INFLUENCE OF DIETARY PROTEIN ON THE FRACTIONATION OF SELENIUM IN THE RUMEN OF SHEEP

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Summary

The effects of diets differing in protein content through soybean meal supplementation on ruminal fractionation of selenium (Se) were studied. A 3×3 Latin square design was used with three Japanese Corriedale wethers (45 kg average body weight), three periods, and three dietary treatments. The three dietary treatments were : Diet 1, without soybean meal supplementation (14% crude protein, CP); Diet 2, with 10% soybean meal supplementation (16.5% CP); and Diet 3, with 20% soybean meal supplementation (19% CP). All the diets had a Se supplementation in the form of sodium selenite at 0.2 mg Se/kg dietary DM. The Se supplement and the concentrate mixture were fed only in the morning before the hay was given. Daily feeding schedule for hay was set at 09:00 and 17:00 h. On the final day of the collection period, ruminal fluid samples were obtained at 0.5, 2, 6, 12 and 24 h post-feeding starting at 09:00 h. Total ruminal fluid Se was markedly higher ($p < 0.05$) in Diet 3 than those in Diets 1 and 2 at almost all sampling time except at 24 h. The proportion of Se in soluble protein to the total ruminal Se was higher ($p < 0.05$) in Diet 3 (40%) followed by Diet 2 (28%) and Diet 1 (21%). The proportion of free inorganic Se to the total ruminal Se was the reverse, especially after two hours where Diet 1 ($p < 0.05$) was higher than the other diets. Bacterial Se was lower ($p < 0.05$) in Diet 1 than those in Diets 2 and 3 at any sampling time. The highest was observed at 2 h postprandially in all diets with a value of 421, 556, 655 μg/kg bacterial DM for Diet 1, 2 and 3, respectively. No differences ($p > 0.05$) were observed on ruminal pH, ammonia and total volatile fatty acids although increasing protein supplementation tended to decline the ruminal pH and increase ruminal ammonia. This study concludes that increasing dietary protein content by soybean meal supplementation can affect the ruminal Se metabolism.

(**Key Words** : Selenium, Selenite, Ruminal Selenium, Bacterial Selenium, Ruminal Ammonia, Volatile Fatty Acid, Sheep)

Introduction

Selenium (Se) nutrition of ruminants is unique compared to those monogastric animals because the presence of rumen of the former. Thus, the dietary Se of the ruminants is not only for the animal *per se* but also including those ruminal microbes. Previous reports (Hidiroglou et al., 1968; Paulson et al., 1968; Whanger et al., 1968; Hudman and Glenn, 1984) indicated that ruminal microbes are capable of incorporating Se into their cells. Upon introducing into the rumen compartment, the Se uptake of ruminal microbes is very fast especially in the first three hours postprandially (Serra et al., 1994). These microbes play a key role in the conversion of dietary Se into unavailable forms (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Lopez et al., 1969; Hudman and Glenn, 1984). As well as bacterial Se, Se can be fractionated into free inorganic Se and Se in soluble protein in the rumen (Serra et al., 1994).

Due to the influence of the rumen environment, Se requirement could vary depending on the type or kind of diet. Diet affects ruminal functions and therefore nutrient metabolism including Se. In the present study, soybean meal was supplemented to increase the dietary protein and studied its effects on ruminal Se metabolism specifically the fractionation of ruminal Se between bacteria, free

organic and soluble protein fractions.

Materials and Methods

The full details of the experimental procedures were discussed in the companion paper (Serra et al., 1996). The salient features are given below :

Experimental animals, diets and design

The study used a 3 × 3 Latin square design with three Japanese Corriedale wethers (45 kg average body weight), three periods (7 d dietary adjustment followed by 5 d total collection of urine and feces), and three dietary treatments. The three dietary treatments were: Diet 1, without soybean meal supplementation (14% crude protein, CP); Diet 2, with 10% soybean meal supplementation (16.5% CP); and Diet 3, with 20% soybean meal supplementation (19% CP). All the diets had a Se supplementation as sodium selenite at 0.2 mg Se/kg dietary DM. The Se was mixed to the carrier, (0.2 g Se/kg wheat flour). The Se supplement and the concentrate mixture were fed only in the morning before the hay was given. Daily feeding schedule for hay was set at 09:00 and 17:00 h.

Sample collection

Feed intake was recorded daily. Subsamples of the diet were obtained several times during the experiment and composited for analysis. Subsamples of feces or urine were obtained daily with total weight or volume recorded. The feed and fecal samples were dried at 60°C for 48 h and ground through a 1 mm screen in a grinding mill and saved for later analysis. Urine samples were acidified with 10% sulfuric acid (100 ml/L urine) and kept in the freezer (−20°C) before analysis.

On the final day of the collection period, ruminal fluid samples obtained at 0.5, 2, 6, 12 and 24 h post-feeding starting at 09:00 h. Time 24 could be considered also as time 0. Immediately after sampling, pH was measured using a pH meter (Model M-8L, Horiba, Tokyo, Japan) and later mercuric chloride drops were added as preservative. The fluid sample was strained using four layers of gauze cloth and then frozen at −20°C and later analyzed.

Laboratory analyses

Diet and fecal samples were analyzed for dry matter (DM), CP and ash following the procedures of AOAC (1984). Urine samples were analyzed for their CP contents. Moreover, the diet, fecal and urine samples were digested with nitric and perchloric acids (3:1 v/v) and their Se contents were analyzed following the fluorometric

procedure of Watkinson (1966) and the fluorescence spectrophotometer used was Hitachi 204 (Hitachi Ltd., Tokyo, Japan). The conditions of the spectrophotometer were presented earlier (Serra et al., 1994).

The ruminal fluid samples were partitioned into various ruminal Se fractions following the previous procedure (Serra et al., 1994). The different fractions were wet ashed and their Se contents were determined by the procedures mentioned earlier. The ruminal samples were also analyzed for their ammonia-nitrogen (Oser, 1965) and VFA content (Erwin et al., 1961).

Statistical analyses

All results are expressed as means ± SEM (n = 3) unless otherwise stated. Data were analyzed by ANOVA in 3 × 3 Latin square (Steel and Torrie, 1980; Statistica for the Macintosh™ Release 4.1, Statsoft, Inc., Tulsa, OK). The sum of squares was partitioned into the main effect of animal, period and treatment. When the main effect was significant (p < 0.05), means were compared using least significance difference. Separate analyses of variance were performed for all Se ruminal fractions and ruminal characteristics at each hour of sampling. Correlation and regression analysis were also done among the measures affecting the bioavailability of Se.

Results

The Se concentration of ruminal fluid fractions are presented in table 1. Higher (p < 0.05) total ruminal Se was found at every sampling time in Diet 3 that those in Diets 2 and 1 except at time 24 or 0. At time 24 or 0, the total ruminal Se was at the lowest. Then, it peaked at 2 h after feeding and declined subsequently. Diet 3 ruminal Se values had increased 37 and 20% over Diet 1 and Diet 2, respectively.

Free inorganic Se in ruminal fluid was lower (p < 0.05) in Diet 3 over Diets 2 and 1 at time 24 or 0. At 2 h after feeding, Diet 1 was higher (p < 0.05) than the other two diets. No significant differences were noted at other sampling times. It should be noted that at 2 h postprandially the free inorganic Se was at its peak.

Selenium in soluble protein of ruminal fluid was different (p < 0.05) across diets at 24 or 0, 0.5 and 2 h postprandially. At time 6 and 12, Diet 3 was higher (p < 0.05) than those in Diets 2 and 1. The highest value obtained was after two hours regardless of the diets.

Selenium content of ruminal bacteria was presented in figure 1. Diet 1 was lower (p < 0.05) in bacterial Se content in every sampling time compared to those Diets 2 and 3. At time 24 or 0, the value obtained for Diets 1, 2,

and 3 was 226, 326, and 344 μg/kg bacterial DM, respectively. It increased to 421, 556, 655 μg/kg bacterial DM at two hours in Diets 1, 2, and 3, respectively. Subsequently, it declined in succeeding time.

TABLE 1. SELENIUM CONCENTRATION OF DIFFERENT RUMINAL FLUID FRACTIONS[1]

Hour	Diet 1	Diet 2	Diet 3
Total ruminal fluid Se, μg/kg ruminal fluid[2]			
0.5	6.04±0.27[a]	6.78±0.18[a]	8.45±0.53[b]
2	6.63±0.19[a]	7.50±0.92[a]	9.13±0.45[b]
6	5.60±0.67[a]	5.92±0.36[a]	8.09±0.22[b]
12	5.17±0.18[a]	5.14±0.42[a]	6.42±0.22[b]
24	4.67±1.17	4.50±0.29	5.30±0.15
Mean	5.62±0.50	5.97±0.31	7.48±0.31
Free inorganic Se, μg/kg ruminal fluid[3]			
0.5	2.55±0.30	2.23±0.12	1.45±0.28
2	3.73±0.74[a]	2.10±0.29[b]	1.24±0.14[b]
6	2.19±0.31	1.67±0.28	1.10±0.10
12	1.60±0.25	1.41±0.28	0.95±0.13
24	1.72±0.06[a]	1.00±0.09[a]	0.57±0.17[b]
Mean	2.36±0.33	1.68±0.21	1.06±0.19
Se in soluble protein, μ/kg ruminal fluid[3]			
0.5	1.17±0.27[a]	2.11±0.17[b]	3.22±0.09[c]
2	1.57±0.19[a]	2.20±0.12[b]	3.72±0.15[c]
6	1.45±0.21[a]	1.80±0.12[a]	3.55±0.12[b]
12	1.21±0.15[a]	1.32±0.04[a]	2.74±0.14[b]
24	0.59±0.18[a]	1.12±0.06[b]	1.76±0.08[c]
Mean	1.20±0.20	1.71±0.10	3.00±0.11

[1] Values are means ± SEM, n = 3 sheep/treatment. Figures within row with different superscript differ (p < 0.05).

[2] Analyzed from the supernatant of ruminal fluid after low centrifugation (2,000 × g); also contains ruminal microbes.

[3] Analyzed from the supernatant of ruminal fluid after high centrifugation (24,000 × g) and then partitioned into free inorganic Se and Se in soluble protein by the addition of 5% trichloracetic acid and centrifuged to 3,000 × g.

Ruminal pH, ammonia and volatile fatty acids characteristics are presented in table 2. No differences (p > 0.05) on various measures of ruminal characteristics were observed although increasing protein supplementation tended to decline the ruminal pH and to increase ruminal ammonia.
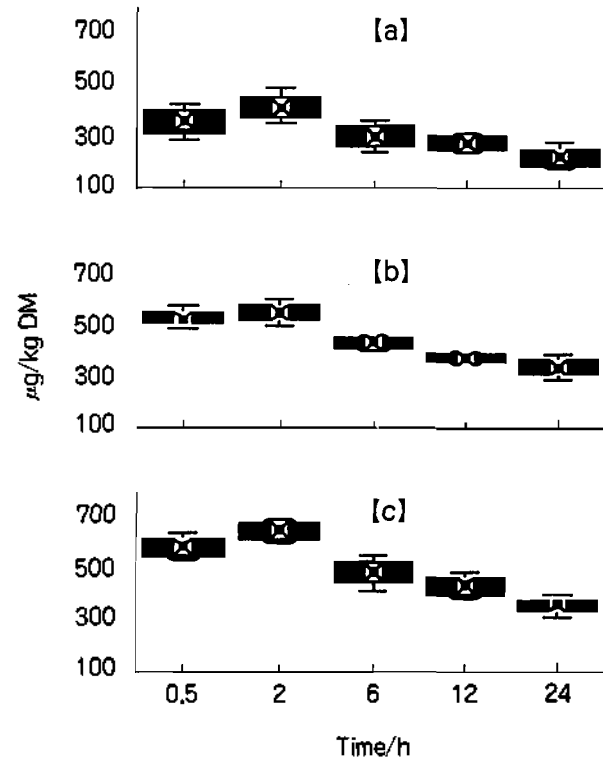

Figure 1. Concentrations of Se in ruminal bacteria as influenced by dietary protein content: a, Diet 1; b, Diet 2; c, Diet 3. Diet 1 was lower (p < 0.05) in bacterial Se content in every sampling time compared to those Diets 2 and 3. Box and whisker plots are Mean, SE and SD.

Discussion

The ruminal environment is being thought as the reason for the low bioavailabilities of Se and other trace elements in ruminants. Selenium present in the rumen is of both dietary and an endogenous origin. Whatever its origin, Se like many trace elements in the rumen forms complexes with anionic ligands which may be either inorganic or organic (Durand and Kawashima, 1980) or reduced into elemental Se (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Lopez et al., 1969; Durand and Kawashima, 1980; Hudman and Glenn, 1984). The forms of Se in the rumen depend on type, kind of diet or feeding management (Gerloff, 1992).

The increase in dietary protein as a result of soybean meal supplementation markedly increased the concentration of Se in ruminal fluid (table 1). A previous study (Serra et al., 1994) indicated that only 10 and 15% of the Se content of the ruminal fluid was associated with

TABLE 2. INFLUENCE OF PROTEIN SUPPLEMENTATION ON SOME RUMINAL CHARACTERISTICS[1]

Hour	Diet 1	Diet 2	Diet 3
·············· Ruminal pH ··············			
0.5	6.93±0.81	6.86±0.05	6.80±0.12
2	6.89±0.01	6.78±0.18	6.55±0.01
6	6.80±0.10	6.72±0.19	6.45±0.05
12	6.40±0.10	6.27±0.22	6.01±0.13
24	6.93±0.15	6.83±0.04	6.67±0.10
Mean	6.79±0.11	6.69±0.14	6.50±0.08
······ Ruminal ammonia (mg/100 ml) ······			
0.5	21.80±4.73	22.37±5.31	27.99±3.84
2	28.96±0.03	29.85±3.71	29.25±2.54
6	15.37±0.60	15.64±1.87	17.22±0.97
12	16.60±1.17	16.78±3.43	20.13±3.43
24	16.11±2.84	16.59±3.62	16.49±0.86
Mean	19.77±1.87	20.25±3.59	22.22±2.33
······ Ruminal total VFA, mmol/100 ml ······			
0.5	6.26±0.57	5.99±0.62	6.87±0.71
2	5.88±0.02	5.63±0.58	5.89±0.38
6	6.21±0.05	6.07±0.97	6.31±0.66
12	7.56±0.70	6.78±1.10	7.25±1.07
24	6.01±0.58	5.91±1.04	6.55±1.05
Mean	6.38±0.38	6.08±0.86	6.57±0.77
········· Acetate : Propionate ratio ·········			
0.5	5.42±0.32	4.50±0.37	4.10±0.14
2	4.51±0.005	4.34±0.37	3.61±0.33
6	4.21±0.12	4.52±0.15	4.06±0.43
12	4.89±0.93	3.84±0.21	3.36±0.50
24	5.15±0.52	5.14±0.68	4.25±0.05
Mean	4.84±0.38	4.47±0.36	3.88±0.29

[1] Values are means ± SEM, n = 3 sheep/treatment. Figures within row are not statistically different (p > 0.05).

soluble protein as influenced by selenate and selenite supplementation, respectively, on a diet without protein supplementation (hay alone). When the diets had protein supplementation as in the case of the present study, the proportion of Se in soluble protein increased to 21, 28 and 40% for Diets 1, 2 and 3, respectively. Thus, there was a great tendency that Se could be incorporated into protein. Previous paper (Favier, 1989) discussed the

synthesis of selenoamino acids from inorganic Se. To accomplish this, sulfur containing amino acids must be present. According to Burk (1991) and Gerloff (1992), inorganic Se was incorporated into the selenoproteins by Se substitution for a sulfur atom in the cysteine residue of the protein. The work of Hudman and Glenn (1984) showed the incorporation of $^{75}[Se]$-selenite into selenocystine, selenoethionine, selenohomocysteine and selenomethionine.

Free inorganic Se of the ruminal fluid showed a decrease in Se concentration as dietary protein increases (table 1). Thus, more available Se for the host animal was observed in diets with low protein content. In the present study, selenite is the form of Se that was supplemented. Selenite is an oxidized form of Se and the biologically active. If the ruminal environment has a higher reducing capacity due to concentrate supplementation, then more of this oxidized Se may be converted into reduced, unavailable state. This is supported by earlier studies (Harrison and Conrad, 1984; Serra et al., 1994; Serra et al., 1996) which showed that Se absorption and retention are affected by concentrate supplementation.

The higher concentrations of bacterial Se is another reason for higher Se concentrations of the ruminal fluid brought about by protein supplementation. Diet 2 (16.5% CP) and Diet 3 (19% CP) had higher (p < 0.05, figure 1) concentrations of bacterial Se than that of Diet 1 (14% CP). The present study found that even at 0.5 h after the introduction of Se in the rumen, bacterial Se fraction increased and peaked at 2 h postprandially. The highest concentration of bacterial Se seen at 2 h postprandially was 362, 556 and 655 μg/kg bacterial DM in Diets 1, 2 and 3, respectively. On hay alone, the highest concentration of bacterial Se obtained was 239 and 249 μg/kg bacterial DM in selenate and selenite supplemented diets upon Se introduction in the rumen up to 1 h postprandially (Serra et al., 1994). Many reports indicated the capability of ruminal bacteria in metabolizing inorganic Se (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Hidiroglou et al., 1968; Paulson et al., 1968; Whanger et al., 1968; Hudman and Glenn, 1984) and ability to actively transport selenite (Hudman and Glenn, 1984). The Se incorporated into bacterial cells and its influence on its Se availability to the host animal is not known (Church et al., 1971; Durand and Kawashima, 1980). However, ruminal microorganisms are considered to be the probable cause of the lower absorption of Se in ruminants than in nonruminants (NRC, 1983).

Ruminal environments are affected by type of diets (de Faria and Huber, 1984) as in the case of the present study. Ruminal pH showed diurnal changes, with lowest value at

12 h after morning feed and the mean pH of animals fed with the Diet 1 (basal diet; 6.79) was slightly higher than those supplemented with soybean meal (Diet 2, 6.69; Diet 3, 6.50). Ruminal ammonia increased with the inclusion of soybean meal in the diet and the trend was Diet 3 > Diet 2 > Diet 1. The distinct peaks of ruminal ammonia values were observed at 2 h across diets and they all exceeded 23 mg/100 ml (suggested value for maximum ruminal fermentation activity according to Mehrez et al., 1977) including the value at 0.5 h of Diet 3. The range of ruminal ammonia obtained in this study was 15.37-29.85 mg/100 ml. Bondi (1981) stated that 2-50 mg/100 ml was the normal range of ruminal ammonia concentrations depending on the diet and time after feeding. Moreover, he cited that the maximal concentration of ammonia could reached about 2 h after ingestion of the food containimg protein as also observed in the present study.

Conclusion

These data indicate that increasing dietary protein content by soybean meal supplementation can affect the ruminal environment including the various fractions of ruminal Se. Increasing the dietary protein content tend to increase the concentration of bacterial Se and Se in soluble protein but tend to decrease free inorganic Se.

Acknowledgements

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