ARTICLES

High sulfur related thiamine deficiency in cattle: A field study

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Abstract

Following development of polioencephalomalacia in one of 105 cattle in a farm in southeastern Saskatchewan, a study was initiated to monitor thiamine (B_1) and copper (Cu) status, and to evaluate interactive nutritional factors which may have been responsible for this occurrence. It was evident that a combination of high sulfur (S) and low Cu intake was responsible for the depletion of blood B_1 and plasma Cu. Supplementation with trace minerals alone resulted in a significant ($p<0.05$) improvement in both B_1 and Cu status of the herd. We recommend that herds exposed to high intakes of S be supplemented with Cu up to 50 mg/kg feed dry matter to alleviate potential deficiencies of B_1 and Cu.

Résumé

Déficience en thiamine reliée à une ingestion excessive de souffre chez les bovins : étude dans un élevage

Une étude a été initiée en vue de déterminer les taux de thiamine (B_1) et de cuivre et d'évaluer les facteurs d'interactions nutritionnelles qui pourraient être impliquées dans le développement de polioencéphalomalacie chez une vache qui faisait partie d'un élevage de 105 bovins dans une ferme du sud-est de la Saskatchewan. Les données recueillies ont démontré qu'une combinaison de facteurs tels qu'un apport alimentaire élevé en souffre (S) et faible en cuivre (Cu) étaient responsables de la diminution des taux sanguins de vitamine B_1 et du cuivre plasmatique. Une supplémentaire en oligo-éléments a produit une amélioration significative ($P<0,05$) à la fois dans les concentrations de vitamine B_1 et de cuivre chez tous les bovins. Nous recommandons une supplémentation de cuivre alimentaire (jusqu'à 50 mg/kg de matière sèche) dans les élevages où l'alimentation fournit un excès de souffre afin de prévenir des déficits possibles en vitamine B_1 et en cuivre.

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Introduction

Ruminants are not considered to require thiamine (B_1) in their diet (1) since sufficient amounts are synthesized in the rumen to meet the demands of the mature animal (2). However, acute (3) and subclinical (4) B_1 deficiencies have been reported. There is little doubt that progressive B_1 deficiency is responsible for field cases of polioencephalomalacia (PEM) which occur in ruminants (1). More recent reports have suggested a possible causal relationship between high intakes of sulfur (S) and PEM (5,6). Such incidents may occur during long-term consumption of high levels of S, and in those instances the whole herd is potentially at risk. Although, in some cases further intake of excessive amounts of S can be reduced, under practical farming conditions this is not always possible. This is especially so when water contains a high content of S, since the cost of water purification is exorbitant. Ruminant livestock in the Canadian Prairies can therefore be regarded as potentially at risk to PEM since water from deep aquifers on many farms contains high levels of S as sulfates (7,8).

Relevance of PEM induced by high levels of S in cattle in Canada is not known, nor is it known whether diets deficient in trace minerals increase the risk of the disease. Adverse effects of high intakes of S on metabolism of copper (Cu) in cattle have been reviewed (7). Although the role of S in decreasing the availability of dietary Cu in sheep is well established (9), the mechanisms underlying S-induced PEM are poorly understood.

The suggested role of S in the induction of both Cu (7) and B_1 (10) deficiency in cattle led us to suggest the existence of a Cu-S-B_1 interaction. In the rumen, S compounds are reduced to sulfides, and these combine readily with Cu to form insoluble Cu sulfides (9). Therefore we believed that in cattle fed moderate to high levels of S, a diet high in Cu alone might be beneficial in reducing the S load in the rumen. If our

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TABLE 1
Trace mineral and sulfur concentrations[a] in feed and deep well water

Sample	Cu	Zn	Fe	Mn	Mo	Se	S[b]
Forage	13.8	22.0	412.7	43.3	0.12	0.60	0.23
Barley	3.9	21.3	51.4	15.5	0.12	0.64	0.13
Canola meal	7.5	52.2	174.0	51.9	0.08	1.71	0.73
Deep well water (sampling 1) (February 6, 1987)	0.01	0.04	0.02	0.007	0.18	0.006	437
Deep well water (sampling 2) (April 25, 1987)	0.004	0.04	0.02	0.005	0.05	0.006	442

[a]Cu, Zn, Fe, Mn, Mo and Se concentrations in feed and water are expressed as mg/kg DM and mg/L respectively
[b]S concentration in feed and water are expressed as % DM and mg/L respectively

TABLE 2
A comparison of manufacturer's guaranteed analysis and laboratory analysis of cattle mineral mix fed to cattle prior to micropremix supplementation

Units	Mineral	Guaranteed analysis by manufacturer[a]	Laboratory analysis of mineral mix[b]
%	Ca	14.0	11.4
	P	14.0	11.6
	Na	8.0	11.6
	S	c	0.57
mg/kg	Cu	3,000	32
	Zn	5,025	332
	Fe	1,000	4940
	Mn	c	155
	Mo	c	0.5
	Se	c	0.32

[a]Guaranteed analysis printed on label by manufacturer. This feed was also guaranteed to contain 1600 mg/kg of iodine, 100 mg/kg of cobalt, and a minimum of 100,000 IU/kg of vitamin A, 50,000 IU/kg of vitamin D_3 and 100 IU/kg of vitamin E
[b]Analysis was carried out by the Feed Testing Laboratory, University of Saskatchewan, Saskatoon
[c]Not available

hypothesis is correct, a high concentration of Cu in the diet alone could be expected to improve indirectly the B_1 status of the herd. In this paper we report a field study carried out in southeastern Saskatchewan to test this hypothesis in cattle at a feedlot with a prior history of PEM. In this study we fed a custom-made trace-mineral supplement to animals of low to marginal B_1 and Cu status, and measured its impact on concentrations of B_1 and Cu in blood and plasma respectively.

History

The farm involved was a 105 beef-cattle unit located in southeastern Saskatchewan. Calves of mixed breeding had been purchased through a local auction market in October 1986. They were separated into two weight classes and housed in two feedlot pens designated as North and South pens.

North pen: Housed 53 heavier calves. Their estimated ages at the time of purchase ranged from nine to eleven months of age and their mean body weight was 340 kg. Many calves in this pen were medium to large frame type and 44% were Simmental-breed crosses.

South pen: Housed the remaining 52 smaller calves. Their estimated ages at the time of purchase ranged from seven to nine months and their mean body weight was 260 kg. Only 18% of the animals in this pen were Simmental crosses.

Feeding and Management: All animals were fed a mixture of forage, barley and canola meal. Based on farmer's records the approximate daily feed intake by steers in the north pen was 2.7 kg forage, 4.1 kg barley and 0.9 kg canola meal per animal. Feed analysis showed this diet to provide approximately 12.8% protein and 2.18 M cal/kg metabolizable energy. This was within National Academy of Sciences–National

TABLE 3
Copper, Zn, Fe, Mo and S intake by cattle prior to and after micropremix supplementation

	Pen	Mineral intake (mg/kg DM) Cu	Zn	Fe	Mo	S %
Prior to supplementation[a]	North and South	7.1	31	257	0.13	0.38
After supplementation[b]	North	57.5	126	268	0.18	0.38
	South	37.0	86	245	0.16	0.38

[a]These were based on average daily intakes of 2.7 kg forage, 4.1 kg barley and 0.9 kg canola meal (see Table 1 for analysis) and 70 g mineral mix 1 (prior to supplementation) (see Table 2), by steers in the north pen, and 10% less by steers in the south pen. Since the concentrations of minerals are expressed in terms of feed DM intake, the values are similar for both pens. Daily water intake was assumed to the 18 L per animal

[b]Feed intake was similar to above[a] but intake of custom made micropremix (see Table 4) varied between the animals in the two pens; 133 g by steers in the north pen compared to 78.5 g by those in the south pen

Research Council (NAS-NRC) (2) recommendations for this type of cattle. Smaller steers in the south pen were offered approximately 10% less feed than those in the north pen. Trace mineral and S analysis of the feed and water are shown in Table 1. The animals were also offered a free-choice commercial mineral (macro and micro) supplement. The mineral supplement had not been analyzed previously, but according to the manufacturer's guaranteed analysis it should have supplied adequate amounts of both macro and micro-minerals (Table 2). Calves in both pens received water from a 40 m well drilled approximately two years previously. The water from this well had not been analyzed previously. Although the calves were consuming all the feed given to them, the owner had noticed that the animals were somewhat depressed and not growing at the expected rate.

Clinical findings

On February 4, 1987 the owner noticed that one animal (Simmental mix breed) in the north pen was scouring and walking with difficulty and sought veterinary advice (GAK). Examination of this steer (approximately 400 kg body wt) revealed a normal body temperature, passage of loose feces, and associated abdominal pain. The animal was treated with sulfamethazine (Spanbolets, Norden Laboratories, Lincoln, Nebraska), sodium sulfosuccinate (Dioctol, rogar/STB, Montreal, Quebec) orally, and oxytetracycline (Liquamycin LP, rogar/STB, Montreal, Quebec) intravenously (IV) for coccidiosis, colic, and thromboembolic meningoencephalitis, respectively. The following day the animal was found recumbent and the veterinarian (GAK) was called again. The steer lay in lateral recumbency with legs extended. There was opisthotonus and the animal was shivering. Examination revealed a normal temperature, rigidity of limbs, and loss of vision. At this time a tentative diagnosis of PEM was made and the steer was given B_1 hydrochloride (400 mg) (thiamine hydrochloride, MTC Pharmaceuticals, Cambridge, Ontario) and oxytetracycline (Liquamycin LP, rogar/STB, Montreal, Quebec) IV. By evening the animal showed some improvement but was unable to get up. The following morning the animal was able to rise and had regained coordination but was walking cautiously and appeared to be visually impaired. Administration of B_1 hydrochloride continued over the next four days, and approximately three weeks elapsed before this animal recovered completely. A blood sample was taken from this animal on day 2 of treatment with B_1 (February 6). This animal became anorectic again on March 3, but recovered prior to any treatment. A blood sample taken from this animal on March 3 was analyzed for levels of Cu, zinc (Zn), iron (Fe) and B_1. Another blood sample was taken from the same animal on April 25.

Experimental Study

Since the sick animal responded readily to B_1 administration, diagnosis of PEM was presumptively confirmed on February 5, and a collaborative study was initiated to monitor the trace mineral [Cu, Zn, Fe] and the B_1 status of this herd. Blood samples were taken (sampling 1) for measurement of concentrations of Cu, Zn, Fe and B_1 from approximately 70% of the animals in both pens on February 6. Samples of all feed ingredients, drinking water and mineral mix (Table 2) were taken for analysis of Cu, Zn, Fe, manganese (Mn), molybdenum (Mo), selenium (Se) and total sulfur (S). Based on analysis of trace minerals (Table 1 and 2), total intake of Cu by the animals was found to be inadequate (Table 3). A custom-made micromix (Table 4) to supplement the existing mineral supplement was advocated on April 2, 1987. Feeding of this newly mixed supplement commenced on April 5, 1987. Three weeks later (on April 25), samples of feed, water and blood (sampling 2) were collected as outlined previously, from approximately 34% and 27% of the animals in north and south pens respectively.

TABLE 4
Manufacturer's guaranteed and laboratory analysis of custom-made micropremix advocated to supplement the existing mineral mix after diagnosis of trace mineral deficiency

Mineral	Guaranteed analysis by manufacturer[a] (mg/kg)	Laboratory analysis (mg/kg)	Daily intake of mineral/animal[b] (mg) after micropremix supplementation North Pen	South pen
Cu	50,000	69,658	359	212
Zn	140,000	131,878	700	413
Fe[c]	d	6,967	429	254
Mo[c]	d	65	0.36	0.21
Mn[c]	d	519	15.0	12.0
Se	400	480	2.48	1.60
S[c]	d	3.18%	0.61g	0.36g

[a]Minimal guaranteed analysis by supplier. This micropremix also contained 3×10^7 IU/kg of vitamin A, 4×10^6 IU/kg of vitamin D_3 and 10,000 IU/kg of vitamin E

[b]Mineral supplementation to cattle from April 5 consisted of a mixture of 1.6 kg of the micropremix, 25 kg of mineral mix (see Table 2), 8.2 kg of calcium carbonate, and 6.8 kg of blue salt (sodium chloride, iodine, cobalt). Daily intakes of minerals have been estimated from intakes of 133 g/d and 78.5 g/d of this mixture by steers in north and south pen respectively

[c]These minerals were not recommended for addition to this micromix but appeared as contaminants

[d]Not available

Analytical Techniques: Concentrations of Cu, Zn and Fe in plasma were determined after dilution with deionized water (v/v; 1:3) followed by precipitation with 1 volume of 20% trichloroacetic acid (TCA) and centrifugation. The plasma supernatants so obtained and farm water samples were measured directly on an atomic absorption spectrophotometer (AAS) (Perkin Elmer, model 5000). Concentrations of Cu, Zn, Fe, Mn, Mo, and Se from samples of forage, barley, canola meal and mineral supplements were also measured on AAS after digestion with a nitric: perchloric acid (3.5:1 v/v) mixture.

Levels of S in forage, barley, canola meal and mineral supplements were analyzed by induction coupled plasma emission spectroscopy (ARL Model 3410) after digestion in nitric and perchloric acids as described by Blanchar *et al* (11).

Determinations of blood B_1 were carried out by the method of Olkowski *et al* (unpublished), a modification of the method of Sarett and Cheldelin (12).

Statistics: Student's *t*-test (13) was used to determine pre- and postsupplementation of micropremix (main effect) on concentrations of Cu, Zn, Fe in plasma and B_1 in blood. The secondary effects between breeds (Simmental-crosses and others), and pens (north and south pen) were tested using analysis of variance (ANOVA) and Duncan's multiple range test (14). Differences of $p<0.05$ are reported as statistically significant.

Results

Composition of diets and trace mineral intakes: Cu and Zn had been omitted inadvertently by the manufacturer from the mineral mix (Table 2). Based on feed analysis (Table 1) and a daily consumption of 2.7 kg forage, 4.1 kg barley, 0.9 kg canola meal, 70 g of mineral supplement (Table 2), and a water intake of 18 L (2), we calculated the daily intake of Cu, Zn, Fe and Mo by each steer in the north pen to be 50, 169, 1800 and 0.92 mg respectively. Intake of S via feed and water were 18.6 and 7.9 g respectively. Values for steers in the south pen were approximately 10% less than values for steers in the north pen. Based on 90% feed DM, we calculated the resulting concentrations of Cu, Zn, Fe, Mo to be 7.1, 31, 257 and 0.13 mg/kg of feed DM (Table 3). Similarly, intake of S was 0.38% feed DM (Table 3).

Supplementation with a micropremix (initiated on April 5) increased the daily intakes of Cu and Zn several fold (Table 4). This was most apparent for animals in the north pen because of their higher consumption of this supplement. The intakes of other microminerals also increased but only slightly, whereas the intakes of macrominerals including S did not change to any great extent following supplementation of the micropremix (Table 3).

Growth and general appearance of animals: Examination of the herd on the date of the first blood sampling (February 6) showed evidence of Cu deficiency including lack of desire to feed, unthriftiness, and loss of condition with typical dull, rough hair coat and loss of pigment in hair (Figure 1).

Three weeks of supplementation with the micropremix was sufficient to markedly improve the overall condition of the animals. All animals showed improved body condition, weight gain, and return of normal coat luster and color of hair (Figure 2).

Copper status of calves: Copper status of calves at the first sampling, as assessed by concentration of plasma Cu, was low (Table 5). Concentration of Cu in plasma

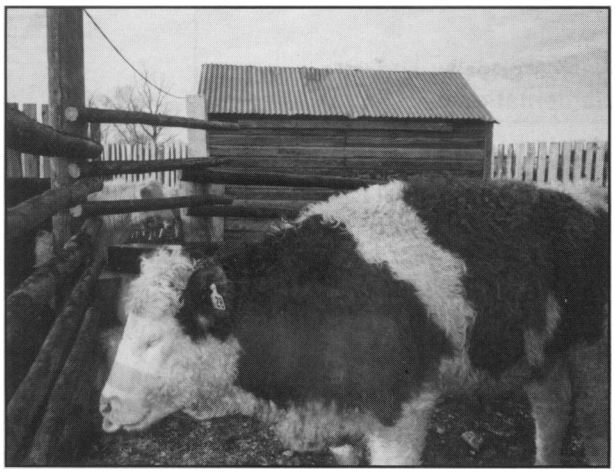

Figure 1. Appearance of steer 28 (which developed polioencephalomalacia) prior to trace mineral supplementation. Note the depressed state of the animal and its dull, rough and depigmented coat.

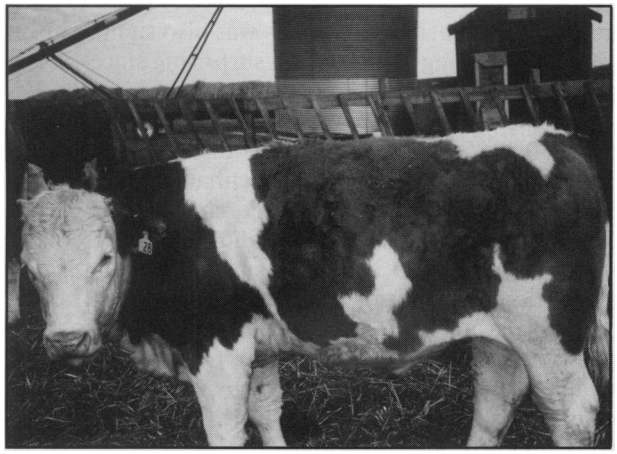

Figure 2. Appearance of the same animal (steer 28) after three weeks of trace mineral supplementation. Note the marked improvement in body condition, coat texture, and coat color.

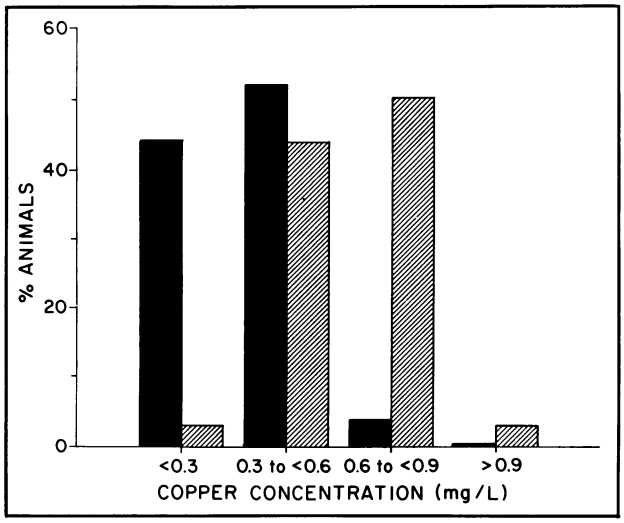

Figure 3. A comparison of plasma copper concentration in cattle prior to (■, sampling 1), and after (▨, sampling 2) micropremix supplementation.

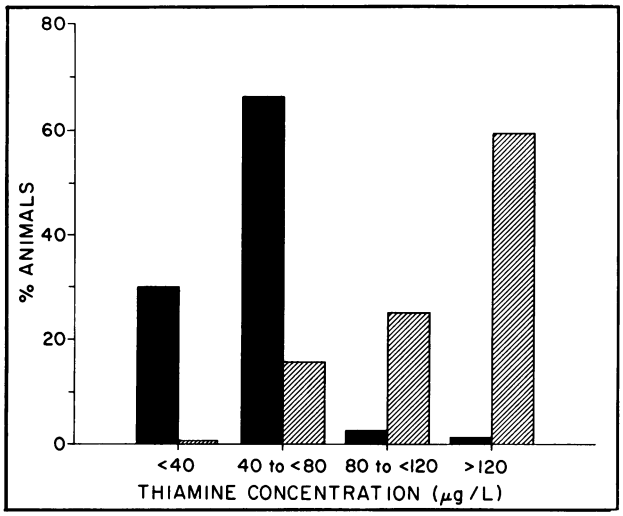

Figure 4. A comparison of blood thiamine concentration in cattle prior to (■, sampling 1), and after (▨, sampling 2) micropremix supplementation.

of less than 0.3 mg/L has been associated with Cu deficiency in cattle (13). Thus, approximately 45% of the animals were Cu deficient (Figure 3). Animals in the north pen appeared to be the most severely affected. Simmental crosses had lower concentration of plasma Cu than the other breeds in the herd. As expected, supplementation of the micropremix resulted in a significant increase ($p < 0.01$) in concentration of Cu in plasma of all steers except the Simmental crosses in the south pen. The latter result may not represent true Cu status of this group since only two Simmental crosses from the south pen were sampled at this time. At sampling 2, following supplementation with the micropremix, only 3% of the animals had Cu levels in plasma less than 0.3 mg/L.

Concentration of Cu in plasma of the steer which developed PEM was 0.35 mg/L at sampling 1 but declined to 0.33 on March 3. After three weeks of supplementation with the micropremix, the concentration of Cu in plasma had increased to 0.55 mg/L.

Iron status of calves: The levels of consumption of Fe before and after supplementation with the micropremix were similar (Table 3). The concentration of Fe (mean ± SD) in plasma of cattle after premix supplementation at sampling 2 were significantly lower (2.15 ± 0.42 mg/L) ($\bar{x}$ ± SD) ($p < 0.05$) compared to the values at sampling 1 (2.73 ± 0.47 mg/L).

Zinc status: Consumption of Zn increased three-to-fourfold after supplementation with the premix, but the mean plasma Zn concentration of all animals increased only slightly ($p > 0.05$); 1.44 ± 0.26 mg/L at sampling 2 compared with 1.37 ± 25 mg/L at sampling 1.

Thiamine status: Mean (± SD) blood B_1 concentration at sampling 1 (prior to micropremix supplementation) was 49.2 ± 14.9 μg/L. Approximately 30% of animals in the herd at this time had concentrations of B_1 less than 40 μg/L in blood (Figure 4). Levels in Simmental cattle and other breeds were similar. After micropremix supplementation, concentration of B_1 in blood increased significantly ($p < 0.05$) in all cattle to 128.2 ± 41.7 μg/L (Figure 4).

TABLE 5
Plasma Cu concentration[a] of calves prior to and after micropremix supplementation

	Plasma Cu concentration (mg/L)				
		North Pen		South Pen	
	Both Pens	Simmental crosses	Others	Simmental crosses	Others
Prior to supplementation[b]	0.42[d] ±0.16 (n=77)	0.34[f] ±0.11 (n=17)	0.38[f] ±0.14 (n=21)	0.42[fg] ±0.16 (n=7)	0.48[g] ±0.16 (n=32)
After supplementation[c]	0.68[e] ±0.15 (n=32)	0.72[h] ±0.14 (n=8)	0.70[h] ±0.19 (n=10)	0.45[fg] ±0.21 (n=2)	0.67[h] ±0.10 (n=12)

[a]Results are expressed as mean ± SD
[b]Prior to supplementation refers to sampling 1 on February 6, 1987
[c]After supplementation refers to sampling 2 on April 25, 1987, taken three weeks after commencement of micropremix supplementation
[de]Values with different letter superscripts differ significantly ($p<0.01$) (Student's *t*-test)
[fgh]Values with different letter superscripts differ significantly ($p<0.05$) (Duncan's Multiple Range Test)

Only the steer which developed PEM received B_1 supplementation. After a single injection of B_1, the concentration of B_1 in blood of this animal increased to 320.3 μg/L, but it declined to 36.1 μg/L four weeks later. No supplementation of B_1 was given after February 10, but three weeks of supplementation with the micropremix alone increased the concentration of B_1 in blood to 96.9 μg/L in this steer.

Discussion

It is evident from the investigation reported here that a combination of excessive intake of S, and a low dietary intake of trace minerals, especially Cu, is detrimental to the B_1 status in cattle. Based on analysis of B_1 in blood of cattle in this study, it might be expected that on the Prairies there are many cattle which suffer from subclinical deficiency of B_1. Supplementation of trace minerals alone resulted in a significant improvement in both the B_1 and Cu status of cattle exposed to an above-normal exposure of S. Although PEM has been associated with a high concentration of S in the diet (6) and drinking water (8), to our knowledge this is the first time a trace mineral-responsive B_1 deficiency has been documented. We believe that the events leading to a deficiency of B_1 as reported here are due to a series of complex interactions of microminerals (Cu, Zn, Fe, Mo), macrominerals (S) and B_1.

Interactions among Cu, Zn, Fe, Mo, and S are widely recognized (15). The bioavailable fraction of each of these elements can be regarded to be "in balance" with that of the other four, and hence the changes in concentration of one would distort the metabolism of the others (15). In this context, it is notable that concentration of Zn in plasma was lower at sampling 2 in spite of a three- to fourfold increase in intake of dietary Zn after supplementation with the micropremix. This may have resulted from a decreased absorption of dietary Zn because the concentration of Cu in the diet of animals in the two pens increased from 7 to 37.0 (south pen) and 57.5 (north pen) mg/kg during this period (Table 3). Similarly, the concentration of Fe in plasma of animals was also significantly lower at sampling 2. This is not surprising since excess dietary Cu, relative to Fe, is known to reduce the Fe status in cattle, and vice versa (16). It has been shown that 250 mg/kg DM of Fe in the diet can induce Cu deficiency in cattle (17) and an enhanced accumulation of Fe has been noted in such animals (18). Copper deficiency produces anemia and a characteristic response to anemia is an enhanced efficiency in Fe absorption (19). Thus a relatively higher concentration of Fe in plasma observed at sampling 1 (prior to supplementation with the micropremix) was probably due to an enhanced absorption of Fe by Cu-deficient animals from the diet which contained low Cu, moderately high Fe, and marginal Zn.

In ruminants, both inorganic and organic S compounds are reduced to sulfides by rumen bacteria. Sulfides bind with a variety of divalent cations to form cationic sulfides. Affinity of S for Cu is higher than other cations and the solubility product of Cu sulfide is extremely low (3.48×10^{-38}). In addition, thiomolybdates (TM) which form in the rumen at moderate to high intakes of S and Mo, form unavailable Cu-TM complexes which exert effects both in the gut and systemically (7), further reducing the availability of Cu to the animal. Breed differences in Cu metabolism are recognized and Simmental cattle appear to be more susceptible to Cu deficiency than other breeds (7). This is supported by the present study in that the concentration of Cu in plasma of the Simmental crosses were lower than those in the other breeds (Table 5). Hypocupremia was evident at sampling 1 (Table 5) with approximately 45% of the animals deficient in Cu (Figure 3). Depigmentation and rough hair coat observed at this time (Figure 1) are typical manifestations of Cu deficiency (20). Reversal of these signs within three weeks of supplementation with the micropremix suggest synthesis of Cu-dependent enzymes such as polyphenyloxidases which are involved in pigmentation and keratinization of hair. It was not surprising to find an improvement in growth and body condition after supplementation with micropremix.

Significant increases in weight gains, have been reported in previously deficient cattle when supplemented with Cu (21) and B_1 (22). Therefore it is reasonable to believe that the improved animal performance observed in the present study was due to a combined improvement in Cu and B_1 status in these animals.

Recent evidence that excess dietary S also exerts an effect on availability of B_1 in ruminants (10) suggests the possible occurrence of a complex interaction of B_1-S-divalent cations in the rumen. If this is true, a low concentration of divalent cations in the diet would indirectly result in a relatively higher concentration of "free" sulfide in the rumen and this may create a B_1 deficiency. We believe that this may have been the reason for the depleted levels of B_1 in blood (sampling 1) observed in the present study prior to supplementation with the micropremix. Conversely, if the diet is supplemented with normal or a slight excess of cations, especially with Cu, it would result in formation of cationic sulfides. This would lower the concentration of free sulfide in the rumen (23), and thereby alleviate the effects of S-induced B_1 deficiency. This is probably the most likely explanation for the significant increase in levels of B_1 observed in blood of animals in the present study after supplementation with the micropremix. The response was immediate, since an improvement in B_1 status was observed within three weeks of supplementation. This improvement in B_1 status occurred in spite of no change in basal diet, drinking water supply or any supplementation with B_1.

Clinical signs of B_1 deficiency have been divided broadly into those attributable to general metabolic disorders, which may be observed at an early stage, and those related to disorders of the central nervous system (24). It is interesting to note that the clinical signs listed as metabolic disorders of B_1 deficiency such as transient scouring, reduced growth rate, anorexia, general unthriftiness and reproductive disorders are also common signs of chronic Cu deficiency. We believe that, under field conditions, deficiencies of both Cu and B_1 may exist concurrently in cattle having access to excessive levels of S. In instances in which high S-related PEM is diagnosed, conventional B_1 therapy (25) is beneficial and clinical recovery occurs within one to three days. However, as observed in the present study, the elevation of concentration of B_1 in blood after IV B_1 therapy is only temporary. As long-term therapy, we recommend that herds exposed to high intakes of S also be supplemented with Cu up to 50 mg/kg feed DM. This could be expected to alleviate deficiencies of both Cu and B_1.

In a previous experiment, two of eight cattle fed 0.5% S and 10 mg Cu/kg DM in the diet, developed PEM within six weeks. Concentrations of B_1 in blood of affected animals were 14.7 and 23.5 μg/L (Gooneratne *et al*, unpublished observations). Based on our experience, we believe that a concentration of less than 40 μg/L of B_1 in blood could be considered as marginal. Thus, approximately 30% of the animals in the present study were deficient at sampling 1. Depression of concentration of B_1 in blood has been reported previously in cattle fed diets adequate in Cu but supplemented with 0.72% sulfate (10). Since ruminants do not require B_1 in their diet, one or several of the following mechanisms may have been responsible for the lower levels of B_1 in plasma as observed in the present study: inadequate microbial synthesis of B_1, impaired absorption and utilization of B_1, presence of B_1 antimetabolites, lack of apoenzyme, increased metabolic demand for B_1, or increased rate of B_1 excretion (3). It is not clear which of these pathways are involved in the induction of S-related B_1 deficiency. However, there is some evidence that diets high in S decrease the amount of B_1 entering the duodenum, probably due to a reduced synthesis of B_1 (10), and increase excretion of B_1 (Olkowski *et al*, unpublished observations). High intakes of S decrease ruminal, duodenal and fecal pH (26). Ruminal acidosis appears to establish conditions conducive to development of PEM (27), such as enhanced production of the B_1-destroying enzyme, thiaminase (1). It is reported that B_1 and other related compounds can repress extracellular enzyme, thiaminase (28), and hence a critical concentration of extracellular B_1 in rumen fluid may be an important factor in the control of thiaminase production. We believe that future research needs to be directed at understanding: (i) whether excess S induces subclinical lactic acidosis in rumen, (ii) whether thiaminase-producing organisms are selected or encouraged preferentially by high intakes of S, and (iii) how high intakes of S increase urinary B_1 excretion. Experiments are in progress to test these hypotheses.

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