



Original Article

Effect of Selenium and Vitamin E Injection during Late Pregnancy on Immune System and Productive Performances of Sanjabi Ewes and Their Lambs

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ABSTRACT

This study was performed to investigate the effect of selenium and vitamin E supplementation during late pregnancy on plasma and colostrum selenium concentrations and immune system of Sanjabi ewes and their lambs. Twenty seven Sanjabi ewes were randomly assigned to three treatments 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. Each ml of the supplement containing of 0.5 mg Se as sodium selenite and 50 mg vitamin E as D, L-alpha-tocopheryl acetate. Plasma and colostrum Se concentrations, colostrum and plasma IgG concentrations, white blood cell and differential leukocyte counts were measured. The results showed that plasma Se concentrations were significantly increased in T2 compared with controls as well as plasma Se concentration of lambs of treatments were significantly increased compared with lambs of control. The colostrum Se concentrations were significantly increased in Se supplemented groups compared with control. White blood cell counts was higher in lambs of T2 when compared with controls ($P<0.05$). The colostrum IgG concentrations at one hour postpartum were higher in T2 compared with controls ($P<0.05$). The mean colostrum production at one hour postpartum did not differ between ewes but the mean colostrum production at 10 and 18 hours postpartum increased in ewes of T2 group ($P<0.05$). The length of restless prepartum and length of gestation in supplemented ewes were shorter than controls.

Selenium and vitamin E injection during late pregnancy at the level of 10 ml could have influenced passive immune system and ewes and lambs performance.

Keywords: Selenium, White blood cell, Immunoglobulin G, Colostrum, production, Sheep.

INTRODUCTION

Selenium (Se) is an important trace mineral, acting in synergism with vitamin E which inhibits the oxidation of membrane polyunsaturated fatty acids and DNA by oxygen radicals produced during aerobic metabolism (Florence, 1995). Selenium has a biological function related to vitamin E in that Se is an essential component of glutathione peroxidase, an enzyme involved in detoxification of hydrogen peroxide and lipid hydro peroxidase. Moreover, Se is a component of selenoproteins and is involved in immune and neuropsychological function in animals (Meschy, 2000). Enjalbert *et al.* (1999) reported that Se and vitamin E supplementation enhances the levels of Se in beef cattle serum. Evidence from the literature and practical experiences suggests that nutritional factors are perhaps the most crucial, in terms of their direct effects on the reproductive phenomenon (Rastogi, 2007). Dietary supplementation of selenium and vitamin E are shown to be highly effective in increasing reproductive performance by improving litter size and total number of piglets born (Kim and Mahan, 2001). Koyuncu and Yerlikaya (2007) observed increased numbers of ewes exhibiting estrus, and higher pregnancy, lambing and twinning rates when ewes were given a selenium injection before breeding. Although it is well established that Se supplementation of deficient ewes enhances ewe reproductive performance, the effects of Se source and supplementation rate on reproductive performance have not been investigated. Moeini *et al.*, (2009) reported that Se supplementation had no effect on gestation lengths, services per conception, retained fetal membrane and open days in heifer injected Se and vitamin E supplementation 4 weeks before expected calving. Selenium and vitamin E status in the parturient period and during early life is important for health and performance of cows and their offspring (Lacetera *et al.*, 1996).

A positive effect of Se on haematological indicators was observed by several authors (Li *et al.*, 1990; Chen and Lin, 2000; Tras *et al.*, 2000), but not confirmed by others (Hu *et al.*, 1984; Pisek *et al.*, 2008). The role of Se and vitamin E as antioxidants may explain its importance in immune responses. Selenium deficiency plays a role in numerous economically important livestock diseases, problems that include impaired fertility, abortion, retained placenta and neonatal weakness (McDowell *et al.*, 1996; Atwill Reynolds., 2004). According to Boland *et al.*, (2005) high Se and vitamin E intake by pregnant ewes reduced the lamb serum IgG values and the efficiency of IgG absorption. Selenium supplementation may boost passive immunity by enhancing IgG absorption in the newborn lambs (Rock *et al.*, 2001). It has also been reported that an adequate supply of Se in combination with vitamin E in cows at late pregnancy, increased colostrum and post-suckle serum IgG concentrations (Sweeker *et al.*, 1995). In recent years, research on the physiological role of Se has been aimed with organic and inorganic forms of Se in the processes of immunity including the functions of white blood cell (WBC) (Kachuee, 2011; Pisek *et al.*, 2008). Most of the available information in this field is based on studies outside the Middle East, where the conditions for livestock are different, for example, in housing systems, feeding, climate, and management. According to Moeini *et al.*, (2011b) a high level of Se and vitamin E in heifers in late of pregnancy had positive effect on immune system. Kachuee, (2011) reported that the WBC counts, neutrophils and lymphocytes counts were higher in kids of goats in Se supplemented group when compared with the control group at birth day and 7 days of age ($P>0.05$), but changes of the mean serum IgG concentrations did not differ among goats and kids ($P>0.05$). Fazaeli and Talebian, (2009) showed that Se and vitamin E supplementation had positive effects on the productive and reproductive performance in Iranian ewes. The objective of this study was to determine the effect of different levels of Se and vitamin E supplementation at the late of pregnancy on plasma Se and IgG concentrations, colostrums Se and IgG concentrations, white blood cell and differential leukocyte counts, production and reproductive performance of Sanjabi ewes and their lambs.

MATERIALS AND METHODS

This experiment was performed at Mehregan Research Station of Jihad in Kermanshah province in west of Iran. Twenty seven fat tail Sanjabi ewes were randomly assigned to three treatments groups. Ewes in three groups were homogeneous for age, weight and were fed alfalfa hay, wheat straw, oat and concentrate (corn, soybean meal, barley, bran) providing 2.93 Mcal ME and 156 g/kg DM and crud protein, respectively according their requirements and body weight (NRC, 1985). The Se concentration of the diet was 0.07 ± 0.01 mg/Kg DM, (Table 1). Estrous was synchronized with two intramuscular injections of prostaglandin given 9 days apart. The estrous behavior was detected by testing the ewes every 12 hour with vasectomies' rams from the day of the second PG injection and from day 15 of the next estrous cycle. Four and two weeks before expected lambing, ewes were injected intramuscularly 0 ml (C), 5 ml (T1), 10 ml (T2) with a Se and vitamin E solution (each ml of the supplement containing of 0.5 mg Se as sodium selenite and 50 mg vitamin E as D, L-alpha-tocopheryl acetate). The length of restless (showing sign of gestation) pre partum and length of gestation in all ewes were monitored. Live weight at birth, weaning weight (after 60 days) and average daily gain of lambs were recorded.

Table 1: Nutrient composition (DM basis) of alfalfa hay, concentrate and oat

Nutrient	Alfalfa hay	Wheat straw	Oat	Concentrate
% DM	92.53	93.66	93.11	88.1
%CP	15.30	3.21	10.33	18
%CF	32.15	40.10	6.03	-
Ca%	1.87	0.43	0.18	0.33
P%	0.20	0.09	0.31	0.29
Se, mg/kg	0.09	-	0.07	0.07

Blood samples were collected from the jugular vein of ewes four weeks before the expected time of lambing (before administration supplement) and on lambing day. Blood samples were collected in vacuum tubes, venoject (Sterile Terumo Europe, Leuven, Belgium), early in the morning before feeding. Blood samples of the lambs were drawn from their jugular vein at birth (before suckling) and at 7 days of age. Blood samples were centrifuged for 15 min at 1000 rpm. The plasma and colostrums samples stored at -20 C until analysis. The ewes were milked by hand and colostrum production was recorded for 18 hours after lambing. Colostrum samples were collected from ewes in 1, 10 and 18 hours after lambing. Selenium concentrations in plasma and colostrums were measured by working standards for inductively coupled plasma optical emission spectrometry (ICP-OES). The standard solutions containing 1000 ppm for each tested element obtained from Perkin elmer (USA) according to the method described by Kachuee *et al* (2012). Colostrums and plasma IgG concentrations were measured by sandwich ELISA method, in accordance with the technique described by John (2009).

Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films using the cross-sectional technique (Jain, 1986). The experimental design randomized the complete block with 9 replicates and three treatments. 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 followed model:

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

Where:

X_{ij} : dependent variable

μ : overall mean

T_j : effect of treatment

ij : random error

The statistical analyses were performed with SPSS package 18 (2009).

RESULTS AND DISCUSSION

Plasma and colostrum Se concentrations of ewes and lambs are shown in Table 2 and indicate plasma Se concentrations of ewes before injection did not differ but by lambing day, the mean plasma Se concentrations were higher in T2 compared with control ewes ($P < 0.05$). The mean values of colostrum Se concentrations in ewes were higher in treated groups compared with controls ($P < 0.05$). Plasma Se concentrations of new born lambs were higher for both supplemented groups compared with controls ($P < 0.05$), although by 7 days of age this was only significant for the T2 lambs ($P < 0.05$).

Table 2: Mean plasma and colostrum Se concentrations of ewes and lambs ($\mu\text{g} / \text{L}$)

Time	Control	T1	T2
Ewes- 4 weeks before Lambing	68 \pm 3.6 ^a	69 \pm 4.1 ^a	67 \pm 3.5 ^a
Ewes-lambing day	54 \pm 4.2 ^b	71 \pm 2.5 ^{ab}	83 \pm 3.8 ^a
Lambs- at birth day	49 \pm 1.7 ^b	62 \pm 3.4 ^a	67 \pm 2 ^a
Lambs-7 days of age	56 \pm 4.6 ^b	65 \pm 5.1 ^{ab}	72 \pm 4 ^a
Colostrum	116 \pm 6.5 ^b	140 \pm 8.2 ^a	142 \pm 7.5 ^a

Mean \pm standard error

Means with different superscripts in the same row differ at $P < 0.05$

The results of the present study demonstrated that Se supplementation enhanced the amount of selenium in milk and colostrum, which is in line with results from other studies (Kachoei *et al.*, 2013; Meyer *et al.*, 2011). 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 and Tortora, 2010). Plasma IgG concentration (Table 3) did not differ significantly between groups at 4 weeks before or at lambing although IgG concentrations did fall in the last 4 weeks of pregnancy.

Table 3: Plasma and colostrums IgG concentrations of ewes and lambs (mg/dl)

Items	Control	T1	T2
Ewes -4 weeks before lambing	2040 \pm 94	1995 \pm 90	2025 \pm 88
Ewes-Lambing day	1815 \pm 87	1860 \pm 83	1880 \pm 68
Lambs at birth	101 \pm 4.43	106 \pm 5.59	110 \pm 6.31
Lambs at 7 days of age	1180 \pm 59	1200 \pm 39	1160 \pm 61
Colostrums; 1 hour postpartum(g/L)	86.48 \pm 0.84 ^b	86.78 \pm 0.81 ^b	90.01 \pm 1 ^a
10 hours postpartum	51.04 \pm 1.32	50.30 \pm 1.06	50.25 \pm 1.19
18 hours postpartum	21.04 \pm 0.92	20.4 \pm 0.81	19.9 \pm 0.74

Mean \pm standard error , Means with different superscripts in the same row differ at $P < 0.05$

(Control = 0 ml Se+VE), (T1 = 5 ml Se+VE), (T2 = 10 ml Se+VE)

The lambs from treated ewes had higher plasma Se concentrations compared with controls ($P < 0.05$), this fact was in agreement with previous reports (Abdelrahman and Kincaid, 1995; Davis *et al.*, 2006). Moeini *et al.*, (2011b) reported that serum selenium 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 selenium concentration increased in the supplemented groups. Knowles *et al.*, (1999) and Juniper *et al.*, (2006) showed a similar result in dairy cows. The plasma Se concentrations of control lambs were slightly above the normal range (40-50 $\mu\text{g} / \text{L}$) reported by other studies (Izadyar 1987, Jalilian *et al.*, 2012). Close correlations existed between the Se concentration in blood of calves and in dams at birth (Kincaid and Rock, 1999). Davis *et al.* (2006) showed Se concentrations in the plasma of lambs were affected by dietary Se concentrations of their dams. Further Kim and Mahan, (2001) reported elevated serum and tissue Se concentrations in neonate pigs when dietary Se levels of sows were increased. Like blood and tissue, Se in milk is affected by dietary Se level (Givens *et al.*, 2004). Placental and colostrums transfer of Se from ewe to lamb has been shown to occur even when the dam is deficient in selenium (Abd El-Ghany and Tortora-

Perez, 2010). The efficiency of placental transfer of Se is highly dependent on the levels and the chemical form of selenium supplementation. Dietary Se supplementation of pregnant beef cows, markedly increased Se concentrations in colostrum and milk (Ammerman *et al.*, 1980). Colostrums Se concentrations were affected by Se supplementation of the ewe's diet and increased linearly as dietary Se increased (Davis *et al.*, 2006). The results of our trial are further supported by Mahan (2000), who demonstrated that colostrums Se concentrations were increased by increasing Se in pre partum and postpartum sow's diet.

The plasma IgG concentrations of treated ewes did not differ significantly at 4 weeks before lambing and at lambing day (Tables 3). The plasma IgG concentrations in all groups of ewes decreased during the last 4 weeks of pregnancy. In present study, treatments did not affect the plasma IgG concentrations of lambs at birth. However, plasma IgG concentration at 7 days of life was higher in supplemented groups than when recorded immediately after birth before the first colostrum feeding. This is not surprising, because the placental structure in ruminants does not permit IgG transportation from mother to fetus. The placenta acts as a barrier to the in utero transmission of IgG in lambs, and lambs are born with negligible quantities of IgG in plasma (Schultz *et al.*, 1973), therefore depend on the successful transfer of colostrum IgG to provide them with immunity in early days and weeks of life, and following the ingestion of colostrums, an increase in plasma IgG titers was observed (Boland *et al.*, 2005).

Adequate Se status of the newborn not only ensures prevention of nutritional myopathy, but also decreases associated losses in lamb productivity. Lambs from Se-supplemented ewes show faster progression to stand and nurse compared to lambs from non Se-supplemented ewes leading to an overall decrease in lamb mortality (Munoz *et al.*, 2009). The mean colostrum IgG concentrations of ewes have been shown in Table 3. The mean of colostrum IgG concentrations at one hour postpartum was higher in T2 compare with controls. Moeini *et al.* (2011b) reported the mean of colostrum IgG concentrations at one hour postpartum did not differ between treatments.

The mean colostrum production is shown in Table 4. The mean colostrum production at one hour postpartum did not differ between ewes but the colostrum production at 10 and 18 hours postpartum was higher in ewes of T2 group ($P < 0.05$). The colostrum production was affected by Se and vitamin E supplement; although Weiss, (2003), Kachuee, (2011) and Bourne *et al.*, (2007) indicated that Se supplementation had no significant effect on milk production.

Table 4: Mean colostrum productions of ewes (ml)

Time	Control	T1	T2
1 hour postpartum	501.66±6.61	507.77±7.02	503.88±5.18
10 hours postpartum	591.11±6.27 ^b	602.77±5.89 ^b	652±5.83 ^a
18 hours postpartum	522.77±6.51 ^b	530±3.22 ^b	608±3.72 ^a

Mean ± standard error, Means with different superscripts in the same row differ at ($P < 0.05$)

(Control = 0 ml Se+VE), (T1 = 5 ml Se+VE), (T2 = 10 ml Se+VE)

The leukocyte count of ewes 4 weeks before lambing and at lambing day is shown in Table 5. The results indicated that the changes of mean values of WBC counts and the percent of types of WBC did not significantly differ between treatments four weeks before expected lambing and at lambing day. The leukocyte count of lambs of treated ewes at birth day and on 7 days of age is shown in Table 6. The WBC counts were higher in lambs of ewes in T2 group compared with control group but the percent of types of WBC did not significantly differ between lambs of treated ewes at 7 days of age ($P > 0.05$). Kachuee, (2011) reported that the WBC counts, neutrophils and lymphocytes counts were higher in kids of goats in selenomethionin group compared with the controls on birth day and 7 days of age ($P < 0.05$). The higher WBC and Lymphocyte cell counts could be related to the protection of cell membrane and intracellular organcles by the antioxidant effects of Se and thus increase

their lifespan. Fazaeli and Talebian, (2009) showed that the combination of vitamin E and Se were more effective than Se supplement to improve immune system.

Table 5: Leukocyte counts in treated ewes four weeks before lambing and at lambing day

Parameters	Control	T1	T2
Ewes before lambing ;			
White blood cell count	9333±494	9355±370	9388 ±385
Neutrophile %	33.3±3.04	31.3 ±1.3	31.2±1.06
Lymphocyte %	65.7±3/2	67.1±1.2	66.8±1.01
Monocyte %	2 ±0.70	2.25 ±0.62	2.25±0.62
Eosinophile %	1±0	1.25±0.25	1.5±0.28
Ewes at lambing day;			
White blood cell count	9966±676	10098 ±541	10100 ±417
Neutrophile %	32.66±4.1	31.83±2.06	30.5±3.3
Lymphocyte %	66.5±1.6	67.33±2	68.5 ±2.4
Monocyte%	1.5 ±0.60	1.5±0.60	2 ±0
Eosinophile%	1±0	1±0	1±0

Mean ± standard error

(Control = 0 ml Se+VE), (T1 = 5 ml Se+VE), (T2 = 10 ml Se+VE)

Faixova *et al.*, (2007) reported that supplementation of lambs with Se-yeast had no positive effect on WBC counts. The results presented here show a positive effect of Se and vitamin E on WBC in lambs of treated ewes at 7 days of age (Table 6). In addition, Se is known to act as a scavenger of free radicals within cell membranes, having a protective effect against oxidative damage (Smith *et al.*, 1997). Mohri *et al.*, (2005) reported that Se and vitamin E supplementation affected on WBC counts in third weeks of calves' life. In another study, WBC counts were significantly higher in Se and vitamin E injected groups of rats than in the control (Cay and Naziroglu, 1999).

Table 6: Leukocyte counts in lambs at birth and at 7 days of age

Parameters	Control	T1	T2
lambs at birth;			
White blood cell count	5060±437	5300±206	5500 ±394
Neutrophile %	58.25±3.7	54.75±4.8	56.8±2.3
Lymphocyte%	40.25±3.2	44 ±4.1	44.2 ±3
Monocyte %	1 ±0	1.5±0.38	2.33 ±0.80
Eosinophile %	1±0	1±0	1±0
lambs at 7 days of age;			
White blood cell count	7925±443 ^b	8600±533 ^{ab}	9176 ±308 ^a
Neutrophile %	30 ±2.7	29.25±1.8	31.4±2
Lymphocyte %	68.75±3.8	69.25±2	68.2±2.25
Monocyte %	1 ±0	1.66 ±0.5	2 ±0
Eosinophile %	1±0	1±0	0

Mean ± standard error

(Control = 0 ml Se+VE), (T1 = 5 ml Se+VE), (T2 = 10 ml Se+VE)

The lambs birth weight, average daily gain; gestation length and length of restless parturition are shown in Table 7. The lamb's birth weight did not differ between treatments. The gestation in T2 group was shorter than control groups ($P<0.05$). The length of restless parturition until lambing in Se supplemented ewes was shorter than control group ($P<0.05$).

Average daily weight gain of lambs up to 60 days of age increased in lambs of supplemented ewes compared with control group ($P < 0.05$) (Table 7).

Table 7: The mean birth weight, daily weight gain, gestation length and length of restless postpartum until lambing

Items	Control	T1	T2
Length of gestation(day)	156±1.2 ^a	154±1.04 ^a	150±0.45 ^b
length of restless pre partum until lambing(minutes)	44±2.3 ^a	39±0.89 ^b	37±0.72 ^b
Birth weight(kg)	3.7±0.21	3.9±0.36	4.1±0.25
Daily weight gain of lambs (g)(for 60 days)	266.8±8.1 ^b	300.9±9.4 ^a	308.5±8.7 ^a

Mean ± standard error

Means with different superscripts in the same row differ at ($P < 0.05$)

Control = 0 ml Se+VE), (T1 = 5 ml Se+VE), (T2 = 10 ml Se+VE)

Dominguez-Vara *et al.*, (2009) found no differences in growth response in weaned lambs supplemented with 0.3 mg/kg Se yeast in the finishing diet, when compared with non Se supplemented lambs. Similar result were found with Christaldi *et al.*, (2005) whereby no effect of Se supplementation on growth rate was observed in growing and finishing lambs supplemented with increasing amounts of sodium selenite. In contrast Abd El-Ghany and Tortora-Perez, (2010) and Gabryszuk and Klewicz, (2002) suggest that growth as a result of Se supplementation is most noticeable in the first two weeks of age, although other studies suggest that enhanced growth from Se supplementation can be detected in lambs up to one year of age (Munoz *et al.*, 2009; Kumar *et al.*, 2009). Koyuncu and Yerlikaya, (2007) suggested that ewes supplemented with Se and vitamin E had a positive effect on daily weight gain and body weight up to 60 days of age.

CONCLUSION

Selenium and vitamin E supplementation at the late of pregnancy of ewes caused a higher Se status at parturition. The colostrums Se concentrations were significantly increased in Se supplemented ewes with higher colostrums production. Lambs born to ewes given Se and vitamin E in late pregnancy had higher Se status at birth and 7 days of age. White blood cell counts were higher in lambs of supplemented ewes, and the mean colostrums IgG concentrations at one hour postpartum were higher in supplemented ewes. In conclusion, it seems selenium and vitamin E supplementation at the levels of 10 ml improves passive immune system and colostrums production.

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