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EFFECTS OF SELENIUM AND VITAMIN E SUPPLEMENTATION ON CONCENTRATIONS OF SELENIUM AND IMMUNOGLOBULINS IN COLOSTRUM AND BLOOD OF BALUCHI EWES AND LAMBS, AND ON LAMB GROWTH

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ABSTRACT

Effects of injections of selenium (Se) and/or vitamin E were studied on concentrations of Se and immunoglobulins in ewe blood and colostrum, immunoglobulin concentrations in lamb blood, incidence of nutritional muscular dystrophy (NMD) and on lamb growth in Baluchi sheep. Blood concentration of Se in ewes increased ($P<0.05$) gradually during pregnancy after injections with Se. Injections of selenium, with or without vitamin E resulted in an increase in concentration of Se in colostrum. Supplemental Se and vitamin E resulted in higher concentration of immunoglobulins in ewe blood as compared with other treatment groups ($P<0.05$). Beta-globulin levels in blood of ewes and lambs were not affected by the treatment. All treatments increased colostral beta-globulin levels as compared with the control group ($P<0.05$). Mean lamb birth weight was not different among various treatment groups; however, lambs injected with

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supplements, were heavier than control (P<0.05). Incidence of NMD was decreased by the treatment (P<0.05).

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اثرمکمل های سلنیوم و ویتامین E بر برخی از پارامترهای ایمیونولوژیکی و فیزیولوژیکی در میش های آبستن و بره های آن ها

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چکیده

اثر مکمل های تزریقی سلنیوم و یا ویتامین E بر غلظت سلنیوم و ایمیونوگلوبولین های خون و آغوز میش ها، انتقال ایمیونوگلوبولین ها به جنین در دوران آبستنی، بروز بیماری ماهیچه سفید و رشد بره های بلوچی بررسی شد. غلظت سلنیوم خون میش ها در دوره آبستنی ، تحت تاثیر سلنیوم ونیز سلنیوم و ویتامین E بتدریج افزایش یافت (P< 0.05) ولی ویتامین E به تنهایی بی تاثیر بود. تزریق سلنیوم همراه با ویتامین E یا بدون آن، غلظت سلنیوم در آغوز را افزایش داد. مکمل سلنیوم با ویتامین E یا بدون آن، غلظت سلنیوم در آغوز را افزایش داد. مکمل سلنیوم با ویتامین E در مقایسه با دیگر تیمارها، غلظت IgG در خون میش ها را افزایش داد. غلظت بتاگلوبولین خون میش ها در مراحل پایانی آبستنی اندکی زیادتر شد. تزریق سلنیوم و یا ویتامین E، میزان بتاگلوبولین آغوز را نسبت به میش های شاهد افزایش داد. تزریق مکمل ها به میش ها تاثیری بر انتقال

ایمیونوگلوبولین ها به بره ها دردوران آبستنی نداشت. میانگین وزن تولـد بـره هـا بیـن گـروه هـای مختلف تفاوتی نشان نداد ولی تزریق مکمل به بره ها موجب افزایش وزن بیشـتری نسـبت بـه گـروه شاهد شد. میزان بروز بیماری ماهیچه سفید در اثر تزریق مکمل ها کاهش یافت (P< 0.05) .

INTRODUCTION

Cell membranes contain polyunsaturated fatty acids (PUFA). Cyclooxygenase and lipoxygenase act on PUFA to produce eicosanoids (31); however, these acids also give rise to detrimental peroxides and free radicals. Glutathione peroxidase (GSH-Px) contains selenium (Se) and destroys peroxides as they are formed in the body, oxidizing glutathione in the process. Therefore, the presence of an adequate supply of Se to keep this enzyme fully operational is an important complementary mechanism to that of vitamin E, which helps to prevent lipid peroxide accumulation (41).

Selenium and vitamin E are involved in growth (2, 9), disease resistance (11, 15, 17, 36) and wool production (9, 16). Insufficient dietary Se, sometimes with concurrent vitamin E and/or excessive PUFA causes various syndromes, one of the best known example in ruminants is nutritional myopathy or muscular dystrophy (NMD) also known as white muscle disease (2, 30).

Selenium deficiency reduces the fertilization rate in cattle (21) and sheep (6), and causes embryonic death at 3 to 4 w postconception in ewes (6). Reduced fertility sometimes accompanies Se deficiency in sheep and cattle, but reports are contradictory (12). Low tocopherol in forages or presence of certain antagonists of Se and vitamin E in feeds may reduce fertility in ruminants in Se-deficient areas (20).

For prevention of NMD in severely deficient areas, ewes are given 5 mg Se parenterally or orally before mating, in midgestation, and 3 w before lambing, and lambs receive 1 mg Se at docking and 2 mg at weaning, repeated 2 to 4 months later (2). Selenium status of soils and plants in Iran has not yet been studied but there are published (32) and unpublished records of NMD in sheep. Sheep owners indiscriminately use Se and vitamin E supplements for disease prevention, and often are faced with Se toxicity. Sheep are mainly range animals in Iran and are rarely hand-fed. Until efforts are made to map the Se-deficient areas in Iran, measurement of blood Se can be used as an indirect method of assessing Se status of soils and plants.

Selenium can be measured by atomic absorption (10) but there are no reports on evaluation and application of this method for determination of blood and colostral Se in any animal species in Iran. Preliminary analysis of selenium concentration of whole blood of mature Baluchi ewes, reared at the Ferdowsi University Animal Husbandry Farm, showed a mean of 69±14 ng ml^{-1}. Lambs born in this station and the nearby farms are frequently afflicted with NMD although no official data are available. The normal values of Se in whole blood of sheep are above 100 ng ml^{-1}; a value of 50 ng ml^{-1} indicates deficiency whereas intermediate values are associated with suboptimal production (2). A recent report cited a normal range of 50-80 ng per ml for new born lambs, and 120-150 ng ml^{-1} for mature sheep (34).

The objective of this work was to study the effects of Se and /or vitamin E injections on immunoglobulin levels in blood and in colostrum of Baluchi ewes and lambs, on lamb weight gain during the suckling period, and on the incidence of NMD in lambs.

MATERIALS AND METHODS

This experiment was carried out on 38 nulliparous Baluchi ewes at the Animal Husbandry Station, College of Agriculture, Ferdowsi University of Mashhad. Means (±SD) for body weight and age of ewes at the beginning of the experiment were 43.9±5.7 kg and 482.2±55.9 d, respectively. Ewes were randomly allotted to four experimental groups (9 or 10 ewes per group) in a 2 by 2 factorial design. Each group received intramuscular injections according to the following protocol:

Group 1: Se and vitamin E (+Se+E)

Group 2: Se (+Se-E)
Group 3: Vitamin E (-Se+E)
Group 4: Saline (-Se-E)

Ewes were offered, *ad libitum* (mean daily feed consumption of 1.8 kg), a total mixed ration containing 2.32 Mcal metabolizable energy kg^{-1} dry matter (DM), and 11.9% crude protein. The ration consisted of (all DM) alfalfa hay (42%), chopped wheat straw (11%), barley grains (28%), dried sugarbeet pulp (14%) and wheat bran (5%). Ration ingredients, except dried sugarbeet pulp, were farm-produced. Ewes had access to barley stubble for 1-2 h each day. Lambs were not creep fed and had access to the same diet as their dams. Lambs were weaned at 100 d of age.

Estrus was synchronized by two injections of a prostaglandin $F_{2\alpha}$ analogue (3) and fertile rams were introduced to the experimental flock. Ewes were injected with saline (control) or 0.083 mg Se kg^{-1} BW and/or vitamin E (100 mg α-tocopherol) on three occasions: approximately 20 d before mating (premating), midgestation, and approximately 20 d before lambing (preterm). Lambs were also injected with 0.076 mg Se kg^{-1} BW and/or α-tocopherol (30 mg) or with saline at 20 d of age. Selenium was in the form of sodium selenite.

Blood samples were collected from the jugular vein at 5, 10, 20 and 40 d after each injection. Blood and serum samples were kept at -20° C until analysis. Colostral samples were obtained after parturition and stored at -20° C until analysis. Blood samples were also collected from lambs at birth and at 5, 20 and 40 d after injecting supplements on 20 d of age.

Selenium concentration in whole blood and colostrum was determined (10) by atomic absorption (Schimadzu, AA-670/670 G & GFA-4A). The procedure was modified by Dr. Chamsaz of the Department of Chemistry, Ferdowsi University. Optimal results were obtained after a 5-stage heating of samples:

1. 110° C for 30 s
2. 110° C for 20 s

3. 1000° C for 30 s
4. 1000° C for 20 s
5. 2600° C for 4 s

Samples were diluted 5 folds with triton to minimize background interference.

Immunoglobulin and betaglobulin concentrations in serum and colostrum were determined by electrophoresis (Titan Pius, Electrophoresis, Helena Laboratory, Germany) (35). Due to high viscosity, colostral samples were diluted with distilled water, and a 1:10 dilution was optimal in allowing separation of protein bands.

Lambs were weighed within 24 h after birth and again every 20 d until 100 d of age. Lambs were observed for occurrence of NMD, and those which showed signs were autopsied to confirm the disease (3).

Blood and colostral data were analyzed by repeated-measures analysis of variance (33) and means were compared by using the Duncan's multiple range test. Occurrence of NMD was analyzed by the Chi Square test. Premating levels of Se and immunoglobulins were used as covariates in analysis of variance for changes in Se and immunoglobulin concentrations. Ewe body weight and age before mating were used as covariates for analysis of lamb birth weight, and lamb birth weight was used as a covariate for analysis of body weight changes. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

Selenium Levels

Twenty d after the first (premating) injection of Se, Se levels in the blood of ewes were higher than in other groups (Table 1) and gradually increased to values within the normal ranges (34). Dietary Se whether as selenate or selenite increased serum Se levels in cattle, sheep and horses (27). Although Se injection increased its blood concentration in ewes which had been fed a purified diet, the presence or absence of vitamin E had no effect on blood Se concentrations or upon tissue glutathione peroxidase activity (29). Two intramuscular injections of Se (25 mg) and/or vitamin E (340 IU) to feedlot steers increased their serum levels of Se and vitamin E (6).

Hayek *et al.* (11) did not observe any changes in whole blood concentration of sows which had received a single intramuscular injection of Se and/or vitamin E on d 100 of pregnancy. However, there was a sharp decrease in blood Se concentrations at parturition that returned to baseline values at the two-week bleeding period.

Table 1. Mean blood concentration of Se (ng ml^{-1}) in control and experimental ewes injected with Se and/or vitamin E at 20 d before mating (premating), midgestation, and 20 d before parturition (preterm)[†].

Time of injection	Days after injection	-Se-E	+Se-E	-Se+E	+Se+E	SEM
Premating	5	70.1[a§]	70.8[a]	76.1[a]	80.9[a]	4.71
	10	76.4[a]	82.9[a]	76.5[a]	87.0[a]	3.05
	20	74.7[a]	88.5[b]	81.2[a]	91.7[b]	2.12
	40	80.9[ab]	87.6[b]	72.0[a]	88.8[b]	3.29
Midgestation	5	67.7[a]	94.2[b]	72.7[a]	94.8[b]	3.87
	10	70.9[a]	106.2[b]	72.1[a]	108.1[b]	3.92
	20	71.2[a]	115.2[b]	69.2[a]	109.2[b]	3.43
	40	69.4[a]	115.5[b]	72.3[a]	115.7[b]	1.55
Preterm	5	71.2[a]	124.5[b]	75.9[a]	121.1[b]	4.74
	10	67.6[a]	123.3[b]	76.0[a]	131.1[b]	3.65
	20	62.0[a]	114. 0[b]	68.9[a]	124.6[b]	4.68
	40	55.5[a]	99.8[b]	58.0[a]	99.7[b]	2.30

† Treatments were: -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E).

§ Within each row, means followed by the same letters are not statistically different ($P<0.05$).

Blood concentration of Se is influenced by dietary Se levels but Se concentration in the whole blood increases more slowly than in serum or plasma (15, 34). Blood Se concentration decreased in all groups after lambing (Table 1) which might be due to excretion of most of blood Se into milk or uptake by the mammary tissue from the blood (7, 18, 22).

Selenium and/or vitamin E injection increased Se concentration in colostrum as compared with control ewes (Table 2). Vitamin E might have

Table 2. Mean concentration of Se (ng ml^{-1}) and gamma- and betaglobulins (% of total proteins) in colostrum of ewes injected with Se and/or vitamin E[†].

Treatment	Se	Gammaglobulins	Betaglobulins
-Se-E	70.5[a§]	34.6[a]	29.7[a]
+Se-E	128.3[c]	43.6[b]	36.3[b]
-Se+E	95.3[b]	39.9[b]	36.5[b]
+Se+E	117.5[bc]	39.1b[c]	34.2[b]

† Treatments were : -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E).

§ Within each column, means followed by the same letters are not statistically different (P<0.05); SEM=7.41

influenced Se transfer by some unknown mechanism. Norton and McCarthy (22) reported that milk Se concentrations increased in response to Se and vitamin E injections. Several intramuscular injections of Se (0.01 mg kg^{-1} initial body weight) or vitamin E (0.9 IU α-tocopherol) before lambing, at parturition and during lactation of the ewes resulted in higher levels of Se and vitamin E in blood plasma and milk (1). Meneses *et al.* (19) injected (i.m.) ewes with vitamin E (2000 IU per ewe) and Se (12 mg sodium selenite per ewe) at lambing and 5 to 6 w after lambing. Ewes supplemented with vitamin E had a significantly higher concentration of vitamin E in their milk as compared with the control group, up to 14 d of lactation. Selenium-supplemented ewes also had higher vitamin E concentrations in milk from d

2 to d 35 of lactation. They suggested that Se was effective in increasing the level of vitamin E in the milk supporting previous evidence that Se can be utilized instead of vitamin E and hence more vitamin E was available for transfer to milk. The first colostrum had about twice the Se content of the milk 1 d later, and 3.5 times the Se content of the milk taken 1 w after lambing. Similar results were reported by Pherson *et al.* (26). Norton and McCarthy (22) suggested that α-tocopherol may have some type of sparring effect on Se utilization. Levels of vitamin E in the present experiment was not determined but Norton and McCarthy (22) reported that lambs receiving injectable vitamin E exhibited higher plasma tocopherol levels in comparison with those lambs that did not. They also noted breed differences in concentrations of α-tocopherol in plasma.

Some reports showed that a small proportion of the dietary Se was transferred to cows milk (7, 18). Perry *et al.* (24) showed that feeding of 5 mg Se (but not 1 and 2 mg) per cow per d during gestation and lactation resulted in higher milk Se as compared with the control group. They concluded that supplementing cows with high doses of Se would increase milk Se level. Conrad and Moxon (7) also were able to increase milk Se levels in cows, but they did that by adding sodium selenite to the diet. They suggested that addition of Se to diets already adequate in naturally occurring Se produced no appreciable increase in milk Se.

Lambs born from ewes receiving supplements had slightly higher blood Se levels at birth but these were not significantly different from the control group (Table 3). Norton and McCarthy (22) found that plasma Se concentrations in lambs were lowest at 1 d of age but gradually increased to d 56. Lambs receiving the injectable vitamin E treatment tended to exhibit higher plasma Se concentrations; however, non-treated lambs from injected ewes had lower plasma Se concentrations in comparison with lambs that received injections. In addition, non-treated lambs from injected ewes exhibited lower plasma Se levels than treated lambs from injected ewes. Prepartum injection of the ewes produced higher lamb plasma Se concentrations at d 1 and the difference diminished by d 18. Similar observations have been reported by Jenkins *et al.* (14). Pherson *et al.* (26)

stated that suckling lambs depended entirely on supply of Se from the colostrum and milk because no storage takes place during fetal life.

Table 3. Mean concentration of Se (ng ml^{-1}) in blood of lambs[†].

Treatment	1-d old	25-d old	30-d old	40-d old	60-d old
-Se-E	68.8[a§]	77.8[a]	76.7[a]	81.4[a]	80.7[a]
+Se-E	85.7[a]	111.7[bc]	115.1[bc]	123.5[b]	114.6[a]
-Se+E	82.8[a]	95.7[ab]	92.8[ab]	113.3[ab]	116.4[a]
+Se+E	97.9[a]	115.7[c]	121.5[c]	125.7[b]	110.8[a]
SEM	8.96	5.74	7.34	12.11	12.33

† Treatments were : -Se-E (no selenium and no vitamin E), +Se-E (selenium alone),-Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E). Injections were given at 20 d of age.

§ Within each column, means followed by the same letters are not statistically different ($P<0.05$).

Perry *et al.* (25) fed Se to cows (0, 1, 2 and 5 mg per cow per d) from 90 d before calving to 7 months of lactation and found that Se levels in the serum of calves at birth reflected maternal intake, being higher in calves from supplemented cows than in calves from unsupplemented ones. They suggested that Se crosses the placenta.

Time × treatment interaction was significant for the effect of supplements on Se level in lambs. Significant differences in blood levels of Se in lambs were observed after they had consumed colostrum and received supplements. The levels remained generally high until 60 d of age (last blood sampling) but differences between treated and control ewes were not significant at this stage (Table 3). This could have been due to decreased milk consumption and increased solid intake (3).

Immunoglobulin levels

Gammaglobulins showed two distinct peaks (IgG1 and IgG2) in the serum of ewes and lambs; however, only one peak (IgG1) was observed in the colostrum. Peak concentrations of gammaglobulins in blood serum of ewes occurred before parturition but decreased after lambing (Tables 4 and 5). This may be due to the transfer of immunoglobulins to colostrum. Injections of Se and vitamin E at midgestation did not affect serum gammaglobulin level in ewes, but premating injection increased its concentration. It seems that one injection of Se and vitamin E at latter stages of gestation was sufficient to increase blood gammaglobulin levels.

Table 4. Percentage of total serum gammaglobulin-I (IgG1) in control and experimental ewes injected with Se and/or vitamin E at midgestation and 20 d before parturition (preterm)†

Time of injection	Days after injection	-Se-E	+Se-E	-Se+E	+Se+E	SEM
Midgestation	5	$13.0^{a§}$	12.1^{a}	12.6^{a}	12.3^{a}	1.17
	20	13.2^{ab}	11.9^{a}	12.3^{a}	16.0^{b}	0.84
	40	13.2^{ab}	13.1^{a}	12.5^{a}	13.9^{a}	1.43
Preterm	5	14.7^{a}	14.6^{a}	14.9^{a}	14.7^{a}	1.61
	20	14.6^{a}	20.7^{b}	15.2^{a}	15.8^{ab}	1.52
	40	12.0^{a}	13.7^{a}	14.8^{a}	12.0^{a}	1.61

† Treatments were : -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E).

§ Within each row, means followed by the same letters are not statistically different ($P<0.05$).

Hayek *et al.* (11) studied the effect of a single intramuscular injection of Se (5 mg) and/or vitamin E (1000 IU) on d 100 of gestation on immunoglobulin levels of sows fed levels of Se and vitamin E which approximated their requirements. The IgA, IgG and IgM concentrations in serum were not altered by the treatment. Selenium injection significantly increased IgM levels in sow colostrum. Responses to vitamin E or vitamin E and Se were not significant, but the observed concentrations were intermediate between those for sows treated with Se and controls. Concentrations of colostral IgG and IgA were not affected by the treatment, but their patterns followed the same trend as that observed for IgM. Immunoglobulin concentrations in serum from sows did not appear to correspond with colostral immunoglobulin changes. However, because significant amounts of IgM are produced by the mammary gland, serum IgM levels may not be indicative of total IgM synthesis. This may also indicate effects of Se and vitamin E on IgM synthesis in the mammary gland. Increased colostral IgM levels resulted in higher IgM levels in serum of the 1-d old pigs.

Table 5. Percentage of total serum gammaglobulin-II (IgG2) in control and experimental ewes injected with Se and/or vitamin E at midgestation and 20 d before parturition (preterm)[†]

Time of injection	Days after injection	-Se-E	+Se-E	-Se+E	+Se+E	SEM
Midgestation	5	27.5[a§]	31.3[b]	28.3[ab]	27.2[a]	1.07
	20	26.5[a]	32.4[b]	27.4[a]	25.0[a]	1.06
	40	29.6[a]	31.1[a]	29.1[a]	28.8[a]	0.99
Preterm	5	27.3[a]	30.6[a]	28.4[a]	30.8[a]	1.49
	20	29.6[a]	27.9[a]	28.7[a]	34.1[b]	0.95
	40	24.3[a]	27.1[ab]	26.7[ab]	29.7[b]	1.23

† Treatments were : -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E).

§ Within each row, means followed by the same letters are not statistically different ($P<0.05$).

Two intramuscular injections of Se (25 mg) and vitamin E (340 IU) to feedlot steers increased their serum IgG concentrations in response to *Pasteurella haemolytica* (8). Reddy *et al.* (28) reported that lymphocyte stimulation indices were significantly higher for calves fed 2800 mg dl-α-tocopheryl acetate or injected with 1400 mg dl-α-tocopherol at weekly intervals as compared with unsupplemented calves. There were no significant differences in the concentrations of IgG1 and IgG2 among treatments.

Concentration of IgM was significantly higher at w 6 in calves given 2800 mg oral supplementation than in all other calves.

Knight and Tyznik (15) depleted ponies of Se and found that 0.22 ppm of Se in the diet increased blood IgG concentrations and glutathione peroxidase activity as compared with 0.02 ppm Se in the diet.

Immunoglobulin levels were higher in colostrum of ewes which were injected with Se and/or vitamin E (Table 2) Colostrum accumulates in mammary glands during the latter stages of pregnancy and immunoglobulins represent the most important class of proteins in colostrum. In cattle, gammaglobulin levels decrease in maternal plasma 2 w before calving but increase in colostrum, as a result of selective transfer (23). Plasma-to-colostrum transfer of gammaglobulins, coinciding with high levels of total estrogens, occur in sows 1 w before farrowing.

Injection of Se and/or vitamin E did not affect the levels of IgG1 in serum of lambs at birth, nor did their injections at 20 d of age affect IgG1 level (Table 6). However, lambs from ewes injected with Se and/or vitamin E

Table 6. Percentage of total serum gammaglobulin-I (IgG1) in lambs[†]

Treatment	1-d old	25-d old	40-d old	60-d old
-Se-E	3.1[a§]	0.9[a]	1.8[a]	0.9[a]
+Se-E	5.2[a]	2.3[a]	3.4[a]	0.6[a]
-Se+E	5.7[a]	1.1[a]	3.4[a]	1.1[a]
+Se+E	9.7[a]	1.1[a]	5.0[a]	0.0[a]
SEM	2.71	1.03	1.72	0.74

† Treatments were -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E). Injections were given at 20 d of age.

§ Within each column, means followed by the same letter are not statistically different (P<0.05).

had a higher level of IgG2 as compared with lambs from control ewes (Table 7). Lambs were fed colostrum within 2 h of birth which increased the immunoglobulin levels in their blood on d 1 of birth. Blood immunoglobulin levels were also increased after lambs received the supplements at 20 d of age, with a combination of Se and vitamin E being more effective than either one alone (Table 7). It has been shown that antibody synthesis in cows are affected by Se and/or vitamin E injections but the mechanism of action is unknown (13). Concentration of betaglobulins in the serum of ewes and lambs were not affected by the treatment (data not shown).

Table 7. Percentage of total serum gammaglobulin-II (IgG2) in lambs†.

Treatment	1-d old	25-d old	40-d old	60-d old
-Se-E	20.1[a§]	19.2[a]	19.1[a]	19.0[a]
+Se-E	25.0[ab]	22.8[ab]	22.0[b]	21.7[ab]
-Se+E	25.0[ab]	23.0[ab]	22.3[b]	21.7[ab]
+Se+E	25.9[b]	23.4[b]	23.2[b]	23.1[a]
SEM	1.59	1.20	0.68	0.81

† Treatments were : -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E). Injections were given at 20 d of age.

§ Within each column, means followed by the same letters are not statistically different (P<0.05).

Reproductive Performance and Lamb Daily Gain

About 55 to 80% of ewes in the four experimental groups lambed but the difference was not significant (Chi Square test). Similarly, the interval from the ram introduction to lambing date was not affected by the treatment.

Lamb birth weight was not different between the experimental groups. After 20 d of age, the differences between weights of lambs from the control and supplemented groups were significant (Table 8). Lamb daily gain was greatest for +Se+E and +Se-E groups, intermediate for -Se+E group and lowest for -Se-E (control) group. This may be due to occurrence of NMD at about 20 d of age. Signs of the disease are generally apparent during the first four w of life (3). There is evidence that Se affects metabolism of sulfhydryl groups in the oxidative tricarboxylic acid cycle and in metabolism of fatty acids and glucose (37). Data on effects of Se and vitamin E supplementation are consistent with those in the literature (9, 16).

Table 8. Mean body weight (kg) and daily gain (g) of lambs from birth to 100 days of age[†]

	-Se-E	+Se-E	-Se+E	+Se+E	SEM
No. of lambs	8	5	6	8	-
1-day old BW	3.99[a§]	4.54[a]	4.11[a]	4.03[a]	0.21
20-day old BW	8.28[a]	8.80[a]	8.45[a]	9.20[a]	0.60
40-day old BW	7.28[a]	12.89[b]	13.06[b]	13.78[b]	1.51
60-day old BW	11.19[a]	18.03[b]	17.20[b]	18.67[b]	1.95
80-day old BW	12.76[a]	21.7[b]	20.85[b]	21.86[b]	2.52
100-day old BW	15.86[a]	26.54[b]	23.2[ab]	26.47[b]	2.97
Daily gain	141.4[ab]	220.0[cd]	184.8[bc]	226.5[cd]	22.07

† Treatments were : -Se-E (no selenium and no vitamin E), +Se-E (selenium alone), -Se+E (vitamin E alone) and +Se+E (both selenium and vitamin E). Injections were given at 20 d of age.

§ Within each row, means followed by the same letters are not statistically different ($P<0.05$).

In a study by Buchanan-Smith (4) in which sheep on a purified diet were weekly injected with Se (5 mg) and/or vitamin E (700 IU), Se improved growth but satisfactory reproductive performance was obtained with both Se and vitamin E. Selenium increased plasma tocopherol concentrations, and Se and vitamin E increased vitamin A concentration in blood plasma. Appeddu *et al.* (1) reported that several injections of Se and vitamin E to ewes before and after lambing did not affect the lamb weaning weight. In a study by Norton and McCarthy (22), ram lambs injected with vitamin E produced greater average daily gains compared with ewe lambs receiving injectable vitamin E as well as ram lambs and ewe lambs not receiving an injection. Whanger *et al.* (38) reported that reproductive performance, lamb birth weight and average gain from birth to 6 w were not different between the control and Se and/or vitamin E supplemented groups.

Occurrence of NMD

Four lambs from the control group showed typical signs (3) of NMD around 20 d of age which persisted for 10-11 d. Two lambs recovered but their daily gains were consistently lower than healthy lambs. Two lambs with severe NMD were autopsied and showed typical signs of the disease including white strips in the myocardium and in leg and shoulder muscles. Selenium and/or vitamin E supplements prevented the occurrence of NMD in lambs as also reported by others (14, 23, 39, 40). Se administration of ewes, as 3 subcutaneous injections during gestation, as aqueous drench or in salt, prevented NMD in lambs (40). Whanger *et al.* (38) studied the effects of bi-weekly injections of Se (5 mg) and/or vitamin E (750 IU) in sheep fed a purified diet. All lambs born from ewes receiving no vitamin E or Se developed NMD, whereas only a few lambs in either the -Se+E or +Se-E groups developed these lesions. Similar results were found by Buchanan-Smith (4). It has been reported that vitamin E is more effective than Se in preventing NMD via a mechanism that involves a non-Se-glutathione peroxidase (39); however, in the present experiment Se and vitamin E, either alone or in combination, were equally effective in preventing NMD. There are many possible interacting factors in maternal feeds, making the interpretation of results obtained with such feeds difficult.

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