Research in Veterinary Science 91 (2011) e116–e120

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effects of dietary Cu, Mo and S on urinary Cu and Zn excretion in Simmental and Angus cattle

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ARTICLE INFO

Article history:
Received 16 August 2010
Accepted 26 January 2011

Keywords:
Cattle
Copper
Molybdenum
Sulphur
Urinary excretion

ABSTRACT

This study determined the effects of dietary copper (Cu), molybdenum (Mo) and sulphur (S) on urinary Cu and zinc (Zn) excretion in cattle. Four Simmental and four Angus heifers were fed low (L) or high (H) levels (mg/kg DM) of Cu (5, 40), Mo (1, 10) and S (0.2, 0.5%). Initially two of each breed was fed either LCu or HCu (2 mo). Then all eight animals were fed sequentially LCu—HS (1.5 mo), HCu—HS, HCu—HMo and HCu—HMo—HS (2 mo each). Simmental had a higher urine flow, increased concentration and total excretion of urinary Cu and Zn compared to Angus, but only total Zn excretion was significantly higher. Urinary Cu excretion was greatest with the HCu—HMo—HS diet. Urinary Zn excretion significantly increased with HS but not HS in combination with HMo and/or HCu. This study, together with previously reported biliary excretion, allows a direct comparison of urinary and biliary Cu and Zn excretion responses to dietary Cu, Mo and S.

1. Introduction

Copper (Cu) is an essential trace mineral and its homeostasis in sheep and cattle is dependent on the concentration of other nutrients, including molybdenum (Mo), sulphur (S) and zinc (Zn). Since the interrelationship between Cu, Mo and S in ruminants was reported (Dick, 1956), Cu deficiency (Hill et al., 1969) and toxicity (Woolliams et al., 1983) in sheep and cattle, particularly as a function of its interrelationship with Mo and S, have been of research interest. Copper deficiency is more common in cattle than Cu toxicity, and in many instances is associated with high Mo and S intakes via formation of thiomolybdates (TM), which have a high affinity for Cu making it unavailable to the animal (Dick et al., 1975). Relative to sheep, few reports exist in the literature on individual genetic differences or breed variation in cattle Cu metabolism (Weiner, 1987); although Smart and Gudmundson (1980) suggested that Simmental cattle appear more susceptible to Cu deficiency than other breeds, and more recently Hansen et al. (2010) also reported of differential trace mineral metabolism between Angus and Simmental calves.

The effects on biliary Cu and Zn excretion of Simmental and Angus cattle fed varying dietary levels of Cu, Mo and S have been published (Gooneratne et al., 1994), but urinary excretion of Cu and Zn as a function of dietary Cu, Mo and S concentrations in that experiment has not yet been reported. Zinc concentrations were measured because it is a transitional element which apparently is not chelated by thiomolybdates. This, then, is the follow-up paper to Gooneratne et al. (1994), allowing a direct comparison of the two routes (biliary and urinary) of Cu and Zn excretion in cattle for the first time within one experiment.

2. Materials and methods

2.1. Experimental animals

Handling and care of cattle were in accordance with the principles and guidelines established by the Canadian Council on Animal Care (CCAC) (1984). Four Simmental and four Angus heifers (10 months old), weighing 209 ± 18 (SD) and 192 ± 34 kg, respectively, were used in the trial. All animals were clinically examined prior to the study. For full details of animal care and diet see Gooneratne et al. (1994). Individually housed cattle were offered a diet containing 5, 1, 52, 43 and 97 mg/kg of Cu, Mo, Zn, manganese (Mn), and iron (Fe), respectively, and 0.2% S within twice-daily rations of brome hay (60%), barley (35%) and canola meal (3%) (1.6 mm pellets), except that Cu was maintained at 5 mg/kg dry matter (DM). Each animal was offered 7 kg of feed daily at the beginning of the experiment, increasing monthly by 0.1–0.2 kg to accommodate body weight gain, and had free access to drinking water. Feed intake did not appear to be affected by the level of Cu, Mo and S in the diet and the heifers consumed all food offered.

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doi:10.1016/j.rvsc.2011.01.024

From 4 weeks after surgery (cannulation of gall bladder and duodenum; Gooneratne et al., 1994), all animals gained weight during the trial at a monthly rate of 21–33 kg.

Six weeks after surgery, two animals of each breed were allocated randomly to either a high (H) Cu (40 mg/kg DM) diet group ($n = 4$) or continued on the low (L) Cu diet ($n = 4$) for 2 months (mo). This was done to examine the effect of initial HCu or LCu loading on subsequent treatments. After the 2 mo, all animals were sequentially given diets containing LCu—HS (0.5% S) for 6 weeks, HCu—HS for 2 mo, HCu—HMo (10 mg kg^{-1} Mo) for 2 mo, and HCu—HMo—HS for 2 mo. The LCu—HS diet was changed to HCu—HS after 6 weeks because during week 4 on this diet, two Simmental and one Angus showed signs of polioencephalomalacia (PEM), becoming anorexic, blind and uncoordinated. All three animals responded readily to intravenous thiamine treatment but sampling was delayed to allow a 2-week recovery period.

2.2. Sampling protocol

Urine was sampled at 1 mo and 2 mo after transfer to each diet, except during the LCu—HS diet when three animals developed PEM and sampling was carried out at 6 weeks instead of the usual 4 weeks. On sampling days the cattle were held in metabolism crates on raised floors, and a Foley catheter inserted into the bladder at least 1 h prior to sampling. Urine was collected into a plastic bucket for consecutive 30-min periods continuously between 0900 and 1500 h for two consecutive days. The excreted volume was noted and a sample taken for Cu and Zn analysis.

2.3. Analytical techniques

Copper and Zn determinations were by atomic absorption spectrophotometry (AAS). The urine samples were digested in nitric, sulphuric and perchloric acids and appropriately diluted with deionised water prior to reading on AAS (Perkin–Elmer, model 5000; Perkin–Elmer Corp., Norwalk, CT).

2.4. Statistical analysis

The data were analysed using the general linear model procedure (Ray, 1982). One-way analysis of variance (ANOVA) indicated no effect of initial dietary Cu level on the parameters (urinary volume, urinary Cu and Zn concentration, total urinary Cu and Zn excretion) measured during subsequent dietary treatment periods. This agrees with the observations of Suttle (1974), who found no evidence of effect of initial Cu status on the nature of Cu—Mo—S antagonism. Firstly, the effect of diet, breed, and day of sampling on the above parameters was analysed as a one-way ANOVA, and secondly, the effect of diet and breed on the same parameters was analysed as a two-way ANOVA. Parameter means, and also means of samples collected on days 1 and 2, were compared using Duncan's multiple-range test (Snedecor and Cochran, 1980). Results are significantly different at $P < 0.05$.

3. Results

3.1. Urinary volume

Urinary flow was generally lower in cattle fed Cu alone (LCu or HCu) and higher when fed Cu in association with HS and HMo, but was significantly higher only in cattle fed the LCu—HS diet compared with the LCu diet (Table 1). Flow was not affected by day of sampling (day 1 vs day 2), on pooled data (Table 2), or after separation by diet for each breed, probably due to the high standard deviation (SD) except that Simmental on the LCu—HS diet had a significantly higher flow rate (3.74L/6 h) than Angus (1.84L/6 h) on the LCu diet. However, on pooled data urinary flow at month 1 – day 1 differed in relation to diet, with significantly higher flow rates in cattle on LCu—HS (3.55L/6 h) and HCu—HMo—HS (3.30) diets compared with cattle on LCu (1.64) and HCu (1.58) diets. There was no difference in urinary flow between the two breeds (pooled data), probably due to the high SD (Table 3).

3.2. Urinary Cu

Urinary Cu concentration was affected by diet (Table 1), being significantly higher in cattle fed the HCu—HMo—HS diet compared with those fed HS or HMo diets, but not with those fed LCu or HCu only. The HCu diet resulted in a non-significantly higher urinary Cu concentration compared with Cu diets in association with either S or Mo (LCu—HS, HCu—HS or HCu—HMo). Total Cu excretion was also affected by diet (Table 1), and followed a somewhat similar pattern to that of urinary Cu concentration, but with the HCu—HMo—HS diet resulting in the highest Cu excretion compared with all other diets, significantly higher than when fed HCu—HS and HCu—HMo diets, the latter being the lowest. Neither Cu concentration nor excretion was different between sampling days 1 and 2, on pooled data (Table 2). Urinary Cu excretion was, however, affected by breed and diet. Highest Cu excretion was in Simmental fed HCu—HMo—HS and significantly higher than in Simmental fed LCu—HS or Angus fed HCu—HS diets (Fig. 1). Cu excretion differed in relation to diet, being significantly higher in day 1 monthly

Table 1
Effect of varied levels of copper (Cu), molybdenum (Mo), and sulphur (S) on urinary flow and urinary Cu, Zn concentration and excretion.

Diet/treatment	Urine volume (L)		Urine Cu				Urine Zn			
			Concentration (mg/L)		Excretion (mg/6 h)		Concentration (mg/L)		Excretion (mg/6 h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low Cu*	2.133[b]	0.911	0.081[a,b]	0.024	0.168[b]	0.069	0.080[b]	0.024	0.161[b]	0.054
High Cu*	2.467[a,b]	1.241	0.099[a,b]	0.035	0.215[a,b]	0.041	0.076[b]	0.023	0.173[b]	0.066
Low Cu, high S†	3.173[a]	1.231	0.075[b]	0.036	0.206[a,b]	0.109	0.114[a]	0.083	0.358[a]	0.315
High Cu, high S‡	2.747[a,b]	1.151	0.075[b]	0.026	0.190[b]	0.062	0.119[a]	0.031	0.329[a]	0.179
High Cu, high Mo‡	2.732[a,b]	0.999	0.064[b]	0.023	0.161[b]	0.051	0.070[b]	0.034	0.170[b]	0.063
High Cu, high Mo, high S‡	2.742[a,b]	0.978	0.116[a]	0.034	0.292[a]	0.060	0.100[a,b]	0.025	0.258[a,b]	0.065

* Results given as mean ± SD for 4 cattle (2 Simmental and 2 Angus) from means of 6-h samplings (collected half-hourly) on 4 occasions per animal (i.e. on 2 consecutive days on each month on each diet for 2 months) ($n = 16$).
† Results given as mean ± SD for 8 cattle (4 Simmental and 4 Angus) from means of 6-h samples (collected half- hourly) on 2 occasions per animal (i.e. on 2 consecutive days after 1 month on diet ($n = 16$).
‡ Results given as mean ± SD for 8 cattle (4 Simmental and 4 Angus) from means of 6-h samplings (collected half-hourly) on 4 occasions per animal (i.e. on 2 consecutive days on each month on each diet for 2 months) ($n = 32$).
a,b,c Values with different letter superscripts within a column differ significantly ($P < 0.05$) (Duncan's multiple-range test).