

NUTRITIONAL FACTORS AFFECTING MINERAL STATUS AND  
LONG-TERM CARRY-OVER EFFECTS IN SHEEP. II TRACE MINERALS<sup>1</sup>

O R Rosero<sup>2</sup> L R McDowell<sup>3</sup> J H Conrad<sup>3</sup> G L Ellis<sup>3</sup>  
and F G Martin

University of Florida, Gainesville 32611 and  
Facultad de Ciencias Veterinarias de la Universidad del  
Zulia, Maracaibo, Venezuela

Forty-eight ewe lambs were assigned to an 18 month factorial experiment, with two dietary levels of minerals (high and low) and two levels of energy-protein to study trace mineral status, storage and long term carry-over effects. Treatments with high minerals were administered for only four months of the growing period. There were no carry-over effects for the concentration of iron (Fe), copper (Cu), zinc (Zn) or selenium (Se) in serum or in liver in ewes fed the original experimental diets which were high in minerals. Under the conditions of this experiment, levels of energy-protein were more important than levels of minerals in influencing tissue mineral concentrations. Milk had higher Fe and manganese (Mn) concentrations than colostrum (4.9 and 0.77 vs 3.3 and 0.51 µg/ml, respectively); in contrast Cu, Zn and Se were higher ( $P < 0.05$ ) in colostrum than in milk (3.7, 65.3 and 0.04 vs 3.5, 40.5 and 0.02 µg/ml, respectively). Serum Fe, Cu and Zn decreased from newborn to weaned lambs (3.59, 0.2 and 1.46 to 1.80, 0.15 and 0.96 µg/ml, respectively).

Key Words: Sheep, mineral status, energy-protein, carry-over.

In a tropical pasture-ruminant animal system, a major part of the animal's requirements in terms of energy, protein and vitamins can be satisfied by pasture. Minerals, however, are often low in tropical pastures resulting in substantial production losses for grazing livestock (McDowell, 1976; McDowell et al, 1983). During the dry season, grasses mature and become dry, consequently forage quality progressively declines and the animals lose weight, at the end of this season they may have lost as much as 30% of their peak weight in the rainy season (Van Niekerk, 1974).

The fluctuation of nutrient content results in the familiar pattern of growth rate of animals on native grasses, that is, rapid growth in the rainy season followed by a loss of body weight during the dry season. The deficiencies result in a poor animal performance either directly or through depression of feed intake.

To have rapid and economical improvement in grazing livestock production in a tropical environment, factors that influence mineral status under

<sup>1</sup>Florida Agr. Exp. Sta. Journal Series No. 4919.

<sup>2</sup>Present address: Facultad de Ciencias Veterinarias de la Universidad del Zulia, Maracaibo, Venezuela.

<sup>3</sup>Dept. of Animal Science, University of Florida, Gainesville 32611

<sup>4</sup>Dept. of Statistics, University of Florida, Gainesville 32611.

grazing conditions must be determined. The objectives of this research were to investigate the effect of two dietary levels of energy-protein on sheep mineral status and to compare the effect of two mineral levels (high and low) on mineral storage and long-term carry-over effects in sheep. The present study evaluates trace elements, while a companion paper (Rosero et al, 1984) evaluates animal performance and macro elements.

### Experimental Procedure

Forty-eight Rambouillet crossbred ewe lambs, 8 to 10 months old, averaging 28.5 kg initial body weight were randomly assigned to four experimental groups in a 2 x 2 factorial arrangement representing two dietary levels of minerals (high and low) and two levels of energy-protein (high and low). The experiment was divided into four periods. Experimental periods and diets were described in the companion paper (Rosero et al, 1984).

Blood samples were collected in ewes at the end of each experimental period (four times) and also, from both newborn and weaned lambs. Blood serum and feed samples were collected, prepared and analysed for minerals by standardized methods described by Fick et al (1979). Liver biopsies were taken only at the end of the growing period. Liver biopsy sample preparation and analysis was carried out as described by Fick et al (1979).

Colostrum was collected within the first 15 hours after birth and milk during the middle of the lactation period. Milk and colostrum samples (15 and 10 ml, respectively) were dried overnight in an oven at 60°C, then ashed overnight in a muffle furnace at 500°C. Solutions of milk and colostrum were prepared in the manner described for liver and analysed for Fe, Cu, Zn, Mn, Mo and Se (Fick et al, 1979).

Statistical procedures have been described previously in the companion paper (Rosero et al, 1984).

### Results and Discussion

*Blood parameters:* Mean serum trace mineral concentrations for ewes fed four experimental diets are presented in Table 1. In period 1 (growing), high energy-protein diets resulted in higher serum Fe ( $P < 0.01$ ) and Zn ( $P < 0.05$ ) than low energy-protein diets (1.66 vs 1.07 and 1.59 vs 1.33  $\mu\text{g/ml}$ , respectively). Significant interactions ( $P < 0.05$ ) between levels of energy protein and minerals were found only for serum Fe in ewes. With increased levels of energy-protein in the low mineral diets serum Fe increased from 1.05 to 1.40  $\mu\text{g/ml}$  whereas with the high mineral diets the increase was from 1.09 to 1.91  $\mu\text{g/ml}$ .

In period 2 (breeding), serum Cu was higher ( $P < 0.05$ ) in ewes fed low energy-protein diets (0.59 vs 0.46  $\mu\text{g/ml}$ ) and serum Zn was higher ( $P < 0.01$ ) for high energy-protein diets (1.65 vs 1.19  $\mu\text{g/ml}$ ). In period 3 (gestation-parturition), serum Fe and Zn were higher ( $P < 0.01$ ) for high energy-protein diets than for low energy-protein diets (2.11 vs 1.71 and 1.59 vs 1.24  $\mu\text{g/ml}$  respectively). The feeding of high mineral diets resulted in higher serum Cu concentrations ( $P < 0.01$ ) than the low mineral diets (0.62 vs 0.48  $\mu\text{g/ml}$ ). No interactions ( $P > 0.05$ ) between dietary levels of energy protein and minerals were found in this period.

In period 4 (lactation), treatment differences were found between high and low energy-protein diets in serum Fe ( $P < 0.01$ ), Cu ( $P < 0.05$ ) and Zn ( $P < 0.01$ ) (2.66 vs 1.91, 0.43 vs 0.27 and 1.01 vs 0.85  $\mu\text{g/ml}$ , respectively).

No interactions ( $P > 0.05$ ) between dietary levels of energy-protein and minerals were found in blood parameters in this period. Underwood (1977) gives the normal value of plasma Cu in mature cattle as 0.93  $\mu\text{g/ml}$ . Serum Cu levels of 0.6  $\mu\text{g/ml}$  were reported to be slightly deficient in cattle (CMN, 1973); also this committee considered plasma a good indicator of Zn status in animals and suggested above 0.6  $\mu\text{g/ml}$  to be a normal level. On this basis, the majority of animals in this experiment would have low Cu but adequate Zn serum concentrations.

Table 1

Means and standard errors (SE) of serum Fe, Cu, Zn and Se in ewes fed four experimental diets ( $\mu\text{g/ml}$ )<sup>a, b</sup>.

	Diet 1		Diet 2		Diet 3		Diet 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>Period 1</b>								
Fe	1.05	0.03	1.09	0.03	1.40	0.07	1.91	0.22
Cu	0.63	0.04	0.65	0.03	0.50	0.05	0.62	0.06
Zn	1.40	0.13	1.26	0.08	1.62	0.03	1.55	0.09
<b>Period 2</b>								
Fe	1.66	0.13	1.62	0.16	1.76	0.21	1.79	0.08
Cu	0.59	0.05	0.59	0.04	0.36	0.07	0.56	0.10
Zn	1.20	0.19	1.18	0.08	1.74	0.16	1.55	0.12
<b>Period 3</b>								
Fe	1.81	0.10	1.61	0.08	1.95	0.08	2.27	0.10
Cu	0.51	0.04	0.63	0.03	0.45	0.05	0.60	0.08
Zn	1.21	0.14	1.26	0.07	1.53	0.12	1.65	0.12
<b>Period 4</b>								
Fe	1.91	0.17	1.91	0.26	2.25	0.21	3.06	0.36
Cu	0.30	0.04	0.23	0.05	0.36	0.07	0.50	0.11
Zn	0.82	0.05	0.87	0.03	1.06	0.05	0.96	0.05
Se	0.14	0.02	0.14	0.02	0.16	0.01	0.15	0.02

<sup>a</sup> Means based on 8 to 12 observations, with 8 observations for Se per treatment only in period 4.

<sup>b</sup> Diet treatment are as follows: 1(low energy-protein + low minerals), 2(low energy-protein + high minerals), 3(high energy-protein + low minerals) and 4(high energy-protein + high minerals).

There were period effects ( $P < 0.01$ ) for serum Fe, Cu and Zn. Serum Fe increased from period 1 to 4 (1.36, 1.71, 1.91 and 2.28  $\mu\text{g/ml}$ , respectively); in contrast, serum Zn decreased from period 1 to 4 (1.46, 1.42, 1.41 and 0.93  $\mu\text{g/ml}$ , respectively). Means of serum Cu were 0.60, 0.53, 0.55 and 0.35  $\mu\text{g/ml}$  for periods 1, 2, 3 and 4, respectively. In relation to period effects Mtimuni (1982) reported that serum Cu was significantly lower in the rainy season than in the dry season in three regions of Malawi (0.04, 0.04, 0.03 vs 0.24, 0.32, 0.40  $\mu\text{g/ml}$ ). Lebdoesoekojo (1977) and Mendez (1977) reported similar results. No carry-over effects were observed

in serum Fe, Cu and Zn in ewes fed the original experimental diets high in minerals for a period of four months.

*Liver trace elements concentrations:* Mean liver mineral concentration for ewe lambs following the growing period are presented in Table 2. Liver Fe and Co (Cobalt) concentrations were higher ( $P < 0.05$ ) in ewes fed low energy-protein than in those fed high energy-protein diets (382 vs 222 and 0.43 vs 0.34 ppm, respectively). Liver Se concentration was higher ( $P < 0.05$ ) for high energy-protein than for low energy-protein diets (1.99 vs 0.77 ppm). Liver from ewes fed high mineral diets had higher ( $P < 0.01$ ) Co and Mo concentrations than that from those fed low mineral diets. Interactions ( $P < 0.001$ ) between dietary levels of energy-protein and minerals were found only for liver Se concentrations. For the low energy-protein diets, increased mineral levels from low to high decreased liver Se (1.16 to 0.38 ppm); in contrast, for the high energy-protein diets, increased minerals from low to high increased liver Se concentrations (1.04 to 2.93 ppm). A number of reports (Underwood, 1977) suggest that the liver is a good indicator of the status of most trace minerals in livestock. In the present experiment, the majority of the animals had normal liver concentrations of trace elements.

Table 2

Means and standard error (SE) of liver mineral concentration (ppm) in growing ewe lambs fed four experimental diets<sup>a, b</sup>.

Mineral	Diet 1		Diet 2		Diet 3		Diet 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fe	321	60	442	72	199	21	244	27
Cu	207	107	64	13	28	7	61	12
Co	0.33	0.04	0.52	0.05	0.24	0.03	0.43	0.03
Mn	7.4	1.1	6.4	0.5	7.1	1.1	8.8	0.6
Zn	219	92	164	41	101	7	96	10
Mo	4.0	0.7	8.4	1.3	2.7	0.4	6.4	1.4
Se	1.16	0.28	0.38	0.18	1.04	0.20	2.93	0.7

<sup>a</sup>Means based on 6 observations except Se (3).

<sup>b</sup>Diet treatments are as follow: 1(low energy-protein + low minerals), 2(low energy-protein + high minerals), 3(high energy-protein + low minerals) and 4(high energy-protein + high minerals).

*Milk and colostrum:* Mean milk and colostrum Fe, Cu, Zn, Mn and Se concentrations, as affected by treatment, are presented in Table 3. Milk had higher ( $P < 0.05$ ) Fe and Mn concentrations than did colostrum (4.9 and 0.77  $\mu\text{g/ml}$  vs 3.3 and 0.51  $\mu\text{g/ml}$  respectively). Copper, Zn and Se concentrations were higher ( $P < 0.05$ ) in colostrum than milk (3.7, 65.3 and 0.04 vs 3.5, 40.5 and 0.02  $\mu\text{g/ml}$ , respectively). Milk Mn concentration was higher ( $P < 0.05$ ) for high energy-protein than for low energy-protein diets (0.91 vs 0.64  $\mu\text{g/ml}$ ). In contrast, the Cu concentration in colostrum was

higher ( $P < 0.05$ ) for the low energy-protein diets (4.47 vs 2.94  $\mu\text{g/ml}$ ). No significant difference ( $P < 0.05$ ) between levels of minerals or significant interactions between energy-protein and minerals were found for milk and colostrum trace elements concentrations. In cows, sows and goats, Fe supplementation does not appreciably affect milk Fe content (Archibald, 1958). In both rats and sheep, consumption of Cu deficient diets resulted in milk significantly lower than normal Cu concentration (Archibald, 1958). In dairy animals and in the sow, the Zn content of milk can be increased by dietary supplementation (Miller et al, 1965). In humans, as well as in cows, the Mn concentration of milk may be elevated by increasing the dietary Mn intake (Archibald, 1958).

Table 3

Means and standard error (SE) of milk and colostrum Fe, Cu, Zn, Mn and Se concentration ( $\mu\text{g/ml}$ ) in ewes fed four experimental diets <sup>a</sup>.

Variable	MILK					COLOSTRUM				
	Fe	Cu	Zn	Mn	Se	Fe	Cu	Zn	Mn	Se
<u>Diet 1</u>										
Mean	5.5	3.4	49.2	0.592	0.018	3.06	4.87	64.71	0.526	0.056
SE	0.8	0.2	26.1	0.099	0.001	0.36	0.83	9.98	0.081	0.018
n	5	5	5	5	2	7	7	7	7	4
<u>Diet 2</u>										
Mean	4.5	2.7	40.3	0.678	0.027	3.32	4.07	82.0	0.392	0.012
SE	0.5	0.0	13.1	0.084	0.006	0.62	0.50	12.2	0.062	0.000
n	4	4	4	4	2	5	5	5	5	1
<u>Diet 3</u>										
Mean	4.5	4.1	43.3	0.988	0.018	2.67	2.37	52.6	0.616	0.020
SE	0.2	0.8	11.3	0.130	0.008	0.51	0.62	15.4	0.152	0.006
n	4	4	4	4	4	5	5	5	5	3
<u>Diet 4</u>										
Mean	4.9	3.6	29.3	0.825	0.016	4.08	3.50	61.8	0.515	0.054
SE	0.7	0.7	7.7	0.197	0.004	0.94	0.89	15.8	0.204	0.034
n	4	4	4	4	4	4	4	4	4	2

<sup>a</sup>Diet treatments are as follows: 1 (Low energy-protein + low minerals), 2 (low energy-protein + high minerals), 3 (high energy-protein + low minerals) and 4 (high energy-protein + high minerals)

*Blood trace elements of newborn and weaned lambs:* Mean serum trace mineral concentrations in lambs from ewes fed the experimental diets are presented in Table 4. Iron, Cu and Zn were analysed at birth and at weaning, and Se only at weaning. Serum Fe, Cu and Zn in newborn lambs were higher ( $P < 0.05$ ) than in weaned lambs (3.59 vs 1.80; 0.20 vs 0.15; and 1.46 vs 0.96  $\mu\text{g/ml}$ , respectively). No sex differences ( $P < 0.05$ ) were found in serum Fe, Cu, Zn and Se concentrations.

Table 4

Means and standard errors (SE) of serum concentrations ( $\mu\text{g/ml}$ ) of Fe, Cu, Zn and Se in lambs from ewes fed four experimental diets<sup>a</sup>.

	Diet 1			Diet 2			Diet 3			Diet 4		
	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
Fe <sup>1</sup>	8	3.09	0.50	5	3.08	0.62	4	4.47	1.32	7	3.71	0.73
Fe <sup>2</sup>	6	1.79	0.20	4	1.39	0.09	2	2.22	0.65	5	1.80	0.15
Cu <sup>1</sup>	8	0.24	0.07	5	0.25	0.08	4	0.14	0.03	7	0.15	0.02
Cu <sup>2</sup>	6	0.15	0.05	4	0.12	0.03	3	0.10	0.03	5	0.22	0.09
Zn <sup>1</sup>	8	1.61	0.24	5	1.57	0.21	4	1.28	0.28	7	1.36	0.14
Zn <sup>2</sup>	6	0.93	0.16	4	0.83	0.21	2	1.10	0.30	5	0.98	0.04
Se <sup>1</sup>	4	0.08	0.02	3	0.08	0.04	1	0.06	0.00	5	0.08	0.001

<sup>a</sup>Diet treatments are as follow: 1(low energy-protein + low minerals), 2(low energy-protein + high minerals), 3(high energy-protein + low minerals), 4(high energy-protein + high minerals).

<sup>1</sup>Sampling at birth.

<sup>2</sup>Sampling at weaning (approximately 60 days).

## Conclusion

No carry-over effects in tissue mineral concentrations were found in ewes fed the high mineral diets. Dietary energy-protein was more important than mineral concentrations in influencing mineral tissue concentrations. Milk had higher Fe and Mn and lower Cu, Zn and Se concentrations than colostrum. Serum Fe, Cu and Zn concentrations on lambs decreased between birth and weaning.

## Acknowledgements

The authors wish to thank the Scientific Bureau of the Agency for International Development and CONDES of the University of Zululana for funds supporting this research, Mancy Wilkinson for laboratory analysis and Maria Morrell for assistance in manuscript preparation.

## References

- Archibald J G 1958 Trace Elements in Milk A Review - Pt II Dairy Science Abstracts 20:325-330
- CMN 1973 Committee on Mineral Nutrition Tracing and Treating Mineral Disorders in Dairy Cattle Center for Agriculture Publishing and Documentation Wageningen Netherlands
- Fick K R McDowell L R Miles PH Wilkinson N S Funk J D & Conrad J H 1979 Methods of Mineral Analysis for Plants and Animal Tissues University of Florida Gainesville Florida
- Lebosoekojo S 1977 Mineral Supplementation of Grazing Beef Cattle in Eastern Plains of Colombia PhD Dissertation University of Florida Gainesville Florida

- McDowell L R 1976 Mineral Deficiencies and Toxicities and their Effect on Beef Production in Developing Countries In A J Smith (ed) Beef Cattle Production in Developing Countries pp 216-241 Edinburgh Scotland University of Edinburgh Centre for Tropical Veterinary Medicine
- McDowell L R Conrad J G & Ellis G L 1983 Mineral Deficiencies and Imbalances and their Diagnosis in: Herbivore Nutrition in Subtropics and Tropics Problems and Prospects South Africa (In press)
- Méndez M O 1977 Mineral Status of Beef Cattle in Northern Part of Mato Grosso, Brazil as Indicated by Age Season and Sampling Technique PhD Dissertation University of Florida Gainesville Florida
- Miller W J Clifton C M Flower P R & Perkins H F 1965 Influence of High Levels of Dietary Zinc on Zinc in Milk Performance and Biochemistry of Lactating Cows Journal of Dairy Science 48:450-53
- Mtimuni J P 1982 Identification of Mineral Deficiencies in Soil, Plant and Animal Tissues as Constraints to Cattle Production in Malawi PhD Dissertation University of Florida Gainesville Florida
- Rosero O R McDowell L R Conrad J H Ellis G L & Martin F G 1984 Nutritional Factors Affecting Mineral Status and Long-Term Carry-Over in Sheep I Macro Elements and Animal Performance Tropical Animal Production
- Underwood E J 1977 Trace Elements in Human and Animal Nutrition Fourth Ed New York Academic Press
- Van Niekerk B H D 1974 Supplementation of Grazing Beef Cattle in Potential to Increase Beef Production in Tropical America Proc Sem CIAT Cali Colombia p 83-97

*Received July 10, 1984*