INFLUENCE OF DIETARY PROTEIN ON THE APPARENT ABSORPTION AND RETENTION OF SELENIUM IN SHEEP

A. B. Serra[1], S. D. Serra and T. Fujihara[2]

Faculty of Life and Environmental Science (formerly Faculty of Agriculture), Shimane University, Matsue-shi, Shimane, 690 Japan

Summary

Selenium (Se) apparent absorption and retention in sheep as influenced by diets differing in protein content through soybean meal supplementation was 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. In each period, 7 d dietary adjustment was followed by 5 d total collection of urine and feces. 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 dietary DM intake of the animals was 2% of their body weight. No significant differences were obtained among the three dietary treatments on the Se balance of the animals. However, as percent of Se intake, only urinary Se concentration of Diet 3 was markedly lower ($p < 0.05$) than the other diets. Fecal Se as percent of Se intake followed the trend of Diet 3 > Diet 2 > Diet 1 resulting a Se absorbed as percent of Se intake of 58.9%, 62.3% and 68.2% for Diets 3, 2 and 1, respectively but their differences among each other were insignificant. No significant differences that were observed either on Se retained as percent of intake (Diet 1, 48.2%; Diet 2, 45.2%; Diet 3, 46.0%) or Se retained as percent of Se absorbed (Diet 1, 70.7%; Diet 2, 72.4%; Diet 3, 77.9%). Significant correlation coefficients among the various measures of Se utilization were also observed. Regression analysis showed the following equation: $Y = 93.8 - 1.86X$ ($p < 0.05$, $r^2 = 0.48$), where Y is the Se absorbed as percent of Se intake (%) and X is the dietary protein content (%). This study concludes that Se requirement in sheep is greater when dietary protein content is high.
(**Key Words** : Selenium, Selenite, Selenium Absorption, Selenium Retention, Dietary Protein, Sheep)

Introduction

The selenium (Se) compounds in seeds or forages consumed by livestock are selenocystine, selenocysteine, selenomethionine and Se-methylseleno-methionine (NRC, 1983). If the dietary Se concentrations are low, supplementation is necessary to ensure optimum animal performance. The most common Se supplement being used is in inorganic form of either sodium selenite or sodium selenate. However, their absorption in ruminants is lesser than those monogastric animals due to the factors in the rumen. The rumen provides a very strong reducing environment which can convert ingested Se into a reduced and insoluble unavailable form, such as selenide or elemental Se (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Lopez et al., 1969; Hudman and Glenn, 1984).

Other factors affecting Se bioavailability are the following: the chemical form of ingested Se, the previous Se status of the animal, and the amounts interfering or enhancing factors in the diet including vitamin E, sulfur, proteins, amino acids, copper, mercury, arsenic and cadnium (NRC, 1983). The chemical form affects the bioavailability of Se because the organic form is more efficiently retained in the animal's body than the inorganic form (Ullrey et al., 1977). Level of supplementation and protein in the diet can possibly increase or decrease Se bioavailability from an inorganic Se. Thus, this study was designed to determine the effects of diets of differing protein content through soybean meal supplementation on

[1] On-leave from Central Luzon State University, Nueva Ecija, 3120 Philippines.

[2] Address reprint requests to Dr. T. Fujihara, Faculty of Life and Environmental Science, Shimane University, Matsue-shi, 690 Japan.

Received November 7, 1995
Accepted May 7, 1996

Se absorption and retention in sheep.

Materials and Methods

Animals and diets

Three Japanese Corriedale wethers with an average body weight of 45 kg were used in this study. They were placed individually on metabolism cage and kept indoors through out the study period. Each animal was fitted with small cannula in the dorsal sac of their rumen.

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). The composition of the diets are shown in table 1 whereas the nutrient contents of its main components (hay, wheat bran and soybean meal) are presented in table 2. The nutrient requirements of wethers (NRC, 1975) was met by ensuring DM intakes of 2% of their body weight.

TABLE 1. COMPOSITION AND ANALYSIS OF THE EXPERIMENTAL DIETS (PERCENT OF DRY MATTER[1])

Item	Diet 1	Diet 2	Diet 3
Composition, % of DM			
Hay	89.0	79.0	69.0
Wheat bran	10.0	10.0	10.0
Soybean meal	0	10.0	20.0
Sodium chloride	0.2	0.2	0.2
Calcium carbonate	0.2	0.2	0.2
Sodium phosphate	0.6	0.6	0.6
Analysis, % of DM			
Crude protein[2]	14.0	16.5	19.0
Total digestible nutrients[3]	52.0	54.0	57.0
Calcium[3]	0.44	0.44	0.44
Phosphorus[3]	0.49	0.49	0.49

[1] Selenium was provided as sodium selenite at 0.2 mg Se/kg dietary DM to all diets.
[2] Actual analysis.
[3] Calculated analysis.

All the diets had a Se supplementation as sodium selenite at 0.2 mg Se/kg dietary DM. The Se was mixed in a carrier, (0.2 g Se/kg wheat flour). The Se supplement and concentrate mixture were fed only in the morning before the hay was given. The hay was fed at 09:00 and 17:00 h.

Experimental design

The experimental design of this study was 3 × 3 Latin square (three dietary treatments, three wethers and three experimental periods). The dietary adjustment period was 7 d followed by 5 d total collection period of urine and feces. The animals were allowed to rest for 14 d between experimental period.

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℃ 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℃) before analysis.

Laboratory analyses and calculations

The three main feed ingredients (hay, wheat bran and soybean meal) were analyzed for their DM contents, proximate composition according to AOAC (1984) and Se content. Fecal samples were analyzed for their DM and Se content whereas the urine samples were analyzed for their Se content. All the samples for Se analysis were wet ashed with nitric and perchloric acids (3:1 v/v). The fluorometric determination of Se by Watkinson (1966) was followed and the fluorescence spectrophotometer used was Hitachi 204 (Hitachi Ltd., Tokyo, Japan). The conditions of the spectrophotometer were presented earlier (Serra et al., 1994).

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. Correlation and regression analysis were also done among the measures related to Se absorption and retention.

Results

Selenium balance

Selenium intake, apparent absorption and retention in Japanese Corriedale wethers as affected by dietary protein content through soybean supplementation are shown in table 3. No marked differences on Se intake were noted due to similar Se supplementation received by the animals. The Se contribution of the diets (hay, wheat bran and soybean meal) was considered to be minimal as their Se content was very low (table 2). This was classified as Se-deficient feed ingredients (< 0.1 mg/kg DM, McDowell, 1985).

TABLE 2. PROXIMATE ANALYSIS AND SELENIUM CONTENTS OF HAY, WHEAT BRAN AND SOYBEAN MEAL

Item	Hay	Wheat bran	Soybean meal
	······	% of DM	······
DM	94.5	92.2	95.8
Ash	9.3	5.2	6.9
Crude protein	13.5	17.8	40.9
Ether extract	2.2	5.4	0.9
Crude fiber	33.3	11.3	8.6
Nitrogen-free extract	41.6	60.4	42.7
	······	μg/kg DM	······
Se	40.0	12.1	3.8

TABLE 3. SELENIUM INTAKE, APPARENT ABSORPTION AND RETENTION IN SHEEP FED DIETS OF VARYING CRUDE PROTEIN CONTENT

Item	Diet 1	Diet 2	Diet 3
Crude protein (%)	14.0	16.5	19.0
Selenium balance (μg/d)			
Intake	186.2±2.67	172.8±31.18	191.7±36.52
Fecal	59.2±1.19	64.0± 8.75	78.8±15.41
Urine	37.1±1.66	30.4± 7.80	24.9± 4.90
Absorbed[2]	127.0±3.86	108.8±23.91	112.9±23.30
Retained[3]	89.9±4.62	78.4±17.53	88.0±18.59
Percentage of Se Intake (%)			
Fecal	31.8±1.07	37.0± 2.92	41.1 ± 3.59
Urine	20.0±1.02[a]	17.6± 2.32[a]	13.0± 0.09[b]
Absorbed	68.2±1.07	63.0± 2.92	58.9± 3.59
Retained	48.3±1.83	45.4± 4.03	45.9± 3.54
Percentage of Se Absorption (%)			
Retained	70.8±1.78	72.1± 4.35	77.9± 1.32

[1] Diet 1, 0% soybean meal; Diet 2, 10% soybean meal; Diet 3, 20% soybean meal. Values are means ± SEM, n = 3 sheep/treatment. Figures within a row with different superscripts differ ($p < 0.05$).

[2] Se absorbed (apparent absorption) = Se intake − fecal Se.

[3] Se retained = Se absorbed − urinary Se.

The daily excretion of Se in feces and urine were insignificant among the three dietary treatments. However, the trend of fecal Se output was Diet 3 > Diet 2 > Diet 1 and the reverse was observed in urinary Se. Insignificant results were also obtained on apparent Se absorption and retention among the three dietary treatments. When fecal Se was expressed as a percent of Se intake, the trend was Diet 3 > Diet 2 > Diet 1, however, their differences were insignificant. Only urinary Se expressed as a percent of Se intake showed significant differences among the three dietary treatments, Diet 3 was lower ($p < 0.05$) when compared diets 1 and 2. No differences were found on Se absorption expressed as percent of Se intake on the three dietary treatments but the trend was Diet 1 > Diet 2 > Diet 3. The same were observed on Se retention expressed as a percent of intake with insignificant differences among the three dietary treatments were obtained and the trend was Diet 1 > Diet 2 and 3. The dietary treatments had no significant differences on Se retention expressed as a percent of Se absorption although the trend was highest in Diet 3 followed by Diet 2 and Diet 1.

Correlation and regression analysis of measures

Table 4 shows the correlation coefficients on the different measures describing the Se utilization in sheep. Selenium intake was significantly correlated to Se in the feces ($p < 0.01$), Se in the urine ($p < 0.05$), Se apparent absorption ($p < 0.01$) and Se retention ($p < 0.01$). A significant correlation was found between Se in the urine and Se absorption ($p < 0.01$). While negative correlation was obtained between Se in the urine and percent Se retention based on absorption ($p < 0.05$). Selenium absorption was also correlated to Se retention ($p < 0.01$). Selenium absorption as percent of intake was correlated to Se retention as percent of intake ($p < 0.05$). The dietary protein content (%) of the diet (X) was related to Se

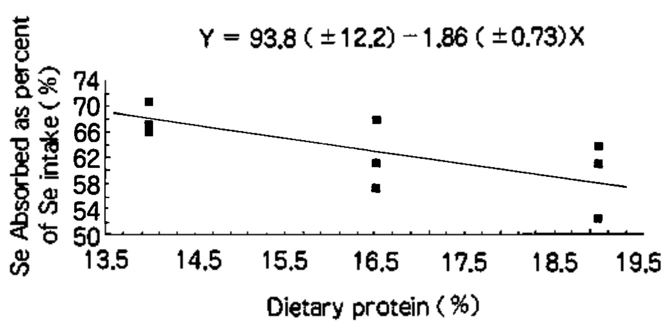

Figure 1. Regression of Se absorption (% intake) [Y] and dietary protein content (%) [X] ($p < 0.05$; $r^2 = 0.48$).

TABLE 4. CORRELATION COEFFICIENTS AMONG VARIOUS MEASURES OF SELENIUM UTILIZATION

Concept	Se intake	Se in feces	Se in urine	Se absorption	Se retention	Percentage Se absorption based on intake	Percentage Se retention based on intake
Percentage Se retention based on absorption	−0.10	−0.02	−0.67*	−0.12	0.12	−0.09	0.57
Percentage Se retention based on intake	0.06	−0.40	−0.03	0.32	0.44	0.77*	
Percentage Se absorption based on intake	0.16	−0.46	0.49	0.50	0.45		
Se retention	0.92**	0.56	0.64	0.97**			
Se absorption	0.93**	0.53	0.81**				
Se in urine	0.72*	0.33					
Se in feces	0.80**						

* $P < 0.05$.
** $P < 0.01$.

absorption expressed as percent Se intake (Y) by the regression equation $Y = 93.8 - 1.86X$; $p < 0.05$; $r^2 = 0.48$ (figure 1). The relationship between the dietary protein content and Se retention as a percent of Se absorption was not significant.

Discussion

Earlier reports identified feces as the main route of Se excretion in ruminants when this element is given orally (Cousins and Cairney, 1961; Paulson et al., 1966; Lopez et al., 1969; Langlands et al., 1986). This means it is highly correlated to Se intake (Harrison and Conrad, 1984) as also shown in the present study ($r = 0.80$, $p < 0.01$; table 4). Excretion of Se in the feces tended to be increased by increasing the dietary protein content as shown in our present study or increasing the intake of organic matter (Langlands et al., 1986). One possible reason is the conversion of dietary Se into a more non-available form in the rumen as dietary protein content increases. Gerloff (1992) suggested that high concentrate diets might be expected to promote a lower pH and greater reducing capacity in the rumen which could be hypothesized to increase the reduction of dietary Se into elemental form. Also, ruminal microbes are capable of reducing Se salts such as sodium selenate and sodium selenite to red elemental Se which in this form may not be utilized by the host animal (Cousins and Cairney, 1961; Peterson and Spedding, 1963; Lopez et al., 1969; Hudman and Glenn, 1984). Moreover, ruminal microbes can incorporate dietary Se into their cells (Hidiroglou et al., 1968; Paulson et al., 1968; Hudman and Glenn, 1984) but the mechanism of their uptake or incorporation into their cells is unknown (Heider and Böck, 1993). Incorporation of dietary Se into bacterial cells is doubled upon its introduction into the rumen (Serra et al., 1994) however there is no information states whether the Se attached to bacterial cells are available to the host animal or not (Church et al., 1971; Durand and Kawashima, 1980). If they are not metabolized, they would pass into the feces, thus increasing the excretion of fecal Se.

Urinary Se is also a major route of excretion in ruminants when this element is given orally (NRC, 1983). In monogastric animals, it is the main route of Se excretion (NRC, 1983). In ruminants, urinary Se expressed as a percent of intake is inversely related to the dietary protein level and this suggests that the absorbed Se was retained effectively in this study ($r = 0.77$, $p < 0.05$; table 4) by increasing the dietary protein level. It could be postulated that the absorbed Se was incorporated into amino acids as selenoproteins by a Se substitution for a sulfur atom in the cysteine residue of the protein. Cousins and Cairney (1961) observed that when supplementing inorganic Se to sheep retained this element by the tissues, particularly by the kidney. The Se is initially protein bound followed by a considerable period over which this protein-incorporated Se is lost. The incorporation of inorganic Se in protein is to be specific to amino acid cysteine (Burk, 1991). However, Hudman and Glenn (1984) observed the incorporation of ^{75}[Se]-selenite into selenocystine, selenoethionine, selenohomocysteine and selenomethionine. Selenium is excreted in urine as trimethyl selenide (NRC, 1983).

The Se absorbed as percent of Se intake as presented in the previous study (Serra et al., 1994), is much higher than what was by Harrison and Conrad (1984). The reason behind this is the type of diet. Only hay was given which possibly resulted in weaker reduction capacity of the rumen in converting the dietary Se into unavailable form. When soybean meal was supplemented to increase dietary protein level (present study), the Se absorbed as percent of intake was lower by 6.5 to 20.6% as dietary protein increased from 14 to 19.5% CP compared to the previous findings (hay alone, Serra et al., 1994). High concentrate diets like those dairy cattle feeds resulted in much lower Se absorbed as percent of Se intake, from 17 to 50% (Harrison and Conrad, 1984). Therefore, increasing dietary protein content (X) has a negative effect on Se absorbed as percent intake (Y) in sheep as illustrated in figure 1.

Selenium retention as percent of Se intake is very low in dairy cattle when fed with various levels and types of concentrate mixtures as Harrison and Conrad (1984) reported Se retention as low as 2% and as high as 41%. Selenium intake and Se retention as percent of Se intake were not significantly correlated as in the present study. Selenium retention as percent of Se intake (45.2-48.2%) was similar for all the dietary treatments regardless of the level of dietary protein.

The findings of the present study agreed with other workers showing that Se requirements in sheep are higher when protein-rich legumes are fed (Whanger et al., 1977), and when intake is high (Gerloff, 1992) especially high intakes of organic matter (Langlands et al., 1986). The present study implies that ruminants fed high protein diets will have higher Se requirement to compensate the loss of dietary Se in feces and urine.

Conclusion

Although insignificant differences were observed in Se balance in diets of increasing dietary protein content, Se absorption tended to decrease as dietary protein content increased. Thus, Se requirement in sheep could be greater when dietary protein content is high.

Acknowledgements

The authors wish to express their apprecation to Ms. M. Horiguchi and Mr. K. Shinchi for their assistance in animal care and laboratory analyses.

Literature Cited

AOAC. 1984. Official Methods Analysis (14th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Burk, R. F. 1991. Molecular biology of selenium with implication for its metabolism. FASEB J. 5:2274.

Church, D. C., S. L. Hansard, J. K. Miller and P. D. Whanger. 1971. The trace elements. In: D. C. Church (Ed.) Digestive Physiology and Nutrition of Ruminants. O & B Books, Inc., Corvallis, OR. pp. 100-156.

Cousins, F. B. and I. M. Cairney. 1961. Some aspects of selenium metabolism in sheep. Aus. J. Agric. Res. 12:927-943.

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.

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

Harrison, J. H. and H. R. Conrad. 1984. Effect of selenium intake on selenium utilization by the nonlactating dairy cow. J. Dairy Sci. 67:219-223.

Heider, J. and A. Böck. 1993. Selenium metabolism in micro-organisms. In: Advances in Microbial Physiology (Vol. 35). Academic Press Ltd., San Diego. pp. 71-109.

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

Hudman, J. F. and A. R. Glenn. 1984. Selenite uptake and incorporation by *Selenomonas ruminantium*. Arch. Microbiol. 140:252-256.

Langlands, J. P., J. E. Bowles, G. E. Donald and A. J. Smith. 1986. Selenium excretion in sheep. Aust. J. Agric. Res. 37:201-209.

Lopez, P. L., R. L. Preston and W. H. Pfander. 1969. Whole-body retention, tissue distribution and excretion of selenium-75 after oral and intravenous administration in lambs fed varying selenium intakes. J. Nutr. 97:123-132.

McDowell, L. R. 1985. Nutrition of Grazing Ruminants in Warm Climates. Academic Press, Inc., San Diego, CA. pp. 165-185.

NRC. 1975. Nutrient Requirements of Domestic Animals, No. 5. Nutrient Requirements of Sheep. Fifth Revised Ed. National Academy of Sciences-National Research Council. Washington, D.C.

NRC. 1983. Selenium in Nutrition. Revised Ed. National Academy of Sciences-National Research Council. Washington, D.C.

Paulson, G. D., C. A. Baumann and A. L. Pope. 1966. Fate of a physiological dose of selenate in the lactating ewe: Effect of sulfate. J. Anim. Sci. 25:1054.

Paulson, G. D., C. A. Baumann and A. L. Pope. 1968. Metabolism of ^{75}Se-selenite, ^{75}Se-selenate, ^{75}Se-selenomethionine and ^{35}S-sulfate by rumen microorganisms *in vitro*. J. Anim. Sci. 27:497-504.

Peterson, P. J. and D. J. Spedding. 1963. The excretion by sheep of ^{75}Selenium incorporated into red clover (*Trifolium pratense* L.): the chemical nature of the excreted selenium and its uptake by three plant species. N. Z. Agric. Res. 6:13-23.

Serra, A. B., K. Nakamura, T. Matsui, T. Harumoto and T. Fujihara. 1994. Inorganic selenium for sheep. I. Selenium balance and selenium levels in the different ruminal fluid fractions. Asian-Australasian J. Anim. Sci. 7:83-89.

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

Ullrey, D. E., P. S. Brady, P. A. Whetter, P. K. Ku and W. T. Magee. 1977. Selenium supplementation of diets for sheep and beef cattle. J. Anim. Sci. 46:559-565.

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, J. A. Schmitz and J. E. Oldfield. 1977. Effects of selenium and vitamin E on blood selenium levels, tissue glutathione peroxidase activities and white muscle disease in sheep fed purified or hay diets. J. Nutr. 107:1298-1307.