POTENTIAL MINERAL DEFICIENCY DISEASES OF INDONESIAN RUMINANT LIVESTOCK: ZINC

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ABSTRACT

Two studies on zinc status of Indonesian ruminants are presented. Most serum zinc levels of slaughterhouse buffalo and grazing sheep fell into the marginal zone, less than 0.8 ppm but above 0.4 ppm, suggesting suboptimal zinc status. It is concluded that further study of ruminant zinc status is warranted.

INTRODUCTION

Mineral deficiencies are a major cause of poor production and disease in ruminants in developing tropical countries where livestock rely entirely on forages to supply nutrient requirements (McDowell, 1985). Mineral deficiencies are poorly understood in Indonesia. The data available from local studies are few and often reported in such a manner that their veracity cannot be determined. In this paper we present recent data on zinc, a micro-element essential for ruminant nutrition, critically review Indonesian data and identify gaps in the local information.

Zinc was recognised as an essential element for food ruminant nutrition in 1960 (Legg and Sears, 1960). Zinc is incorporated into a number of metalloenzymes, including carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases, alkaline phosphatase, thymidine kinase and RNA and DNA polymerases. Zinc is also involved in vitamin A metabolism. Zn deficiency is characterised by poor appetite, reduced growth, abnormalities of skin and hair and poor reproductive performance. Development of the male reproductive system is particularly sensitive to Zn. Parakeratosis, alopecia, bowing of hind limbs, foot soreness and testicular hypoplasia are seen (Underwood, 1981; Nelson *et al.*, 1984).

The minimum dietary requirements of Zn for ruminants have been estimated at between 12—50 ppm on a dry matter basis. Conrad *et al.* (1984) and Tejada *et al.* (1987) considered 30 ppm the critical level below which deficiency might occur while McDowell (1985) suggested a critical level of 40 ppm. Other studies indicated that 22 ppm was adequate for maximum weight gain and testicular development of young rams fed a high fibre diet (Hatch *et al.*, 1987).

Although the concentration of Zn in pastures and fodders may vary from 5 to 200 ppm, most plants growing on normal soils are reported to contain 25 to 50 ppm (Underwood, 1981). While legumes are generally higher than grasses, there appears to be no difference in Zn content of tropical versus temperate grasses (Norton, 1981). Higher levels have been found in pastures exposed to contamination from industrial sources (Parada *et al.*, 1987). The importance of forage sampling was emphasized in a detailed study of minerals in dwarf elephant grass (*Pennisetum purpureum*), in which Montalvo *et al.* (1987) found mean Zn levels in leaves around 20 ppm and levels of 57—124 ppm in stems.

Two studies are reported here. The first was a preliminary study to examine the relationship between serum Zn concentration and testicular histology in slaughterhouse buffalo in Bogor. The second study established baseline serum Zn levels in grazing sheep in the Cirebon area.

MATERIALS AND METHODS

Sera were collected specifically for Zn analysis. Precautions were taken to prevent contact of blood with rubber stoppers on evacuated blood collection tubes as these stoppers can be a source of zinc contamination (Fick *et al.*, 1979).

The buffalo sera were obtained at Bogor slaughterhouse as part of a survey on diseases of the male reproductive tract. The sera were analysed "blind" so that the analyst did not know which samples came from normal or diseased animals. The sheep sera were obtained from grazing animals at Cirebon at the end of the dry season. The 47 samples came from 19 different farms.

Zinc was determined by atomic absorption spectrophotometry (Varian AA-1275) in sera diluted with double distilled water (Fick *et al.*, 1979). Standards (BDH) were diluted in 10% glycerol solution. A homemade bulk serum standard and commercial reference sera (Precinorum U,

Boehringer Mannheim; Seronorm, Nycomed AS Diagnostics) were used to check accuracy and precision of analyses.

RESULTS AND DISCUSSION

The possibility of Zn contamination by anticoagulants, blood collection and serum separation procedures was minimized. Major problems in Zn assessment are reported to be contamination from rubber stoppers or anticoagulant and concentration through loss of water in samples stored in "frost-free" freezers (Smith *et al.*, 1985). Since the Zn levels we report are low rather than high, it is unlikely that significant contamination or concentration was involved. Accuracy and precision and/or repeatability of the analytical method were established by repetitive analysis of certified reference materials and a pooled bench standard.

In our studies reported here, only sera were analyzed. Sera from buffalo with hypoplastic or degenerative (noninfectious) testicular lesions contained an average of 0.6 ppm Zn, identical to the Zn level in sera of buffalo with normal testicular architecture (Table 1). Most of the Zn values in this study fell into the marginal zone, below the normal range but above the critical level.

Table 1. Buffalo zinc level and testicular histology

Testicular histology	# Buffalo	Serum zinc level*
Normal	8	0.61 (0.04)
Hypoplastic or degenerative	10	0.62 (0.04)

*ppm, mean (S.E.)

Although the number of animals is small, so is the variance. Since these buffalo sampled at the Bogor slaughterhouse originate from a variety of areas, the results may be fairly representative. In addition, the failure to find an association between zinc level and testicular histology may indicate a real lack of relationship between zinc status and the testicular lesions observed.

In the study on serum zinc levels in grazing sheep, a mean level of 0.75 ppm was found; sixty-eight percent of animals had serum levels in the marginal zone, but none were below the critical level of 0.4 (Table 2).

Table 2. Zinc status of grazing sheep

% of animals		
Deficient (< 0.4 ppm)	Marginal (0.4–0.8 ppm)	Normal (> 0.8 ppm)
0	68	32

The serum zinc measurements in this sample were not normally distributed, but showed a degree of positive skewness. However, logarithmic (base e) transformation of the data allowed analysis using parametric statistical techniques. For this population, a sample size of 16 would allow an estimation of the mean to $\pm$ 0.1 ppm with 95% confidence. The data from these samples gave sigma = 0.2, or 25% of the mean. Thus, it would be possible to detect a difference of 0.14 ppm with 95% confidence and a power of 80% if group sizes of 24 were used.

Earlier studies from this institute reported high levels (averages > 2 ppm) in cattle and buffalo sera (Ginting *et al.*, 1985; Stoltz *et al.*, 1985). These are likely due to contamination from rubber stoppers on blood collection tubes.

Although response to Zn supplementation is the definitive diagnostic criterion, the Zn concentration in blood serum or plasma is commonly used to diagnose Zn deficiency. The normal plasma concentration range is considered to be 0.8—1.2 ppm Zn and 0.4 ppm is considered the critical value for diagnosis of deficiency (Underwood, 1981). Goats with a mean plasma Zn of 0.62 ppm exhibited clinical disease and response to Zn supplementation (Reuter *et al.*, 1987). Similarly, sheep with clinical deficiency and serum levels of 0.44 ppm Zn responded to supplementation with loss of symptoms and a rise in serum Zn to 0.78 ppm (Suliman *et al.*, 1988). The zinc content of serum has been reported to be 16% higher than in plasma and has been attributed to liberation of Zn from platelets (Foley *et al.*, 1968). For a detailed discussion of serum versus plasma analysis of Zn and additional references see Smith *et al.* (1985). The zinc concentration in liver may also be used to assess Zn status. The normal range is reported to be 84—132 ppm (dry matter basis).

Little (1986) has reported results of hundreds of analyses on Indonesian ruminant feeds. A summary of his data, given in Table 3, indicates potentially marginal supplies of Zn, especially for grazing ruminants. Higher Zn levels were reported for cassava leaves (83 ppm) and miscellaneous weeds (41 ppm). According to soil scientists and plant

nutritionists, "the micro-element most likely to be deficient in Indonesian soils is zinc, followed by copper, boron and molybdenum" (Soepardi, 1982).

Table 3. Mean Zn concentration in feeds

Feed	# Samples	Zinc*
Legumes	132	25
Grasses	211	30
Roughages	57	24

(adapted from Little, 1986)
*ppm: dry matter basis

Prabowo *et al.* (1983) studied several trace elements in sheep at 2 locations in West Java in both wet and dry seasons. Zn levels in forage were lower in Cirebon but liver concentrations were higher (Table 4). In both locations, liver Zn levels could be considered low.

Table 4. Zinc in forage and sheep liver

Location/Season	Zinc*	
	Forage	Liver
Cirebon		
wet	18	71
dry	17	84
Garut		
wet	37	38
dry	43	44

(adapted from Prabowo *et al.*, 1983)
*ppm: dry matter basis

Panggabean *et al.* (1985) reported Zn levels in native grass at Ciawi, West Java, mainly *Paspalum conjugatum* and *Axonopus compressus*, and in blood and liver of sheep fed this grass with and without a mineral supplement containing Zn (Table 5).

Table 5. Zinc in forage and sheep ± mineral supplement

Sample	Zn concentration* in animals eating	
	Grass only	Plus minerals
Blood	2.9	3.0
Liver	160	133
Forage	60	

(adapted from Panggabean *et al.*, 1985)
*ppm: dry matter basis assumed for liver and forage

The mean forage level (60 ppm) is higher than reported by other local investigations, but the number of samples is not stated. The blood levels may be high but it was not stated whether whole blood, serum or plasma was analyzed. Such values suggest contamination from rubber stoppers on evacuated blood collection tubes or from anti-coagulant (Fick *et al.*, 1979; Smith *et al.*, 1985).

In another report, Panggabean and Little (1987) give a mean Zn concentration of 44 ppm (range 35—58) in roadside grasses at Ciawi.

Sutrisno *et al.* (1982) have reported liver Zn levels in slaughterhouse cattle in Central Java (Table 6). These results suggest Zn deficiency, but the number of samples analysed was not stated.

Table 6. Liver zinc in slaughter cattle

District	Mean Zn concentration*
Temanggung	33
Semarang	35
Rembang	47
Magelang	75
Grobogan/Purwodadi	79

(adapted from Sutrisno *et al.*, 1982)
*ppm: dry matter basis

There are several problems in relying on critical element levels in the diet to predict likely deficiency. Firstly, there is considerable difference of opinion regarding the actual critical level, which is estimated to lie between 12 and 50 ppm Zn (Little, 1981; Underwood, 1981; Conrad *et al.*, 1984; McDowell, 1985; Tejada *et al.*, 1987). Secondly, the Zn requirement depends upon the level of competing or interacting minerals such as copper and iron (Little, 1981; Rosa *et al.*, 1986) and may also depend upon exposure to certain toxins to which Zn has been shown to exert a protective effect (Smith *et al.*,1977; Seagrave *et al.*, 1983). Thirdly, the utilization of ingested Zn may be influenced by the degree and type of intestinal helminthiasis, as has been demonstrated for other minerals (Little, 1981; Hegarty and Gray, 1987). And finally, variables such as species and breed as well as physiological state, for example growth, maintenance, lactation, and parturition, likely have contributed to the wide range of estimated critical dietary levels of Zn.

Similarly, there is some disagreement regarding the serum or plasma critical Zn level indicating deficiency. Whereas we have used a reasonably

conservative critical level of 0.4 and a wide marginal zone of 0.4 to 0.8 ppm in the evaluation of our studies, McDowell (1985) and Tejada *et al.* (1987) recommended a critical level of 0.6 to 0.8 ppm Zn in serum and Mills *et al.* (1967) have opted for plasma concentrations less than 0.3 ppm in two analyses. As suggested by Underwood (1981), blood Zn levels lack sensitivity as diagnostic criteria of Zn status. Nevertheless, estimation of dietary intake and blood level of Zn assists in diagnosis of Zn deficiency. But, as stated by Suttle (1986), "for all elements the surest diagnosis (of dificiency) is an improvement in growth or health in response to a specific supplement".

CONCLUSIONS

The studies we report indicate that Zn deficiency may be a problem in Indonesian ruminant livestock. Other local studies discussed here indicate low Zn levels, high Zn levels and possibly some regional differences.

Further study of Zn (forage analyses, animal analyses, response to supplementation) is definitely warranted to clarify the Zn status of livestock. The possibility of regional differences should be investigated.

Quality control in laboratory analysis needs attention. Laboratories doing mineral analyses should make bulk house standards for day to day use, and these should be checked routinely against certified analytical standards. Contamination of blood samples by Zn from rubber stoppers on blood collection tubes and in anticoagulants must be avoided.

Published reports should contain essential information on the number of samples analyzed, fresh vs dry weight expression of results and the use of standards.

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