Effects of Supplemental Calcium Carbonate on the Distribution of Iron, Copper, Zinc and Manganese in Digestive Tract of Sheep

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Abstract Six sheep were divided into three groups. Each group was given a ration containing respectively 0.1, 0.6 and 1.2% calcium. The dietary calcium level was adjusted by the addition of calcium carbonate. Polyethylene glycol was utilized as a marker. After 67 days feeding, all sheep were sacrificed 2 hours after the morning feeding. Digestive contents were taken from the rumen, omasum, abomasum, upper small intestine, middle small intestine, lower small intestine, cecum and colon. Iron and copper concentrations in dry matter of digesta sampled from the upper part of small intestine decreased with the elevation of dietary calcium. In the sites of rumen, omasum and abomasum, the copper was also lower in sheep given the highest calcium ration than in animals fed with the lowest. The decrease in iron and copper secretion into the digestive tract might be induced by a consecutive high calcium intake. Zinc in the rumen, the abomasum and the upper small intestine tended to be decreased by the addition of calcium carbonate. Manganese in dry matter of digesta taken from the colon had a trend to increase and those in supernatant from the cecum and the colon, on the contrary, lowered with the elevation of dietary calcium. These results suggested that a high calcium intake might result in the changes of zinc and manganese metabolism in ruminants.

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It was found in our previous experiment using sheep that retentions of iron and copper were significantly decreased by the addition of calcium carbonate to rations¹⁾. The changes of absorption or secretion of trace minerals in the gastro-intestinal tract may occur when calcium carbonate is given. Trace mineral concentrations in the digestive tract of ruminant have been investigated by several researchers²⁻⁴⁾. However, few information is available about the effect of dietary calcium levels on trace mineral concentrations in digesta.

This experiment was conducted to study the distribution of trace minerals in the digestive tract as dietary calcium was increased.

Materials and Methods

Six corriedale wethers, weighing about 35 kg, were divided into three groups, two animals each. The each group was given the ration containing respectively 0.1, 0.6

and 1.2% calcium. Dietary calcium levels were obtained by the supplementation of calcium carbonate (laboratory reagent grade) to the basal ration. The dietary phosphorus level was adjusted to 0.60% by the addition of dibasic sodium phosphate (Na_2HPO_4). All sheep were given the same ration as reported in the previous experiment¹⁾, which contained 56% of barley, 24% of wheat bran and 20% of chopped rice straw. The mineral composition of the basal ration was the following; 0.10% of calcium, 0.53% of phosphorus, 0.22% of magnesium, 409 $\mu\text{g/g}$ of iron, 6 $\mu\text{g/g}$ of copper, 54 $\mu\text{g/g}$ of zinc and 142 $\mu\text{g/g}$ of manganese. Vitamin premix was added to furnish 3,000 and 200 I. U. per kilogram feed of vitamin A and D_3 , respectively.

The ration was fed twice a day at a level of 1% of body weight. Water was offered *ad libitum*. After 67 days of feeding, all sheep was slaughtered 2 hours after the morning feeding. Polyethylene glycol 4000 (PEG) was given at a level of 2% of concentrate ration as a marker for 7 days before sacrifice. Digestive contents were collected from the rumen, omasum, abomasum, upper small intestine, middle small intestine, lower small intestine, cecum and colon. After taking the samples, pH in digesta were immediately determined with pH test paper. Supernatant fluid in digesta was obtained after centrifugation for 1 hour at $15,000 \times g$. Corrected mineral concentrations in supernatant fluid were determined by the following calculation method⁹⁾.

Corrected mineral concentration in supernatant fluid (μg nutrient/g feed ingested)

$$= \frac{\text{Mineral concentration in supernate } (\mu\text{g/ml})}{\text{Marker concentration in supernate } (\%)} \times \frac{\text{Marker concentration in feed } (\%) }{\text{Marker concentration in supernate } (\%)}$$

Analytical samples for feed, digesta and supernatant fluid in digesta were prepared by a dry-ashing method. After the process of ashing, mineral contents were extracted by 10 ml of 3N hydrochloric acid solution and 15 ml of purified water on a steam bath. Iron, copper, zinc and manganese concentrations in these samples were determined with an atomic absorption spectrophotometry (Jarrell-Ash, Model AA-1). PEG concentrations in supernatant fluid were determined by the method of SMITH⁹⁾. Data were treated statistically as an arranged factorial design consisting of 3 diets and 7 or 8 sampling locations. After the data were analyzed by the use of variance, significant differences among means were detected by DUNCAN's multiple range test⁶⁾.

Results and Discussion

As shown in table 1, pH values of digesta were lowest in the abomasum and appeared to become higher along with the small intestine. In the sites of abomasum, the pH values tended to increase slightly with the elevation of dietary calcium level. WHEELER and NOLLER⁷⁾ indicated that the addition of limestone increased the fecal pH in dairy cattle given high energy rations.

Dry matter concentrations in the sites of colon and omasum were higher and those in the abomasum and the small intestine were comparatively lower values. The effects of dietary calcium level on dry matter concentrations were not so obvious although the average values in the abomasum became lower slightly and those in the

CaCO₃ Supplementation and Trace-Elements in Digesta

Table 1. Dry matter concentrations and pH values in digesta sampled from various sites of digestive tract.

Dietary calcium levels (%)	Rumen	Omasum	Abomasum	Small intestine			Cecum	Colon	S.E.
				Upper	Middle	Lower			
Dry matter concentration, %									
0.1	16.2	23.2	13.0	12.1	10.0	9.4	16.5	35.7	±0.4
0.6	16.8	22.7	12.2	12.3	11.7	11.8	16.5	32.5	
1.2	15.5	21.3	9.3	11.5	12.3	13.3	18.6	40.4	
Mean	16.2 ^w	22.4 ^x	11.5 ^y	12.0 ^y	11.3 ^y	11.5 ^y	17.2 ^w	36.2 ^z	
Value of pH									
0.1	6.0	5.7	2.4	5.3	7.3	8.5	8.3	—	±0.1
0.6	6.2	5.9	2.9	5.3	7.2	8.5	8.4	—	
1.2	5.9	5.9	3.7	5.6	7.8	8.4	8.1	—	
Mean	6.0 ^w	5.8 ^w	3.0 ^x	5.4 ^w	7.4 ^y	8.5 ^z	8.3 ^z	—	

w, x, y and z: Means in the same line with different superscript letters differ significantly (P<.05).

Table 2. Trace mineral concentrations in dry matter of digesta sampled from the various sites of digestive tract.

Dietary calcium levels (%)	Rumen	Omasum	Abomasum	Small intestine			Cecum	Colon	S.E.
				Upper	Middle	Lower			
Iron, mg/g									
0.1	0.21	0.52	5.28	3.37	0.71	4.98	3.19	0.83	
0.6	0.15	2.37	3.68	1.66	0.63	0.78	0.79	1.02	±0.3
1.2	0.26	0.65	3.57	0.72	0.72	1.20	0.68	1.04	
Mean	0.21	1.18	4.18	1.92	0.69	2.32	1.55	0.96	
Copper, µg/g ¹⁾									
0.1	7.3	9.0	26.6	28.7	3.9	10.4	6.7	12.2	
0.6	5.8	4.5	29.1	22.3	12.1	4.7	8.9	11.3	±0.7
1.2	3.8	3.6	8.9	10.5	6.4	9.6	14.0	13.3	
Mean	5.6 ^x	5.7 ^x	21.5 ^y	20.5 ^y	7.5 ^{xz}	8.2 ^{xz}	9.9 ^{xz}	12.3 ^z	
Zinc, µg/g									
0.1	144	179	159	478	63	148	141	216	
0.6	171	85	209	586	127	65	122	152	±13
1.2	89	219	99	269	103	95	104	158	
Mean	135 ^x	161 ^x	156 ^x	444 ^y	98 ^x	103 ^x	122 ^x	175 ^x	
Manganese, µg/g									
0.1	132	238	423	272	125	165	272	409	
0.6	78	136	168	244	222	341	163	422	±30
1.2	100	104	252	143	267	229	209	849	
Mean	103 ^x	159 ^x	281 ^x	220 ^x	205 ^x	245 ^x	215 ^x	560 ^y	

1): There were significant differences (P<.05) among means of three diet groups; 1.2% < 0.6% and 0.1%. x, y and z: Means in the same line with different superscript letters differ significantly (P<.05).

Table 3. Trace mineral concentrations in supernatant fluid of digesta sampled from the various sites of digestive tract¹⁾ ($\mu\text{g/g}$ feed ingested).

Dietary calcium levels (%)	Rumen	Omasum	Abomasum	Small intestine			Cecum	Colon	S.E.
				Upper	Middle	Lower			
Iron									
0.1	10.7	44.8	5.8	36.3	12.7	25.3	10.3	11.1	
0.6	9.5	105.5	4.5	24.9	11.1	18.3	37.0	13.4	±2.0
1.2	9.2	50.5	5.6	19.0	15.3	12.6	7.5	10.7	
Mean	9.8 ^x	66.9 ^y	5.3 ^x	26.7 ^z	13.0 ^{xz}	18.7 ^{xz}	18.3 ^{xz}	11.7 ^{xz}	
Copper									
0.1	0.5	4.5	0.8	2.6	0.9	0.9	0.8	0.9	
0.6	0.5	6.0	0.7	4.9	1.0	1.2	0.8	1.8	±0.4
1.2	1.6	10.2	0.4	2.8	0.9	0.9	0.4	0.5	
Mean	0.9 ^x	6.9 ^y	0.6 ^x	3.4 ^x	0.9 ^x	1.0 ^x	0.7 ^x	1.1 ^x	
Zinc									
0.1	9.4	16.3	7.5	115.3	9.8	9.1	7.8	8.1	
0.6	3.1	55.6	14.4	70.9	7.0	15.4	16.1	9.6	±3.0
1.2	5.4	18.0	23.9	100.7	15.5	7.1	6.6	14.3	
Mean	6.0 ^x	30.0 ^x	15.3 ^x	95.6 ^y	10.8 ^x	10.5 ^x	10.2 ^x	10.7 ^x	
Manganese									
0.1	9.6	8.0	8.0	14.2	2.7	3.5	4.5	5.8	
0.6	7.0	11.7	8.3	12.6	1.6	1.5	2.7	2.4	±0.5
1.2	8.8	11.8	10.7	8.8	1.3	1.2	0.9	1.0	
Mean	8.5 ^x	10.5 ^x	9.0 ^x	11.9 ^x	1.9 ^y	2.1 ^y	2.7 ^y	3.1 ^y	

1): Mineral concentrations in supernatant fluid were corrected with the marker. x, y and z: Means in the same line with different superscript letters differ significantly ($P < .05$).

lower small intestine became higher with the elevation of dietary calcium level.

Iron and copper concentrations in supernatant fluid were likely to increase in the omasum (Table 3). The digesta sampled from the omasum had a very low water content in comparison with those from the rumen, abomasum and small intestine. OMORI⁸⁾ and PERRY *et al.*⁹⁾ indicated that digesta taken from the omasum of calves contained low water. It seems probable that iron and copper are driven out of solid phase of digesta.

Iron concentrations in digesta and supernatant fluid collected from the upper part of small intestine tended to decrease with the elevation of dietary calcium (Table 2 and 3). It is well established that iron is secreted via bile into the digestive tract^{10, 11)}. Therefore, it seems that net secretion of iron is decreased by the elevation of dietary calcium.

As increasing the dietary calcium, copper concentrations in dry matter of digesta taken from the rumen, omasum, abomasum and upper small intestine appeared to lower (Table 2). It was reported in rats that copper was largely absorbed from the stomach¹²⁾. Recently, IVAN and GRIEVE⁴⁾ indicated that net absorption of copper took place in the stomach region of calves. Although it was not known clearly whether the

decreasing of copper concentrations in these regions resulted from the increase of absorption or the decrease of secretion, the elevation of dietary calcium appeared to reduce copper concentrations in these sites of the digestive tract.

In the trial of mineral balance¹¹, negative retentions of iron and copper were observed in sheep given the highest calcium ration. From the data of the present experiment, causes as to why negative retentions of these minerals were induced were not clarified. However, it is conceivable that the elevation of dietary calcium results in the decrease in body stores of iron and copper, which is followed by the reduction of secretion of these minerals into the digestive tract of sheep.

MILLER and CRAGLE¹⁸) demonstrated that zinc was absorbed from the abomasum and the lower small intestine, and secreted into the upper part of small intestine of calves. The results that zinc concentrations of supernatant fluids elevated in the upper small intestine and lowered in the middle and lower small intestine (Table 3) supported the observations by them. However, no obvious trend was found for the zinc concentration of supernatant fluid in the abomasum.

In the sites of rumen, abomasum and upper and lower small intestine, the zinc concentrations in digesta from sheep given the highest calcium ration tended to be lower than those from animals given the other rations (Table 2). These changes of zinc were similar to the cases of iron and copper of which requirements were enhanced by the elevation of dietary calcium^{14,15}). Although a high dietary calcium level reduces zinc absorption in monogastric animals¹⁶), the effects of dietary calcium on zinc requirement are not established in ruminants.

MILLER *et al.*¹⁷) reported that the digesta from the cecum and colon had high manganese concentrations in calves. IVAN and GRIEVE⁴) also found a considerable net secretion of manganese into the cecum of calves. The supernatant manganese concentrations corrected with the marker appeared to elevate to a little extent in the cecum and colon when the lower calcium rations were given to sheep (Table 3).

The manganese in digesta taken from the colon had a trend to increase and those in supernatant from the cecum and colon, on the contrary, lowered with the elevation of dietary calcium level (Table 2 and 3). SCHAIKLE and BANDEMER¹⁸) showed that the increasing of calcium phosphate concentrations removed ionic manganese from solution through adsorption by the insoluble portion of the compound. VAGG¹⁹) demonstrated using a radioisotope technique that the endogenous loss of manganese was increased by the elevation of dietary calcium in dairy cows.

On the other hand, OGURA²⁰) concluded that feeding with an excessive amount of calcium had no inhibitory effect upon the absorption of manganese in chicks. In the previous balance trial¹), the retention of zinc and manganese were insignificantly affected by the addition of calcium carbonate. Further study, therefore, will be required to determine the effect of calcium carbonate on the metabolism of zinc and manganese in cattle and sheep.

References

- 1) YANO, H., M. NOKATA and R. KAWASHIMA, *Jap. J. Zootech. Sci.*, **49**: 625-631. 1978.
- 2) BREMNER, I., *Br. J. Nutr.*, **24**: 769-783. 1970.
- 3) GRACE, N. D., *Br. J. Nutr.*, **34**: 73-82. 1975.
- 4) IVAN, M. and C. M. GRIEVE, *J. Dairy Sci.*, **59**: 1764-1768. 1976.
- 5) SMITH, R. H., *J. Agric. Sci.*, **52**: 72-78. 1959.
- 6) DUNCAN, D. B., *Biometrics*, **11**: 1-42. 1955.
- 7) WHEELER, W. H. and C. H. NOLLER, *J. Dairy Sci.*, **59**: 1788-1793. 1976.
- 8) OMORI, S., *Bull. Nat. Inst. Anim. Ind.*, **24**: 51-58. 1971.
- 9) PERRY, S. C., R. G. CRAGLE and J. K. MILLER, *J. Nutr.*, **93**: 283-290. 1967.
- 10) THOMAS, J. W., *J. Dairy Sci.*, **53**: 1107-1123. 1970.
- 11) UNDERWOOD, E. J., *Trace Elements in Human and Animal Nutrition*. 3rd ed. 77-78. Academic Press. New York and London. 1971.
- 12) VAN CAMPEN, D. R. and E. A. MITCHELL, *J. Nutr.*, **86**: 120-124. 1965.
- 13) MILLER, J. K. and R. G. CARGLE, *J. Dairy Sci.*, **48**: 370-373. 1965.
- 14) KIRCHGESSNER, M., *World Rev. Anim. Prod.*, **4**: 41-50. 1965.
- 15) O'DELL, B. L., *Annals N.Y. Acad. Sci.*, **199**: 70-81. 1972.
- 16) MILLER, W. J., *J. Dairy Sci.*, **53**: 1123-1135. 1970.
- 17) MILLER, W. J., M. W. NEATHERY, R. P. GENTRY, D. M. BLACKMON, J. W. LASSITER and F. M. PATE, *J. Anim. Sci.*, **34**: 460-464. 1972.
- 18) SCHABBLE, P. J. and S. L. BANDEMER, *Poultry Sci.*, **21**: 8-14. 1942.
- 19) VAGG, M. J., *Mineral Studies with Isotopes in Domestic Animals*. 121-124. International Atomic Energy Agency. Vienna. 1971.
- 20) OGURA, Y., *Nat. Inst. Anim. Hlth Quart.*, **17**: 163-170. 1977.

飼料中への炭酸カルシウム添加がめん羊の消化管内容物中 鉄, 銅, 亜鉛, マンガン濃度に及ぼす影響

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6頭の去勢めん羊を2頭ずつ3区に分けて、飼料中カルシウム含量が0.1, 0.6, 1.2%である飼料をそれぞれ各区のめん羊に給与した。飼料中のカルシウム水準は炭酸カルシウムの添加により調整し、ポリエチレングリコールをマーカーとして用いた。67日間飼養した後、朝の給餌後2時間目に殺した。消化管内容物は第1胃, 第3胃, 第4胃, 小腸上部, 小腸中部, 小腸下部, 盲腸, 結腸から採取した。小腸上部では消化管内容物乾物中の鉄および銅濃度は飼料中カルシウム濃度の増加により低下した。高カルシウム飼料を与えると第1胃, 第3胃, 第4胃での消化管内容物中銅濃度は低くなる傾向にあっ

た。高カルシウム飼料をめん羊に長期間給与すると、消化管内への鉄, 銅の再排泄は低下するものと考えられた。飼料中への炭酸カルシウム添加により、消化管内容物中亜鉛濃度は、第1胃, 第4胃, 小腸上部で低下する傾向にあった。結腸での消化管内容物マンガン濃度は、飼料中カルシウム濃度の増加にともない増加する傾向にあり、逆に盲腸, 結腸内容物の上澄み液中マンガン濃度は低下した。これらの結果から高カルシウム飼料を反すう動物に給与すると、亜鉛, マンガン代謝にも変化を及ぼすように思われた。

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