

Mineral deficiencies in beef cattle from southern Brazil Deficiências minerais em gado de corte no sul do Brasil

Stella de Faria VALLE¹
Félix Díaz GONZÁLEZ¹
Dimas ROCHA¹
Haydée Beatriz SCALZILLI¹
Rómulo CAMPO¹
Verônica Lima LAROSA¹

1- Departamento de Patologia Clínica Veterinária da Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul, Porto Alegre - RS

Abstract

Minerals play an important role in the cattle metabolism with great impact in productive and reproductive performance of animals raised on extensive grasslands. At present, there is a lack of information concerning mineral deficiency throughout the analysis of biological fluids in Rio Grande do Sul State (southern Brazil). The Central Valley region of this State is characterized by extensive production on natural pastures with poor mineral supplementation. The low levels of minerals found by previous studies on pastures in this area motivated this research. The aim of the present work was to detect possible mineral deficiencies in beef cows by determining concentrations of minerals in biological fluids and in pasture. There were determined concentrations of phosphorus, calcium, copper, zinc, glutathione peroxidase (as indicator of selenium status) and thyroxine (as indicator of iodine status) in blood, sodium and potassium in saliva, and calcium, phosphorus, potassium, zinc, copper, sodium and iron in pastures, in four different periods of the reproductive cycle as follows: service period (artificial insemination), clean up bulls period, end of gestation and early lactation. Four herds were selected in Cachoeira do Sul County for this study which included 112 animals. The results showed marginal deficiencies of phosphorus, copper, iodine and selenium. Calcium concentration was below normal level and could be related to the low protein content of pasture. Most critical periods were the end of gestation and the early lactation.

Key-words:

Mineral profile.
Mineral deficiency.
Southern Brazil.
Beef cattle.

Correspondência para:
FÉLIX DÍAZ GONZÁLEZ
Departamento de Patologia Clínica Veterinária
Faculdade de Veterinária
Universidade Federal do Rio Grande do Sul
Av. Bento Gonçalves, 9090
91540-000 - Porto Alegre - RS
felixgon@orion.ufrgs.br

Received: 16/05/2003
Accepted: 19/02/2004

Introduction

Brazil holds the second greatest livestock in the world (155 million heads). Rio Grande do Sul (southern Brazil) is the fourth greatest Brazilian State in beef cattle (13.7 million heads). This activity occupies 56% of the State area.¹ Almost all cattle in this State are raised on native pastures with poor mineral supplementation along the year. Grass native species are predominantly of low productivity and have low protein and mineral levels.² Consequently, productivity indicators of beef cattle in the State are poor.³

Body weight losses of 30% have been reported during winter season (June to August) in cattle on native pasture without supplementation.⁴

Previous works have indicated mineral deficiencies in Rio Grande do Sul State, namely in phosphorus^{5,6}, copper^{5,7,8,9}, selenium¹⁰, as well as molybdenum excess which predispose to copper deficiency.⁸ Almost all those works have diagnosed mineral deficiencies on pathological or plant analysis basis. Identification of mineral deficiencies strategy must include, besides, animal tissues and fluid analysis of mineral

components, as well as performance response to supplementation.^{6,11,12}

The aim of the present work was to detect possible mineral deficiencies in beef cows from a region of Rio Grande do Sul State by determining blood and saliva levels of mineral indicators along the reproductive cycle.

Materials and Methods

The Central Depression Region of Rio Grande do Sul State (southern Brazil) was selected for this work. This region holds 20% of beef cattle of the State (2.8 million heads). Its soils are classified as acid, moderately fertile and poor in phosphorus.² Native predominant pastures are *Paspalum notatum*, *Desmodium sp* and *Eryngium sp*.¹³ Livestock is raised in extensive conditions, grazing continuously native pastures with poor mineral supplementation. Mineral deficiency in pasture reported by Cavalheiro and Trindade² and Senger et al.^{14,15} and the economic relevance of this region were factors determining the choice of this geographic area of the State for this study.

Four similar herds were selected among representative farms in terms of breeds, husbandry, number of animals and type of native pasture (Table 1). The farms were not fertilized or used previously for agricultural purposes. All herds grazed native pastures and received weekly common salt (NaCl) *ad libitum*. Pasture, blood and saliva samples were collected in four periods during the 2001-2002 reproductive season, as follows: service period (artificial insemination: summer), clean up bulls period (autumn), end of pregnancy (winter) and early lactation (spring). Autumn and winter seasons are characterized by lesser forage production and quality. In each period, seven cows of each herd were aleatory selected. A total of 112 samples were collected.

Blood samples were collected from coccygeal venipuncture using vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ, USA) with and without heparin. Samples were kept refrigerated during transportation to the

laboratory. One aliquot of 50 mL of total blood was hemolized in a diluting agent (Randox, Antrim, United Kingdom) for determination of glutathion peroxydase (GSH-Px) activity in erythrocytes by UV method¹⁶, as indicator of selenium status. Hemoglobin (Hb) concentration was also determined in total blood samples by cyanate colorimetric method (Labtest Diagnóstica, Lagoa Santa, MG, Brazil) in order to express activity of GSH-Px in U/g Hb. Remaining blood samples were centrifuged to obtain plasma (heparinized samples) or serum (non-heparinized samples), which were frozen (-20°C) until analysis of metabolites.

Calcium was determined by flame purple method and phosphorus by phosphomolibdenum colorimetric method (Labtest Diagnóstica, Lagoa Santa, MG, Brazil). Copper and zinc were analyzed by atomic absorption spectrophotometry using dilutions described by Fick et al.¹⁷. Serum thyroxine (T₄), as indicator of iodine status, was determined by radioimmunoassay (Diagnostic Products Co., Los Angeles, USA).

Saliva samples were collected from the lateral region of oral cavity with a 19 cm length steel probe connected to a plastic cannula and a 60 ml syringe. At the laboratory, saliva samples were centrifuged (2800 rpm, 10 min) and supernatant kept in eppendorf tubes at -20°C. Sodium and potassium were determined in saliva by in a Perkin Elmer instrument (Analyst 100) in acetylene/antioxidant flame emission mode.

Pasture samples were collected manually with stainless steel scissors, kept in plastic bags and dry at 75°C in an air circulating stove until constant weight. Samples were grounded in stainless steel mill to determine calcium, phosphorus, sodium, potassium, zinc, copper and iron by atomic absorption spectrophotometry.

All obtained data were organized in casualized blocks considering period sampling as treatment for analysis of variance using SAS program version 6.12 (SAS Institute, Cary, NC, USA). Tukey test was used for mean comparisons.

Results and Discussion

Values of blood and saliva components indicating mineral metabolism in the four periods of the reproductive cycle are shown in table 2. Mineral contents in pastures are shown in table 3. General mean of plasma phosphorus was 1.55 ± 0.32 mmol/L. Kaneko, Harvey and Bruss¹⁸ reported reference range values for this mineral between 1.80 and 2.10 mmol/L. Plasma phosphorus values are good indicator of phosphorus intake in ruminants.¹⁹ The results obtained in all periods, except in the service period (AI), showed that cows might be under phosphorus deficiency, which is worsened during early lactation period, when mean reached 1.32 mmol/L. This value is considered as critical by Timm²⁰ and worth mineral supplementation. González et al.²¹ found a mean of 1.68 mmol/L for plasma phosphorus in beef heifers from a neighbor region, indicating phosphorus deficiency in native pastures along the year. Lisboa et al.²² consider that cows with plasma phosphorus level below 1.16 mmol/L may show deficiency symptoms.

Phosphorus content in pasture was $0.15\% \pm 0.05$, which is not adequate to fulfill recommended requirements of 0.16% for beef cattle²³. Cavalheiro and Trindade² and Senger et al.¹⁴ have found mean phosphorus of 0.13% in native pastures of southern Brazil. Correlation coefficient between phosphorus in plasma and in pastures was not significant ($r=0.04$), suggesting that variations of plasma phosphorus levels were more related to mineral metabolic demand than to mineral intake. Lactation seems to be the period when phosphorus demand is higher and deficiency is more frequent. These finding is in according to Gióvine²⁴, who mentioned that plasma phosphorus levels are low in beef cows during lactation.

Mean plasma calcium was 1.94 ± 0.16 mmol/L. The level of this mineral was below minimum values (2.43 mmol/L) reported by Kaneko, Harvey and Bruss¹⁸ and by Bauer, Santos and Mancuso⁵ in southern

Brazil (2.16 mmol/L). Plasma calcium concentration was significantly higher in cows during service period (2.43 mmol/L) and lower value was seen in lactation cows (1.86 mmol/L, possibly related to higher calcium demand in milk synthesis (Table 2).

Mean calcium content in pastures was $0.59\% \pm 0.11$ (dry matter basis), which is similar to the value (0.60%) found by Senger et al.¹⁵ but higher than that of Cavalheiro and Trindade² in the same region (0.25%). The requirements of Ca in feed recommended by NRC²³ for beef cows are 0.19-0.25%. Calcemia values below reference range found in this work in spite of Ca content in pastures, might reveal poor availability of the mineral in native pastures. Also, this may be due to low protein intake, which may reduce calcium intestinal absorption and calcium circulating levels bound to albumin.²⁵ Cavalheiro and Trindade² reported great fluctuations of protein quality and contents in native pastures of southern Brazil.

Mean plasma zinc was 15.93 ± 2.75 mmol/L. Mc Dowell⁶ reported reference range of plasma zinc between 9.19 and 12.24 mmol/L. Cows in service period had the highest values of zinc (18.96 mmol/L). Although zinc contents in pastures (mean 21.5 ppm) were below NRC²³ requirements of 30 ppm (Table 3), plasma concentrations of this mineral do not indicate deficiency. Senger et al.¹⁴ have found 16 ppm of zinc in pastures of the same region. This result may indicate that zinc requirements for beef cattle of the observed region could be lower than values reported by NRC²³. This finding is in agreement with Morais et al.²⁶ who observed normal serum values of zinc in cows feeding pastures with low levels of this mineral in Brazil. In another work²⁷ was evidenced an increase in zinc plasma concentration of cows receiving zinc supplementation. Lower levels of plasma zinc in pregnant and lactating cows (Table 2) could suggest a higher demand of the mineral in those periods. Wittwer et al.²⁸ in Chile mentioned that lactating cows had lower plasma zinc than cows in another period.

Mean plasma copper was 10.93 ± 2.26 mmol/L. McDowell⁶ cited reference range for plasma copper of 9.42 to 23.55 mmol/L. There was a significant lower copper value in pregnant cows (9.09 mmol/L), which may be considered in a deficient level as McDowell⁶ describes (deficiency less than 9.42 mmol/L). Riet-Correa²⁹ mentioned that deficiency situations are observed when copper content in pasture is under 3 ppm. In the present work mean copper content in pastures was 7.31 ± 1.49 ppm and Cavalheiro and Trindade² have found 6 ppm in the same region. Wittwer et al.²⁸ affirmed that pregnant cows showed significant decrease of blood copper as gestation progress. The results evidence that, although copper contents in pasture is apparently adequate, pregnant cows may suffer marginal deficiency of this element.

Molybdenum is an important antagonist of copper, especially if feed level is above 10 ppm. In this work, mean Mo in pastures was 0.15 ppm, which is below levels reported by Cavalheiro and Trindade² for the same region (0.32 ppm). Nevertheless, mean iron content was 722 ppm, value that is beyond the maximum value (500 ppm) recommended by NRC²³. Cavalheiro and Trindade² found similar iron levels in pastures of this area. It is possible that a marginal copper deficiency condition may be configured due to the high level of iron in pastures of the studied region, since iron interferes with copper metabolism.³⁰

Glutathione peroxidase (GSH-Px) activity has been used as indicator of selenium balance.³¹ This enzyme is abundant in red blood cells and may be measured by photometric techniques.¹⁶ Mean GSH-Px activity obtained in this work was 33.1 ± 21.7 U/g Hb. According to Wittwer³¹ this value may be considered as compatible with selenium deficiency (<60U/g Hb). There are no works evaluating selenium content in pastures in southern Brazil, but the results obtained here may be suggesting a marginal deficiency of selenium in the studied region. Barros et al.¹⁰ described a case of white muscle disease in cattle, possibly linked to

selenium or vitamin E deficiency. Cows in service period had significant higher values of GSH-Px, revealing less selenium demand in that period. It is recommended more research to evaluate biological response to selenium supplementation in cattle from southern Brazil.

Serum thyroxine (T_4) levels are used as indicator of iodine balance because this mineral is almost exclusively used in thyroid hormone synthesis.¹⁸ Mean value of serum thyroxine was 44.2 ± 15.4 nmol/L. Kaneko, Harvey and Bruss¹⁸ cited a reference range for this hormone of 54 to 110 nmol/L in bovines. This finding strongly suggests iodine marginal deficiency in cattle of this region of southern Brazil, especially in gestation and lactation cows, which had significant lesser values of T_4 (Table 2). This result is in agreement with the values of T_4 reported by González, Dias and Ricco³² for beef heifers in Rio Grande do Sul State (mean 30.24 nmol/L). Contreras et al.³³ found lower values of T_4 in lactating cows compared to other groups of cows in the same feed regime, and Dayrell³⁴ mentioned that lactating cows have higher iodine requirements as a consequence of iodine excretion by milk.

Sodium:potassium (Na:K) ratio in saliva has been used as indicator of sodium balance by several workers^{19,35}, who mentioned that in sodium deficiency, Na:K ratio in saliva may diminish below 10. Underwood and Suttle³⁰ observed a Na:K ratio of 0.45 in saliva from bovines with sodium deficiency. In the present work, mean saliva sodium (88.19 ± 31 mEq/L) and potassium (22.3 ± 17.1 mEq/L) made a mean Na:K ratio of 9.8. This suggests a marginal sodium deficiency, mainly in pregnant cows (Table 2).

Sodium content in pastures had a mean of 147 ± 85 ppm, which is below the value reported by Cavalheiro and Trindade² for the same region (300 ppm). In any case, sodium content of native pastures did not fulfill recommended requirements of 600-1000 ppm²³. Potassium mean content in pastures was $0.51\% \pm 0.1$.

Cavalheiro and Trindade² obtained 1% and Senger et al.²⁸ found 0.9% of K in pastures in the same area. It is necessary to perform more studies using supplementation response on saliva Na:K ratio to describe precisely the degree of sodium deficiency in beef cattle from this region of southern Brazil.

In abstract, using analysis of mineral indicators in blood and pastures, the present work detected deficiency of phosphorus in

beef cows, more severely in early lactation period. Plasma calcium level was below normal reference range, possibly associated to low protein intake. It is necessary to perform more research to test this hypothesis. Although zinc content in pasture was below recommended level, plasma concentration was within normal reference values. There was detected a marginal (without evident symptoms) copper deficiency in pregnant

Table 1

Characteristics of studied beef herds in Rio Grande do Sul State, southern Brazil, 2002

Herd characteristic	Number of herd			
	A	B	C	D
Total area (Ha)	1200	270	700	1500
Number of animals	600	315	450	665
Grazing load (animal/Ha)	0.8	0.6	0.7	0.8
Pregnancy rate (%)	55	45	40	56
Predominant breeds	Zebu x Charolais	Zebu crossbred	Zebu x Angus	Zebu x Shorthorn

Table 2

Mean values of metabolites indicating mineral status of beef cows during four different periods of the reproductive cycle in southern Brazil, Rio Grande do Sul, 2002

Metabolite	Sampling period			
	Service (AI)	Clean up bulls	End pregnancy	Early lactation
N	28	28	28	28
Plasma calcium (mmol/L)	2.43 ^a ± 0.20	1.90 ^b ± 0.12	1.94 ^b ± 0.14	1.86 ^b ± 0.12
Plasma phosphorus (mmol/L)	1.87 ^a ± 0.14	1.47 ^b ± 0.08	1.56 ^b ± 0.10	1.32 ^c ± 0.19
Plasma Ca/P ratio	1.11 ^a ± 0.19	1.32 ^{b,c} ± 0.20	1.27 ^{a,b} ± 0.21	1.48 ^c ± 0.36
Plasma zinc (mmol/L)	18.96 ^a ± 2.09	15.47 ^b ± 1.64	14.21 ^c ± 2.08	14.86 ^{b,c} ± 2.42
Plasma copper (mmol/L)	11.66 ^a ± 0.71	12.06 ^a ± 2.10	9.09 ^b ± 1.17	10.94 ^a ± 1.36
Erythrocyte GSH-Px (U/g Hb)	52.27 ^a ± 25.52	30.03 ^b ± 20.52	20.26 ^b ± 7.25	22.94 ^b ± 9.44
Serum thyroxine (nmol/L)	52.53 ^a ± 13.72	51.24 ^a ± 16.44	40.28 ^b ± 12.07	32.83 ^b ± 9.75
Saliva sodium (mEq/L)	*	89.16 ^a ± 26.24	78.02 ^b ± 29.19	97.38 ^a ± 35.02
Saliva potassium (mEq/L)	*	21.15 ± 17.03	22.89 ± 17.22	22.84 ± 17.12
Saliva Na/K ratio		8.18	9.83	11.44

Values with different letters significantly differ ($p < 0.05$) among sampling periods. * Unavailable samples

Table 3

Mean values of mineral components of native pastures during four different periods of the reproductive cycle in southern Brazil, Rio Grande do Sul, 2002

Mineral (dry matter basis)	Sampling period			
	Service (AI)	Clean up bulls	End pregnancy	Early lactation
Calcium (%)	0.55 ± 0.13	0.66 ± 0.18	0.60 ± 0.12	0.61 ± 0.04
Phosphorus (%)	0.16 ± 0.07	0.13 ± 0.04	0.15 ± 0.04	0.15 ± 0.05
Potássio (%)	*	0,65 ± 0,36	0,46 ± 0,12	0,44 ± 0,07
Zinc (ppm)	21.50 ± 6.70	22.75 ± 6.08	20.00 ± 4.26	21.75 ± 14.85
Copper (ppm)	9.00 ± 1.15	6.75 ± 1.26	6.50 ± 0.58	7.00 ± 1.83
Sódio (ppm)	*	246,5 ± 158,5	96,3 ± 9,0	101,0 ± 26,2
Ferro (ppm)	810 ± 647	726 ± 536	637 ± 278	714 ± 254

* Unavailable samples

cows, in spite of adequate copper levels in pasture. High levels of iron in pastures may be involved in copper availability.

This work reports for the first time GSH-Px activity in red blood cells and

thyroxine plasma levels in beef cows from southern Brazil and the results suggest marginal deficiency of selenium and iodine, which must be experimented by supplemental response works.

Resumo

Os minerais são de grande importância no metabolismo dos bovinos, tendo impacto no desempenho produtivo e reprodutivo dos animais. Atualmente há poucos relatos no Rio Grande do Sul com relação ao diagnóstico de deficiências minerais mediante a análise de fluidos biológicos em animais a campo. A região da Depressão Central do Rio Grande do Sul é caracterizada pela produção extensiva de gado de corte em campo nativo com manejo precário da suplementação mineral, apresentando baixos níveis de alguns minerais nas pastagens. O objetivo do presente trabalho foi diagnosticar possíveis deficiências minerais em vacas de corte na região da Depressão Central mediante a dosagem de indicadores minerais em fluidos biológicos e na pastagem. Foram determinadas concentrações de fósforo, cálcio, cobre, zinco, glutathion peroxidase (como indicador de selênio) e tiroxina (como indicador de iodo) no sangue, sódio e potássio na saliva e sódio, e cálcio, fósforo, potássio, zinco, cobre, sódio e ferro na pastagem. Foram estudadas quatro propriedades em Cachoeira do Sul, em quatro momentos do ciclo reprodutivo: período de serviço (inseminação artificial), período de repasse de touros, final da gestação e início da lactação, num total de 112 animais. O perfil mineral indicou deficiência marginal de fósforo, cobre, iodo e selênio. O cálcio sérico apresentou-se diminuído, possivelmente relacionado com o baixo conteúdo de proteína na pastagem. Os períodos mais afetados foram o final da gestação e o início da lactação indicando que essas categorias de produção têm maior exigências metabólicas.

Palavras-chave:

Perfil mineral.
Deficiência mineral.
Gado de corte.
Sul do Brasil.

References

1. CACHAPUZ, J. M. Perspectivas da pecuária de corte gaúcha frente ao mercado futuro. In: CICLO DE PALESTRAS EM PRODUÇÃO E MANEJO DE BOVINOS – PERSPECTIVAS DA PECUÁRIA GAÚCHA FRENTE AO NOVO MILÊNIO, 6., 2001, Canoas. **Anais...** Canoas: Editora da ULBRA, 2001. p. 49-56.
2. CAVALHEIRO, A. C. L.; TRINDADE, D. S. **Os minerais para bovinos e ovinos criados em pastejo.** Porto Alegre: Sagra-DC Luzzato, 1992. 141 p.
3. OSPINA, H. P.; SILVEIRA, A. F. Exigências nutricionais e manejo da alimentação de bovinos de corte em pastejo. In: CICLO DE PALESTRAS EM PRODUÇÃO E MANEJO DE BOVINOS DE CORTE, 4., 1999, Canoas. **Anais...** Canoas: Editora da ULBRA, 1999. p. 7-26.
4. BARCELLOS, J. O.; PRATES, E. R.; OSPINA, H. Suplementação mineral de ruminantes nos campos nativos do Rio Grande do Sul: uma abordagem aplicada à pecuária de corte, In: ENCONTRO ANUAL SOBRE NUTRIÇÃO DE RUMINANTES DA UFRGS, 1., 1999, São Gabriel. **Anais...** Porto Alegre: Gráfica da UFRGS, 1999. p. 81-110.
5. BAUER, A. C.; SANTOS, A. G.; MANCUSO, P. C. Algumas observações sobre uma doença de bovinos no município de Santa Vitória do Palmar. In: CONFERÊNCIA DA SOCIEDADE DE VETERINÁRIA DO RIO GRANDE DO SUL, 1., 1964, Porto Alegre. **Anais...** Porto Alegre: Sociedade dos Veterinários do Rio Grande do Sul, 1964. p. 153-161.
6. MCDOWELL, L. R. **Minerais para ruminantes sob pastejo em regiões tropicais, enfatizando o Brasil.** 3. ed. Gainesville: Universidade da Florida, 1999. 92 p.
7. BONDAN, E. F.; RIET-CORRÊA, F.; GIESTA, S. M. Níveis de cobre em fígados de bovinos no Sul do Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 11, p. 75-80, 1991.
8. MORAES, S. S.; TOKARNIA, C. H.; DÖBEREINER, J. Deficiências e desequilíbrios de microelementos em bovinos e ovinos em algumas regiões do Brasil. **Pesquisa Veterinária Brasileira**, v. 19, p. 19-33, 1999.
9. RIET-CORRÊA, F. et al. Efeito da suplementação com

cobre e doenças associadas à carência de cobre em bovinos no Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 13, p. 45-49, 1993.

10. BARROS, C. S. L. et al. Miopatia nutricional em bovinos no Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 8, p. 51-55, 1988.
11. TOKARNIA, C. H.; DÖBEREINER, J.; MORAES, S. S. Situação atual e perspectivas da investigação sobre nutrição mineral em bovinos no Brasil. **Pesquisa Veterinária Brasileira**, v. 8, p. 1-16, 1988.
12. TOKARNIA, C. H. et al. Deficiências e desequilíbrios minerais em bovinos e ovinos - revisão dos estudos realizados no Brasil de 1987 a 1998. **Pesquisa Veterinária Brasileira**, v. 19, p. 47-62, 1999.
13. BRASIL. MINISTÉRIO DA AGRICULTURA. **Levantamento de reconhecimento dos solos do Estado do Rio Grande do Sul**. Recife, 1973. p. 267-269.
14. ENGER, C. C. D. et al. Teores minerais em pastagens do Rio Grande do Sul. I: Cálcio, fósforo, magnésio e potássio. **Pesquisa Agropecuária Brasileira**, v. 31, p. 897-904, 1996.
15. ENGER, C. C. D. et al. Teores minerais em pastagens do Rio Grande do Sul. II: sódio, enxofre, zinco, cobre, ferro e manganês. **Pesquisa Agropecuária Brasileira**, v.32, p. 101-108, 1997.
16. PAGLIA, E.; VALENTINE, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. **Journal of Laboratory Clinical Medicine**, v. 70, p. 158, 1967.
17. FICK, K. R. et al. **Métodos de análises de minerais em tecidos de animais e de plantas**. Gainesville: Universidade da Florida, 1980. 79 p.
18. KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. **Clinical biochemistry of domestic animals**. 5. ed. San Diego: Academic Press, 1997. 932 p.
19. MUFARREGE, D. J. El fósforo y la sal en la alimentación de vacunos de carne. In: ENCONTRO ANUAL SOBRE NUTRIÇÃO DE RUMINANTES DA UFRGS, 1., 1999, São Gabriel. **Anais...** Porto Alegre: Gráfica da UFRGS, 1999. p. 111-142.
20. TIMM, C. D. Carências Minerais. In: RIET-CORRÊA, F.; SCHILD, A. L.; MENDEZ, M. D. C.; LEMOS, R. A. A. **Doenças de ruminantes e eqüinos**. 2. ed. São Paulo: Varela, 2001. p. 301-312.
21. GONZÁLEZ, F. H. D. et al. Variações sanguíneas de uréia, creatinina, albumina e fósforo em bovinos de corte no Rio Grande do Sul. **Hora Veterinária**, v. 20, p. 59-62, 2000.
22. LISBÔA, J. A. N. et al. Resultados de patologia clínica e dosagens de elementos minerais em bovinos acometidos pelo botulismo epizoótico no estado de São Paulo. **Pesquisa Veterinária Brasileira**, v. 16, p. 91-97, 1996.
23. NATIONAL RESEARCH COUNCIL **Nutrient requirements of beef cattle**. 7. ed. Washington, D.C.: National Academy Press, 1996. 242 p.
24. GIÓVINE, N. Estudo clínico da deficiência de fósforo nos bovinos de Minas Gerais. **Arquivos da Escola Superior de Veterinária**, v. 1, p. 17-23, 1943.
25. GONZÁLEZ, F. H. D. Indicadores sanguíneos do metabolismo mineral em ruminantes. In: GONZÁLEZ, F. H. D.; BARCELLOS, J.; PATIÑO, H. O.; RIBEIRO, L. A. (Eds.) **Perfil metabólico em ruminantes – seu uso em nutrição e doenças nutricionais**. Porto Alegre: Gráfica da UFRGS, 2000. p. 31-51.
26. MORAIS, M. G. et al. Variação sazonal de eletrólitos no sangue de vacas aneladas sob pastejo contínuo de *Brachiaria decumbens*. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 52, p. 105-110, 2000.
27. MORAES, S. S. et al. **Avaliação da deficiência subclínica de zinco em vacas de cria e a relação com a hígidez de seus bezerras**. Embrapa - Gado de Corte, 2001. 10 p. Comunicado Técnico nº 65.
28. WITTEWER, F. et al. Concentraciones de zinc y cobre en forrajes y suero sanguíneo de 40 predios lecheros de la X Region-Chile. **Archivos de Medicina Veterinária**, v. 20, p. 118-125, 1988.
29. RIET-CORRÊA, F. Deficiência de Cobre. In: RIET-CORRÊA, F.; SCHILD, A. L.; MENDEZ, M. D. C.; LEMOS, R. A. A. **Doenças de ruminantes e eqüinos**. 2. ed. São Paulo: Varela, 2001. p. 312-320.
30. UNDERWOOD, E. J.; SUTTLE, N. F. **Mineral nutrition of livestock**. 3. ed. Edinburgh: CAB International, 1999. 624 p.
31. WITTEWER, F. Estrés oxidativo y selenio en bovinos. In: GONZÁLEZ, F. H. D.; OSPINA, H. P.; BARCELLOS, J. O. (Eds.). **Nutrição mineral em ruminantes**. 2ª ed. Porto Alegre: Gráfica da UFRGS, 1998. p. 111-131.
32. GONZÁLEZ, F. H. D.; DIAS, M. M.; RICCÓ, D. Taxa de gestação e perfil metabólico em novilhas tratadas ou não com fósforo mais vitamina B12 e iodo na temporada de monta em Mostardas, RS, Brasil. In: CONGRESSO ESTADUAL DE MEDICINA VETERINÁRIA, 14., 1999, Gramado. **Resumos...** Gramado: Sociedade Veterinária do Rio Grande do Sul, 1999. p. 114.
33. CONTRERAS, P. A. et al. Valores sanguíneos de triyodotironina y tiroxina en vacas Frisón Negro a pastoreo. **Archivos de Medicina Veterinaria**, v. 31, p. 205-210, 1999.
34. DAYRELL, M. S. **Teores de minerais no tecido animal, plantas e solos do Brasil**. Coronel Pacheco: Embrapa-CNPGL, 1986. 37 p. Documentos, nº 24.
35. URPHY, G. M.; CONNELL, Q. D. A. A simple method of collecting saliva to determine the sodium status of cattle and sheep. **Australian Veterinary Journal**, v. 46, p. 595-598, 1970.