Comparative effects of dietary molybdenum concentration on distribution of copper in plasma in sheep and red deer (Cervus elaphus)

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ABSTRACT

Differences between sheep and deer in the effects of molybdenum (Mo) and sulphur (S) on copper (Cu) metabolism were investigated in two dietary experiments each using 8 animals of each species. The animals were offered a hay diet supplemented (Expt 1) to contain 3, 6, 12 or 24 mg Mo/kg DM and 2.3 g S/kg DM or (Expt 2) to contain 6, 12, 24 or 48 mg Mo/kg DM and 5.3 g S/kg DM. The duration of the feeding periods was 35 and 16 days, respectively.

With the low S diet (Expt 1) total plasma Cu was changed from the pre-experimental level of 0.75 to 0.63, 0.77, 0.81 and 1.03 mg/litre , respectively, in sheep and from 0.95 to 0.77, 0.79, 0.67 and 1.01 mg/litre in deer. The proportion of trichloroacetic acid (TCA) soluble/total Cu was reduced by 0.05, 0.09, 0.07 and 0.21 in sheep and by 0.01, 0.05, 0.08 and 0.11 in deer.

With the high S diet (Expt 2) total plasma Cu concentration changed from 0.81 to 0.82, 0.97, 1.43 and 1.41 mg/litre in sheep and from 1.08 to 0.72, 0.96, 0.88 and 1.21 mg/litre in deer. The proportion of TCA-soluble/total Cu was reduced by 0.20, 0.50, 0.60 and 0.63 in sheep and by 0.05, 0.10, 0.23 and 0.48 in deer.

This study has shown that Cu metabolism in sheep is more sensitive than in deer to increasing dietary Mo concentration. While plasma total Cu increased with increasing Mo in the diet in sheep, concentrations declined in deer. This was associated with a greater induction of insoluble Cu in plasma in sheep. In both species increased dietary S enhanced the effect of dietary Mo on Cu metabolism, but deer appeared to be more sensitive than sheep to increasing dietary S concentration.

Keywords Dietary; molybdenum; sulphur; copper; plasma copper fractions; sheep; deer

INTRODUCTION

Dietary molybdenum (Mo) and sulphur (S) interact to impair copper (Cu) metabolism in sheep. Systematic work with deer has not been undertaken. However, a preliminary study by Mason *et al.* (1984) showed that the effect of molybdates on Cu metabolism in deer may be less than in sheep. This conclusion was also reached by Freudenberger *et al.* (1987) who were unable to detect any trichloroacetic acid (TCA)-insoluble Cu in the plasma of deer offered a silage diet containing 6 mg Mo, 5.4 g S and 9-10 mg Cu/kg DM but which did induce significant of TCA-insoluble plasma Cu in sheep. The present experiments were designed to confirm and extend these observations.

MATERIALS AND METHODS

Animals

Eight sheep and 8 deer were used in the studies which comprised two experiments. The animals were divided randomly within species into four groups of two animals. Meadow hay, containing (/kg DM) 5.9 mg Cu, 0.4 mg Mo and 2.3 g S was offered during a seven week period to both animal species at levels estimated to provide maintenance energy. Each group within species was then allocated to one of four dietary levels of Mo (Expt 1) vis 2.4 (Mo3), 5.4 (Mo6), 11.4 (Mo12) and 23.4 (Mo24) mg/kg DM which were maintained for 35d. The animals were returned to the basal diet for 3 weeks before random re-allocation (Expt 2) to dietary concentrations of Mo of 5.4 (Mo6S), 11.4 (Mo12S), 23.4 (Mo24S) and 47.4 (Mo48S) mg/kg DM which were maintained for 16 days. The levels were achieved by carefully sprinkling, daily, an aqueous solution (100 ml) of sodium molybdate onto the hay of each animal and mixing by hand. In the latter experiment Na_2SO_4 was also added to provide dietary S concentration of 5.6 g

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S/kg DM in all diets. Blood samples were collected three times each week in Expt 1 and twice weekly in Expt 2, from the jugular vein into 10 ml heparinized (100 USP; sodium heparin, Sigma Chemical Co., vacutainer tubes and the plasma was separated by centrifugation at 2000 rpm. The plasma samples were stored at -20°C in Expt 1 but were analysed immediately in Expt 2.

Chemical Analyses

Food

Food samples were freeze-dried and milled (1 mm stainless steel sieve) before analysis for Cu and S (0.5 and 0.1 g DM, respectively) after wet digestion in a mixture (4:1 v:v) of nitric and perchloric acids. The dry digest was reconstituted by the addition of 5 ml of 5% HCl. For S the digest was further made up to 20 ml with water. Sulphur was measured in the digest as described by Quin and Woods (1976) and Cu was determined by Atomic Absorption Spectro-photometer (AAS), (Shimadzu, Model AA-670).

Molybdenum in food samples was determined after wet ashing 0.25 g DM in 5 ml of a mixture (1:4, v:v) of perchloric and nitric acids. The mixture was initially heated at 100°C until brown fumes disappeared, and then at 210°C until dry. Nitric acid (1 ml) was added and heated at 210°C and the procedure repeated until the ash was colourless. The digest was then washed one to three times with 'nanopure' water and the vial taken to dryness after each wash. Molybdenum in the digest was measured by the catalytic method of Bradfield and Stickland (1975) in a centrifugal analyzer (IL model Multistat III plus).

Total and TCA-soluble Plasma Copper

Total and TCA-soluble plasma copper were determin-ed by AAS. For total Cu, plasma (1 ml) was diluted in 6% (v/v) butanol (2 ml) before aspiration and for TCA-soluble Cu 1 ml plasma was added to 3 ml 6% TCA (w/v), centrifuged at 200 g and Cu in the supernatant determined.

Statistical Analysis and Presentation

Analysis of variance procedures were used to study the differences in initial values and the changes in plasma Cu parameters within individuals between treatments and animal species. All results presented in the text are given as deviations from the initial values (means of two animals) with vertical bars that denote the standard error.

TABLE 1 Initial values and changes in total Cu (mg/l) in sheep and deer offered a hay diet containing 2.3 g S/kg DM and with Mo content of 3, 6, 12 or 24 mg/kg DM for 35 days (Expt 1) or 5.5 g S/kg DM and 6, 12, 24 or 48 mg Mo/kg DM for 16 days (Expt 2).

	Experiment 1			Experiment 2		
	Initial	0-16 days	0-35 days	Initial	0-3 days	0-16 days
Treatment comparisons						
Mo3	0.94	-0.05	-0.10			
Mo6	0.82	-0.06	-0.03	0.98	0.06	-0.17
Mo12	0.85	0.04	-0.06	0.89	0.21	0.01
Mo24	0.81	0.21	0.22	0.98	0.35	0.20
Mo48				0.93	0.45	0.36
Significance	NS	**	**	NS	**	***
SED	0.130	0.054	0.060	0.183	0.082	0.086
Species comparisons						
Sheep	0.75	0.08	0.07	0.81	0.27	0.34
Deer	0.95	-0.01	-0.04	1.08	0.18	-0.14
Significance	¶	¶	¶	¶	¶	***
SED	0.092	0.038	0.042	0.129	0.060	0.061

¶ $P<0.1$

RESULTS

Total Copper

Experiment 1

Initially, sheep and deer had similar total plasma Cu concentrations (Table 1). Both sheep and deer on Mo24 diet showed an increase in total plasma Cu compared to the other treatments, the change tending to be more marked in sheep whether comparisons were made between 0-16 or 0-35 days ($P<0.05$ during both periods). Deer, however, showed a smaller ($P<0.10$) effect of treatment after 35 days. A general trend for a difference between the animal species in the changes between 0-16 and between 0-35 days ($P<0.10$) was due to general reductions and increases in plasma total Cu in deer and sheep, respectively.

Experiment 2

The changes in total plasma Cu in sheep and deer during Expt 2 are given in Table 1. Initially total plasma Cu concentration was marginally higher in deer than in sheep ($P<0.10$). Within the first three days of treatment both sheep and deer showed Mo- dependent increases in total plasma Cu. Within deer the change between 0-3 days was small and similar in groups on Mo6S and Mo12S but was much greater on Mo48S ($P<0.001$). The difference between treat-ments in sheep, however, was not significant ($P>0.10$) reflecting the large between-individual variation. While concentrations in sheep subsequently increased until day 16, values in deer decreased on all treatments ($P>0.10$). The overall difference ($P<0.001$) in response between the animal species was mainly due to a general reduction in total Cu concentration in deer and an increase in sheep.

Proportion of TCA-soluble Copper

Experiment 1

The proportion of TCA-soluble to total plasma Cu (TCA- soluble/total Cu) in Expt 1 (Table 2) was not clearly affected in either animal species. There was a tendency, however, for greater reduction in sheep than in deer after 35 days on the Mo24 on which the greatest change was generally seen.

Experiment 2

During Expt 2, there was a trend for reduction in the proportion of TCA-soluble Cu in sheep on the Mo6S diet but large reductions on the other treatments, which occurred predominantly within the first three days (Table 2). In deer no clear change was found on either the Mo6S or Mo12S

TABLE 2 Initial values and changes in ratio of TCA-soluble/total Cu (mg/litre) in sheep and deer offered a hay diet containing 2.3 g S/kg DM with Mo content of 3, 6, 12 or 24 mg/kg DM for 35 days (Expt 1) or 5.5 g S/kg DM and 6, 12, 24 or 48 mg Mo/kg DM for 16 days (Expt 2).

	Experiment 1			Experiment 2		
	Initial	0-16 days	0-35 days	Initial	0-3 days	0-16 days
Treatment comparisons						
Mo3	0.98	-0.08	-0.03			
Mo6	0.98	0.07	-0.07	1.02	0.01	-0.13
Mo12	1.03	-0.12	-0.08	1.02	-0.29	-0.30
Mo24	0.98	-0.08	-0.16	0.99	-0.23	-0.41
Mo48				1.01	-0.47	-0.55
Significance	NS	*	¶	NS	NS	***
SED	0.027	0.045	0.038	0.014	0.177	0.050
Species comparisons						
Sheep	0.97	-0.06	-0.10	1.00	-0.33	-0.48
Deer	1.01	-0.04	-0.06	1.02	-0.12	-0.21
Significance	*	NS	NS	¶	NS	***
SED	0.1465	0.0451	0.0269	0.01006	0.1252	0.0354

¶ $P<0.1$

diet but deer on the two higher Mo treatments showed reductions after three days and no further change subsequently. There was no difference between the animal species during the first three days ($P > 0.10$) but by the end of the experiment the overall reduction in the proportion of TCA-soluble Cu was clearly greater in sheep than in deer ($P < 0.001$).

DISCUSSION

These studies have provided further evidence that Cu metabolism in deer, as judged by the appearance of TCA-insoluble Cu in plasma, is less affected by dietary Mo and S intake than in sheep. This confirms the suggestions of Mason *et al.* (1984) and the finding of Freudenberger *et al.* (1987). Sheep in Expt 1 were expected to show substantial changes in total and TCA-soluble plasma Cu when they were offered diets containing 2.3 g S/kg DM and 2.4 to 23.4 mg Mo/kg DM since Smith and Wright (1975a,b) reported such changes in sheep on diets containing 0.75 g S/kg DM and 8 to 16 mg Mo/kg DM. The difference between the present results and those of Smith and Wright (1975a,b) is surprising, especially since dietary S was higher in the present experiment (2.3 g/kg DM). Differences between breeds of sheep (Robinson *et al.*, 1987) may have been important. An effect of diet, simply in terms of its formulation (Suttle, 1983), seems less likely, however, since Bremner and Young (1978), using the same diet as Smith and Wright (1975a,b), a semi- purified diet described by Suttle and Field (1968), presented results in line with the present study. In a previous report from this laboratory, in which sheep offered a silage diet containing only 6.2 mg dietary Mo/kg DM showed an increase in TCA-insoluble and total plasma Cu while deer showed simply a reduction in total plasma Cu (Freudenberger *et al.*, 1987), a considerably higher dietary S concentration (5.6 g/kg) obtained. This lead to the conclusion that dietary S may be of crucial importance in determining the response of the two animal species and to the second experiment.

The general pattern of change in total plasma Cu, after dietary S concentration was increased, differed considerably between sheep and deer. Whereas total plasma Cu increased continuously in sheep on all diets (5.4-47.4 mg Mo/kg DM) (Table 1), increase occurred in deer for only three days before it fell below the initial levels, with the exception of deer on the high Mo diet (Mo48S). Similarly the reduction in TCA solubility of Cu showed similar trends in both species. This is suggestive of similar initial response in the two species to the effects of dietary S and Mo but that metabolism in deer could adapt rapidly to mitigate against such effects.

A conclusion of lower susceptibility of deer than sheep to high dietary Mo, supports the findings of Freudenberger *et al.* (1987). It seems probable that this reflects differences in the formation of thiomolybdates (TMs) (Suttle, 1974; Dick *et al.*, 1975) in the rumen, in their absorption into the plasma or subsequent degradation. Three species of thiomolybdate - di- (TM2), tri-(TM3) and tetra-(TM4) are produced in the rumen, the ability to complex with plasma Cu increasing with degree of thio-substitution. In this regard Mason *et al.* (1982) and Mason *et al.* (1984) detected both TM2 and TM3 in the plasma of sheep injected intraruminally with ^{99}[Mo]molybdate but only TM2 in the plasma of deer similarly treated. Subsequent studies (Osman, 1988) have shown that while both sheep and deer are capable of formation and absorption of the three species of TM, greater proportions of the higher thiosubstituted compounds occur in sheep than in deer. In the same series of studies TM2 was clearly shown to be less effective than both TM3 and TM4 in reducing TCA-solubility of plasma Cu and was most rapidly cleared from the plasma.

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