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SHORT NOTE

Prepartum urea serum concentration and the effect on the mineral metabolism of dairy cows in the immediate postpartum

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KEYWORDS

Ammonia.
Calcium.
Hypocalcemia.
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SUMMARY

The objective of this study was to verify if the urea serum concentrations in prepartum dairy cows influence the mineral metabolism of magnesium (Mg) and calcium (Ca). Twenty-seven multiparous Holstein cows were selected from two properties. Urinary pH measurement was performed, and blood samples were collected 7 days before the expected calving date, and in the immediate postpartum period, blood samples were collected at 24, 48 and 72 hours. In the blood, serum urea, Ca and Mg concentrations were determined. The data were subjected to analysis of variance and correlation and categorized according to the median prepartum urea concentration into high urea group (HUG) and low urea group (LUG). There was positive correlation between prepartum Ca and Mg (r= 0.85; P<0.05), 24 hours postpartum Ca and Mg (r= 0.74; P<0.05) and prepartum Ca and 48 hours Ca (r= 0.62; P<0.05). HUG animals (46.01 mg/dL) showed subclinical hypomagnesemia at 72 hours postpartum (P<0.05). Higher serum urea concentrations in the prepartum, influenced the mineral metabolism of dairy cows in the immediate postpartum, being detected subclinical hypomagnesemia 72 hours after calving.

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Amônia.
Cálcio.
Hipocalcemia.
Magnésio.

Concentração sérica de ureia no pré- parto e o efeito no metabolismo mineral de vacas leiteiras no pós- parto imediato

RESUMO

O objetivo deste estudo foi verificar se as concentrações séricas de ureia no pré-parto de vacas leiteiras, influenciaram no metabolismo mineral do magnésio (Mg) e do cálcio (Ca). Foram selecionadas 27 vacas multíparas da raça Holandês oriunda de duas propriedades. Foi realizada aferição do pH urinário e coleta sanguínea 7 dias antes da data prevista do parto, e no pós-parto imediato coleta de sangue nas 24, 48 e 72 horas. No sangue foi determinado a concentração sérica de ureia, Ca e Mg. Os dados foram submetidos análise de variância e correlação, sendo categorizados de acordo com a mediana da concentração de ureia pré- parto em grupo de ureia alta (GUA) e grupo de ureia baixa (GUB). Houve correlação positiva entre o Ca e Mg pré-parto (r= 0,85; P<0,05), Ca e Mg 24 horas pós-parto (r= 0,74; P<0,05) e Ca pré-parto e Ca 48 horas (r= 0,62; P<0,05). Os animais do GUA (46,01 mg/dL) apresentaram hipomagnesemia subclínica, nas 72 horas após o parto (P<0,05). Concentrações séricas de ureia maiores no pré-parto, influenciaram no metabolismo mineral da vaca leiteira no pós-parto imediato, sendo detectado hipomagnesima subclínica 72 horas após o parto.

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INTRODUCTION

Among the metabolic diseases, hypomagnesemia and hypocalcemia are extremely important, especially in the immediate postpartum period where the dairy cow goes through several abrupt changes in its metabolism (McArt & Neves 2019, p. 690). There are several reasons that can trigger these ailments, among them, an unbalanced diet since its prepartum such as the use of feeds that increase the amount of ammonia within the rumen environment due to excess non-protein nitrogen sources in the diet in relation to carbohydrates (Heidari *et al*, 2022, p. 2).

Pastures rich in non-protein nitrogen such as fertilized pastures with liquid swine manure, increase ammonia and ammonium ion levels (Flores *et al*. 2014, p.103), which precipitate magnesium, within the rumen, decreasing its absorption (Marín & Cárdenas 2001, p. 62). Increased nitrogen concentration increases the pH of the rumen fluid and forms insoluble hydroxides with magnesium (Gabel & Martens 1986, p.285). Magnesium is a cofactor on the parathormone

receptor (PTH) acting directly on calcium metabolism (Goff 2014, p.370).

The relationship between calcium and magnesium levels in the dairy cow's metabolism is that in cases of hypomagnesemia, there may be a decrease in the action of tissue PTH, reducing bone clearance and renal reabsorption of calcium, thus increasing the risk of hypocalcemia (Goff 2014, p.371). Ammonia when in excess in the rumen is absorbed through the rumen wall and converted to urea in the liver, and in turn increases its serum concentration, and is then eliminated through milk, saliva, urine, and feces (Ariyarathne *et al*. 2019, p.183).

In this context, the objective was to verify whether increased serum urea concentrations in prepartum dairy cows influence the mineral metabolism of magnesium (Mg) and calcium (Ca) in the recent postpartum period.

MATERIAL AND METHODS

All the procedures performed in this study were approved by the Ethics Committee on Animal Experimentation of the Catarinense Federal Institute, Araquari Campus, under protocol n. 233/2017.

The experiment was carried out in the municipality of Seara (27º 08' 56" S, 52º 18' 39" O) located in western Santa Catarina, from July to December. Two dairy farms with Holstein cattle breed (Property A and Property B) and average production of 28 kg milk/animal/day, average of 3.07± 1.36 lactations, ECC of 3.44 ± 0.16, under pasture, whose forages offered to pre-lactation cows were fertilized with liquid swine manure (LSM) were previously selected. Thirty days before the expected calving date, 27 multiparous cows were separated from the other animals (15 cows from property A and 12 from property B), and kept in an easily observable area of the property to which they belonged. During the experiment, all animals were subjected to similar management and nutrition (**Table I**). Bromatological analysis of the forage supplied during the pre-labour period was performed in both properties, composed of Tifton 85 (Cynodon spp) and Jiggs (Cynodon dactylon) monthly, totaling 5 samples which were compiled and the average contents of Crude Protein was 139, 78 ± 15.76 g/kg dry matter (DM), Nitrogen 22.37 g/kg DM 2.52), Neutral Detergent Fiber 47.09 ±4.69 g/kg DM, Acid Detergent Fiber 22.41 ± 1.31 g/kg DM and Total Digestible Nutrients 64.15 ± 1.95 g/kg DM.

Urine samples were collected by perineal stimulation, 7 days from the expected date of parturition, for pH measurement with a portable pH meter (model K39-0014PA, KASVI, Brazil). Blood samples were collected by puncture of the coccygeal arteriovenous complex using a vacuum tube with clot activator gel, in the prepartum (7 days from the expected date of delivery) and postpartum (24, 48 and 72 hours after delivery) periods.

In the laboratory, the samples were centrifuged for 20 minutes at 1800 rpm, the serum was stored in identified eppendorf® tubes and later frozen at -20°C for further biochemical evaluation. All analyses were performed in a semi-automatic biochemical analyzer (T-3000 VET, Tekna, Brazil), for determination of magnesium serum, calcium and urea concentrations (Labtest®, Brazil), by colorimetric methods.

Animals were categorized into two groups according to the median herd urea serum concentration at prepartum, high urea group (HUG= ≥36.09 mg/dL, mean= 43.33 ± 2.28 mg/dL urea) and low urea group (LUG= <36.09 mg/dL, mean= 27.40 ± 1.69 mg/dL urea).

The data were analyzed using the MIXED procedure in a model that included the urea group as a fixed effect and the cow as a random effect, with ownership used as the subject. Using Akaike's information criterion, the variance component (VC) structure was found to be the best model for the residual covariance structure. The data were also subjected to correlation analysis (PROC CORR) to assess the relationships between the variables studied. The analyses were performed in the Statistical Analysis System program (SAS Inst. Inc.,

Table I. Composition of ingredients (kg of dry matter) of the pre- and postpartum diet available daily to dairy cows on farms A and B respectively (Composición de los ingredientes (kg de materia seca) de la dieta pre y postparto disponible diariamente para las vacas lecheras en las granjas A y B respectivamente).

Ingredients	Prepartum Diet (kg)		Postpartum Diet (kg)	
	Property A	Property B	Property A	Property B
Pasture	Ad libitum	Ad libitum	Ad libitum	Ad libitum
Corn Silage	4,5	3,0	7,5	4,5
Concentrated kg*	3,9	3,48	8,7	6,96
Mineral prepartum***	0,150	0,200	---	---
Mineral postpartum***	---	---	Ad libitum	Ad libitum

*Property A 55.1% corn meal, 40.9% soybean meal, 4% mineral. Property B 68,8% corn bran, 27,2 % soybean meal, 4% mineral.
** Calcium (min) 136.90 g/kg, Calcium (max) 149.50 g/kg, Phosphorus (min) 20.80 g/kg, Sulfur (min) 53.20 g/kg, Magnesium (min) 43.40 g/kg, Sodium (min) 25.00 g/kg, Cobalt (min) 10.00mg/kg, Copper (min) 500.00 mg/kg, Chromium (min) 25.00 mg/kg, Iron (min) 500.00 mg/kg, Iodine (min) 50.60 mg/kg, Manganese (min) 1,333.40 mg/kg, Selenium (min) 13.30 mg/kg, Zinc (min) 2,000.00 mg/kg, Saccharomyces cerevisiae 1.25 x 109 CFU/kg, Vitamin A (min) 400,000.00 I.U. /kg, Vitamin D3 (min) 400,000.00 I.U./kg, Vitamin E (min) 10,000.00 I.U./kg, Biotin (min) 66.70 mg/kg, D-Limonene (min) 2,533.40 mg/kg, Fluorine (max) 208.00 mg/kg.
*** Calcium 145.00 g, Phosphorus 78.00 g, Sulfur 26.00 g, Magnesium 20.00 g, Sodium 114.00 g, Cobalt 90.00 mg, Copper 1,238.00 mg, Chromium 20.00 mg, Iron 2,000.00 mg, Iodine 80.00 mg, Manganese 2,057.00 mg, Selenium 27.50 mg, Zinc 4,896.00 mg, Fluorine (max) 780.00mg.

Cary, NC, version 9.3), where the significance level of 5% (P<0.05) was considered.

RESULTS AND DISCUSSION

There was a positive correlation between prepartum Ca and Mg (r= 0.85; P<0.05), Ca and Mg 24 hours postpartum (r= 0.74; P<0.05) and prepartum Ca and Ca 48 hours (r= 0.62; P<0.05). The HUG animals, showed lower magnesium concentrations 72 hours after calving (1.44 ±0.26 mg/dL; P< 0.0299) (**Table II**). From the results obtained, it was possible to verify that the prepartum urinary pH in both groups is close to what is expected, since values between 5.8 and 6.3 are indicative of the effectiveness of the anionic diet as a preventive strategy for clinical hypocalcemia (Constable *et al*. 2019, p.11377).

The lower magnesium concentrations in the HUG 72 hours after calving, can be explained by the lower absorption of this mineral in the ruminal environment (Care *et al*. 1984, p.587), coming from the increased nitrogen metabolism in the rumen (Flores *et al*. 2014, p.104), that when in excess, ruminal ammonia is converted into urea by the liver, increasing its serum concentrations. Serum magnesium oscillates its homeostasis due to its absorption and excretion metabolism (Martens *et al*. 2018, p.1), directly related to dietary intake (Goff, 2014, p.370). Hypomagnesemia is more frequent in multiparous cows (Reinhardt *et al*. 2011, p.122), due to reduced efficiency of absorption and reabsorption of this mineral (Van Mosel *et al*. 1991, p.199).

Our findings show that the higher blood urea in the prepartum period did not influence calcium metabolism, however both groups have subclinical hypocalcemia after calving. As blood calcium concentration reduces, the animal exhibits subclinical hypocalcemia (<8.6 mg/dL calcium) (Melendez et al. 2023, p.1) and the body tends to increase parathormone (PTH) secretion in order to maintain calcium homeostasis (Goff 2014, p.361).

PTH acts by increasing renal reabsorption, bone clearance and intestinal absorption of calcium, however, these mechanisms are often not sufficient to meet the Ca demand in the peripartum. This failure to maintain calcium homeostasis is usually associated with a tissue resistance to PTH, which may be associated with Mg concentration that acts as a cofactor on the parathormone receptor (Goff 2014, p.362; Goff et al. 2014, p.1527). The positive correlations between the minerals calcium and magnesium in the prepartum and 24 hours after calving, demonstrate that the metabolism of these minerals is interconnected and that possibly the lower levels of calcium are influenced by the lower absorption of magnesium in the rumen environment arising from the high levels of ammonia.

CONCLUSIONS

The higher urea serum concentrations in prepartum dairy cows influenced their mineral metabolism

Table II. Parameters assessed (mean ± standard error of the mean) in multiparous dairy cows categorized by median (of prepartum urea concentration ≥ or < 36.09 mg/dl urea), into high urea group (HUG, n=14) or low urea group (LUG, n=13) (Parámetros evaluados (media ± error estándar de la media) en vacas lecheras multíparas categorizadas por mediana (de concentración de urea preparto ≥ o < 36,09 mg/dl de urea), en grupo de alta urea (HUG, n=14) o grupo de baja urea (LUG, n=13)).

Variables	Groups			
	HUG	LUG	MEAN	Pr>F
Cow's age (months)	65,35±7,08	58,22±7,19	61,74	0,4841
Lactation number	3,42±0,35	2,69±0,36	3,07	0,1640
Urinary pH	6,41±0,16	6,38±0,13	6,47	0,8819
Urea (mg/dL)				
Urea 24h pre calving	46,01±1,99	27,96 ± 2,06	37,32	<0,001
Urea 24h postpartum	40,29±3,41	30,80±2,44	35,72	0,0590
Urea 48 hours postpartum	37,82±3,44	30,77±3,57	34,42	0,1698
Urea 72h postpartum	33,84±2,69	30,88±2,79	32,42	0,4535
Magnesium (mg/dL)				
Mg pre-birth	2,09±0,25	2,25±0,26	2,17	0,6646
Mg 24h postpartum	2,29±0,31	1,77±0,29	2,07	0,2243
Mg 48h postpartum	1,81±0,15	1,64±0,12	1,80	0,3209
Mg 72h postpartum	1,44±0,26	2,04±0,21	1,86	0,0299
Calcium (mg/dL)				
Ca pre-birth	8,65±0,55	8,84±0,44	8,27	0,7386
Ca 24h postpartum	8,39±0,85	8,27±0,88	8,33	0,9205
Ca 48h postpartum	7,44±0,34	7,87±0,29	7,74	0,1351
Ca 72h postpartum	7,77±0,21	7,80±0,18	7,70	0,8692

Pr>F: probability. Values of P<0.05 indicate that there was a significant difference.

in the postpartum period, leading to subclinical hypomagnesemia 72 hours after calving. However, further studies considering serum urea in the mineral metabolism of cows in the immediate postpartum period should be elucidated in the future.

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