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# Studies on the Trace Elements in Soil-Plant-Animal System

# IV. Improvement of copper status of cattle resulting from application of copper sulfate to grazing field

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# Summary

The mineral compositions of blood and tissues of cattle were examined on the Cu deficient field with the surface application of either copper sulfate or lime in the Kawatabi farm of Tohoku University. The improvement of Cu status of cattle was observed in the Cu level of blood and tissues with the copper sulfate application treatment, although the daily gains of cattle were not improved by the copper supplementation. The mean Cu concentration in the whole blood of cattle increased with the copper sulfate application, and other mineral concentrations in the blood did not show obvious variation with both treatments. The mineral concentrations in tissues differed among tissues. The accumulation of Cu was observed in liver with the copper sulfate application. At the end of the last grazing, Cu concentration in cattle livers was about 37 times higher with the copper sulfate application than with the lime application treatment. Lime application increased Ca and Fe in spleens, but did not affect the levels of other minerals in tissues. The mineral concentrations in tissues after housing for 4.5 months from the end of grazing was similar to that at the end of last grazing, except that Cu in the liver and Fe in the kidney with the copper sulfate application and Fe and Ca in the spleen with the lime application treatment decreased.

A previous paper (1) showed that the Cu level in herbage was improved by the topdressing of copper sulfate to the grazing field in the Kawatabi farm of Tohoku University. The lime application slightly raised the pH value of surface soil and the Ca concentration in herbage, but did not affect the Cu level in herbage.

Some workers have reported on the improvement of the copper concentration in blood and tissues of ruminants which were supplied Cu compound in Cu deficient regions (2-5).

This study reports on how the application of copper sulfate or lime to the grazing field shows effects on the mineral status of the grazing cattle, namely the mineral concentration in blood and tissues such as brain, liver, kidney, spleen and heart.

#### Materials and Methods

The treatment methods on the applications of copper sulfate and lime to the grazing field and the cattle used were as described in the previous paper (1). Cattle grazing procedures were carried out through the two-season grazing, from 20 April to 4 December in 1974 and 21 April to 14 October in 1975. During the housing phase, the cattle were fed on hay alone which was harvested from neighbouring fields, 1.5 ha each, with treatments similar to grazing fields, copper sulfate or lime application. The Cu concentration in hay was  $11.7 \pm 2.7$  and  $4.9 \pm$ 0.9 ppm and Ca in hay  $0.39 \pm 0.05\%$  and  $0.51 \pm 0.05\%$  with the copper sulfate and lime application treatment, respectively. The mean body weight of the cattle used was  $476.0 \pm 20.8$  kg at the end of the last grazing. The difference between treatments on the mean body weight was not significant, and the daily gains of cattle were not improved by copper supplementation. Blood samples were collected from all of the cattle once every one or two months. Two cattle for copper sulfate application, and one for lime application treatment at the end of last grazing, and one cattle each for treatments after a subsequent housing for 4.5 months were slaughtered, and the tissues such as brain, liver, kidney, spleen and heart were sampled. Tissues were stored as frozen samples until analysis. Whole blood and tissue samples were wet-ashed and the concentrations of Cu, Zn, Fe, Ca and Mg in them were measured by the method as mentioned previously (6).

#### Result and Discussion

## 1. Minerals in blood of cattle

The mean mineral concentrations in whole blood of cattle reared with treatments either of copper sulfate or lime application are exhibited in Table 1. Data were arranged in such order as those in the whole experimental period from April

Table 1. Mineral concentrations in blood of cattle. Whole period (Apr. 1974 ~ Mar. 1976)

Treatment Cu		Cu	Zn	${f Fe}$	Ca	Mg			
Copp.	appl.	$76\pm 7$	$380\pm138$	$34.6 \pm 5.0 \times 10^{3}$	$50.5 \pm 12.8 \times 10^{2}$	$20.2 \pm 1.7 \times 10^{2}$			
$_{ m Lime}$	appl.	$68 \pm 14$	$347\pm 93$	$36.3 \pm 6.2 \times 10^{3}$	$51.5 \pm 11.1 \times 10^{2}$	$19.9\pm1.4 imes10^{2}$			
Grazin	Grazing phase in 1975 (Jun. ~Oct.)								
Copp.	appl.	$83 \pm 5$	$378\pm77$	$35.3 \pm 3.7 \times 10^{3}$	$39.2 \pm 3.4 \times 10^{2}$	$21.2 \pm 0.5 \times 10^{2}$			
Lime	appl.	$48\pm10$	$395 \pm 49$	$35.2 \pm 2.0 \times 10^{3}$	$42.1 \pm 6.1 \times 10^{2}$	$20.1\pm0.9\times10^{2}$			

(µg per dl)

in 1974 to March in 1976 and in the grazing phase of 1975. The mean Cu concentration in the whole period of the experiment was higher with the copper sulfate application than with the lime application. The difference between treatments on Cu concentration was particularly distinguished in the data from the grazing phase of 1975, 83  $\mu$ g/dl for copper sulfate application and 48  $\mu$ g/dl for lime application. The other minerals in blood did not show obvious differences in concentration between treatments, but Ca concentration in the grazing phase of 1975 was lower than that in the whole experimental period,  $39.2-42.1 \times 10^2 \,\mu\mathrm{g/dl}$ in the grazing phase of 1975 and  $50.5-51.5\times10^2 \,\mu\text{g/dl}$  in the whole experimental period. Table 2 shows the time course variation of Cu, Ca and Mg concentrations in blood of cattle from beginning to finish of experiment. The Cu in blood with the copper sulfate application became higher than that with the lime application on and after 30 October, 1974, despite the fact that Cu in herbage with the copper sulfate application had been already higher than that with the lime application treatment since spring of 1974 as shown in the previous report (1). The great improvement of the Cu level of blood with the copper sulfate application treatment was observed in the grazing phase of 1975, and the Cu concentration on 21 August was about 2.3 times the concentration of that with the lime application. The variation of Cu level in the grazing phase of the lime application was similar to that of an untreated field (6). Ca in blood of cattle did not change significantly by the application of lime to the grazing field in spite of an increase of the Ca level in herbage (1). Ca concentration in blood changed similarly on the range from about  $30 \times 10^2$  to  $75 \times 10^2 \,\mu\text{g/dl}$  in both treatments. Mg in blood did not show the remarkable change between treatments through the experimental period and its seasonal change was also small, although it decreased slightly in the housing phase.

## 2. Minerals in tissues of cattle

The mean mineral concentrations in wet tissues of cattle are shown in Table 3 for the end of last grazing and in Table 4 for that after a subsequent housing. At the end of last grazing, the mineral concentrations in tissues differed among tissues, and on minerals except Cu and Ca a serious difference was not observed between treatments. Cu concentrations in tissues were in the following order: liver>kidney>heart>brain>spleen with the copper sulfate application treatment and kidney>heart>brain>liver>spleen with the lime application treatment. Such differences in the order caused by the difference of Cu status of cattle was in agreement with data reported by Bingley and Anderson (2). It is notable that the Cu accumulation in liver was due to the copper sulfate topdressed to the grazing field, and that the topdressing was equally as effective as injection and doses of Cu supplementation for preventing copper deficiency in cattle (2-4). The copper sulfate application (68.13 ppm) resulted in a Cu concentration 37

· · · · · · · · · · · · · · · · · · ·		1974		
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		Apr. 25	Jun. 5	Jul. 31
Cn. (a./dl)	Copp. appl.	95	76	69
$\mathrm{Cu}\;(\mu\mathrm{g}/\mathrm{dl})$	Lime appl.	96	87	70
Co. (a/dl)	Copp. appl.	$44.7 \times 10^{2}$	$76.6 \times 10^{2}$	$51.1 \times 10^{2}$
$Ca (\mu g/dl)$	Lime appl.	$45.0 \times 10^2$	$71.1 \times 10^{2}$	$49.7 \times 10^{2}$
Ma (a/dl)	Copp. appl.	$21.8 \times 10^{2}$	$23.6 imes10^{2}$	$21.7 \times 10^{2}$
$Mg (\mu g/dl)$	Lime appl.	$21.0 \times 10^2$	$22.6\! imes\!10^{2}$	$22.4 \times 10^2$
		1975		
	,	Mar. 26	Apr. 21	Jun. 11
C., ( m/dl)	Copp. appl.	73	75	82
$\mathrm{Cu}\;(\mu\mathrm{g}/\mathrm{dl})$	Lime appl.	69	75	49
Co. (	Copp. appl.	$39.6 \times 10^2$	$41.5 \times 10^{2}$	$38.9 \times 10^{2}$
$\mathrm{Ca}\;(\mu\mathrm{g}/\mathrm{dl})$	Lime appl.	$40.8 \times 10^2$	$48.1 \times 10^{2}$	$43.2 \times 10^{2}$
Ma (a /d1)	Copp. appl.	$19.7 \times 10^{2}$	$20.0 \times 10^{2}$	$21.7 \times 10^{2}$
${ m Mg}~(\mu{ m g/dl})$	Lime appl.	$19.8 \times 10^2$	$20.0\! imes\!10^{2}$	$20.4 \times 10^{2}$

Table 2. Seasonal variation of Cu, Ca

Table 3. Mineral concentrations in tissues of cattle at the end of last grazing.\*

	Copper sulfate application				Lime application					
	Cu	Fe	Zn	Ca	Mg	Cu	Fe	Zn	Ca	Mg
Brain	2.33	41.3	12.1	11.7	117	2.08	39.4	12.1	18.0	119
Liver	68.13	92.5	43.4	11.0	179	1.83	93.8	34.7	11.4	148
Kidney	3.94	137.5	22.5	40.6	161	3.95	93.8	21.3	50.0	155
Spleen	0.70	275.0	20.9	15.8	167	0.70	468.8	19.6	68.8	163
$\overline{\text{Heart}}$	3.25	56.3	18.8	9.8	228	3.01	58.8	18.4	9.8	208

<sup>\* 15</sup> October, 1975 (ppm in wet tissue)

Table 4. Mineral concentrations in tissues of cattle\* housed for 4.5 months after grazing.

	Copper sulfate application				Lime application					
	Cu	Fe	Zn	Ca	Mg	Cu	Fe	Zn	Ca	Mg
Brain	2.20	25.9	11.6	18.1	114	1.88	32.5	7.8	16.4	127
Liver	475.0	75.0	27.7	8.4	131	5.00	90.6	29.1	10.9	153
Kidney	3.25	41.3	17.2	43.8	144	3.15	55.0	18.0	37.5	148
Spleen	0.70	225.0	19.1	12.1	163	0.70	312.5	20.3	17.4	169
Heart	3.25	51.6	16.6	11.3	217	3.20	85.6	17.5	10.5	216

<sup>\* 3</sup> March, 1976 (ppm in wet tissue)

times higher in liver than with the lime application treatment (1.83 ppm). Ca concentration in tissues was found to be in the following order: kidney>spleen> brain>liver>heart with the copper sulfate application treatment and spleen>

1975 Oct. 30 Dec. 4 Dec. 18 Sep. 4 Jan. 22 70 73 69 76 74 61 64 68 69 76  $62.0 \times 10^{2}$  $66.7 \times 10^2$  $67.7\times10^{2}$  $64.4 \times 10^2$  $30.7 \times 10^2$  $62.8\!\times\!10^{\scriptscriptstyle2}$  $64.6 \times 10^{2}$  $66.3 \times 10^2$  $64.7\times10^{2}$  $32.4\times10^{2}$  $21.0 \times 10^{2}$  $19.3 \times 10^{2}$  $16.8 \times 10^{2}$  $17.8 \times 10^{2}$  $21.3 \times 10^{2}$  $18.9\times10^{2}$  $20.6 \times 10^{2}$  $20.8 \times 10^{2}$  $19.9 \times 10^{2}$  $17.0 \times 10^{2}$ 1976 Oct. 14 Dec. 9 Jan. 23 Mar. 2 Aug. 21 73 88 78 71 71 58 66 69 38 75  $42.7 \times 10^{2}$  $35.9 \times 10^2$  $50.0 \times 10^{2}$  $47.5 \times 10^2$  $49.4 \times 10^{2}$  $53.1\times10^{2}$  $46.9\times10^{\rm 2}$  $47.6 \times 10^{2}$  $35.5 \times 10^{2}$  $51.6 \times 10^{2}$  $20.8 \times 10^{2}$  $\overline{19.4 \times 10^2}$  $21.0 \times 10^2$  $18.4 \times 10^2$  $18.4 \times 10^2$  $19.1 \times 10^2$  $20.7 \times 10^{2}$  $18.4 \times 10^{2}$  $18.8 \times 10^{2}$  $18.4 \times 10^{2}$ 

and Mg concentrations in blood of cattle.

kidney>brain>liver>heart with the lime application treatment. It was identified that Ca and Fe accumulated in spleens of cattle with lime application. Especially Ca in spleen (68.8 ppm) attained to a level 4 times as much with the lime application treatment as that of copper application treatment (15.8 ppm). Zn concentration in tissues was in the following order: liver>kidney>spleen>heart>brain in both treatments and Mg: heart>liver>spleen>kidney>brain for copper sulfate and heart>spleen>kidney>liver>brain for lime application treatment. Zn and Mg concentrations in every tissue did not differ extremely between treatments.

Data from cattle which were fed on hay for 4.5 months after the close of grazing showed that the mineral concentrations in tissues were similar to those at the end of grazing, although Cu in liver and Fe in kidney with the copper sulfate application and Fe and Ca in spleen with the lime application treatment decreased.

Blood copper concentrations between 0.70 and 1.1 ppm and liver copper concentrations greater than 9.3 ppm in wet tissues (30 ppm dry weight) were accepted as characteristic of the normal copper status of cattle (3, 7). The cattle at Kawatabi farm were below the optimal levels for copper status, though none showed signs of bad health, and did not keep the satisfactory level of Cu in blood and tissues without Cu supplementation. The surface application of copper sulfate to the grazing field increased the Cu level in blood and liver of cattle. The accumulation of Cu in liver was especially significant. Such a high Cu accumulation in tissues of cattle are liable to cause Cu toxicosis. It was suggested that the application rate and the kind of Cu compounds applied to the grazing field should be considered in order to prevent a copper toxicity to the grazing

cattle caused by feeding excessive Cu.

Further investigation is needed to identify other factors influencing the health and good daily gains of cattle in Kawatabi farm of Tohoku University.

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