ANALYSIS OF COPPER AND ITS STATUS IN CATTLE FROM MOROBE PROVINCE, PAPUA NEW GUINEA

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ABSTRACT

The copper status of cattle from 16 properties in the Morobe Province was investigated by the analysis of liver and serum for copper levels. It was found that fifteen properties had animals with copper concentrations in serum and liver samples which were below normal. The normal serum and liver copper concentration in cattle was 0.60 ppm and 100 ppm Dry Matter. Haemolysed serum samples were often found to give higher results than non-haemolysed serum samples. Samples stored for nine days at room temperature gave lower values than samples stored in the refrigerator or freezer. No significant differences were found between trichloroacetic acid (TCA) and glycerol procedures in sample preparation. Treatment of a test serum with the concentrations of 5, 8, 11, 15, 20 percent TCA, did not give any significant differences in copper concentration.

Key words: Cattle, Copper status, Serum, Liver, Atomic Absorption Spectrophotometer, haemolysis and storage conditions.

INTRODUCTION

Research has shown that copper plays an important role in the biochemistry of ruminants. Because copper is a constituent of many enzymes and other biological catalysts, it is described as one of the prime-movers of the "biochemical machine" (Frieden 1978). It plays a vital role in the processes of pigmentation of hair and wool, formation of blood and bone, reproduction and myelination of the spinal cord (Blood et al. 1979).

The level of copper in biological materials is well documented (Underwood 1971; Bull 1980). The concentration of copper in pasture and animal tissues varies with species and age. For animal tissues, copper levels are generally highest in liver, brain, kidney, heart and hair (Bull 1980).

Deficiencies of copper in animals have been well studied since the 1930s. In the United States of America, Holland and Australia ruminant diseases such as 'salt sick', 'echsucht' and enzootic ataxia' were investigated and shown to be caused by copper deficiency (Bull 1980; Grace 1983; Jones and Hunt 1983). In New Zealand, two cattle diseases, 'teart' and 'peat scours', were shown to be caused by the combination of very high molybdenum levels in pasture forages and depletion of copper in the body tissues (Bull 1980). The interrelationships between copper and molybdenum and with other elements has shown that the effects of the total diet must be considered on the metabolism of copper (Bull 1980; Suttle 1986 a).

Animals deficient in copper vary with respect to the extent of the deficiency. The degree of copper insufficiency may be categorised as either severe or marginal. Marginal insufficiency results in a reduction in tissue enzyme activities and copper concentrations but no impairment of biochemical processes within the tissues. Severe copper in-

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sufficiency results in significant impairment of biochemical processes and alterations in tissue structure. Clinical signs such as depigmentation of wool or hair, severe scouring, enzootic ataxia and bone fractures are likely to be displayed (Jones and Hunt 1983; Paynter 1987).

Several studies have been performed to determine the copper status of ruminants in Papua New Guinea (Glasgow 1966; Mayall 1973; Holmes 1981; Holmes *et al.* 1986). These studies concentrated on major ruminant grazing areas and usually involved copper supplementation for some of the animals. Previous field observations, together with biochemical analyses, indicate that deficiencies exist in a number of areas including the Markham Valley in Morobe Province (Mayall 1973).

Analysis of biological samples such as serum or plasma for copper levels are usually performed by Atomic Absorption Spectrophotometry (AAS). Various methods of sample preparation have been used to overcome some of the problems associated with this technique.

Viscosity of samples has been postulated as a major source of error in flame AAS methods (Weinstock and Uhlemann 1981). If the viscosity of standards and samples differ, then the aspiration rates and hence the absorbances differ (Makino and Takahara 1981). The viscosity effect in biological fluids is due mainly to proteins, particularly albumin (Carthrew and Dey 1985).

There are two ways to counteract the effect of viscosity. One is to increase the viscosity of the standards to match that of the serum or plasma samples. Glycerol can be used for this. Another is to reduce the effects of viscosity of the serum or plasma samples by adding trichloroacetic acid (TCA) to precipitate the proteins (Clark 1971; Osheim 1983). Various TCA concentrations have been used by different authors (Saunderman and Roszel 1967; Mayall 1973; Healy *et al.* 1978). Therefore, the effect of different TCA concentrations on the determination of copper in serum was studied.

Sample handling is an important factor in obtaining accurate results for trace elements in biological samples. There is a particular problem with the collection of blood samples from animals in the field, where refrigeration may not be available. Samples are sometimes left at ambient temperature for long periods of time or may be transported over rough roads which may lead to haemolysis.

Haemolysis and storage are important aspects of sample handling. Haemolysis will affect the results if the intra-cellular concentration of elements differs from the concentration in the serum or plasma. Such effects of tissue membrane leakage into plasma, are particularly seen where the intra to extra cellular concentration differences are in excess of 10 fold e.g. with potassium and magnesium or with spectrophotometric assays where haemoglobin may be absorbed directly. In the case of copper measured by AAS, where in normal bovines, plasma and read cell concentrations are similar, and interferences by haemoglobin per sera should be very low. The following experiments investigated how haemolysis and the storage regime affect copper concentration.

There were three aims in this study. The first was to assess the copper status of cattle from selected properties in the Morobe Province; the second was to compare different methods of sample preparation, including effects of contamination from tubes and different methods of deproteinising serum samples; and the third was to establish the effects of haemolysis and storage conditions on copper results.

MATERIALS AND METHODS

The following test tubes and blood or serum containers were used:

(a) Sterile blood container without anticoagulant, 10mL, disposable plastic tube.

(b) Serum container, 5mL, disposable plastic tube.

(c) Centrifuge plastic tube, 10mL.

(d) Round bottom glass tube, 10mL.

All glassware was thoroughly washed with detergent, rinsed with tap water, and rinsed well with distilled water. Excess water was removed and the glassware was soaked in 5 percent analytical reagent grade nitric acid, for 24 hours. Finally it was washed with tap water, rinsed three times with distilled water and dried in an oven.

The following eight solutions were prepared simultaneously using both Glycerol and TCA procedures:

1. 1 mL distilled H_2O + 1 mL 10 percent Glycerol+
2. 1 mL deionised H_2O + 1 mL 10 percent Glycerol*
3. 1 mL control serum+ + 1 mL distilled H_2O
4. 1 mL control serum* + 1 mL deionised H_2O
5. 1 mL distilled H_2O + 1 mL 8 percent TCA+
6. 1 mL deionised H_2O + 1 mL 8 percent TCA*
7. 1 mL control serum+ + 1 mL 8 percent TCA+
8. 1 mL control serum* + 1 mL 8 percent TCA*

\+ : prepared with distilled H_2O

* : prepared with deionised H_2O

All samples were prepared in duplicates.

After pipetting each of the eight solutions the contents in the test tubes were immediately mixed on a vortex mixer. The TCA prepared control serum samples were centrifuged to separate the supernatant from the protein precipitate. All the test tubes were left to stand for 10 minutes, then spun for 10 minutes and aspirated without disturbing the precipitated plug. The absorbances were measured and the copper concentrations calculated. Blanks containing TCA and Glycerol solutions were analysed with the samples in each run and no contamination was found.

Atomic Absorption Spectrophotometer and Standard Reference Materials

A Varian model A-1475 atomic absorption spectrophotometer and Varian Spectra AA-40 spectrophoto meter with air/acetylene flame were used at 324.8 nm. A range of settings of the AA-1475 including use of single beam (SB), single beam/ background correction (SB/BC), double beam (DB) and DB/BC were investigated.

A standard reference material, Gilford QCS Normal (freeze-dried human serum), with an established concentration of 0.94 ± 0.15 ppm copper was used as a control to test the recovery of copper when comparing the Glycerol and TCA procedures. The National Bureau of Standards (NBS), Standard Reference Material SRM 909 human serum and a certified NBS Bovine Liver control SRM 1577a were included in the sample analysis.

Sample Collection

In 1985 and 1986 serum samples were collected from slaughtered animals at the abattoir and from live animals in the field by officers of the Department of Agriculture and Livestock and staff of the National Veterinary Laboratory (NVL). However the precise time of the year and methods of collection, storage and transportation are not known. Analyses were carried out by the staff at NVL after sera were separated.

Serum and liver samples were collected at the Lae Central Abattoir in 1987 from slaughtered cattle. Figure 1 shows the farms in the Morobe Province from which the slaughtered animals came.

Serum samples were collected at the abattoir in sterile blood containers without anticoagulant and transported to the laboratory in an esky cooled by

an ice pack. These samples were left on the bench for 30 - 60 minutes to allow the blood to clot. They were then centrifuged to obtain clear serum samples. If the serum samples could not be analysed immediately they were stored in a freezer at - 23°C until required for analysis.

Comparison of Methods

Glycerol and TCA procedures as defined by Ross (1984) and as used by Mayall (1973) were evaluated.

A stock copper solution of 1000 ppm cupric nitrate, Spectrosol, British Drug House (BDH), was used for copper standard. The working standard solutions of copper was made up to 100 mL with 10 percent glycerol to copper concentrations of 0.0, 0.25, 0.50, 1.00, 1.50, and 2.00 ppm. An intermediate copper solution of 20 ppm was made up with 8 percent TCA from the stock solution to prepare working standard copper solutions. The working standard copper solutions of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ppm were prepared with 8 percent TCA. These standards were used for standard curve.

In one experiment a range of TCA concentration of the samples from 5 - 20 percent were tested. The volume of TCA added to sera was kept constant at a ratio of 1:1.

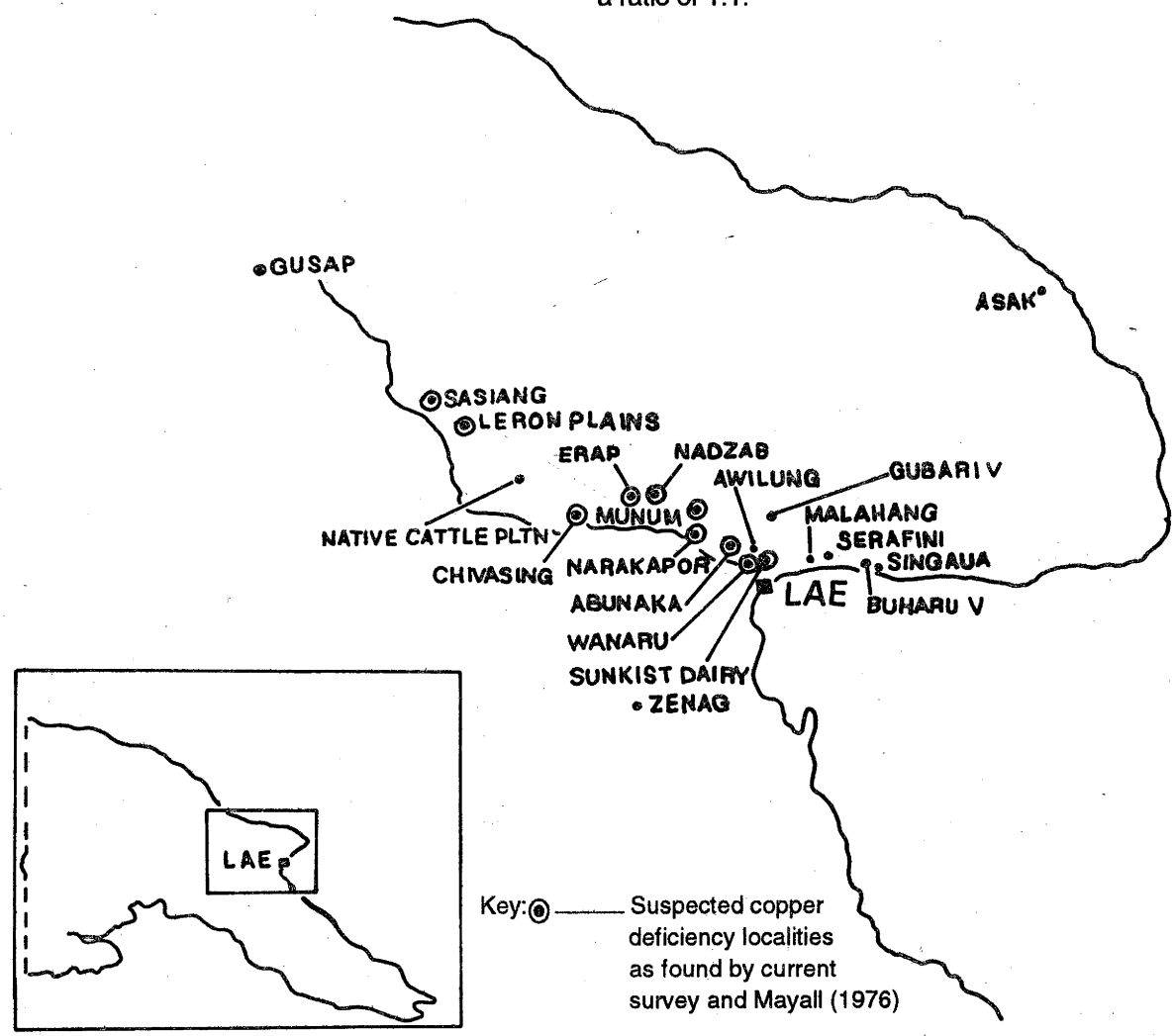

Figure 1. **Map showing locations in Morobe Province of beef and dairy cattle farms investigated for copper status.**

Haemolysis and Storage

The effects of haemolysis on the copper content prior to: (1) sera separation and (2) storage method of serum were studied under two types of conditions (non-haemolysed and haemolysed), three storage systems (freezer, refrigerator and ambient temperature) over five time periods (1,3,5,7,9 days post collection). Blood from one animal (A) was collected into 60 ten mL, tubes and centrifuged. Thirty tubes were allocated as clean non-haemolysed sera. One or two drops of concentrated red blood cells (RBC) (one of the tubes of centrifuged blood) was added with a pasteur pipette to each of the other 30 tubes and shaken vigorously to give haemolysed sera. Both non-haemolysed and haemolysed sera were stored in a freezer on the day of collection (Day 1). On day 1 twelve sera from both haemolysed and non-haemolysed serum groups were removed from the freezer and three of each groups were placed in each of the storage systems referred to above. This procedure was repeated on days 3, 5, 7, and 9. On day 10 all samples (60) were analysed for their copper content. Blood from a second animal (B) was analysed with Animal (A) at the same time and following the same procedure.

The liver samples were stored in a deep freezer until they were ready to be digested and analysed for their copper content.

Sample Analysis

One millilitre (mL) of 8 percent TCA was added to an equivalent amount of serum in a test tube and the contents were vortexed thoroughly to mix the TCA with the serum. The test tubes were allowed to stand for 10 minutes, then centrifuged for a further 10 minutes to separate the supernatant from protein precipitate and aspirated without disturbing the precipitated plug. National Bureau of Standards (NBS) Certified Standard Reference Material SRM 909, dried human serum, was included as an external serum standard.

Digestion of Liver Samples

Samples of thawed out liver weighing one to three grams were digested with 15 - 20 mL of a mixture of analytical grade nitric and perchloric acids (4 : 1). The digestions were carried out in 150 mL pyrex conical flasks. Anti-bumping granules were placed in the digestion flasks to prevent violent boiling. The temperature of the digestions was in the range of 120 - 200°C. A certified NBS Bovine liver control SRM 1577a was included as an external liver control. The digested samples were diluted into 50 ml volumetric flasks and analysed using the serum method. The results were determined on a dry matter (DM) basis.

Statistics

The data were analysed by one or four way analysis of variance (ANOVA) (Sokal and Rohlf 1981). The four treatments (SB, SB/BC, DB AND DB/BC) that have eight figures each were compared for their significant differences equally at one time by using four way analysis (Table 1). One way analysis was used between two tubes (CPT and RBGT), deionised and distilled waters, reagents (TCA and Glycerol) and different handling systems under various conditions for different periods (Table 1 and 2).

RESULTS

The results indicate that the washing procedure was adequate and no significant copper contamination detected from the tubes, reagents or the type of water used. In all cases the blank absorbances were in the range of 0.000 - 0.005 which were within the limits of the machine noise.

Different absorbance settings (SB, SB/BC, DB and DB/BC) at 324.8 nm of the AAS did not affect the accuracy or precision in copper analysis. Copper concentrations were all within the expected range 0.94 ± 0.15 ppm copper for the control serum. The AAS method was very suitable because of its

simplicity, accuracy and precision in determining copper at trace levels.

No significant differences in copper concentrations were found between the use of trichloroacetic acid or glycerol procedures. The mean of sixteen values for sera using TCA was 0.91 ppm and sixteen values for sera using glycerol was 0.89 ppm. the analysis of variance of these methods gave an experimental F ratios of 2.219 and 1.816 which are not significant because these F ratios were lower than the value of 4.35 obtained from the statistical tables. The TCA concentrations from 5 - 20 percent gave similar results for copper levels in a serum sample. The TCA procedure was used in subsequent analyses of serum and liver samples.

The mineral concentrations of control serum under different conditions are shown in Table 1. The analysis of variance was calculated assuming there were no interactions as the concentrations of the control serum were not affected by interference effects.

The four way analysis of variance (ANOVA) gave no significant differences at 5 percent F Critical

Table 1. **Comparison of copper concentrations in control serum obtained from Trichloroacetic acid and Glycerol procedures.**

Proceedures	Water type	Tubes	Copper concentration (ppm) SB	SB/BC	DB	DB/BC
TCA	Deionised H_2O	CPT	0.80	0.94	0.94	0.90
		RBGT	0.92	0.94	0.98	0.98
	Distilled H_2O	CPT	0.94	0.90	0.94	0.84
		RBGT	0.92	0.86	0.94	0.86
Glycerol	Deionised H_2O	CPT	0.94	0.80	0.86	0.86
		RBGT	0.92	0.94	0.96	0.92
	Distilled H_2O	CPT	0.88	0.86	0.86	0.94
		RBGT	0.86	0.80	0.86	0.96

SB - Single Beam; BC - Background Correction; DB - Double Beam; CPT - Centrifuge Plastic Tube; RBGT - Round Bottom Glass Tube.

Points under various conditions.

Table 2 shows results of two sera analysed for two haemolysis types under three different storage systems. Samples stored at ambient temperature gave lower copper concentrations than those stored in the refrigerator or freezer. For Serum A, a mean copper concentration of 0.55 ppm was obtained for non-haemolysed and haemolysed samples stored in the refrigerator or freezer whereas a mean copper concentration of 0.51 ppm was obtained for samples stored at ambient temperature. In Serum B the concentration was 0.72 ppm and 0.66 ppm for the refrigerated or ambient temperature samples respectively. Although the change in copper concentration over time differed for the different storage systems no discernible trends were observed.

The results for the two types of sera with different handling systems under various conditions for dif-

Table 2. **The mean copper concentrations of two sera, both haemolysed, and non-haemolysed, stored under three diferent storage systems for one to nine days. Significant levels of ANOVA for this table are shown below.**

	Copper Concentration (ppm)											
	SERUM A						SERUM B					
Days (Periods)	Non-Haemolysed			Haemolysed			Non-Haemolysed			Haemolysed		
	Frz.	Ref.	A.T.	Frz	.Ref.	A.T.	Frz.	Ref.	A.T.	Frz.	Ref.	A.T.
1	0.53	0.54	0.50	0.55	0.55	0.49	0.69	0.74	0.57	0.69	0.69	0.65
3	0.51	0.53	0.49	0.54	0.56	0.51	0.75	0.69	0.65	0.73	0.73	0.66
5	0.54	0.58	0.52	0.55	0.56	0.52	0.72	0.71	0.71	0.69	0.71	0.64
7	0.55	0.53	0.51	0.55	0.53	0.53	0.71	0.64	0.67	0.77	0.71	0.66
9	0.55	0.55	0.51	0.53	0.53	0.49	0.68	0.73	0.65	0.72	0.71	0.69

Key: Frz.-Freezer Ref.-Refrigerator A.T.-Ambient Temperature

ANOVA

Between Serum A and Serum B - Significant at 0.1%
Between different storage systems - Significant at 0.1%
Period by storage system interactions - Significant at 0.1%
Period by Haemolysis interactions - Significant at 5.0%
Between different periods - Not Significant
Between two haemolysis types - Not Significant
Haemolysis by storage systems interactions - Not Significant

Table 3. **Copper status of cattle from different farms in the Morobe Province.***

Owner Cattle Farms	Sample Type	No. & Sex Type	Observed copper Range (ppm)	Mean ± SEM copper (ppm)
	serum	5 steers	0.70 - 1.10	0.98 ± 0.08
	liver		36.0 - 152.0	91.00 ± 20.97
		8 steers		
	serum	6 cows	0.70 - 1.20	0.86 ± 0.04
Chivasing#		5 steers		
	serum	8 cows	0.40 - 1.20	0.71 ± 0.06
	liver		17.00 - 246.0	121.5 ± 18.08
		8 steers		
	serum	4 cows	0.52 - 1.57	0.77 ± 0.08
	liver	1 heifer	43.00 - 212.00	108.4 ± 13.81
	serum	7 heifer	0.52 - 0.90	0.78 ± 0.05
Ex-Narakapor@	serum	6 cows	0.49 - 0.73	0.59 ± 0.04
Native Cattle@		1 bull		
Plantation	serum	6 cows	0.59 - 1.10	0.83 ± 0.07
Erap@	serum	18 -	0.71 - 1.74	1.06 ± 0.07
Leron@		7 -		
	serum	6 cows(D)	0.60 - 1.50	1.12 ± 0.12
Ex-Leron#	serum	12 cows	0.35 - 0.77	0.58 ± 0.04
	serum	6 weaners	0.40 - 1.00	0.73 ± 0.09
Sasiang@		4 weaners		
Cattle Range	serum	7 heifers	0.30 - 0.90	0.71 ± 0.05
	serum	7 heifers	0.52 - 0.90	0.78 ± 0.05
		4 weaners		
Markham@		6 cows(D)		
Farmers	serum	2 cows(W)	0.60 - 1.60	0.80 ± 0.08

(Contd.)

	serum	6 -	0.62	-	2.10	1.34 ± 0.27
Zenag@	serum	2 -	0.80	-	1.00	0.90 ± 0.10
Swiss@ Mission, Lae	serum	4 cows	0.94	-	1.73	1.26 ± 0.17
Ex-Munum#	serum	7 bulls	0.44	-	0.65	0.54 ± 0.03
Zimu Markis@	serum	4 heifers	0.52	-	0.79	0.65 ± 0.06
Yamaku Timas@	serum	1 bull 3 cows	0.52	-	0.75	0.58 ± 0.06

* - **These results were obtained by staff at NVL in 1985 and 1986**

@ - **Samples collected at Lae Central Abattoir**

\# - **Samples collected in the field,**

D - Dry and W - Wet

ferent periods and the ANOVA (assuming no serum interaction or no real order of interactions) are presented in Table 2.

The copper concentration for the control liver was 158 ± 10 ppm DM while the certified value was 158 ppm DM. The copper concentration in the control serum was analysed to be 1.10 ± 0.04 ppm whereas its certified value was 1.10 ± 0.10 ppm. The results from samples submitted to the National Veterinary Laboratory (NVL) in 1985 and 1986 are summarised in Table 3. Copper values for serum and liver samples (DM) in this project are presented in Table 4.

It was expected that the available serum copper concentration should correlate well with the liver copper concentration. However in correlating the copper values of liver and serum, no real relationship has been observed. A poor correlation existed between serum and liver copper (Figure 2).

DISCUSSION

The normal range of copper concentration in serum is 0.60 - 1.60 ppm (Campbell 1983; Grace 1983; Whitelaw 1985). Most of the mean serum values fell within this range (Table 3 and Table 4). However the mean values of serum copper in cattle from Abunaka, Narakapor, and Nadzab were below normal, indicating that there was probably a copper deficiency among cattle in these places (Fig 1). Field supplementation would be necessary to quantify the effects of this deficiency.

Although the mean correlation is within the normal range on many farms, there are some values below normal. This indicates that the copper status may be marginal in most of the existing farms mentioned in Table 3 and Table 4. It is recognised that animals with marginal copper deficiency grow more slowly and as a result reduce their apparent copper requirement. Because of this, these animals may still maintain "normal" serum/plasma values, but would

Table 4. **Copper levels in serum and liver samples analysed rom individual beef cattle killed at Lae Abattoir.**

Owner/Farms	Sample Type	No/Sex or Type	Observed Range copper (ppm)	Mean copper (ppm)
Chivasing	serum	7 -	0.61 - 0.72	0.66
	serum	9 steers	0.46 - 0.77	0.62
	liver		55.6 - 397.5	166.6
Narakapor	serum	10 -	0.32 - 0.62	0.47
Abunaka	serum	4 -	0.12 - 0.46	0.27
	serum	4 cows	0.38 - 0.53	0.41
	liver		7.8 - 107.7	36.0
Native Cattle Plantation	serum	2 bulls	0.63 - 0.67	0.65
	serum	2 cows	0.65 - 0.79	0.72
	liver		52.2 - 266.7	159.5
A & J Beline Nadzab	serum	10 -	0.38 - 0.68	0.58
	Liver		38.5 - 159	83.4
Boiyo Kaninga Gubari Village	serum	4 steers	0.62 - 1.02	0.89
	liver		132.8 - 265.9	205.5
	serum	2 cows	0.56 - 1.10	0.83
	liver		36.0 - 63.5	49.8
Yanamahu Buharu Village	serum	5 steers	0.74 - 1.12	0.94
	liver		63.2 - 159.4	95.1
	serum	1 cow	0.98	
	liver		105.7	
Livestock Development Corporation ERAP	serum	7 cows	0.42 - 1.05	0.71

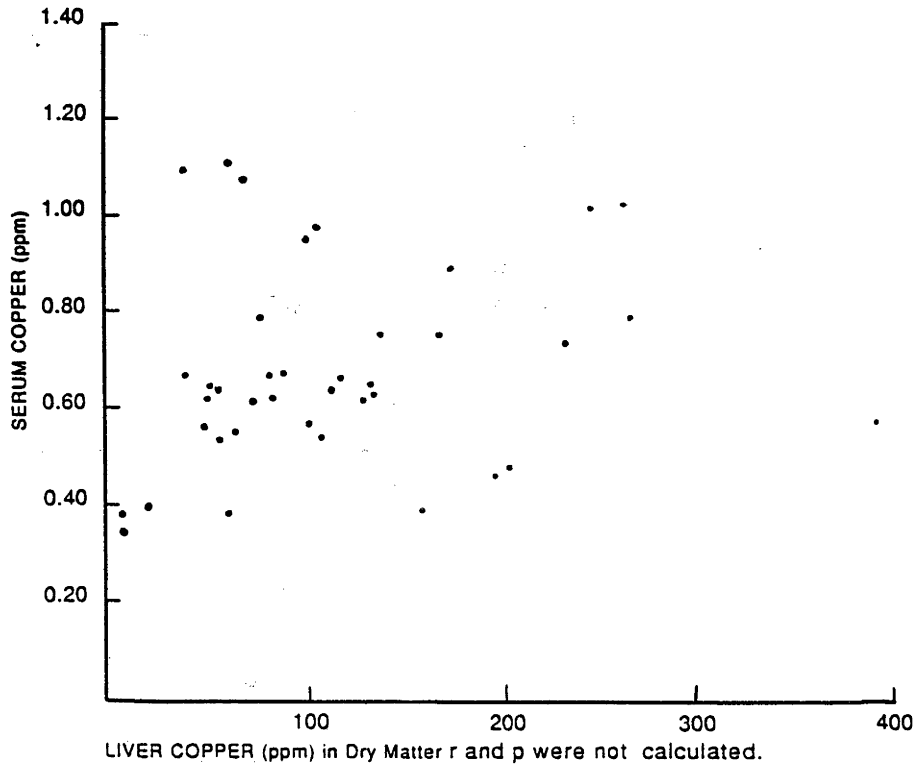

Figure 2. **Scatter diagram of Serum Copper concentration with Liver Copper concentration of individual Cattle from Morobe Province.**

respond to copper supplementation (Hill *et al*, 1962; Glasgow 1966; Mayall 1973; Reddy and Mahadevan 1976; Gallagher and Cottrill 1985; Langlands *et al.* 1986; Suttle 1986b). Although many of the animals slaughtered were in good condition and had no impairment, they were also mature adults. If the copper status was improved it might have been possible to slaughter at a younger age. Steers also have lower requirements for minerals than lactating or heavily pregnant cows. Lactating or pregnant cows are likely to have lower copper concentrations. However to confirm the lower copper concentrations in lactating cows a more detailed investigation of animals in the Morobe Province is necessary.

The mean copper concentrations of liver samples from cattle obtained from Abunaka, Nadzab and some smallholder cattle farmers at Buharu and Gubari villages are below the normal range (100 - 300 ppm DM) (Campbell 1983; Hill 1985). As with the serum results, some liver samples had very low copper concentrations of 7.8 - 635 ppm DM even though the mean concentrations were within the normal range.

Some authors (Campbell 1983; Grace 1983; Hall 1985; Paynter 1987) state that copper concentration in the liver is a better indicator of copper status than serum copper concentration because the former is the body's main reservoir of coper. Copper is drawn into the blood from this reservoir when it is required to maintain copper level. Therefore, the blood copper levels should not fall until the reservoir copper levels are exhausted. Field (1984); Suttle (1986 b) and Paynter (1987), indicated that there is

a relationship between plasma copper and liver copper in sheep, cattle and deer *(Axis porcinus)*, that normal plasma concentrations are maintained from the reservoir. However Hill *et al.* (1962) found no relationship between serum and liver values existed in cattle or buffalo. *Figure 2* indicates that in the 1985 - 87 survey there was no clear relationship between serum copper and liver copper, although animals from Abunaka had the lowest average serum and liver values. The data on the graph may agree with the trend observed by Paynter (1987) but there are many more outlying points and it would not be cautious to say that the data show this trend. This suggests that our understanding of copper metabolism is still incomplete.

The results obtained in 1985 - 87 in this study are consistent with those of Mayall (1973), although she recorded extremely low levels in animals from several cattle farms. Mayall's survey found that the Wanaru cattle in Markham Valley had copper concentration ranging from 0.04 - 0.12 ppm in serum, while Singaua Plantation, Bulolo and Sunkist Dairy cattle had low serum copper concentrations ranging from 0.17 - 0.40 ppm. Munum Plantation, Malahang and Serafini cattle had serum copper concentrations above 0.50 ppm. The liver copper concentrations in the cattle studied were found to be as low as 7.8 ppm DM which could suggests a primary copper deficiency as indicated in studies by Blood *et al.* (1979). The results of this study reconfirm that low serum and liver copper levels are occurring in the Morobe Province which indicates that production responses to copper supplementation may occur.

To more fully interpret the copper concentrations in cattle, factors such as growth and production rates, soil pH, cattle management systems, soil and pasture copper status need to be known. One very important factor is the molybdenum and sulphur status of the pasture (Hall 1985). These elements can cause a large reduction of copper availability for normal metabolic functions (Cunningham 1955; Hogan *et al.* 1971; Anon 1972; Smart *et al.* 1981; Paynter *et al.* 1982; Paynter 1984; Whitelaw 1985; Allen and Gawthorne 1987). It is unlikely that these elements are having an effect on the copper concentration because levels of molybdenum in soil and pasture in the Morobe Province are low (B. Kaupa, personal communication). Limited information is available on the copper status of soils in the Morobe Province and therefore further investigation is necessary to evaluate the cause of copper deficiency. No consideration was made on the seasonal effect of the copper status in this study.

The analysis indicates that the copper level was not affected by the different concentrations of trichloroacetic acid used to deproteinize the serum. Trichloroacetic acid at the concentration of 8 percent was probably sufficient to completely deprotenize the serum.

From the study of sample handling techniques it can be seen that non-haemolysed serum samples are required for copper analysis, as haemolysed serum samples gave slightly higher copper concentration readings. However there was no real trend of increased copper concentration being observed over time. The actual contribution of copper, to that already present in serum, from one or two drops of normal blood cells should be insignificant in AAS measurement. If serum and liver samples are not analysed immediately after collection, storage under freezing conditions is essential. Refrigeration, using ice packs, is necessary for transportation of samples, but care is required during transportation of whole blood to prevent haemolysis of red cells which may cause an increase in copper values of the serum.

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