PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 43:e07137, 2023
DOI: 10.1590/1678-5150-PVB-7137

Original Article
Livestock Diseases

Micromineral concentrations (copper, cobalt, iron, molybdenum and zinc) in the liver of dairy cows from Campos Gerais Region, Paraná state, Brazil[1]

Kialane C.A. Pagno[2], José D. Barbosa[3], Felipe M. Salvarani[3], Henrique A. Bomjardim[4], Kelson C.F. Faial[5], Renato S. Sousa[2], Aldo Gava[6], João H. Perotta[2] and Ivan R. Barros Filho[2]*

ABSTRACT.- Pagno K.C.A., Barbosa J.D., Salvarani F.M., Bomjardim H.A., Faial K.C.F., Souza R.S., Gava A., Perotta J.H. & Barros Filho I.R. 2023. **Micromineral concentrations (copper, cobalt, iron, molybdenum and zinc) in the liver of dairy cows from Campos Gerais Region, Paraná state, Brazil.** *Pesquisa Veterinária Brasileira 43:e07137, 2023*. Setor de Medicina Veterinária, Universidade Federal do Paraná, Rua dos Funcionários 1540, Curitiba, PR 80035-050, Brazil. E-mail: ivanbarf@ufpr.br

This study collected samples from 50 Holstein cows, most intensively bred, and from the Campos Gerais region, Paraná, with an average milk production of 30.21L/day. Samples of the liver, spleen and lymph nodes were collected to determine the levels of copper, cobalt, iron, molybdenum and zinc. Spleen and lymph nodes were subjected to histological analysis and evaluation of the degree of hemosiderosis. The average concentrations of copper (495.05ppm), molybdenum (4.19ppm), and zinc (274.49ppm) were higher than those established for the bovines. For cobalt 26% of the animals presented levels below the established level, which characterized cobalt deficiency. Only iron (299.12ppm) exhibited an adequate average level. Histopathologically hemosiderosis was observed mainly in the spleen (78%) and less frequently in the lymph nodes (20%). The observation of hemosiderin in the spleen and lymph nodes is not related to copper deficiency. Still it may be related to high levels of molybdenum, zinc, iron, or other undetermined causes.

INDEX TERMS: Cattle, mineral deficiency, liver, microminerals, hemosiderosis, Brazil.

RESUMO.- [**Concentrações de microminerais (cobre, cobalto, ferro, molibdênio e zinco) no fígado de vacas leiteiras da Região de Campos Gerais, Paraná, Brasil.**] Para este estudo foram coletadas amostras de 50 vacas, da raça Holandesa, a maior parte criada intensivamente e oriunda da região dos Campos Gerais/PR, com média individual de produção de leite de 30,21L/dia. Foram coletadas amostras de fígado, baço e linfonodos para determinação dos níveis de cobre (Cu), cobalto (Co), ferro (Fe), molibdênio (Mo) e zinco (Zn). Baço e linfonodo foram submetidos à análise histológica e avaliação do grau de hemossiderose. A média das concentrações de Cu (495,05ppm), Mo (4,19ppm) e Zn (274,49ppm) encontrava-se acima dos níveis estabelecidos para bovinos. Para o Co, observou-se que 26% dos animais apresentaram níveis abaixo do estabelecido, o que caracterizou deficiência de Co. Apenas o Fe (299,12ppm) apresentou níveis médios adequados. Na histopatologia, hemossiderose foi observada, principalmente no baço (78%) e com menos frequência nos linfonodos (20%). A observação de hemossiderina no baço e linfonodos não está relacionada à deficiência de Cu, porém pode estar relacionada a elevados níveis de Mo, Zn e Fe ou a outras causas não determinadas.

TERMOS DE INDEXAÇÃO: Bovinos, deficiência mineral, microminerais, fígado, hemossiderose, Brasil.

[1] Received on July 22, 2022.
Accepted for publication on August 8, 2022.

[2] Departamento de Medicina Veterinária, Universidade Federal do Paraná (UFPR), Rua dos Funcionários 1540, Curitiba, PR 80035-050, Brazil. *Corresponding author: ivanbarf@ufpr.br

[3] Instituto de Medicina Veterinária, Universidade Federal do Pará (UFPA), Campus Castanhal, BR-316 Km 61, Saudade II, Castanhal, PA 68740-970, Brazil.

[4] Instituto de Estudos do Trópico Úmido (IETU), Universidade Federal do Sul e Sudeste do Pará (Unifesspa), Xinguara, PA 68557-335, Brazil.

[5] Laboratório de Toxicologia, Setor de Meio Ambiente, Instituto Evandro Chagas, BR-316 Km 7, Levilândia, Ananideua, PA 67000-000, Brazil.

[6] Departamento de Medicina Veterinária, Universidade do Estado de Santa Catarina (UESC), Av. Madre Benvenuta 2007, Florianópolis, SC 88035-901, Brazil.

INTRODUCTION

Few studies have been conducted on mineral imbalances in dairy cows in Brazil, although it is essential for selective mineral supplementation in specialized herds. Many factors affect mineral requirements, such as production level, age, interrelationship with other nutrients, level and chemical formula of minerals, breed, animal intake, and adaptation (Underwood & Suttle 1999, Peixoto et al. 2005). In areas where the requirement for a specific mineral is high, commercial mineral mixes may not meet animals' needs, as they are formulated for general situations (Peixoto et al. 2005). Minerals play essential roles in the animal's body, such as participating in the form of structural components of body tissues (calcium, phosphorus), acting on tissues and body fluids as electrolytes to maintain the acid-base balance, osmotic pressure, and cell membrane permeability (calcium, phosphorus, sodium, chlorine); activators of enzymatic processes (copper, manganese); components of metal-enzyme structures (zinc, manganese); and vitamins (cobalt) (Underwood & Suttle 1999, Tokarnia et al. 2000, 2010). Diets with insufficient amounts of minerals or unbalanced rations that result in a deficiency of one or more elements should be corrected so that the animals that consume them can develop their genetic potential and remain healthy (Tokarnia et al. 2000, Peixoto et al. 2005).

In many parts of the world, ruminants ingest insufficient amounts of minerals because foods are often poor in specific elements or contain them in unbalanced proportions to each other (Tokarnia et al. 2000, 2010, Valle et al. 2003, Peixoto et al. 2005, Marques et al. 2013, Dermauw et al. 2014). Brazil ranks sixth in the world for milk production. The Southeast of the country concentrates milk production by 35.9%, the south by 33.2%, and the midwest by 14.9%. Paraná (PR) ranks third in milk production in Brazil, with 3,968,506L/day. Regarding dairy farming, the Campos Gerais/PR region Castro/PR is the municipality with the highest milk production in the country, with 226,800L. Carambeí/PR is in fifth place with 129,600L. Regarding productivity, Castro, Arapoti, and Carambeí occupy the second, third, and fifth national places, respectively (IBGE 2020). Therefore, the objective of the present study was to evaluate the micromineral reserves of dairy cows in municipalities in Paraná.

MATERIALS AND METHODS

Animals and sample collection sites. Samples of the liver, spleen, and lymph nodes from 50 Holstein cows, black and white, mainly from the Campos Gerais/PR region, with an average milk production of 30.21L/day, were collected. Most of the animals (86%) were kept in confinement systems, and the diet consisted of rations, corn silage, corn meal, cottonseed hulls, pre-dried ryegrass, and mineral salt. The remaining cows (14%) were kept in semi-confinement and were given the same diet plus a small amount of pasture. The collection was carried out in a slaughterhouse in the municipality of Araucária/PR, and 25 properties were obtained from the cities of Carambeí, Castro, Arapoti, and Santo Antônio da Platina, all from the state of Paraná. Samples were collected from 49 animals in the slaughterhouse and from one animal during necropsy (Table 1). The animals were transported directly from their city of origin to the slaughterhouse and were slaughtered for consumption. In the case of animals in which necropsy was performed, the procedure was performed at the farm of origin. Data on the cause of the disposal of the animals slaughtered in the slaughterhouse were obtained from the owners. Since the animals were sent for slaughter by the owners, this work was exempt from the need to issue an opinion from the animal experimentation ethics committee for working with samples of slaughterhouse materials.

Sample collection. Liver, spleen, and lymph node samples were collected at the slaughterhouse, at the slaughter line, and during the necropsy. Approximately 300g of liver fragments were collected and stored in plastic bags, identified individually, and frozen at -20°C. Approximately 100g of spleen fragments were collected, stored in containers containing 10% buffered formalin, and identified individually. Approximately two mesenteric lymph nodes were collected from each animal, stored in containers containing 10% buffered formalin, and identified individually.

Determination of mineral concentrations in the liver. Biochemical assays in the liver for copper, cobalt, molybdenum, iron, and zinc were carried out at the "Instituto Evandro Chagas" (IEC), Pará. Liver samples were prepared by cutting the liver into thin slices with clean and stainless-steel razor blades, stored in 50mL Falcon tubes, and subjected to lyophilization. For the lyophilization process, the frozen liver samples at -20°C were placed in trays and then in an automated LIOTOP® device (model L101), previously connected for 10 min, at a temperature of 55°C. They remained for 12 hours for the complete drying of the samples. They were then ground in a mortar and pestle. The ground liver samples were weighed between 0.25 and 0.26g and placed in a Teflon digestion tube (model Xpress) to determine minerals (copper, cobalt, iron, molybdenum, and zinc). Then, 3mL of 65% nitric acid P. A. (QUIMEX), 1mL of 30% hydrochloric acid (MERCK), and 1mL of 30% hydrogen peroxide P. A. (BIOTEC) were added. The samples were left to rest for two hours before being pre-digested and then placed for final digestion for 50 min in a closed system using microwave radiation (MARSXpress, CEM Corp. Matthews, NC, USA). After the digestion of the minerals, the samples were analyzed by optical emission spectrometry with inductively coupled plasma (ICP OES), in the ICP-OES equipment (Vista-MPX simultaneous, axial VARIAN), in an automatic sampling system (SPS-5). The operational conditions of the ICP-OES were controlled using the ICPExpert Vista software. The analytical blanks were prepared using the same procedures without adding liver samples (adapted from the techniques of Nomura et al. 2005). The following contents were considered excessive, adequate, sub-deficient, or deficient based on the data obtained by Underwood (1977) and used by Tokarnia et al. (2010) to interpret the analytical values of the hepatic tissue. These contents are, for copper: 0-50ppm indicate a deficiency, 51-100ppm sub-deficiency, from 101-300ppm adequate index and 300ppm in excess; cobalt: up to 0.05ppm indicate a deficiency, 0.06-0.12ppm, sub-deficiency and from 0.13ppm adequate index; iron: normal values of 180-380ppm; molybdenum: normal values of 2-4ppm; zinc: normal values of 100-200ppm. All parameters were subjected to the Kolmogorov-Smirnov normality test and classified as Gaussian.

Table 1. Number of samples collected by municipality in Campos Gerais, Paraná State, Brazil

Municipality	Liver	Lymph nodes	Spleen
Arapoti	20	20	20
Carambeí	13	13	13
Castro	13	13	13
Santo Antônio da Platina	4	4	4
TOTAL SAMPLES	50	50	50

Means and standard deviations were determined descriptively. The reference extensions obey two standard deviations relative to the average. The parameters were analyzed using the t-test. The level of significance was set at $p<0.05$. All analyses were performed using BioEstat 5.0, a computer program (Ayres et al. 2007).

RESULTS

The causes of discarding and the number of animals studied are shown in Table 2. The average values of copper, cobalt, iron, molybdenum, and zinc in liver tissue are presented in Table 3. From the analysis of the liver tissue of the dairy cows, it was found that there was a deficiency of cobalt in 24% (12/50) and iron in 16% (8/50) of the animals. High levels of copper, molybdenum, zinc and iron were observed in 86% (43/50), 60% (30/50), 44% (22/50), and 7% (14/50) of animals, respectively. The quantity and percentage of deficient, sub-deficient, adequate, and excessive minerals in liver tissue are presented in Table 4. The degree of hemosiderosis in the spleen and lymph node samples is shown in Table 5.

DISCUSSION

Considering that the detection limit was 0.003ppm and that, according to Underwood (1977), the critical level of cobalt is 0.05ppm, it was confirmed that the samples not detected by the methodology were deficient in this element. Of the 50 liver samples studied, 22% (11/50) did not have a reading of cobalt in the methodology employed. The average level of cobalt detected by the methodology was 0.47±0.09ppm, a value considered adequate. However, considering the levels that were not detected by the methodology, which are known to be lower than 0.05ppm, 24% (12/50) of samples had values lower than 0.05ppm, only 2% (1/50) had values of 0.06ppm, and 37 74% (37/50) had values above 0.13ppm, which, according to Underwood (1977) and Tokarnia et al. (2010), represent a deficiency, sub-deficiency, and adequate values, respectively. Therefore, if deficient and sub-deficient levels were added, we observed that 26% (13/50) of animals had low cobalt reserves in the organism. A similar scenario was described by Moraes et al. (1999) in a survey of microelement imbalance in cattle and sheep in various regions of Brazil, such as in the states of Santa Catarina (SC) and Rio Grande do Sul (RS), where they obtained an average cobalt level of 0.3±0.1ppm in samples of liver tissue from 21 cattle, with one (4.8%) animal showing deficiency, seven (33.3%) sub-deficiency and 13 (61.9%) normal levels. Dermauw et al. (2014) reported normal levels of minerals (0.47ppm) in the beef cattle liver in Ethiopia. Still, in SC, Tokarnia et al. (1989) described cobalt sub-deficiency in four out of six liver samples of beef cattle, with an average of 0.12ppm.

Animals with cobalt deficiency may not present clinical signs for weeks or even months until the cobalt reserves in the organism are exhausted (Tokarnia et al. 2010). The main clinical sign is loss of appetite, which can be followed by death from starvation, and is caused by the inability to metabolize propionic acid (Riet-Correa 2007). In addition, animals show deficient growth, reduced immunity, rapid loss of muscle mass, dried feces and begin to consume tree bark or pieces of wood, an act called allopathy (Suttle & Jones 1989, Tokarnia et al. 1999, Tokarnia et al. 2010). In more severe cases, patients develop anemia and fatty liver disease. In milder cases, only signs of underdevelopment, low production, and infertility can be observed (Underwood & Suttle 1999). In this study, most (60%, 7/12) of the co-deficient animals had reproductive problems, leading to infertility and consequently reduced milk production, a loss of the milk production system, and the animal being discarded. The average copper level in the liver had an average of 495.05±135.802ppm, which, according to Underwood (1977), is considered an excess value. These values differed from Enjalbert et al. (2006), who studied hepatic samples from 332 dairy cows from France

Table 2. Causes of culling and number of cows in Campos Gerais, Paraná State, Brazil

Causes of culling	Amount	%
Reproductive disorders	24	48
Mastitis	14	28
Hoof problems	4	8
Fracture	3	6
Heart disease	2	4
Abomasal volvulus	2	4
Senility	1	2
TOTAL	50	100

Table 3. Means and standard deviations of copper, cobalt, iron, molybdenum and zinc in the liver of cows from Campos Gerais, Paraná State, Brazil

Mineral (ppm)	Mean (ppm)	Standard deviation (ppm)
Copper	495.05	135.80
Cobalt	0.47	0.09
Iron	299.12	290.82
Molybdenum	4.19	1.01
Zinc	274.49	80.80

Table 4. Number and percentage of animal's deficiency, deficiency sub, adequate and excess of copper, cobalt, iron, molybdenum and zinc in the liver of cows from Campos Gerais, Paraná State, Brazil

Mineral	Deficiency		Deficiency sub		Adequate		Excess	
	Number	%	Number	%	Number	%	Number	%
Copper	-	-	-	-	7	14	43	86
Cobalt	12	24	1	2	37	74	-	-
Iron	8	16	-	-	35	70	7	14
Molybdenum	-	-	-	-	20	40	30	60
Zinc	1	2	-	-	27	54	22	44

Table 5. Degree of hemosiderosis in the spleen and lymph nodes of dairy cows from Campos Gerais, Paraná State, Brazil

Degree of injury	Spleen	Number of samples/organs		%
		%	Lymph node	
-	11	22	40	80
(+)	9	18	3	6
+	7	14	3	6
+ +	18	36	3	6
+ + +	5	10	1	2

- Absence, (+) discret, + mild, ++ moderate, +++ severe.

and Belgium. Marques et al. (2003) obtained mean values below the parameters (3.6±1.6ppm) of hepatic copper for grazing beef cattle. Tokarnia et al. (1999) reviewed mineral deficiency studies conducted in Brazil from 1987 to 1998 and obtained deficient values for the mineral in 27 (17.31%) and sub-deficient values in 30 samples (19.23%) of the liver, for a total of 156 collected.

The average level of zinc found was 274.49±80.80ppm, which was considered excess. This result agrees with Moraes (1998), who determined an average of 279.61±167.02ppm for the mineral in the state of SC in liver samples. Valle et al. (2003) studied cattle in RS and obtained adequate values for the mineral. Enjalbert et al. (2006), in a retrospective study of French dairy cows, determined the same deficiency in liver samples. Similar to the results obtained in this study, cattle with excessive copper and zinc in liver tissue were described by López-Alonso et al. (2005) in Spain. According to the authors, copper and zinc compete for binding to metallothionein (MT) protein, which is necessary for the excretion of copper through bile. Since zinc has a higher affinity for MT, in case of an excess of the two minerals, zinc would bind to MT, initiating the accumulation of copper in the organ because of an excess of both minerals. Animals that receive dietary supplements that lead to excessive copper in the liver, even slightly above normal levels, have adverse effects on performance, with reduced food intake and daily weight gain (Engle & Spears 2000). In this study, animals with excessive copper and zinc were discarded, for the most part, from production systems because of reproductive problems and mastitis, which may be related to low-calorie intake, weight gain, and immunity.

For the mineral molybdenum, the average value observed was 4.19±1.01ppm, slightly higher than the level recommended by Underwood (1977) and Tokarnia et al. (2010). Excessive levels were obtained by Marques et al. (2003) in RS, with an average of 6.5±1.3ppm. Dermauw et al. (2014) verified adequate values (average of 3.8ppm) for beef cattle in Ethiopia. Molybdenum and sulfur have been identified as the most important copper antagonists (Gooneratne et al. 1989, Underwood & Suttle 1999, Rosa & Mattioli 2002, Picco et al. 2012). A slight change in the concentration of molybdenum and S in forage, which naturally occurs in foods, can cause a change in the absorption, distribution, and excretion of copper, leading to clinical signs of deficiency or intoxication (Gooneratne et al. 1989, Henry & Miles 2000, Picco et al. 2012, Marques et al. 2013). In the present study, despite the slight increase in molybdenum, the antagonistic effect was not observed, leading to copper deficiency, which could be directly related to the data on excess zinc, which, according to Engle & Spears (2000), has a greater affinity for MT, which is necessary for the excretion of copper, determining excess copper in the organism.

In determining iron in the liver, an average of 299.12 ± 290.82 ppm was observed, which is considered adequate for the mineral. A similar average (273.66±68.94ppm) was obtained by Moraes (1998) in an evaluation of iron concentrations in the liver of cattle in SC. In studies in the same state, Moraes et al. (1999) verified excessive mineral levels (1111.13±715.80ppm). Hemosiderin is a granular brown pigment that contains iron and is usually found in macrophages. It is commonly found in the liver, spleen, lymph nodes, bone marrow, and any site of hemorrhage. If it is sufficient to constitute a lesion, the term hemosiderosis is used (Thomson 1983, Cheville 2004).

Histopathologically marked hemosiderosis was observed, mainly in the spleen (Fig.1) and less frequently in the lymph nodes. Of the 50 samples studied, 39 (78%) were in the spleen, and 10 (20%) were in the lymph nodes of the lesion. Only eight animals did not present with simultaneous hemosiderosis in the spleen and lymph nodes. Tokarnia & Dobereiner (1998) also evaluated the degree of hemosiderosis in cattle affected by "ronca", an obscure disease in cattle with noisy breathing. In this study, hemosiderin was found in the spleen in 100% of the animals studied and in the lymph nodes in 81.8% of the animals, which was attributed to the deficiency of copper. In the present study, the hemosiderosis identified in the spleen and lymph node is not related to the deficiency of copper. Still, it may be associated with the excesses of molybdenum, zinc, and iron detected in the animals or even with other factors not clarified by this study, indicating the need, as a perspective, for new research to elucidate this limiting point.

CONCLUSIONS

Among dairy cows from the Campos Gerais region of Paraná, 86% (43/50), 14% (7/50), 60% (30/50), and 44% (22/50) had excess copper, iron, molybdenum, and zinc, respectively. Cobalt deficiency was detected in 24% (12/50), iron in 16% (8/50), and zinc in 14% (1/50) of the animals.

The observation of hemosiderin in the spleen and lymph nodes is not related to copper deficiency. Still, it is believed to be related to excess molybdenum, zinc, iron, or other causes not determined in the present experiment.

Acknowledgments.- The authors thank the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) Finance Code 001, the "Instituto Evandro Chagas" (IEC), and "Pró-Reitoria de Pesquisa e Pós-Graduação" of the "Universidade Federal do Pará" (PROPESP-UFPA) for financing the publication of this article via the Qualified Publication Support Program (PAPQ/2022) call for proposals.

Conflict of interest statement.- The authors have no conflicting interests.

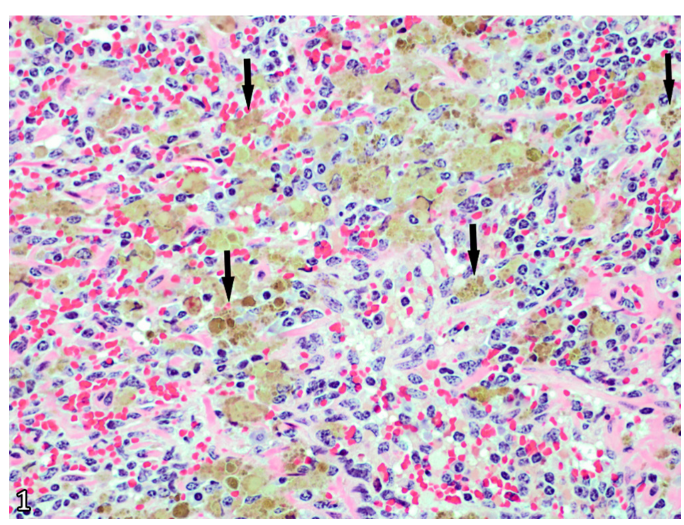

Fig.1. Spleen hemosiderosis. Spleen: macrophages with large amounts of hemosiderin with multifocal distribution. HE, obj.40x.

REFERENCES

Ayres M., Ayres Jr M., Ayres D.L. & Santos A.A.S. 2007. BioEstat: aplicações estatísticas nas áreas das Ciências Bio-médicas. Versão 5.0. Sociedade Civil Mamirauá, Belém, Pará. 324p.

Cheville N.F. 2004. Introdução à Patologia Veterinária. 2ª ed. Roca, São Paulo, p.334.

Dermauw V., Alonso M.L., Duchateau L., Laing G.D., Tolosa T., Dierenfeld E., Clauss M. & Janssens G.P.J. 2014. Trace element distribution in selected edible tissues of zebu (*Bos indicus*) cattle slaughtered at Jimma, SW Ethiopia. PLoS One 9(1):e85300. <https://dx.doi.org/10.1371/journal.pone.0085300> <PMid:24465529>

Engle T.E. & Spears J.W. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78(9):2446-2451. <https://dx.doi.org/10.2527/2000.7892446x> <PMid:10985420>

Enjalbert F., Lebreton P. & Salat O. 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. J. Anim. Physiol. Anim. Nutr. 90(11/12):459-466. <https://dx.doi.org/10.1111/j.1439-0396.2006.00627.x> <PMid:17083426>

Gooneratne S.R., Buckley W.T. & Christensen D.A. 1989. Rewiew of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. 69:819-845. <https://dx.doi.org/10.4141/cjas89-096>

Henry P.R. & Miles R.D. 2000. Interactions among trace minerals. Ciênc. Anim. Bras. 1(2):95-106.

IBGE 2020. Pesquisa da Pecuária Municipal. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, RJ.

López-Alonso M., Prieto F., Miranda M., Castillo C., Hernández J. & Benedito J.L. 2005. The role of metallothionein and zinc in hepatic copper accumulation in cattle. Vet. J. 169(2):262-267. <https://dx.doi.org/10.1016/j.tvjl.2004.01.019> <PMid:15727919>

Marques A.P., Riet-Correa F., Soares M.P., Ortolani E.L. & Giuliodori M.J. 2003. Mortes súbitas em bovinos associadas à carência de cobre. Pesq. Vet. Bras. 23(1):21-32. <https://dx.doi.org/10.1590/S0100-736X2003000100005>

Marques A.P.L., Botteon R.C.C.M., Amorim E.B. & Botteon P.T.L. 2013. Deficiência de cobre condicionada a altos teores de zinco, manganês e ferro na região do Médio Paraíba, RJ, Brasil. Semina, Ciênc. Agrárias 34(3):1293-1300.

Moraes S.S. 1998. Avaliação das concentrações de zinco, manganês e ferro no fígado de bovinos e ovinos de várias regiões do Brasil. Pesq. Vet. Bras. 18(3/4):107-110. <https://dx.doi.org/10.1590/S0100-736X1998000300003>

Moraes S.S., Tokarnia C.H. & Döbereiner J. 1999. Deficiências de microelementos em bovinos e ovinos em algumas regiões do Brasil. Pesq. Vet. Bras. 19(1):19-33. <https://dx.doi.org/10.1590/S0100-736X1999000100004>

Nomura C.S., Silva C.S., Nogueira A.R.A. & Oliveira P.V. 2005. Bovine liver sample preparation and micro-homogeneity study for Cu and Zn determination by solid sampling electrothermal atomic absorption spectrometry. Spectrochim. Acta Part B, Atomic Spectroscopy. 60(5):673-680. <https://dx.doi.org/10.1016/j.sab.2005.02.021>

Peixoto P.V., Malafaia P., Barbosa J.D. & Tokarnia C.J. 2005. Princípios de suplementação mineral em ruminantes. Pesq. Vet. Bras. 25(3):195-200. <https://dx.doi.org/10.1590/S0100-736X2005000300011>

Picco S., Ponzzinibio M.V., Mattioli G., Rosa D., Minatel L., Fazzio L. & Seoane A. 2012. Physiological and genotoxic effects of molybdenum-induced copper deficiency in cattle. Agrociencia 46(2):107-117.

Riet-Correa F. 2007. Deficiência de cobre, p.239-248. In: Riet-Correa F., Schild A.L., Lemos R.A.A. & Borges J.R.J. (Eds), Doenças de Ruminantes e Eqüídeos. Vol.2. Pallotti, Santa Maria.

Rosa D.E. & Mattioli G.A. 2002. Metabolismo y deficiência de cobre en los bovinos. Analecta Vet. 22(1):7-16.

Suttle N.F. & Jones D.G. 1989. Recent developments in trace element metabolism and function: trace elements, disease resistence and immune responsiveness in ruminants. J. Nutr. 119(7):1055-1061. <https://dx.doi.org/10.1093/jn/119.7.1055> <PMid:2666601>

Thomson R.G. 1983. Patologia Geral Veterinária. Guanabara Koogan, Rio de janeiro, p.412.

Tokarnia C.H. & Dobereiner J. 1998. Sobre o "ronca", doença de etiologia obscura em bovinos, caracterizada por respiração ruidosa. Pesq. Vet. Bras. 18(3/4):93-98. <https://dx.doi.org/10.1590/S0100-736X1998000300001>

Tokarnia C.H., Dobereiner J. & Peixoto P.V. 2000. Deficiências minerais em animais de fazenda. Pesq. Vet. Bras. 20(3):91-138. <https://dx.doi.org/10.1590/S0100-736X2000000300007>

Tokarnia C.H., Dobereiner J., Moraes S.S. & Peixoto P.V. 1999. Deficiências e desequilíbrios minerais em bovinos e ovinos. Revisão dos estudos realizados no Brasil de 1987 a 1998. Pesq. Vet. Bras. 19(2):47-62. <https://dx.doi.org/10.1590/S0100-736X1999000200001>

Tokarnia C.H., Gava A., Peixoto P.V., Stolf L. & Moraes S.S. 1989. A "doença do peito inchado" (edema na região esternal) em bovinos no Estado de Santa Catarina. Pesq. Vet. Bras. 9(3/4):73-83.

Tokarnia C.H., Peixoto P.V., Barbosa J.D., Brito M.F. & Döbereiner J. 2010. Deficiências Minerais em Animais de Produção. Helianthus, Rio de Janeiro. 200p.

Underwood E.J. & Suttle N.F. 1999. The Mineral Nutrition of Livestock. 3th ed. CABI Publishing, London, p.614.

Underwood E.J. 1977. Trace Elements in Human and Animals Nutrition. 4th ed. Academic Press, New York, p.545.

Valle S.F., González F.D., Roch D., Scalzilli H.B., Campo R. & Larosa V.L. 2003. Mineral deficiencies in beef cattle from Southern Brazil. Braz. J. Vet. Res. Anim. Sci. 40(Supl.1):47-53. <https://dx.doi.org/10.1590/S1413-95962003000700007>