The influence of breed and dietary molybdenum on the concentration of copper in tissues of sheep

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The accumulation and retention of copper (Cu) in the livers of Ile de France and South African Mutton Merino lambs were compared at four levels of dietary molybdenum (Mo) supplementation, *viz.* 0, 2, 4 and 8 mg Mo/lamb/day in a 2 × 4 factorial experiment. Individual lambs consumed an average of 36,3 mg Cu and 3,7 g sulphur per day. The accumulation of Cu and Mo in the kidney cortices and in the livers of the lambs was measured. At each level of Mo intake the Ile de France accumulated and retained more (59,8% $P<0,01$) dietary Cu in their livers than the Mutton Merinos. In the 0 Mo treatment the Ile de France retained 1,96% of their dietary Cu in their livers and the Mutton Merinos 1,30% ($P<0,01$); at the 2 Mo level 1,64% vs 1,04% ($P<0,05$); at 4 Mo 0,77% vs 0,40% ($P>0,05$) and at 8 Mo intake 0,25% vs 0,16% ($P>0,05$) respectively. The slopes of the regressions depicting Mo intake vs percentage hepatic Cu retention were not significantly different between the two breeds. Significant ($P<0,05$) increases in kidney cortex Cu and Mo, liver Mo and plasma Cu concentrations were observed at the 8 mg Mo intake above the other Mo treatments although no breed effect was observed.

Die akkumulering en retensie van koper (Cu) in die lewers van Ile de France- en Suid-Afrikaanse Vleismerinolammers is by vier peile van molibdeen(Mo)-byvoeging nl. 0, 2, 4 en 8 mg Mo/lam/dag in 'n 2 × 4-faktoriale-proefontwerp vergelyk. Die lammers het gemiddeld 36,3 mg Cu en 3,7 g swawel per dag ontvang. Die opbou van Cu en Mo in die skape se nierkortekse en lewers is bepaal. Op elke peil van Mo-inname het die Ile de France-lammers meer (59,8% $P<0,01$) Cu in hul lewers versamel as die Vleismerino-lammers. Op die 0 Mo-byvoegingspeil was die retensie van Cu in die lewers van die Ile de France-lammers 1,96% teenoor 1,30% ($P<0,01$) in die Vleismerino-lammers op die 2 Mo-peil 1,64% teenoor 1,04% ($P<0,05$), by 4 Mo-inname 0,77% teenoor 0,40% ($P>0,05$) en by 8 Mo-inname 0,25% teenoor 0,16% ($P>0,05$) respektiewelik. Die hellings van die regressielyne wat Mo-inname teenoor persentasie lewer-Cu-retensie uitdruk, het nie betekenisvol tussen die twee rasse verskil nie. Op die 8 Mo-behandelingspeil is betekenisvolle ($P<0,05$) verhogings in die Cu- en Mo-peile van die nierkorteks, Mo-peile in die lewers en Cu in plasma teenoor die ander behandelings waargeneem. Geen raseffek is in die metings waargeneem nie.

Keywords: Copper metabolism, copper retention, sheep breeds, molybdenum-copper nutrition

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Introduction

In 1941 Edgar, Hindmarsh, Keast & Rose reported that British sheep breeds and their crosses were more susceptible to copper (Cu) toxicity than Merino types. Marston & Lee (1948) confirmed this finding with a comparison between the Merino and Border Leicesters. Since then the existence of genetic differences between sheep breeds in Cu metabolism has been well established (Wiener & Woolliams, 1983). Many British and European sheep breeds have been compared for their ability to accumulate Cu in their livers and kidneys (Wiener & Field, 1969; Luke & Marquering, 1972; Herbert, Wiener & Field, 1978 ; Woolliams, Suttle, Wiener, Field & Woolliams, 1982; van der Berg, Levels & van der Schee, 1983) and in their capacity to absorb Cu from the digestive tract (Wiener, Suttle, Field, Herbert & Woolliams, 1978). Distinct breed differences were observed with the Welsh Mountain breed showing a much higher ability to absorb and accumulate Cu in the liver than the Scottish Blackface (Wiener & Field, 1969; Herbert, *et al.*, 1978; Woolliams, Wiener, Suttle & Field, 1983), the Texel higher than the Finnish Landrace and other European breeds (van der Berg, *et al.*, 1983) and the Texel higher than the Scottish Blackface and other British and European crosses (Woolliams, *et al.*, (1982). Wiener, *et al.* (1978) found that the North Ronaldsay breed from the Orkney Islands has such a high ability to absorb dietary Cu that the breed will suffer from Cu toxicosis on a 'normal' sheep diet.

Differences in blood and plasma Cu levels were also observed between sheep breeds. The breeds showing the higher liver Cu levels also had the highest blood Cu and plasma Cu levels (Wiener & Field, 1969, 1970, 1974; Wiener & Woolliams, 1983). Woolliams, Wiener, Woolliams and Suttle (1985) bred different strains according to plasma Cu concentration, a high and a low line, and found from hepatic Cu levels that a corresponding high Cu-accumulating and low Cu-accumulating strain had resulted.

In South African sheep breeds Bath (1979) suggested that a chronic copper toxicity is observed more frequently in Dorpers than in Merinos. It has also been observed, but never documented that the Ile de France breed is the most susceptible of all the breeds to copper toxicity in South Africa (Bath, 1984 personal communication). In Holland, van der Berg, *et al.* (1983) compared the Ile de France × Finnish Landrace cross with other pure and crossbred sheep and found this cross to have a low rate of Cu accumulation in the liver relative to the Texel, Texel crosses, and the Flemish milk sheep. Visscher, Garssen & Zaalmink (1980) reported a similar

observation that the Ile de France showed intermediate rates of hepatic Cu retention compared to the high Cu retention of the Texel and the low retention of the Finnish Landrace.

The present trial was conducted to compare the ability of the Ile de France and the South African Mutton Merino to retain Cu in their livers. The lambs were fed different levels of molybdenum (Mo) to compare the magnitude of the possible breed effect at the different levels of Mo and thus possible differences in response when Mo is used to counter Cu toxicity.

Materials and Methods

Animals, treatments and procedure

Liver biopsies (Dick, 1944) were taken from 30 Ile de France and 33 South African Mutton Merino ewe lambs aged 9 months with live masses of *ca* 38 kg. Six Ile de France and nine Mutton Merino lambs with the most extreme liver Cu concentrations (following biopsy assay) were sacrificed as a pre-experimental slaughter group to determine the relationship between liver mass and body mass of the lambs at the onset of the trial. The remaining lambs (24 per breed) were ranked (within breed) according to liver Cu levels and divided into six groups of four animals. Within each group the lambs were allocated at random to four dietary treatments, thereby ensuring that the mean liver Cu concentrations of the treatment groups within a breed were approximately equal at the onset of the trial. The treatments were Mo (as $(NH_4)_6 Mo_7O_{24}.4H_2O$) included in the diet to supply 0, 2, 4, and 8 mg added Mo per lamb per day and were designated the 0 Mo, 2 Mo, 4 Mo and 8 Mo treatments.

The lambs were fed individually and feed intakes were recorded. Each lamb received 400 g of a concentrate to which the minerals were added and 600 g milled hay (*Themeda triandra*). Copper sulphate (as $Cu SO_4.5H_2O$) and sodium sulphate (as Na_2SO_4) were added to the concentrate to provide at least 30 mg Cu per sheep per day and to establish a final dietary concentration of *ca* 0,5% S. A second liver biopsy sample was collected from each sheep 45 days after commencement of the trial, and the lambs slaughtered on day 125. Bodymasses were recorded every 3 weeks and jugular blood samples were taken periodically throughout the trial. At slaughter the livers and kidneys were collected and their fresh masses and dry matter contents were determined. Dried samples from these organs were stored for further analyses.

Analytical methods

The Cu,iron (Fe), zinc (Zn) and manganese (Mn) concentrations in the feed and tissues and the Cu concentration in plasma were determined by atomic absorption spectrophotometry after acid digestion. The calcium (Ca), phosphorus (P) and crude protein content of the feeds were determined with an auto analyzer (Technicon Auto Analyzer II, New York). Molybdenum was measured by the method as modified by van Ryssen (1979) and S by the magnesium nitrate method (AOAC, 1980). The method of Smith & Wright (1975) was followed for the determination of trichloro-acetic acid (TCA) soluble Cu in plasma. The haemoglobin (Hb) content, using a Sigma cyanometh-hemoglobin kit, and packed cell volume (PCV) using a microhematocrit, were measured on fresh whole blood. Plasma aspartate transaminase (AST; EC 2.6.1.1.) levels were estimated using a commercial kit (Boehringer Mannheim GmbH Diagnostica, West Germany).

Statistical analyses

The experimental results were analysed as a 2 × 4 factorial design, with breed and dietary Mo constituting the treatments. The results from three lambs that died after the second biopsy were excluded from calculations. Results were subjected to analysis of variance by the 'Genstat' statistical package (Genstat V Mark 4.03 C, 1980. Lawes Agricultural Trust, Rothamstead Experimental Station). Logarithmic transformations were employed to reduce differences in variance between treatments where results showed a skewed distribution.

Results

Intakes, body and liver masses

All the lambs consumed their daily concentrate portion of the diet completely. Mean daily hay intakes were 534, 519, 521 and 519 g dry matter and the mean Mo intakes were 2,4; 4,1; 6,5 and 10,3 mg per lamb per day for the 0 Mo, 2 Mo, 4 Mo and 8 Mo groups respectively. Each lamb consumed on average 36,3 mg Cu; 458 mg Fe; 50 mg Zn; 160 mg Mn; 3,7 g S; 3,4 g Ca; 2,8 g P and 169 g crude protein per day.

At the end of the trial the dry mass of the livers of the Ile de France lambs tended to be greater than those of the Mutton Merinos, though the differences were not significant within the Mo treatments (Table 1). The mass of the dry livers of the Mutton Merino lambs slaughtered at the onset of the trial constituted 0,381% of their body mass and that of the Ile de France lambs 0,378% of body mass. The relationship between dry liver mass and live

Table 1 The live mass of the lambs at the onset and at the end of the trial and the dry liver mass at the end of the experiment.

Treatment[a]		Initial[c]	Final mass[c]	
Mo	Breed	bodymass (kg)	Body (kg)	Dry liver (kg)
0 Mo	MM	40,0	49,2	132,0
	IF	38,0	45,2	136,2
2 Mo	MM	39,4	49,6	128,9
	IF	39,2	44,8	131,4
4 Mo	MM	41,0	47,8	133,6
	IF	40,0	45,8	135,3
8 Mo	MM	39,0	47,2	135,9
	IF	39,7	45,8	130,5
SED[b]		2,01	2,18	5,30

[a] MM = SA Mutton Merino IF = Ile de France

[b] *SED* = Standard error of differences of means

[c] Within columns differences were not significant ($P>0,05$)

Table 2 Effect of dietary molybdenum (Mo) on the content and percentage retention of copper (Cu) in the livers of SA Mutton Merino and Ile de France lambs (expressed on a dry basis)

Treatments[a]		Copper content (mg) of livers at days			Dietary Cu retention[e]	
Mo	Breed	1[d]	45[d]	125	(mg)	(%)
0 Mo	MM	24	44	81	58,3	1,30
	IF	24	48	111	87,7	1,96
2 Mo	MM	25	41	71	47,2	1,04
	IF	26	44	100	74,6	1,64
4 Mo	MM	24	31	42	18,9	0,40
	IF	26	33	62	36,2	0,77
8 Mo	MM	25	27	31	7,0	0,16
	IF	26	27	36	11,0	0,25
SED[b]		5,1	3,8	12,5	10,3	0,23
LSD[c]	5%	10,3	7,7	25,5	21,0	0,58
	1%	13,8	10,3	34,1	28,1	0,62

[a] MM = SA Mutton Merino; IF = Ile de France

[b] *SED* = Standard error of differences of means

[c] *LSD* = Least significant differences

[d] Calculated from liver Cu concentration (biopsy samples) and estimated dry liver mass

[e] Measured between days 1 and 125

mass calculated from the pre-experimental slaughter group and the three lambs that died during the second biopsy were :

Dry liver mass (g) = 90,79 + 0,9439 × live mass (kg), $n = 18$, $r = 0,90$.

Liver copper

With increasing levels of dietary Mo, the rate of accumulation of Cu in the liver of the lambs was found to decrease. This was evident at both days 45 and 125. The differences in liver Cu content (Table 2) and liver Cu concentrations (Figure 1) on day 125 were not significant between the 0 Mo and 2 Mo treatments or between the 4 Mo and 8 Mo treatments. Differences between the 0 Mo/2 Mo groups and the 4 Mo/8 Mo groups were, however, significant ($P<0,01$). These differences were also more pronounced in the Ile de France lambs than in the Mutton Merino lambs (Figure 1). The retention of Cu in the liver (Table 2), shown by liver Cu content and Cu concentration, showed a similar trend resulting from Mo treatment. The response of Ile de France lambs to Mo treatment appears to be more pronounced but the slopes of the regression equations of percentage liver Cu retention versus Mo intake (Figure 2) were not significantly different. At the intercepts (zero Mo intakes, Figure 2) the differences between these two breeds in percentage

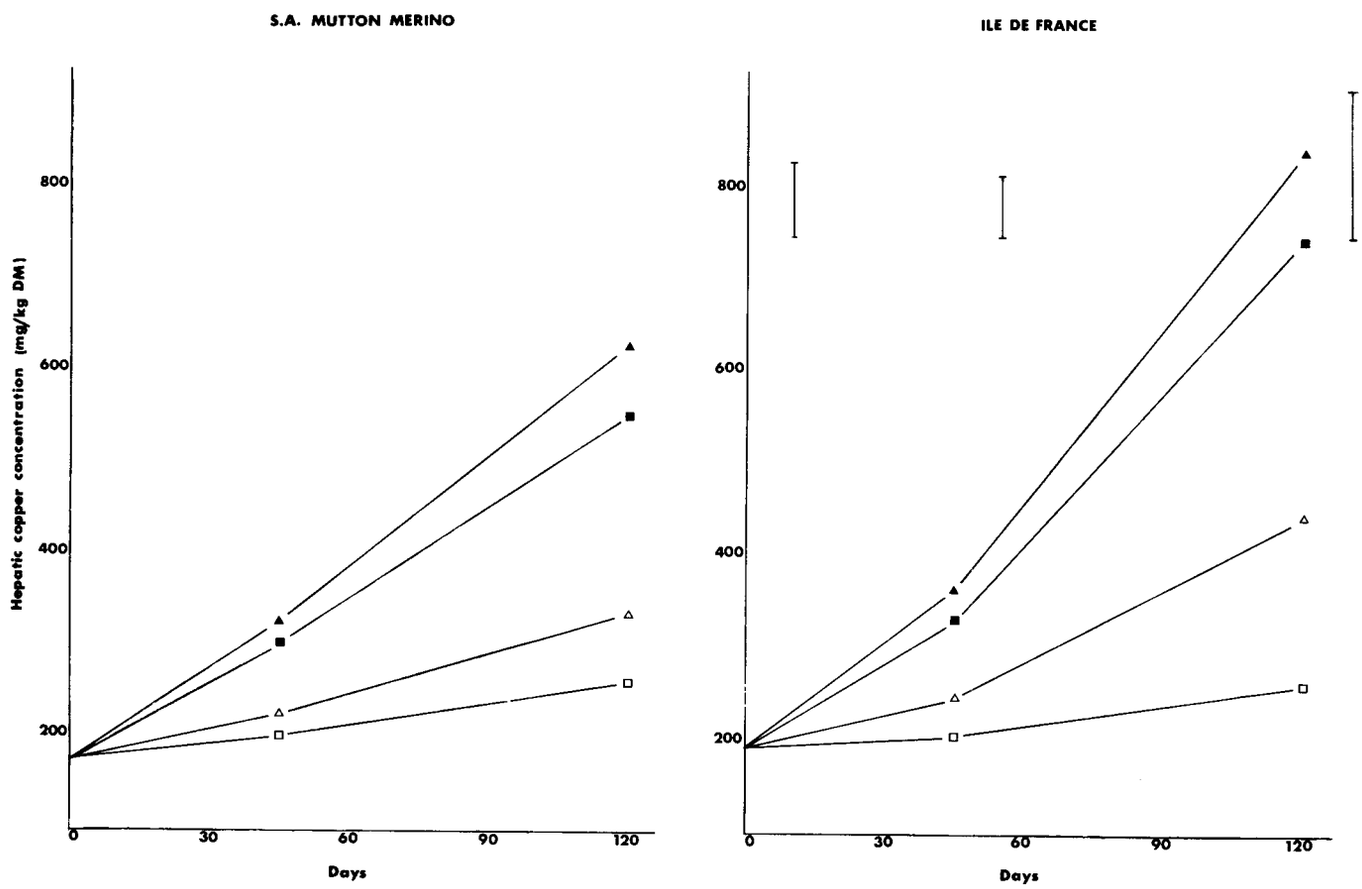

Figure 1 The influence of dietary molybdenum and breed on the concentration of copper in the livers of sheep at various stages of the trial. Treatments: ▲—▲ 2,4 mg Mo, ■—■ 4,1 mg Mo, △—△ 6,5 mg Mo and □—□ 10,3 mg Mo. Vertical bars indicate least significant differences (5%) applicable to both breeds.

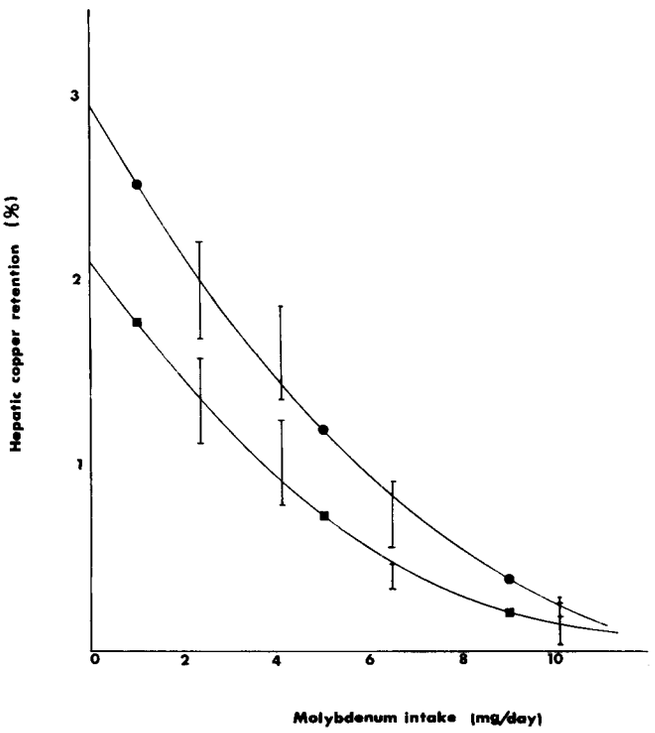

Figure 2 Effect of dietary molybdenum on the percentage retention of copper in sheep breeds. ●—● Ile de France (Y = 2,959 – 0,417x + 0,0147x^2 . ■—■ SA Mutton Merino (Y = 2,116 – 0,358x + 0,0162x^2). Vertical bars denote standard deviations. Intercepts between breeds were significantly different. Slopes of curves were not significantly different.

Cu retention in the liver were significant (P<0,01). A positive quadratic response of dietary Mo on percentage Cu retention in the liver was observed at the higher Mo intakes, though this quadratic effect was not statistically significant. When calculated at all Mo intakes in the factorial analysis, the Ile de France lambs retained an average 52,4 mg dietary Cu in their livers which was 59,8% higher (P<0,01) than the 32,8 mg Cu retained by the Mutton Merinos.

Minerals in the liver and kidneys

The concentration of Mo in the livers and kidney cortices and Cu in the kidneys showed a sharp increase (P<0,01) in the 8 Mo treatment above that in the other Mo treatments (Table 3). No breed effect was apparent. Iron, Zn and Mn levels of the livers and kidneys cortices were variable but no significant differences were observed owing to either breed or dietary treatment.

Plasma Cu and TCA soluble Cu

Plasma Cu concentrations tended to be higher in both breeds in the 8 Mo treatments than in the other treatments (Table 4). Neither dietary Mo nor breed had any significant effect on TCA soluble Cu. A slight decrease with time was evident in both plasma Cu and TCA soluble Cu.

Haemoglobin, PCV and AST

No significant differences in the haemoglobin level in whole blood (10,8 g/100 m ± 0,64) and the plasma AST concentrations (68,6 IU/± 6,54) were measured which could be ascribed to dietary Mo or breed of sheep. The PCV levels in the whole blood of the Ile de France lambs (33,7% ± 1,71) were, throughout the trial higher (P<0,01) than that of the Mutton Merinos (30,9% ± 1,69). No dietary Mo effect on PCV levels was evident.

Discussion

Liver Cu retention — breed differences

The results from the present trial confirmed the unpublished observations of a substantial difference (± 60%) between the S.A. Mutton Merino and Ile de

Table 3 Effect of molybdenum (Mo) on the concentration of copper (Cu) and Mo in the livers and kidney cortices of SA Mutton Merino and Ile de France lambs at slaughter

Treatment[a]		Liver (mg/kg DM)[c]		Kidney cortex (mg/kg DM)[c]
Mo	Breed	Mo	Cu	Mo
0 Mo	MM	3,2[e]	16,5[e]	1,45[e]
	IF	3,4[e]	17,2[e]	1,64[e]
2 Mo	MM	3,7[e]	16,0[e]	1,39[e]
	IF	3,7[e]	16,7[e]	1,42[e]
4 Mo	MM	4,3[e]	17,8[e]	1,97[e]
	IF	4,7[e]	16,0[e]	1,73[e]
8 Mo	MM	10,2[f]	27,4[f]	6,29[f]
	IF	9,7[f]	25,3[f]	5,43[f]
SED[b]		0,51	1,61	0,45

[a] MM = SA Mutton Merino; IF = Ile de France

[b] *SED* = Standard error of differences of means

[c] Values within columns with superscripts e – f differ significantly (P<0,01)

Table 4 The effect of diet and breed on the total plasma copper concentration at blood collections 1, 7, 23, 42, 71, 98 and 112 days after onset

Treatment[a]		Plasma copper concentration (mg/ml) on days						
Mo	Breed	1	7	23	42	71	98	112
0 Mo	MM	0,95	1,07	0,95	0,91	0,98	0,66	0,71
	IF	0,93	1,07	0,94	0,94	0,93	0,65	0,58
2 Mo	MM	1,13	1,13	0,98	0,98	1,38	0,71	0,66
	IF	1,02	1,11	0,93	0,96	1,00	0,71	0,73
4 Mo	MM	1,02	1,21	1,00	0,96	1,25	0,76	0,77
	IF	1,07	1,19	0,99	0,89	1,12	0,74	0,74
8 Mo	MM	0,87	1,13	0,99	1,10	1,25	0,91	0,84
	IF	0,95	1,11	0,94	1,02	1,36	0,93	0,95
SED[b]		0,18	0,13	0,11	0,13	0,17	0,09	0,09
LSD[c] 5%		0,36	0,26	0,22	0,26	0,34	0,18	0,18
LSD[c] 1%		0,49	0,35	0,29	0,35	0,46	0,24	0,24

[a] MM = Mutton Merino; IF = Ile de France

[b] *SED* = Standard error of differences of mean

[c] *LSD* = Least significant differences

France sheep breeds in ability to accumulate Cu in their livers. The effect of differences in Cu requirements due to differences in bodymass and liver size, as pointed out by Herbert, *et al.* (1978) and Wiener & Woolliams (1983) was minimal in the present trial. The reasons for the differences in hepatic Cu retention between the Ile de France lambs and Mutton Merinos could thus be a difference in ability to absorb Cu from the digestive tract (Wiener, *et al.*, 1978), though differences in endogenous excretion of Cu and in ability to accumulate hepatic Cu have also been suggested as possible reasons for breed differences (Woolliams, *et al.*, 1983).

Although the difference in ability to accumulate hepatic Cu between the Ile de France and Mutton Merino is highly significant, e.g. 1,96% vs 1,30% at the 0 Mo treatment, much larger differences were reported among British and European breeds. Van der Berg, *et al.* (1983) observed that the Finnish Landrace retained 2,5% of dietary Cu in its liver compared to 7,5% in the case of Texel sheep and 4,2% for a Finnish Landrace × Ile de France cross. Woolliams, *et al.* (1982) measured a difference of 13,7% vs 5,6% in hepatic Cu retention between Blackface × Texel and Scottish Blackface sheep respectively whilst Suttle (1977) found a hepatic Cu retention of 2,05% in the Scottish Blackface and 1,03% in the Finnish Landrace. Changes in mg Cu stored in livers per kg food consumed of 0,46 vs 0,66 were observed between Scottish Blackface and Welsh Mountain respectively (Herbert, *et al.*, 1978), and hepatic Cu retentions of 4,2% vs 7,3% between these two breeds respectively (Woolliams, *et al.*, 1983).

These differences between sheep breeds in ability to accumulate dietary Cu in their livers emphasize the greater care that must be taken in controlling dietary Cu levels in some sheep breeds to avoid the risk of Cu toxicity than in others. Van der Berg, *et al.* (1983) claimed that even a level of 10 mg Cu/kg feed is too high to prevent the accumulation of Cu in the livers of some sheep breeds. The danger of high levels of Cu in diets is especially evident in the case of concentrate rations, where it may be difficult to formulate a ration low in Cu (van der Berg & van der Schee, 1973; Hartmans, 1975; Harker, 1976).

Liver Cu retention — Mo effect

In the present trial dietary S levels were kept relatively high (3,7g/lamb/day) to minimize the chance that S may be a restricting factor in the Cu-Mo-S interaction. Suttle (1977) observed a curvilinear reduction in the percentage Cu retained in the livers of sheep with increased Mo intakes. Such a response, though not statistically significant, was also evident in the present trial, accepting that the highest Mo level in the feed was *ca* 10 mg/kg DM compared with a Mo level of 16,7 mg/kg DM in Suttle's trial. Dick (1956) measured no change in hepatic Cu retention in sheep at Mo intakes above 20 mg/sheep/day. This was suggested to be due to a low level of dietary S in their ration (van Ryssen & Stielau, 1981). Although the Ile de France breed showed a higher ($P<0,01$) Cu retention than the Mutton Merinos at the lowest level of dietary Mo in the present trial, the degree of response to Mo, as measured by the slope of the regression lines, was not significant. The differences between breeds were still present at the higher Mo intakes, but were not statistically significant. These results may therefore suggest that breed differences in Cu retention may be important at low levels of Mo intake, but become less pronounced at higher dietary Mo levels. Formulae, using Mo and S levels in feed to predict Cu absorption in sheep (Suttle & Mclauchlan, 1976; Suttle, 1983) may therefore be breed dependent at low dietary Mo levels, but far less so at higher Mo intakes. A possible explanation, assuming no breed effect in the Cu-Mo-S interaction *per se*, is that each level of dietary Mo and S may render a specific amount of Cu unavailable for absorption. At each level of Mo intake different amounts of Cu, inversely related to the Mo levels, will be available for absorption. The proportion of this available Cu being absorbed, will be breed dependent.

Copper in plasma and kidneys

The sharp rise in the Cu levels of the kidneys and plasma at the highest Mo intake as compared to the other treatments could be a 'systemic' manifestation of the Cu-Mo-S interaction in sheep (Suttle, 1974; van Ryssen & Stielau, 1980, 1981). This may indicate that a threshold level of Mo + S is required for systemic manifestation of the Cu-Mo-S interaction in the kidney, as suggested by van Ryssen & Stielau (1980).

No breed difference in plasma Cu levels was observed in the present trial, which is contrary to reports by Wiener & Field (1969, 1974), Herbert, *et al.* (1978) Woolliams, *et al.* (1983, 1985) , but in agreement with observations by van der Berg, *et al.* (1983). Significant differences in the Cu concentration of the kidneys between breeds were measured by Woolliams, *et al.* (1982, 1983) and van der Berg, *et al.* (1983), contrary to that observed in the present trial. Breed differences in kidney Cu levels observed by Woolliams, *et al.* (1982) and van der Berg, *et al.* (1983) were most pronounced with high dietary Cu treatments and corresponded with elevated plasma enzyme concentrations used to depict necrosis of tissues such as the liver. These high kidney Cu levels could in those cases reflect a more advanced pre-haemolytic crisis stage of Cu toxicity (Gopinath & Howell, 1975). rather than a breed difference in kidney Cu levels *per se*. The lack of difference between the Ile de France and Mutton Merinos in kidney Cu levels observed in the present trial as compared to the breed effect observed by Woolliams, *et al.* (1982, 1983) and van der Berg, *et al.* (1983) could also be due to the possibility that the breeds used by these workers had a greater ability to metabolize Cu than the Ile de France and Mutton Merinos used in the present trial.

Molybdenum in tissues

The concentration of Mo in the tissues of cattle and sheep was found to be related to dietary Mo levels (Lesperance & Bohman, 1963; van Ryssen & Stielau, 1981). In the present trial the dietary Mo levels were

much lower than that used by van Ryssen & Stielau (1981) and Mo concentrations in the tissues did not correspond with changes in dietary Mo. The rise in Mo levels in the liver and kidneys at the highest Mo intake could be related to the systemic effect, the accumulation of Cu present in poorly exchangeable compounds with Mo (Grace & Suttle, 1979) in the kidneys as well as the liver. This supports the suggestion by van Ryssen & Stielau (1981) that Cu in this inert form could also accumulate in the liver at high Mo intakes, but will normally not be detected. If this is the case the observed curvilinear response of hepatic Cu retention with increased dietary Mo as observed by Suttle (1977) and as indicated in the present trial, may be due partly to the accumulation of Cu in an unavailable form in the liver.

Haematology

Differences between breeds in PCV and the haemoglobin content of whole blood as observed in the present trial have been reported (Bradley, Radhakrishnan, Putil-Kulkarni & Loggins, 1973). Whether lower inherent levels could be related to an ability to withstand a haemolytic crisis period of low oxygen supply is not known.

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