Effects of Supplemental Calcium Carbonate on the Metabolism of Iron, Copper, Zinc and Manganese in Sheep

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Abstract Nine sheep were divided into three groups, three sheep in each. 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. From the 30th and the 60th day after the beginning of trial, urine, fecal and serum samples were taken for seven days. After a 67-days feeding period, two sheep from each group were sacrificed, and the livers and spleens were examined to determine trace mineral concentrations. The amount of all trace minerals excreted via urine were too little to affect the mineral balances. There was no significant difference in serum mineral concentrations among treatment groups. Fecal excretion of iron and copper significantly increased and retentions of these trace minerals became negative when the highest calcium ration was given. Liver iron and copper concentrations did not consistently alter, but spleen iron and copper showed a decreasing trend as the dietary calcium was increased. It may be possible that iron and copper requirements increase when a high level of calcium carbonate is given to cattle and sheep.

In recent years wheat bran and rice bran, which are rich in phosphorus, have been frequently used for fattening cattle and simultaneously calcium carbonate is added to rations in order to adjust a dietary ratio of calcium to phosphorus. It has been shown in sheep that liver-iron¹⁾ or liver-copper stores²⁾ decreased when dietary calcium was elevated. UNDERWOOD³⁾ described that, in cattle, the availability of copper lowered when there was an increase in the dietary calcium content. It was also reported in monogastric animals that a high calcium ration decreased zinc absorption in the intestine⁴⁻⁷⁾ or induced manganese deficiency⁸⁾.

However, it seems that only a little consideration has been paid to the availability of trace minerals when a high level of calcium carbonate is given to cattle and sheep. This experiment was conducted to study the influence of high dietary calcium ration on the metabolism of iron, copper, zinc and manganese in sheep.

Materials and Methods

Nine corriedale wethers averaging 42 kg were divided, on a weight basis, into three groups, three sheep in each. 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 and mineral

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Table 1. Composition of basal ration and its mineral concentrations.
(air dry matter basis)

Barley	56%	Calcium	0.10%	Iron	409 µg/g
Wheat bran	24	Phosphorus	0.53	Copper	6
Rice straw	20	Magnesium	0.22	Zinc	54
				Manganese	142

contents of the basal ration are shown in Table 1. Chopped rice straw was given as a roughage source.

Sheep were placed in metabolism cages throughout the experimental period. The ration was given twice a day at the level of 1% of the body weight. Water was available at all times. From the 30th and the 60th day after the beginning of trial, urine, fecal and serum samples were taken for seven days. Urine and fecal samples were taken everyday from 24-hour collections during the sampling period. Serum samples were obtained twice in the same period.

After the feeding period, two sheep from each group were sacrificed, and samples of liver and spleen were taken to determine the concentrations of iron, copper, zinc and manganese.

Analytical samples for urine, feces, rations and organs were prepared by ashing with nitric acid and perchloric acid. That is, 15 ml of nitric acid solution and 10 ml of perchloric acid solution were used for ashing a sample. After the process of ashing, mineral components were extracted by 5 ml of hydrochloric acid solution (HCl/H₂O; 1/1) and 20 ml of purified water. Trace mineral concentrations in these samples were analyzed by an atomic absorption spectrophotometry. Dietary phosphorus concentrations were determined by the method of FISKE-SUBBAROW⁹.

A serum sample of 3 ml was mixed with 1.5 ml of 1N hydrochloric acid solution, and then the sample was boiled for 5 minutes. After being added by 1.5 ml of 10% trichloric acid solution, the serum sample was centrifuged at 3000 rpm for 10 minutes. The supernatant was analyzed for trace mineral concentrations.

Results

Table 2 shows the amount of urine excretion of trace minerals. There was no obvious difference in urine excretion of trace minerals among three rations. In the urine excretion at the percentage basis, all trace minerals examined in this experiment were under 1.0%. When compared with iron, copper and zinc, the percentage of dietary manganese excreted in urine appeared to be less.

Fecal excretion of iron significantly increased when the highest calcium ration was fed ($P < .05$). Although no great difference was observed among three rations at one month after the beginning of trial, the amount of copper excretion via feces was larger in the highest calcium ration than that in the lowest calcium ration at two months after ($P < .05$). Fecal manganese excretion in the ration containing 0.6% calcium appeared to be lower than those in the other two rations. Fecal zinc excretion also slightly decreased when the ration containing 0.6% calcium was given.

As shown in Table 4, iron and copper retentions became lower with the increasing of dietary calcium. And significant differences were found in these mineral retentions between the lowest and the highest calcium rations ($P < .05$). Retentions of zinc and manganese showed no obvious tendency though a little increase was found in manganese in the ration of 0.6% calcium.

Adding CaCO₃ on Trace Mineral Metabolism

Table 2. Effects of supplemental calcium carbonate on urine excretion of iron, copper, zinc and manganese.

	Dietary calcium, (%)					
	1 month after			2 months after		
	0.1	0.6	1.2	0.1	0.6	1.2
Urine excretion, mg/day						
Iron	0.41	0.25	0.52	0.41	0.36	0.44
Copper	0.04	0.03	0.05	0.04	0.04	0.04
Zinc	0.20	0.19	0.22	0.26	0.29	0.19
Manganese	0.05	0.04	0.06	0.07	0.08	0.08
% of dietary intake excreted in urine						
Iron	0.12	0.07	0.15	0.12	0.11	0.13
Copper	0.83	0.65	0.98	0.80	0.88	0.80
Zinc	0.41	0.45	0.47	0.56	0.69	0.41
Manganese	0.04	0.04	0.05	0.06	0.07	0.07

Table 3. Effects of supplemental calcium carbonate on fecal excretion of iron, copper, zinc and manganese.

	Dietary calcium, (%)					
	1 month after			2 months after		
	0.1	0.6	1.2	0.1	0.6	1.2
Fecal excretion, mg/day						
Iron	324 ^a	346 ^a	488 ^b	295 ^a	254 ^a	431 ^b
S.D.	±47	±58	±87	±58	±54	±89
Copper	4.7	5.3	5.3	4.7 ^a	5.0 ^{ab}	6.3 ^b
S.D.	±1.0	±1.1	±1.3	±0.9	±1.7	±1.3
Zinc	44	36	43	43	38	45
S.D.	±4	±7	±10	±6	±5	±11
Manganese	102	82	110	106 ^a	81 ^b	99 ^{ab}
S.D.	±21	±7	±43	±6	±7	±16

a and b: Means with different superscript letters differ significantly ($P < .05$).

Table 5 shows serum concentrations of iron, copper and zinc. Among the three rations, no significant difference was found in serum concentrations of iron, copper and zinc. Serum iron and copper tended to be a little higher in sheep given 0.6% calcium ration than in animals given the other rations. Serum manganese concentrations were too low to be determined by the method used in this experiment.

The results of trace mineral concentrations in livers and spleens are presented in Table 6. Liver iron and copper had large individual variations and showed no consistent tendency when dietary calcium levels were changed. Though large variations were also found, spleen iron and copper showed a trend to decrease with an increase in the dietary calcium concentration. Spleen

Table 4. Effects of supplemental calcium carbonate on retentions of iron, copper, zinc and manganese.

	Dietary calcium, (%)					
	1 month after			2 months after		
	0.1	0.6	1.2	0.1	0.6	1.2
Retention, mg/day						
Iron	15 ^a	-23 ^{ab}	-136 ^b	55 ^a	63 ^a	-86 ^b
Copper	0.1	-0.7	-0.2	0.3 ^a	-0.5 ^{ab}	-1.3 ^b
Zinc	1.1	6.2	4.5	3.7	3.6	1.3
Manganese	15.9	29.9	13.4	15.6	29.0	22.0
% of intake						
Iron	4 ^a	-8 ^a	-39 ^b	16 ^a	19 ^a	-25 ^b
Copper	2	-15	-4	7 ^a	-11 ^{ab}	-26 ^b
Zinc	2	14	10	8	8	3
Manganese	14	27	11	13	26	18

a and b: Means with different superscript letters differ significantly ($P < .05$).

Table 5. Effects of supplemental calcium carbonate on serum concentrations of iron, copper and zinc.

	Dietary calcium, (%)					
	1 month after			2 months after		
	0.1	0.6	1.2	0.1	0.6	1.2
Serum values, $\mu\text{g/ml}$						
Iron	2.89	2.99	2.57	2.57	3.28	2.37
S. D.	± 0.74	± 0.86	± 0.65	± 0.61	± 0.50	± 0.16
Copper	0.67	0.94	0.83	0.72	1.05	0.85
S. D.	± 0.06	± 0.28	± 0.19	± 0.13	± 0.30	± 0.18
Zinc	0.59	0.65	0.62	0.70	0.68	0.68
S. D.	± 0.02	± 0.10	± 0.05	± 0.05	± 0.03	± 0.01

iron concentrations in the highest calcium ration were about a half of those in the lowest calcium ration. No obvious difference was found in zinc and manganese concentrations of the livers and spleens among three rations.

Discussion

In the percentage of the dietary intake excreted via urine, all trace minerals were under 1.0%. Therefore, the amount of urine excretion of trace minerals was too little to affect the retentions. It is described in nonruminants and ruminants that high percentage of iron⁽¹⁰⁾, copper⁽¹¹⁻¹³⁾, zinc⁽¹⁴⁻¹⁶⁾ and manganese⁽¹⁷⁻¹⁹⁾ excreted from the body are mainly through feces and very little by way of urine.

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Table 6. Effects of supplemental calcium carbonate on iron, copper, zinc and manganese concentrations of liver and spleen. ($\mu\text{g/g}$, dry matter basis)

Sheep No.	Dietary calcium, (%)					
	0.1		0.6		1.2	
	1	2	3	4	5	6
Iron						
Liver	75	105	100	63	94	81
Spleen	2760	1275	1530	236	535	750
Copper						
Liver	788	262	309	688	180	803
Spleen	2.3	1.8	2.1	2.1	1.4	1.3
Zinc						
Liver	109	81	69	79	69	119
Spleen	112	119	76	96	68	101
Manganese						
Liver	5.0	7.3	5.6	5.9	7.0	5.8
Spleen	3.1	3.0	3.1	—	2.9	3.4

In this experiment, fecal iron excretion became higher, and iron retentions and spleen iron concentrations became lower with the elevation of dietary calcium. It is conceivable from these results that the amount of iron requirement is increased when a high calcium ration is given to ruminants. However, liver iron concentrations were not affected by the administration of calcium carbonate. HUBER and PRICE²⁾ also found a similar result using cattle. Fontenot *et al.*¹⁾ found, to the contrary, that the liver iron level was lower for the highest level of dietary calcium than for the two lower levels in sheep.

There was no substantial difference in serum iron concentrations among three rations and these values were similar to the data shown by KOONG *et al.*²⁰⁾ and HIDIROGLOU and JENKINS²¹⁾. It is well established that serum iron concentrations are decreased in severe cases of iron deficiency³⁾. On the other hand, KAWASHIMA stated in his review on trace minerals²²⁾ that, in general, trace mineral concentrations in blood do not strictly correlated with body stores. In the sheep used in this experiment, iron deficiency might not be so severe as serum iron became lower.

Some studies^{3,23,24)} indicated that the utilization of dietary copper was decreased by the elevation of dietary calcium. In accordance with their observations^{3,23,24)}, copper retentions decreased as calcium carbonate was added to rations.

In an experiment by HUBER and PRICE²⁾, liver copper stores decreased when dietary calcium was elevated. However, FONTENOT *et al.*¹⁾ found that liver copper in sheep was not affected by increased dietary calcium level. Liver copper concentrations in which there were many variations, fell into a normal range³⁾. Furthermore, among three rations, consistent change was not found in liver copper. Liver copper concentrations are useful aids in the diagnosis of copper deficiency²⁵⁾. Therefore, it is conceivable that a severe copper deficiency would not be induced in sheep of this experiment.

Spleen copper concentrations tended to decrease with the increasing of dietary calcium content. The significance of decrease in spleen copper is not obvious, but the copper requirement

of ruminant may be elevated as calcium carbonate is added to rations.

Among three rations, no obvious difference was found in the fecal zinc concentration and zinc concentrations of serum, liver and spleen. From the work of OTT *et al.*²⁶⁾ and MILLS *et al.*²⁷⁾, it appears that phytic acid, in association with high concentrations of calcium in the diet, does not interfere with the utilization of zinc in ruminants. MILLER²⁸⁾, in his description of zinc metabolism, described this situation as a breakdown of phytic acid in the rumen.

It was indicated by THOMAS⁸⁾ that high dietary calcium and phosphorus induced the incidence of manganese deficiency in animals. O'DELL's description²⁹⁾ said that high dietary levels of calcium and phosphorus markedly increased the manganese requirement of growing chickens and the phenomenon was best explained by the increase of manganese adsorption on large surface area produced by calcium phosphate complex in the gut. The increase of dietary calcium did not affect manganese balances and liver and spleen manganese concentrations in this experiment. It may be possible that the dietary phosphorus content was not sufficient to form the complex of calcium, phosphorus and manganese in the gut of sheep.

The results obtained in this experiment suggested that iron and copper requirements would be elevated by the addition of calcium carbonate to rations. But causes as to why calcium carbonate decreases the availability of iron and copper in sheep are not well known. Therefore further studies will be required to clarify these problems.

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飼料中への炭酸カルシウム添加がめん羊の鉄, 銅, 亜鉛, マンガン代謝に及ぼす影響

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平均体重 42 kg の9頭のめん羊を3頭ずつ3区に分けて, 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飼料添加により調整した。供試飼料は濃厚試料を主体とし, 粗飼料としては稲わらを用いた。試験開始後, 30日目と60日目から1週間, 尿, 糞, 血液のサンプルを採取した。その後, 肝臓と脾臓を採取するために各区の3頭のめん羊のうち2頭をと殺した。尿中の重金属含量は非常に少なく, 尿を通しての

排泄は重金属の出納にはほとんど影響を及ぼさないようであった。血清中の重金属濃度は, 各区の間で明らかな違いは見られなかった。飼料中のカルシウム含量が高くなると糞中の鉄と銅排泄量は増加し, 体内保有量は低下した。肝臓中の鉄と銅含量は一定の変化を示さなかったが, 脾臓中の鉄と銅含量は, 炭酸カルシウム添加により低下する傾向であった。以上の結果より高水準の炭酸カルシウムを反すう動物に与えると, 鉄と銅の要求量が増加するものと考えられた。