STUDIES ON THE CAUSE OF A LOW COPPER STATUS IN CATTLE IN SOUTH-EASTERN QUEENSLAND

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SUMMARY

Four experiments were made to compare the copper/molybdenum/inorganic sulphate relationship in the diets of sheep and cattle and to determine if this relationship was responsible for the low copper status in cattle grazing pastures at the Animal Husbandry Research Farm at Rocklea, near Brisbane.

Experiment 1 measured changes in liver copper concentrations in four groups of two sheep on (1) a basal diet of oaten and lucerne chaff, (2) this diet supplemented with molybdenum, (3) this diet supplemented with copper plus molybdenum, and (4) this diet supplemented with copper plus molybdenum plus inorganic sulphate. The findings confirmed that sheep can increase their liver copper reserves on a ration containing 2.5 p.p.m. Cu, that a high concentration of molybdenum (23 p.p.m. Mo) does not interfere with copper metabolism when the diet is low in inorganic sulphate (0.02 per cent.) and crude protein (4.4 per cent.) and that the addition of inorganic sulphate to provide 0.45 per cent. SO_4 permits the molybdenum to interfere with copper metabolism.

Experiment 2 was similar in design to Experiment 1 but included a comparison of sheep and cattle. The basal diet contained 9 per cent. linseed meal to give a productive ration of 8.2 per cent. crude protein. Findings were similar for sheep and cattle. Both species showed little decline in liver copper on a diet containing 4 p.p.m. Cu, 0.25 p.p.m. Mo and 0.02 per cent. SO_4 The addition of molybdenum to provide 11.5 p.p.m. Mo in this protein supplemented diet caused a marked fall in liver copper although the diet was low in inorganic sulphate. The further addition of inorganic sulphate as sodium sulphate to give 0.45 per cent. SO_4 caused an increased interference with copper metabolism.

Experiment 3 included a stall feeding treatment to examine the copper/molybdenum/sulphate relationship at the levels found in freshly cut pasture, a comparison of the changes in liver copper levels in cattle fed freshly cut pasture in stalls and in cattle grazing similar pasture, and a long-term experiment to determine changes in liver copper concentrations in cattle grazing these pastures over one year. Essential findings were (1) grazing cattle showed a marked fall in liver copper reserves throughout the year, (2) this rate of decline in liver copper levels in grazing cattle was greater than in cattle stall-fed on freshly cut pasture and (3) inability to metabolize copper did not appear to be related either to the copper content or to the molybdenum level in these pastures.

Experiment 4 was firstly a measure of the rate of decline in liver copper levels in cattle grazing these pastures for 12 weeks up to the time at which the pasture was conserved as hay. The second stage was a stall-feeding experiment to examine the copper/molybdenum/sulphate relationship in a diet of pasture hay. Essential findings were (1) grazing cattle, as

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in Experiment 3, showed a marked fall in liver copper levels, (2) the decline in liver copper levels was greatest in animals during the grazing period, less in animals fed paspalum hay and least in animals fed a basal diet with a copper, molybdenum, inorganic sulphate and crude protein content similar to that of the paspalum hay and (3) as in Experiment 3 inability to metabolize copper could not be related either to the copper content or to the molybdenum level in these pastures.

From the findings of all four experiments the conclusion is drawn that the predominantly paspalum pasture at the Animal Husbandry Research Farm, Rocklea, contains some factor or factors, other than molybdenum in the presence of inorganic sulphate, which interfere with metabolism of copper by cattle.

I. INTRODUCTION

It has been known for some years that copper deficiency, as indicated by low liver copper reserves and/or low blood copper concentrations, is widespread in both sheep and cattle in Queensland (Harvey 1952). The occurrence and correction of copper deficiency in sheep in north-western Queensland were described by Moule, Sutherland, and Harvey (1959). These workers stated that copper deficiency in sheep in this locality was due partly to the low copper and partly to the high molybdenum and inorganic sulphate levels in the predominant vegetation.

Copper deficiency in cattle in south-eastern Queensland was described by Sutherland (1952). Alexander and Harvey (1957) showed that a low copper status in cattle occurred on 95 of 127 farms situated on the alluvial areas in south-eastern Queensland. Sutherland (1956) reported that cattle grazing the predominantly *Paspalum dilatatum* pasture at the Animal Husbandry Research Farm at Rocklea, Brisbane, showed very low liver copper levels. He stated also that since the copper content of the dominant pasture species was adequate, it was apparent that there must be some interference with the metabolism of copper by cattle.

Cunningham (1950) confirmed and amplified the findings of Dick and Bull (1945) that a high molybdenum intake could reduce liver copper storage in both sheep and cattle. Dick (1952, 1953) established that molybdenum in the presence of inorganic sulphate interferes with copper metabolism in sheep. However, Allcroft and Lewis (1956) reported that molybdenum and inorganic sulphate were not the only factors which limit copper storage in sheep and cattle on diets of apparently adequate copper content. Mylrea (1958) showed that the addition of sulphate to diets low in sulphate (0·03 per cent.) and containing 2·4 p.p.m. molybdenum and 7·6 p.p.m. copper caused a decline in liver copper levels in cattle. Cunningham, Hogan, and Lawson (1959) stated that cattle are more sensitive than sheep to high molybdenum levels in pasture. They suggested that the greater sensitivity of cattle is related to the low level of accompanying inorganic sulphate that is necessary to cause loss of copper.

The investigations reported in this paper were undertaken to examine the cause of the low copper status in cattle grazing the predominantly *Paspalum dilatatum* pastures at the Animal Husbandry Research Farm, Rocklea. Four experiments are reported. Experiment 1 was designed to examine the copper/molybdenum/sulphate relationship in sheep on a diet of oaten chaff plus 5 per cent. lucerne chaff. Experiment 2 was to compare the copper/molybdenum/sulphate relationship for sheep and cattle on a diet of oaten chaff, lucerne chaff and linseed meal. Experiment 3 was to examine the copper/molybdenum/sulphate relationship at the levels found in the predominantly *Paspalum dilatatum* pasture. This pasture, which was cut and fed daily, showed considerable variation in chemical composition during the experimental period. Experiment 4 was designed to evaluate the copper/molybdenum/sulphate relationship at the levels encountered in hay made from this pasture in early summer.

II. METHODS AND MATERIALS

(a) Experimental Animals

The sheep in Experiment 1 were selected from a uniform line of Merino wethers, approximately 14 months of age and obtained three months previously from the Roma district in southern Queensland. The sheep in Experiment 2 were from a uniform line of full-mouth Merino wethers which had been in the experimental flock for three years.

All cattle used in these experiments were Hereford steers sired by the same bull and born and reared on the pastures of the Animal Husbandry Research Farm, Rocklea. In Experiment 2, weaners aged 6–7 months were used. In Experiment 3 the experimental animals were 11 steers aged approximately 12 months and four steers aged 24 months. The former included the eight steers used previously in Experiment 2. The nine steers used in Experiment 4 were approximately 12 months of age.

As all cattle grazing the pastures on the Animal Husbandry Research Farm show low copper levels in liver, copper therapy by intravenous injection of copper sulphate was given approximately three weeks prior to the commencement of each experiment.

Liver biopsies on sheep were made by the techniques of Dick (1944). Liver samples from cattle were obtained by the biopsy technique of Loosmore and Allcroft (1951). The instruments described by Dick (1952) were used for biopsies in both sheep and cattle.

Blood samples were drawn from the jugular vein, using stainless steel needles; the blood was collected in copper-free bottles containing potassium oxalate.

(b) Experimental Facilities

The sheep were housed in wooden metabolism crates, and the cattle in individual stalls provided with concrete floors and individual concrete feeding troughs.

(c) Body-weight

Body-weight of sheep in Experiments 1 and 2 was measured with a clock-face spring balance with an accuracy of $\pm$ 0·5 lb. Cattle were weighed on a cattle weighing scale with an accuracy of $\pm$ 2 lb. Both sheep and cattle were weighed on all occasions at a standard time before the morning feed and prior to obtaining liver samples.

(d) Chemical Methods

Methods used in analyses of feeds were essentially those of the Association of Official Agricultural Chemists (1955). Copper in feed, blood and liver was determined by the method of Clare, Cunningham, and Perrin (1945). The method of Dick and Bingley (1951) was used for the determination of molybdenum. Inorganic sulphate was determined by the modification of the benzidene sulphate method of J. B. Bingley and A. T. Dick (personal communication 1953).

(e) Experimental Rations

A quantity of oaten chaff, lucerne chaff, linseed meal and pulverized limestone sufficient for all experiments was purchased prior to the commencement of these studies. The oaten chaff was a uniform line of poor quality containing virtually no grain. The lucerne chaff was of fair quality. Both were individual consignments from a property.

Rations were compounded in a rotary feed mixer. Additional copper, molybdenum and inorganic sulphate were in the form of chemically pure copper sulphate, ammonium molybdate and sodium sulphate respectively. The appropriate chemical was dissolved in water and the solution sprayed as a fine mist on the ration as it rotated in the feed mixer. Analyses of samples confirmed the adequacy of mixing.

The paspalum used in Experiment 3 was harvested, chaffed and fed daily. This material was cut from the paddock in which the experimental grazing group was maintained.

The paspalum hay used in Experiment 4 was prepared from the paddock in which all animals in this experiment were maintained prior to the stall-feeding portion of this study.

Representative samples for chemical analyses were collected at intervals during the course of these experiments. In Experiment 3 the dry-matter content was determined daily on the freshly cut paspalum; these dried samples were bulked and analysed at weekly intervals; all feed residues were sampled and determined as dry matter.

The proximate analyses on a dry-matter basis are shown in Table 1 for individual feeds, compounded rations, paspalum pasture and paspalum hay used in Experiments 1–4. The levels recorded for paspalum and rations in Experiment 3 are average values from weekly analyses; figures in parentheses show the range of crude protein and copper levels.

III. EXPERIMENT 1

(a) Experimental

The eight sheep were distributed at random to four groups of two sheep, and the groups were allotted at random to the treatments as follows:—

Group I: Basal ration of 94 per cent. oaten chaff, 5 per cent. lucerne and 1 per cent. limestone.

Group II: Basal ration + ammonium molybdate to increase the molybdenum content to 23 p.p.m. Mo.

Group III: Basal ration + ammonium molybdate + copper sulphate to provide 23 p.p.m. Mo and 21 p.p.m. Cu.

Group IV: Basal ration + ammonium molybdate + copper sulphate + sodium sulphate to give 23 p.p.m. Mo, 21 p.p.m. Cu and 0·45 per cent. SO_4.

Sheep were fed *ad lib.* for the 10 weeks of this experiment. Individual daily feed consumption was determined.

Liver biopsy samples for copper analyses were taken and body-weight was recorded at the commencement and at the conclusion of this experiment.

Blood samples for the determination of copper and molybdenum were drawn initially, after four weeks and at the conclusion of the 10 weeks of this experiment.

(b) Results

The concentration in the diet and the average daily intake of copper, molybdenum and inorganic sulphate of sheep are shown in Table 2.

Table 1

COMPOSITION ON DRY-MATTER OF INDIVIDUAL FEEDS, COMPOUNDED RATIONS, PASTURE AND HAY FED IN EXPERIMENTS 1 TO 4

—	Protein (%)	Fat (%)	Fibre (%)	NFE (%)	Ash (%)	Calcium (% Ca)	Phosphorus (% P)	Sulphate (% SO_4)	Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Manganese (p.p.m. Mn)
Individual Feeds—											
Oaten chaff ..	3·7	2·7	30·3	56·8	6·5	0·10	0·11	0·01	2·5	0·1	25·7
Lucerne chaff ..	17·8	1·5	30·8	41·4	8·5	0·89	0·36	0·07	6·0	0·9	33·7
Linseed meal ..	40·2	3·9	9·0	40·8	6·1	0·23	1·07	0·22	17·4	0·7	38·1
Limestone ..						39·2		0·01	2·0	0·4	17·5
Experiment 1—											
Basal ration ..	4·4	2·6	30·0	55·6	7·4	0·47	0·12	0·02	2·5	0·2	25·8
Experiment 2—											
Basal ration ..	8·2	2·7	28·3	53·7	7·1	0·56	0·22	0·02	4·0	0·2	26·9
Experiment 3—											
Basal ration (Av.)	5·5 (5·0–6·4)*	2·6	30·0	55·2	6·7	0·21	0·14	0·02	5·8 (5·0–6·5)*	0·2	26·9
Paspalum (Av.) ..	5·2 (4·2–7·0)*	1·6	37·3	48·2	7·7	0·25	0·16	0·35	5·9 (5·0–6·5)*	0·2	25·0
Experiment 4—											
Basal ration ..	7·1	2·7	29·1	54·4	6·7	0·17	0·18	0·02	6·5	0·2	28·0
Paspalum hay ..	7·2	1·2	37·6	45·4	8·6	0·15	0·22	0·35	6·5	0·1	27·4

* Figures in parentheses show the range in values obtained from weekly analyses.

Table 2

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF SHEEP IN EXPERIMENT 1

Group	Sheep No.	Concentration in Diet			Mean Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	i	2·5	0·2	0·02	0·8	<0·01	0·07
	ii	2·5	0·2	0·02	0·7	<0·01	0·06
II	iii	2·5	23·0	0·02	0·6	5·0	0·04
	iv	2·5	23·0	0·02	0·9	8·2	0·07
III	v	21·0	23·0	0·02	7·1	7·8	0·07
	vi	21·0	23·0	0·02	6·7	7·4	0·06
IV	vii	21·0	23·0	0·45	8·0	8·7	1·71
	viii	21·0	23·0	0·45	5·9	6·4	1·26

Despite the inclusion of 5 per cent. lucerne chaff and 1 per cent. limestone in the basal ration, feed intake was most unsatisfactory and it was not possible to maintain the planned daily intake of 10 mg copper and 10 mg molybdenum even though the high concentrations of 21 p.p.m. Cu and 23 p.p.m. Mo were used in the supplemented rations. Because of the unsatisfactory feed intake the experiment was terminated after 10 weeks.

Changes in body-weight and in liver copper concentrations of the individual sheep are shown in Table 3. In spite of the low copper content in the basal ration, the sheep in Group I tended to at least maintain liver copper levels. This is emphasised in Group II where although additional molybdenum was added to the basal ration the sulphate level was sufficiently low for the molybdenum to have no influence and sheep increased liver copper levels.

Table 3

BODY-WEIGHT AND COPPER CONCENTRATIONS IN LIVER OF SHEEP IN EXPERIMENT 1

Group	Sheep No.	Body-weight (lb)			Copper Concentration (p.p.m. Cu)			
		Initial	After 10 Weeks	Mean Group Change	Initial	After 10 Weeks	Change	Mean Group Change
I	i	46	42	−4	428	373	− 55	+ 53
	ii	44	40		228	389	+161	
II	iii	42	35	−5	429	496	+ 67	+ 73
	iv	46	44		281	359	+ 78	
III	v	46	43	−4	297	611	+314	+313
	vi	42	38		224	536	+312	
IV	vii	44	45	−4	487	541	+ 54	+ 60
	viii	50	43		306	372	+ 66	

In Group III the increased copper intake caused a marked increase in liver copper levels in spite of the high molybdenum content of the diet. As in Group II the inorganic sulphate level was low—0·02 per cent.

In Group IV the addition of sulphate to a ration similar to that of Group III prevented the increase in liver copper shown by sheep in Group III.

The copper and molybdenum levels in blood are shown in Table 4. The blood copper levels were similar in all groups and would be considered normal. The blood molybdenum levels were high in sheep from Groups II and III, where the diet was high in molybdenum and low in sulphate. These high levels were not attained in sheep from Group IV, where additional sulphate was added to the high molybdenum diet.

Table 4

COPPER (mg Cu/100 ml) AND MOLYBDENUM (μg Mo/100 ml) IN BLOOD OF SHEEP IN EXPERIMENT 1

Group	Sheep No.	Initial		After 4 Weeks		After 10 Weeks	
		Cu	Mo	Cu	Mo	Cu	Mo
I	i	0·09	2·0	0·12	2	0·12	2
	ii	0·11	2·0	0·12	2	0·10	11
II	iii	0·12	2·0	0·10	360	0·09	735
	iv	0·09	2·0	0·12	875	0·10	1300
III	v	0·11	2·0	0·12	1025	0·12	1675
	vi	0·12	2·0	0·12	1025	0·12	1085
IV	vii	0·13	2·0	0·14	17	0·12	30
	viii	0·10	2·0	0·13	27	0·12	30

IV. EXPERIMENT 2

(a) Experimental

The eight sheep and eight cattle were distributed at random to four groups each containing two sheep and two cattle. The groups were allotted at random to the treatments as follows:—

Group I: Basal ration of 81 per cent. oaten chaff, 9 per cent. lucerne, 9 per cent. linseed meal and 1 per cent. limestone.

Group II: Basal ration + ammonium molybdate to increase the molybdenum concentration to 11·5 p.p.m. Mo.

Group III: Basal ration + ammonium molybdate + copper sulphate to provide 11·5 p.p.m. Mo and 13 p.p.m. Cu.

Group IV: Basal ration + ammonium molybdate + copper sulphate + sodium sulphate to provide 11·5 p.p.m. Mo, 13 p.p.m. Cu and 0·45 per cent. SO_4.

Both sheep and cattle were fed slightly below appetite to minimize feed residues and thus selectivity. An attempt was made to maintain comparable feed intake for all sheep and for all cattle. Individual daily feed consumption was determined.

Body-weight was recorded initially and again at the conclusion of the 12 weeks of this experiment.

Liver samples for copper analysis were obtained at the commencement and at the conclusion of the experiment. An additional sample from cattle was taken when the animals had been on the experimental ration for six weeks.

Blood samples for the determination of copper and molybdenum were drawn initially, after four weeks, after eight weeks and at the conclusion of the 12 weeks of this experiment.

(b) Results

The concentration in the diet and the average daily intake of copper, molybdenum and inorganic sulphate of sheep and cattle are shown in Table 5. The mean feed intake of sheep was 800 g daily except for the lighter animal in Group III, which consumed an average of 700 g per day. The mean daily feed intake of cattle ranged from 4·01 kg to 4·08 kg.

Table 5

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF SHEEP AND CATTLE IN EXPERIMENT 2

Group	Animal No.	Concentration in Diet			Mean Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	Sheep 1	4·0	0·25	0·02	3·2	0·2	0·16
	Sheep 2				3·2	0·2	0·16
	Cattle 1				16·1	1·0	0·80
	Cattle 2				16·2	1·0	0·81
II	Sheep 3	4·0	11·5	0·02	3·2	9·2	0·16
	Sheep 4				3·2	9·2	0·16
	Cattle 3				16·1	46·3	0·80
	Cattle 4				16·3	46·9	0·82
III	Sheep 5	13·0	11·5	0·02	10·4	9·2	0·16
	Sheep 6				9·1	8·1	0·14
	Cattle 5				53·1	47·0	0·82
	Cattle 6				52·9	46·8	0·81
IV	Sheep 7	13·0	11·5	0·45	10·4	9·2	3·6
	Sheep 8				10·4	9·2	3·6
	Cattle 7				52·1	46·1	18·0
	Cattle 8				53·1	46·9	18·4

As the protein level in the diet and the feed consumption had been unsatisfactory in Experiment 1 it was considered desirable to increase the lucerne chaff content from 5 per cent. to 9 per cent. and to include 9 per cent. linseed meal. This gave a palatable, productive ration. The increased molybdenum concentration (11·5 p.p.m. Mo) in rations for Groups II, III and IV and the increased copper levels (13·0 p.p.m. Cu) in rations for Groups III and IV were to maintain a mean daily intake of approximately 10 mg molybdenum and 10 mg copper in sheep.

Changes in body-weight and in liver copper concentration of individual animals are shown in Table 6. In all groups the mature sheep used in this experiment maintained body-weight, whereas the weaner steers gained weight at the rate of approximately 1 lb per head per day.

Table 6

BODY-WEIGHT AND COPPER CONCENTRATION IN LIVER OF SHEEP AND CATTLE IN EXPERIMENT 2

Group	Animal No.	Body-weight (lb)			Copper Concentration (p.p.m. Cu)					
		Initial	After 12 Weeks	Change	Initial	After 6 Weeks	After 12 Weeks	Total	Change Group Mean Sheep	Change Group Mean Cattle
I	Sheep 1	88	88	0	453		452	− 1	− 5	
	Sheep 2	88	85	− 3	367		359	− 8		
	Cattle 1	367	479	+112	112	105	105	− 7		−14
	Cattle 2	371	462	+ 91	123	118	102	− 21		
II	Sheep 3	83	81	− 2	373		211	−162	−129	
	Sheep 4	82	86	+ 4	414		320	− 94		
	Cattle 3	427	510	+ 83	124	68	40	− 84		−94
	Cattle 4	357	452	+ 95	155	87	52	−103		
III	Sheep 5	80	81	+ 1	492		537	+ 45	+ 80	
	Sheep 6	72	73	+ 1	473		587	+114		
	Cattle 5	369	474	+105	137	166	189	+ 52		+49
	Cattle 6	396	471	+ 75	119	133	165	+ 46		
IV	Sheep 7	85	85	0	360		210	−150	− 99	
	Sheep 8	88	88	0	412		365	− 47		
	Cattle 7	414	500	+ 86	136	108	91	− 45		−54
	Cattle 8	337	447	+110	147	104	85	− 62		

In spite of the low copper content of the basal ration (4 p.p.m. Cu), both sheep and cattle in Group I showed little change in liver copper reserves during the 12 weeks of this experiment.

In Group II the addition of molybdenum caused a marked decrease in liver copper reserves in both sheep and cattle. This occurred in spite of the low level of inorganic sulphate. These findings are contrary to those of Group II in Experiment 1, where a marked increase occurred in liver copper reserves on a diet equally low in sulphate, even lower in copper and containing approximately twice the concentration of molybdenum. An essential difference between the two experiments was the twofold increase in the crude protein content of the basal ration in Experiment 2.

In Group III both sheep and cattle showed an increase in the liver copper reserves due to the higher copper level in the diet. The magnitude of this increase was much lower than for Group III in Experiment 1.

In Group IV both sheep and cattle showed a marked fall in liver copper levels due to the additional sulphate in the rations fed to this group.

The copper and molybdenum levels in blood are shown in Table 7. The blood copper levels tended to be higher in sheep than in cattle. As in Experiment 1, the blood molybdenum levels were high in animals from Groups II and III, where the diet was high in molybdenum and low in sulphate. Similarly, these high levels were not attained in animals in Group IV on the same high molybdenum diet but with additional sulphate.

Table 7

COPPER (mg Cu/100 ml) AND MOLYBDENUM (μg Mo/100 ml) IN BLOOD OF SHEEP AND CATTLE IN EXPERIMENT 2

Group	Animal No.	Initial		After 4 Weeks		After 8 Weeks		After 12 Weeks	
		Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo
I	Sheep 1	0·12	<2	0·11	2	0·10	2	0·09	6
	Sheep 2	0·12	<2	0·10	2	0·09	4	0·09	6
	Cattle 1	0·08	<2	0·08	<2	0·06	<2	0·06	<2
	Cattle 2	0·08	<2	0·06	<2	0·06	<2	0·06	<2
II	Sheep 3	0·10	<2	0·09	654	0·10	756	0·10	698
	Sheep 4	0·10	<2	0·10	716	0·09	756	0·08	708
	Cattle 3	0·09	<2	0·06	160	0·06	40	0·06	47
	Cattle 4	0·09	<2	0·08	164	0·06	49	0·06	53
III	Sheep 5	0·08	<2	0·09	522	0·07	837	0·08	961
	Sheep 6	0·09	<2	0·13	383	0·10	656	0·09	749
	Cattle 5	0·08	<2	0·08	137	0·06	91	0·06	71
	Cattle 6	0·08	<2	0·08	119	0·06	36	0·06	62
IV	Sheep 7	0·09	<2	0·12	12	0·10	12	0·08	8
	Sheep 8	0·09	<2	0·11	16	0·07	8	0·07	10
	Cattle 7	0·08	<2	0·08	8	0·06	4	0·06	10
	Cattle 8	0·09	<2	0·08	7	0·10	2	0·06	8

V. EXPERIMENT 3

(a) Experimental

The 15 Hereford steers were distributed to give five groups of three animals. For Groups I, II and III, nine steers aged approximately 12 months were allocated on a body-weight basis. Groups IV and V each contained two steers aged approximately 24 months and one steer aged about 12 months and allocation within these two groups was on a body-weight basis.

Group I: Basal ration of 90 per cent. oaten chaff and 10 per cent. lucerne chaff, plus sufficient linseed meal and copper sulphate to give the same protein and copper levels as those found in the paspalum pasture mown and fed to Groups III and IV. As this pasture changed in chemical composition with stage of growth, samples were taken daily, oven-dried and bulked to give a weekly composite sample. This was analysed and the necessary adjustments were made at weekly intervals to the amount of linseed meal and copper sulphate added to the basal ration.

Group II: As for Group I, plus sodium sulphate to provide a sulphate level equal to that in paspalum fed to Groups III and IV. As in Group I, adjustments were made at weekly intervals to simulate in this ration the protein, copper, molybdenum and sulphate levels found in the cut paspalum pasture. Additional ammonium molybdate was not required as the molybdenum content of the basal ration was comparable with that of paspalum.

Group III: Paspalum pasture cut and fed daily. The amount fed was slightly below appetite and was adjusted so that all animals in Groups I, II and III had approximately the same daily dry-matter intake.

Group IV: Paspalum pasture cut daily and fed *ad lib.*

Group V: Grazing the same pasture from which the paspalum fed to Groups III and IV was taken.

Individual daily dry-matter feed consumption was measured for each of the animals in Groups I-IV.

Body-weight was determined initially and at the conclusion of the 12 weeks of the experiment.

Liver biopsy samples for copper analyses were taken initially, after six weeks and at 12 weeks when the experiment was concluded.

At the conclusion of the stall-feeding experiment the three animals from Group IV were transferred to grazing on Rocklea pastures. Liver samples were taken for analysis for copper at approximately monthly intervals. Whenever liver copper concentrations fell below 50 p.p.m. copper therapy by intravenous injection of copper sulphate was given to raise the liver copper reserves to about 150 p.p.m. Cu. The observation continued for 12 months. Body-weight was recorded at approximately monthly intervals.

(b) Results

The concentration in the diet and the average daily intake of copper, molybdenum and inorganic sulphate of the experimental cattle are shown in Table 8. These are average figures, as the chemical composition varied with changes in the stage of growth during the experimental period from February to May.

Table 8

COPPER, MOLYBDENUM AND INORGANIC SULPHATE IN THE DIET OF CATTLE IN EXPERIMENT 3

Group	Tag	Concentration in Diet			Average Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	A	5·8	0·2	0·02	23·8	0·8	0·82
	B				24·4	0·8	0·84
	C				24·4	0·8	0·84
II	D	5·8	0·2	0·35	24·4	0·8	14·7
	E				24·4	0·8	14·7
	F				24·4	0·8	14·7
III	G	5·9	0·2	0·35	23·5	0·8	14·0
	H				23·5	0·8	14·0
	I				24·3	0·8	14·4
IV	J	5·9	0·2	0·35	32·9	1·1	19·5
	K				32·9	1·1	19·5
	L				29·9	1·0	17·8
V	M	Cattle grazing pasture from which diets of Groups III and IV were taken					
	N						
	O						

Changes in body-weight and in liver copper concentrations are shown in Table 9. Cattle tended to maintain or gain weight in Groups I, II and V and to lose weight in Groups III and IV. These findings suggest that the basal ration was of higher nutritive value than the paspalum. They also indicate that the grazing animals in Group V were able to select a better quality diet than the pasture cut and fed *ad lib.* to comparable animals in Group IV.

Table 9

COPPER CONCENTRATIONS IN LIVER AND BODY-WEIGHT OF CATTLE IN EXPERIMENT 3

Group	Tag	Body-weight (lb)				Copper Concentration (p.p.m. Cu)				
		Initial	After 12 Weeks	Change		Initial	After 6 Weeks	After 12 Weeks	Change	
				Total	Group Mean				Total	Group Mean
I	A	620	632	+12		133	128	94	−39	
	B	560	552	− 8	+ 7	114	95	107	− 7	−22
	C	492	508	+16		135	107	114	−21	
II	D	520	564	+44		199	129	109	−90	
	E	548	580	+32	+20	170	128	115	−55	−63
	F	708	692	−16		201	175	158	−43	
III	G	552	512	−40		176	139	184	+ 8	
	H	572	556	−16	−25	221	177	173	−48	−16
	I	524	504	−20		189	160	182	− 7	
IV	J	852	796	−56		175	128	135	−40	
	K	816	788	−28	−27	162	179	177	+15	+ 8
	L	720	724	+ 4		205	193	253	+48	
V	M	816	808	− 8		154	118	107	−47	
	N	856	912	+56	+32	158	117	105	−53	−57
	O	732	780	+48		163	119	92	−71	

The mean changes in liver copper reserves showed a slight fall in Groups I and III, a marked fall in Groups II and V and a small gain in Group IV. Thus there is an indication that the addition of sodium sulphate to the diet of Group II adversely affected the availability of the dietary copper. This finding was not confirmed subsequently in Experiment 4. There is also an indication that the moderately low copper (5.9 p.p.m.) in freshly cut paspalum pasture at this season (February to May) is sufficient to permit stalled cattle on *ad lib.* feeding to remain in copper balance. A comparison of data from Groups IV and V suggests that grazing cattle select a diet which is higher in total digestible nutrients but lower in "available" copper.

The mean changes in body-weight and in liver copper concentration of cattle grazing Rocklea pastures from June 21, 1957, to June 20, 1958, are shown in Figure 1. Cattle gained in body-weight from August to May. All animals showed a marked fall in liver copper throughout the year, the rate of decline tending to decrease as the copper concentration in liver became less.

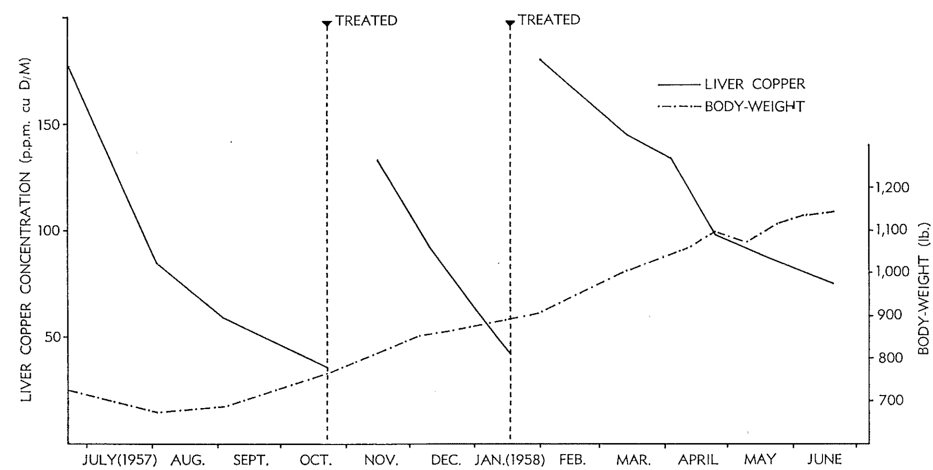

Fig. 1.—Mean changes in body-weight and liver copper concentrations in grazing cattle.

VI. EXPERIMENT 4

(a) Experimental

Nine Hereford steers approximately 12 months of age were treated intravenously with copper sulphate on 9th, 13th and 16th October, 1958. On each occasion the dose rate was 250 ml of 0·125 per cent. $CuSO_4 \cdot 5H_2O$ in physiological saline. On October 30, liver samples were taken for copper analysis and the animals were placed in the paddock to be mown subsequently for hay. Liver biopsies were taken at 3-weekly intervals up to January 28, 1959. The pasture was mown for hay on February 3. Intravenous copper therapy was repeated to ensure adequate liver copper reserves at the commencement of the stall-feeding trial.

For the stall-feeding portion of this experiment, the nine steers were distributed by stratified randomization on a body-weight basis to three groups each of three animals. The groups were allotted at random to the treatments as follows:—

Group I: Basal ration of 85 per cent. oaten chaff, 9 per cent. lucerne chaff, 6 per cent. linseed meal, plus sufficient copper sulphate to raise the copper content of the ration to that of the paspalum hay.

Group II: As for Group I, plus sufficient sodium sulphate to raise the inorganic sulphate level to that of the paspalum hay.

Group III: Paspalum hay.

All animals were fed the same amount of dry matter, which was slightly below the intake of the poorest eater.

Body-weight was recorded initially and at the conclusion of the 12 weeks of stall feeding.

Liver samples were taken initially and thereafter at 4-weekly intervals for the 12 weeks of stall feeding.

(b) Results

The average daily intake of copper, molybdenum and inorganic sulphate and the concentrations of these constituents in the diet of the experimental cattle are shown in Table 10. The copper level in paspalum hay, 6·5 p.p.m. Cu on dry-matter, was slightly greater than the mean value for the freshly cut paspalum fed in Experiment 3. The inorganic sulphate concentration of 0·35 per cent. was similar to that of the freshly cut paspalum used in the previous experiment. The molybdenum content of 0·1 p.p.m. Mo was even lower than that of the basal ration for Groups I and II.

Table 10

Copper, Molybdenum and Inorganic Sulphate in the Diet of Cattle in Experiment 4

Group	Tag	Concentration in Diet			Mean Daily Intake		
		Copper (p.p.m. Cu)	Molybdenum (p.p.m. Mo)	Sulphate (% SO_4)	Copper (mg)	Molybdenum (mg)	Sulphate (g)
I	a	6·5	0·2	0·02	23·6	0·8	0·79
	b				23·6	0·8	0·79
	c				23·6	0·8	0·79
II	d	6·5	0·2	0·35	23·4	0·8	13·6
	e				23·6	0·8	13·8
	f				23·6	0·8	13·8
III	g	6·5	0·1	0·35	23·6	0·4	13·8
	h				23·6	0·4	13·8
	i				23·6	0·4	13·8

Changes in liver copper concentration in the nine experimental steers grazing the pasture from which the hay was subsequently made are shown in Table 11. All animals showed a marked and consistent fall in liver copper reserves on this pasture for the 12 weeks immediately prior to the time in which the paddock was mown for hay.

Table 11

COPPER IN LIVER OF CATTLE GRAZING THE PASTURE UP TO THE TIME OF MAKING HAY

Tag	Copper Concentration (p.p.m. Cu)					
	30.x.58	20.xi.58	11.xii.58	6.i.59	28.i.59	Change
a	233	156	122	86	51	−182
b	286	209	149	83	66	−220
c	207	150	119	60	29	−178
d	227	168	137	97	61	−166
e	212	134	97	44	29	−183
f	215	168	143	62	35	−180
g	238	179	152	89	51	−187
h	258	193	141	106	65	−193
i	192	136	89	50	28	−164
Mean	230	166	128	75	46	−184

Changes in body-weight and liver copper concentration of the cattle during the stall-feeding period are shown in Table 12. Cattle tended to make slight weight gains in all groups. Animals in Groups I and II showed slight reductions in liver copper levels during the 12 weeks on stall feeding, whereas greater falls were recorded in animals from Group III. There appears to be a factor in this paspalum hay which interferes with copper metabolism, and as the molybdenum content of this hay is even lower than in the basal ration this factor does not appear to be molybdenum in the presence of inorganic sulphate.

Table 12

BODY-WEIGHT AND COPPER CONCENTRATION IN LIVER OF STALL-FED CATTLE IN EXPERIMENT 4

Group	Tag	Body-weight				Copper Concentration (p.p.m. Cu)					
		Initial	After 12 Weeks	Change		Initial	After 4 Weeks	After 8 Weeks	After 12 Weeks	Change	
				Total	Group Mean					Total	Group Mean
I	a	560	584	+24		184	177	..	176	− 8	
	b	592	616	+24	+29	142	136	118	119	−23	−29
	c	622	658	+38		138	136	109	112	−26	
II	d	542	570	+28		224	236	204	220	− 4	
	e	624	646	+22	+20	154	132	117	125	−29	−11
	f	600	610	+10		244	247	..	243	− 1	
III	g	588	596	+ 8		143	104	94	86	−57	
	h	600	586	−14	+ 5	175	156	117	130	−45	−56
	i	636	658	+22		182	161	136	117	−65	

VII. DISCUSSION

The essential findings on sheep from Experiments 1 and 2 are:—

(1) On a basal diet of oaten chaff plus limited lucerne chaff sheep were able to at least maintain liver copper levels in spite of the low intake of copper.

(2) The addition of molybdenum to this diet of low inorganic sulphate content markedly increased blood molybdenum levels but did not interfere with the metabolism of copper by sheep.

(3) The addition of molybdenum to the diet in Experiment 2, which contained additional protein in the form of linseed meal, did interfere with the metabolism of copper by sheep.

(4) The addition of inorganic sulphate to the diet lowered blood molybdenum levels, allowed molybdenum to interfere with the metabolism of copper by sheep on the low protein diet of Experiment 1, and enhanced the molybdenum interference with copper metabolism in sheep on the higher protein diet of Experiment 2.

These findings are in agreement with those of Dick (1953, 1956).

In Experiment 2, sheep and cattle were fed similar rations. A comparison of the data shows that for the limited diets tested a similar copper/molybdenum/sulphate relationship is apparent in both species.

The rapid rate of decline in the liver copper status of cattle on pastures at Rocklea is shown in the data on grazing animals in Experiment 3. This rate of decline was greater in grazing cattle than in similar animals fed freshly cut pasture. Moir (1960) showed that grazing cattle on this property selected a diet containing 12 per cent. crude protein when the total available pasture contained 7·9 per cent. protein. The body-weight data in Experiment 3 lend further support to the selectivity of grazing cattle. Grazing cattle tended to gain weight while similar animals, stall-fed with freshly cut pasture, lost weight.

A number of reasons can be advanced to explain the difference in copper status between stall-fed and grazing cattle:—

(1) The animal gaining in body-weight could have a greater requirement for copper.

(2) The weight of liver, being proportional to body-weight, might be expected to be greater in the animal gaining weight than in the animal losing weight. If the same amount of copper was metabolized, a lower concentration of copper could occur in the liver of the heavier animal.

(3) The copper content could be lower in the selected diet.

(4) The copper could be less available in the selected diet.

The first reason seems unlikely to fully explain the difference. Cattle in Experiment 2 on a diet containing 4 p.p.m. Cu and $0 \cdot 2$ p.p.m. Mo gained more than 1 lb per head per day and remained in copper balance.

The second reason also appears inadequate. Body-weight gains in the grazing cattle in Experiment 3 were less than 4 per cent., whereas the decline in liver copper levels in these animals was more than 30 per cent.

Unpublished data on pastures on this property do not indicate a lower content of copper in the selected diet. There is little variation in copper content between pasture species in this sward, and new growth, which appears to be preferentially selected, tends to show a higher copper content. Further, the copper content of the total pasture available in Experiment 3 is $5 \cdot 9$ p.p.m. Cu; cattle in Experiment 2 tended to remain in copper balance on a compounded ration containing 4 p.p.m. Cu.

The fourth reason, availability of copper in selected pasture, must be regarded with favour until further evidence becomes available.

In Holland, van der Grift (personal communication) has found that hay made from pasture on which cattle are unable to maintain liver copper reserves usually provides sufficient available copper for animals to at least remain in copper balance. In Experiment 4 the hay-fed animals showed a decline in liver copper levels. However, the rate of decline was much less in the hay-fed group during the 12 weeks of stall feeding than in the same animals during the previous 12 weeks, when they were grazing the pasture from which the hay was made.

In the stall-feeding portion of Experiment 4 the decline in liver copper levels was greater in animals fed paspalum hay than in comparable animals fed a ration compounded from oaten chaff, lucerne chaff and linseed meal. The concentration and daily intake of copper and inorganic sulphate were the same but the molybdenum level in the hay was even lower ($0 \cdot 1$ p.p.m. Mo) than in the compounded ration ($0 \cdot 2$ p.p.m. Mo). Thus molybdenum in the presence of inorganic sulphate cannot be incriminated as the factor in the paspalum hay which interferes with the metabolism of copper by cattle.

From Experiment 3 it would appear that the addition of inorganic sulphate to the maintenance diet of oaten chaff, lucerne chaff and linseed meal caused a further decline in the liver copper levels of cattle although the molybdenum content of the diet was low ($0 \cdot 2$ p.p.m. Mo). This did not occur in Experiment 4 when the same level of inorganic sulphate was added to a diet slightly better than maintenance containing different proportions of oaten chaff, lucerne chaff and linseed meal. No satisfactory explanation is apparent for this anomaly.

The findings from the experiments reported in this paper are in agreement with published data on the ability of molybdenum in the presence of inorganic sulphate to interfere with the metabolism of copper by sheep and cattle. The results also support the conclusion that the factor or factors which interfere with the metabolism of copper by cattle grazing the predominantly paspalum pasture at the Animal Husbandry Research Farm at Rocklea is not molybdenum in the presence of inorganic sulphate.

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