Journal of Animal and Veterinary Advances 7 (1): 61-67, 2008
ISSN: 1680-5593

Effect of Pre- and Postpartum Selenium Supplementation in Sheep

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Abstract: In this study, the effect of pre- and postpartum Selenium (Se) supplementation on Se levels of the allantoic fluid, colostrum, milk and plasma as well as on the body weight gain of ewes and their newborn lambs was investigated. A total of 32 pregnant primiparus Pelibuey ewes were selected and classified randomly into 3 groups. The 1st group received Subcutaneous (SC) Se supplementation with 0.1mg of sodium selenite/Kg BW at 7th, 4th week prepartum and 1st week postpartum. The 2nd group was treated with oral Se supplementation 3 mg of sodium selenite/head/week, for 7 weeks prepartum, while the 3rd group remained as a control, without Se supplementation. Maternal plasma samples were collected weekly for 8 weeks pre- and postpartum and at parturition, while samples of the allantoic fluid were collected for 5 weeks prepartum. Plasma samples of the newborn lambs were collected at 48 h of the age and weekly for 8 weeks. Samples of colostrum and then that of the milk were collected weekly for 8 weeks for determination of Se levels. The body weight of the lambs born to the SC Se supplemented ewes was higher than that of the control ($p<0.05$) group for the first 2 weeks postpartum. Allantoic fluid, colostrum, milk, maternal and lamb plasma Se concentrations were significantly increased in Se supplemented groups than that of the control ($p<0.05$). Positive relationships were observed between gestational age and Se concentration of the allantoic fluid ($r = 0.92$- 0.96; $p<0.05$) and between milk and lamb plasma Se concentration ($r = 0.57$- 0.73; $p<0.05$). Positive relationships were also detected between maternal plasma and milk Se concentration ($r = 0.66$-95; $p<0.05$) in Se supplemented groups, while negative relationship occurred in the control group ($r = -0.60$; $p<0.05$). Milk and lamb plasma Se concentrations were higher in the SC group than that of the oral group ($p<0.05$), for the first 2 weeks postpartum. Prepartum sodium selenite supplementation was important to maintain the maternal plasma Se level during gestation and postpartum Se supplementation was important for maintaining milk and lamb plasma Se concentrations and improving the body weight gain of the newborn lambs. Allantoic fluid changes were confirmed as a good indicator of fetal Se status and may act as storage of Se and play a role in Se metabolism between the dam and fetus.

Key words: Selenium (Se), allantoic, colostrum, milk, plasma

INTRODUCTION

Selenium (Se) deficiency could be prevented by oral supplementation, but digestive Se absorption in the ruminants varies between wide ranges, from 10-16% (Koenig *et al*., 1991) to 51% (Harrison and Conard, 1984). Since lambs and ewes are more prone to disease when dams were deficient in Se during pregnancy, it is therefore believed that supplementation of pregnant animals with Se would minimize the occurrence of diseases. Se is transferred from the dam to the fetus across the placenta (Koller *et al*., 1984; Van Suan *et al*., 1989) and is present in the colostrum and milk (Cuesta *et al*., 1995), according to the mother availability. Results of a previous study suggested that, fetus requirements were covert by the dam with regardless of their Se status, dam sacrificed its Se levels to maintain fetus (Abd Elghany *et al*., 2007). The total diet for sheep should contain 0.10-0.30 ppm of Se (Smith and Sherman, 1994; Ullery *et al*., 1978). The choice of the treatment of Se deficiency will depend on the circumstances of each case, including cost, husbandry system and ease of administration (McPherson and

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Chalmers, 1984; Kott *et al*., 1983). The allantoic sac was traditionally considered to be a deposit for fetal wastes (Alexander and Williams, 1968). However, studies in pigs and sheep have shown that the allantoic sac may play an important role in the homeostasis of nutrients and metabolites used by the fetus (Bazer, 1989; Hyukjung *et al*., 2003). Results of a previous study demonstrated that Se concentration in the allantoic fluid may be used as a good indicator of the fetal Se status throughout the gestational period (Abd Elghany *et al*., 2007).

This research evaluated the effect of pre and postpartum Se supplementation in sheep on the Se levels of the maternal plasma, allantoic fluid, colostrum, milk and plasma Se and on the body weight gain of the newborn lambs.

MATERIALS AND METHODS

A total of 32 primiparus pregnant Pelibuey ewes were selected after ultrasound examination approximately 90 days of pregnancy. The ewes were 1.5-2 years of age with an average body weight of 41.09±0.8 kg. These ewes were divided into 3 groups, the 1st group (n = 11) were supplemented with Subcutaneous injection (SC) of sodium selenite, 0.1mg/kg BW, at the 7 and 4th weeks prepartum and at 1st week postpartum, at the same time weekly blood sample was collected. The 2nd group (n = 11) was orally supplemented with sodium selenite 3 mg/head in deionized water once weekly for 7 weeks prepartum while the 3rd group was left as a control (n = 10). Animals received feed and water *Ad libitum*. The diet was composed of alfalfa, ground corn, soybean as in (Table 1) and a mineral salt without Se, nevertheless diet Se contents were determined.

Sampling: Blood samples were collected by the jugular vein puncture weekly for eight weeks pre- and postpartum from the ewes. Newborn lambs were sampled at 48 h of the age and then weekly for 8 weeks. Blood samples were centrifuged immediately (2000 xg, 15 min) to obtain plasma which was stored at -20°C Allantoic fluid was collected by puncture with ultrasonographic guide at the 8, 7, 6, 5 and 4th weeks prepartum (Zeppertiz and GrÜn, 1991). Blood and colostrum were sampled at the day of the parturition as well as milk samples were collected in the morning weekly for 8 weeks postpartum. The first nipple was cleared and then 2 mL of the milk were collected. All the samples were kept at (-20°C) until the analysis.

Table 1: Dietary supplementation of the ewes during gestation and lactation period (kg/head/day)

Item	Gestation	Lactation
Roughage	0.4	0.5
Alfa alfa	1.3	0.7
Soybean	0.2	0.4
Ground corn	0.3	0.9
Mineral salt	0,006	0,007
Water	*Ad libtium*	*Ad libtium*
Crude protein	15%	15%
Energy (Mcal/kg)	2.3	2.7

Preparation and analysis of the samples: The plasma, allantoic fluid, colostrum and milk samples were processed by mixing 1 mL of each sample with 10 mL of deionized water, 5 mL of concentrated nitric acid and 2 mL of hydrogen peroxide (30%) (JT. Baker, Phillipsburg, N J.) keeping the solution at room temperature for 30 min in sealed Teflon vessels (Koening *et al*., 1997). Subsequently, the samples were placed in a microwave digester (Mars 5 CEM Corporation USA) with an increasing temperature ramp of 5 min to reach 120°C for plasma and allantoic fluid and 100°C for colostrum and milk and it was held in this temperature for 2 min for plasma and allantoic fluid and 5 min for colostrum and milk. The temperature was then increased to 170°C for plasma and allantoic fluid and 140°C for colostrum and milk within 5 min and maintained for 2 min for plasma and allantoic fluid and 10 min for colostrum and milk with a maximum pressure of 350 psi for plasma and allantoic fluid and 66 psi for colostrum and milk (Ortman and Pehon, 1997). The samples were allowed to cool for 5 min in the oven and then left to obtain room temperature for 1 h. The samples were then transferred to 50 mL volumetric flasks and filled to the top with 7M HCl and left overnight (4°C) to be analyzed the following day. Se concentrations were determined with the aid of atomic absorption spectrophotometer (Varian, model Spectra AA-800).

Statistical analysis: Means Pearson correlation coefficient, Analysis of Variance (ANOVA) and multiple regression analysis were performed using the Microsoft excel and Statgraphic plus v. 4.

RESULTS

One ewe from the control group was aborted and it may be attributed to the method of allantoic fluid extraction and another one of the oral supplemented group was non pregnant and was eliminated. The dietary Se concentrations were 0.22 and 0.23 ppm during pregnancy and lactation respectively.

No significant (p>0.05) differences in the maternal Body Weight (BW) were detected between Se supplemented and control groups. Nevertheless newborn lambs from SC Se supplemented ewes demonstrated a

Table 2: Mean (± SEM) of the body weight (kg) of the lambs born to S/C, oral Se supplemented and control ewes

	Age of the lambs (week)								
Group	0 day	1	2	3	4	5	6	7	8
S/c	2.8±0.1	4.4±0.1[a]	5.5±0.2[a]	5.9±0.2	6.6±0.2	7.5±0.2	8.2±0.3	9.1±0.4	9.6±0.4
Oral	2.6±0.2	4.1±0.3	5.2±0.3	5.8±0.3	6.7±0.4	7.7±0.5	8.7±0.7	9.7±1	10±0.8
Control	2.7±0.2	3.6±0.2[b]	5.3±0.3	4.5±0.3[b]	6.1±0.5	7.1±0.4	8.1±0.5	9.2±0.6	9.6±0.6

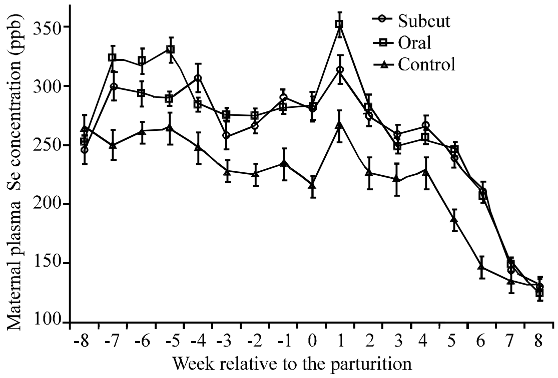

Fig. 1: Maternal plasama Se concentration in Se supplemented and control groups.Arrows indicate each S/C injection

Table 3: The relationship between milk, maternal plasma and lamb plasma Se concentrations in Se supplemented and control group

Milk Se	Maternal plasma Se	Lamb plasma Se
S/C group	0.95 **	0.57 *
Oral group	0.66*	0.6 *
Control group	-0.60*	0.73 *

*p<0.05 ** p<0.01

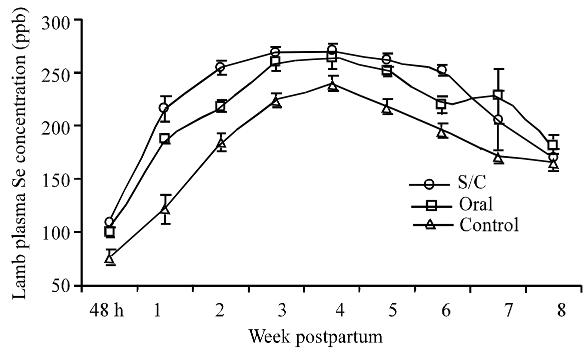

Fig. 2: Lamb plsama Se concentration in Se supplemented and control group

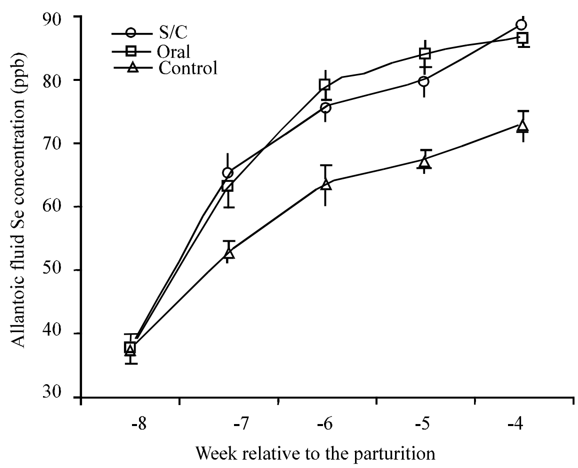

Fig. 3: Allantoic fluid Se concentration in Se supplemented and control group

greater BW at the first 2 weeks of age than those born from the control group (p<0.05). The sex of the newborn lambs did not affect the BW. During the time of the study there were no significant differences in the BW in the lambs neither between both Se supplemented groups nor between the oral supplemented and control one (Table 2).

Maternal plasma Se concentration was significantly (p<0.05) higher in the oral supplemented than that of the SC supplemented group at the 6 and 5th weeks prepartum and at 1st week postpartum. At the 4th week prepartum, this situation was reversed and the maternal plasma Se concentration of the SC supplemented group was significantly (p<0.05) higher than that of the oral group. Maternal plasma Se concentrations in both Se supplemented groups were higher than that of the control group (p<0.01), until the 7th week postpartum when there was no significant difference between the supplemented and control groups. At parturition, maternal plasma Se concentrations were significantly decreased (p<0.01) in the control group, while that of the supplemented groups were maintained without significant difference from the prepartum levels (Fig. 1). Maternal plasma and milk Se concentrations relationship in the supplemented groups was positive (r = 0.66-95; p<0.05), while this relationship was negative in the control group (r = - 0.60; p<0.05) (Table 3).

A positive relationship (r = 0.57-0.73; p<0.05) was detected between milk and plasma Se concentrations in the lambs that born from Se supplemented and control groups (Table 3). Nevertheless, plasma Se concentration was higher in the lambs that born from supplemented ewes (p<0.01), until the 7th week postpartum Plasma Se concentration of the lambs that born to SC supplemented ewes was greater than that of the oral group in the 1st, 2nd, 6th week (p<0.01) (Fig. 2).

Allantoic fluid Se concentration increased in the supplemented groups than that of the control group

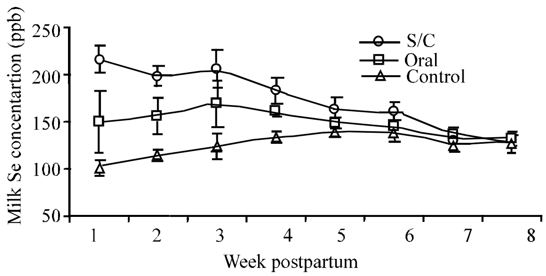

Fig. 4: Milk Se concentration in Se supplemented and control group

($p<0.01$) and positive relationship ($r = 0.92$ to 0.96; $p<0.05$) was demonstrated between allantoic fluid Se concentration and age of gestation in the supplemented and control groups (Fig. 3).

Se supplemented ewes produced colostrum with higher Se concentration than that of the control group ($p<0.01$) while, differences in the colostrum Se concentrations in both Se supplemented groups were not significant. Colostrum Se values were 407.3±31.2, 381.3±19.4 and 270.9±32.7 ppb in oral, SC Se supplemented and control group, respectively. Milk Se concentrations in the supplemented groups were higher than that of control group ($p<0.01$), until 7 and 8th weeks postpartum (Fig. 4). Nevertheless, milk Se concentrations were greater in the SC supplemented group than that of the oral group in the 1st and 2nd weeks postpartum ($p<0.01$).

DISCUSSION

Maternal SC Se supplementation significantly ($p<0.05$) increased the BW gain of the newborn lambs for the first two weeks. Similar results have been reported in the lambs from birth to 28 days when their dams received Se supplementation by injection (Rock *et al*., 2001; Gabryszuk and Klewiec, 2002). Similar condition occurred in the newborn calves from birth until 70 days or 5 months of age, when cows were supplemented with Se (Castellan *et al*., 1999; Wichtel *et al*., 1996). Nevertheless, other reports indicated that, Se supplementation did not influence the BW gain in cows and their calves (Awdeh *et al*., 1998; Gunter *et al*., 2003; Rowntree *et al*., 2004).

Maternal plasma Se concentration, as expected, was significantly greater in the Se supplemented than that of the control group ($p<0.01$) and decreased at the parturition, especially in the control group ($p<0.01$). These results were supported by previous observations that indicated a great mobilization of the Se from the dam to the fetus at the end of gestation (Abd Elghany *et al*., 2007). Se in colostrum and milk added to the rapid fetal growth and this suggest increased Se requirements in the late stage of pregnancy (Van Saun *et al*., 1989). This observation may conclude the importance of the prepartum (Enjalbert *et al*., 1999) and postpartum (Rowntree *et al*., 2004). Se supplementation for maintaining the maternal Se levels. Weiss *et al*. (1984) found that cows fed Se supplemented diet during the dry period increased serum Se concentrations of their calves at the birth. In sheep oral Se supplementation resulted in adequate Se level in the dam to ensure effective transfer of the element to the fetus and provide to the neonate appropriate blood concentration to prevent Se-deficient associated syndromes (Paulson *et al*., 1968; Horton *et al*., 1978; Loren *et al*., 1984).

Se supplemented ewes produced colostrum with higher Se concentration than that of the control ($p<0.01$), previous works reported the same results in sows (Mahan, 2000) and cows (Overnes *et al*., 1985; Cuesta *et al*., 1995; Rowntree *et al*., 2004). Schingoethe *et al*. (1982), reported that dietary administration of Se (0.1 or 2 ppm Se) or selenite injection (5 mg Se/45.4 kg BW) did not influence dairy cow colostrum Se concentrations in normal cows.

In this study supplemented ewes demonstrated a positive relationship between maternal plasma and milk ($r = 0.66$- 0.95; $p<0.05$) and their milk Se concentration was significantly higher than that of the control one ($p<0.01$), while the relationship was significantly negative in the control group ($r = -0.60$; $p<0.05$). As expected milk and lamb plasma Se concentrations relationship was positive in all groups ($r = 0.57$-0.73; $p<0.05$), these results were similar to that obtained in previous works (Perhson *et al*., 1999; Davis *et al*., 2005). Maternal and lamb plasma Se concentrations did not correlate ($p>0.05$) and this suggest that lambs mainly depend upon the milk Se source. Milk and lamb plasma Se concentrations in the SC supplemented group were greater than the oral ($p<0.05$) in the first 2 weeks postpartum. This change probably was influenced by the Se injection (0.1 mg kg^{-1} BW) to this group in the 1st week postpartum. Absence of differences in lamb plasma between supplemented groups at 3, 4 and 5th weeks postpartum may be related with the declining milk production in this period. Cardellino and Benson (2002) reported that, the milk production of 2 years old ewe rearing twin lambs peaked at 21 days of the lactation. Similar results were previously reported in sheep supplemented orally or by injection (Gardner and Huge, 1967; Givens *et al*., 2004; Norton and McCarthy, 1986).

Maus *et al*. (1980) demonstrated a close relationship between Se concentrations in the plasma and milk in dairy cows. Dietary Se supplementation of pregnant beef cows markedly increased concentrations of Se in the colostrum and milk. This increase caused, the suckling calves to have higher Se concentrations in their plasma (Koller *et al*., 1984; Ammerman *et al*., 1980; Salih *et al*., 1987). Milk is normally the sole source of nutrients consumed by the lambs (Jenkins and Hidiroglou, 1971) and these reinforce the importance of the maternal postpartum Se supplementation.

Allantoic fluid extraction was a simple, rapid and secure procedure, nevertheless authors never used it previously, only one animal probably aborted for its use. Se concentration in this fluid was modified by the supplementation indicating a better contribution of the fetal Se status (Abd Elghany *et al*., 2007). Allantoic fluid Se levels was higher in the Se supplemented than that of the control group ($p<0.01$). Positive relationship ($r = 0.92$-0.96; $p<0.05$) was demonstrated between allantoic fluid Se concentrations and gestational stage in both supplemented and control groups and this result confirmed with the previous findings (Grace *et al.*, 1986; Abd Elghany *et al*., 2007) and hierarchies the use of alantoic fluid as indicator of the fetal Se status. Hyukjung *et al*. (2003) demonstrated that, sheep allantoic fluid volume was increased progressively ($p<0.01$) from days 40-120 and did not differ between days 120-140 of gestation, but at this period allantoic cysteine concentration was increased, considering Se-cysteine metabolic relationship, allantoic fluid may act as a storage of Se in the late stage of pregnancy and plays a role in Se metabolism between the dam and fetus.

Significant increase in the dam and lamb plasma Se concentration in the supplemented groups than that of the control one indicated that, the supplementation may allow adequate plasma Se levels which was higher than that of the normal values (110.1±5.8 ppb) according to Mustafa *et al*. (1998). The diet used in the experiment had adequate Se levels and control group had normal plasma Se concentrations however the supplementation improves this with a good result. It has been reported that, sheep receiving 12 mg kg^{-1} dietary Se gave birth to lambs with up to three-fold higher plasma Se than did in the non-supplemented (Davis *et al*., 2005). Similar results have been reported in calves born from supplemented cows (Enjalbert *et al*., 1999; Rowntree *et al*., 2004). In this study, lambs born to Se supplemented ewes had greater plasma Se concentrations until the 6th week postpartum compared with lambs born from control ewes ($p<0.05$). Significant increase in the maternal plasma Se concentration in the oral Se supplemented group than that of the SC group ($p<0.05$) in the 6, 5th pre- and 1st week postpartum indicates that, the oral method is better for supplementing Se and that is more available when supplemented as sodium selenite, despite the lower absorption and retention of Se in sheep (Koenig *et al*., 1997).

CONCLUSION

While prepartum Se supplementation was important to maintain the maternal plasma Se levels, postpartum Se supplementation may improve the Se status in the milk and plasma of the newborn lambs. Supplementation of the newborn lamb is recommended to start at least in the 7th week postpartum even thought they born from ewes supplemented with Se. Allantoic fluid Se levels are a good indicator of the fetal Se status and may act as storage of the element and plays a role in the Se metabolism between the dam and fetus.

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