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CAUSES OF VARIATION OF COPPER, IRON, MANGANESE, ZINC AND MAGNESIUM LEVELS IN BOVINE LIVERS*

3. THE EFFECTS OF LOCALITY

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SUMMARY

The effects of locality on the copper, iron, manganese, zinc and magnesium levels in 407 bovine caudate lobe liver samples preserved in formalin for differing storage periods were examined. The mineral determinations, expressed on the wet basis (WB), were made by atomic absorption spectrophotometry after wet ashing of the liver. Two hundred and ten of the liver samples were from cattle from one farm (Farm 1) the remaining 197 cattle being from another farm (Farm 2).

The copper, iron and magnesium levels were taken as indicative of the hepatic concentrations at slaughter. Locality had a significant effect ($P < 0{,}05$) on the copper, iron and magnesium levels. All copper levels on Farm 1 fell well below the accepted minimum (33,0 mg/kg). The deficiency appeared to be secondary with the possible implication of sewage effluent.

In terms of biological variation the different iron levels appeared of minor importance and no inverse relationship was found between iron and copper.

The manganese and zinc levels were interpreted with caution due to the significant differences reported in their hepatic concentrations after six months of storage in formalin. Extremely high zinc levels in individual animals could have been associated with sewage effluent.

INTRODUCTION

The liver is the main storage organ for copper and its copper concentration has been found to vary enormously[35]. Low levels of copper in the livers of cattle are the result of a primary deficiency, when the diet is inadequate, or a secondary (conditioned) deficiency when the dietary intake is sufficient, but the utilization of the copper is impeded, for example by the interaction of molybdenum and sulphate[5 27 31 32]. Copper deficiency in cattle has been associated with unthriftiness, anaemia[26 31], a low incidence of spontaneous bone fractures[31] and neonatal ataxia[31]. Depigmentation and defective keratinization of hair, fibrosis of the myocardium with associated sudden death, severe diarrhoea and low fertility[2 26 31] have also been described in copper deficient areas. More recently copper supplementation in cattle has produced significant increases in conception rates[1], reduced intercalving periods[15] and improved semen quality[14].

The liver is also the main storage organ for iron[35] and its content is affected by an interaction between the developmental or productive status and the diet of the animal concerned, but may also be due to the influence of certain pathological conditions. Iron-deficiency anaemia leads to a diminution in the amount of iron stored in the liver. This primary deficiency has been recorded in calves consuming an exclusive milk diet[17] and there is a positive correlation between the blood haemoglobin level and liver iron in calves[28]. There is, however, no convincing evidence that iron deficiency ever occurs in grazing stock under natural conditions, except possibly in circumstances involving severe blood loss or a disturbance in iron metabolism, as a consequence of parasitic infestation or disease[5 32].

Manganese is not concentrated in any particular organ or tissue. The concentrations in the liver are, however, higher than most other tissues and can be raised or lowered with varying manganese intake. The manganese storage capacity of the liver is limited when compared with the remarkable capacity of this organ to accumalate iron and copper[31 35]. Although a primary manganese deficiency is rare under farm conditions[5], a deficiency of manganese in cattle may cause poor growth, skeletal abnormalities and depressed or disturbed reproductive function[3 9 25 31 32]. Improved fertility has been recorded with manganese supplementation in cattle grazing on manganese deficient herbage[36].

According to Underwood[32] the capacity of the animal to store zinc in any of its organs other than bones, is extremely limited so that animals do not normally carry large reserves of zinc. More recently van Leeuwen and van der Grift[33] and Miller, Blackmon, Gentry and Pate[19] have shown that high dietary levels of zinc give rise to large increases in bovine liver zinc levels. As a state of zinc deficiency develops there is usually, but not invariably, a small decline in the concentration of zinc in the liver and certain other tissues[32]. In cattle, zinc deficiency has been associated with subnormal growth, alopecia and parakeratosis particularly of the muzzle, ears, neck, genitalia, back of the hind legs and knee-folds, a stiff gait and swelling of the hocks and knees and retarded testicular development[4 20 21 24 32 33]. Zinc supplementation has been associated with a marked improvement in conception rate in cows[22].

A lowered serum-magnesium concentration is implicated in the metabolic hypomagnesaemic tetanies of cows and calves. Some workers claim that the magnesium content of milk, soft tissue and bones of cows with hypomagnesaemic tetany remain within normal limits[32 34]. Others claim that although there is no large mobilizable store of magnesium in the body, the reserves which do exist in the bones and soft tissues can be of importance under stress situations[5]. Low

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magnesium intake has been associated with the calcification of certain soft tissue, in particular blood vessels, as well as renal lesions, in laboratory animals[18], calves[8] and more recently in sheep[6]. From his investigations in sheep, Boyazoglu[6] found that the occurrence of these lesions was not only directly correlated with low magnesium levels in the diet but also with low magnesium levels in the liver. Furthermore in his studies on calcium and phosphorus inadequacy, Boyazoglu[5] has encountered high magnesium levels in the liver in the presence of normal dietary magnesium intakes.

An earlier pilot investigation conducted on 80 beef cattle owned by the Johannesburg City Council, in which the analysis of liver samples for copper, zinc and manganese concentration was undertaken brought to light an apparent copper deficiency. This deficiency was more marked in cows from one of the two farms, from which the samples were drawn[10]. In view of the implications regarding decreased productivity, more extensive investigations were instituted so that corrective procedures could be applied should the results of the pilot trial be confirmed. It was decided to include iron and magnesium assays in the studies.

MATERIALS AND METHODS

A total of 407 liver samples from cross-bred beef cattle of various ages were taken at slaughter and submitted to a laboratory for analysis for copper, iron, manganese, zinc and magnesium content[29]. The cattle, slaughtered over a period of 15 months, came from two intensive beef enterprises which were ancillary to sewage and waste-water purification plants operated by the Johannesburg City Council.

EXPERIMENTAL ANIMALS

Of the 407 liver samples 210 were from animals from Farm 1 situated 40 kilometres north of Johannesburg. Apart from the areas occupied by the purification plant and that used in the production of preserved communal cattle feed, the farm is divided into five, separate cattle sections. Three of these, namely 1, 2 and 3 are cow breeding and calf raising sections. Section 4 comprises a heifer breeding and young stock section and is the section to which the weaned calves from sections 1, 2 and 3 are sent at approximately 7 months of age. Section 5 is a separate feedlot.

The remaining 197 liver specimens were from animals from Farm 2 situated 24 kilometres south of Johannesburg. As with Farm 1 a considerable portion of Farm 2 is not grazed by cattle. The five cattle sections are once again strictly separated and also comprise three cow breeding and calf raising sections (sections 1, 2 and 3), a heifer breeding and young stock section (section 4) and a separate feedlot (section 5).

The origin of the 407 liver samples is given in table 1.

All animals with the exception of the weaners, steers and 10 breeding heifers from section 1 of Farm 2 which were recently transferred from section 4, had been in their sectional localities for at least 6 months prior to slaughter.

The breeding females slaughtered were animals culled for failure to conceive, but because a very strict culling procedure was in force it should not be concluded that all the cows were infertile. The maiden heifers and weaners were culled because they fell below the operative group selection indices calculated mainly on a daily weight gain. The steers were slaughtered when ready for market.

Table 1: ORIGIN OF BOVINE LIVER SAMPLES

Type	FARM 1					FARM 2				
	Section					Section				
	1	2	3	4	5	1	2	3	4	5
Cows	50	59	10			59	42	48		
Breeding heifers				12		10			8	
Maiden heifers				20					20	
Weaners				32						
Steers					27					10
Total	210					197				

During the sampling period the cattle population of Farm 1 averaged 3 479 with an average of 1 825 breeding females. During the same period the average population on Farm 2 was 4 187 with 2 002 breeding females. The respective production figures for Farms 1 and 2 for the three breeding seasons from which the sampled cows were drawn is given in Table 2. The number of calves weaned, over the total number of females bred, is expressed as a percentage. Breeding on both farms was by artificial insemination.

Table 2: PRODUCTION OF CALVES ON FARMS 1 AND 2, FOR THE BREEDING SEASONS OF SAMPLED BREEDING CATTLE.

Breeding Season	Calf Production %	
	Farm 1	Farm 2
June/July/August 1968	58,8	71,0
Nov./Dec. 1968 Jan. 1969	57,7	79,0
June/July/August 1969	61,6	73,0

MANAGEMENT AND FEEDS

Extensive use is made of effluent flood irrigation in the production of the cattle feed grown on these farms. The effluent used on Farm 1 is mainly from domestic sewage with the direction of flow from section 4 through sections 2 and 1 and finally to section 3. The irrigation effluent used on Farm 2 comes from two purification works, one of which handles both industrial and domestic waste-water and supplies the irrigation effluent for sections 4 and 1. The other works handles mainly domestic sewage and supplies first section 2 and then section 3.

The feeds produced on both farms were similar. The pastures consisted predominantly of rye-grasses (a mixture of perennial rye-grass *(Lolium perenne)* Italian rye-grass *(L. multiflorum)* and tetraploid rye *(L. multiflorum sel.)*, a mixture of rye-grass, small areas of clover *(Trifolium spp.)* and particularly on Farm 2, fescue grasses *(Festuca spp.)* and cocksfoot *(Dactylis glomerata L.)*. In addition, large *Eragrostis curvula* pastures, and particularly on Farm 2, kikuyu pastures *(Pennisetum clandestinum)*, plus small areas of naturally occurring veld grasses were available. *E. curvula* hay and a limited amount of lucerne hay *(Medicago sativa)* was produced. Maize *(Zea mays)* and some hybrid sorghum species were grown and ensiled.

The only additional feed used during the period of this study was a maize/sorghum brewers grain mixture.

A lick consisting of equal quantities of salt (NaCl) and dicalcium phosphate to which 0,25 per cent copper sulphate and 0,125 per cent cobalt sulphate was added was continually available to all animals. The intake of the lick was not monitored and an adequate intake is questionable owing to its low acceptability.

Drinking water from a domestic source was provided in all overnight camps and feeding pens, but grazing animals on occasion had access to effluent water from the irrigation canal and holding dam system on the two farms.

The animals in both farms feedlots were on zero grazing. All other animals had access to pastures for the bulk of their nutritional requirements. The nutritional requirements were however controlled and were not on an *ad libitum* basis.

The access to grazing is important in so far as both farms were infested with liver fluke (mainly *Fasciola hepatica*) and consequently grazing animals were exposed to varying degrees of infestation depending upon the locality.

COLLECTION OF LIVER SAMPLES

All liver samples were removed at the time of evisceration which was approximately 45 minutes after stunning and bleeding. Annotation of degree of liver damage due to fascioliasis was made and only those livers not showing extensive damage were sampled. Each sample in the form of an approximate 20 g blunt ended wedge was taken from a portion of caudate lobe which appeared normal. The sample was immediately placed in a glass bottle containing sufficient 10 per cent formalin to cover the specimen completely. The formalin solution was prepared from laboratory reagent formaldehyde solution (37 to 41 per cent (W/V) diluted with distilled water. After collection the bottles were tightly sealed and stored until required for the analysis of the various elements[29]. The period of storage varied from 4 to 19 months. The methods for the determination[29] and the dispersion of the metals in the liver together with the effects of storage in formalin on the mineral concentrations and moisture content of liver samples have been previously described[30].

The mineral concentrations are expressed on the wet basis (WB)

ANALYSIS OF DATA

Data pertaining to the mineral content of the livers was arranged as detailed below. The possible interactions of the age of animal, time of slaughter and varying degrees of liver damage on the mineral concentrations in the livers were ignored.

Comparisons were made between:-

(i) the overall results of the two farms;

(ii) the composite results of the cows of the two farms;

(iii) the composite results of the breeding females of the two farms

(iv) the individual cow and heifer sections within each farm.

The 10 recently introduced breeding heifers from section 1 on Farm 2 were excluded from the comparisons made in sections (iii) and (iv) above.

Analysis of variance (Anova) was used to determine the statistical significance of the above comparisons. In the formal Anova the analysis terminates with the calculation of the F-ratio and it is left to the experimenter to determine which treatments led to the rejection of the null hypothesis (Ho) if this was the case. The computer however was programmed to perform the Scheffe multiple comparison procedure (S-method) and in this way isolate the out of range means.

The comparisons were tested at the 95 per cent level of confidence ($P < 0,05$).

RESULTS

The comparison between the overall results of the 210 cattle from Farm 1 and the 197 cattle from Farm 2 (Table 3) showed that the levels of copper, iron and zinc of Farm 1 were significantly lower ($P < 0,05$) than those of Farm 2.

Table 3: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg BETWEEN FARMS 1 AND 2

Farm	N	Mineral concentrations mg. per kg. Wet Basis									
		Cu		Fe		Mn		Zn		Mg	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
1	210	15,2	13,6	120,1	63,7	3,6	1,0	39,9	26,6	136,4	26,1
2	197	32,1	20,0	133,2	46,6	3,7	1,1	51,3	58,5	135,0	35,3
Comparison $P < 0,05$		*		*		N.S.		*		N.S.	

Cu = copper. Fe = iron. Mn = manganese. Zn = Zinc. Mg = Magnesium
N = Number of samples $\bar{X}$ = Mean; S.D. = Standard deviation;
* = Significant (at the 95 per cent level of confidence);
NS = Not significant (at the 95 per cent level of confidence)

The comparison between the composite results of the cows of the two farms (Table 4) showed that the 119 cows of Farm 1 had significantly lower ($P < 0,05$) copper and iron levels than the 149 cows from Farm 2. levels than either the 50 cows from section 1 or the 59 cows from section 2; there was no significant difference between the heifers' magnesium levels and those of the 10 cows from section 3.

Table 4: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF THE COWS OF FARMS 1 AND 2

Farm	N	Mineral concentrations mg/kg. Wet Basis									
		Cu		Fe		Mn		Zn		Mg	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
1	119	11,1	11,6	107,2	34,4	3,6	1,0	41,3	32,1	128,7	23,0
2	149	30,7	20,4	133,4	48,0	3,6	1,2	49,8	61,1	130,5	23,4
Comparison $P<0,05$		*		*		N.S.		N.S.		N.S.	

The comparison of the composite result of the breeding females of Farm 1 and Farm 2 is given in Table 5. It was found that the inclusion of the breeding heifers of section 4 did not alter, to any degree, the figures shown in Table 4 above. Once again the copper and iron results of Farm 1 were significantly lower ($P < 0,05$) than the results of Farm 2.

The results and comparisons of the cows from sections 1, 2 and 3 and the heifers from section 4 of Farm 2 are given in Table 7. Significant diferrences ($P < 0,05$) occurred in the copper, manganese and magnesium levels. The 59 cows from section 1 had the lowest copper levels which were significantly lower than the 42 cows from section 2, the 48 cows from section 3 and the 28 heifers from section 4. However,

Table 5: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF THE COWS AND BREEDING HEIFERS OF FARMS 1 AND 2

Farm	N	Mineral concentrations mg/kg. Wet Basis									
		Cu		Fe		Mn		Zn		Mg	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
1	131	10,7	11,3	107,2	33,1	3,5	1,0	41,4	32,0	128,8	22,6
2	157**	31,2	20,4	132,9	47,2	3,6	1,2	52,4	61,8	131,1	23,5
Comparison $P<0,05$		*		*		N.S.		N.S.		N.S.	

** 10 breeding heifers ex section 1 of Farm 2 not included.

there were no significant differences between the copper levels of section 2, 3 and 4. The 28 heifers had the highest manganese levels which were significantly ($P < 0,05$) different from the results of sections 1 and 3 but not different from section 2. The magnesium levels for the 28 heifers were significantly higher than those of the three cow sections. The levels for zinc, although ranging from a mean of 37,3±22,6 mg/kg for section 3 to a mean of 73,2±60,1 mg/kg for the 28 heifers were not significantly different ($P < 0,05$). The levels for iron showed little variation and were not statistically different ($P < 0,05$).

The results and comparisons of the cows from sections 1, 2 and 3 and the breeding and maiden heifers from section 4 of Farm 1 are given in Table 6. The only significant difference ($P < 0,05$) between the sections occurred in the copper and magnesium levels. The 59 cows from section 2 had the lowest copper levels, which were significantly different ($P < 0,05$) from the levels of the 50 cows from section 1 and the levels of the 10 cows from section 3, but were not significantly different from the levels of the 32 heifers. The 32 heifers had significantly higher ($P < 0,05$) magnesium

Table 6: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF COWS FROM SECTIONS 1, 2 AND 3 AND HEIFERS FROM SECTION 4 OF FARM 1

Farm 1 Sections	N	Mineral concentrations mg/kg. Wet Basis									
		Cu		Fe		Mn		Zn		Mg	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
1	50	13,5	11,7	100,4	26,6	3,5	1,1	33,1	7,9	131,3	24,5
2	59	7,4	8,6	114,3	40,1	3,7	0,9	46,5	43,8	126,1	22,8
3	10	21,3	17,9	99,5	25,3	3,7	0,5	51,6	13,1	131,7	16,0
4	32	11,2	8,4	102,0	15,2	3,7	1,0	42,2	24,4	152,4	28,2
Comparison $P<0,05$		1 * 2 3 * 2		N.S.		N.S.		N.S.		1 * 4 2 * 4	

Table 7: COMPARISON OF LIVER LEVELS OF Cu, Fe, Mn, Zn AND Mg OF COWS FROM SECTIONS 1, 2 AND 3 AND HEIFERS FROM SECTION 4 OF FARM 2

Farm 2 Sections	N	Mineral concentrations mg/kg. Wet Basis									
		Cu		Fe		Mn		Zn		Mg	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
1	59	19,5	13,7	131,7	55,8	3,6	1,3	65,8	88,3	132,0	26,7
2	42	35,6	18,3	136,5	49,8	3,9	1,0	41,5	35,0	133,0	23,0
3	48	40,2	22,7	132,6	35,1	3,2	1,1	37,3	22,6	126,3	19,0
4	28	33,4	16,8	135,6	51,8	4,6	0,9	73,2	60,1	157,1	25,2
Comparison P<0,05		1 * 2		N.S.		1 * 4		N.S.		1 * 4	
		1 * 3				3 * 4				2 * 4	
		1 * 4								3 * 4	

DISCUSSION

The copper, iron and magnesium levels were taken as indicative of the hepatic concentrations at slaughter[30]. However, the manganese and zinc levels were interpreted with caution due to the significant differences in their hepatic concentration reported after 6 months of storage in formalin[30].

COPPER LEVELS

Blood and Henderson[5] state that normal concentrations of copper in the livers of cattle should be above 100 mg/kg when calculated on the dry basis (DM) but are usually above 200 mg/kg (DM). These values have been confirmed by Underwood[32] who states that concentrations of copper in the livers of normal adult cattle are consistently high, within a range of 100 to 400 mg/kg on the dry basis with a high proportion lying between 200 and 300 mg/kg. When these values are converted to a wet basis (using a 66 per cent moisture content[30]) it would appear that normal levels should all be above 33 mg/kg (100 mg/kg DM) with the majority being between 66 and 99 mg/kg (200 to 300 mg/kg DM) Boyazoglu, Barrett, Young and Ebedes[7] in their recently completed survey of 190 bovine livers collected from South Africa found that the mean copper content was 48,8 ±31,9 mg/kg on the wet basis.

In terms of the above results it is quite obvious that all the copper levels of Farm 1 fell well below the accepted minimum. The overall mean of 15,2 ±13,6 mg/kg was less than half of this value. The mean of the breeding females was less than a third and the mean of the 59 cows from section 2 (7,4 ±8,6 mq/kg) was less than a quarter of the accepted minimum level. Whereas the values for the overall farm, cows and breeding females of Farm 2 can be regarded as just falling into the minimum accepted level, the sectional comparisons showed that the levels of the 59 cows from section 1 (mean 19,5 ±13,7 mg/kg) were well below normal limits.

As stated in the introduction, various workers have associated a copper deficiency with certain clinical manifestations in cattle. Clinical signs observed amongst cattle on Farm 1 suggest that they may have been associated with a copper deficiency. Unthriftiness, harsh stary hair coat, scours and impaired fertility were seen. As a parameter of impaired fertility one can compare the low overall calf production percentage of Farm 1 relative to Farm 2. Although some black coated animals had a slight reddish tinge, which could have been genetic, no specific symptoms were observed of copper deficiency. In fact the clinical signs observed were indistinguishable from those resulting from unbalanced nutrition or from helminthiasis, both of which were distinct possibilities. However, the low levels of copper demonstrated in the liver confirmed that copper was in fact involved.

The cumulative data of this investigation and the investigation into the effect of the age of the animal on the mineral concentrations[11, 12] suggest that the low copper levels were secondary (induced) rather than primary. Blood and Henderson[5] point out that extensive deposits of haemosiderin can be found in the liver, spleen and kidney in most cases of primary copper deficiency. However, no inverse relationship between iron and copper was observed in low copper status animals. In addition the demonstration that the younger animals on Farm 1 had significantly higher ($P < 0,05$) copper levels[11, 12] adds further weight to the deficiency having been an induced one. According to Blood and Henderson[5] "Apart from falling disease which occurs only in adult cattle, young animals are much more susceptible to primary copper deficiency than are adults. Calves on dams fed deficient diets may show signs at 2 to 3 months of age. As a rule the signs are severe in calves and yearlings, less severe in 2 year-olds and of minor degree in adults."

Of interest as possible aetiological factors of an induced copper deficiency was the presence of fair amounts of sulphates and nitrates in the sewage effluent[23]. The interaction of sulphate with molybdenum in the inducement of a copper deficiency has been mentioned. Nitrogen fertilization has been shown to bring about a decrease in copper concentration in plants[27] and the presence of nitrates in the irrigation effluent plus the liberal use of inorganic nitrogen fertilizer on the farm could have aided the manifestation of copper deficiency amongst the cattle.

The importance of balanced nutrition and elemental interaction as given in Mulder's interaction chart[27] should be borne in mind. More detailed analysis of the constituents of the effluent and various forages, and of the animals would have been necessary to confirm whether the deficiency was in fact induced, and if so, by what possible factors.

The association between the copper levels of the sections on Farm 1 with the flow of the irrigation effluent is interesting. With the exception of section 4 which was closest to the works, the copper levels were found to increase with increasing distance from the works. This relationship would seem to support the possible presence in the effluent of some conditioning factor, which decreased with increasing distance from the purification works. In this regard it can be mentioned that although the sulphate concentration of the effluent remained fairly constant, the nitrate content decreased with increasing distance from the purification works[23]. The levels of copper in the livers of cows from sections 2, 1 and 3 (given in order of proximity to the purification works) were 7,4 ±8,6 : 13,5 ±11,7 and 21,3 ± 17,9 mg/kg respectively. However, section 4, which was closest to the sewage works had higher copper concentrations (11,2 ±8,4 mg/kg for 32 sampled heifers) than the section next in line (section 2: 7,4 ±8,6 mg/kg for 59 cows). If the other animals of section 4, namely the weaners[11] had been taken into consideration, the values would have been even higher. A possible explanation for these relatively higher levels, appears to be a combination of age of animal and their brief stay in the locality. Of further interest, in support of the possible incrimination of the sewage effluent with the low concentrations of copper in the livers, is the fact that the steers (section 5) on both farms, but particularly on Farm 1, had consistently higher liver copper levels than the other sub-groups[11]. As stated earlier these animals were on zero grazing and did not come into direct contact with the sewage effluent.

IRON LEVELS

Iron deficiency states are rare in farm animals. The iron content of herbage plants although very variable as a consequence of species differences and soil effects is invariably much higher than the normal requirement for this element by the grazing animal. In addition the intake of soil under most grazing conditions, provides considerable opportunity for the grazing animal to obtain additional iron[32].

Boyazoglu *et al*[7] found the iron content of bovine livers to be 149 ±82,2 mg/kg (WB). These values together with the means for Farms 1 and 2 of 120,1 ±63,7 mg/kg and 133,2 ±46,6 mg/kg, as well as the sectional variations of 99,5 ±25,3 mg/kg for the 10 cows from section 3 of Farm 1 up to the mean of 136,5 ±49,8 mg/kg for the 42 cows of section 2, Farm 2, would all appear in the light of the earlier remarks to fall within normal limits. In terms of this apparent wide biological variation the demonstration of statistically significant ($P<0,05$) inter-farm differences therefore appears meaningless. Although variation in the concentrations in individual animals was extreme (39,9 to 729,9 mg/kg), the majority fell within, or close to, the above mean values[11].

Of interest is the examination of the copper : iron relationships both on group level and on individual animal level[11]. Marston, Lee and McDonald[16] demonstrated extensive deposits of iron in the liver and other tissues of sheep confined to copper deficient grazing. More recently Boyazoglu *et al*[7] drew attention to a similar inverse relationship between copper and iron liver levels in certain individual cattle. However, the examination of the various mean values given above show that the lower copper values are invariably coupled with the lower iron values. Only in the case of the sectional comparisons on Farm 1 were the lowest copper levels (mean 7,4 ±8,6 mg/kg) coupled to the highest iron levels (mean 114,3 ±40,1 mg/kg). Furthermore perusal of the data of individual animals showed no consistent relationship[11].

MANGANESE LEVELS

Primary manganese deficiency is uncommon under farm conditions. However, a manganese dificient diet usually results in a lowering of the limited amount stored in the body, located mainly in the bones and to a smaller extent in the liver and other tissues[32]. According to Underwood[32] the liver levels of manganese are useful but not entirely reliable indicators of manganese deficiency, unless the deficiency is severe. Gessert, Berman, Kastelic, Bentley and Phillips[13] state that the livers of cattle generally contain 8 to 10 mg/kg manganese on the dry basis (2,7 to 3,3 mg/kg WB) whereas Blood and Henderson[3] state that the manganese content of the liver in normal animals is about 12 mg/kg on the dry basis (4 mg/kg WB) and about 8 mg/kg (DM) (2,7 mg/kg WB) in calves. However Boyazoglu *et al*[7] found a mean of 8,1 ±2,6 mg/kg (WB) for 188 bovine livers, which included the bulk of the 80 livers from Farms 1 and 2 (mean 9,3 ±2,9 mg/kg) examined in the pilot investigation[10].

The various mean values for Farms 1 and 2 in this study fell within the range of normality given by Gessert *et al*[13]. and Blood and Henderson[3]. However, the range for individual animals was quite wide, from 1,2 mg/kg to 10,2 mg/kg[11].

The investigations into the effect of prolonged storage in formalin on manganese concentrations revealed a statistically significant increase (P± 0,05)[30]. This is 'however' difficult to equate with the variations of individual animals[11]. Groups from different farms sampled at the same time and therefore subjected to similar storage conditions showed extreme variation. This was well illustrated in a group from Farm 2 where the 19 animals had a mean of 3,65 mg/kg and varied from 2,0 to 9,2 mg/kg[11]. Although this variation was extreme, the pattern of high and low within a group was constant[11]. This was interpreted as indicating an inconsistent loss or gain of manganese due to storage in formalin and it was concluded that the various comparisons were valid.

The statistically significant ($P<0,05$) sectional differences on Farm 2 where the heifer section had the highest levels (mean 4,6 ±0,9 mg/kg) and cow section 3 the lowest (mean 3,2 ±1,1 mg/kg) were interpreted as insignificant in terms of accepted normal variation

ZINC LEVELS

As stated earlier there is definite evidence of a naturally occurring zinc deficiency in cattle.

Gessert *et al.*[13] reported a mean of 125 mg/kg zinc on the dry basis for the livers of 30 milking cows (42 mg/kg on the wet basis) and van Leeuwen and van der Grift[33] after working on zinc metabolism concluded that normal liver values for cattle lay between 100 to 200 mg/kg (DM), that is between 33 and 66 mg/kg (WB). However Boyazoglu *et al.*[7] recorded a mean of 158,0 ±75,9 mg/kg (WB) for 189 cattle in South Africa. Included in the latter analyses are the bulk of the 80 livers from Farms 1 and 2 (mean 103,3 ±43,0 mg/kg) examined in the pilot investigation[10] Thus, as with manganese, the current zinc levels on the farms were lower than those demonstrated in the pilot investigation[10].

However, it is difficult to interpret the values demonstrated in this study in view of the significant ($P < 0,05$) loss of zinc content shown to occur during 6 weeks to 6 months storage in 10 per cent formalin[30]. Thus no conclusions were drawn, even though no group levels were found to be below the 33 mg/kg (WB) given by van Leeuwen and van der Grift[33] as the normal minimal value. No clinical signs indicative of a zinc deficiency were observed on either farm.

In contrast to the variation of manganese concentrations, the groups on the farms showed consistent zinc levels with the particular exception of some groups from sections 1 and 4 of Farm 2[11]. This is interesting in view of the fact that these sections were the only sections supplied by the sewerage works handling the greatest volume of industrial effluent. Some of the levels demonstrated on these two sections were extremely high (up to 501,8 mg/kg). This observation plus the marked variation encountered may have been associated with the zinc content of the effluent.

MAGNESIUM LEVELS

Boyazoglu *et al.*[7] in their analysis of 188 bovine livers for magnesium content demonstrated a mean of 208 $\pm$60,7 mg/kg on the wet basis. The levels demonstrated on Farms 1 and 2 were, however, lower, (overall farm means: Farm 1 136,4 $\pm$26,1 mg/kg and Farm 2 135,0 $\pm$35,3 mg/kg with the overall mean of the cows and breeding females showing hardly any deviation from the overall mean). However, the sectional comparisons within a farm showed that the heifers on both farms had the significantly highest levels ($P < 0,05$). Although the variation in the levels of magnesium of individual animals was extreme, from 46,2 to 463,0 mg/kg (a breeding heifer from section 1 of Farm 2), the majority fell close to, or within their respective mean values[11].

Boyazoglu[6] has found high concentrations of magnesium in the liver to be indicative of inadequate calcium : phosphorus nutrition and in terms of this finding the demonstrated sectional differences may indicate that the calcium and phosphorus intake of the heifers was relatively inadequate despite the fact that all magnesium levels fell below those of Boyazoglu *et al.*[7]

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