

A COMPARISON OF THE COPPER STATUS OF SHEEP AND CATTLE GRAZING A PREDOMINANTLY *PASPALUM DILATATUM* PASTURE IN SOUTH-EASTERN QUEENSLAND

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SUMMARY

A study was made, over a period of three years, to compare the liver and blood copper concentrations of adult Hereford cattle with those of adult Merino sheep simultaneously grazing the same predominantly *Paspalum dilatatum* pastures. Blood and liver samples were taken on 15 occasions during this period.

Mean liver copper levels in cattle were less than 30 p.p.m. (D.M.) at all except one sampling, when 34.5 p.p.m. (D.M.) was recorded. Levels in sheep were greater than 100 p.p.m. for 12 of the 15 samplings. Mean blood copper levels in cattle were less than 0.08 mg/100 ml on 13 occasions, and on only 3 occasions for sheep. There was no consistent seasonal pattern in the changes in copper concentrations in liver and blood of either cattle or sheep.

Comparisons in two successive years indicated that pregnancy did not influence liver or blood copper concentrations in sheep.

Liver copper concentrations were determined in calves and lambs and their dams within 24 hr of parturition. In both cattle and sheep, liver copper concentrations in the new-born were significantly higher than in their dams. Cows with mean liver copper concentrations of 17.1 p.p.m. (D.M.) in 1957 and 9.8 p.p.m. (D.M.) in 1958 gave birth to calves with mean liver copper levels of 361 and 329 p.p.m. (D.M.) respectively. In 1957, ewes with mean liver copper levels of 103 p.p.m. (D.M.) produced lambs with mean liver copper levels of 157 p.p.m. (D.M.). In 1958, ewes giving birth to single lambs had mean liver copper levels of 134 p.p.m. (D.M.) and the levels in their lambs were 239 p.p.m. (D.M.). In the same year, the six ewes with twin or triplet lambs had liver copper levels of 141 p.p.m. (D.M.) and their lambs averaged 262 p.p.m. (D.M.).

The high liver copper concentrations in new-born calves declined rapidly in both the years studied. The rate of decline appeared to be more rapid in the year that calves grew at a faster rate. There was little decline in liver copper concentration in the growing lambs in 1957. In the next year, lambs had higher liver copper concentrations at birth, but these declined during the first four months to approximately 47 per cent. of the initial values.

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I. INTRODUCTION

Lee and Moule (1947) first recorded the presence of copper deficiency in livestock in Queensland, and Harvey (1952) defined a number of localities where a low copper status had been found in sheep or cattle. Sutherland (1952) reported a beneficial response to oral therapy with copper sulphate in cattle in some areas of south-eastern Queensland. The results of a survey reported by Alexander and Harvey (1957) showed that low liver or blood copper levels were found in cattle from 74.8 per cent. of 127 farms examined in coastal Queensland south of Brisbane.

Sutherland (1956) summarized the results of studies at Rocklea, near Brisbane, in which cattle with very low liver copper concentrations, grazing paspalum/white clover pastures, showed no response to parenteral copper therapy. Subsequently, when sheep were introduced to this area at Rocklea, it was noted that their liver copper concentrations remained much higher than those of the cattle.

The studies to be reported in this paper commenced in 1955. The original aim was to compare and study the seasonal variation in the liver and blood copper concentrations of sheep and cattle grazing the same paddock located on the Animal Husbandry Research Farm at Rocklea. It was noted that a marked fall in liver and blood copper concentrations occurred in ewes at the time of lambing in the first year and in consequence the experiment was modified to include studies on the effect of pregnancy and lactation on liver copper reserves in sheep. In the last two years of the experiment, copper concentrations were determined in livers of lambs and calves at birth and changes in these levels with growth were studied.

Body-weights of the animals were recorded and determinations of blood haemoglobin, packed cell volume and blood inorganic phosphate were made to define more adequately the conditions under which results were obtained.

II. MATERIALS AND METHODS

(1) *Experimental Area*.—The experimental paddock of 28.2 ac was located on the Animal Husbandry Research Farm, Rocklea, approximately 5 miles south-west of the centre of Brisbane, Queensland. The dominant pasture species in the paddock was *Paspalum dilatatum*. Line transect data obtained in June 1954 showed the following botanical composition: *Paspalum dilatatum* (paspalum) 79.3 per cent., *Digitaria didactyla* (blue couch) 3.9 per cent., *Bothriochloa decipiens* (pitted blue grass) 2.3 per cent., *Trifolium repens* (white clover) 10.2 per cent., and miscellaneous species 4.3 per cent. The proportion of white clover varied seasonally and was negligible in unfavourable seasons. The copper content obtained at monthly intervals from March 1954 to February 1955 varied from 6.5 to 8.6 p.p.m. (dry-matter basis) for paspalum and from 8.0 to 8.8 p.p.m. (dry-matter basis) for white clover.

Except for two short periods in 1956 and 1957, when portion of the ewe flock was mated, all the experimental animals continuously grazed this paddock. During joining in those years, the group to be mated and the unmated sheep were alternated at weekly intervals between the experimental and an adjacent paddock having pastures of similar botanical composition.

(2) *Experimental Animals*.—Sixteen Merino ewe weaners were obtained from the Roma district in Queensland during March 1954. They had been born during September-October 1953 and had been weaned after shearing in March 1954. No evidence of copper deficiency had been recorded on the property of origin. The animals were vaccinated against tetanus and blackleg and transferred to the experimental paddock soon after arrival. They were pregnant when the experiment commenced in June 1955, having been mated from April 22 to May 5. They had been shorn in March 1955. During the experiment, five ewes died from various causes, mainly associated with liver biopsy, and results for the portion of the experiment comparing sheep with cattle are based on the 11 ewes surviving at the end of the experiment. Most of the progeny of these ewes were retained in the experimental paddock for examination of other aspects associated with the study.

Six Hereford cows were introduced to the experiment on June 7, 1955. These animals were born in 1951 and were from a group of 46 purchased from a property at Texas in south-western Queensland and brought to Rocklea in June 1953. Since their arrival they had been grazing on pastures similar to those in the experimental area. They were non-lactating and 1-3 months pregnant when introduced to the experiment. These six cows were retained throughout the experiment and the results from them have been used in the comparisons of sheep and cattle. On July 4, 1956, a further six cows from the group purchased in 1953 were introduced to the area. These animals were non-pregnant, had calved during November-December 1955 and had recently weaned their calves. They were therefore comparable with the six original cows. They were introduced in order to increase the stocking rate and to provide larger numbers for studying liver copper concentrations in new-born calves and their dams. All calves born during the experiment were removed from the paddock after weaning at 7-9 months.

(3) *General Management*.—The animals were observed three times per week. Cattle tick, *Boophilus microplus*, populations were controlled on the cattle by regular spraying or dipping in 0.5 per cent. DDT mixtures. As a precaution against tick fever under these conditions of low tick infestation, cattle were inoculated at intervals of approximately three months with blood containing *Babesia argentina* and *B. bigemina*. The sheep and calves were drenched at intervals with phenothiazine to control internal parasites. Blowfly strikes were treated as they occurred, and in March 1956 all sheep were sprayed with dieldrin as a preventive measure during a blowfly wave. Calves and lambs were vaccinated with tetanus toxoid and blackleg vaccine 2-3 weeks before castration, dehorning or marking.

The sheep were shorn during March 1956, January 1957, September 1957 and October 1958. Crutching was not carried out during the experiment.

Both ewes and cows were "paddock mated". A raddled ram was introduced to the experimental paddock in 1958, when all ewes were mated. In 1956 and 1957, when only portion of the ewe flock was mated, a raddled ram was allowed to run with the group selected for mating. Ewes were examined three times per week for evidence of service. Mating, as indicated by raddling, took place during the following periods: April 20-28, 1956; May 22-31, 1957; and June 23 to July 18, 1958.

The cows were mated for a period of six weeks, dates of introduction of the bull being July 4, 1956, and February 25, 1958. In each year, mating was delayed until after weaning of the calves.

(4) *Body-weight*.—Body-weights of cows, calves and adult sheep were obtained on a cattle weighing scale with an accuracy of ± 1 lb.

(5) *Sampling and Analytical Methods*.—Liver samples were obtained at intervals of approximately three months by biopsy with the instruments described by Dick (1952). The procedure of Loosemore and Allcroft (1951) was used for cattle and that of Dick (1944) for adult sheep. An open laparotomy technique reported by Watson (1960) was used to obtain liver samples from new-born lambs. Liver samples were obtained from the new-born animals and their dams within 24 hr of parturition. The liver samples were analysed for copper by the method of Clare, Cunningham, and Perrin (1945), and the results have been expressed as dry matter (D.M.).

Blood samples were obtained with stainless steel needles from the external jugular vein on the same day as liver sampling. Analytical methods employed were as follows: blood copper, Clare, Cunningham, and Perrin (1945); blood inorganic phosphate, Moir (1954); and blood haemoglobin, Donaldson *et al.* (1951). The method of determination of packed cell volume was essentially that of Wintrobe (1947), using a relative centrifugal force of approximately 2067 g.

(6) *Duration of the Experiment*.—The portion of the experiment designed to compare liver and blood copper levels in sheep with those of cattle commenced in June 1955 and continued until June 1958. Both the sheep and cattle remained in the experimental area until the studies on liver copper levels of new-born lambs and calves were completed in March 1959.

III. RESULTS

(a) Liver and Blood Copper Levels in Sheep and Cattle

The liver and blood copper levels and body-weight of both sheep and cattle are presented in Figure 1. Except at the first three sampling periods, sheep had mean liver copper levels greater than 100 p.p.m. On only one occasion were levels below 60 p.p.m. With the exception of one sampling period, cattle had

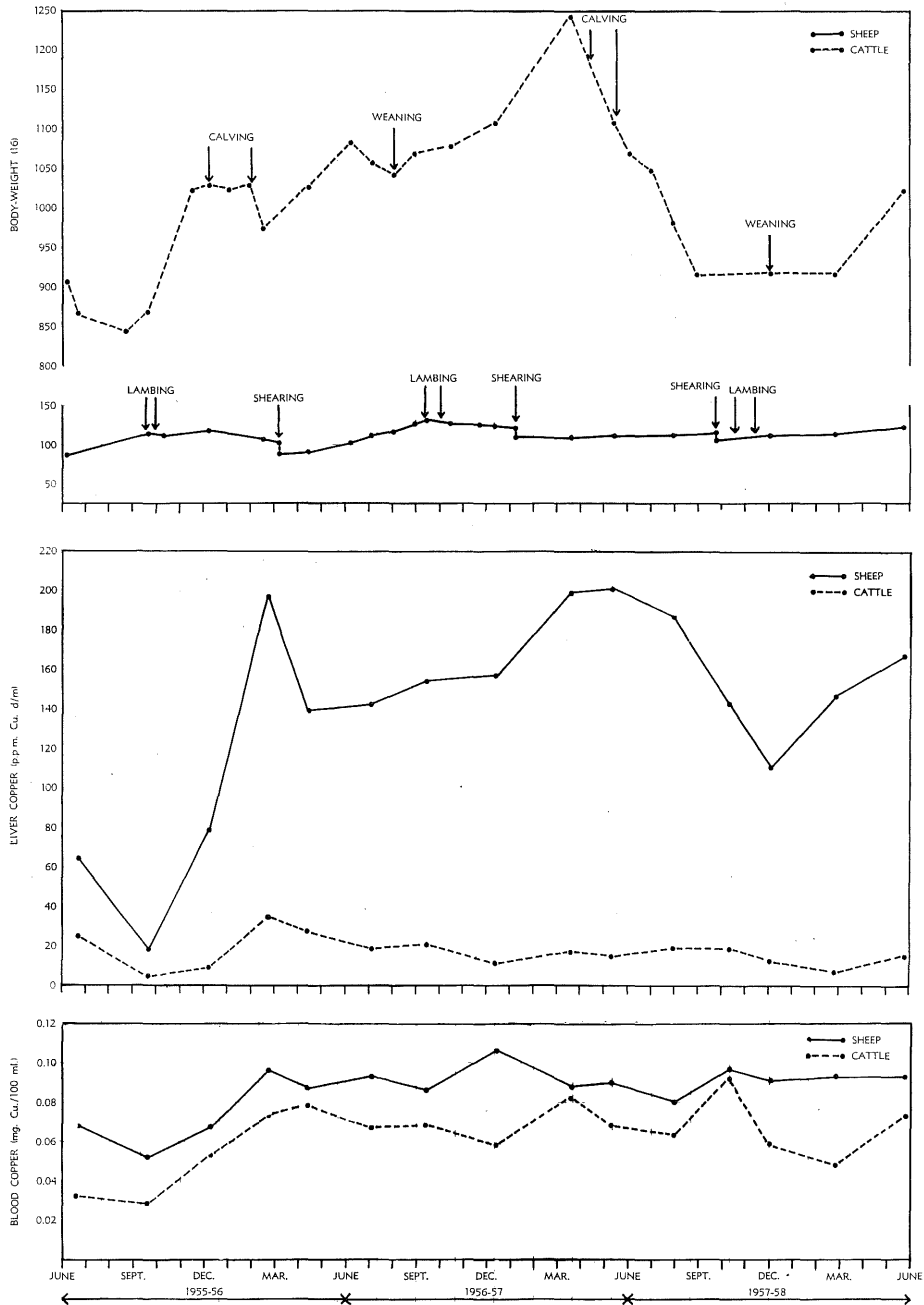


Fig. 1.—Mean changes in body-weight and in the concentration of copper in liver and blood of sheep and cattle grazing the same pasture.

mean liver copper concentrations which were less than 30 p.p.m. On three occasions the mean levels were less than 10 p.p.m. Cu. One of the six cows had liver copper reserves that were higher than those of all other cows for 13 of the 15 samplings. The mean liver copper concentration of this animal for all 15 samples was 41.3 p.p.m., compared with an average of the means for the other five of 12.2 p.p.m.

If the data on liver concentrations are considered for the periods July 1955 to June 1956, July 1956 to June 1957, and July 1957 to June 1958, there does not appear to be any marked consistent seasonal trend. In both sheep and cattle the maximum concentration in 1955-56 was recorded in late summer and the minimum level in spring. The data for 1956-57 show little seasonal variation. In 1957-58 there was a more marked seasonal variation than in the previous year and minimum values for sheep and cattle were found during the summer period.

Blood copper levels in sheep were below 0.08 mg Cu/100 ml for only the first three sampling times in 1955, whereas cattle had concentrations above this figure on only two occasions. From these data it would appear that the lowest levels in cattle were associated with the spring and summer periods, mean levels recorded in the three December samplings being 0.053, 0.058 and 0.058 mg Cu/100 ml. The lowest mean level recorded for cattle was 0.28 mg/100 ml in the spring of 1955.

In sheep, the relationship between mean blood and liver concentrations was $r = +0.74$ ($P < 0.01$). The lack of independence of successive measurements could have been an influence in this result. The comparable relationship between blood and liver copper concentration in cattle was not significant ($r = +0.33$). Ignoring the lack of independence among successive measurements, the correlation between liver copper concentration for sheep and cattle was only $r = +0.43$ ($P < 0.1$). For mean blood copper levels, the correlation was $r = +0.78$ ($P < 0.01$).

(b) Effect of Pregnancy and Lactation on Liver and Blood Copper Concentrations in Sheep

The mean liver and blood copper concentrations of pregnant and non-pregnant ewes in 1956 and 1957 are shown in Table 1. Sampling times were approximately 3-monthly, the intervals being related to the pregnant group, viz., prior to mating, at mid-pregnancy, close to lambing, and during lactation. In 1956, the liver copper reserves increased during pregnancy and lactation, whereas during 1957 the levels decreased, but at a rate similar to that in the non-pregnant ewes. There was little change in blood copper concentrations during either year. There were no significant differences in either liver or blood copper concentrations between pregnant and non-pregnant ewes in either year. However, the difference between the blood copper levels of lactating and non-lactating ewes in 1956 was significant at the 5 per cent. level.

TABLE 1
EFFECT OF PREGNANCY AND LACTATION ON LIVER AND BLOOD COPPER CONCENTRATIONS IN SHEEP

	Mating Period	Lambing Period	No. of Ewes	Mean Liver Copper Concentration (p.p.m. D.M.)				Mean Blood Copper Concentration (mg/100 ml)			
				13.iv.56	4.vii.56	14.ix.56	11.xii.56	13.iv.56	4.vii.56	14.ix.56	11.xii.56
Pregnant ewes ..	20.iv.56 to 5.v.56	10.ix.56 to 2.x.56	8	134	136	162	175	0.086	0.089	0.082	0.090
Non-pregnant ewes	7	147	148	148	152	0.087	0.087	0.087	0.116
Difference†	+24±13†	+30±17†	-0.005 ± 0.009	-0.026 ± 0.011*
				10.v.57	29.vii.57	10.x.57	3.xii.57	10.v.57	29.vii.57	10.x.57	3.xii.57
Pregnant ewes ..	22.v.57 to 31.v.57	18.x.57 to 14.xi.57	8	165	153	103	79	0.094	0.086	0.091	0.085
Non-pregnant ewes	8	180	172	127	99	0.095	0.084	0.100	0.089
Difference†	-14±18†	-13±11†	-0.009 ± 0.007	-0.004 ± 0.006

* Difference significant at 5% level.

† Difference between means of pregnant and non-pregnant ewes adjusted for initial differences on 13.iv.56 for 1956 data and on 10.v.57 for 1957 data (mean and standard error)

TABLE 2
MEAN AND STANDARD ERRORS OF MEAN LIVER COPPER CONCENTRATION IN NEW-BORN CALVES AND LAMBS AND IN THEIR DAMS AT PARTURITION
CATTLE

Calving Period	Number		Mean Liver Copper Concentration (p.p.m. D.M.)		
	Cows	Calves	Cows at Calving	Calves at Birth	Difference between Calf and Cow
1957 (4.iv.57 to 16.v.57)	11	12†	17.1 ± 2.5	361 ± 33	344 ± 33***
1958 (6.xi.58 to 28.xi. 58)	8	8	9.8 ± 1.7	329 ± 58	319 ± 57***

SHEEP

Lambing Period	Number		Mean Liver Copper Concentration (p.p.m. D.M.)		
	Ewes	Lambs	Ewes at Lambing	Lambs at Birth	Difference between Lamb and Ewe
1957 (18.x.57 to 14.xi.57)	8	8	103.0 ± 12.3	157 ± 15	54 ± 15**
1958 (14.xi.58 to 23.xii. 58) ..	9‡	9	134 ± 12	239 ± 27	} 109 ± 21***
	6§	13	141 ± 15	262 ± 24	

† One cow gave birth to twins.
‡ Ewes giving birth to single lambs.
§ Ewes giving birth to twin or triplet lambs.
** Significant at 1% level.
*** Significant at 0.1% level.

(c) Liver Copper Concentrations in New-born Calves and Lambs

The mean liver copper concentrations in new-born calves and lambs, together with those of their dams, are shown in Table 2. In both species, the level in the new-born animals was significantly higher than in their dams, the difference in concentration between dam and offspring being more marked in cattle than in sheep.

There were no significant correlations between levels in the dams and their offspring for either sheep or cattle when the data for the two years were considered either separately or collectively.

(d) Liver Copper Concentrations in Calves during Early Growth

The body-weights and liver copper concentrations recorded at intervals for calves born in 1957 are shown in Table 3. Those for calves born in 1958 are shown in Table 4. In 1957, 10 of the 12 calves born alive completed the suckling period. One died five days after birth and one cow would not allow its calf to suckle. All six live calves born during 1958 completed the experiment.

In 1957, the calves were born in late autumn. Their mean growth rate in the 56 days from June 4 (mean age 47.3 days) to July 29 was 1.29 lb per head per day. The liver copper concentrations fell rapidly, but in general did not decline to levels comparable with those found in the cows until after the calves were three months of age.

The calves born in early summer in 1958 had a mean growth rate, in the 56 days from the following January 6 (mean age 51.8 days) to March 3, of 1.91 lb per head per day. Liver copper concentrations fell at a more rapid rate than in the previous year, levels having declined in three months to concentrations comparable with those of the cows.

Despite the rapid reduction in liver copper concentrations, these calves continued to grow at satisfactory rates.

(e) Liver Copper Concentrations in Lambs during Early Growth

In lambs born in 1957, the mean fall in liver copper concentration for the first four months of life was 13 p.p.m., whereas their dams over the same period showed a mean decline of 8 p.p.m. Lambs born in 1958 had much greater liver copper levels at birth (Table 4) and showed a mean fall in liver copper concentration for the first four months of life of 137 p.p.m., whereas the liver copper reserves in their dams increased by 46 p.p.m. over the same period.

TABLE 3
LIVER COPPER CONCENTRATION IN CALVES AND THEIR DAMS AND BODY-WEIGHT OF CALVES BORN IN 1957

Calf No.	Date of Birth	Liver Copper Concentration (p.p.m. D.M.) and Body-weight† (lb)								
		Dam	Calf at Birth	10.v.57	4.vi.57	1.viii.57	29.vii.57	26.viii.57	10.x.57	3.xi.57
1‡	4.iv.57	5	374	103	50	21	32	12	8	6
2‡	4.iv.57		228	200	42	27	16	19	15	14
3	9.iv.57	20	128	155	57	45	28	19	12	20
4	15.iv.57	30	336	160	98	91	77	55	36	...
5	17.iv.57	22	445	145	62	71	51	54	38	11
6	18.iv.57	17	367	121	98	17	13	15	15	10
7	27.iv.57	31	562	383	218	114	89	61	46	17
8	9. v.57	12	336	136	76	56	40	22	22	10
9	15. v.57	11	482	—	130	40	110	93	65	33
10	16. v.57	9	338	—	190	116	98	59	40	20
Mean		17.4	360		120	162	206	232		382

† Body-weight in italics.
‡ Calves 1 and 2 were male twins.

... No liver sample obtained.
— Calves not born.

TABLE 4
LIVER COPPER CONCENTRATION IN CALVES AND THEIR DAMS AND BODY-WEIGHT OF CALVES BORN IN 1958

Calf No.	Date of Birth	Liver Copper Concentration (p.p.m.) and Body-weight †(lb)					
		Dam	Calf at Birth	6.i.59	3.ii.59	3.iii.59	31.iii.59
1	6.xi.58	10	433	41	19	14	18
			<i>68</i>	<i>206</i>	<i>272</i>	<i>316</i>	<i>370</i>
2	9.xi.58	5	300	19	13	17	18
			<i>66</i>	<i>182</i>	<i>240</i>	<i>296</i>	<i>340</i>
3	14.xi.58	9	213	17	11	26	23
			<i>56</i>	<i>154</i>	<i>200</i>	<i>248</i>	<i>292</i>
4	17.xi.58	17	305	12	9	. . .	10
			<i>56</i>	<i>162</i>	<i>212</i>	<i>262</i>	<i>304</i>
5	17.xi.58	4	135	19	8	12	6
			<i>56</i>	<i>144</i>	<i>206</i>	<i>264</i>	<i>316</i>
6	28.xi.58	15	670	72	25	14	14
			<i>70</i>	<i>138</i>	<i>192</i>	<i>242</i>	<i>284</i>
Mean		10	343				

† Body-weight in italics.
. . . No liver sample.

(f) Blood Haemoglobin, Packed Cell Volume (P.C.V.) and Blood Inorganic Phosphate Levels in Experimental Animals

The blood haemoglobin, P.C.V. and inorganic phosphate levels for cattle at the 15 sampling periods varied from 12.3 to 14.9 g/100 ml, 31.4 to 44.0 per cent., and 3.1 to 5.3 mg/100 ml respectively. The range of these constituents for sheep were: haemoglobin 10.2 to 13.2 g/100 ml, P.C.V. 29.0 to 42.2 per cent., and blood inorganic phosphate 2.9 to 5.8 mg/100 ml.

IV. DISCUSSION

Studies during a period of three years indicate that in adult sheep grazing predominantly paspalum pastures on the Animal Husbandry Research Farm, Rocklea, the liver copper concentrations are in general greater than 100 p.p.m., whereas those in adult cattle are usually less than 30 p.p.m. There are at least three factors which could be responsible for these differences. Firstly, there is evidence of a species difference in the ability of cattle and sheep to store copper. Cunningham (1931, 1946), Bennetts and Chapman (1937), and Beck (1956) have recorded mean liver copper levels in "normal" adult sheep of 236, 599, 360 and 305 p.p.m. respectively, whereas Cunningham (1931, 1946) obtained levels of 77 p.p.m. and 200 p.p.m. respectively for adult cattle. Secondly, differences in the method of prehension by cattle and sheep may result in differential selection of pasture. It is therefore possible that the sheep were selecting a diet higher in copper than that obtained by the cattle. Thirdly, it is possible that cattle and sheep are not affected similarly by factors interfering with copper metabolism. In the year preceding these studies, copper levels were determined at monthly intervals on the predominant pasture species on this property. Levels ranged from 6.5 to 8.8 p.p.m. Cu on a dry-matter basis. A low copper status in grazing cattle suggests some factor or factors which interfere with the availability of this copper to cattle. Harvey, Ryley, Beames, and O'Bryan (1961) showed that molybdenum in the presence of high levels of inorganic sulphate exerted a similar effect on the storage of copper in livers of both sheep and cattle, but that this factor was unlikely to be responsible for the low levels of liver copper found in cattle at Rocklea.

The generally low concentration of copper in the liver and blood of cattle did not appear to result in anaemia, clinical symptoms of copper deficiency, or poor growth of suckling calves. These findings are in agreement with those reported by Sutherland (1956) in which no response in growth rate was recorded following parenteral copper therapy of cattle on this property.

The lack of correlation between blood and liver copper concentrations in the cattle is in agreement with the results obtained by Loosemore and Allcroft (1951). However, because of the small number of experimental animals in our studies, only a very strong relationship could be expected to be statistically significant.

In our experiment, pregnancy did not affect liver or blood copper concentrations in ewes. This is contrary to published data for pregnant cows. van der Grift (1955) found that the amount of copper in the liver decreased during the last four months of pregnancy. Allcroft and Uvarov (1959) stated that the copper concentration in the livers of pregnant cows falls during gestation. In the latter case, the small number of animals and the fact that comparisons were made between different animals biopsied at varying intervals before parturition may have influenced the findings.

There is general agreement that, in the bovine, new-born animals have higher liver copper concentrations than adults. Cunningham (1931, 1946), on data from slaughtered animals, reported higher liver copper levels in new-born calves than in adult cows. In a herd in which copper deficiency occurred, Allcroft and Uvarov (1959) found higher concentrations in calves slaughtered within four days of birth than in their untreated or treated dams. Pryor (1960) found that copper concentrations in livers of bovine foetuses were significantly higher than in their dams. Our findings with new-born calves are in agreement with these published data.

With sheep our results show liver copper concentrations in new-born lambs significantly higher than those of their dams at parturition. This would appear to be contrary to the findings of Cunningham (1931, 1946). However, his results were not obtained in a direct comparison of the copper levels in livers of new-born lambs with those of their dams.

The rapid decrease in liver copper concentration in growing calves is in agreement with the findings of Cunningham (1946). The marked fall in liver copper concentration suggests that total liver reserves declined, although it was not possible to determine changes in total dry-matter weight of liver in our studies.

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REFERENCES

- ALEXANDER, G. I., and HARVEY, J. M. (1957).—A survey of the incidence of copper deficiency in dairy cattle in coastal Queensland south of Brisbane. *Qd J. Agric. Sci.* 14:23-7.
- ALLCROFT, RUTH, and UVAROV, OLGA (1959).—Parenteral administration of copper compounds to cattle with special reference to copper glycine (copper amino-acetate). *Vet. Rec.* 71:797-810.
- BECK, A. B. (1956).—The copper content of the liver and blood of some vertebrates. *Aust. J. Zool.* 4:1-18.
- BENNETTS, H. W., and CHAPMAN, F. E. (1937).—Copper deficiency in sheep in Western Australia: A preliminary account of the etiology of enzootic ataxia of lambs and an anaemia of ewes. *Aust. Vet. J.* 13:138-49.

- CLARE, N. T., CUNNINGHAM, I. J., and PERRIN, D. D. (1945).—The determination of copper in pastures and livers. *N.Z.J. Sci. Tech.* A26:340-50.
- CUNNINGHAM, I. J. (1931).—Some biochemical and physiological aspects of copper in animal nutrition. *Biochem. J.* 25:1267-94.
- CUNNINGHAM, I. J. (1946).—Copper deficiency in cattle and sheep on peat lands. *N.Z.J. Sci. Tech.* A27:381-96.
- DICK, A. T. (1944).—Aspiration biopsy of the liver in sheep. *Aust. Vet. J.* 20:298-303.
- DICK, A. T. (1952).—Improved apparatus for aspiration biopsy of the liver in sheep. *Aust. Vet. J.* 28:234-5.
- DONALDSON, R., SISSON, R. B., KING, E. J., WOOTTON, I. D. P., and MACFARLANE, R. G. (1951).—Determination of haemoglobin VII. Standardised optical data for absolute estimations. *Lancet* 260:874-81.
- HARVEY, J. M. (1952).—Copper deficiency in ruminants in Queensland. *Aust. Vet. J.* 28:209-16.
- HARVEY, J. M., RYLEY, J. W., BEAMES, R. M., and O'BRYAN, M. S. (1961).—Studies on the cause of a low copper status in cattle in south-eastern Queensland. *Qd J. Agric. Sci.* 18:85-104.
- LEE, H. J., and MOULE, G. R. (1947).—Copper deficiency affecting sheep in Queensland. *Aust. Vet. J.* 23:303-9.
- LOOSEMORE, R. M., and ALLCROFT, RUTH (1951).—Technique and use of liver biopsy in cattle. *Vet. Rec.* 63:414-6.
- MOIR, K. W. (1954).—The preservation of bovine blood for the determination of inorganic phosphate in the diagnosis of aphosphorosis. *Qd J. Agric. Sci.* 11:143-7.
- SUTHERLAND, A. K. (1956).—Copper supplementation trials with cattle in Queensland. *Proc. Aust. Soc. Anim. Prod.* 1:18-9.
- SUTHERLAND, D. N. (1952).—Copper deficiency in cattle in Queensland. *Aust. Vet. J.* 28:204-8.
- WATSON, J. W. (1960).—A note on open laparotomy to obtain liver samples from new-born lambs. *Aust. Vet. J.* 36:472.
- PRYOR, W. J. (1960).—Copper storage in the bovine foetus. *Proc. Aust. Soc. Anim. Prod.* 3:162-6.
- VAN DER GRIFT, J. (1955).—*Diss. Utrecht* (Cited by Allcroft, Ruth, and Uvarov, Olga (1959) *Vet. Rec.* 71:797-810).
- WINTROBE, M. E. (1947).—“Clinical Haematology”. 2nd Ed. (Lea and Febiger: Philadelphia.)

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