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Experimentally induced chronic copper toxicity in cattle

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ABSTRACT

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Eight Bonsmara bulls and eight Bonsmara heifers, having masses of between 210 and 266 kg when selected, were randomly allocated to four groups, each comprising two bulls and two heifers. Group 1 received 0,6 mg of copper (Cu)/kg of body mass per day (bm/d), group 2, 10 mg of Cu/kg of bm/d and group 3, 20 mg of Cu/kg of bm/d as a copper sulphate solution, given orally, 5 d a week over 745 d. Group 4 was the control group. One bull from group 3 was euthanased on day 679 of the trial, a heifer from group 3 and a bull from group 2, on day 695 of the trial, and a heifer from group 2, on day 731 of the trial, after they had shown clinical signs.

During the course of the trial, clinical signs, serum gamma glutamyltransferase and aspartate aminotransferase activity, blood urea nitrogen, and plasma copper, zinc and iron concentrations were monitored. Live mass was recorded weekly to determine any effect on mass gain. The liver and kidney copper, zinc, iron and manganese concentrations at the time of death or slaughter are given.

From the results it was concluded that subclinical damage to the liver and eventual copper toxicity can occur when cattle are continually exposed to oral doses ≥12 mg of Cu/kg of bm/d. It was also concluded that cattle can probably tolerate oral doses of ≤0,6 mg of Cu/kg of bm/d for an indefinite period, provided there are no other sources of copper, such as may occur with air-pollution, or provided no other adverse mineral interactions occur, such as may occur with molybdenum deficiency.

Keywords: Cattle, chronic copper toxicty, experimentally induced

INTRODUCTION

Copper mining plays a major role in the South African economy and the economy of other African countries, and the possibility of cattle coming into contact with copper mining operations or high background concentrations of copper, remains distinct. In 1989, a descriptive epidemiological investigation showed that cattle near Phalaborwa in the northern Transvaal had died of chronic copper poisoning caused by air pollution (Gummow, Botha, Basson & Bastianello 1991). Despite a number of reports of field outbreaks of chronic copper poisoning in cattle (Shand & Lewis 1957; Stogdale 1978; Perrin, Shiefer & Blakely 1990), no precise data could be found on the tolerance levels for cattle chronically exposed to oral ingestion of copper (Cu). A trial was therefore designed to determine the effects in cattle, of continual exposure to low doses of copper administered *per os*.

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MATERIALS AND METHODS

Eight Bonsmara bulls and eight Bonsmara heifers, weighing between 210 and 266 kg when selected, were randomly allocated to four groups each comprising two bulls and two heifers. Group 1 received 0,6 mg of Cu/kg of body mass per day(bm/d), group 2, 10 mg of Cu/kg of bm/d and group 3, 20 mg of Cu/kg

of bm/d as a copper sulphate solution, given orally, 5 d a week over 745 d. Group 4 served as a control group and received no copper. The eight bulls were housed in a separate outdoor camp, adjacent to the eight heifers. Both camps had concrete floors. All animals received the standard ration fed to Onderstepoort Veterinary Institute (OVI) experimental cattle. This ration consisted mainly of maize silage supplemented with lucerne or *Eragrostis* hay, and no mineral supplements were supplied. Samples of the ration were analysed sporadically throughout the trial period for background copper concentrations.

Each animal was observed daily for signs of ill health. Once a week, beginning on day –7 of the experiment, each animal was weighed on an electronically calibrated scale with a standard deviation of less than 100 g, and their masses were recorded. Starting on day –39 until day 114 of the trial, 20 mℓ of venous blood was collected from the jugular vein of each animal once a month, by means of standard evacuated blood-collecting tubes. Ten millilitres of the 20 mℓ aliquot of blood was collected into heparinized vacutubes, and 10 mℓ, into standard glass vacutubes. Each animal was similarly bled once every 2 weeks between days 114 and 212 of the experiment and after that, once a week until the end of the experiment (day 745) or until the animal had been euthanased owing to copper toxicity.

Plasma or serum, depending on the type of vacutube used, was separated from the whole blood samples as soon as practically possible after the bleeding. The plasma samples were analysed within 2 d for copper (Cu), zinc (Zn), and iron (Fe) concentrations, by means of an atomic absorption spectrophotometry method (Boyazoglu, Barrett, Young & Ebedes 1972), and the serum samples were analysed on the same day, for gamma glutamyltransferase (GGT) and aspartate aminotransferase (AST) activity and blood urea nitrogen (BUN) concentrations. Boehringer Mannheim, France SA, CBR kits were used for the determination of GGT and AST activity.

Semen was collected from the bulls approximately every 35 d, by means of electro-ejaculation, with the use of a bipolar rod ejaculator. The purpose of this collection was to determine whether Cu had any effect on semen quality and quantity. Unfortunately, during the course of the experiment, the bulls became infected with ureaplasmosis (Gummow, Staley & Gouws 1992) and, since the long-term effects of these infections could not be quantified, it was decided to regard the results of this aspect of the experiment as unreliable. This aspect of the experiment will therefore not be discussed further.

Four animals showing symptoms of Cu poisoning were euthanased by an overdose of pentobarbitone sodium (Eutha-naze, Centaur Labs) administered intravenously. Complete necropsies were performed on these animals shortly after euthanasia. Liver and kidney specimens from each animal were analysed for Cu, Zn, Fe and manganese (Mn) concentrations. The remainder of the animals were slaughtered at the end of the trial at the OVI abattoir (with the exception of one control animal which was found to be 6 months pregnant). The slaughtered animals were examined at the abattoir for evidence of pathology, and liver, kidney and lung specimens were taken in formalin for histopathology and the determination of Cu, Zn, Fe and Mn concentrations. The organ mineral determinations were carried out by means of a standard atomic absorption spectrophotometry method (Boyazoglu *et al.* 1972).

Statistical procedures

The area under the "concentration" (AUC) versus the time curve was calculated by the trapezoidal method (Rowland & Tozer 1980) for mass, GGT, AST, BUN and plasma Cu, Zn and Fe concentrations. A two-sided student *T*-test was then used to determine whether the null hypothesis (H_0)—that no difference existed between the mean AUC for groups 1, 2 or 3 and the control group—could be accepted or not. AUC was chosen for comparison since it reflected both the rate and extent of change over time, which the mean, alone, failed to do. The AUC was calculated up to 681 d, at which stage the first animal died.

In addition to the AUC, the student *T*-test was used to test for any difference between the Cu dosed groups and the control animals, with respect to the average daily gain (ADG). Average daily gain was calculated as:

$$\text{ADG} = \frac{[(\text{Maximum mass achieved}) - (\text{mass at } -7 \text{ d})]}{[\text{The time taken to reach maximum mass}]}$$

Differences in AST and GGT enzyme activity, and BUN and plasma Cu, Zn and Fe concentrations between the Cu dosed groups and the control animals, were tested for by use of the student *T*-test. In addition, the student *T*-test was used to compare liver, kidney and lung concentrations of Cu, Zn, Fe and Mn. Comparisons were made with respect to the mean enzyme activity or concentration obtained over the experimental period, as well as the mean AUC.

RESULTS

Symptoms

One bull from the high-dose group 3 was euthanased on day 679 of the trial, after it had shown rumen stasis, bloat, depression and congested mucous membranes. A heifer from group 3 and a bull from group 2 were euthanased on day 695 of the trial after they had shown symptoms similar to those of the first bull, as well as a green, watery, mucoid diarrhoea and a

mucopurulent nasal discharge. These two animals began showing signs of depression, diarrhoea and erratic rumen movements approximately 15–17 d before they were euthanased. The severity of the signs increased until they were euthanased. A fourth animal, a heifer from group 2, was euthanased on day 731 of the trial after it had shown only moderate symptoms similar to those already described. The rectal temperature in all four cases was normal (38,1–39,1 °C) and their heart rates ranged from 40–71 beats per min.

Effects of Cu on mass

The average mass per group over time for bulls and heifers is shown in Fig. 1. Heifer mass gains were lower in all groups receiving Cu, as compared with the control heifer group. The bulls, however, showed little difference between the middle- or high-dose Cu groups and the control bulls, but the low-dose group appeared to gain more mass than the controls (Fig. 1). The distinct drop in mass seen in all groups from about day 630 was attributed to change in the ration at that time, from lucerne to *Eragrostis* hay supplement.

With respect to AUC for mass and ADG, there was no statistically significant difference ($P < 0,05$) between groups receiving Cu and the control groups (Table 1).

Effects of Cu on AST activity

Fig. 2 shows the average AST activity over time per group for heifers and bulls. No significant ($P < 0,05$) difference in AST activity could be found between heifers and bulls within each group, with respect to AUC. The bull and heifer data were therefore combined for each group, and the pooled data were used to test for a difference between the Cu-receiving groups and the control animals. Table 2 gives a summary of the statistics applicable to each group.

The rate and extent of AST activity (i.e. AUC) in the group receiving 10 mg of Cu/kg of bm/d was found to be significantly greater than that in the control animals at the 90% confidence level and in the group receiving 20 mg of Cu/kg of bm/d at the 99% confidence level (Table 3). No difference in AUC could be found between the control animals and the group receiving 0,6 mg of Cu/kg of bm/d. All the Cu-receiving groups had increased mean AST activity, calculated for the duration of the experiment (Table 3), as compared with the control animals at the 95% confidence level.

Effects of Cu on GGT activity

Fig. 3 shows the GGT activity for heifers and bulls over time. No significant difference could be found between the GGT activity for the heifers that received Cu and the controls, when the AUC for each group was compared (Tables 4 and 5). There was, however, a significantly increased AUC for GGT in the bulls that received 10 and 20 mg of Cu/kg of bm/d ($P < 0,10$), as compared with the control bulls (Tables 4 and 5). When the AUC results of the heifers and bulls were combined, no difference in GGT activity could be shown between the Cu-receiving groups and the control animals (Tables 4 and 5). There was, however, a significant difference ($P < 0,05$) between the means of the groups that received 10 and 20 mg of Cu/kg of bm/d, and the control animals. Table 4 gives a summary of the statistics relevant to GGT activity.

Effects of Cu on plasma Cu concentrations

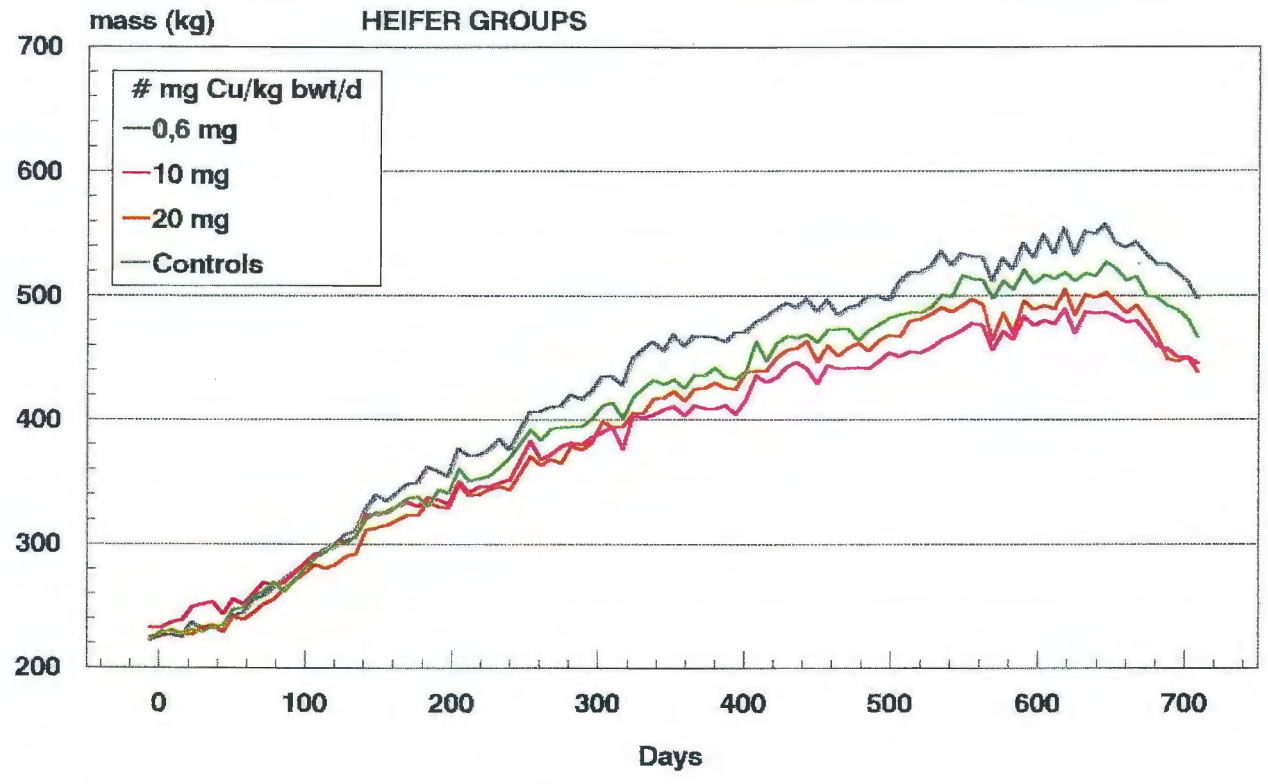
Fig. 4 shows the plasma Cu concentrations for heifers and bulls over time. Summary statistics for Cu plasma concentrations as measured over time for each group are shown in Table 6, and the results of the *T*-tests for the comparison of plasma Cu concentrations and AUC means are shown in Table 7. Significant differences ($P < 0,05$) in mean plasma Cu concentrations and AUC were seen between the control cattle and those that received 10 mg of Cu/kg of bm/d or 20 mg of Cu/kg of bm/d. Data stratified by sex showed that only the groups that received 20 mg of Cu/kg of bm/d differed significantly ($P < 0,10$) from the controls with respect to higher mean plasma Cu concentrations and mean AUCs (Table 7).

Effects of Cu on blood urea nitrogen, plasma Zn and plasma Fe concentrations

A statistical summary of the BUN, Zn and Fe data is shown in Table 8. No significant difference ($P < 0,05$) could be found between Cu dosed groups and control groups with respect to mean concentrations or AUCs for BUN, Zn and Fe, with one exception; the mean plasma Fe concentration during the trial period in the group that received 0,6 mg of Cu/kg of bm/d, was significantly lower than that in the control group. The BUN concentrations for heifers and bulls over time are shown in Fig. 5.

Organ Cu concentrations

Comparison (with the use of the *T*-test) of the liver Cu concentrations at slaughter or euthanasia (Table 9) of the different groups showed that the group that received 0,6 mg of Cu/kg of bm/d, and the group that received 20 mg of Cu/kg of bm/d had significantly higher ($P < 0,01$) concentrations of Cu in their livers than did the control group. No conclusion could be reached regarding the group that received 10 mg of Cu/kg of bm/d, because of the large variance within this group. No difference could be demonstrated between groups for Zn, Fe and Mn concentrations in the liver ($P < 0,01$) and no difference could be demonstrated between groups for kidney Cu concentrations. Lung Cu concentrations were ≤ 2 ppm for all groups.



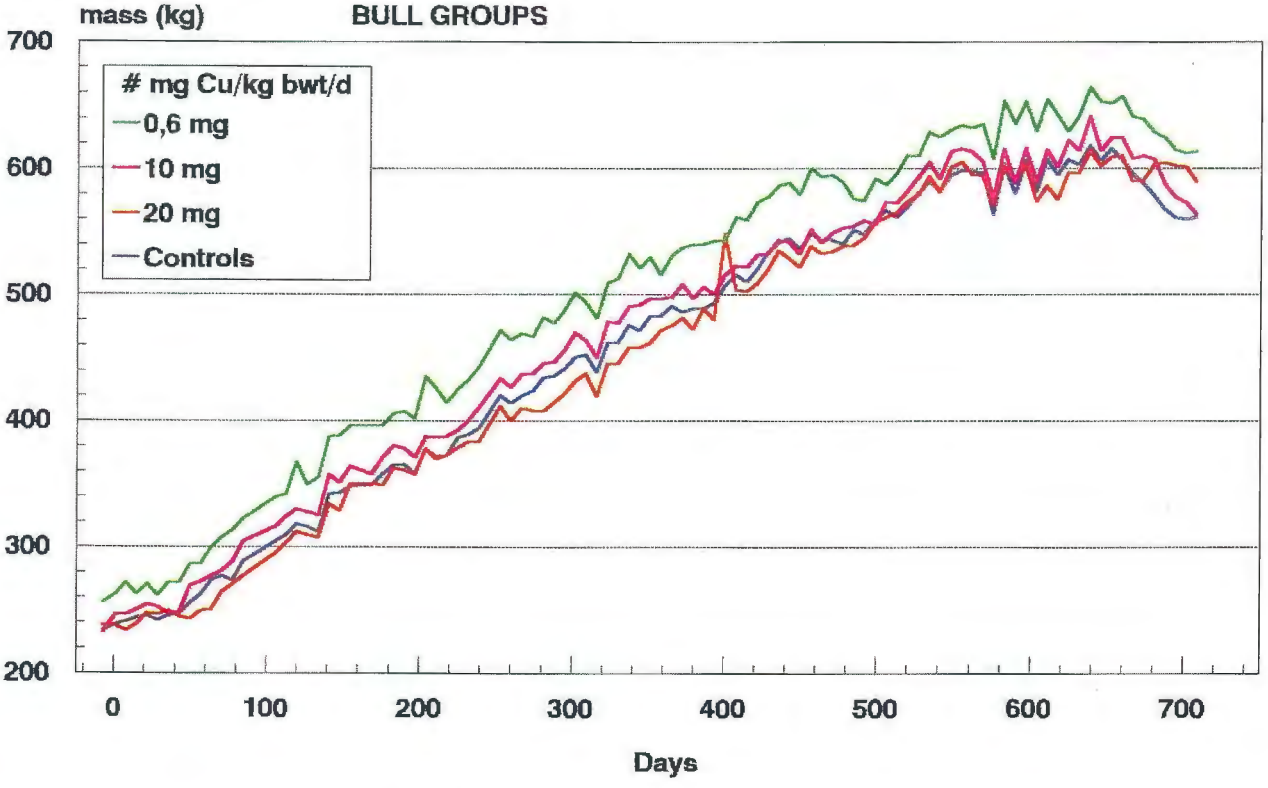
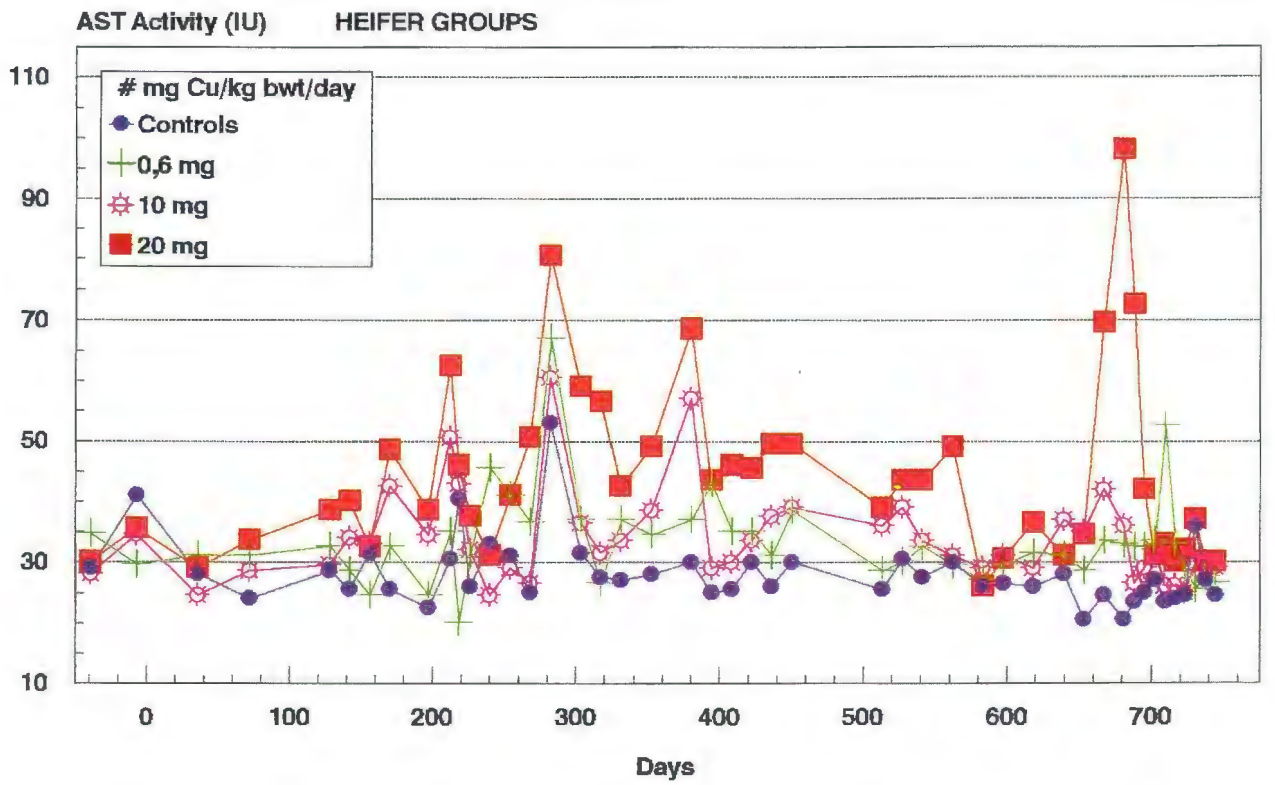


FIG. 1 The average mass per group over time



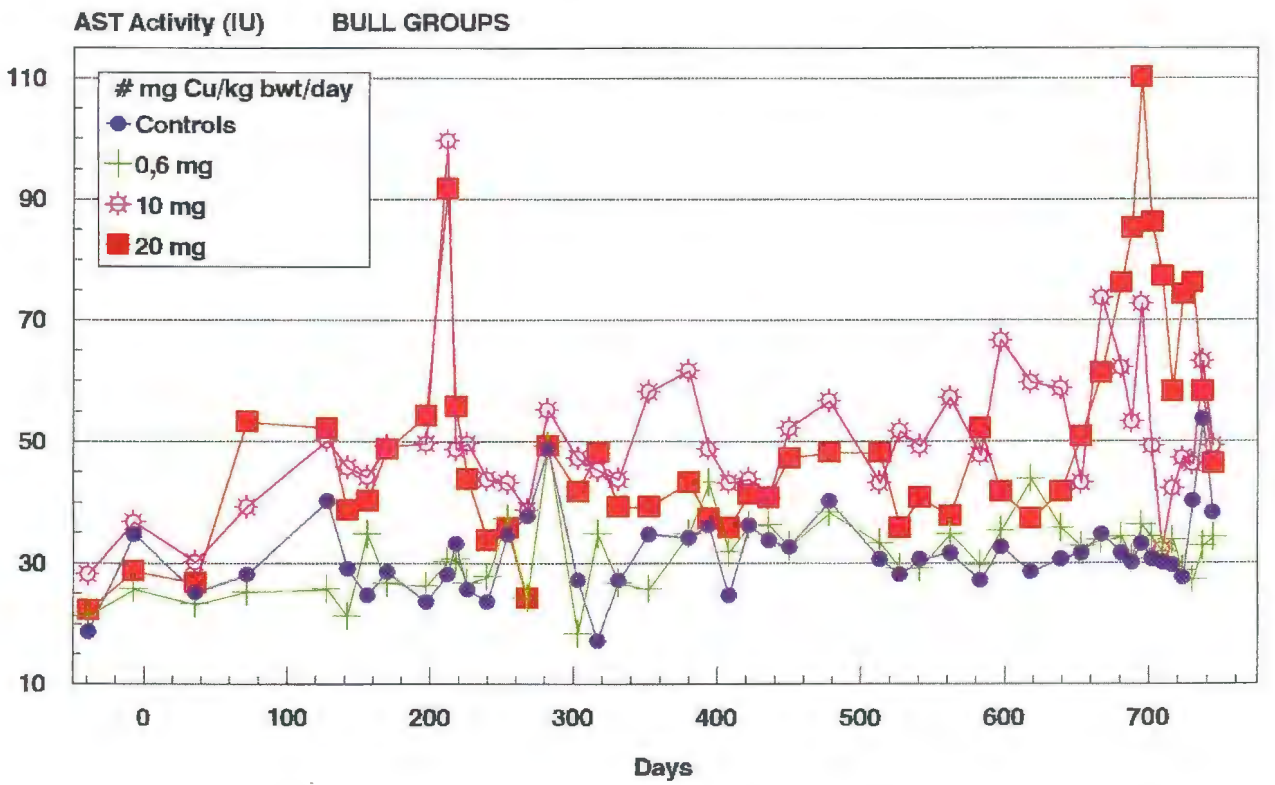
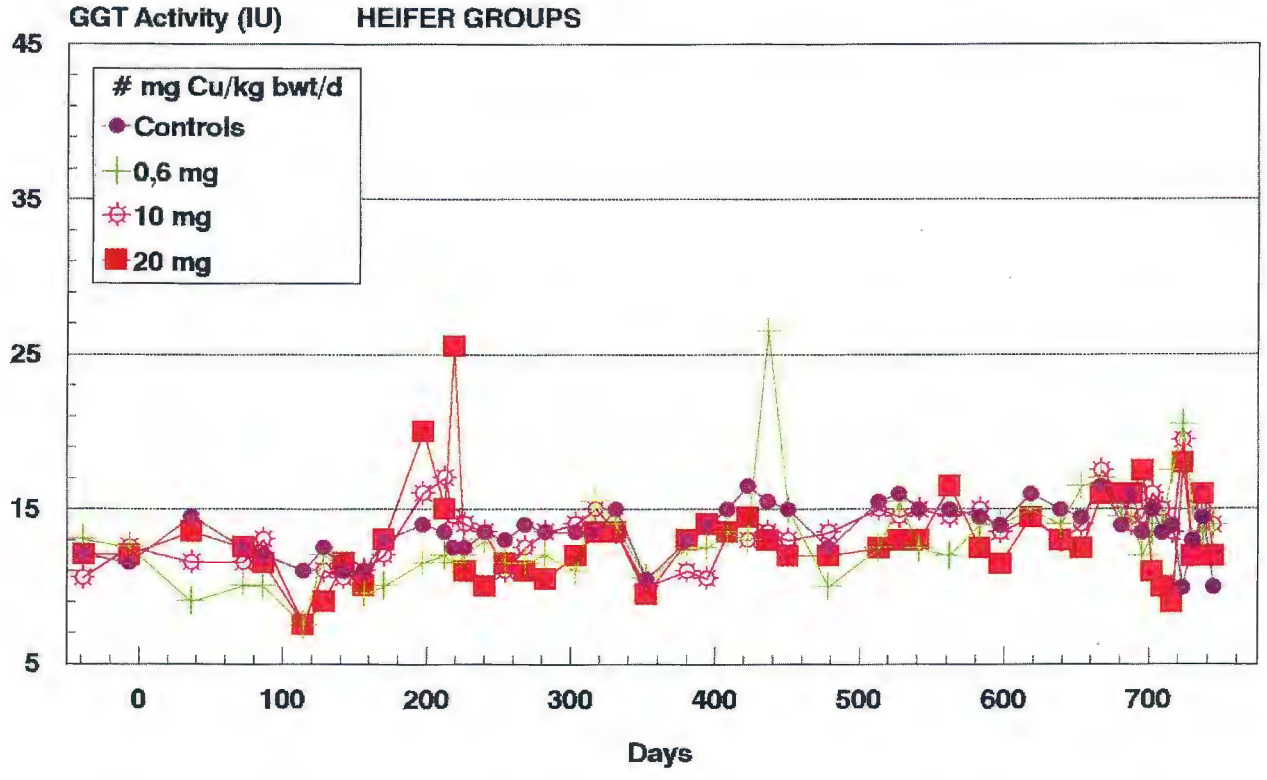


FIG. 2 The average AST activity over time per group



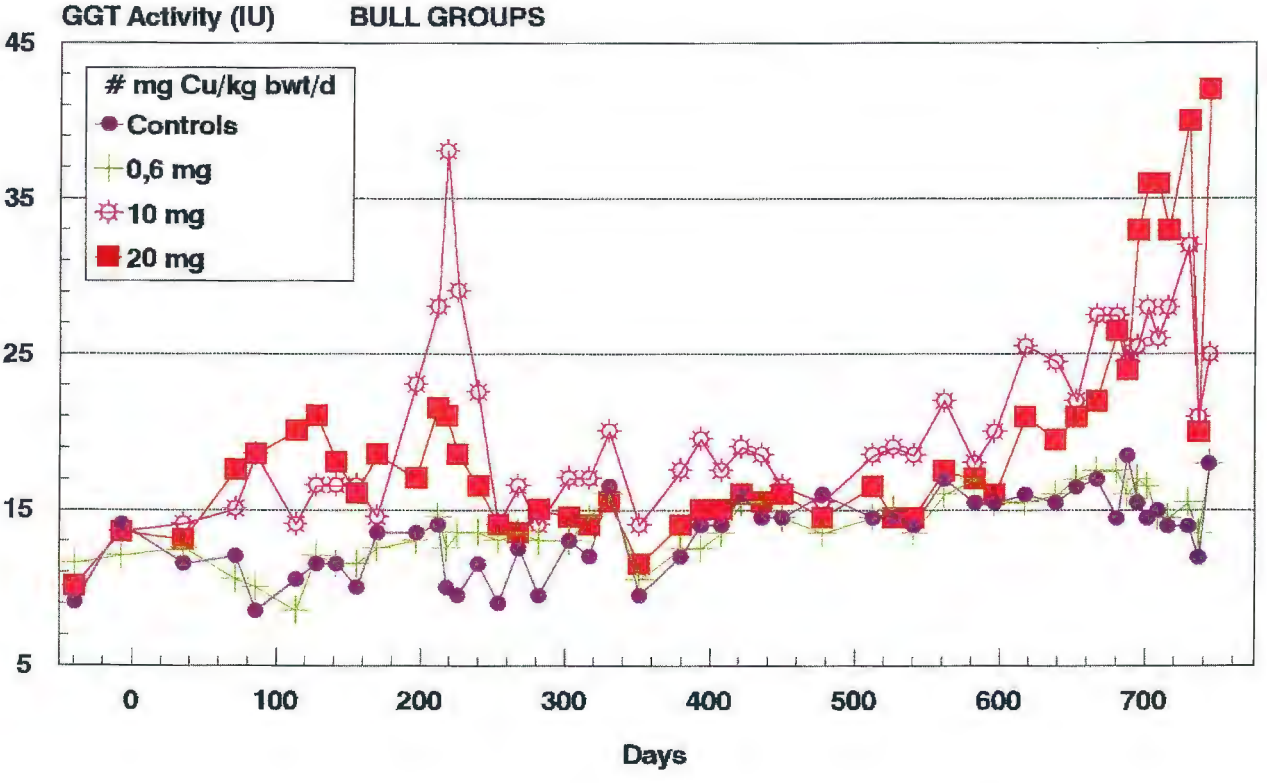
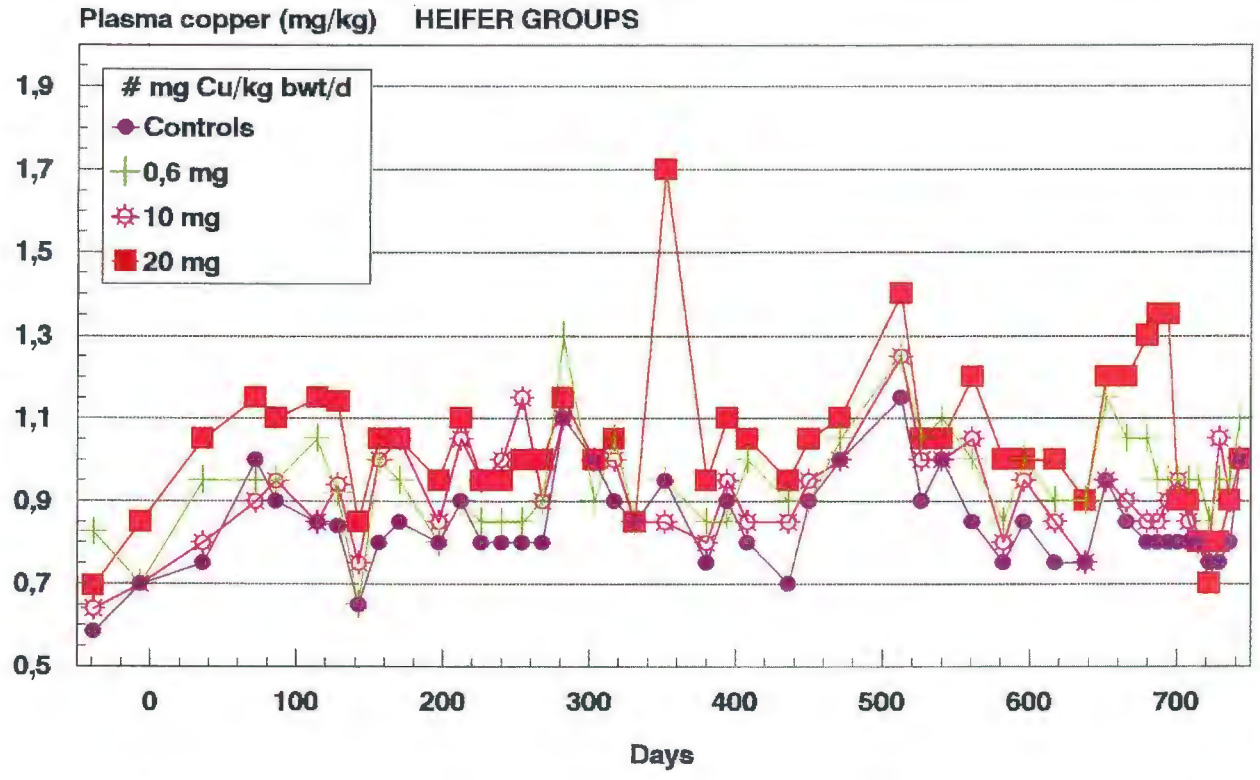


FIG. 3 The average GGT activity over time per group



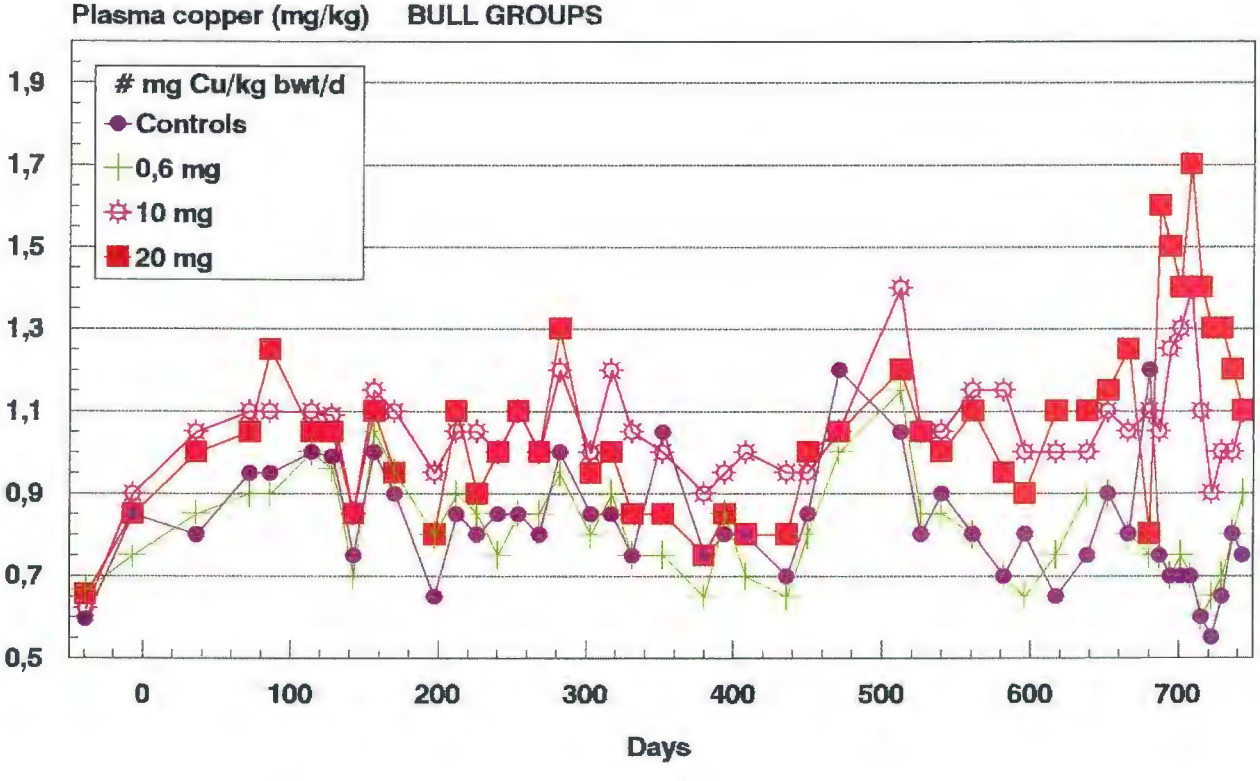
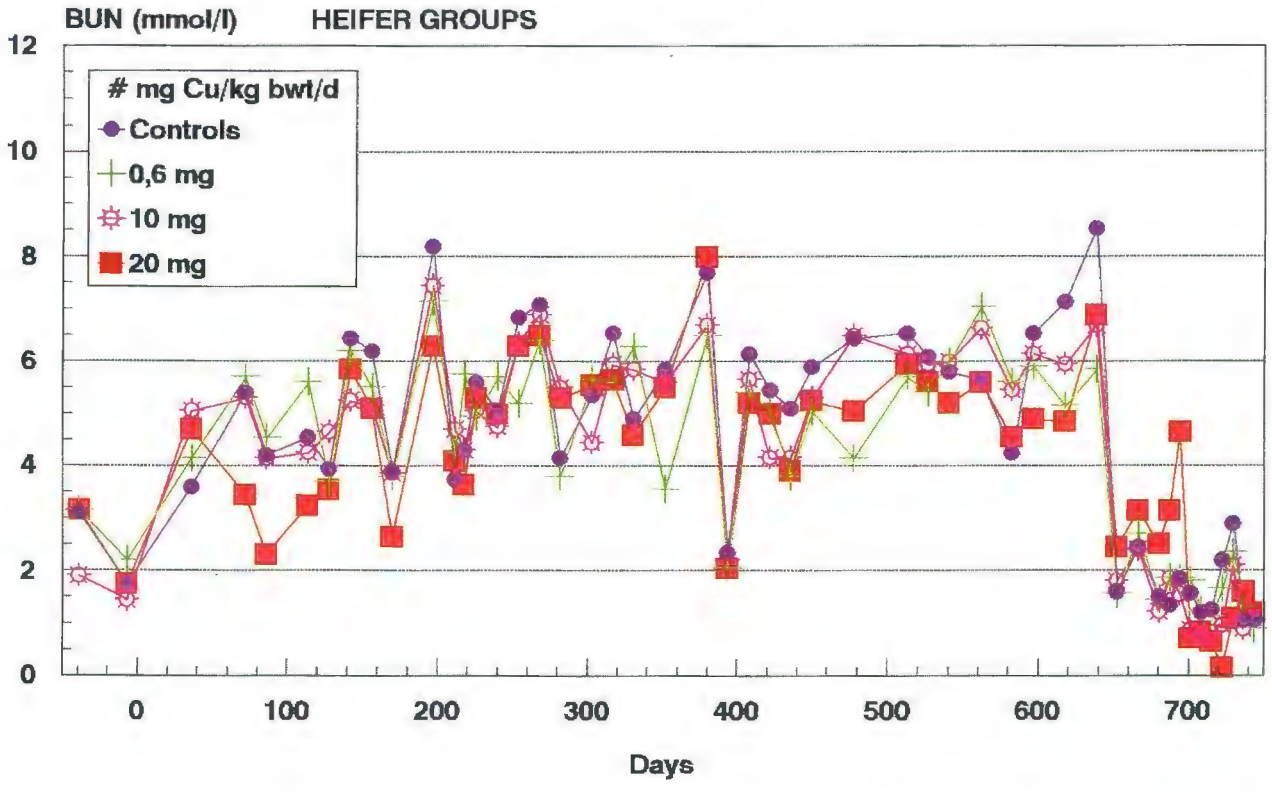


FIG. 4 The plasma-copper concentrations over time per group



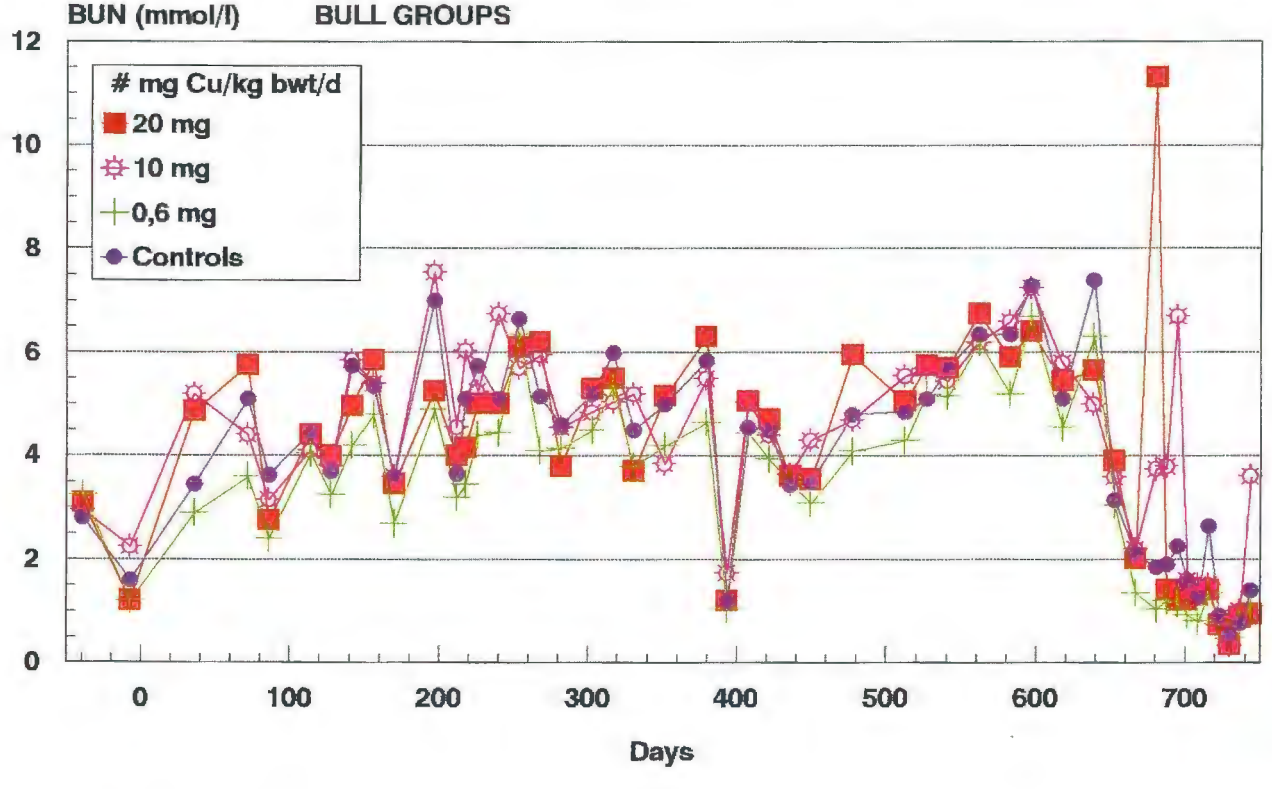


FIG. 5 The BUN concentrations over time per group

TABLE 1 Parameters used for testing whether there was a difference in mass between groups receiving copper (Cu) and the control animals

Dosage group (mg Cu/kg bm/d)	AUC (kg/d)	*P*-values	ADG (kg/d)	*P*-values
Heifers				
Control	289 380		0,520	
0,6	276 739	0,360	0,503	0,113
10	264 952	0,100	0,406	0,183
20	265 706	0,160	0,448	0,293
Bulls				
Control	310 121		0,604	
0,6	317 709	0,423	0,643	0,670
10	336 562	0,082	0,643	0,668
20	306 312	0,786	0,592	0,891

AUC = area under mass vs time curve; ADG = average daily gain

TABLE 2 Summary statistics for AST activity

Variable	Controls	0,6 mg Cu/kg bm/d	10 mg Cu/kg bm/d	20 mg Cu/kg bm/d
Sample size	186	186	177	170
Mean	29,76	32,16	41,94	46,15
Median	29	31	40	42
Mode	30	30	33	41
Geometric mean	28,90	31,21	39,10	43,09
Standard error	0,57	0,61	1,25	1,49
Minimum	14	13	17	18
Maximum	73	74	140	164
Range	59	61	123	146
Lower quartile	25	27	30	33
Upper quartile	33	36	50	53
AUC	21462,50	22867,60	29891,80	31074,50

TABLE 3 Calculated significance levels for accepting the null hypothesis that no difference exists between the means of the listed AST variables

Variable	0,6 mg Cu/kg bm/d	10 mg Cu/kg bm/d	20 mg Cu/kg bm/d
AUC	0,264	0,097	0,001
Mean	0,029	< 0,001	< 0,001

TABLE 4 Summary statistics for GGT activity

Variable	Controls	0,6 mg Cu/kg bm/d	10 mg Cu/kg bm/d	20 mg Cu/kg bm/d
Sample size	204	204	191	184
Mean	13,58	13,72	16,56	15,72
Median	14	13	16	14
Mode	13	12	13	13
Geometric mean	13,28	13,27	15,61	14,82
Standard error	19	27	0,43	0,45
Minimum	6	6	6	7
Maximum	22	38	49	42
Range	16	32	43	35
Lower quartile	12	11	13	12
Upper quartile	15	16	19	17
AUC	9633,75	9294,25	11217	10437,60

Feed Cu concentrations

The mean Cu concentration in the feed for the period of the trial, was 29 ± 17 ppm (DM) ($n = 9$) and ranged from 9,9–64 ppm.

Necropsy findings

It was decided to publish these findings in a separate article.

DISCUSSION

It could not be statistically proved that Cu has any effect on mass gain in cattle. It would appear that Cu does not adversely affect mass gain ($P < 0,05$), but the low *P*-values obtained for some of the AUC comparisons (Table 1) suggest that larger sample sizes need to be used to prove this conclusively.

The AST results show that animals that received 10 mg of Cu/kg of bm/d or more, experienced liver damage that became detectable from about day 40, and persisted throughout the trial (Fig. 2). The pattern of liver damage, however, is interesting as spikes of damage appears to have occurred roughly every 80 d. They were interspersed with periods of almost normal enzyme activity. It is postulated that this pattern may reflect episodes of severe cellular damage, during which individual hepatocytes are destroyed, these being followed by replacement with non-active fibrous tissue. The result is a decrease in the functional mass of the liver and an apparent temporary normalization of liver enzyme activity. According to Duncan & Prasse (1986), such a pattern can be typical of chronic, progressive liver disease. Correlation between serum activity and clinical manifestation of hepatic insufficiency is poor. In chronic, progressive liver diseases typically fewer hepatocytes undergo necrosis at any specific time, and serum AST activity may be unimpressive (Duncan & Prasse 1986). Even with acute hepatic sublethal injury or necrosis with very high serum AST activity, signs of hepatic insufficiency may be minimal. This could explain why AST activity could be high in the bulls, without there being any apparent influence on mass. The AST results could also reflect that not all hepatocytes accumulate Cu simul-

TABLE 5 Calculated significance levels for accepting the null hypothesis that no difference exists between the means of the listed GGT variables

Variable	0,6 mg Cu/kg bm/d	10 mg Cu/kg bm/d	20 mg Cu/kg bm/d
Heifers			
AUC	0,616	0,824	0,611
Bulls			
AUC	0,847	0,022	0,105
Combined heifers and bulls			
AUC	0,653	0,286	0,415
Mean	0,754	< 0,0001	0,006

TABLE 7 Calculated significance levels for accepting the null hypothesis that no difference exists between the mean plasma copper concentrations or mean AUCs of the test versus control group

Variable	0,6 mg Cu/kg bm/d	10 mg Cu/kg bm/d	20 mg Cu/kg bm/d
Heifers			
AUC	0,427	0,251	0,050
Bulls			
AUC	0,536	0,147	0,061
Combined heifers and bulls			
AUC	0,552	0,036	0,009
Mean	0,0006	< 0,0001	< 0,0001

TABLE 6 Summary statistics for plasma copper concentrations (ppm)

Variable	Controls	0,6 mg Cu/kg bm/d	10 mg Cu/kg bm/d	20 mg Cu/kg bm/d
Sample size	184	183	174	165
Mean	0,835	0,893	0,988	1,05
Median	0,8	0,9	1	1
Mode	0,8	0,9	1	1,1
Geometric mean	0,82	0,88	0,97	1,03
Standard error	0,011	0,012	0,013	0,018
Minimum	0,5	0,5	0,6	0,65
Maximum	1,7	1,4	1,6	2,4
Range	1,2	0,9	1	1,75
Lower quartile	0,7	0,8	0,9	0,9
Upper quartile	0,9	1	1,1	1,1
AUC	621,01	647,87	703,7	741,78

taneously or at the same rates, causing some cells to be damaged before others. The fluctuating nature of the AST activity, however, makes this enzyme an unreliable diagnostic tool for chronic Cu poisoning.

Unlike AST, GGT activity was increased only in the bulls that received 10 and 20 mg of Cu/kg of bm/d. The pattern of GGT peaks corresponded with the AST peaks, as expected, since both enzymes are released with liver damage. GGT is associated with microsomal membranes and is usually released with lethal cell necrosis—unlike AST, of which the activity increases with changes in hepatocellular permeability (sublethal injury and necrosis) (Duncan & Prasse 1986). The degree of liver damage may therefore explain why there was a rise in AST activity in the heifer group that received 20 mg of Cu/kg of bm/d, but no rise in GGT activity. Why liver damage in bulls should be more severe than in heifers, is not known. This is the converse of what may have been expected, given the reduced mass gains of the heifers that had received high doses of Cu. Another explanation may be related to the fact that GGT is most active in the canalicular surfaces of hepatocytes and bile duct epithelium and increases with cholestasis. If these cells in the liver are therefore minimally affected as opposed to the other hepatocytes, then GGT activity need not parallel the magnitude of AST activity. The results could therefore suggest that the canalicular surfaces of hepatocytes and bile duct epithelium are not the primary sites of hepatocellular damage with Cu poisoning. At the time of the field outbreak of Cu poisoning (Gummow *et al.* 1991) 43% of the affected herd had elevated GGT activity. Owing to budget constraints, however, the prevalence of AST activity in the herd was never determined.

When the plasma Cu concentrations were examined, it was found that plasma Cu concentrations in the groups that had received 10 and 20 mg of Cu/kg of bm/d, were significantly elevated as compared to the controls. It has been postulated (Gummow *et al.* 1991) that during the terminal stages of Cu poisoning, Cu is released from damaged liver cells in large quantities, thus precipitating the characteristic haemolytic crises that contribute to the death of the animal. The results show that Cu-plasma concentrations are elevated throughout the accumulation phase of the pathogenesis, and that the pattern of elevation is similar to that seen for AST and, to a lesser extent, GGT enzyme activity. This supports the postulation that there is a correlation between liver cell damage and plasma Cu concentrations, but further suggests that the cellular damage need not be that severe before Cu-plasma concentrations rise. Plasma Cu concentrations could act as an indicator for chronic exposure to Cu if the analytical method were

TABLE 8 Summary statistics for plasma blood urea nitrogen (BUN), iron (Fe) and zinc (Zn) concentrations

Variable	Controls			0,6 mg Cu/kg bm/d			10 mg Cu/kg bm/d			20 mg Cu/kg bm/d		
	BUN (mmol/ℓ)	Zn (ppm)	Fe (ppm)	BUN (mmol/ℓ)	Zn (ppm)	Fe (ppm)	BUN (mmol/ℓ)	Zn (ppm)	Fe (ppm)	BUN (mmol/ℓ)	Zn (ppm)	Fe (ppm)
Sample size	196	45	45	196	45	45	186	45	45	180	45	45
Mean	4,28	1,15	1,18	3,82	1,15	0,99	4,43	1,25	1,17	4,39	1,13	1,09
Median	4,55	1,18	1,15	4,05	1,13	0,95	4,80	1,28	1,08	4,70	1,10	1,10
Mode	5,70	1,13	1,05	4,40	1,10	0,78	5,40	1,35	0,88	4,90	1,03	1,15
Standard error	0,15	0,05	0,43	0,13	0,04	0,04	0,14	0,05	0,06	0,16	0,07	0,07
Minimum	0,32	0,57	0,35	0,32	0,60	0,30	0,34	0,60	0,35	0,15	0,43	0,10
Maximum	8,80	2,82	1,80	8,00	1,70	1,80	10,50	1,90	2,40	21,30	2,88	1,90
Range	8,48	2,25	1,50	7,68	1,10	1,50	10,16	1,30	2,00	21,15	2,45	1,80
AUC	3527	917	786	3157	887	786	3482	1016	9866	3384	960	911

TABLE 9 Summary statistics of organ mineral concentrations

Concentration in liver (ppm wet mass)	Cu	Zn	Fe	Mn
Control group				
Mean	23,33	27,33	179,66	2,30
Standard error	5,20	0,88	22,98	0,10
Median	24	27	198	2,40
Standard deviation	9,02	1,52	39,80	0,17
Range	18	3	73	0,3
Minimum	14	26	134	2,10
Maximum	32	29	207	2,40
Count	3	3	3	3
0,6 mg Cu/kg bm/d group				
Mean	58,25	28	112,75	1,50
Standard error	1,75	2,27	44,35	0,34
Median	58,5	29	95,50	1,50
Standard deviation	3,50	4,54	88,71	0,69
Range	8	10	206	1,20
Minimum	54	22	27	0,90
Maximum	62	32	233	2,10
Count	4	4	4	4
10 mg Cu/kg bm/d group				
Mean	212,25	73	154	3,67
Standard error	105,67	33,22	50,18	1,38
Median	120,50	47,50	140,50	2,55
Standard deviation	211,35	66,44	100,36	2,77
Range	442	143	237	6
Minimum	83	27	49	1,80
Maximum	525	170	286	7,80
Sum	849	292	616	14,70
Count	4	4	4	4
20 mg Cu/kg bm/d group				
Mean	152,50	42	174,75	2,17
Standard error	28,30	13,24	56,79	0,51
Median	173,50	32,50	179	1,95
Standard deviation	56,61	26,49	113,59	1,02
Range	125	59	265	2,40
Minimum	69	22	38	1,20
Maximum	194	81	303	3,60
Sum	610	168	699	8,70
Count	4	4	4	4

more sensitive. The relatively low sensitivity of the analysis method would make it difficult to detect the small differences between normal and exposed animals under field conditions. This is illustrated by the small difference between the means of the experimental groups shown in Table 6.

The fact that no significant difference could be found between groups with respect to BUN concentrations, implies that no kidney damage occurred during the course of the trial; hence urinary excretion rates of Cu were probably the same for all groups. This precludes the possibility of plasma accumulation of Cu due to reduced excretory rates. However, shortly before the three terminally ill cattle were euthanased, the concentrations of BUN did rise sharply. This paralleled a rise in AST activity and plasma Cu concentrations in these three animals but not GGT activity, and probably reflects the early stages of a haemolytic crisis where haemoglobin casts begin to block the kidney tubules.

Of further interest, was the drop in BUN (Fig. 5) and Fe concentrations for all groups from around day 645 to the end of the trial. This correlates with a drop in mass over the same period. Both these decreases are ascribed to a change in ration at that point in time. The ration change constituted a decrease in available protein and an increase in roughage as a result of lucerne (≈15 % protein) in the diet being replaced by *Eragrostis* hay (≈8 % protein). The decrease in protein available for use by rumen flora probably results in more efficient use of rumen urea by these organisms, and thus a decrease in the quantity of urea being absorbed across the rumen wall. Hence the drop in BUN. It is also reported that lucerne tends to have higher Fe concentrations than does *Eragrostis* hay (Ensminger, Oldfield & Heinemann 1990).

It is interesting to note that the liver Cu concentrations in the group that received 0,6 mg of Cu/kg of bm/d were significantly higher than those in the controls, since there was no indication of clinical signs or clinical pathology in this group. This would suggest

that liver Cu concentrations in cattle are probably the most sensitive indicators of exposure to high oral concentrations of Cu, and may therefore still be the best diagnostic tool currently available. The knowledge that very low concentrations of Cu occurred in the lungs of these cattle, including those animals euthanased, may be useful as an epidemiological tool for field investigations where air pollution is suspected, since one may find higher concentrations of Cu in the lungs among field cases where air pollution is a source of Cu.

Before any conclusion can be drawn from this trial regarding the tolerance levels of cattle to Cu exposure, the background Cu concentration in the feed needs to be taken into account. Working on an assumption that a 500 kg cow would consume 12 kg of feed per day, the amount of Cu such an animal would have consumed in this trial from the feed, would have ranged from 0,24–1,55 mg of Cu/kg of bm/d (mean = 0,7 mg of Cu/kg of bm/d). Thus, at times, the amount of Cu dosed to the low-dose group would have been exceeded. The fairly wide range in background Cu concentrations could explain some of the variability seen in the results. To some extent, these background concentrations would have been compensated for by the lack of oral dosing over week ends. However, the fact remains that all animals, including the controls, were receiving a noticeable amount of extra Cu during the trial.

CONCLUSION

From the results it can be concluded that subclinical damage to the liver, and eventual Cu toxicity, can occur when cattle are continually exposed to oral doses of $\geq$12 mg of Cu/kg of bm/d (this value includes 2 mg of Cu/kg of bm/d to compensate for background concentrations in the feed). It can also be concluded that cattle can probably tolerate oral doses of $\leq$0,6 mg of Cu/kg of bm/d for an indefinite period, provided there are no other sources of Cu, such as may occur with air pollution, or provided no other adverse mineral interactions occur, such as may occur with molybdenum deficiency.

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