pm$ 48
Calves- at 7 days	604 $\pm$ 43 <sup>b</sup>	581 $\pm$ 63 <sup>b</sup>	681 $\pm$ 61 <sup>ab</sup>	635 $\pm$ 63 <sup>ab</sup>	734 $\pm$ 58 <sup>a</sup>
CuSe/Cucol	0.66	0.63	0.61	0.69	0.63

Different letters within rows relate to significant (\*=  $P < 0.05$ )

Cuse/Cucol: Ratio of Cu concentration in serum/Cu concentration in colostrums.

(Underwood and Suttle, 1999).

The relatively important and rapid increase in serum Cu concentrations of newborn calves during the period of colostrum nutrition may therefore be associated with increasing Cu colostrum concentration and improving the ability of the body to produce caeroluplasmin and, thus, distribute Cu from the liver (Pavlata *et al.*, 2004; Enjalbert *et al.*, 1999). These results were in agreement with the finding of another study in which supplementary administration of Se to sheep on copper deficient pastures increased copper status (Cristaldi *et al.*, 2005). The occurrence of changes in calves' Cu serum concentrations in treatment groups compared with the control group was probably due to selenium supplementation given to pregnant heifers. The present results showed that the positive effect of Se administration to the pregnant heifers on their copper status and that of their offspring could increase the Cu status of newborn calves. This finding is in agreement with the

result of Kojouri and Shirazi (2007).

Kojouri and Shirazi (2007) indicated that the vitamin E and Se supplementation increases serum copper concentration in lambs of treated ewes from birth to the fourth week of age. This is, however, in contrast with the results of Gooneratne and Christensen (1989) and Muehlenbein *et al.*, (2001). They reported that organ Cu concentrations of calves during gestation and soon after delivery are higher than those of their mothers. Based on previous studies and this one, copper crosses the placental and mammary barrier is partly limited (Pavlata *et al.* 2005).

The serum Zn concentrations at parturition day decreased and tended to be lower for the Se treated heifers compared with the control (Table 4). Serum Zn concentrations in calves of treated heifers also were lower than calves of heifers in the control group ( $P < 0.05$ ). In contrast with the Cu level, the Zn concentration in their colostrum decreased in treated heifers (Table 4).

**Table 4.** Zinc concentrations of heifers and their calves ( $\mu\text{g l}^{-1}$ ).

	Control	T1	T2	T3	T4
Heifers serum before calving	1432 $\pm$ 134	1748 $\pm$ 176	1256 $\pm$ 124	1313 $\pm$ 150	1516 $\pm$ 167
After calving	1045 $\pm$ 98	1302 $\pm$ 102	997 $\pm$ 75	917 $\pm$ 125	1011 $\pm$ 140
Colostrum	14366 $\pm$ 1200	15127 $\pm$ 1100	13478 $\pm$ 879	12602 $\pm$ 850	13388 $\pm$ 980
Calves at birth	1505 $\pm$ 140	1360 $\pm$ 149	1442 $\pm$ 120	1134 $\pm$ 158	1092 $\pm$ 135
Calves-7 Day	1814 $\pm$ 190 <sup>b</sup>	1398 $\pm$ 135 <sup>a</sup>	1505 $\pm$ 160 <sup>ab</sup>	1364 $\pm$ 180 <sup>a</sup>	1107 $\pm$ 143 <sup>a</sup>
Znc1/Cuc1	3.51 <sup>b</sup>	2.37 <sup>ab</sup>	2.44 <sup>ab</sup>	1.86 <sup>a</sup>	1.61 <sup>a</sup>

Different letters within rows relate to significant (\*=  $P < 0.05$ )

Znc1/Cuc1: Ratio of Zn concentration in calves' serum/Cu concentration in calves at 7 days.

Although the general rule is that the microelement status of newborn calves is a result of the status of their mother during gestation, but the situation is different for each individual microelement and therefore no generalizations should be made (Pavlata *et al.*, 2004). Goff and Stable (1990) stated that in the last stage of pregnancy and around calving time, dams lose more Zn than they ingest as a normal physiological process and the blood Zn level declines around calving, thus their Zn reserves are depleted. It seems the large quantities of Zn are used up by the developing foetus but high concentration of Zn are present in colostrums and milk (Pavlata *et al.*, 2004; 2003).

The interaction between Cu and Zn assumes practical significance when more copper is added to ration (Underwood and Suttle, 1999). The absorption of Zn is impaired by elements such as copper and cadmium, through an increase the mucosal binding of Zn by methallothionein (Bremner, 1993). In the present study, the serum Zn concentrations of calves of treated heifers before the first intake of colostrums on calving day showed a higher mean Zn concentration compared with control. Se injection increased Cu absorption while Cu changes may have an indirect effect of Se on serum Zn concentrations. Kojouri and Shirazi (2006) reported that changes in the Zn:Cu ratios at the end of the fourth week, in turn, might have led to Zinc reduction. The serum 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. Abdelrahman and Kincaid (1995) determined colostrum Zn concentration of cows with a different Se status at 6,200 and 7,610  $\mu\text{g l}^{-1}$ , respectively, for the control and

treated groups but colostrum Zn concentration was not affected by Se supplementation.

Iron is an essential nutrient for the growth of micro organisms and could decrease in serum after exposing the host to the infection and a stress status. Iron deficiencies, except in young ruminants and milk-fed calves, do not normally occur in ruminants (Mollerberg, 1975). At birth serum concentrations of Fe in calves of all treatment groups were similar in the range of 160 – 270  $\mu\text{g dl}^{-1}$  (Table 5). Kojouri and Shirazi (2006) indicated that administration of vitamin E and Se affected iron concentrations in lambs. In that study, the iron of treated lambs was increased from birth to 4 weeks of life. In our study, the iron concentrations in all calves were similar and in the normal range and Se injection did not affect the iron concentrations in calves of the treatment groups (Table 5).

## CONCLUSIONS

Se and Cu serum concentrations increased in treated heifers compared with those in controls but no changes were seen in serum Zn concentrations. The selenium and copper concentrations also were significantly increased in the colostrum of treated heifers; however, Zn concentration of colostrum decreased in treated heifers compared with the controls. The occurrence of significant changes in Se, Cu and Zn concentrations in calves' colostrums and serum was probably due to vitamin E and selenium supplementation given to pregnant heifers. It seems that large doses of Se injected in pregnant heifers may inhibit zinc levels in their colostrum which, in turn, leads to zinc reduction in heifers and their newborn calves. It can be concluded that a higher

**Table 5.** Serum Fe concentrations in newborn calves ( $\mu\text{g l}^{-1}$ ).

	Control	T1	T2	T3	T4
Calves at birth	2200±270	2103±244	1951±189	1972±214	1951±153
Calves-7 day	1998±320	2009±170	2001±201	2023±168	2097±266



amount of Zn should be supplemented when more than 40 ml Se supplements are administered to pregnant heifers.

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### اثر مکمل سلنیوم بر مقادیر سلنیوم، مس، آهن و روی در تلیسه ها و گوساله های آنها

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#### چکیده

در این مطالعه اثر تزریق مکمل سلنیوم به تلیسه های آبستن بر مقادیر روی، مس و آهن سرم تلیسه ها و گوساله های آنها بررسی شد. پنجاه راس تلیسه هشتاین به طور تصادفی به پنج گروه تقسیم و ۲ و ۴ هفته قبل از زایش به ترتیب ۰، ۱۰، ۲۰، ۳۰ و ۴۰ میلی لیتر مکمل تجارتي سلنیوم و ویتامین E تزریق شد. هر میلی لیتر از مکمل حاوی ۵/ میلی گرم سلنیوم بصورت سلتات سدیم و ۴۰ واحد آلفا توکوفرول بود. چهار هفته قبل از زایش و در روز زایش از تلیسه ها و در روز زایش و یک هفته پس از زایش از گوساله های آنها خونگیری شد. مقادیر سلنیوم و مس سرم خون تلیسه های تیمار شده افزایش یافت همچنین مقادیر سلنیوم و مس آغوز نسبت به گروه شاهد افزایش یافت ( $P < 0.5$ ). اما در مقدار روی سرم تلیسه های تیمار شده کاهش معنی داری مشاهده شد. مقادیر عناصر معدنی سلنیوم، مس و آهن در گوساله های تلیسه های تیمار شده در مقایسه با شاهد افزایش یافت اما مقدار روی (Zn) کاهش یافت. با توجه به نتایج فوق به نظر میرسد که تزریق مکمل سلنیوم و ویتامین E به تلیسه های آبستن سنگین باعث افزایش مس و کاهش سطح Zn سرم خون تلیسه ها و گوساله های آنها و تغییر و اختلال در نسبت Zn:Cu می شود. در نتیجه گیری کلی؛ در صورت دادن بیش از ۸۰ میلی گرم مکمل سلنیوم به تلیسه های آبستن باید مقدار روی جیره نیز افزایش یابد.