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Effects of short term acidogenic diet feeding on metabolic parameters of dairy cows with induced subclinical hypocalcemia

Efeitos da alimentação acidogênica de curto prazo sobre parâmetros metabólicos de vacas leiteiras com hipocalcemia subclínica induzida

Efectos de la alimentación con dieta acidogénica a corto plazo sobre los parámetros metabólicos de vacas lecheras con hipocalcemia subclínica inducida

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Abstract

The objective of was to evaluate whether the provision of an acidogenic diet in late gestation influences the metabolism of cows with experimentally induced subclinical hypocalcemia. Nine multiparous Holstein cows were divided into 3 groups according to the duration of supply of the acidogenic diet: G0 = without acidogenic diet; G9 = nine days and G15 = fifteen days. All animals underwent an experimental induction of subclinical hypocalcemia for 6 hours, performed after the last day of feeding with the diet. Urine samples were collected weekly to evaluate the effectiveness of the diet and blood samples were collected performed one day (-1) and immediately (0) before the induction, every hour until 6 h after the induction and at the end of 72 hours for the evaluation of metabolic parameters. During INDUCTION, G15 had the lowest concentration of CaT, globulins, TP, HCO¬¬ 3, pCO2, K and the highest concentration of creatinine (P < 0.05); G9 and G15 had the lowest concentration of iCa and higher concentration of Mg (P <0.05). In the POST-INDUCTION period, G15 again had the lowest concentration of globulins, TP, HCO¬- 3 and lowest serum pH, in addition to having the highest concentration of creatinine, glucose, and Na (P <0.05). G9 and G15 had the lowest CaT and K concentration and the highest Mg (P < 0.05) in that period. Provision of 15 days of an acidogenic diet to cows with induced hypocalcemia modulated other parameters besides calcium, demonstrated may not be suitable for animals.

Keywords: Acidosis; Calcium; Prepartum.

Resumo

O objetivo foi avaliar se o fornecimento de uma dieta acidogênica no final da gestação influencia o metabolismo de vacas com hipocalcemia subclínica induzida experimentalmente. Nove vacas Holandesas multíparas foram divididas em 3 grupos de acordo com a duração do fornecimento da dieta acidogênica: G0 = sem dieta acidogênica; G9 = nove dias e G15 = quinze dias. Todos os animais foram submetidos à indução experimental de hipocalcemia subclínica por 6 horas, realizada após o último dia de alimentação com a dieta. Amostras de urina foram coletadas semanalmente para avaliar a eficácia da dieta e amostras de sangue foram coletadas um dia (-1) e imediatamente (0) antes da indução, a cada hora até 6 h após a indução e ao final de 72 horas para a indução. avaliação de parâmetros metabólicos. Durante a INDUÇÃO, o G15 apresentou a menor concentração de CaT, globulinas, TP, HCO¬¬3, pCO2, K e a maior concentração de creatinina (P < 0,05); G9 e G15 apresentaram a menor concentração de iCa e maior concentração de Mg (P<0,05). No período PÓS-INDUCÇÃO, o G15 novamente apresentou a menor concentração de globulinas, TP, HCO¬¬3 e menor pH sérico, além de apresentar a maior concentração de creatinina, glicose e Na (P<0,05). G9 e G15 apresentaram as menores concentrações de CaT e K e as maiores de Mg (P < 0,05) nesse período. Fornecimento de 15 dias de dieta acidogênica para vacas com hipocalcemia induzida em outros parâmetros além do cálcio, demonstra que pode não ser adequada para animais.

Palavras-chave: Acidose; Cálcio; Pré-parto.

Resumen

El objetivo fue evaluar si la provisión de una dieta acidogénica al final de la gestación influye en el metabolismo de las vacas con hipocalcemia subclínica inducida experimentalmente. Nueve vacas Holstein multíparas fueron divididas en 3 grupos según la duración del suministro de la dieta acidogénica: G0 = sin dieta acidogénica; G9 = nueve días y G15 = quince días. Todos los animales fueron sometidos a una inducción experimental de hipocalcemia subclínica durante 6 horas, realizada después del último día de alimentación con la dieta. Se recolectaron muestras de orina semanalmente para evaluar la efectividad de la dieta y se recolectaron muestras de sangre un día (-1) e inmediatamente (0) antes de la inducción, cada hora hasta las 6 h después de la inducción y al final de las 72 horas para la inducción. evaluación de parámetros metabólicos. Durante la INDUCCIÓN, G15 presentó la menor concentración de CaT, globulinas, TP, HCO¬¬ 3, pCO2, K y la mayor concentración de creatinina (P < 0,05); G9 y G15 tuvieron la menor concentración de iCa y la mayor concentración de Mg (P <0.05). En el periodo POST-INDUCCIÓN, G15 volvió a tener la menor concentración de globulinas, TP, HCO¬¬ 3 y menor pH sérico, además de tener la mayor concentración de creatinina, glucosa y Na (P<0.05). G9 y G15 tuvieron la concentración más baja de CaT y K y la más alta de Mg (P < 0.05) en ese período. La provisión de 15 días de una dieta acidogénica a vacas con hipocalcemia inducida moduló otros parámetros además del calcio, lo que demostró que puede no ser adecuado para los animales.

Palabras clave: Acidosis; Calcio; Preparto.

1. Introduction

Hypocalcemia is a metabolic disease that mainly affects high producing dairy cows (Goff, 2014; Amanlou et al., 2016) and may be classified as clinical or subclinical (Goff, 2014); both forms can be directly related to the occurrence of several diseases in the postpartum period, such as ketosis, mastitis and metritis, in addition to having negative effects on milk production, fertility and premature culling (Martinez et al., 2014). These relationships ensue as a result of calcium being an important intra- and extracellular mineral that participates in processes such as proliferation, differentiation, cellular motility (both in defense cells and in the control of muscle contraction) (Kimura et al., 2006), as well as in cell adhesion processes, maintenance of bone integrity and regulation of cellular function (Martinez et al., 2012). The clinical form of hypocalcemia is most readily diagnosed as it presents with distinct clinical signs, but its prevalence is lower (affecting 10% of animals), while the subclinical form may show a prevalence of up to 54%, depending on the lactation number of the animal (Reinhardt et al., 2011).

The anionic diet is a nutritional strategy used during the pre-calving period and involves providing a diet that has a higher prevalence of anions than cations, thus activating the calcium homeostasis system that regulates the levels of this mineral (Cardoso, 2020; Seifi et al., 2010). Thus, with an anionic diet, calcium mobilization can be increased during the postpartum period, preventing hypocalcemia (TCa < 8.5 mg/dL) (Goff, 2014; Feijó et al., 2017). The acidogenic diet, because it is rich in anions, causes an increase in blood H^+ concentration via the body's compensation mechanisms that reduce excess negative charge. Consequently, blood pH is reduced. This reduction in pH is responsible for activating the cascade of events that constitute the calcium homeostasis mechanism (Albornoz et al., 2016). For a diet to be considered acidogenic, the total mEq must be less than or equal to -10 mEq/g of dry matter (DM) (Shanchez, 1995). Currently, this diet is recommended from 30 days prepartum, but it is known that the cow responds quickly to the addition of acidogenic salts, noted by a drop in urine pH within 48 h. Blood pH is also slightly increased and activation of calcium homeostasis mechanisms takes around 5 to 7 days (Goff, 2014; Santos, 2011).

Metabolic acidification caused by an acidogenic diet may contribute to the occurrence of other postpartum diseases because, as demonstrated in an in vitro study, acidification of serum reduces intracellular pH and calcium levels, reducing cellular activity (Campion et al., 2015; Fernandez et al., 2005), and exemplified by (Martinez et al., 2012) who showed that cows with decreased intracellular calcium displayed reduced immune cell activity). Moreover, studies have shown that low palatability of the diet leads to a reduction in dry matter intake (DMI) (Vagnoni and Oetzel, 1998), although it is now known that this reduction is a consequence of metabolic acidosis (Santos et al., 2019; Zimpel et al., 2018). In addition, a recent study showed that prolonged feeding of the diet (up to forty-two days) reduced milk production at the beginning of lactation and its use was therefore not recommended for more than 21 days, even though this duration of feeding was not effective in reducing the risk of hypocalcemia (Lopera et al., 2018). This may be an indication that an optimal duration of feeding, effective in reducing disease occurrence but without negative impact on other metabolic parameters has not yet been determined.

The metabolic effects of short term feeding of the acidogenic diet are not known. It is possible that shortening the period of feeding could be equally effective in the activation of calcium homeostasis mechanisms without negatively affecting other metabolic processes. Hence, this study aimed to evaluate the metabolism of multiparous cows subjected to subclinical hypocalcemia and fed an acidogenic diet for a maximum of 15 days.

2. Methodology

This study used animals from a dairy farm located south of Rio Grande do Sul, geographic coordinates 32º 16' S, 52° 32' W, and was approved by the Animal Ethics and Experimentation Committee of the Federal University of Pelotas (23110).

Nine pregnant dry Holstein cows were used in the study. The cows were raised in a semi-extensive rearing system, and grazed on ryegrass (Lolium multiflorum spp.), clover (Tripholium spp.), and birdsfoot trefoil (Lotus corniculatus spp.) pasture, supplemented with 5 kg of concentrate per day, composed of 28.3% soybean meal, 28.3% ground corn, 24.5% soybean hull, 10% rice bran, 3% acidogenic salt, 4.4% calcareous, 1.5% amino acids, offered twice daily. The animals were divided into three groups, separated by the number of lactations (three to five), body condition score (BCS) (Edmonson et al., 1989) and milk production in the last lactation, between 15 and 21 days prepartum and according to the supplement of an acidogenic diet. All groups were induced to subclinical hypocalcemia.

Group G0 (n = 3) did not receive acidogenic diet supplementation (dietary cation-anion difference (DCAD) = +10.03 mEq/100g DM) when subclinical hypocalcemia was induced. Group G9 (n = 3) was induced to subclinical hypocalcemia after receiving acidogenic diet supplementation for a period of 9 days (DCAD = -37.49 mEq/100g DM) and group G15 (n = 3) was induced to subclinical hypocalcemia after 15 days of acidogenic diet supplementation (DCAD = -37.49 mEq/100g DM). The minerals contained in the pasture and concentrate were considered in the calculation of the mEq in the diet. To evaluate the efficacy of the acidogenic diet, the urinary pH of the animals was measured by urine collection, acquired by manual stimulation, every 3 days (Spanghero, 2004). The pH was measured after collection, using a bench pH meter (MPA 210, MS Tecnopon, Brazil).

For the induction of subclinical hypocalcemia, all animals were catheterized in the marginal vein of the ear. For this, ear trichotomy and local antisepsis were performed with 2% povidone-iodine (PVPI) solution. Subsequently, the vessel was punctured using a 20G x 12 intravenous catheter (BD I-Cathtm, BD, 1 Becton Drive, Franklin Lakes, NJ, USA). The three groups underwent a subclinical hypocalcemia induction protocol, by endovenous infusion of a solution of ethylenediaminetetraacetic acid disodium (EDTANa2) (Jorgensen et al., 1999) for a period of 6 hours. Subclinical hypocalcaemia was considered when animals reached levels of ionized calcium (iCa) below or equal to 4 mg/dl, which was monitored hourly, for 10 hours.

Blood samples were collected by coccygeal vein puncture, using the Vacutainer system (BD Diagnostics, São Paulo, Brazil). Ionized calcium was monitored by the portable I-Stat apparatus (Abbott Laboratories, Abbott Park, IL USA) using a CG8+ cartridge (Abbott Laboratories, Abbott Park, IL USA). Immediately after collection, the cartridge was filled with blood and the iCa reading was verified by the I-Stat. At the same time, carbonic gas pressure (pCO2), blood pH, bicarbonate (HCO3-), sodium (Na), potassium (K) and glucose were also analyzed. The remaining blood was centrifuged (in 10ml tubes without anticoagulant) at 1800 g. Serum was divided into two 1.5ml tubes and stored at -80 °C until further analysis.

Plasma levels of total calcium (tCa), magnesium (Mg), albumin (ALB), total serum proteins (TP) and creatinine were analyzed by colorimetry, using Plenno enzyme kits (Labtest Diagnóstica SA, Brazil). Globulins were quantified with the formula Globulins = TP - ALB. Parameters were analyzed in three different periods: pre-induction, in which blood samples were collected 1 day before the induction protocol, to evaluate the baseline level of blood calcium (-1), followed by collection on the day of induction immediately before its start (0); induction, starting 1 h after infusion of the calcium chelator and following at hours 2, 3, 4, 5 and 6; and post-induction at hours 7, 8, 9, 10, 12, 18, 24, 48 and 72.

Dependent variables, iCa, tCa, ALB, GLOB, Mg, TSP, pH, HCO3-, pCO2, Na and K, were analyzed throughout the study, by the Shapiro-Wilk test (P>0.90) and those with normal distribution were analyzed using Tukey-Kramer analysis, using the ANOVA PROC MIXED model in the SAS statistical program (SAS Studio 3.5, SAS Institute Inc., Cary, NC, USA). The comparison between groups considered all time points (hours -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, 24, 48 and 72). Subsequently, the analysis was separated into three periods, to isolate the effect of induction: pre-induction (hours -1 and 0), induction (hours 1, 2, 3, 4, 5 and 6) and post-induction (hours 7, 8 , 9, 10, 12, 18, 24, 48 and 72). The analysis was used to compare the main effects, and their interactions were included in the models and the P-value less than 0.05 were considered significant. The precision was evaluated by six analyses of the same sample at the same day (intraassay precision) and at different days (intraassay precision). Precision was assumed when the coefficient of variation was 5%. The variance of components structure was used, due to lower values in the Bayesian information criterion for all variables.

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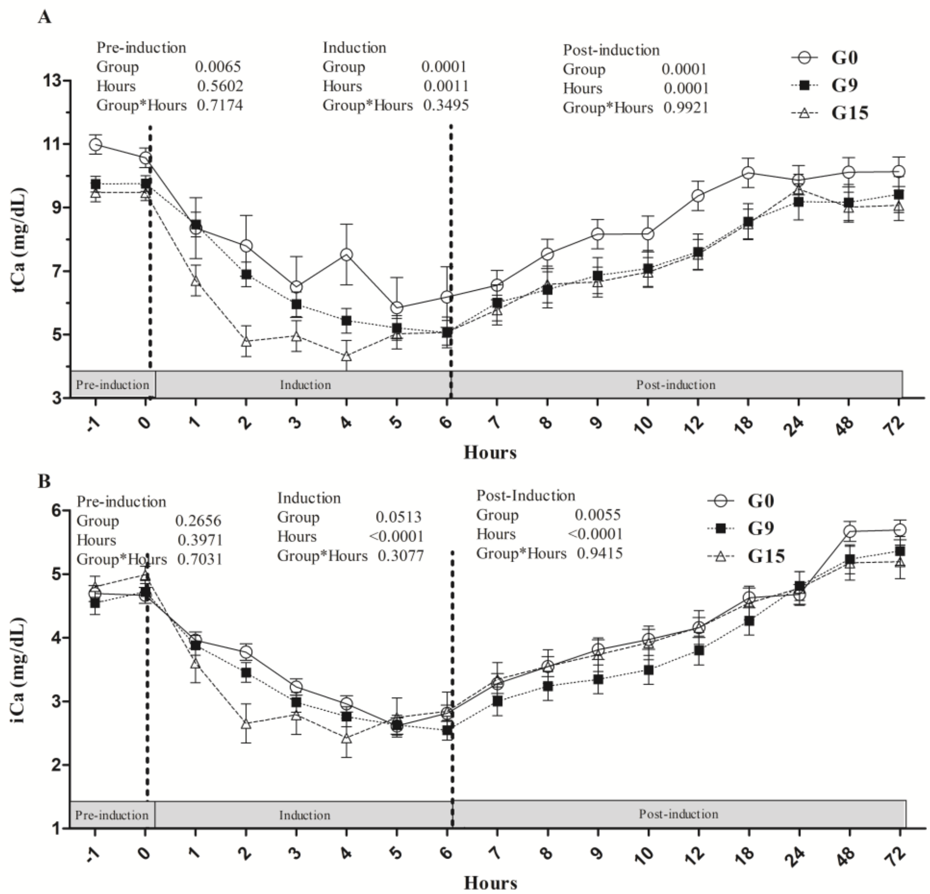
3. Results

We observed that the urine pH of animals differed significantly among groups (P <0.01). Group G0 had a mean urine pH of 8.52 ± 0.24, while the groups that received the diet for 9 and 15 days had urine pH of 6.53 ± 0.15 and 5.39 ± 0.04, respectively.

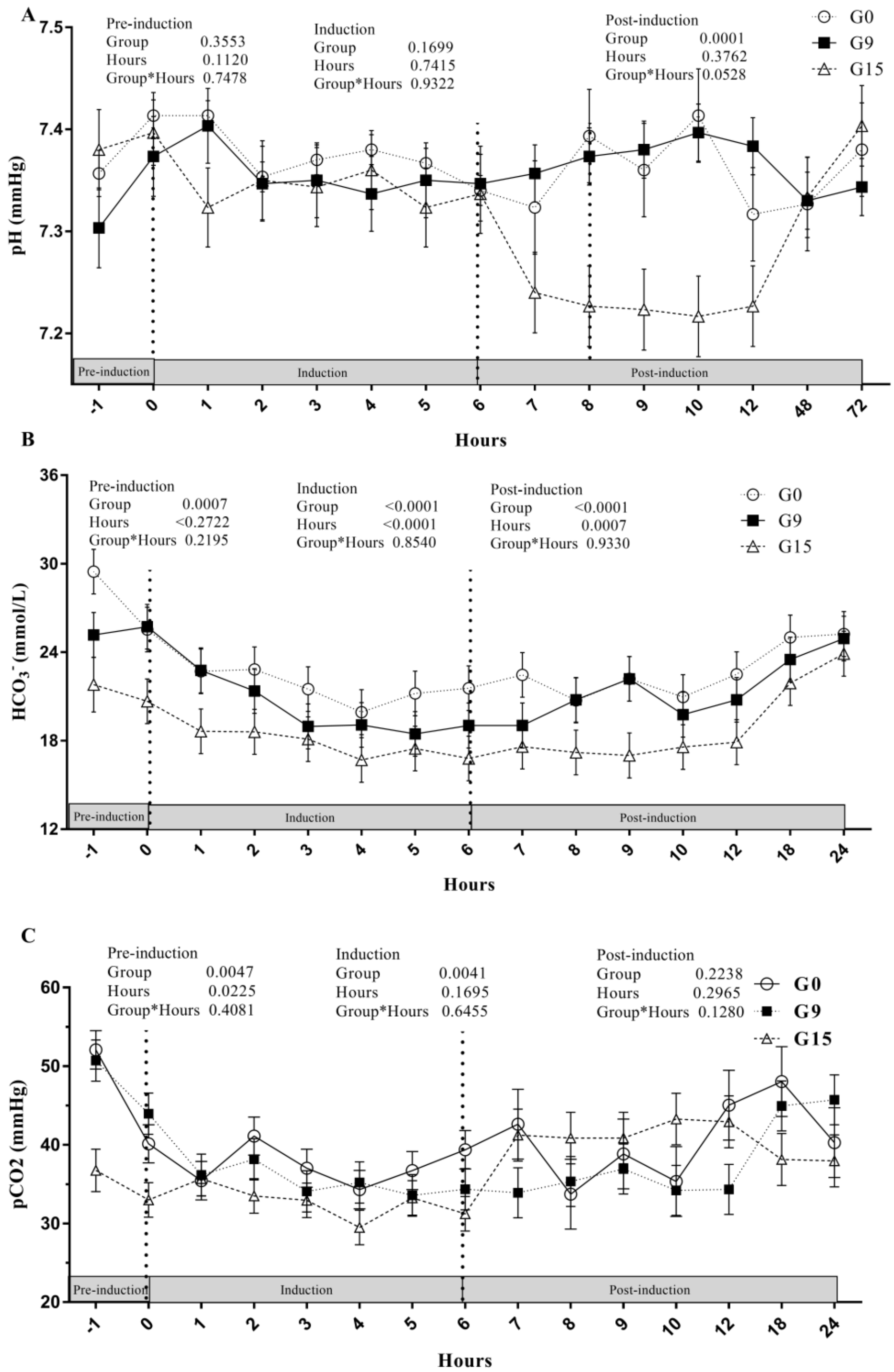
The analyses of the groups and the group x period interaction, throughout the study, are not reported. The observed differences were expected, since an experimental induction was performed. The calcium concentration during the experiment is presented in Figures 1 (A and B), the success of induction can be observed during hours one to six. The Figure 2 presented the serum pH (Figure 2A), HCO3- (Figure 2B) and the pCO2 (Figure 2C) during the experiment

Figure 1: Total Calcium (A), Ionized Calcium (B) of cows during Pre-induction, Induction and Post-induction of subclinical hypocalcemia, receiving anionic diet during prepartum for different periods.



[1]G0 - animals that underwent induction to subclinical hypocalcemia without addition of anionic diet. [2]G9 - animals that underwent induction to subclinical hypocalcemia after already receiving the anionic diet for 9 days. [3]G15 - animals that underwent induction to subclinical hypocalcemia after receiving the anionic diet 15 days ago. Statistical difference with P <0.05. Each period was analyzed separately.
Source: authors.

Figure 2: Serum pH (A), Bicarbonate (B), Gas Pressure (C) of cows during Pre-induction, Induction and Post-induction of subclinical hypocalcemia, receiving anionic diet during prepartum for different periods.



[1]G0 - animals that underwent induction to subclinical hypocalcemia without addition of anionic diet. [2]G9 - animals that underwent induction to subclinical hypocalcemia after already receiving the anionic diet for 9 days. [3]G15 - animals that underwent induction to subclinical hypocalcemia after receiving the anionic diet 15 days ago. Statistical difference with P <0.05. Each period was analyzed separately.
Source: authors.

When analyzing each period separately, we observed that during the PRE-INDUCTION there was a difference between the groups in some metabolites; groups G9 and G15 showed lower concentrations of CaT compared to G0 (9.74 ±

0.17; 9.47±0.19; 10.77±0.21) ($P<0.05$) and higher Na (141.17±1.39; 140.42± 0.28; 139.33±0.16; $P<0.04$), G15 also showed lower concentrations of TP (7.51±0.08; 8.46±0.25; 8.58±0.14), HCO^-_3 (21.23±0.50; 25.45±1.68; 27.50± 0.58) and pCO_2 (34.87±1.70; 47.33±2.97; 46.10±2.33) in relation to G9 and G0 ($P<0.01$) and higher glucose levels (57.91±2.25; 47.83±2.96; 49.00±2.13) ($P<0.05$). There were no differences between groups in the other metabolites during PRE-INDUCTION.

During INDUCTION the G15 had the lowest values of CaT (5.14±0.19; 6.17±0.15; 7.03±0.39), Globulins (5.03±0.06; 5.97±0.17; 5.46±0.17), TP (7.83±0.06; 8.76±0.14; 7.83±0.06), HCO^-_3 (17.71±0.54; 19.94±0.81; 21.62±0.30), pCO^2 (32.68±0.90; 35.25±0.75; 37.32±0.84) and K (4.01±0.09; 4.39±0.13; 4.40±0.07) ($P<0.01$); and the highest values of Creatinine (1.40±0.016; 1.20±0.07; 1.27±0.04) and Na (145.67±0.28; 143.39±0.67; 142.33±0.42) ($P<0.01$). In addition, the groups that received the anionic diet (G9 and G15) had the lowest concentrations of iCa (3.04±0.06; 2.84±0.12; 3.22±0.05) ($P<0.05$) and highest concentrations of Mg (1.57±0.06; 1.62±0.16; 0.97±0.04) ($P<0.01$).

At POST-INDUCTION, the groups that received the anionic diet (G9 and G15) had lower values of CaT (7.80±0.18; 7.73±0.16; 8.88±0.15), K (4.00±0.08; 4.14±0.05; 4.35±0.09) and higher levels of Mg (2.17±0.05; 2.11±0.07; 1.53±0.04) ($P<0.01$). G9 had the lowest iCa concentration when compared to G0 (4.06±0.07; 4.38±0.05; 4.26±0.08;) and G15 also presented the lowest Globulin values (4.85±0.09; 5.70±0.09; 5.38±0.10), TP (7.68±0.06; 8.37±0.09; 8.24±0.06), HCO^-_3 (19.00±0.50; 21.56±0.81; 22.72±0.81), serum pH (7.26±0.01; 7.36± 0.01; 7.35± 0.01) ($P<0.01$) and higher Creatinine values (1.47±0.03; 1.17±0.05; 1.18±0.02), Glucose (58.80±1.17; 55.33±0.73; 54.71±1.49) and Na (145.85±0.36; 141.95± 0.55; 143.33± 0.47) ($P<0.05$) compared to G9 and G0.

When the periods of PRE-INDUCTION, INDUCTION and POST-INDUCTION were compared, there was a difference in several metabolites. The CaT value differed between the periods, being lower during INDUCTION (6.11±0.33; 9.99±0.33; 8.16±0.15) compared to PRE-INDUCTION and POST-INDUCTION respectively. INDUCTION iCa was also lower (3.03±0.09; 4.74±0.17; 4.23±0.08), as was HCO^-_3 (19.76±0.38; 24.69±0.68; 21.1 ±0.35). Mg showed the lowest concentration during INDUCTION, but there was no difference between PRE-INDUCTION and POST-INDUCTION (1.38 ± 0.05; 1.99 ± 0.09; 1.93 ± 0.04); similarly for PCO_2 (35.08± 0.77; 42.64± 1.39; 39.73± 0.72) ($P<0.01$).

Blood glucose concentrations differed between periods, whereby higher concentrations were observed during INDUCTION, followed by POST-INDUCTION and PRE-INDUCTION (59.61± 0.83; 56.28± 0.77; 51.94± 1.49) ($P<0.01$). Levels of Na increased during INDUCTION and POST-INDUCTION (143.80±0.29; 143.71±0.27; 140.37±0.52) ($P<0.01$). Concentration of TP was higher in INDUCTION versus POST-INDUCTION, while PRE-INDUCTION did not differ from the other periods (8.31±0.06; 8.09±0.05; 8.18±0.10). Serum pH did not differ between the PRE-INDUCTION and INDUCTION periods but was lower during the POST-INDUCTION (7.37 ± 0.01; 7.35 ± 0.008; 7.33 ± 0.007) ($P <0.02$).

4. Discussion

Several studies have already evaluated the effects of an anionic diet fed for the recommended period to cows that develop spontaneous hypocalcemia (Boudon et al., 2016; Goff et al., 2014; Weich et al., 2013); however, the metabolic effects of shorter term feeding of the diet to cows with experimentally induced hypocalcemia have yet been little examined.

Urine pH was evaluated as a measure of effectiveness of the acidogenic diet and demonstrated that the group that did not receive the diet (G0) maintained its physiological values (Dirksen et al., 1993), while G9 and G15 presented acidification. The reduction of urine pH is an attempt by the body to buffer the blood, eliminating H+ (Spanghero, 2004).This systemic response is typically seen following the addition of anionic salts to the prepartum diet as a way to prevent postpartum hypocalcemia and a drop in urine pH has been noted within 24 hours after the start of feeding, followed by increased iCa concentration after the third day (Vieira-Neto et al., 2019). Several authors report that pH values between 5.5 - 6.8 are sufficient to consider that the diet is being effective, however values lower than these may be harmful to the urinary epithelium (Goff et al., 2014; Spanghero, 2004); furthermore, (Melendez et al., 2019) observed that cows with urine pH <6 were 2.39 times more likely to have stillborn fetuses.

In the present study, we observed that 15 days of anionic diet feeding resulted in lower values than those recommended for urine pH, possibly due to the fact that the diet presents a high degree of acidogenesis, with -37.49mEq/100g, demonstrating the importance of evaluating urine pH prepartum, as this can inform adjustments to the diet that could help minimize negative effects on the animal's health. As observed by Santos et al. (2019), diets up to -256 mEq/kg were beneficial to animals, increasing milk production and reducing the occurrence of postpartum diseases.

In the present study, we observed that the supply of an acidogenic diet for 15 days (G15) affected relevant metabolic alterations in animals evaluated since PREINDUCTION. The lowest concentration of HCO^-_3 in G15 during the three periods and pCO_2 in the PRE-INDUCTION and INDUCTION might have been observed due to the high concentration of diet anions. Similar results were found by Feijó et. Al (2021) referring to an acid diet, corroborating the finding. Martinez et al. (2016) consider that HCO^-_3 is one of the main serum buffer and CO_2 the main product eliminated when the body is in the process of maintaining neutrality in the event of metabolic acidosis. Therefore, in the present study, acid-base homeostasis was maintained during PRE-INDUCTION and INDUCTION, not enabling the pH to be changed. In post-INDUCTION, G15 showed a reduction in blood pH, probably due to the accumulation of CO_2 that could have been compensating the pH previously. Induction of subclinical hypocalcemia was able to reduce the concentration of HCO^-_3 and pCO_2 compared to the other two periods, especially due to the product used being an acid.

The groups that received the acidogenic diet (G9 and G15) already presented lower concentrations of calcium during PRE-INDUCTION, due to the effect that the diet has on urine pH and, consequently, on calcium excretion, going from 0.5 g/day to 8 to 11 g/day (Santos et al., 2019). This increase in calcium excretion occurs because of the high concentration of H^+ in the glomerular filtrate, which interferes with the kidneys' ability to reabsorb calcium (Goff, 2014). Furthermore, we observed that in the POST-INDUCTION period, G0 continued to present a higher concentration of CaT and iCa. This could have happened due to a rebound effect, similar to that caused by treatment of hypocalcemic cows with intravenous calcium, i.e. a transient increase in the mineral that generally does not persist following milking, often resulting in disease recurrence (Oetzel, 2013). During the INDUCTION, the difference present between the groups could have been caused by the protocol used for the hypocalcemia induction.

The higher concentration of Mg in groups G9 and G15 during INDUCTION and POST-INDUCTION could be explained by the fact that the acidogenic diet stimulates secretion of parathyroid hormone, which increases the renal threshold of excretion of this mineral, preventing a decrease in its serum levels (Reinhardt et al., 1988) Mg is a fundamental cofactor in the activation of cyclic AMP, is responsible for stimulating bone calcium resorption, and can directly interfere with the activation of homeostasis mechanisms (Goff et al., 2014). Although higher Mg values were maintained in the groups fed an acidogenic diet, when only the periods were compared, Mg was lower during INDUCTION, different from what was observed by several authors that used the EDTA induction protocol and reported that Mg did not vary during induction, due to EDTA's low affinity for Mg (Desmecht, D.J-M et al., 1996; Heron, V.S. et al., 2009; Mellau et al., 2001)The higher concentrations of Na in the G15 group across all periods could also be a consequence of the urine pH of this group being lower throughout the study. The Na concentration changes in this situation as it is one of the main regulatory ions of the kidneys and has an important role in intracellular fluid pH homeostasis, being reabsorbed while H+ ions are excreted through the Na^+/H^+ cotransport pump (Reece, 2006). In addition, specifically during the INDUCTION period, its levels increased due to the product used in the induction protocol containing sodium in its composition. Similarly to Na, K also acts at the renal level and its main absorption route is through the Na/K pump, which explains why the concentration of the metabolite was opposite to that of Na throughout most of the experimental period; moreover, the reduction of K levels in animals with EDTA-induced hypocalcemia was also observed by Heron et al. (2009), and it may also occur in cases of milk fever due to stress, whereby increased production of ACTH contributes to the reduction of K levels.

In the present study, G15 showed the highest concentration of creatinine, both during INDUCTION and POST-INDUCTION periods. Although an increase in serum creatinine can be indicative of renal failure (KANEKO et al., 2008), in our study, the increase was most likely associated with greater protein catabolism induced by low serum pH (Lecker et al., 2006).

Group G15 showed a reduction in TP beginning at PRE-INDUCTION and this was maintained throughout the other periods. Little has been reported about the effects of the acidogenic diet on protein metabolism; some authors believe that the lower concentration of TP may happen because the diet causes mild ruminal acidosis (Kaneko et al., 2008; Razzaghi et al., 2012) that would culminate in decreased DMI (Santos et al., 2019) and, as a consequence, decreased protein intake. Another hypothesis is that the acidosis caused by this diet impairs protein transcription, as has been described in humans (Lecker et al., 2006). Furthermore, since INDUCTION, we observed a reduction in the globulin concentration in G15, which may be a negative indicator related to the immune response, since immunoglobulins are constituents of this group.

Glucose values were higher in G15 in PRE-INDUCTION and POST-INDUCTION, possibly because of the effects of the acidogenic diet in the body, which may reduce glucose absorption. Moreover, during INDUCTION, there was an increase in the concentration of glucose as a direct consequence of the fall in calcium during this period. Low calcium levels prevent insulin from being secreted by blocking glucose from entering cells, increasing its concentration in the serum (Martinez et al., 2014). Because of this, cows that experience subclinical hypocalcemia in the postpartum period may have transient diabetes (Martinez et al., 2014).

Although the variability between animals would have had an effect on treatments due to the limited number of experimental units combined with the non-measurement of DMI and consequently the assessment of daily DCAD, we believe that the results could be replicated with a greater number of animals in an environment with controlled intake, as the metabolic changes found are consistent with the physiological changes expected under the conditions tested here. In view of the results of the study, it is believed that although the acidogenic diet is described as a good tool for the prevention of hypocalcemia (Goff et al., 2014; Santos et al., 2019a), its use for a period of 15 days could already have detrimental effects on the animals' metabolic function and contribute to the occurrence of diseases in the postpartum period.

5. Conclusion

In conclusion, the provision of an acidogenic diet for different periods in late gestation dairy cows experiencing hypocalcemia can alter multiple metabolic parameters in addition to calcium levels, and its use for 15 days can excessively lower the urine pH. Short term feeding of the acidogenic diet may therefore not be appropriate for periparturient cows susceptible to hypocalcemia. Still, however, further studies are needed to better clarify the form of action and other possible effects.

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