INORGANIC SELENIUM FOR SHEEP
Ⅱ. ITS INFLUENCE ON RUMEN BACTERIAL YIELD, VOLATILE FATTY ACID PRODUCTION AND TOTAL TRACT DIGESTION OF TIMOTHY HAY

A. B. Serra, K. Nakamura, T. Matsui[1], T. Harumoto and T. Fujihara[2]

Faculty of Agriculture, Shimane University, Matsue 690, Japan

Summary

This study was conducted to determine the effect of inorganic selenium (Se) sources on rumen bacterial yield, ruminal volatile fatty aicd (VFA) production and total tract digestion of timothy hay (*Phleum pratense* L.) in Japanese Corriedale wethers. A 3 × 3 Latin square design was used with three wethers, three periods and three treatments. In each period, there was 7 d dietary adjustment followed by 5 d total collection of urine and feces. Ruminal fluid samples were obtained at 0, 1, 3, 5 and 7 h postprandially on the final day of the collection period. The three dietary treatments were: (1) without Se supplementation (control); (2) with Se supplementation as sodium selenate; and (3) sodium selenite at a rate of 0.2 mg Se/kg dietary DM. The basal diet was timothy hay fed at 2% of body weight/d. Results indicated that there was slight decrease in rumen bacterial yield of animals supplemented with inorganic Se, however, differences over the control were insignificant. It was found that Se content of ruminal fluid was negatively correlated ($p < 0.05$) to rumen bacterial yield. The various VFA contents and acetate and propionate ratio of the different ruminal fluid samples were insignificant across treatment means and the same manner was observed to the different digestibilities (DM, OM, CP, NDF, ADF and NDS). This study concludes that Se supplementation at 0.2 mg Se/kg dietary DM either from sodium selenate or sodium selenite could not significantly influence rumen bacterial functions.

(**Key Words**: Selenium, Wethers, Rumen Microorganisms, VFA, Digestibility)

Introduction

Selenium is an important dietary constituent of animal feed. It is a component of glutathione peroxidase (GSH-Px) which acts to destroy damaging peroxides that accumulate in tissues (Rotruck et al., 1973). It also prevents white muscle disease (nutritional muscle dystrophy) and improves the growth of young animals and the reproductive performance of older animals (Ammerman and Miller, 1975). Also, recent development revealed that dietary Se can decrease the incidence of metritis in cattle and improve the antibody response in sheep (Suttle and Jones, 1989).

In ruminant nutrition, one aspect that needs attention is its influence on rumen bacterial functions. Because of its immediate incorporation to the bacterial protein upon its introduction into the rumen (Hidiroglou et al., 1968; Whanger et al., 1978; Serra et al., 1993), bacterial functions might be affected. The study of Durand and Kawashima (1980) on the influence of trace elements (iron, manganese, molybdenum, zinc and cobalt) to rumen microorganism functions showed that the trace elements optimized microbial metabolism at certain levels in *in vitro* results. On the other hand, copper (Cu) and Se even at low concentrations (less than 1 ml/l) inhibited cellulolytic activity. However, *in vivo* results on these trace elements describing its influence in rumen microbial functions are lacking especially in Se. Because of this, this study was conducted to determine the influence of dietary Se sources on rumen bacterial yield, ruminal VFA production and total tract digestion of timothy hay (*Phleum pratense* L.) in sheep when fed only to meet their maintenance requirements.

Materials and Methods

The full details of the experimental procedures were discussed earlier (Serra et al., 1993). However, salient features are given below:

[1]Faculty of Agriculture, Kyoto University, Kyoto 606, Japan.

[2]Address reprint requests to Dr. T. Fujihara, Faculty of Agriculture, Shimane University, Matsue 690, Japan

Received June 4, 1993

Accepted October 7, 1993

Experimental animals, diets and design

The study used a 3 × 3 Latin square design with three Japanese Corriedale wethers (average body weight of 47 kg), three periods and three dietary treatments. In each period, had 7 d duration for dietary adjustment followed by 5 d total collection period of urine and feces. The three dietary treatments were: (1) without Se supplementation (control); (2) with Se supplementation as sodium selentate; and (3) sodium selenite. The Se was mixed to the carrier, (20 g pure Se/kg wheat flour) and was fed to the wethers to provide 0.2 mg of Se/kg dietary DM. The basal diet was timothy hay (*Phleum pratense* L.) [Nutrient composition (DM basis): 8.43% CP, 10.76% ash, 60.23% NDF, 38.91% ADF, 39.77% NDS and 31.74 ppb Se.] fed to the animals at a rate of 2% of body weight/day.

Sample collection

Feed intake was recorded daily with no orts. Subsamples of the diet were obtained several times during the experiment and composited for analysis. Wet feces was obtained daily, dried at 50 ℃ for 48 h. An aliqout sample was obtained from the 5 d collection, ground through a 1 mm screen in a grinding mill and saved for later analysis. Urine sample was collected daily and preserved in the freezer at −20℃, also for later analysis. On the final day of the collection period, ruminal fluid samples obtained at 0, 1, 3, 5 and 7 h postprandially. Immediately after sampling, mercuric chloride drops were added as preservative. The fluid sample was strained using four layers of gauze cloth and then frozen at −20℃ and later analyzed.

Laboratory analyses

Diet and fecal samples were analyzed for dry matter (DM), crude protein (CP), and ash following the procedures of AOAC (1984); neutral detergent fiber (NDF), acid detergent fiber (ADF) and neutral detergent solubles (NDS) using the procedures of Goering and Van Soest (1970). Also, the diet, feces and ruminal fluids were analyzed for their Se content (Watkinson, 1966) using fluorescence spectrophotometer (Hitachi 204, Hitachi Ltd., Tokyo, Japan). The different ruminal fluid samples were injected into a gas chromatograph (Hitachi 164 Gas Chromatograph, Hitachi Ltd., Tokyo, Japan) for the determination of VFA (Erwin et al., 1961).

To obtain a bacterial-rich precipitate, ruminal fluid was centrifuged at 2,000 × g for 20 min. The resulting supernatant was transferred to another centrifuge tube and centrifuged at 24,000 × g for 20 min. The precipitate was washed in an acid solution (pH 2.8 to 3) and oven-dried at 70℃ for 12 h and then weighed. A portion of the first supernatant was analyzed for its Se content.

Statistical analysis

Data were analyzed by using Analysis of Variance in 3 × 3 Latin square (Steele and Torrie, 1980). 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 significant difference. Correlation coefficients among rumen bacterial yield, ruminal total VFA content and Se content of ruminal fluid were also computed.

Results

Rumen bacterial yield

Table 1 shows the postprandial effect of Se intake on rumen bacterial yield of ruminal fluid. Although the results were not significant across treatment means, Se intake either as selenate or selenite decreased rumen bacterial yield by 19 and 27%, respectively. Selenium content of the ruminal fluid was negatively correlated ($p < 0.05$) to rumen bacterial yield ($r = -0.78$, table 2). However, bacterial yield was positively correlated to ruminal total VFA content ($r = 0.46$).

Ruminal VFA content

The effects of Se intake on VFA concentration and acetate and propionate (A:P) ratio of ruminal fluids are shown in table 3. Slightly higher acetate and valerate and slightly wider A:P ratio were observed on the Se supplemented diets, however, statistical analysis showed insignificant differences among the treatment means.

Figure 1 shows the time course of ruminal total VFA content and Se content of the different treatments. The peaks of total VFA content in all treatments were seen to be evident after one hour. In contrast, the peaks of Se content of ruminal fluids in the treated diets were noted

TABLE 1. POSTPRANDIAL EFFECT OF SE INTAKE ON BACTERIAL YIELD ($\bar{X} \pm SD$) OF RUMINAL FLUID (g DM / kg RUMINAL FLUID)[1]

Hour	Control	Selenate	Selenite
0	0.51 ± 0.21	0.45 ± 0.33	0.42 ± 0.07
1	0.62 ± 0.19	0.44 ± 0.15	0.41 ± 0.24
3	0.64 ± 0.32	0.44 ± 0.23	0.45 ± 0.25
5	0.70 ± 0.38	0.56 ± 0.13	0.50 ± 0.41
7	0.64 ± 0.27	0.60 ± 0.35	0.44 ± 0.14
Mean	0.62 ± 0.23	0.50 ± 0.22	0.45 ± 0.21

[1] Figures in each row are not statistically different.

TABLE 2. CORRELATION COEFFICIENTS AMONG BACTERIAL YIELD, RUMINAL TOTAL VFA CONTENT AND SE CONTENT OF RUMINAL FLUID

Concept	Se content of ruminal fluid	Ruminal total VFA content
Rumen bacterial yield	−0.78*	0.46
Ruminal total VFA content	−0.28	

* ($p < 0.05$).

TABLE 3. EFFECT OF SE INTAKE ON VFA CONCENTRATIONS (mo / 100 mol) AND ACETATE AND PROPIONATE RATIO (A:P) OF RUMINAL FLUID ($\bar{X} \pm SD$)[1]

Item	Control	Selenate	Selenite
Acetate	66.76 ± 1.84	69.91 ± 3.43	68.04 ± 2.19
Propionate	20.40 ± 2.40	20.21 ± 1.31	19.29 ± 1.16
Butyrate	11.41 ± 1.52	11.38 ± 1.38	11.85 ± 2.05
Valerate	0.69 ± 0.18	1.00 ± 0.60	0.83 ± 0.37
A:P	3.31 ± 0.48	3.47 ± 0.22	3.54 ± 0.27

[1] Figures in each row are not statistically different.

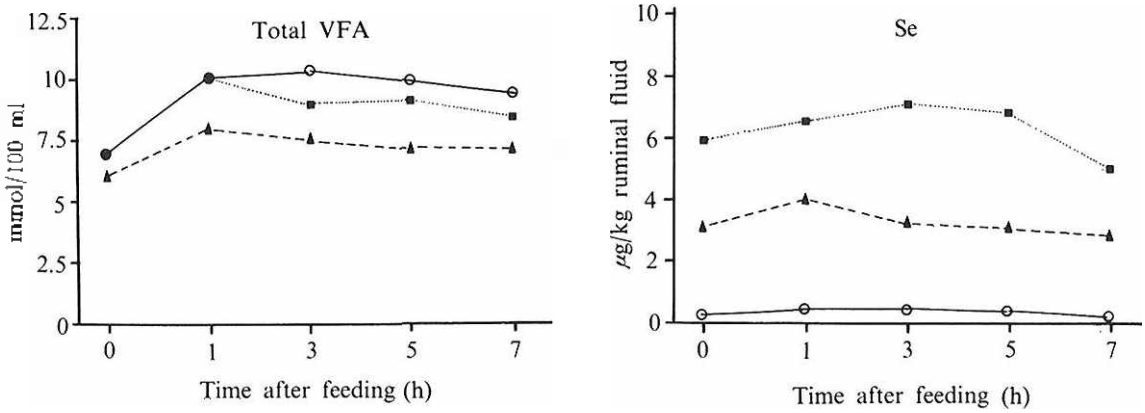

Figure 1. Total VFA concentrartion and Se content of ruminal fluid postprandially in wethers supplemented without and with inorganic Se (○ , control; ▲ , selenate; ■ , selenite).

after one to three hours unlike the control which remained more or less constant. The two parameters (ruminal total VFA content and Se content of ruminal fluid) were negatively correlated (r = −0.28, table 2) to other.

The time course of individual ruminal VFA value is shown in figure 2 (a and b). The animals under the control had higher individual VFA value over time except for valeric acid. Comparing the two Se sources, the animals supplemented with selenite had higher VFA values than the animals supplemented with selenate.

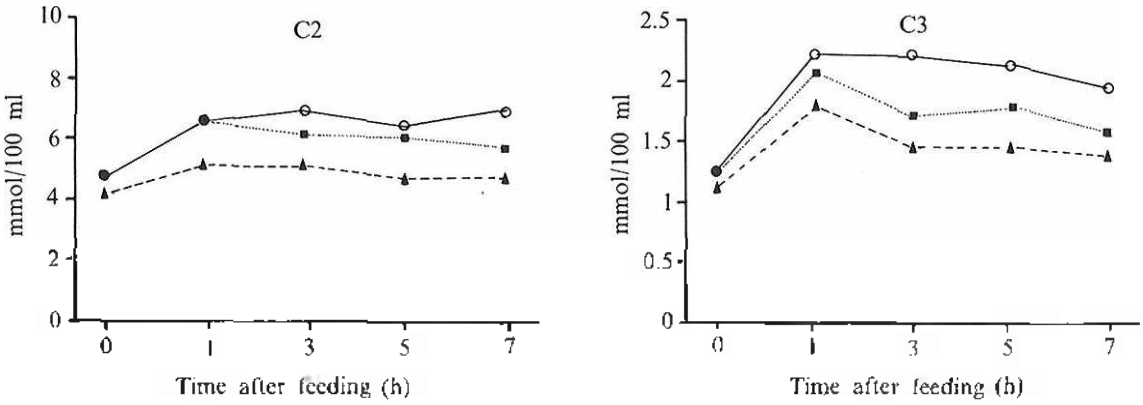

Figure 2a. Acetate (C2) and propionate (C3) concentrations of ruminal fluid postprandially in wethers supplemented without and with inorganic Se (o , control; ▲ , selenate; ■ , selenite).

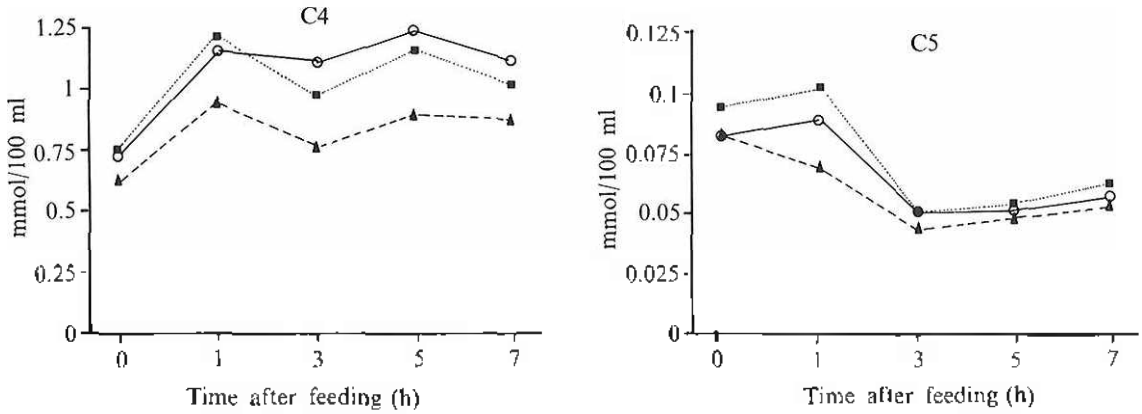

Figure 2b. Butyrate (C4) and valerate (C5) concentrations of ruminal fluid postprandially in wethers supplemented without and with inorganic Se (o , control; ▲ , selenate; ■ , selenite).

Nutrient digestibilities

The different nutrient digestibilities were not affected significantly by inorganic Se supplementation (table 4). However, CP digestibility was slightly improved while the fiber components (NDF and ADF) were slightly changed.

Discussion

A little change in VFA concentration and nutrient digestibilities might have been the end result of a complicated adjustment in the rumen due to the presence of dietary Se. The change in the diet had a marked impact on the number and kind of microorganisms in the rumen contents. This was shown by the slight decrease in rumen bacterial yield which subsequently affected VFA production as well as fiber and protein digestion. Previous study indicates that Se supplementation in the purified diet of sheep

TABLE 4. EFFECT OF SE INTAKE ON TOTAL TRACT ($\bar{X} \pm$ SD) DIGESTION OF TIMOTHY HAY[1]

Item	Control	Selenate	Selenite
DM intake (g/d)	948.10 ± 14.08	948.10 ± 14.08	948.10 ± 14.08
Se intake (μg/d)	30.09 ± 0.44[a]	230.09 ± 0.44[b]	230.09 ± 0.44[b]
Digestibility (%)			
DM	56.25 ± 5.87	55.10 ± 5.88	56.17 ± 4.90
OM	56.70 ± 4.87	56.06 ± 5.82	57.33 ± 5.66
CP	64.64 ± 5.47	64.54 ± 8.21	68.08 ± 1.63
NDF	51.14 ± 5.74	48.25 ± 8.59	49.57 ± 7.62
ADF	42.27 ± 9.30	39.63 ± 6.65	41.74 ± 2.18
NDS	65.70 ± 10.22	65.50 ± 5.48	66.17 ± 0.88

[1] Figures in each row having the same superscript are not significantly different ($p < 0.05$).

alters the VFA production (Hidiroglou and Lessard, 1976). The present study showed that the VFA concentration failed to show significant changes possibly due to optimum level of Se supplementation. As presented earlier, the fiber digestibilities were slightly affected due to Se supplementation both in the forms of selenate and selenite. These findings conformed with the *in vitro* study of Martinez and Church (1970) which revealed low Se supplementation (0.01 to 5 ppm), slightly reduced digestion. While, higher supplementation (7 up to 20 ppm) caused a significant depression ($p < 0.05$). According to McDowell (1985), dietary Se above 5 ppm is considered toxic to the animals. The toxic level of dietary selenium can significantly decrease rumen bacterial yield. This bacteria play a key role in the conversion of dietary Se into unavailable forms in the rumen (Durand and Kawashima, 1980). Therefore, if there are few bacteria then most of the dietary Se is absorbed in the gut, making toxic to the animal. Based on the results of the present study and the previous evidence presented (Martinez and Church, 1970), Se is not an essential element for rumen cellulolytic microorganisms.

While Se inhibits cellulolytic microorganisms, it probably stimulates proteolytic microorganisms as indicated in the slight improvement in the protein digestibility. This improvement might also be attributed the increased turnover rate of bacterial protein into the abomasum. This uncertainty therefore necessitates further study on the effect of protein to Se metabolism.

Se supplementation in ruminants should be made with precaution. Factors like the type of the diet should be considered. If the animal is nourished on hay, straw or with low concentrate supplementation, then low Se supplementation rate should be followed so that fiber digestion is not affected. This study showed that Se supplementation at 0.2 mg/kg dietary DM did not significantly alter fiber digestion. On the other hand, high concentrate diet requires higher Se supplementation rate (Gerloff, 1992). This type of diet will result in high reducing capacity of the rumen environment making the dietary Se mostly unavailable to the animal. Podoll et al. (1992) presented a Se supplementation of 0.3 mg/kg dietary DM in cattle, sheep and horses. Above 0.5 mg Se/kg dietary DM should be avoided because its effects as explained earlier. Thus, Se supplementation either as selenate or selenite at the rate of 0.2 mg Se/kg dietary DM does not have significant influence on the different rumen bacterial functions.

Acknowledgements

The senior author is indebted to the Japan Solidarity Committee for Asian Alumni (JASCAA, Tokyo, Japan) for his scholarship at Shimane University, Japan.

Literature Cited

Ammerman, C. B. and S. M. Miller. 1975. Selenium in ruminant nutrition: A review. J. Dairy Sci. 58:1561-1577.

AOAC. 1984. Official Methods Analysis (14th Ed.).

Association of Official Analytical Chemists, Arlington, VA.

Durand, M. and R. Kawashima. 1980. Influence of minerals in rumen microbial digestion. In: Y. Ruckebusch and P. Thivend (Ed.) Digestive Physiology and Metabolism in Ruminants. MTP Press Ltd., Lancaster, England. pp. 375-408.

Erwin, E. S., G. J. Marco and E. M. Emery. 1961. Volatile fatty acids analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44: 1769-1774.

Gerloff, B. J. 1992. Effect of selenium supplementation on dairy cattle. J. Anim. Sci. 70:3934-3940.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook 379, ARS, USDA, Washington, D.C.

Hidiroglou, M., D. P. Heaney and K. I. Jenkins. 1968. Metabolism of inorganic selenium in rumen bacteria. Can. J. Physiol. Pharmacol. 46:229-232.

Hidiroglou, M. and J. R. Lessard. 1976. The effect of selenium or Vitamin E supplementation on volatile fatty acid content of rumen liquor in sheep fed a purified diet. Int. J. Vit. Nutr. Res. 46: 458-463.

Martinez, A. and D. C. Church. 1970. Effect of various mineral elements on *in vitro* rumen cellulose digestion. J. Anim. Sci. 31:982-990.

McDowell, L. R. 1985. Cobalt, iodine and selenium. In: L. R. McDowell (Ed.) Nutrition of Grazing Ruminants in Warm Climates. Academic Press, Inc., San Diego. pp. 275-286.

Podoll, K. L., J. B. Bernard, D. E. Ullrey, S. R. DeBar, P. K. Ku and W. T. Magee. 1992. Dietary selenate versus selenite for cattle, sheep and horses. J. Anim. Sci. 70:1965-1970.

Rotruck, J. T., A. L. Pope, H. E. Ganther, D. G. Hafeman and W. G. Hoekstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588-590.

Serra, A. B., K. Nakamura, T. Matsui, T. Harumoto and T. Fujihara. 1993. Inorganic selenium for sheep. I. Selenium balance and selenium levels in the different ruminal fluid fractions. Submitted to Asian-Aust. J. Anim. Sci.

Steele, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Book Co., New York.

Suttle, N. F. and D. G. Jones. 1989. Recent developments in trace element metabolism and function: Trace elements, disease resistance and immune responsiveness in ruminants. J. Nutr. 119:1055-1061.

Watkinson, J. H. 1966. Fluorometric determination of selenium in biological material with 2, 3 diaminonaphthalene. Anal. Chem. 38:92-97.

Whanger, P. D., P. H. Weswig and J. E. Oldfield. 1978. Selenium, sulfur and nitrogen levels in ovine rumen microorganisms. J. Anim. Sci. 46:515-519.