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Blood concentrations of calcium, phosphorus and magnesium and dietary cation-anion difference in dairy cows

[*Concentrações sanguíneas de cálcio, fósforo e magnésio e diferença cátion-ânion da dieta de vacas leiteiras*]

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ABSTRACT

The objective of this study was to identify the effect of pre and postpartum DCAD on serum levels of calcium, phosphorus, and magnesium in peripartum Holstein cows. Blood samples were collected from 56 cows (18 primiparous and 38 multiparous) on days -7, +7, +14 and +28 relative to parturition, to determine serum levels of total and ionized calcium, phosphorus, magnesium, and albumin; diet samples for determination of calcium, phosphorus, magnesium and DCAD were collected at the same moments and urine samples were collected at day –7 for measurement of urinary pH. Prepartum DCAD was positively correlated with total calcium (p = 0.02) and with corrected total calcium (p = 0.01) at day -7. There was a negative correlation between prepartum DCAD and magnesium levels at day -7 (p = 0.02). No influence of prepartum DCAD on phosphataemia was observed. Postpartum DCAD did not correlate with serum mineral levels at any time point. Our results demonstrate that prepartum DCAD can influence calcium and magnesium homeostasis, but not phosphorus. In addition, parity and time period should be considered when evaluating serum levels of these minerals in Holstein cows during transition period.

Keywords: reference ranges, DCAD, subclinical hypocalcemia.

RESUMO

Objetivou-se identificar o efeito da DCAD pré e pós-parto nos níveis séricos de cálcio, fósforo e magnésio, em vacas Holandesas em periparto. Foram coletadas amostras de sangue de 56 vacas (18 primíparas e 38 multíparas) nos dias -7, +7, +14 e +28 em relação ao parto, para determinação dos níveis séricos de cálcio total e ionizado, fósforo, magnésio e albumina; amostras de alimento foram coletadas nos mesmos momentos para determinação dos níveis cálcio, fósforo, magnésio e DCAD dietético; e amostras de urina foram coletadas no dia -7, para mensuração do pH urinário. A DCAD pré-parto foi positivamente correlacionada com cálcio total (P = 0,02) e com cálcio total corrigido (P = 0,01) no dia -7. Houve correlação negativa entre a DCAD pré-parto e os níveis de magnésio no momento -7 (P = 0,02). Não houve influência da DCAD pré-parto na fosfatemia. A DCAD pós-parto não se correlacionou com os níveis séricos de minerais em nenhum momento. Os resultados demonstram que a DCAD pré-parto pode influenciar na homeostase do cálcio e do magnésio, mas não do fósforo. Além disso, as variáveis paridade e tempo devem ser consideradas ao se avaliarem os níveis séricos desses minerais em bovinos leiteiros da raça Holandesa em transição.

Palavras-chave: intervalos de referência, DCAD, hipocalcemia subclínica

INTRODUCTION

Early studies demonstrated the great potential of prepartum acidogenic diets in reducing the incidence of clinical or subclinical hypocalcemia in the fresh cow period of dairy cattle. In addition to the beneficial effects on calcium homeostasis, data from latter studies showed that prepartum diets could influence performance for weeks or months postpartum, increasing milk

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yield, reproductive performance and reducing overall incidence of infectious or metabolic diseases during the transition period (Lean *et al.*, 2006, 2019; Santos *et al*., 2019).

Despite the large number of studies published in the literature, further research is necessary to clarify some other possible physiological and metabolic effects caused by the reduction of the Dietary Cation-Anionic Difference (DCAD) in dairy cattle (Santos *et al*., 2019). For instance, the effects of DCAD on minerals such as phosphorus and magnesium, have not yet been explored in depth, even though, those minerals are closely related to calcium (Lean *et al*., 2006; Shahzad and Sarwar, 2008; Razzaghi *et al*., 2012).

Investigating the relationship between calcium, phosphorus and magnesium and the diet cation-anion difference (DCAD) during peripartum, allows a better understanding of the dynamics of these elements, and enable a better understanding of when and where cut-off points in their homeostasis occurs. When studies are applied in a specific geographic region, data provides a better understanding of the dynamics of these minerals and associated factors in that specific population. This overview allows the establishment of more precise preventive measures that are more suitable for each reality.

The objective of this study is to describe the influence of pre and postpartum DCAD on serum levels of calcium (total, corrected and ionized), phosphorus and magnesium during the peripartum period of dairy cows located in the Middle Plateau region of the state of Rio Grande do Sul, and describe the serum levels of these minerals in this local population.

MATERIALS AND METHODS

The procedures in this study were approved by the Ethics Committee on the Animals Use of the Federal University of Rio Grande do Sul under the protocol nº 29966 and authorized by those responsible for the herds.

The study was carried out in six commercial dairy herds located in the plateau region of Rio Grande do Sul, during the cold season of the year (autumn-winter). A total of 56 healthy Holstein cows (18 primiparous and 38 multiparous) were enrolled in this study. According to data from the National Institute of Meteorology (Dados..., 2021), between March and July 2021, the average air temperature (dry bulb) recorded in the region was 16.3ºC (minimum temperature of -1.1 and maximum of 33ºC), the average humidity was 77.5% and the average monthly rainfall was 123.4mm.

Animals were housed in compost-bedded pack barn (4 farms) or free-stalls (2 farms), with an average of 105 lactating cows. They were milked twice or thrice a day (3 farms for each system) and average milk yield at peak lactation was 39.7 liters/cow/day. Animals were fed twice a day *ad libitum*. The composition of pre and postpartum diets are shown in Table 1.

The prepartum diet was formulated to achieve a negative DCAD, through restriction of cations and supplementation of anionic salts containing the following composition: Ca 12%, P 1.25%, Mg 7%, Na 2%, Cl 13.8%, S 4%, Cu 0.045%, Zn 0.16%, Mn 0.08%, I 0.0018%, Se 0.0013%, Co 0.003%, vitamin A 300,000 IU/kg, vitamin D3 60,000 IU/kg, vitamin E 6,750 IU/kg and sodium monensin 750mg/kg.

Milk yield data at the peak was obtained in the Ideagri platform (Belo Horizonte, MG) used by farmers. Diseases occurrence, diagnosed by veterinary practitioners, were also recorded. Through the puncture of the coccygeal vessels with a *vacutainer* system, blood samples were obtained at days -7, +7, +14 and +28, in relation to calving, in tubes with separator gel and clot activator. Blood samples were immediately centrifuged at 1.000 g for 10 minutes and the serum stored in duplicate at −20°C until analysis. Serum levels of total calcium, phosphorus, magnesium, and albumin were analyzed by spectrophotometry with an automatic biochemical analyzer (CM200, Wiener lab, Argentina) using commercial reagent kits (Labtest, Brazil). Ionized calcium levels were measured using the NM-BAPTA method using a Cobas 8000 device.

Table 1. Ingredients and nutritional composition of pre and postpartum diets from each farm

	Farm 1		Farm 2		Farm 3		Farm 4		Farm 5		Farm 6	
Item	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Barley (%)												2.2
Citrus pulp (%)				13.5				12		13.5		14
Corn grain (%)	6.1		3.75	6	3.8	6	8.1	1.5	8.1	5.3	8.1	1.5
Corn silage (%)	83.3	46.6	83.3	58.4	24	46.2	50	66		47.5	40	42
Oat grain (%)		3.8		2		5.6		3		3.4		4.2
Oat hay (%)		1.16								3.4		
Soy hulls (%)	1.25	2.9	2.5	6.3		3.2		3		2.5		5
Soybean meal (%)	3.75	5.1	5.25	10		7	8		8	6.7	8	5
Triticale silage (%)		4.1			24	11.3		6	80		40	
Wet brewery residue (%)		26.2				19.3		7.5		17		22.5
Wheat bran (%)					2.4		2.4		2.4		2.4	
Wheat straw (%)	1.5		4.2	1.5	45		30					1.5
Postpartum mineral supplement (%)		0.7		1		0.8		0.7		0.3		0.8
Prepartum mineral supplement (%)	1.4		1		1		1.5		1.5		1.5	
Buffer (%)		0.3		0.4		0.4		0.2		0.2		0.3
Urea (%)		0.3		0.3		0.2		0.2		0.2		0.3
Protected fat (%)				0.7								0.7
Ca (%)	0.3	0.5	0.7	1.0	0.2	0.6	0.5	0.6	1.2	0.9	0.4	0.7
P (%)	0.2	0.4	0.4	0.4	0.2	0.4	0.3	0.4	0.6	0.4	0.3	0.4
Mg (%)	0.2	0.3	0.3	0.3	0.2	0.3	0.5	0.5	0.7	0.4	0.3	0.3
Na (%)	0.1	0.3	0.2	0.3	0.1	0.5	0.1	0.3	0.3	0.3	0.1	0.2
K (%)	1.2	1.0	1.4	1.2	1.2	1.0	1.2	1.1	1.2	1.0	0.7	1.0
Cl (%)	0.1	0.2	0.4	0.2	0.2	0.4	0.4	0.3	0.6	0.3	0.3	0.2
S (%)	0.2	0.2	0.5	0.3	0.2	0.3	0.4	0.3	0.6	0.3	0.3	0.2
DCAD (meq/100 g/DM)	15.7	18.3	0.8	18.9	17.6	18.4	-0.2	12.5	-9.2	13.5	11.3	15.2

Albumin levels (g/L) were used to corrected total calcium levels, according Figge *et al*. (1988), using the following formula:

Corrected calcium = Total Ca + 0,08 * (38 – albumin)

Urine samples were collected on day −7, through perineal stimulation, for immediate measurement of urinary pH with a portable pH meter (PH-1900 + EPC-70, Instrutherm). Pre and postpartum diet samples were collected (–7, +7, +14 and +28 days in relation to calving), so that for each blood sample there was a corresponding diet sample. These samples were obtained at multiple points, homogenized to form a pool, from which a final sample was taken. Samples were identified and frozen for later measurement of calcium, phosphorus, magnesium and DCAD levels, utilizing inductively coupled plasma optical emission spectroscopy (ICP-OES). The following formula was applied to estimate DCAD:

DCAD (mEq/100g): $[(\%Na^{+}/0{,}023 + \%K^{+}/0{,}039) – (\%Cl^{-}/0{,}0355 + \%S^{2-}/0{,}016)]$

Considering the 56 animals enrolled, 48 had all samples and analysis successfully executed (–7, +7, +14 and +28), and in 8 animals there was a failure in at least one evaluated moment, mainly due to the occurrence of hemolysis. These samples were removed from the study, keeping other evaluated moments, to maintain an adequate sample number. Results of metabolites in primiparous and multiparous cows were expressed together, to maintain a larger sample number. These data were used to calculate confidence intervals to demonstrate the levels of these metabolites in the population used in the study. Reference range intervals based on the analyzed time periods was determined using the Excel Reference Value Advisor add-in (freeware v2.1; http://www.biostat.envt.fr/spip/spip.php?article63). Outliers were identified using Tukey's criteria, the variables normality was verified with Anderson-Darling test, the confidence interval

(90% confidence limit) was calculated using a non-parametric method, when the variables did not meet the requirements of normality and symmetry. The relationship between DCAD at day −7 DCAD and total calcium, corrected total calcium, ionized calcium, phosphorus, magnesium, and calcium:phosphorus ratio was determined using Spearman's correlation. Levels of total calcium, corrected total calcium, ionized calcium, phosphorus, magnesium and calcium:phosphorus ratio were analyzed as repeated measures, using the generalized estimation equation (GEE) model, including parity as fixed factors (Primiparous and Multiparous), sampling period (days -7, 7, 14 and 28) and the interaction between them. For each modeled dependent variable, the Gaussian and gamma distributions were tested, both with identity linkage function, with better convergence and quality associated with the Gaussian distribution, identified through the lowest values of the quasi-likelihood criterion under the independence model (QICc). The pairwise comparison used the estimated marginal means, with Tukey's multiplicity adjustment. The results were significantly different considerations with probability ($p<0.05$). The R statistical package was used for statistical analysis and graphic composition.

RESULTS AND DISCUSSION

The DCAD reduction of diets impacts on calcium metabolism through increased sensitivity to PTH, greater renal production of DHCC and consequent increase in intestinal absorption of calcium, greater bone resorption of calcium and increase ionized serum calcium concentrations (Degaris and Lean, 2008, Goff, 2018). In addition to the benefits in peripartum calcium homeostasis, the use of acidogenic diets during prepartum also results in an improvement in postpartum productive and reproductive performance and a reduction in the incidence of infectious and metabolic diseases during the transition period (Lean *et al*., 2019; Santos *et al*., 2019).

Although the relationship between DCAD prepartum and postpartum calcemia is well established, few studies have evaluated the influence of DCAD in minerals such as phosphorus and magnesium, which metabolism is related to calcium (Lean *et al*., 2006; Li *et al*., 2008; Shahzad and Sarwar, 2008; Wu *et al*., 2008; Razzaghi *et al*., 2012). For example, hyperphosphatemia can reduce calcemia (Lean *et al*., 2006), by inhibiting the conversion of 25-OH-vitamin D to DHCC (Goff, 2014), while magnesium has a protective effect on calcium levels (Degaris and Lean, 2008), due to its role in PTH releasing process (Goff, 2014).

According to Lean *et al*. (2006), low levels of magnesium in prepartum diets may result in failures of calcium homeostasis during postpartum. Hypomagnesemia affects serum calcium levels in two major ways: decreasing PTH secretion and reducing the sensitivity of target tissues to PTH. Magnesium is required for the release of PTH by the parathyroid and is necessary in the synthesis process of intracellular enzymes such as adenyl cyclase and phospholipase C, which signal the expression of PTH receptors in bone and kidney tissue (Goff, 2018). Thus, magnesium is an important factor related to calcium variations in dairy cattle (Degaris and Lean, 2008). Nevertheless, there is no homeostatic mechanism for maintenance of serum magnesium levels and adequate serum levels depends primarily on the ingestion and excretion of this mineral (Goff, 2018). According to Lager & Jordan (2012), plasma magnesium should be maintained between 1.92-2.6 mg/dL. In this study, the interval of serum magnesium found was 1.85 – 1.95 mg/dL (95% CI). It was possible to observe the effect of parity and time (day 7 vs. day 28, in the primiparous category $p < 0.05$; day -7 vs. day 28, in the multiparous category $p < 0.05$) on serum levels of this mineral (Fig. 1).

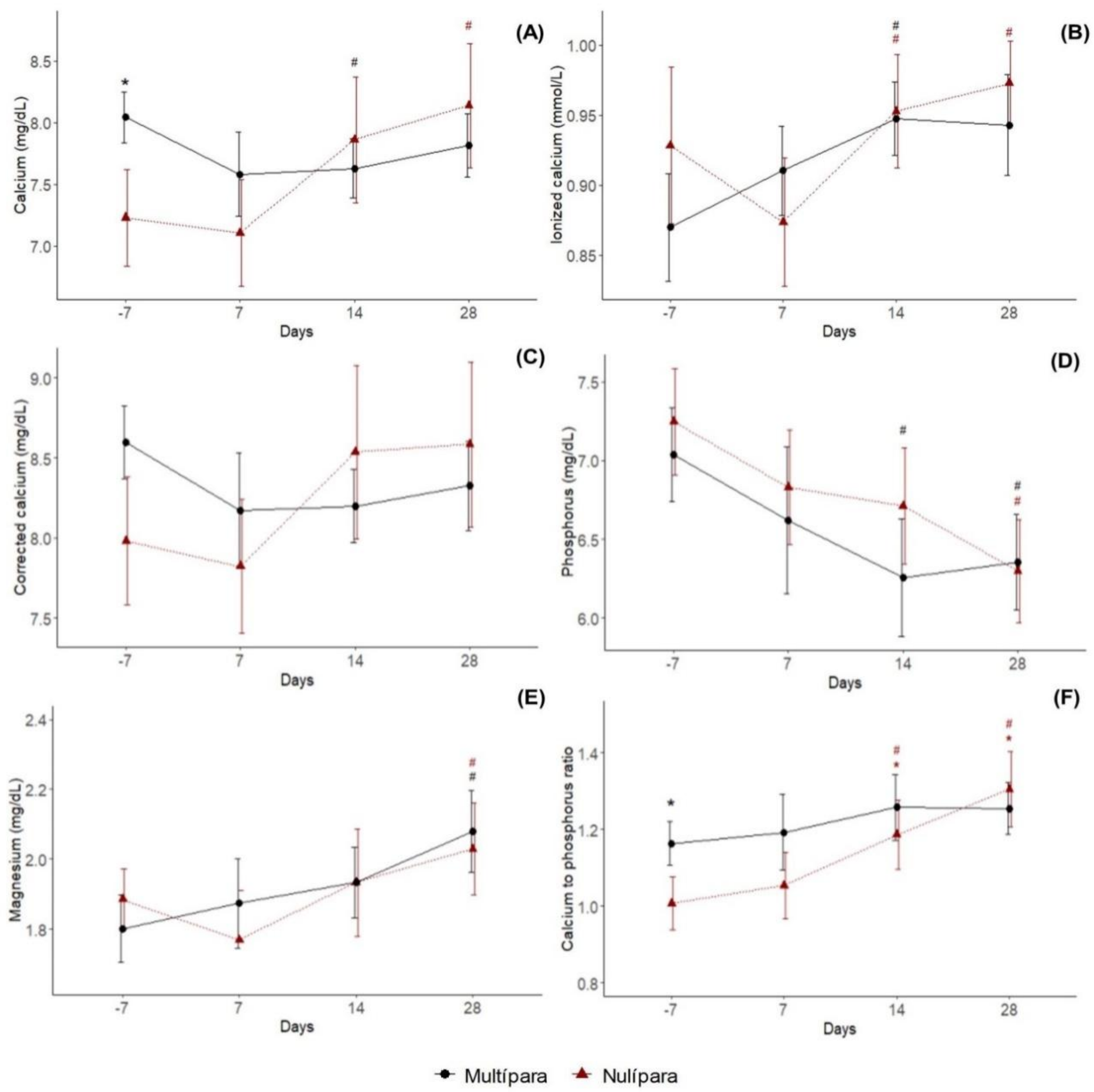

Figure 1. Estimated marginal means of different metabolites in dairy cows (multiparous ●, primiparous ▲) and 95% asymptotic confidence intervals (┬, ┴) for calcium (A), ionized calcium (B), corrected calcium (C), phosphorus (D), magnesium (E) and Ca:P ratio.

Differently to magnesium, high levels of dietary phosphorus reduce the levels of serum calcium. Despite phosphorus being an anion and acidify the metabolic pH, hyperphosphatemia reduces calcemia (Lean *et al*., 2006) by inhibiting the renal alpha-hydroxylase enzyme, which converts 25-OH-vitamin D to 1.25 -diOH-vitamin D (DHCC), resulting in decreased intestinal calcium absorption (Goff *et al.*, 2014). Lager & Jordan (2012) suggest a range for phosphorus between 4.8-7.4 mg/dL. In this study, the phosphorus range found was 6.4 – 6.7 mg/dL (95% CI), varying according to time and parity (day -7 vs. day 28, in the primiparous category $p < 0, 05$; day -7 vs. day 14 and day-7 vs. day 28, in the multiparous category $p < 0.05$). The levels of minerals found in this experiment, according to time (-7, 7, 14 and 28 days in relation to parturition) are shown in below (Table 2).

Table 2. Median, lower, and upper limit of total calcium, corrected total calcium, ionized calcium, phosphorus, magnesium, and calcium:phosphorus ratio of cows at different moments (-7, 7, 14 and 28) in relation to parturition

Period	Parameter	Median	Lower limit (CI 90%)	Upper limit (CI 90%)
Day -7	Total calcium (mg/dL)	7.90	5.44 (5.2-6.32)	8.98 (8.87-8.99)
	Corrected total calcium (mg/dL)	8.30	5.95 (5.43-6.64)	10.44 (9.86-11.44)
	Ionized calcium (mmol/L)	0.90	0.61 (0.60-0.65)	1.11 (1.06-1.13)
	Phosphorus (mg/dL)	7.13	5.30 (5.18-5.63)	9.30 (8.29-9.48)
	Magnesium (mg/dL)	1.82	1.06 (0.98-1.41)	2.25 (2.21-2.25)
	Calcium:phosphorus ratio	1.13	0.72 (0.71-0.80)	1.57 (1.38-1.57)
Day 7	Total calcium (mg/dL)	7.46	5.00 (4.89-6.10)	10.55 (9.09-10.76)
	Corrected total calcium (mg/dL)	8.25	5.98 (5.43-6.64)	10.48 (10.01-11.44)
	Ionized calcium (mmol/L)	0.90	0.64 (0.59-0.76)	1.10 (1.06-1.11)
	Phosphorus (mg/dL)	6.52	4.00 (3.77-4.88)	10.51 (8.64-11.05)
	Magnesium (mg/dL)	1.85	1.04 (1.03-1.16)	2.59 (2.34-2.61)
	Calcium:phosphorus ratio	1.07	0.68 (0.67-0.80)	1.99 (1.66-2.08)
Day 14	Total calcium (mg/dL)	7.58	5.42 (5.24-6.31)	9.81 (9.07-9.95)
	Corrected total calcium (mg/dL)	8.23	6.05 (5.43-6.67)	10.47 (9.86-11.44)
	Ionized calcium (mmol/L)	0.96	0.77 (0.76-0.83)	1.13 (1.06-1.14)
	Phosphorus (mg/dL)	6.29	4.55 (4.51-4.81)	8.57 (8.05-8.63)
	Magnesium (mg/dL)	1.89	1.38 (1.36-1.48)	2.80 (2.47-2.91)
	Calcium:phosphorus ratio	1.27	0.75 (0.74-0.83)	1.93 (1.60-2.04)
Day 28	Total calcium (mg/dL)	7.93	5.76 (5.67-6.44)	10.54 (9.61-10.99)
	Corrected total calcium (mg/dL)	8.26	6.11 (5.43-6.67)	10.60 (10.21-11.44)
	Ionized calcium (mmol/L)	0.96	0.72 (0.72-0.79)	1.19 (1.10-1.20)
	Phosphorus (mg/dL)	6.28	4.41 (4.29-4.88)	8.48 (7.50-8.76)
	Magnesium (mg/dL)	2.04	1.45 (1.44-1.52)	2.92 (2.62-2.94)
	Calcium:phosphorus ratio	1.26	0.85 (0.84-0.93)	1.80 (1.63-1.81)

A study by Razzaghi *et al*. (2012), showed that a negative DCAD (−100mEq/kg DM) increased the prepartum plasma calcium and magnesium concentration, when compared with a positive DCAD (+100mEq/kg DM), On the other hand, Shahzad and Sarwar (2008) did not observe any effect of DCAD on magnesium levels. For phosphorus, the same authors did not observe any effects related to DCAD. According to Lean *et al*. (2006) and Li *et al*. (2008), the increase in plasma magnesium concentrations in animals submitted to prepartum acidogenic diets could be attributed to higher levels of magnesium in the diet itself, and not to a possible physiological effect. This study agrees with Razzaghi *et al*. (2012), as there was a negative correlation (−0.29) between prepartum DCAD and magnesium at −7 (p = 0.02), because as DCAD reduced, magnesium levels increased, suggesting that DCAD may positively influenced prepartum magnesemia (Table 3). It is possible that the restriction of potassium in anionic diets favors the absorption of dietary magnesium, resulting in the higher serum levels of this mineral observed during prepartum (Goff, 2018). There was no influence of prepartum DCAD on phosphatemia in any period evaluated.

Table 3. Spearman correlation (rho) between dietary cation-anion difference (DCAD) in the prepartum period (Day -7) and serum levels of total calcium, corrected total calcium, ionized calcium, phosphorus, magnesium, and the Ca:P at different periods in relation to parturition (Days -7, 7, 14 and 28)

Period	Parameter	DCAD (mEq/100 g/DM) at -7	
		rho	*P*
Day - 7	Total calcium (mg/dL)	0.31	0.021
	Corrected total calcium (mg/dL)	0.33	0.010
	Ionized calcium (mmol/L)	0.19	0.159
	Phosphorus (mg/dL)	-0.17	0.205
	Magnesium (mg/dL)	-0.29	0.028
	Calcium:phosphorus ratio	0.25	0.065
Day 7	Total calcium (mg/dL)	0.10	0.474
	Corrected total calcium (mg/dL)	0.08	0.600
	Ionized calcium (mmol/L)	0.21	0.137
	Phosphorus (mg/dL)	0.06	0.633
	Magnesium (mg/dL)	0.01	0.958
	Calcium:phosphorus ratio	-0.01	0.992
Day 14	Total calcium (mg/dL)	-0.19	0.172
	Corrected total calcium (mg/dL)	-0.25	0.080
	Ionized calcium (mmol/L)	0.32	0.021
	Phosphorus (mg/dL)	-0.15	0.291
	Magnesium (mg/dL)	0.10	0.486
	Calcium:phosphorus ratio	0.03	0.831
Day 28	Total calcium (mg/dL)	0.24	0.073
	Corrected total calcium (mg/dL)	0.15	0.300
	Ionized calcium (mmol/L)	0.19	0.160
	Phosphorus (mg/dL)	0.08	0.567
	Magnesium (mg/dL)	0.01	0.973
	Calcium:phosphorus ratio	0.08	0.566

It is expected that DCAD correlate negatively with calcemia, that is, the higher the DCAD, the lower the calcium levels and vice versa. In this study, there was a positive correlation (+0.31) between prepartum DCAD and tCa (p = 0.02) and corrected tCa (+0.33) (p = 0.01) at day -7. It is possible that the lower prepartum tCa in animals on acidogenic diets, can be attributed to the higher urinary losses of calcium due to metabolic acidosis (Goff, 2018), or even the fact that the reduction in metabolic pH is correlated with an increase in serum iCa levels (Oetzel, 1991). As the calcium homeostasis system is activated by fluctuations in iCa and not tCa, it is possible that animals on either anionic or cationic diets had similar prepartum iCa levels (Goff, 2014). In this study, it was possible to observe that DCAD influenced the serum levels of tCa and corrected tCa, but not of iCa. There was no significant correlation between prepartum DCAD and calcemia in any postpartum period evaluated (Day 7, 14 and 28), and it is likely that the beneficial effect of DCAD on calcemia occurs only in the immediate postpartum, or even that the ideal range of DCAD has not been achieved (Lean *et al*., 2019; Santos *et al*., 2019). No correlations were observed between postpartum DCAD and levels of calcium, phosphorus and magnesium in any period evaluated.

The range of tCa found in this study was 7.6 – 7.9 mg/dL (95% CI), varying according to time and parity ($p < 0.05$, Nulliparous vs. Multiparous, in each time period), with $p<0.05$ (day -7 vs. day 14, in the multiparous category) and $p < 0.05$ (day 7 vs. day 28, in the nulliparous category). Although these values are lower compared to some other studies in the literature, it is difficult to determine the percentage of hypocalcemic animals in this study, due to the various cut-off points proposed. For instance, to diagnosis of subclinical hypocalcemia Chapinal *et al*. (2012) proposed the cut-off point 8.4mg/dL of serum tCa; for Roche and Berry (2006) it is 8.0mg/dL and for Jawor *et al*. (2012), the ideal cut-off point would be 7.5mg/dL. Other studies inferred that the use of iCa would be more appropriate for identifying hypocalcemic individuals, due to the fact that this is the active form of calcium necessary for several biological functions (Jawor *et al*., 2012; Goff, 2018). In this case, Jawor *et al*. (2012), suggest the cut-off point of 1.0mmol/L for iCa. In this work, the iCa range was 0.91 – 0.94 mmol/L (95% CI), varying in line with parity and time (day -7 vs. day 14, in the multiparous category $p<0.05$; day 7 vs. day 14 and day 7 vs. day 28, in the nulliparous category $p<0.05$).

Furthermore, it has been proposed to correct tCa values with serum albumin levels. According to Figge *et al*. (1998), serum calcium values may be underestimated in patients with hypoalbuminemia, since at least 50% of calcium is bound to albumin in the circulation. Corrected tCa values were 8.2 – 8.5mg/dL (95% CI), being considerably higher than tCa values (7.6 – 7.9mg/dL). In this study, albumin range was 29.8±30.8g/L (95% CI). In comparison, Lager and Jordan (2012) suggest values between 40-50g/L and Cozzi *et al*. (2011) 33-41g/L. A study carried out by Silva *et al*. (2022), who compiled metabolic profile data in multiparous Holstein cows in the state of Rio Grande do Sul, showed that there is a trend towards lower albumin levels in individuals from this region. Therefore, the lower levels of calcemia found in this study could be attributed, at least in part, to the lower levels of albumin described.

Prevalence data of subclinical hypocalcemia (SCH) in Holstein dairy cows are highly variable. In the USA, Reinhardt *et al*. (2011) reports an average prevalence of 54%, in New Zealand, Roche *et al*. (2003) reports 33%, In Brazil, Fiorentin *et al*. (2018) reports 17.1%. The great variability between studies may be attribute to differences between herds and production systems, or even, differences in the methodology, such as cut-off points considered or period evaluated (day in relation to calving), making comparison between studies difficult. For comparison purposes, the results of the overall prevalence of SCH in our study, using different cutoff points for tCa, corrected tCa and iCa, are presented in Table 4.

By definition, subclinical hypocalcemia consist in a state of which calcemia is reduced, without progress to a clinical state of puerperal paralysis, even though, there is a greater predisposition to occurrence of other infectious or metabolic diseases, due to low serum levels of serum calcium, or the excessive period of time that calcium levels remain inadequate (Martinez *et al*., 2012). In this study, 28.6% (16/56) of the animals had some type of postpartum disease, as follows: retained placenta 8.9% (5/56), metritis 23.2% (13/56), dystocia 1.7% (1/56) and cattle tick fever 1.7% (1/56). A total of 4 animals showed a combination of retained placenta and metritis. These indices are similar to other studies in the literature (Martinez *et al*., 2012). Despite this, it is not possible to conclude that calcium levels were adequate, because of the modest number of individuals enrolled, in addition to the fact that this was not the main objective of this experiment.

Table 4. Prevalence of subclinical hypocalcemia (SCH) in cows, considering total calcium (tCa), corrected total calcium (tCa) and ionizable calcium (iCa), with three different cut-off points

Cut-off point	Prevalence of SCH with tCa	Prevalence of SCH with corrected tCa	Prevalence of SCH with iCa	References
8.4mg/dL	80%	53.5%	-	Chapinal *et al*. (2012)
8.0mg/dL	64%	33.4%	-	Roche and Berry. (2006)
7.5mg/dL	40%	17.5%	-	Jawor *et al*. (2012)
1.0mmol/L	-	-	78%	Jawor *et al*. (2012)

Further, the experiment was carried out on commercial farms, and because of that, it was not possible to interfere in the management practices. Therefore, the DCAD was measured as such, not allowing to obtain specific ranges of DCAD. A meta-analysis performed by Santos *et al*. (2019) indicates that the optimal range of DCAD can be close to –10 meq/100g/DM. However, we found DCAD values sometimes negative, close to zero and sometimes positive, which in most cases were not sufficient to reach the ideal DCAD range recommended in the literature. For example, the average urinary pH in prepartum was 7.0 (ranging between 6.3 to 8.2). Urinary pH is usually above 8.2 on cationic diets. To prevent hypocalcemia, it is necessary to obtain a range of urinary pH between 6.2 to 6.8 in Holsteins, or even lower (Lean *et al*., 2019; Santos *et al*., 2019). Considering these values it can be concluded that the use of anionic salts was moderately effective in acidifying the urinary pH.

CONCLUSIONS

Pre-partum DCAD was positively correlated with the levels of tCa and corrected tCa and negatively correlated with Mg in the same period. No correlation was observed between prepartum DCAD and P in any evaluated period. Postpartum DCAD did not correlate with serum levels of calcium, phosphorus, and magnesium at any time. Furthermore, it was possible to observe significant fluctuations in serum levels of tCa, corrected tCa, iCa, P, Mg and the Ca:P ratio, according to the variables time and parity (primiparous and multiparous). Serum calcium levels obtained in this study were lower compared to other studies in the literature. It is necessary to develop more specific reference ranges that consider individual factors (age, breed, and physiological state) and environmental factors (production system, climate, and geographic region). Lastly, our data showed the importance to correct calcium according to variations of serum albumin between different populations, for a more precise diagnosis of subclinical hypocalcemia.

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