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A cross-sectional study of serological levels of minerals in lactating dairy cows of Paraná, Brazil

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Abstract: Serum macrominerals calcium (Ca), phosphorus (P), and magnesium (Mg) and microminerals copper (Cu), iron (Fe), and zinc (Zn) were measured in lactating Holstein, Brown Swiss, Jersey and zebu cows managed under pasture-based and confinement-based systems in the state of Paraná, Southern Brazil. The cross-sectional study was performed with 323 lactating cows, aged ≥2 years, from 34 dairy herds of Paraná State. Cows were classified into two groups: Group 1 (G1, n=208) consisted of cows raised in confinement systems, while Group 2 (G2, n=115) included cows from pasture-based herds supplemented at feed troughs. Biochemical analyses were performed by spectrophotometry using commercial kits. The overall mean values (n=323) for each blood parameter were: Ca = 8.1 ± 0.9 mg/dL; P = 6.1 ± 1.2 mg/dL; Mg = 2.3 ± 0.4 mg/dL; Cu = 83.6 ± 18.8 µg/dL; Zn = 94.0 ± 23.4 µg/dL; and Fe = 129.2 ± 44.2 µg/dL. The most prevalent deficiency was found for Ca, whereas the macromineral with the lowest prevalence of deficiency was P. Most dairy cows evaluated were not at risk of Cu intoxication. Cows from the G2 group had lower ($p<0.001$) mean serum minerals and a higher number of cows considered deficient than G1, except for Mg ($p=0.202$). The conclusion is that dairy cows in Paraná State are at risk for mineral deficiencies, mainly cows from pasture-based herds. This emphasizes the need for diagnostic monitoring to identify affected cattle and herds, thereby optimizing animal health and production.
Keywords: mineral deficiency, cattle, pasture, confinement.

1. Introduction

Minerals perform diverse roles in animal physiology, including bone tissue formation, membrane stabilization, DNA transcription, cell cycle control, nerve impulse generation, energy metabolism, muscle contraction, and antioxidant processes. Insufficient availability of one or more minerals can lead to various clinical conditions, and depending on the mineral and duration of deficiency, it may even result in bovine mortality (Goff, 2018; Ribeiro et al., 2019; Ensley, 2020; Weiss and Hansen, 2024).

Deficiencies in essential minerals such as phosphorus (P), copper (Cu), calcium (Ca), zinc (Zn), and magnesium (Mg) have been observed in Brazil (Tokarnia et al., 2010; Fiorentin et al., 2018; Silva et al., 2020; Malafaia et al., 2023). Excessive mineral intake is primarily clinically relevant due to its inhibitory effects on other minerals, with natural intoxications being rare except for Cu (Byrne and Murphy, 2022).

While tissue-specific measurements or mineral-dependent enzyme assays are ideal for diagnosing mineral deficiencies, practical limitations arise when multiple deficiencies are suspected (Ensley, 2020). In such cases, serum mineral levels serve as a valuable indicator of the current mineral status in cattle (Macmillan et al., 2020).

Despite its significance in dairy cattle farming, the mineral status of bovine herds in Paraná State remains unknown, particularly regarding regional variations and specific minerals such as Ca (Frigotto et al., 2009) or lameness (Barbosa et al., 2016). In the literature, we did not find any studies related to deficiencies or excesses of minerals that cover various regions and dairy herds in the state of Paraná. Regional surveys, as emphasized by Malafaia et al. (2014), are essential for determining the actual need for mineral supplementation. Therefore, the objective of this article was to assess the status of macrominerals (Ca, P, and Mg) and microminerals (Fe, Cu, and Zn) in the blood serum of lactating dairy cows managed under confinement-based systems with known mineral supplementation and under pasture-based systems with unknown mineral supplementation in the State of Paraná, Southern Brazil.

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2. Materials and Methods

A total of 323 lactating cows (≥4 weeks postpartum with unknown lactation number) from Holstein, Brown Swiss, Jersey, and zebu cattle breeds, aged ≥2 years, were sampled from 34 dairy herds across 18 counties in the Central-Eastern, North, Northwest, and metropolitan region of Curitiba city, the capital of Paraná State. The study area spanned longitudes –49.0653 to –50.8506 and latitudes –23.0819 to –25.9202, with altitudes ranging from 467 to 1054 meters. Cows were selected for convenience and ease in obtaining blood samples, ensuring that at least 5% of the lactating cows from each herd were sampled. No clinical or physical evaluations were performed except for the blood analyses. The herds were subdivided into herds confined or kept on pasture, based on a previous contact with the farmers. During the talk, the adoption or non-adoption of mineral supplementation in the herd was discussed. The study was conducted over seven months, during which data collection took place.

The samples were classified into two groups: Group 1 (G1, n = 208) consisted of cows managed in confinement systems (free-stall herds) with known and daily mineral supplementation. Group 2 (G2, n=115) included cows from pasture-based herds supplemented at feed troughs, with unknown and voluntary mineral supplementation.

Blood samples were collected via coccygeal vessel puncture using mineral-contaminant-free Falcon tubes. After centrifugation at 2500 rpm for 20 minutes, serum was collected and frozen at –20°C until analysis. Biochemical analyses were performed using commercial kits (Randox©, UK) at the Clinical Laboratory of Ruminants of the University of São Paulo in Pirassununga, SP.

Transferrin saturation (TS) was used to interpret Fe results. A minimum TS value of 29% was considered normal (Herdt and Hoff, 2011). Cows with low Fe concentrations and TS below 29% were classified as deficient in this micromineral. In the absence of specific reference values for Paraná State, we established average ranges (minimum and maximum) based on national and international literature. Seven books (Hofmann, 2005; Puls, 1994; Smith, 2009; Kaneko et al., 2008; Díaz González et al., 2000; Díaz González and Silva, 2006; Tokarnia et al., 2010) and two international articles (Gadberry et al., 2003; Herdt and Hoff, 2011) were used to calculate an average value range used as reference for the following measured minerals (Table 1).

Literature	Zn (µg/dL)		Cu (µg/dL)		Fe (µg/dL)		Ca (mg/dL)		P (mg/dL)		Mg (mg/dL)	
(1)	50.0	150.0	50.0	150.0	57.0	162.0	9.7	12.4	5.6	6.5	1.8	2.3
(2)	60.0	190.0	60.0	110.0	110.0	250.0	-	-	-	-	-	-
(3)	80.0	140.0	65.0	150.0	-	-	-	-	-	-	-	-
(4)	80.0	120.0	80.0	120.0	-	-	-	-	-	-	-	-
(5)	70.0	120.0	50.0	120.0	57.0	230.0	7.4	13.0	2.0	9.6	1.8	3.0
(6)	-	-	-	-	100.0	-	7.6	10.5	3.0	6.0	1.9	2.4
(7)	-	-	-	-	-	-	-	-	3.5	-	1.8	3.2
(8)	80.0	140.0	60.0	150.0	100.0	200.0	8.0	11.0	4.5	7.0	1.8	3.5
(9)	-	-	79.4	120.7	-	-	8.0	10.2	4.6	9.3	2.2	3.2
Average	**70.0**	**143.3**	**63.5**	**131.5**	**84.8**	**210.5**	**8.1**	**11.4**	**4.2**	**7.6**	**1.9**	**2.9**

Table 1 – Minimum and maximum reference values for minerals in bovine serum were compiled from the literature and adopted to compose an average value. (1) Kaneko et al. (2008); (2) Herdt & Hoff (2011); (3) Gadberry et al. (2003); (4) Díaz González & Silva (2006); (5) Díaz Gónzalez et al. (2000); (6) Smith (2009); (7) Tokarnia et al. (2010); (8) Puls (1994); (9) Hofmann (2005).

3. Statistical methods

Statistical analyses were performed using GraphPad Prism 6© software to assess serum mineral levels in dairy cows. The normal distribution of data was evaluated using the D'Agostino & Pearson test. For the comparison of Ca, P, Mg, Fe, and Zn levels between groups, the Mann-Whitney test was employed. The non-paired T-test was used to compare Cu levels between groups. To evaluate the percentages of deficient cows in both groups, Fisher's exact test was utilized.

4. Results

In this study, we conducted a comprehensive assessment of serum mineral levels in dairy cows, considering Ca, P, Mg, Cu, Fe, and Zn. Among the sampled cows, 167 individuals (51.7%) exhibited serum Ca levels below the value of 8.1 mg/dL, and the overall mean serum Ca concentration was 8.1 ± 0.9 mg/dL. We identified 17 cows (5.3%) with serum P concentrations below 4.2 mg/dL, and the average serum P level was 6.1 ± 1.2 mg/dL. A total of 47 cows (14.5%) had serum Mg values below 1.9 mg/dL, and the mean serum Mg concentration was 2.3 ± 0.4 mg/dL. Among the sampled cows, 41 individuals (12.7%) exhibited serum Cu levels below 63.5 µg/dL, and the average serum Cu concentration was 83.6 ± 18.8 µg/dL. A total of 49 cows (15.2%) had serum Fe concentrations below 84.8 µg/dL, and the mean serum Fe level was 129.2 ± 44.2 µg/dL. And lastly, among the sampled cows, 49 individuals (15.2%) had serum Zn levels below 70 µg/dL, and the average serum Zn concentration was 94.0 ± 23.4 µg/dL. Detailed results for both G1 and G2 are provided in Table 2, categorized by their respective counties.

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Counties	Number of cows	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	Cu (µg/dL)	Fe (µg/dL)	Zn (µg/dL)
G1							
Arapoti	66	**8.1±1.2**	6.4±1.1	2.2±0.3	89.2±16.2	117.1±39.1	104.6±24.9
Castro	65	**8.0±1.5**	6.1±1.3	2.2±0.5	79.8±21.1	136.4±50.7	95.8±21.2
Carambeí	20	8.3±0.2	7.1±0.1	2.0±0.1	72.9±3.5	134.3±8.2	106.7±2.6
Palmeira	9	8.7±0.6	7.5±0.6	2.3±0.2	77.5±9.1	186.3±21.9	127.6±12.0
Piraquara	31	8.4±0.4	6.0±0.3	2.6±0.1	92.5±6.0	163.9±13.7	86.0±7.0
Pinhais	15	8.4±0.3	5.7±0.3	2.4±0.1	103.3±5.5	162.7±12.1	86.9±6.0
Fazenda Rio Grande	11	8.9±0.1	6.1±0.3	2.3±0.1	100.1±2.0	153.6±4.7	87.0±5.7
G2							
Reserva	37	**7.6±0.6**	4.9±0.6	2.0±0.2	77.1±15.9	100.9±23.7	**68.2±10.6**
Ortigueira	7	**7.9±0.5**	6.3±0.4	**1.9±0.1**	84.0±6.9	99.9±16.5	83.4±8.9
Tomazina	18	**7.5±0.5**	5.4±0.4	2.3±0.1	78.5±6.7	101.8±15.5	88.2±8.2
Wenceslau Braz	10	**7.9±0.4**	5.3±0.4	2.2±0.1	**60.8±6.4**	119.1±14.8	83.8±7.8
Conselheiro Mairinck	3	**7.6±0.3**	6.4±1.2	2.2±0.1	74.8±4.5	**50.7±8.4**	80.1±8.1
Siqueira Campos	3	**7.7±0.6**	6.6±1.0	2.9±0.4	82.9±5.7	**67.5±11.4**	98.8±25.9
Quatiguá	3	**8.0±0.1**	6.4±0.4	2.0±0.2	103.84±1.0	107.2±3.2	88.4±11.2
Paranavaí	8	**8.0±0.2**	6.6±0.3	2.4±0.1	78.6±4.3	129.9±6.7	128.1±6.3
Araucária	4	8.6±0.2	6.4±0.4	2.5±0.2	**61.6±2.0**	141.9±3.4	88.5±9.5
Tijucas do Sul	6	9.0±0.2	7.5±0.4	2.7±0.1	77.9±2.1	127.9±4.5	102.1±7.6
São José dos Pinhais	7	**8.0±0.2**	6.1±0.3	2.5±0.1	96.3±2.0	153.0±4.3	98.8±7.0

Table 2 – Means and standard deviations of serum concentrations of macro- and microminerals of cows belonging to counties in the Paraná State. G1: cows raised in confinement systems with known mineral supplementation, G2: pasture-based dairy systems without known mineral supplementation. Values outside the average established as standard are in bold.

Serum Ca levels were below reference values in 10 out of the 18 counties. Serum Cu and Fe levels were below reference values in two counties each. Serum Mg and Zn levels were below reference values in one County each, and no county showed values below normal for P. A higher number of counties in G2 had average mineral levels below reference values.

Lower Ca values were observed on pasture-based dairy systems (G2) than confinement systems (G1). Mg did not show a significant difference between production systems, neither for serum concentration nor for the percentage of serologically deficient cows within the groups. For P, 4.6% of cows were below the standard of 4.2 mg/dL. G2 had lower mean serum P levels than G1, and the percentage of deficient cows tended (p=0.054) to be higher in G2 than in G1 (2.9% vs. 7.8%). Thirty-four cows (10.5%) were found with P values above the average reference values. The correlations were positive between P and Mg in both production systems.

For Cu, 36 cows (11.1%) had serum values lower than 63.5 µg/dL. G2 presented lower mean serum Cu values, as well as a higher percentage of deficient cows. For Zn, 49 cows (15.2%) were found to have values below 70 µg/dL. G2 had a lower serum average and a higher number of cows considered deficient than G1 (G2: 23.5% vs. G1: 10.5%). Overall, the mean serum mineral concentrations, occurrence of low serological levels, and observed differences between the groups are summarized in Table 3.

Groups	Ca	P	Mg	Cu	Zn
G1 (mg/dL)	8.2 ± 1.0^a	6.2 ± 1.1^a	2.3 ± 0.4^a	86.8 ± 19.2^a	97.4 ± 22.8^a
G2 (mg/dL)	7.8 ± 0.7^b	5.7 ± 1.3^b	2.2 ± 0.3^a	77.9 ± 16.8^b	85.7 ± 23.6^b
p	<0.001	<0.001	0.202	<0.001	<0.001
G1 (% mineral deficient cows)	43.1^a	2.9^a	13.4^a	7.2^a	10.5^a
G2 (% mineral deficient cows)	66.1^b	7.8^b	13.9^a	18.3^b	23.5^b
p	<0.001	0.054	0.869	0.005	0.003

Table 3 – Mean values and standard deviations of serum mineral concentrations and frequency (%) of mineral levels below reference values in dairy cattle (n=323) in the state of Paraná. G1: cows raised in confinement systems with known mineral supplementation, G2: pasture-based dairy systems without known mineral supplementation. Different letters indicate significant differences ($p < 0.05$) between the group's means.

When verifying the occurrence of single or multiple serological deficiencies in individual cows, it was found, depending on the group, between 50.2% (G1) and 83.5% (G2) of cows with some mineral deficiency serologically detected at the moment of analysis. A subset of cows (n=30) in both G1 and G2 had low serum Fe levels concomitant with TS% below 29% (Table 4). Pasture-based cows (G2) showed a lower (p = 0.026) Fe serum average value than cows from confinement-based dairy systems.

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	G1	G2	p	Literature
Fe (µg/dL)	136.9±44.8	115.3±39.7	0.026	(1), (2), (3), (4), (5)
% Fe below 84.8 µg/dL; TS% < 29%	9.6	11.3	0.702	(1), (2), (3), (4), (5)
% Fe below 100 µg/dl e TS% < 29%	13.5	16.5	0.510	(4), (5)
% Fe below 110 µg/dL; TS% < 29%	11.3	16.5	0.340	(2)

Table 4 – Comparison of serum Fe values and the relative number of Fe-deficient cows between groups. (1) Kaneko et al. (2008); (2) Herdt & Hoff (2011); (3) Díaz González et al. (2000); (4) Smith (2009); (5) Puls (1994). G1: cows raised in confinement systems with known mineral supplementation, G2: pasture-based dairy systems without known mineral supplementation.

5. Discussion

The results obtained here reveal that Ca remains the macromineral with the highest prevalence of deficiency. As 22.6% of all analyzed cows had serum calcemia below 7.5 mg/dL, and therefore, they can be considered latent hypocalcemic. The lower serum Ca average in pasture-based systems compared to confinement-based systems may indicate the ingestion of oxalate-rich compounds (Goyal 2018), as observed in degraded pastures, or irregular feeding practices in extensively raised ruminants (Cao et al., 2024). Other factors, such as hypoproteinemia and deficiencies in Mg, can also influence the observed Ca values (Ali and Gomaa, 2016; Fu et al., 2022).

We found that only 5.3% of the cows had low P serum concentrations, categorizing them as hypophosphatemia. Deficiencies in P levels can lead to bone alterations, such as joint enlargement, deviations, and osteomalacia (Schild et al., 2023). The deficiency of P in cows is linked to the concentration of P in forage, which, in turn, depends on the soil's P content. To address this mineral deficiency, soil fertilization with phosphate-based fertilizers is recommended (Malafaia et al., 2023).

Mg exhibited serum values within the average reference range (>1.9 mg/dL) and aligned with those reported by Fadlalla et al. (2020) in Sudan. Excessive potassium (K) fertilization of pastures interferes with Mg absorption from the diet, reducing Mg content in forage. Overconsumption of K can also decrease the activity of the sodium pump responsible for Mg transport and absorption in gastrointestinal cells (Martens et al., 2018).

In the present study, 41 cows (12.7%) had serum values below 63.5 µg/dL of Cu. Pasture-based lactating cows exhibited more than double the prevalence of Cu deficiency compared to confinement-based animals. Serum Cu levels are influenced by absorption capacity, estimated in only 5% when Cu is mainly supplemented as copper sulphate (NASEM, 2021). This bioavailability is also determined by the presence of other minerals, such as sulfur, iron, molybdenum, and zinc (Sinclair et al., 2017; Skalny et al., 2019; Byrne and Murphy, 2022). Additionally, low Cu values should be carefully evaluated, as the coagulation process during serum collection sequesters a significant portion of Cu; therefore, the use of plasma is recommended (Ensley, 2020).

Regarding Zn, among the cows monitored, 15.2% had values below 70 µg/dL. Morales Almaráz et al. (2007) found in their study that 79% of blood serum samples analyzed had less than 80 µg/dL, which coincided with the 92% of deficiencies observed in forage, which can be used to explain the deficiencies found. However, no bromatological analyses were carried out. Once again, pasture-based lactating cows presented more than twice the prevalence of Zn deficiency than confinement-based animals. Forages in alkaline soils (pH above 6.5) and fertilized with high concentrations of nitrogen and phosphorus can cause Zn deficiency in cattle (Alloway, 2009).

Concerning Fe, 30 cows were found to have low serum Fe levels, concurrently with TS indices below 29%. This finding is surprising, given that Brazil is considered a country with soils and pastures abundant in Fe (Tokarnia et al., 2010). Additionally, 49 cows (15.2%) had serum values below 84.8 µg/dL. Chronic blood loss, and consequently Fe deficiency, can occur due to parasitism, especially in cases of intense infections by hematophagous parasites (Guizelini et al., 2023). Although the cows analyzed in the study did not exhibit severe parasitism upon inspection, it cannot be ruled out that parasitism occurred before blood collection. Serum Fe levels can decrease during systemic inflammatory processes (Baydar and Dabak, 2014; Tsukano et al., 2020), and even apparently healthy dairy cows may have some level of systemic inflammation (Bernarski et al., 2019).

These results, however, do not allow for a conclusive determination of the causes of the observed deficiencies. The lower proportion of lactating animals diagnosed as mineral deficient in confinement-based systems, despite their higher milk production, is noteworthy. Their higher milk yield obligates their owners to supplement with concentrate and mineral supplementation, both of which include essential minerals in accordance with NASEM (2021) recommendations, thereby reducing the likelihood of deficiency. This study serves as an initial broader survey in the state of Paraná and a warning for livestock management practices in other Brazilian states.

6. Conclusion

The macromineral with the highest prevalence of deficiency was Ca, while the lowest prevalence of deficiency was P. Iron (Fe) deficiency was also identified in cows raised in Paraná, justifying the inclusion of this mineral in routine investigations. Cows from confined herds with regular mineral supplementation, despite higher lactation demands, exhibited lower deficiencies for the minerals Ca, P, Cu, Fe, and Zn when compared to cows from pasture-based herds without periodic mineral supplementation. Half of the evaluated animals showed mineral deficiencies, emphasizing the need for a routine diagnostic to identify individual affected cows.

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