THE EFFECT OF MINERAL GLASS BOLUSES ((COSECURE®) SUPPLEMENTATION ON PLASMA COPPER, CERULOPLASMIN, GLUTATHIONE PEROXIDASE AND OTHER PARAMETERS IN GRAZING HEIFERS.

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SUMMARY

The effect of supplementation of slow releasing intra - ruminal mineral glass boluses (COSECURE®) containing 13.4% copper (Cu) as CuO w/v, 0.3% selenium (Se) as Na_2SeO_4 and 0.5% cobalt (Co) as Co_3O_4 w/w on plasma Cu, ceruloplasmin (CP) and glutathione peroxidase(GSH.Px) activities, body weight gain (LBW), packed cell volume (PCV) and haemoglobin (Hb) was assessed in grazing heifers for eight months. Heifers were weighed and PCV, Hb, plasma Cu, CP, GSH.Px measured before supplementation started and thereafter every two months. There were no significant statistical differences (P< 0.05) between control and supplemented heifers in terms of PCV (30.3% vs 31.0%), Hb (11.4 vs 11.8 g/100 ml), plasma Cu (0.93 vs 0.91 mg/l) and CP (55 vs 54 U/l). However significant statistical differences (P< 0.05) was observed on live body weight (LBW) (236 vs 248 kg) and GSH.Px activity (384 vs 462 U/gHb).. It was concluded that the intra - ruminal glass boluses were not as effective in providing immediate boost to Cu reserves in the body as anticipated but were sufficient in releasing significant quantities of Se.

INTRODUCTION

In Tanzania pasture contains very low level of trace elements such as Cu, Se, Zn and Co, (Phiri, 1995; Sendalo, 1986;Maro *et al.*, 1980; Mwakatundu, 1977; Rodgers, 1975). Inspite of this many people do not offer mineral supplements to their animals. Failure or improper supplementation could be the probable cause for the poor performance indicated by low reproduction rates, reduced growth rates, reduced feed conversion efficiency, reduced production of milk, meat, increased susceptibility to disease infections and sometimes death, (McDowell, 1992), observed in the traditional herds in the country.

The principal methods of increasing Cu and Se intake by grazing livestock include Se and / or Cu pasture fertilization, injections of Se or Cu drench and Se or Cu ruminal pellets (heavy boluses) (McDowell, 1992). Copper - selenium controlled intra - ruminal releasing glass boluses have been used successfully (Lawson *et al.*, 1991; Ritchie et al., 1991; Duncan and

Ellis, 1988; Judson *et al.*, 1985; Care *et al.*, 1985). However these intra- ruminal mineral release glass boluses have not yet been used in Tanzania.

The objective of this study was to evaluate the effectiveness of the intra-ruminal mineral glass boluses (COSECURE®) on the balance of Cu and Se, body weight gain, PCV and Hb in grazing heifers at ASAS farm in Iringa Region. The owner of the farm had for some time been experiencing reduced growth, delayed conception, long calving intervals, abortions and poor milk production despite the regular dipping, deworming and normal prophylactic practices. Thus there was a need for investigating mineral imbalances problems at this farm as a probable cause of this animal's poor performance. Plasma Cu and ceruloplasmin activity were used to monitor levels of Cu whereas the activity of GSH.Px was used to monitor the level of Se in the animals.

MATERIALS AND METHODS

Experimental site

The study was carried out in Iringa region Tanzania between June 1994 - February 1995, on a private farm, called ASAS. The farm had a total of 380 cattle and they were grazed throughout the day with concentrate given only to milking herd during milking. Grass hay was supplemented to all animals during the dry season June - November. The dominating pasture grass species included *Hyperrhenia spp* , *Heteropogon spp, Cynodon dactylon, Penicum maximum, Pennisetum purpureum, Brachiaria spp, Chloris spp* and legumes such as *Desmodium intortum* and *Sesbania sesibani.*

Animals, experimental design and treatments

Thirty four grazing heifers of mixed breeds between Aryshire, Zebu, Friesian, Jersey and Boran aged 18 - 28 months were used. The heifers were divided into two groups of 17 each, Group I served as control and Group II was supplemented with the minerals. All heifers were grazed on the same pasture grounds and supplemented with concentrate (45 kg) and hay (18 kg) per group per day during the dry season. The concentrate mixture contained maize bran and cotton seed cake at a ratio of 2:1. The nutrient composition of the concentrate, pasture and hay is given in (Table 1). The heifers in Group I were only maintained on the feeding regime described above whereas heifers in Group II, each in addition was supplemented with two monolithic glass boluses (COSECURE®)(Pilkington Controlled Release Systems Limited, United Kingdom), containing 13.4% Cu as CuO w/v, 0.3% Se as Na_2SeO_4 and 0.5% Co_3O_4 w/w. The boluses were administered into the rumen using a boling gun and their life span in the animal was stated to be more than one year.

Sampling and laboratory analysis

Blood samples were collected from each animal via the jugular vein into heparinized vacutainer tubes on the initial day of the experiment and subsequently at an interval of two months. Whole blood sample for determination of plasma Cu and ceruloplasmin was collected into tubes washed in 6M nitric acid to prevent exogenous Cu contamination and was centrifuged at 4500 rpm for 10 minutes to obtain plasma.

The packed cell volume was measured by the microhaematocrit and Hb by cyanmethaemoglobin method using Drabkin's diluent (Baker and Silverton, 1976). Plasma Cu was determined by atomic absorption spectrophotometer following acid digestion and precipitation of protein by trichloroacetic acid (Paynter 1987). Ceruloplasmin activity was measured according to the method described by Schosinky (1974). Glutathione peroxidase activity was determined by RANSEL reagent kit (RANDOX laboratories, North Ireland). For control of precision and accuracy control samples provided with the kits were analyzed together with the test samples for each analysis run.

Statistical analysis

Data was analyzed by SAS analysis of variance (ANOVA) as described by Schlotzhauer and Litell (1987). P - values of less than 1% and 5% were considered statistically significant. Duncan multiple range test were used for mean comparison.

RESULTS AND DISCUSSIONS

The mean body weight gain (LBW), PCV and Hb are presented in Table 2, whereas plasma Cu, CP and GSH.Px activities are presented in Table 3. Significant Statistical differences ($P<0.05$) between control and supplemented heifers in terms of overall means of PCV, Hb, plasma Cu and ceruloplasmin activity were not detected. However significant statistical difference ($P>0.05$) on mean live weight gain and GSH.Px activity was observed.
Supplemented heifers gained weight at a faster rate than control heifers. The mean gain in body weight per day for the control and supplemented heifers for the study period was 360 g and 460 g respectively. The results in body weight gain are similar to those of Ricciardino et al. (1993) who observed body weight gain in grazing heifers at 240 g/day as a result of Cu and Se supplementation. In the present study the supplemented heifers gained 100 g/day over and above the control group.
The normal Hb levels reported in the literature are highly variable and range from 11- to 12 g/dl (Underwood, 1977). Levels above 11.0 g/dl Hb were detected in this study without obvious signs of anemia indicating normal level of Hb in all groups.

Table 1: Chemical composition of the concentrate mixture and hay given to experimental animals in dry matter basis.

Mineral element	Concentrate	Hay
Macro-element (%)		
Calcium	1.72	0.36
Phosphorus	0.86	0.28
Sodium	0.21	0.13
Potassium	2.90	2.33
Magnesium	1.60	0.12
Micro-element (ppm)		
Manganese	28.0	240
Zinc	34.0	16
Copper	22.0	4
Iron	1000.0	400
Sulphur	708.0	400
Dry matter (%)	95.5	90.9
Ash	5.2	7.9
Crude protein	21.5	3.2
Crude fibre	...... 5.9	39

Plasma Cu concentrations and CP activity reflect the Cu status of cattle. The normal range of plasma Cu is wide, 0.5 - 1.5 mg/l (Underwood, 1977). It is accepted that plasma Cu value of 0.6 mg/l of plasma (MacDowell, 1992) and CP activity below 40 U/l of plasma (Paynter, 1987) is indicative of deficiency in sheep and cattle.

In this study the initial plasma Cu concentration and CP activity in some heifers were below 0.6 mg/l and 40 U/l without any obvious sign of deficiency, suggesting marginal deficiency. The absorption of Cu is higher in the presence of Cu deficiency than it is in adequate nutritional status (McDowell, 1992). After supplementation with concentrate and minerals the plasma Cu concentration was raised in all groups further suggesting that the animals were initially in marginal deficiency of Cu. Apart from low Cu in the diet or problem in absorption CP activity can be reduced due to low protein (McDowell, 1992; Paynter, 1987).

The diet of all the heifers before experiment consisted of pasture and hay with crude protein of 6.1 and 3.2% respectively.

Table 2: Mean monthly live weight body (LBW) (kg), packed cell volume PCV(%) and haemoglobin (mg/l) of grazing heifers at ASAS farm, Iringa, Tanzania.

Months	Animals	Live body weight (LBW)) Kg	PCV%	Haemoglobin (Hb) mg/l
June 1994	CON	194 ± 25a	28.6±3.0a	11.2 ± 1.4a
	SUP	189 ± 24a	29.4±3.6a	11.8 ± 1.6a
August 1994	CON	212 ± 26a	29.9 ± 3.0a	11.3 ± 1.0a
	SUP	219 ± 27a	31.2 ± 3.0a	11.5 ± 1.2a
October 1994	CON	239 ± 28a	32.2 ± 2.3a	11.6 ± 0.9a
	SUP	253 ± 29b	32.8 ± 4.3a	12.6 ± 1.7a
December 1994	CON	255 ± 30a	30.7 ± 3.3a	11.8 ± 1.4a
	SUP	274 ± 31b	31.5 ± 3.7a	11.7 ± 1.6a
February 1995	CON	282 ± 30a	30.0 ± 3.3a	11.0 ± 1.2a
	SUP	304 ± 32b	30.0 ± 2.5a	11.3 ± 1.1a
Overall means	CON	236 ± 28a	30.3 ± 3.0a	11.4 ± 1.2a
	SUP	248 ± 29b	31.0 ± 3.5a	11.8 ± 1.5a

CON = Control; SUP = Supplemented; Means with the same subscript in the same column have no significant difference at $P<0.05$.

This was below the critical level (7%) as suggested by McDowell, (1992) and therefore a factor in the initial low (14.2 U/l) ceruloplasmin activity. The concentrate mixture given to the experimental heifers contained 21.5% crude protein and 8 ppm Cu hence a possible reason for increased plasma Cu and CP activity after two months in all groups. The relative low concentration of plasma Cu in October in all groups was probably due to a decrease in pasture Cu concentration as this is within the dry period for the area with October being more drier. The amount of pasture grass is reduced and lignification of pasture occurs hence digestibility is also reduced. Reduced plasma Cu and CP activity in Group II in December might have been due to unknown factors present in pasture which interact with Cu released from the glass boluses. It has been reported that zinc (Zn), iron (Fe), Ca and P when ingested at high dietary levels may depress Cu absorption (McDowell, 1992; Davis and Mertz, 1987). Iron (755ppm and 1000ppm), calcium (0.64% and 1.72%) in pasture grass and concentrate respectively were high during this period hence a possible cause of depressed Cu absorption.

The mean initial level of GSH.Px in grazing heifers was above 251 IU/l. The normal range of GSH.

Table 3: Mean monthly plasma copper (Cu)(mg/l), ceruloplasmin (IU/l) and glutathione peroxidase GPx (IU/l) activity of grazing heifers, ASAS farm, Iringa Tanzania.

Months	Animals	Plasma copper (Cu) mg/l	Ceruloplasmin (IU/l)	Glutathione peroxidase (IU/l)
June 1994	CON	0.78 ± 0.34a	13.5 ± 5.6a	254 ± 67a
	SUP	0.76 ± 0.40a	14.9 ± 5.0a	251 ± 55a
August 1994	CON	1.15 ± 0.29a	66.8 ± 29.3b	411 ± 85b
	SUP	1.08 ± 0.24a	52.0 ± 16.6b	499 ± 69d
October 1994	CON	0.69 ± 0.31a	55.9 ± 15.3b	471 ± 96c
	SUP	0.71 ± 0.35a	72.7 ± 28.8b	523 ± 85d
December 1994	CON	1.01 ± 0.24a	70.1 ± 17.4b	466 ± 80c
	SUP	1.02 ± 0.28a	67.2 ± 25.6b	503 ± 88b
February 1995	CON	1.02 ± 0.28a	68.5 ± 12.5b	318 ± 52a
	SUP	0.99 ± 0.30a	62.2 ± 14.4b	534 ± 69d
Overall means	CON	0.93 ± 0.29a	55.0 ± 16.5a	384 ± 75b
	SUP	0.91 ± 0.32a	54.0 ± 18.2a	462 ± 70c

CON = Control; SUP = Supplemented; Means with the same subscript in the same column have no significant difference at $P<0.05$.

Px in cattle is above 130 U/gHb as stated by RANSEL kit manufacturers. This implies that the heifers were in their normal GSH:Px activity which corresponds to Se sufficiency. It has been reported by MacDowell (1992) and Underwood (1981) that animals consuming a limited amount of Se accumulator plants during a period of weeks or months, suffer loss of appetite, lameness due to pain and condition of the elongated hooves, lack of vitality, emaciation and roughness of hair coats. These clinical signs were not observed in the experimental heifers however, they were observed in some of the milking cows on the farm and neighbouring farms. From the records on this farm and neighbouring farms the above described signs become severe during the month of June - November, a dry period.

The period in this study corresponded well with high levels of GSH.Px in heifers in both groups (Table 3). Accumulator plants usually remain green during dry season when other forages are not available (McDowell, 1992). Common accumulator plants such as *Astragalus racemosus* were not identified on the farm suggesting probable existence of unidentified accumulator plants. Sipolla (1979) reported that clay soils contains high amount of Se, part of this farm is characterized by clay soil which may favor accumulator plants.

CONCLUSIONS

By comparing the levels of Cu in plasma and ceruloplasmin activity it is evident that heifers had marginal deficiency of Cu throughout the study period. The clinical signs in the milking cows and the level of GSH.PX in heifer indicated that Se is adequate and there is a possibility of having chronic selenosis on the farm. It is possible that deficiemcy of Cu and excess of Se might contribute to the observed reduced growth rate and delayed conception in animals at this farm. An increase in mean body weight gain over the dry season has emphasized the superiority of the glass boluses. The glass boluses were not effective in providing an immediate boost to Cu reserves in the animal body but were sufficient in releasing significant quantities of Se. It is recommended that the degradability of the glass boluses in relation to absorption and interaction of Cu with other minerals be studied.

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