INORGANIC SELENIUM FOR SHEEP
I. SELENIUM BALANCE AND SELENIUM LEVELS IN THE DIFFERENT RUMINAL FLUID FRACTIONS

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Summary

The effects of inorganic selenium (Se), selenate and selenite on Se balance and Se levels in the different ruminal fluid fractions were studied using Japanese Corriedale wethers with an average body weight of 47 kg. A 3 × 3 Latin square design was used with three animals, 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 (*Phleum pratense* L.) fed at 2% of body weight/d.

Results indicated that Se balance were higher ($p < 0.05$) for those animals under supplementation than those animals under control. Overall data gathered showed a similar digestion balance of selenate and selenite in sheep. Inorganic Se, both selenate and selenite produced positive Se contents of the ruminal feed particles and protozoa. Bacterial Se increased ($p < 0.05$) on the first three hours postprandially in Se supplemented diets. Gross ruminal fluid fraction, although there was improvement on their Se content under the supplemented diets, the changes were insignificant over the control. Free inorganic Se and Se in soluble protein of the ruminal fluid were not significantly different for selenate and selenite. Most of the Se in the ruminal fluids of the animals under supplementation were insoluble, indicating the influence of rumen environments on Se bioavailability.

(Key Words: Selenium, Wethers, Se Balance, Ruminal Se, Bacterial Se)

Introduction

The two most common supplemental forms of Se for ruminants are sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3). Comparing these two inorganic sources of Se, the former has higher relative bioavailability value (133 vs 100) using tissue uptake by wethers (Henry et al., 1988). However, the recent study of Podoll et al. (1992) showed the small advantage of selenate over selenite through Se serum concentration in lactating dairy cow but not in sheep. Moreover, nonruminant like poults with Se intake of 0.20 mg/kg dietary DM fed with selenate significantly increased the concentration of Se in plasma and whole blood but not poults fed with selenite (Cantor and Tarino, 1982).

Selenium in ruminant diets is not solely for the animal per se but also for rumen bacteria capable of metabolizing part of it. Both *in vivo* and *in vitro* studies showed the incorporation of Se in bacterial protein (Hidiroglou et al., 1968; Paulsen et al., 1968; Whanger et al., 1978) which contribute in the conversion of dietary Se to unavailable forms (Durand and Kawashima, 1980) and its eventual loss in the feces by adsorption to indigestible particles or as insoluble elemental Se or selenides (Podoll et al., 1992).

Low concentrations of soluble trace elements are found in the supernatant fraction of rumen fluid due to the formation of insoluble complexes and accumulation of protein (Durand and Kawashima, 1980). The chemical form apparently affects this since organic form, selenomethionine, is efficiently retained in the animal's body than the inorganic form, selenate or selenite (Ullrey et al., 1977). These two inorganic forms of Se have inconsistent results when compared in terms of better bioavailability. Thus, this study was conducted to evaluate their bioavailability through

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the digestion balance technique in Japanese Corriedale wethers and to assess the Se content of the ruminal fluid fractions including the bacteria present in it.

Materials and Methods

Animals and diets.

Three Japanese Corriedale wethers with an average body weight of 47 kg were used in this study. They were placed individually on metabolism cage and kept indoors throughout the study period. Each animal was fitted with smal' cannula in the dorsal sac of their rumen.

The three dietary treatments were: (1) without Se supplementation (control); (2) with Se supplementation as sodium selenate; and (3) with Se supplementation as sodium selenite. The inorganic Se had 97 and 90% purity for sodium selenate and sodium selenite, respectively and obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 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% crude protein, 10.76% ash, 60.23% neutral detergent fiber, 38.91% acid detergent fiber, 39.77% neutral detergent solubles and 31.74 ppb Se] fed to the animals at a rate of 2% of body weight/day. This could meet the nutrient requirements for maintenance of wethers (NRC, 1975). The animals were given *ad libitum* access to water and mineralized salt block without Se. The pelleted wheat flour-Se mix was fed only in the morning before the hay was given. Daily feeding schedule for hay was set at 09:00 and 17:00 h.

Experimental design

The experimental design of this study was 3 × 3 Latin square. The dietary adjustment period was 7 d duration followed by 5 d total collection period of urine and feces. However, the animals were allowed to rest for 11 d before undergoing another set of preliminary and collection period.

Sample collection

Feed intake was recorded daily (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 analysis later. Urine sample was collected daily and preserved at −20℃ freezer, also for analysis later. 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 through four layers of gauze cloth. It was frozen at −20℃ and later analyzed.

Laboratory analysis

Diet and feces samples were analyzed for dry matter and Se contents. The same was done for the urine samples for its Se contents. The different ruminal fluids were partitioned and analyzed for Se content. Each rumen fluid was centrifuged at 2,000 × g for 20 min, to separate feed particles and protozoa (1). The resulting supernatant (A) was transferred to another centrifuge tube and centrifuged at 24,000 × g for 20 min to obtain a bacteria-rich precipitate (2). The supernatant (B) from that second centrifugation was again saved and 5 ml was transferred to centrifuge tube. Two ml of 5% trichloracetic acid was added and centrifuged to 3,000 × g for 20 min. The resulting supernatant (C) and precipitate (3) were also saved for later analysis.

The supernatant (A) was used to determine the total Se in the ruminal fluid while the precipitate (1) was used to determine the Se in feed particles and protozoa in DM basis. The precipitate (2) was washed in an acid solution (pH 2.8 to 3.0), to estimate the strength of Se binding to microorganisms (Durand and Kawashima, 1980) and later oven-dried before analysis. The supernatant (C) and precipitate (3) was used to determine the free inorganic Se and Se in soluble protein, respectively. All the samples were weighed before these were analyzed.

All the samples for Se analysis were digested with nitric and perchloric acids. The fluorometric determination of Se by Watkinson (1966) with 2, 3-diaminonaphthalene (Aldrich Chemical Company, Inc., Milwaukee, WI) was followed. The fluorescence spectrophotometer used was Hitachi 204 (Hitachi Ltd., Tokyo, Japan) with the conditions of 377 nm excitation and 520 nm emission.

Statistical analysis

Data were analyzed by 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 significance difference. Where appropriate, student's t-test was also used to compare means.

Results

Selenium balance

Selenium intake, apparent absorption and retention in Japanese Corriedale wethers are shown in table 1. Obviously, higher daily Se intake ($p < 0.05$) was found in the animals under supplementation than those animals without. The difference was 200 μg. Also, Se absorption and its relation to intake were higher ($p < 0.05$) for those animals under supplementation than those animals under control. Moreover, retention was higher in the supplemented diets ($p < 0.05$) but in relation to intake did not differ significantly among treatment means. Overall data gathered showed a similar digestion balance of selenate and selenite in sheep.

TABLE 1. SELENIUM BALANCE ($\bar{X} \pm SD$) IN WETHERS AS INFLUENCED BY INORGANIC SOURCES[1]

Item	Control	Selenate	Selenite
Se balance (μg/day)			
Intake	30.09 ± 0.44[a]	230.09 ± 0.44[b]	230.07 ± 0.41[b]
Fecal	9.79 ± 3.61[a]	62.30 ± 6.58[b]	59.08 ± 10.35[b]
Urine	8.75 ± 0.99[a]	37.45 ± 18.97[b]	37.60 ± 10.17[b]
Absorbed[2]	20.30 ± 3.89[a]	167.80 ± 6.23[b]	171.01 ± 10.44[b]
Retained[3]	11.56 ± 4.70[a]	130.35 ± 24.48[b]	133.41 ± 7.75[b]
Percentage of Se intake (%)			
Fecal	32.61 ± 12.38	27.07 ± 2.92	25.68 ± 4.50
Urine	29.07 ± 3.38	16.27 ± 8.23	16.34 ± 4.39
Absorbed	67.39 ± 12.38[a]	72.93 ± 2.82[b]	74.32 ± 4.51[b]
Retained	38.32 ± 15.43	56.66 ± 10.69	57.98 ± 3.56
Percentage of Se absorption (%)			
Retained	55.15 ± 14.07[a]	77.43 ± 11.85[b]	78.14 ± 5.04[b]

[1] Figures in each row having the same superscript are not significantly different ($p > 0.05$).
[2] Se absorbed (apparent absorption) = Se intake − fecal Se.
[3] Se retained = Se absorbed − urinary Se.

Selenium content of ruminal feed particles and microorganisms

Table 2 presents the selenium content of ruminal feed particles and microorganisms at different time interval postprandially. Although the different treatment means failed to show statistical difference, inorganic Se both selenate and selenite produced positive Se contents of the ruminal feed particles and protozoa. Comparison of the two inorganic Se sources showed selenite had readily dissolved, reduced or metabolized in the rumen than selenate although difference was insignificant.

Bacterial Se increased significantly ($p < 0.05$) on the first three hours postprandially in Se supplemented diets, indicating the rapid incorporation of dietary Se to bacterial protein. Due to its chemical form, Se as selenite was right away metabolized by bacteria after feeding but as time progressed both had been incorporated to bacterial cell equally.

Selenium contents of ruminal fluid and its different fractions

The Se contents of ruminal fluid and its different fractions are presented in table 3. Selc-

TABLE 2. SELENIUM CONTENT ($\bar{X}$ ± SD) OF RUMINAL FEED PARTICLES AND MICROBES POSTPRANDIALLY (μg / kg DM)[1]

Hour	Control	Selenate	Selenite
	..... Feed Particles and Protozoa[2]		
0	0.01 ± 0.02	0.21 ± 0.03	0.15 ± 0.06
1	0.00	0.16 ± 0.06	0.12 ± 0.05
3	−0.03 ± 0.03	0.14 ± 0.03	0.08 + 0.05
5	−0.06 ± 0.03	0.14 ± 0.03	0.07 ± 0.04
7	−0.06 ± 0.02	0.14 ± 0.02	0.06 ± 0.01
Mean	−0.03 + 0.03	0.16 ± 0.04	0.12 ± 0.12
	..... Bacteria[3]		
0	64.86 ± 16.78[a]	178.83 ± 58.87[ab]	248.73 ± 22.37[b]
1	69.85 ± 9.47[a]	238.56 ± 36.75[b]	218.41 + 28.29[b]
3	65.86 ± 5.81[a]	221.67 ± 87.34[b]	171.21 ± 66.76[ab]
5	66.95 ± 19.45	147.89 ± 10.69	177.66 ± 75.88
7	68.35 ± 5.75	131.78 ± 16.08	175.63 ± 86.57
Mean	67.17 ± 10.58	184.36 + 57.11	198.33 ± 56.03

[1] Figures in each row having the same superscript are not statistically different ($p > 0.05$).
[2] Analyzed from the precipitate of ruminal fluid after low centrifugation (2,000 × g).
[3] Analyzed from the precipitate of ruminal fluid after high centrifugation (24,000 × g); washed with acid (pH 2.8-3.0).

nium contents of the ruminal fluid of those in control had lower mean values. Selenite produced higher Se value of the ruminal fluid than selenate. However, their difference was statistically insignificant.

Free inorganic Se and Se in soluble protein were slightly higher in selenite supplemented diets than in selenate supplemented diets. However, their difference was not significant.

Fractionation of Se in ruminal fluid is shown in figure 1. Selenium concentration in the ruminal fluid due to selenite supplementation was higher but not statistically different from the concentration of the same due to selenate supplementation. Also, most of the Se in the ruminal fluid was in insoluble forms (bacterial Se, Se attached to bacterial cell and insoluble compounds).

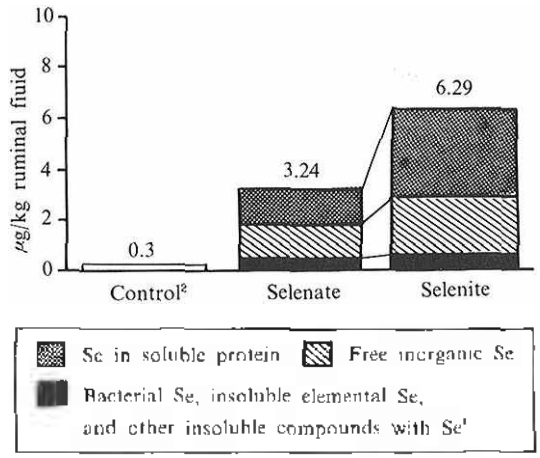

Figure 1. Fractionation of Se in the ruminal fluid.
[1]Insoluble Se = Gross Se of ruminal fluid - (Se insoluble protein + free inorganic Se)
[2]Not fractionated due to extreme low Se content

Discussion

Selenium balance technique used in this study showed equal apparent absorption and retention of selenate and selenite in wethers when supplemented at 0.2 mg/kg dietary DM. Although selenite in its chemical form is easily reduced or metabolized, the two Se sources were subjected to the very complex rumen's environment which resulted in almost equal metabolism. This conforms through their almost the same serum Se concentration when supplemented at 0.3 mg of

TABLE 3. SELENIUM CONTENT ($\bar{X} \pm SD$) OF DIFFERENT RUMINAL FLUID FRACTIONS POSTPRANDIALLY (μg / kg ruminal fluid)[1]

Hour	Control[2]	Selenate	Selenite
		Gross Ruminal Se[3]	
0	0.22 ± 0.22	3.06 ± 1.33	5.91 ± 3.29
1	0.40 ± 0.11	4.00 ± 1.17	6.52 ± 3.15
3	0.38 ± 0.15	3.20 ± 0.79	7.14 ± 2.59
5	0.34 ± 0.16	3.10 ± 1.24	6.85 ± 2.80
7	0.22 ± 0.20	2.86 ± 1.26	5.05 ± 2.88
Mean	0.30 ± 0.17	3.24 ± 1.04	6.29 ± 2.52
		Free Inorganic Se[4]	
0		1.12 ± 0.48	2.09 ± 0.84
1		1.44 ± 0.35	2.26 ± 0.68
3		1.49 ± 0.43	2.66 ± 0.37
5		1.25 ± 0.67	2.20 ± 0.52
7		1.27 ± 0.73	1.62 ± 0.51
Mean		1.31 ± 0.47	2.17 ± 0.57
		Se in Soluble Protein[4]	
0		0.49 ± 0.00	0.63 ± 0.27
1		0.56 ± 0.27	0.59 ± 0.21
3		0.49 ± 0.17	0.63 ± 0.13
5		0.39 ± 0.11	0.62 ± 0.12
7		0.45 ± 0.11	0.60 ± 0.30
Mean		0.48 ± 0.12	0.62 ± 0.15

[1] Figures in each row are not statistically different ($p > 0.05$).

[2] No data for free inorganic Se and Se in soluble protein because of there extreme low concentration in the ruminal fluid.

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

[4] 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.

Se/kg dietary DM to wethers (Podoll et al., 1992). Higher supplementation to wethers (6 mg/kg dietary DM), however, showed that selenate gave higher bioavailability than selenite (Henry et al., 1988). The amount of Se in the diet, composition of the diet, species involved are only some factors that can influence the relative bioavailability of selenate and selenite. Nevertheless, at normal recommended level in the sheep's diet, both have the same bioavailability.

The level of dietary Se influences its absorption and retention. Absorption, according to Behne (1989), does not appear to be homeostatically controlled. The amount of Se absorbed (apparent) and retained increased as dietary Se intake increased (Harison and Conrad, 1984).

Selenium loss via expired air through the lungs as dimethyl selenide was not measured in this present study. Selenium excretion in that pathway is a mechanism to achieve homeostasis during the period of toxicity (Hansard, 1983). The data of Handreck and Godwin (1970) showed that Se intake of 0.5 to 1.3 mg/d resulted in 1% Se loss through expired air. In the present study, the wethers consumed an average of 0.23 mg Se/d under the supplemented diets. Thus, volatile Se is not a substantial factor in the Se balance.

The higher data gathered on Se absorption

and retention in the present study compared to the observation of Harison and Conrad (1984) could be attributed to the type of diet used. Only timothy hay without concentrate was used and this type of diet had a low reduction capacity of the ruminal environments. Thus, the ingested selenate or selenite (oxidized Se) was converted into minimum, reduced and unavailable state. As presented by Gerloff (1992), high concentrate diets are expected to promote a lower pH and greater reducing capacity in the rumen, thereby decreasing the efficiency of Se absorption.

The Se contents of the ruminal fluid and its different fractions were greatly influenced by the rumen environments. Upon introduction to the rumen compartment, Se was metabolized by the bacteria. An increase of 2 to 4 fold bacterial Se was obtained in Se supplemented diets but not in the control. It was also the main bulk (bacterial Se and other insoluble elemental Se and Se compounds) of Se in the ruminal fluid. Earlier reports indicated that ruminal bacteria are capable of metabolizing inorganic Se (Hidiroglou et al., 1968; Paulsen et al., 1968; Whanger et al., 1978). The degree of incorporation is very fast with 30 % of the ruminal liquid constituting ^{75}Selenium acitivity which was found bound to bacterial protein in just one hour of administration (Hidiroglou et al., 1968). Possibly, this was the reason for the rapid dissapearance of plant Se in the ruminal feed particle of the control diet aggravated by its low dietary level. Whether bacterial Se could be metabolized by the host animal or not needs to be verified. Earlier report (Church et al., 1971) indicated that ruminant animal is contributory to the loss of Se in Se biological cycle due to the action of rumen microorganisms.

The influence of selenite to the Se concentration of the ruminal fluid was much faster than selenate. However, as time progressed both showed almost equal values in their ruminal fractions. This supported the findings of Paulsen et al. (1968) that selenate is reduced to selenite in their different fractionation studies when ^{75}Selenium added as selenate and selenite produced almost identically after three-hour incubation period.

Selenium in soluble protein found in this study was only 10 and 15% of the Se content of the ruminal fluid as influenced by selenate and selenite supplementation, respectively. Incorporation of inorganic Se to protein was possibly low due to low level of crude protein in the diet which was only at the wether's maintenance level.

Synthesis of selenoamino acid from inorganic Se is discussed (Favier, 1989). Sulfur containing amino acids must be present to accomplish this. Evidence suggests that inorganic Se is incorporated exclusively into the selenoproteins by a Se substitution for a sulfur atom in the cysteine residue of the protein (Gerloff, 1992).

Based on the results which supported previous studies, equal bioavailability of selenate and selenite could be attained in wethers given at normal recommended level. Rumen environments including its microorganisms affected the bioavailability value of the two Se sources to ruminants.

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