Effect of different levels of dietary molybdenum on copper and Mo metabolism in sheep fed on high levels of Cu

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1. Distribution of copper and molybdenum was followed in the body tissues of sheep fed on high levels of Cu (82 mg Cu/sheep per d), sulphur (3·77 g S/sheep per d) and different levels of Mo (0·6 20·8, 38·4 and 58·5 mg Mo/sheep per d).

2. Liver Cu content decreased as Mo intake increased from 0·6 to 38·4 mg/d, but increased again at high intakes of Mo. With an Mo intake of 58·5 mg/d, the Cu content of liver, kidney, lung, spleen and muscle tissue was significantly higher than with an intake of 20·8 mg Mo/d. The trend of increased Cu concentrations in kidneys and plasma was already evident at an Mo intake of 38·4 mg/sheep per d.

3. High positive correlations were observed between Cu and Mo in both the kidney cortex and medulla of the sheep at the two highest Mo treatments.

4. At constant S intake, Mo concentrations in the tissues tended to increase in proportion to Mo intakes. No indication of any detrimental effect due to the accumulation of Mo in the tissues was observed.

5. It was suggested that in the presence of an abundance of Mo, Cu and S, compounds containing these minerals in metabolically unavailable forms accumulate in the body, first in the kidneys, but eventually also in the other tissues of the sheep.

It is well established that molybdenum in the presence of sulphur reduces the copper concentration in the livers of sheep (Dick, 1954; Wynne & McClymont, 1956; Suttle, 1974). Suttle (1975) pointed out that the extent and risk of the depression of Cu concentration in liver by Mo supplementation depends on the Cu status of the liver, the duration of Mo feeding and the dietary levels of Cu and Mo during this period. However, with Mo intakes of between 20 and 100 mg/sheep per d Dick (1954) observed very little change in hepatic Cu content, though in cattle Vanderveen & Keener (1964) and Huber *et al.* (1971) observed decreased levels of hepatic Cu at higher Mo intakes.

A further consequence of the Cu–Mo–S interaction in the body is the increase in Cu concentration of the plasma and kidneys at high Mo intakes (Dick, 1956; Suttle & Field, 1968; Smith & Wright, 1975; Van Ryssen, 1979). These changes in plasma and kidney Cu levels were described by Suttle (1974) as true systemic effects of the Cu–Mo–S interaction in sheep. However, the relative importance of these so-called systemic effects was considered to be rather small (Suttle, 1975).

It has been observed that hepatic Cu concentrations may remain at high levels (Ross, 1970) and that sheep can die of Cu toxicity months after the withdrawal of all additional Cu from their diets (Bracewell, 1958; Barden & Robertson, 1962). The reduction of hepatic Cu content is therefore essential if Cu accumulation has already taken place in the liver. This can be achieved by exploiting the systemic interactions between Cu, Mo and S.

Relatively little information is available on the effect of different levels of Mo supplementation on Cu metabolism under conditions of Cu toxicity where results have not been confounded by other treatment effects. A trial was carried out in which sheep received different levels of dietary Mo as well as high levels of Cu and S to follow the effect of these

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treatments on the Cu and Mo metabolism in the body once Cu had accumulated in the liver.

EXPERIMENTAL

Animals, treatments and procedure

Forty South African Mutton Merinos (twenty wethers and twenty ewes), approximately 1 year of age, were randomly allocated within sex to five groups. During a pre-experimental period of 42 d all groups received a basic ration high in Cu and S but low in Mo. At the end of this period one group, the pre-experimental slaughter group (Pr–X), was slaughtered, while the other groups were fed on rations containing different levels of Mo for a further 193 d: no-Mo, low-Mo, medium-Mo, high-Mo.

The groups were group-fed once daily in concrete-floored pens. Tap water was available *ad lib.* The ration consisted of milled hay (predominantly *Themeda triandra*) and a concentrate mixture comprising (g/kg): 355 maize meal, 299 sunflower-oil-cake meal, 299 sterilized poultry manure, 30 urea, 15 hydrated sodium sulphate, 0·955 cupric sulphate pentahydrate. Different levels of Mo in the form of $(NH_4)_6Mo_7O_{24}.4H_2O$ were included in the concentrate mixture according to treatment resulting in Mo concentrations (mg/kg) in the experimental rations of: 0·6 no-Mo, 21·4 low-Mo, 39·6 medium-Mo, 60·3 high-Mo.

The sheep were weighed once monthly before the daily feeding and jugular blood samples were taken at regular intervals throughout the trial. At the end of the trial a wool sample was collected from the side of each sheep, the dirty tips were cut off and the remainder washed and the fat extracted. At slaughter, livers, kidneys, spleens, lungs, hearts and 50 × 50 mm muscle samples from the M. longissimus dorsi were collected from the sheep. Samples were dried at 80° for 4 d. These dried samples were ground and kept for further analyses. Fat extractions with diethyl ether were done on the heart and muscle tissues before analysis.

Analytical methods

The following analyses were performed according to the methods described by Van Ryssen & Stielau (1980): dietary Mo, Cu, zinc, iron, manganese, S, calcium, phosphorus and crude protein (nitrogen × 6·25); Mo and total Cu in organs and plasma; packed cell volume (PCV) and haemoglobin (Hb) in whole blood.

Erythrocyte (RBC) counts on whole blood were done using a Coulter counter (Coulter Electronics, Inc. Hialeah, Florida). Serum aspartate aminotransferase (*EC* 2.6.1.1; GOT) and lactate dehydrogenase (*EC* 1.1.1.27; LDH) levels were determined using Boehringer Mannheim standard kits (Boehringer Mannheim GmbH Diagnostica) on a Gemsaec Fast Analyser (Electro-Nucleonics). Plasma arginase (*EC* 3.5.3.1) was estimated according to the method described by Schwartz (1971), total serum protein and albumin by the Biuret method described by Cornelius & Karneko (1963) and direct-reacting Cu in plasma according to the method of Suttle & Field (1968).

Statistical analyses

Statistical analyses (F test and Student's *t* test) were done according to the methods described by Rayner (1967). Where differences were large and obviously different, no statistical comparisons were considered necessary, though for the sake of simplicity in Tables 1 and 2, these differences are indicated as being significant according to the F test.

RESULTS

Food intakes and composition of ration

Each sheep consumed an average of 274 g dry matter (DM) in the form of the concentrate mixture and 698 g DM as hay/d. The average Mo intakes (mg/d) were: 0·6 no-Mo, 20·8

Table 1. *The influence of different levels of molybdenum intake on the concentration of copper in the tissues and wool of sheep fed on high levels of Cu*

Treatment groups ...	Pr-X	No-Mo	Low-Mo	Medium-Mo	High-Mo	
Treatment no. ...	1	2	3	4	5	Effect of
Mo intake (mg/d) ...	0·2	0·6	20·8	38·4	58·5	treatment**
Liver						
mg/kg DM	608	987	812	660	835	4 < 2 1 < 2, 5*
SEM	48	80	58	62	63	
Kidney cortex						
mg/kg DM	16	26	26	94	236	5 > 4 > 3, 2, 1
SEM	0·8	1·9	2·3	15·2	36·6	
Kidney medulla						
mg/kg DM	—	8·2	8·4	14·9	40·4	5 > 4 > 3, 2
SEM	—	0·9	0·3	1·5	4·4	
Lungs						
mg/kg DM	13·0	11·5	11·5	12·5	17·4	5 > 4, 3, 2, 1
SEM	0·8	0·6	0·4	0·5	0·9	
Spleen						
mg/kg DM	3·7	2·2	4·1	3·7	13·0	5 > 4, 3, 2, 1 2 < 4*, 3, 1*
SEM	0·4	0·3	0·4	0·5	1·8	
Muscle, L dorsi (fat-free)						
mg/kg DM	6·2	5·0	4·6	5·6	6·1	5 > 3, 2* 1 > 3*
SEM	0·5	0·3	0·3	0·4	0·2	
Muscle, heart (fat-free)						
mg/kg DM	19·4	18·8	18·8	22·6	27·4	5 > 4* > 3, 2, 1
SEM	0·5	0·9	0·6	0·6	0·8	
Wool (fat-free)						
mg/kg DM	—	6·5	6·0	6·8	5·7	NS
SEM	—	0·6	0·5	0·9	0·3	

Pr-X, pre-experimental slaughter group; NS, not significant; DM, dry matter.
* $P < 0.05$, ** $P < 0.01$.

low-Mo, 38·4 medium-Mo, 58·5 high-Mo. Cu intakes of approximately 82 mg/sheep per d were recorded. The mean daily mineral intakes per sheep were (g): 0·06 Zn, 0·30 Fe, 0·24 Mn, 3·8 S, 10·2 Ca, 4·3 P. The average Cu:Mo values in the rations were: 3·9 low-Mo, 2·2 medium-Mo and 1·4 high-Mo. The average Ca:P in the food was 2·4:1 while the crude protein intake per sheep was 122 g/d during the trial.

Body-weight and clinical condition

The final average body-weights (kg) of the sheep were: 33·9 no-Mo, 35·1 low-Mo, 34·8 medium-Mo, 33·4 high-Mo; the differences were not statistically significant.

One sheep from the high-Mo group and one from the medium-Mo group developed diarrhoea on the 2nd day after the inclusion of Mo in the diets. This diarrhoea lasted for 2 d. No further incidence of diarrhoea was observed in any sheep for the rest of the trial. One ewe (low-Mo) died, apparently due to cardiovascular shock. No apparent clinical abnormality due to the high Cu intakes was evident.

Organs and tissues

No trend in the accumulation of minerals in organs could be related to the sex of the sheep. Values from both sexes were, therefore, pooled in the analyses.

Table 2. *The influence of different levels of molybdenum intake on the concentration of Mo in the tissues and wool of sheep fed on high levels of copper*

Treatment groups ...	Pr-X	No-Mo	Low-Mo	Medium-Mo	High-Mo	
Treatment no. ...	1	2	3	4	5	Effect of
Mo intake (mg/d) ...	0·2	0·6	20·8	38·4	58·5	treatment**
Liver						
mg/kg DM	2·9	2·5	5·6	11·0	21·2	5 > 4 > 3 > 2, 1
SEM	0·2	0·4	0·3	1·4	2·5	
Kidney cortex						
mg/kg DM	1·5	1·7	6·5	54·7	136·5	5 > 4 > 3 > 2, 1
SEM	0·1	0·1	0·7	8·6	20·5	
Kidney medulla						
mg/kg DM	—	1·4	2·3	7·1	24·1	5 > 4 > 3 > 2
SEM	—	0·2	0·4	0·9	3·1	
Lungs						
mg/kg DM	0·19	0·58	1·23	2·39	6·25	5 > 4 > 3 > 2 > 1
SEM	0·07	0·03	0·10	0·16	0·55	
Spleen						
mg/kg DM	0·49	0·24	0·84	3·42	11·28	5 > 4 > 3 > 2, 1
SEM	0·02	0·03	0·19	0·36	1·30	
Muscle, L dorsi (fat-free)						
mg/kg DM	0·20	0·18	0·15	0·44	0·94	5 > 4 > 3, 2, 1
SEM	0·10	0·07	0·07	0·07	0·15	
Muscle, heart (fat-free)						
mg/kg DM	0·12	0·05	0·43	1·22	1·74	5, 4 > 3 > 2, 1
SEM	0·05	0·02	0·06	0·37	0·55	
Wool (fat-free)						
mg/kg DM	—	0·38	0·37	0·45	1·01	5 > 4, 3, 2
SEM	—	0·15	0·10	0·15	0·09	

Pr-X, pre-experimental slaughter group; DM, dry matter.
** $P < 0·01$

Table 3. *The influence of level of molybdenum intake on copper:Mo in the kidneys of sheep fed high levels of Cu*

	Cortex		Medulla	
Treatments	Cu:Mo	*r*	Cu:Mo	*r*
Pr-X	10·67	0·03	—	—
No-Mo	15·29	−0·02	5·86	−0·33
Low-Mo	4·54	0·84	3·65	0·06
Medium-Mo	1·71	0·98	2·10	0·87
High-Mo	1·73	0·92	1·66	0·88

Pr-X, pre-experimental slaughter group.

Cu. The addition of Mo up to a level of 38 mg/sheep per d resulted in marked reductions in liver Cu content (Table 1). Despite wide variations in liver Cu concentrations within treatment groups, differences between the no-Mo and medium-Mo groups were statistically significant ($P < 0·001$). However, at an Mo intake of 58 mg/sheep per d elevated Cu levels were again observed, higher than the liver Cu levels of the low-Mo group. At the 38 mg Mo/kg per d level increased kidney Cu levels were recorded. A slight increase in Cu also occurred in the heart muscle. This trend of Cu accumulation was obvious in all tissues with 58 mg Mo/d and was very pronounced in the kidneys, especially in the kidney cortex.

Table 4. *The influence of different levels of molybdenum on the average concentrations during the experiment of total plasma copper (mg/l), direct-reacting Cu (DR Cu; mg/l) and total plasma Mo (mg/l) of sheep fed on high levels of Cu*

Treatment groups ...		No-Mo	Low-Mo	Medium-Mo	High-Mo	
treatment no. ...		2	3	4	5	Effect of
Mo intake (mg/d) ...		0·6	20·8	38·4	58·5	treatment**
Cu						
Pr-X period:						
Total Cu	Mean	0·90	0·90	0·98	0·92	NS
	SEM	0·02	0·02	0·06	0·03	
Experimental period:						
Total Cu	Mean	0·95	0·96	1·25	1·65	5 > 4 > 3, 2
	SEM	0·02	0·02	0·03	0·05	
DR Cu	Mean	0·22	0·27	0·59	0·97	5 > 4 > 3, 2
	SEM	0·01	0·01	0·02	0·04	
Difference		0·73	0·69	0·66	0·68	NS
Mo						
Pr-X period	Mean	0·13	0·12	0·12	0·11	NS
	SEM	0·08	0·04	0·02	0·04	
Experimental period	Mean	0·06	0·12	0·54	0·92	5 > 4 > 3, 2
	SEM	0·02	0·03	0·04	0·05	

Pr-X, pre-experimental slaughter group; NS, not significant
** $P < 0·01$

Mo. Any increase in level of Mo supplementation was reflected in the concentrations of Mo in the organs and tissues (Table 2). An increase of approximately 20 mg Mo/d was sufficiently high to cause a statistically significant increase ($P < 0·01$) in the Mo concentration of the tissues. The most pronounced increase in Mo concentration was recorded in the kidney cortex. In this respect, Cu and Mo behaved similarly.

Cu:Mo. High positive correlations were observed between Cu and Mo concentrations in the kidney cortex in the groups receiving additional Mo (Table 3). Similarly, high correlations between Cu and Mo in the renal medulla were observed at the two highest Mo intakes.

Blood analyses

Plasma Cu and Mo. Significant increases in total plasma Cu levels were obtained during the experimental period in the medium- and high-Mo treatments when compared with the low- and no-Mo treatments (Table 4). These differences seemed to be due mainly to the direct-reacting Cu fraction of plasma because the differences between total and direct-reacting Cu remained practically constant for all treatments.

The concentration of Mo in plasma (Table 4) followed the same pattern as the Mo concentrations in other tissues. At higher Mo intakes Mo concentrations in plasma were significantly ($P < 0·01$) increased, with a greater difference between the medium- and high-Mo intakes than between the low- and medium-Mo intakes.

Haematological measurements. Throughout the experiment the PCV, Hb levels (g/l) and RBC counts ($\times 10^6$/ml) remained relatively constant without any statistically significant differences between treatments or any indication of anaemia in the sheep. The average values for the experimental period were: PCV, 0·29; Hb, 109; RBC count, 8·6.

Serum protein and enzyme levels. Total protein and albumin levels in the serum were determined on four occasions. These values remained practically constant at all analyses (Table 5). None of the differences due to treatment was statistically significant.

Serum GOT, LDH and plasma arginase levels were determined at various stages during

Table 5. *Average serum protein fractions (g/l), serum aspartate aminotransferase* (EC 2.6.1.1; GOT), *serum lactate dehydrogenase* (EC 1.1.1.27; *LDH*) *and plasma arginase* (EC 3.5.3.1) *levels in the blood of sheep receiving high levels of dietary copper and different levels of molybdenum*

Treatment groups	Protein		GOT U/l*		LDH U/l*		Arginase IU†
	Total	Albumin	First 125d	Last 110d	First 125d	Last 110d	Last 130d
No-Mo							
Mean	61	36	97	161	952	1134	240
SEM	0·83	1·00	15	57	90	153	—
Low-Mo							
Mean	60	34	90	98	871	923	119
SEM	0·79	0·96	16	15	86	62	—
Medium-Mo							
Mean	62	34	70	75	792	772	113
SEM	1·37	1·23	12	5	38	32	—
High-Mo							
Mean	63	37	75	88	812	879	122
SEM	0·99	1·00	10	12	23	46	—

* 1 U indicates the amount of enzyme which brings about the reaction of 1 μM substrate/min.
† 1 IU of arginase activity is the amount of arginase required to form 1 μM urea/h.

the trial. Average values per treatment at different stages of the trial are presented in Table 5. No statistical analyses were done on these results because individual animals within groups showed dramatic increases in enzyme levels, resulting in non-uniform distribution of the results. Elevated enzyme levels were observed in five of the eight sheep in the high-Cu, no-Mo treatments. In two of these sheep the increases were quite dramatic and were first recorded 110 d before the end of the trial. The result was that the mean enzyme levels of this group were well above the levels observed in the other groups. However, none of the sheep succumbed to or showed any other signs of Cu toxicity. Slightly-elevated enzyme levels were measured in one sheep each from the low- and high-Mo treatment groups; the sheep in the high-Mo group showed the elevated enzyme levels from the onset of the trial. This result appears to be unrelated to any treatment effect.

DISCUSSION

An increase in hepatic Cu content due to Mo supplementation, as observed at the high levels of dietary Mo, is contrary to the accepted behaviour of the Cu–Mo–S interaction in sheep (Dick, 1954; Wynne & McClymont, 1956; Ross, 1966). The fact that this increase was observed only at the higher dietary levels of Mo, suggests that it corresponds with the so-called systemic effects of the Cu–Mo–S interaction as observed in the plasma and kidneys (Dick, 1956; Suttle, 1974; Van Ryssen & Stielau, 1980). These elevated Cu levels in the kidneys and plasma have been suggested to be the result of the accumulation of Cu–Mo-containing compounds in these tissues in forms unavailable to the body (Suttle, 1974). The high correlations between Cu and Mo in the kidneys as observed by Bremner & Young (1978), Van Ryssen & Stielau (1980) and in the present trial support the suggestion of Suttle (1974).

In the present trial, at a fixed level of S, Mo concentrations in the tissues followed a pattern of accumulation which corresponded to the daily Mo intake, an observation similar to that of Lesperance & Bohman (1963). Grace & Suttle (1979) demonstrated that poorly-

exchangeable Mo compounds, formed with high Mo plus S intakes, were ineffectively excreted by either the urinary or faecal routes in sheep and suggested that the poor excretion of Mo was due to the formation of Cu-containing thiomolybdates in ruminants.

The results of the present trial may suggest that complexes containing Cu and Mo tend to accumulate mainly in the kidneys but at high Mo intakes also in the other organs, provided high levels of Cu, Mo and S are fed to sheep. In the present trial the accumulation of Cu in tissues took place at Mo intakes less than 60 mg/sheep per d. When high concentrations of the proposed Cu–Mo complexes accumulate in tissues, e.g. in the kidneys, there is a close relationship between Cu and Mo concentrations. However, the presence of other forms of Cu in other tissues will mask any measurable relationship between Cu and Mo in these tissues, e.g. the liver.

The accumulation of Cu in tissues, especially the liver, at high Mo intakes has been reported in non-ruminants (Miller *et al.* 1956; Mills, 1960; Arthur, 1965; Dale *et al.* 1973). The formation of compounds containing Cu and Mo in the proportion 1·5:1 was observed by Mills & Mitchell (1971) in liver tissues of rats receiving high levels of Cu and Mo. Suttle (1974) concluded from work on non-ruminants, that dietary Mo concentrations below 100 mg/kg food would decrease the Cu status of the animal, but above 100 mg Mo/kg food the more familiar response, namely increased tissue Cu concentrations accompanied by symptoms of clinical Cu deficiency, pertains.

In ruminants the concept that Cu and Mo may be present in the body in unavailable forms seems to be accepted (Cook *et al.* 1966; Ward, 1978). However, if Cu levels are only measured in the liver, kidney and plasma and at only two levels of Mo supplementation (Van Adrichem, 1965; Ross, 1966, 1970; Marcilese *et al.* 1969; Van der Berg & van der Schee, 1973; Bremner & Young, 1978), the accumulation of Cu in tissues, except the kidneys and plasma, may not be detected. Any accumulation of bound Cu in the liver will not be noticeable due to a concomitant decrease in total live Cu content brought about by Mo supplementation; this decrease may be more rapid than the Cu accumulation. The continuous decrease in hepatic Cu content in cattle at increasing levels of dietary Mo, as reported by Vanderveen & Keener (1964) and Huber *et al.* (1971) may have been due to the lack of Cu in the diet. In the present trial no sign of Cu deficiency was observed in any of the haematological factors tested or in the Cu content of any tissues.

Dick (1954) observed a constant hepatic Cu retention in sheep at Mo intakes of between 20 and 100 mg/sheep per d. With an S intake of 0·46 g/sheep per d in that trial, it is possible that S was a limiting factor for the full expression of the Cu–Mo–S interaction. In contrast S intake in the present trial was more than 3 g/sheep per d.

Although liver Cu levels are considered to be useful indicators of the Cu status of ruminants (MacPherson & Hemingway, 1965; Ross, 1966), it is clear from the present trial that liver Cu levels may become unreliable factors at very high Mo intakes.

After the commencement of Mo feeding in the present trial, two sheep experienced diarrhoea for a few days. Severe diarrhoea was observed by Suttle & Field (1968) in sheep receiving 50 mg Mo and 10 g sulphate/kg food. Diarrhoea as a sign of molybdenosis is usually observed only in cattle fed on high-Mo rations (Cunningham & Hogan, 1959; Underwood, 1977; Ward, 1978). A reduction in plasma protein was suggested by Smith & Wright (1975) as indicating that Mo supplementation affected protein metabolism. No differences in plasma protein or albumin were observed in the present trial. Bremner & Young (1978) suggested that the accumulation of Mo in the tissues and plasma had a toxic effect and was the reason for reduced growth rates observed in sheep receiving additional Mo. It is possible that the forms in which Mo accumulated in tissues in sheep in the present trial were different from those observed by Bremner & Young (1978) with low S intakes, explaining why growth was not affected.

It is believed that the Mo in the tissues of the sheep in the present trial accumulated in forms unavailable to the body. Any form of tissue damage or interference with protein metabolism due to the presence of Mo therefore seems unlikely. This is supported by evidence from the plasma enzyme levels where little change was observed in any of the Mo-supplemented groups. Elevated enzyme levels were observed in some of the sheep on the high-Cu and no-Mo treatments. From the plasma arginase levels it may be concluded that tissue damage took place mainly in the livers of the affected sheep (Van Adrichem, 1965; Ross, 1966). The extended period during which these elevated enzyme levels were observed without the occurrence of a haemolytic crisis due to Cu toxicity is contrary to the maximum period of 8 weeks reported before a haemolytic crisis can be expected (Todd & Thompson, 1963; Ross, 1966).

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REFERENCES

Arthur, D. (1965). *J. Nutr.* **87**, 69.
Barden, P. J. & Robertson, A. (1962). *Vet. Rec.* **74**, 252.
Bracewell, C. D. (1958). *Vet. Rec.* **70**, 342.
Bremner, I. & Young, B. W. (1978). *Br. J. Nutr.* **39**, 325.
Cook, G. A., Lesperance, A. L., Bohman, V. R. & Jensen, E. H. (1966). *J. Anim. Sci.* **25**, 96.
Cornelius, C. E. & Karneko, J. J. (1963). *Clinical Biochemistry of Domestic Animals.* New York: Academic Press.
Cunningham, I. J. & Hogan, K. G. (1959). *N.Z. Jl agric. Res.* **2**, 134.
Dale, S. E., Ewan, R. C., Speer, V. C. & Zimmerman, D. R. (1973). *J. Anim. Sci.* **37**, 913.
Dick, A. T. (1954). *Aust. J. agric. Res.* **5**, 511.
Dick, A. T. (1956). In *Inorganic Nitrogen Metabolism*, p. 445 [W. D. McElroy and B. Glass, editors]. Baltimore: Johns Hopkins Press.
Grace, N. D. & Suttle, N. F. (1979). *Br. J. Nutr.* **41**, 125.
Huber, J. T., Price, N. O. & Engel, R. W. (1971). *J. Anim. Sci.* **32**, 364.
Huisingh, J. & Matrone, G. (1976). In *Molybdenum in the Environment*, vol. 1, p. 125 [W. R. Chappell and K. K. Petersen, editors]. New York: Marcel Dekker Inc.
Lesperance, A. L. & Bohman, V. R. (1963). *J. Anim. Sci.* **22**, 686.
MacPherson, A. & Hemingway, R. G. (1965). *J. Sci. Fd Agric.* **16**, 220.
Marcilese, N. A., Ammerman, C. B., Valsecchi, R. M., Dunavant, B. G. & Davis, G. K. (1969). *J. Nutr.* **99**, 177.
Miller, R. F., Price, N. O. & Engel, R. W. (1956). *J. Nutr.* **60**, 539.
Mills, C. F. (1960). *Proc. Nutr. Soc.* **19**, 162.
Mills, C. F. & Mitchell, R. L. (1971). *Br. J. Nutr.* **26**, 117.
Rayner, A. A. (1967). *Biometry for Agriculture Students.* Pietermaritzburg: University of Natal Press.
Ross, D. B. (1966). *Br. vet. J.* **122**, 279.
Ross, D. B. (1970). *Res. vet. Sci.* **11**, 295.
Schwartz, M. K. (1971). Clinical aspects of arginase. In *Methods in Enzymology*, vol. 17B, p. 857 [P. Colowick and N. O. Kaplan, editors]. New York: Academic Press.
Smith, B. S. W. & Wright, H. (1975). *Clinica chem. Acta* **62**, 55.
Suttle, N. F. (1974). *Proc. Nutr. Soc.* **33**, 299
Suttle, N. F. (1975). *Br. J. Nutr.* **34**, 411.
Suttle, N. F. & Field, A. C. (1968). *J. comp. Path.* **78**, 351.
Todd, J. R. & Thompson, R. H. (1963). *Br. vet. J.* **119**, 161.
Underwood, E. J. (1977). *Trace Elements in Human and Animal Nutrition*, 4th ed. New York: Academic Press.
Van Adrichem, P. W. M. (1965). *Tijdschr. Diergeneesk.* **90**, 1371.
Van der Berg, R. & van der Schee, W. (1973). *Tijdschr. Diergeneesk.* **97**, 378.
Vanderveen, J. E. & Keener, H. A. (1964). *J. Dairy Sci.* **47**, 1224.
Van Ryssen, J. B. J. (1979). *S. Afr. J. Anim. Sci.* **9**, 21.
Van Ryssen, J. B. J. & Stielau, W. J. (1980). *S. Afr. J. Anim. Sci.* **10**, 49.
Ward, G. M. (1978). *J. Anim. Sci.* **46**, 1078.
Wynne, K. N. & McClymont, G. L. (1956). *Aust. J. agric. Res.* **7**, 45.

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