

Copper responsive infertility in British cattle: the use of a blood caeruloplasmin to copper ratio in determining a requirement for copper supplementation.

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Abstract

Signs of clinical copper deficiency in ruminant animals include infertility. Blood copper concentrations alone are not an adequate indicator of copper status, as they take no account of the thiomolybdate challenge to the animal. The use of the ratio of caeruloplasmin activity to plasma copper concentration should provide a better indicator of molybdenum induced copper problems. The objectives of this study were to obtain veterinary feedback on the University of Leeds Trace Element Laboratory's (ULTEL) assessment of copper status and responses to any recommended supplementation.

Blood samples from 713 female cattle from 114 herds were assessed for copper status, including plasma copper concentrations, serum caeruloplasmin activities and the ratio between the caeruloplasmin and plasma copper (CP/PICu ratio). On the basis of only plasma copper analysis 95.0 % of the cattle were classified as being of normal copper status using Veterinary Laboratory Agency reference ranges. However, when the CP/PICu ratio was also considered only 35.6 % of the cattle would be classed as having normal copper status. Appropriate copper supplementation would be required by 30.7 % of the cattle and a further 27.8 % could also benefit from additional copper.

Veterinary responses to a questionnaire (80 % returned) indicated that infertility was a major problem in their clients' herds. Where copper supplementation was recommended and administered an increase in fertility was observed. In herds showing poor or lack of bulling behaviour/ later embryo loss (signs of clinical copper deficiency), 91.7 % reported that these signs were reduced by copper supplementation.

The relevance of the CP/PICu ratio as a diagnostic aid in the identification of copper responsive infertility has been supported by the responses of the veterinary surgeons.

Introduction

Signs of clinical copper deficiency that have been described in ruminant animals include the alteration of hair pigmentation (ginger-brown rough coats, spectacles) and infertility (depressed or absence of oestrus activity and / or later embryo loss) (Underwood, 1981). Work by Phillipou *et al.* (1987), Moeini (1997) and Mackenzie and Telfer (2000) has shown that clinical copper deficiency is due to the toxic effect of thiomolybdates (MoS_n) rather than a lack of copper for metabolic function. The amount of copper available to detoxify the thiomolybdates will be reduced by the intra-ruminal interactions of copper, iron and sulphur.

The efficacy of copper supplementation of ruminant animals should be assessed by the ability to correct the signs of clinical copper deficiency and not the ability to raise the copper content of the body (liver or total blood copper).

In the presence of free thiomolybdates the activity of caeruloplasmin is significantly reduced (Lannon and Mason, 1986), disproportionately to the level of copper in the blood. The ratio of serum caeruloplasmin activity to plasma copper concentration can be used to assess the functional copper status (the ability to correct the signs of deficiency) in cattle (Mackenzie *et al.*, 1997a). Free thiomolybdates within the blood would result in a reduction of the ratio from a theoretical norm of 1.9 (:1).

The objectives of this study were to obtain veterinary feedback on the University of Leeds Trace Element Laboratory's (ULTEL) assessment of copper status and responses to any recommended supplementation.

Material and methods

This study used data obtained throughout 1998 from trace element analysis of blood samples from 713 female cattle (> 18 months old) in 114 herds, mainly of dairy type and unknown herd size. Veterinary practices (n=15) sending in three or more sets of samples (> 5 cattle per herd) were included. The veterinary surgeons obtained two blood samples by veni-puncture per heifer/ cow, one containing lithium heparin as an anticoagulant and one with no anticoagulant. The samples were dispatched from the veterinary practices, usually on the day of sampling, to the laboratory.

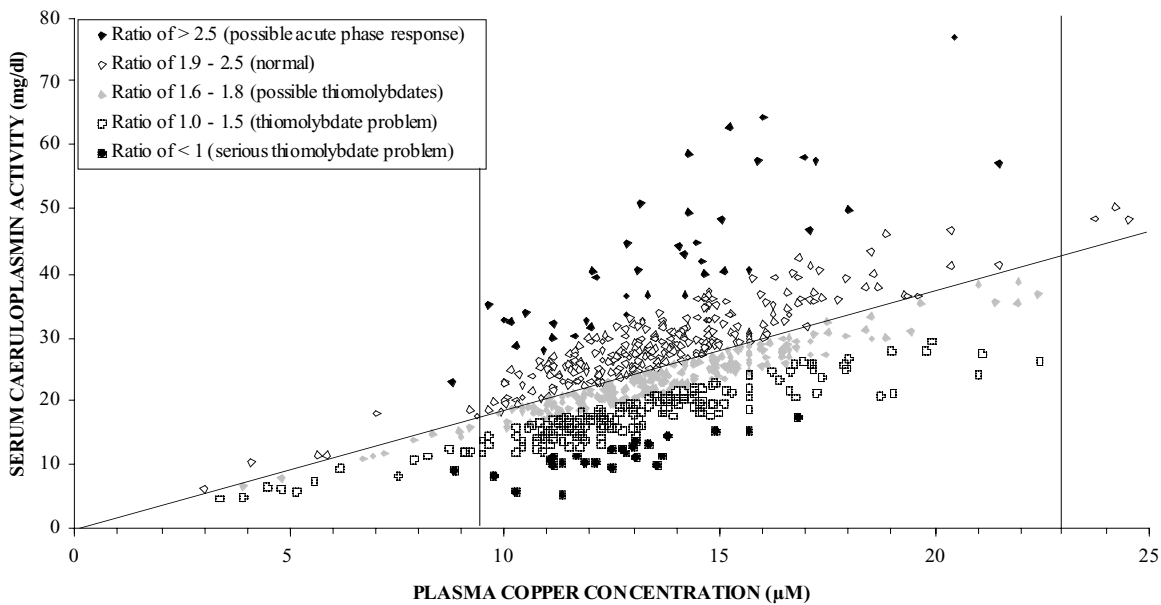
The University of Leeds Trace Element Laboratory's routine copper analytical set consisted of plasma copper concentration (PICu), trichloroacetic acid (TCA-Cu) soluble copper concentration, serum caeruloplasmin activity (CP), serum diamine oxidase activity, erythrocyte superoxide dismutase activity and the ratio between serum caeruloplasmin activity and plasma copper concentration (CP/PICu) using the methods described by Mackenzie *et al.* (1997a,b) and Mulryan and Mason (1992).

Subsequently the veterinary surgeons were asked to fill in a short questionnaire relating to the farms using the diagnostic service. The questionnaires included sections on suspected causes of infertility, the manifestation of the infertility signs of clinical copper deficiency, subsequent treatments with copper, the efficacy of the copper treatments in reducing infertility and any other comments.

Results

The distributions of serum caeruloplasmin activities and plasma copper concentrations are illustrated in Figure 1 and Table 1.

Figure 1 Caeruloplasmin activity vs plasma copper concentration grouped into ratio ranges, with plasma copper concentrations of 9.4 μ M and 23 μ M and a ratio of 2.0 indicated



The serum diamine oxidase, erythrocyte superoxide dismutase activities and plasma TCA soluble copper concentrations are not relevant to this study and are therefore not reported.

The responses of the veterinary surgeons are shown in table 2. The additional comments included: two practices quoting the ratio as the only truly reliable test for copper responsive conditions, four practices indicating that the type and amount of copper are important and that supplementation must be continued to prevent recurrence of the infertility, two practices wanting further research into the area, one practice which treated a lack of oestrus with copper containing soluble glass boluses with “dramatic and immediate response”, whilst another stated the service was “a very useful diagnostic analysis with fertility seen to improve after copper supplementation”.

Veterinary Laboratory Agency reference ranges for PICu (μ M)	ULTEL CP/PICu ratio ranges					Row totals
	< 1.0	1.0-1.5	1.6-1.8	1.9 – 2.5	> 2.5	
<9.4 (deficient)	1	14	10	7	1	33
9.4 – 12.0 (previously classified as marginal)	9	54	41	55	10	169
12.1 – 23.0 (Normal)	16	107	157	199	29	508
>23.0 (High)	-	-	-	3	-	3
Column totals	26	175	208	264	40	713

Table 1 A comparison of Veterinary Laboratory Agency (VLA) plasma copper reference ranges to the University of Leeds Trace Element Laboratory (ULTEL) CP/PICu ratio ranges for all cattle in this study.

	YES	NO	?
Do you regard infertility as a major problem in your client’s herd?	11	1	
What do you regard as the major cause of this infertility? (groupings not provided)	energy imbalance / dietary management	11	
	minerals / copper	7	
	poor oestrus detection	3	
	disease	2	
Did the University of Leeds blood copper analysis prove helpful?	10		2
For herds with infertility where copper supplementation was advised	Was copper supplemented?	12	
	Was fertility improved?	10	1
Was poor or lack of bulling behaviour/ later embryo loss observed?	12		
Were the above signs reduced if copper was supplemented?	11	1	

Table 2 Response to questionnaire from veterinary surgeons (12 of 15 replied)

Discussion

From Table 1, it can be seen that 4.6% of the cattle have plasma copper concentrations below 9.4 μM and would be considered as being deficient using the VLA parameters. These cattle would all benefit from additional copper regardless of the CP/PICu ratio, as they have very little copper in the blood to counter any thiomolybdate challenge. The majority of the cattle according to VLA parameters have 'normal' plasma copper status. However, the veterinary responses suggest that a much higher proportion have fertility problems that are copper responsive. If the ratio is used as an additional diagnostic aid then a further 3.5 % (25) of the cattle would be considered to have a serious thiomolybdate problem (ratio < 1), a further 22.6 % (161) with a definite thiomolybdate problem (ratio 1.0-1.5) and 27.8 % (198) having a possible thiomolybdate problem (ratio 1.6-1.8). Normal animals with no thiomolybdate problem or acute phase response account for 35.6 % (254) cattle. Previous studies have shown that animals with low CP/PICu ratios benefit from copper supplementation (Moeini, 1997; Mackenzie and Telfer, 2000).

The overall distribution of serum caeruloplasmin activity vs plasma copper concentration (Figure 1) has a correlation coefficient (r^2) of 0.42, which indicates a divergence from the strong correlation expected if the CP/PICu ratio was not effected by thiomolybdates. It must be remembered that this distribution will be skewed by the fact that most samples are suspected to have copper problems and therefore the proportion of normal animals will be lower than in the UK as a whole.

The number of blood samples within the VLA normal reference range for plasma copper concentrations (9.4 – 23.0 μM) suggests that there should be a low response from the cattle to copper supplementation. However, the responses from the veterinary surgeons do not support this, with >80% reporting improvements after copper supplementation. Hence, the use of the CP/PICu ratio seems to be a more definitive diagnostic tool for determining copper responsive conditions in the field situation.

The serum diamine oxidase and erythrocyte superoxide dismutase activities are mainly responsive in severe copper deficiency conditions in cattle and are not ideal primary indicators of marginal or molybdenum induced low copper status (Mulryan and Mason, 1992). PICu minus TCA-Cu concentrations were greater than 1 μM in less than 10% of the cattle sampled, indicating that there was no significant binding of free amino acid copper to thiomolybdate in the blood.

Conclusions

The ULTEL copper status analysis was helpful to the majority of the responding veterinary surgeons.

The majority of veterinary surgeons regarded infertility as a major problem in their clients' herds.

The veterinary responses showed that, in the majority of cases where copper supplementation was recommended and administered an increase in fertility was observed.

All responding veterinary surgeons reported herds that showed poor or lack of bulling behaviour/ later embryo loss (signs of clinical copper deficiency) and all but one reported that these signs were reduced by copper supplementation.

The relevance of the CP/PICu ratio as a diagnostic aid in the identification of copper responsive infertility has been supported by the responses of the veterinary surgeons.

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