

Haematological, Blood and Rumen Chemistry Changes in Lambs Following Supplementation with Se-yeast

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Abstract

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The effects of feed supplementation with organic form of selenium (Se) on ruminal enzyme activities (ALT, AST, GGT, ALP and GDH), blood enzyme activity (GPx), serum enzyme activities (LDH, CK) and haematological indicators were examined in lambs. Ten animals were divided into two groups and fed experimental diets for 3 months. The first group received a basic diet (BD) providing a daily intake 50.6 µg of Se only. The diet for the second group consisted of BD supplemented with selenium 0.3 mg·kg⁻¹ DM in the form of Se-enriched yeast and giving a total daily intake 278 µg of Se per animal. Lambs of the second group which were fed additional Se had increased concentrations of Se in plasma ($P < 0.001$), greater activity of blood glutathione peroxidase (GPx) ($P < 0.001$) and lower activity of creatine kinase (CK) ($P < 0.05$) in serum. The activity of alkaline phosphatase (ALP) ($P < 0.001$) and glutamate dehydrogenase (GDH) ($P < 0.001$) in ruminal fluid were found to be significantly higher in Se-yeast group of lamb compared with the group given BD with no differences for Se concentration in ruminal fluid and ALT, AST and GGT activities. Total erythrocyte count and osmotic resistance of red blood cells were significantly higher ($P < 0.01$) in selenium-supplemented animals. White blood cell count was increased in lambs given BD ($P < 0.05$). It was concluded that Se supplementation can influence ruminal enzyme activities and cell membrane resistance of lambs.

Selenium; haematology; ruminal enzyme activity; sheep

Nutritional deficiencies of selenium in sheep cause white muscle disease in lamb (Muth et al. 1958), loss of glutathione peroxidase activity (GSHPx; Rotruck et al. 1973), reduced selenoproteins (Croteau et al. 1996; Yeh et al. 1997), and suppression of immunity (Yamini and Mullaney 1985).

According to NRC Standards (1989) feeds used for ruminants should contain from 0.1 to 0.3 mg selenium/kg DM.

The availability of selenium from mineral compounds (sodium selenite, sodium selenate) in the gastro-intestinal tract of ruminants is poor. Low absorption of selenium in ruminants is believed to result from reduction of dietary selenium to insoluble forms such as elemental selenium or selenides in the rumen environment (Peterson and Spedding 1963; Várady et al. 2005).

It was found that organic selenium in selenized yeast resulted in much larger increases in blood and milk selenium concentrations than selenite (Knowles et al. 1999; Orthman and Pehrson 1999). Lambs fed selenomethionine also had higher selenium concentrations in skeletal muscle and in a number of other tissues than lambs fed selenite (Ehling et al. 1967). Selenomethionine is the predominant form of selenium that occurs naturally in feedstuffs and selenized yeast. Incorporation of selenomethionine into non-specific body

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proteins in place of methionine (Behne et al. 1991) probably explains the higher selenium concentrations in tissues and milk of ruminants that were fed organic compared with selenite selenium.

Absorption of selenium in ruminants depends on several factors. Limited research suggests that either high or low dietary calcium may reduce selenium absorption (Harrison and Conrad 1984). Gutzwiller (1993) reported that ewes fed a white-clover variety that was high in cyanogenetic glycosides had much lower selenium status. Later Koenig et al. (1997) found that selenium absorption and retention were greater in sheep fed a concentrate-based (barley) diet than in those fed a forage-based (alfalfa hay) diet. A number of studies indicate that increasing dietary sulfur reduces the bioavailability of selenium (Ivancic and Wiess 2001). Recently Pavlata et al. (2005) reported that increased iodine supplementation may have a negative effect on selenium metabolism and/or status in kids.

The objective of this study was to determine the effect of feed supplementation with selenized yeast on selected haematological indicators and blood, serum and ruminal enzyme activities in lambs.

Materials and Methods

The experiment was carried out on 10 male lambs of Valaška breed at the age of four months divided into 2 groups of 5 animals and kept on diets that differed in the content of Se supplemented. Animals were housed in individual pens with free access to water and fed both the diets used for 3 months before sampling. The lambs weighed from 18 to 20 kg at the end of experiment.

The composition of the daily ration of basic diet (BD) per lamb and the daily delivery of Se by BD is presented in Table 1.

Table 1. The composition of the daily ration of basic diet per lamb and Se intake

Component	Amount (g)	Dry mater (g)	Content of Se ($\mu\text{g}/\text{kg}$ of DM)	Se intake ($\mu\text{g}/\text{day}$)
Hay	500	440.0	61.9	27.2
Rapeseed oilmeal	40	36.3	166.5	6.1
Barley	300	259.0	66.8	17.3
Total	840	735.3	295.2	50.6

The first group was given BD with the daily Se content of 50.6 μg coming from the natural occurrence of Se in the dietary component only. The second group received BD supplemented with 0.3 mg of Se in the form of Se-yeast extract (Sel-Plex, Alltech Inc., USA) giving the daily Se intake 278.6 μg . The diet for the 1st group was fortified with adequate amounts of the yeast extract without Se (NUPRO, Alltech Inc., USA) to obtain the same final levels of the yeast extract as in the 2nd group (daily intake 1.04 g of NUPRO in feed).

EU requirements related to laboratory animal welfare were met.

Sample analysis

One heparinized and one non-heparinized tube of blood were collected from each lamb at 06:00 h before the morning feeding. Plasma was removed after blood centrifugation at 1, 180 $\times g$ for 15 min. Blood collected in non-heparinized tubes was centrifuged at 2,000 $\times g$ for 20 min and the serum was decanted and frozen.

The concentrations of selenium in dietary components, samples of plasma and rumen fluid were measured by fluorometric method of Rodriguez et al. (1994).

The enzymes, lactate dehydrogenase LD (LD 50, BIO-La-TEST, CzR) and creatine kinase CK (CK 50, BIO-LACHEMA-TEST, CR) were assayed in serum according to procedures outlined in respective commercial kits using a spectrophotometer set at 500 and 400 nm wavelength. The enzyme, glutathione peroxidase, GPx (GPx, RANSEL, RANDOX, UK) was assayed in blood according to procedure outlined in commercial kit using a spectrophotometer set at 340 nm wavelength.

The haemocytometer method was used for total erythrocyte and total leukocyte count determination. Erythrocyte osmotic fragility test was performed according to the method of Coles (1986).

Ruminal fluid collection

Ruminal fluid was obtained by means of a silicone tube attached to a vacuum source through each ruminal cannula 2 hours after the morning feeding. The ruminal fluid was strained through four layers of cheesecloth within 30 min of collection.

The enzymes, alanine aminotransferase, ALT (ALT 360, BIO-LACHEMA-TEST, CzR), aspartate aminotransferase, AST (AST 360, BIO-LACHEMA-TEST, CzR), gamma-glutamyltransferase, GGT (GGT 100, BIO-LACHEMA-TEST, CzR), alkaline phosphatase, ALP (ALP 120, BIO-LACHEMA-TEST, CR) and glutamate dehydrogenase, GDH (GL 442, RANDOX, UK) were assayed in rumen fluid according to procedures outlined in respective commercial kits using a spectrophotometer set at 510, 510, 430, 420 and 340 nm wavelength, respectively.

The results are expressed as mean \pm S.E.M. Statistical significance was evaluated by unpaired Student's *t*-test.

Results and Discussion

The selenium concentration in plasma was significantly higher in lambs given Se supplement (Table 2). This agrees with a study by Chen and Lin (2000) who reported that the Se concentration in serum was significantly increased after SeO₂ treatment compared to control. Later Rock et al. (2001) found that Se supplementation to pregnant ewes either from sodium selenite or selenized yeast increased the concentration of Se in serum of both pregnant ewes and lambs of ewes given Se.

Table 2. Effect of supplemental Se on haematological indicators of lambs

Parameter	Control	Selenized yeast	
RBC (T/l)	5.65 \pm 0.17	7.77 \pm 0.45	<i>P</i> < 0.01
WBC (G/l)	7.32 \pm 0.92	4.57 \pm 0.33	<i>P</i> < 0.05
Fragility of RBCs			
a, minimal	0.64 \pm 0.02	0.61 \pm 0.01	NS
b, maximal	0.50 \pm 0.0	0.42 \pm 0.02	<i>P</i> < 0.01

Results are expressed as mean of five determinations \pm S.E.M. Statistical significance of the differences between groups was determined by unpaired Student's *t*-test.

Table 3. Effect of supplemental Se on plasma concentration of selenium and activities of lactate dehydrogenase and creatine phosphokinase in serum and glutathione peroxidase in blood of lambs

Indicator	Control	Selenized yeast	
Se (μ mol/l)	0.39 \pm 0.02	1.78 \pm 0.05	<i>P</i> < 0.001
LDH (μ kat/l)	7.40 \pm 0.51	7.50 \pm 0.52	NS
CK (μ kat/l)	2.63 \pm 0.09	1.73 \pm 0.29	<i>P</i> < 0.05
GPx (U/g Hb)	142.02 \pm 21.72	830.85 \pm 69.23	<i>P</i> < 0.001

Results are expressed as mean of five determinations \pm S.E.M. Statistical significance of the differences between groups was determined by unpaired Student's *t*-test.

Activity of GPx was greater (*P* < 0.001) in lambs supplemented with Se (Table 3). A high and linear correlation between the Se concentration and GPx activity of blood has been reported by several authors (Pavlati et al. 2001; Rock et al. 2001). Our results confirm the positive correlation between blood Se content and the activity of this selenoenzyme.

However, Se concentrations in rumen fluid of lambs were not affected by Se supplementation (Table 4).

The fate of seleno-methionine in rumen depends on whether Se released from seleno-methionine by rumen degradation is further degraded into inorganic Se or re-incorporated into microbial protein as seleno-methionine. Once absorbed, seleno-methionine can be incorporated into protein non-specifically in place of methionine; an excess of seleno-methionine incorporation into protein can influence protein function of seleno-methionine replaces methionine at the active site of an enzyme (Schrauzer 2000). The extent of seleno-methionine incorporation into protein is dependent upon the methionine status of the animal: when methionine is limiting, incorporation of seleno-methionine is increased. Although seleno-methionine incorporated in protein has no known function, protein-bound

Table 4. Effect of supplemental Se on ruminal concentration of selenium and activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ - glutamyl transferase and glutamate dehydrogenase in ruminal fluid of lambs

Indicator	Control	Selenized yeast	
ALT(μ kat/l)	1.24 \pm 0.05	1.41 \pm 0.17	NS
AST(μ kat/l)	2.3 \pm 0.13	2.48 \pm 0.17	NS
ALP(μ kat/l)	1.7 \pm 0.08	11.88 \pm 0.81	$P < 0.001$
GGT(μ kat/l)	2.47 \pm 0.39	3.34 \pm 0.44	NS
GDH (μ kat/l)	0.16 \pm 0.009	1.77 \pm 0.12	$P < 0.001$
Se (μ mol/l)	0.012 \pm 0.003	0.021 \pm 0.004	NS

Results are expressed as mean of five determinations \pm S.E.M. Statistical significance of the differences between groups was determined by unpaired Student's *t*-test.

seleno-methionine may act as a source of Se in deficiency providing possible local stores for muscle.

Activities of alkaline phosphatase and glutamate dehydrogenase in ruminal fluid were significantly higher ($P < 0.001$) in Se-supplemented lambs compared to control lambs (Table 3).

Numerous studies have addressed the effects of selenium on the digestibility of nutrients. Naziroglu et al. (1997) reported that combined supplementation of Se and vitamin E increased acetic, propionic, butyric and total volatile fatty acid concentration, the total counts of protozoa of the ruminal fluid of lambs *in vivo*. However, there was no statistically significant difference between the control and selenium and vitamin E-supplemented group in the ammonia nitrogen level. Kim et al. (1997) investigated the effect of several forms of selenium on ruminal microbial fermentation *in vitro* using rumen microflora from fistulated dairy cow. They reported that selenium supplementation could influence rumen microbial fermentation and that Se compounds tested, the amounts of short-chain fatty acids were greater with Se-Met treatment, which yielded a higher proportion of acetate compared to elemental Se and sodium selenite.

However, there is only scarce information about the effect exerted by selenium on rumen enzyme activities. Significantly higher activity of alkaline phosphatase and glutamate dehydrogenase in ruminal fluid in Se-supplemented group can be explained by its supportive effect on rumen microbial population, increasing their resistance and activity. In addition, selenium is known to act as a scavenger of free radicals within cell membranes, having a protective effect against oxidative damage.

Total erythrocyte count and osmotic resistance of RBCs were increased by Se supplementation ($P < 0.01$). On the other hand, white blood cell count was increased in lambs given basic diet ($P < 0.05$). A positive effect of selenium on haematological indicators was observed by several authors (Horton et al. 1978; Sehgal et al. 1980; Doni et al. 1984; Li et al. 1990; Chen and Lin 2000; Tras et al. 2000), but not confirmed by others (Hu et al. 1984; Bednarek et al. 1996). Fragility of erythrocyte membranes was significantly decreased in SeO₂-treated rats compared to control (Chen and Lin 2000). However, Tras et al. (2000) found that none of the haematological indicators (red blood cell count, haemoglobin, packed cell volume) were affected by a diet supplemented with vitamin E + selenium in male broiler chicken but ascorbic acid + aspirin + vitamin E and selenium supplementation significantly decreased the white blood cell counts.

A divergence of opinion as regards the effects of Se on haematological indicators was often presented in literature. One of the reasons is the fact that the effect of selenium was mostly examined simultaneously with that of vitamin E and/or ascorbic acid, which made the interpretation difficult.

The results presented here show a positive effect of selenium on the membrane stability of red blood cells and the red blood cell count.

Our study shows that there was an increase in CK activity in the animals in the control group ($P < 0.05$). However, creatine phosphokinase activity in both control and Se-supplemented lambs was within the physiological norm. There were no clinical cases of nutritional muscular dystrophy. Sekin et al. (1996) reported that CK activity is correlated with intensive degenerative changes in the muscle, whereas Bradley et al. (1987) reported that the detection of CK activity is particularly useful in the diagnosis of subclinical states of dystrophy. Later Sobiech and Kuleta (2002) reported significantly higher CK activity in Se-deficient lambs.

Selenium-yeast supplementation to lambs increased the concentration of Se in plasma and blood glutathione peroxidase activity. In addition, Se supplementation increased the stability of red blood cells and red blood cell count and glutamate dehydrogenase and alkaline phosphatase activity in ruminal fluid of lambs.

Zmeny v hematológii a v bachore po podaní selenizovaných kvasníc u jahniat

Vplyv krmiva obohateného organickým selénom na aktivitu enzýmov v bachore (ALT, AST, GGT, ALP a GDH), v krvi (GPx), v sére (LDH a CK) a na hematologické parametre bol sledovaný u jahniat. Desať jahniat bolo rozdelených do dvoch skupín a kŕmených diétou počas troch mesiacov. Prvá skupina zvierat bola kŕmená základnou diétou, ktorá obsahovala 50,6 µg selénu na 1 deň. Druhá skupina zvierat bola kŕmená základnou diétou suplementovanou o selén v dávke 0,3 mg/kg sušiny vo selenizovaných kvasníc a predstavovala dennú dávku 278 µg na 1 zviera. U jahniat druhej skupiny, ktoré boli kŕmené kŕmnom dávkou so zvýšeným obsahom selénu bola nameraná vyššia koncentrácia selénu v plazme ($P < 0,001$), vyššia aktivita enzýmu glutathionperoxidáza (GPx) ($P < 0,001$) v krvi a nižšia aktivita enzýmu kreatinkináza (CK) ($P < 0,05$) v sére. Bolo zistené, že enzymatická aktivita alkalickéj fosfatázy (ALP) ($P < 0,001$) a glutamát dehydrogenázy (GDH) ($P < 0,001$) v bachorovej tekutine bola vyššia u jahniat kŕmených diétou obsahujúcou selenizované kvasnice v porovnaní so skupinou kŕmenou základnou kŕmnom dávkou. Enzymatická aktivita ALT, AST a GGT v bachorovej tekutine oviec sa nelíšila medzi jednotlivými skupinami. Celkový počet erytrocytov a osmotická rezistencia erytrocytov boli signifikantne vyššie ($P < 0,01$) u zvierat suplementovaných selénom. Celkový počet bielych krviniek bol vyšší u jahniat ($P < 0,05$) kŕmených základnou diétou. Možno zhrnúť, že suplementácia diéty selénom môže ovplyvniť enzymatickú aktivitu v bachore a odolnosť bunkových membrán u jahniat.

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