



## Metaphylactic effect of calcium on milk composition and animal health in post-partum dairy cows

KÁSSIO D. ALBANI<sup>1</sup>, LEANDRO S. LOPES<sup>1,2</sup>, GABRIELA CAMPIGOTTO<sup>1</sup>,  
MATHEUS D. BALDISSERA<sup>3</sup> and ALEKSANDRO S. DA SILVA<sup>1,4</sup>

<sup>1</sup>Programa de Pós-Graduação em Zootecnia, Universidade do Estado de Santa Catarina/UEDESC,  
Rua Beloni Trombeta Zanim, 680, Santo Antônio, 89815-630 Chapecó, SC, Brazil

<sup>2</sup>Departamento de Zootecnia, Universidade Federal de Minas Gerais, Avenida Presidente  
Antônio Carlos, 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil

<sup>3</sup>Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria,  
Avenida Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

<sup>4</sup>Departamento de Zootecnia, Universidade do Estado de Santa Catarina/UEDESC, Rua  
Beloni Trombeta Zanim, 680, Santo Antônio, 89815-630 Chapecó, SC, Brazil

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**Abstract:** The aim of this study was to evaluate the effects of subcutaneous calcium administration in post-partum dairy cows with respect to carbohydrate, protein and lipids metabolism, as well as its effects on milk composition. Twenty post-partum dairy cows were randomly divided into two groups (n=10): a control and a treated group with a single dose (150 mL) of calcium. Blood collection was performed on post-partum days 1, 2, 3, 7 and 10. In addition, we measured serum levels of total calcium, total proteins, albumin, globulins, glucose, cholesterol, triglycerides, as well as creatine kinase (CK) activity. We determined milk composition on post-partum days 5, 10 and 20. Serum total calcium levels was higher on post-partum days 3 and 7 in the treated group. Serum urea (day 3) and triglycerides (days 7 and 10) levels were higher in the treated group, as were CK activity levels at post-partum days 2 and 3. Serum glucose and cholesterol levels were lower on post-partum day 2 in treated animals. Fat (day 20) and lactose (day 10) content in milk samples were higher than in the treated animals. We concluded that treatment with calcium in cows during the post-partum period changes biochemical variables related to metabolism.

**Key words:** animal health, dairy cattle, pre-delivery, subcutaneous calcium, hypocalcemia.

### INTRODUCTION

Livestock production, including milk production,

is one of the largest agribusiness sub-sectors, with a global asset value of at least \$1.4T (Thornton 2010). Some phases of production are critical to animal health, including the peripartum period, defined as the period 21 days before parturition and 21 days after parturition. During this period, several metabolic and endocrine alterations provoke metabolic disturbances in the post-partum animal;

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Correspondence to: Leandro Sâmia Lopes  
E-mail: [leandrosamia@uol.com.br](mailto:leandrosamia@uol.com.br)  
ORCID: <https://orcid.org/0000-0002-0641-6598>  
Aleksandro Schafer da Silva  
E-mail: [dasilva.aleksandro@gmail.com](mailto:dasilva.aleksandro@gmail.com)  
ORCID: <https://orcid.org/0000-0002-9860-1933>

it is a period when the animal has a negative energy balance, using a large portion of her reserves for production of colostrum and milk (Hernández-Castellano et al. 2017).

Hypocalcemia, associated with placental retention, is the principal disturbance found in cows during the post-partum period, since the animals do not maintain physiological concentrations of serum calcium (Goff and Koszewski 2018). According to McLaren et al. (2006), approximately 5 % of cases are diagnosed as clinical hypocalcemia, while subclinical hypocalcemia occurs principally in multiparous cows, affecting up to 54 % of the herd (Reinhardt et al. 2011). This condition results from excessive animal effort during parturition, as well as from the large demand of calcium in the generation of colostrum (McGrath et al. 2016).

Hypocalcemia can contribute to dystocia, uterine prolapse, retention of fetal membranes, endometritis, reduction of fertility, mastitis and reduction of ruminal motility, frequently requiring pharmacological interventions (Curtis et al. 1983, Borsberry and Dobson 1989). According to Santos (2011), these disturbances can be prevented by two main methods, i.e., the use of Ca deficient diets, which stimulate activity and parathormonium secretion (PTH) (negative feedback) (Albani and Da Silva 2017); or one second method, easier to implement and more effective in the control of subclinical hypocalcemia, is the manipulation of the cationic-anionic balance of the diet, which influences PTH activity and, therefore, the mechanism of active absorption of calcium (Albani et al. 2017). Another option would be oral calcium supplementation, which was beneficial even for dairies with a very low incidence of hypocalcemia (Oetzel and Miller 2012, Oetzel 2013), however, there is controversy as to the beneficial effects of this practice (Martinez et al. 2016).

According to literature, the subclinical hypocalcemia compromised appetite, altered metabolism, and impaired function of immune

cells in dairy cows (Martinez et al. 2012, 2014). The same research group had already verified that the relative risk of developing metritis decreased by 22 % for every 1mg/dL increase in serum calcium (Martinez et al. 2014), which shows the importance of maintaining in levels of this adequate mineral. Calcium application in the post-partum period as a preventive measure is a common practice on many farms, but the effects of its application on metabolism of carbohydrates, lipids and proteins needs more studies. Thus, the aim of this study was to evaluate whether administration of subcutaneous calcium soon after parturition had positive effects on animal's health, as well as on maintenance of physiological serum calcium levels, consequently avoid hypocalcemia, characterized in cows when calcium levels are lower 8.0 mg/dL (Blanc et al. 2014).

#### MATERIALS AND METHODS

The experiment was approved by the Ethics Committee on Animal Research of the Universidade do Estado de Santa Catarina (UDESC), under protocol number 3317190417.

The present study was conducted on a farm in Pinhalzinho (Santa Catarina, Brazil), where milk production is the principal activity. The farm includes approximately 50 lactating animals. There is a pasture production system, a prepartum confinement sob compost barn system, with animals individually fed and maintained in yokes. The prepartum cows were separated 21 days before parturition and received a diet (pre-dried, silage and concentrate) (Table I) provided twice a day. After parturition, the animals received a post-partum diet (Table I) twice a day individually, and they were observed daily. Importantly, on the farm, all ingredients were added in a device known as a "wagon mixer", and after homogenization, feed was offered to the individual animals. Per cow, the total food supply was 20 kg and 30 kg/day, with

pre-dried oats at 9 kg and 7 kg/day, in the pre- and post-partum periods, respectively.

Twenty multiparous Holstein cows (two to four lactations) and milk production ( $28 \pm 4$  L) were divided into two groups of ten animals each: the control group (CG) and animals that received treatment with injectable calcium to aid in the prevention of subclinical hypocalcemia. The treated animals received a single dose of 150 mL of calcium subcutaneously after parturition (2 hours). The drug used was composed of calcium gluconate monohydrate (22.83 g), D-calcium saccharide tetrahydrate (0.0387) and calcium lactate pentahydrate (3.03 g) for each 100 mL of product.

During the experiment, homogenized food samples (silage, pre-dried and concentrate) were collected, packed and sent to the laboratory for analysis (Table I). Blood samples were collected from the caudal vein using tubes without

anticoagulant on days 1 (parturition), and post-partum days 2, 3, 7 and 10. Samples were transported to laboratory and centrifuged (3500 rpm, 10 min) to obtain serum. The serum was collected and stored at  $-20$  °C until assay. Milk samples were collected on post-partum days 5, 10 and 20 for analysis of milk composition (fat, protein, lactose and total solids) using automatic equipment (LactoStar Funke Gerber®).

Serum levels of total calcium, total protein, albumin, cholesterol, triglycerides, glucose and urea, as well as creatine kinase (CK) activity, were measured using commercial kits (Analisa®) using a semi-automated analyzer (Bio Plus 2000®) according to manufacturers' instructions. The serum globulin levels were obtained by subtracting albumin from total protein levels.

On the day of blood collection, a drop of blood collected from caudal vein was used to quantify ketone bodies levels using a portable digital meter

**TABLE I**  
**Ingredients and chemical composition of diet provided to the cows in the pre and post-partum.**

<b>INGREDIENTS *</b>	<b>Prepartum diet (%)</b>	<b>Post-partum diet (%)</b>
Corn silage	60.6	69.3
Pre-Dried Oats	27.3	16.2
Commercial Concentrate	12.1	13.9
Commercial Mineral	-	0.6
<b>CHEMICAL COMPOSITION (% DR) AT TOTAL DIET</b>		
Crude protein	14.2	12.0
Neutral detergent fiber (NDF)	21.4	39.0
Acid detergent fiber (ADF)	14.1	21.8
Calcium	0.81	0.73
Phosphor	0.40	0.38
Magnesium	0.25	0.19
Sodium	0.16	0.13
Potassium	1.16	1.21
Chlorine	0.63	0.17
Sulfur	0.54	0.14
DCAD	-120.60	231.39

Note:  $(\text{Na}+\text{K}) - (\text{Cl}+\text{S})$  in mEq/kg of dry matter. \* All ingredients are mixed, and offered to animals within 30 min after homogenization.

(KetoVet TD-4235<sup>®</sup>) following the manufacturer's recommendations.

The data were subjected to normality testing (Shapiro-Wilk), and the data that did not present normality were transformed to logarithms for normalization. Subsequently, the data were compared using the Student's *t* test, with  $P < 0.05$  between groups (control and treated) and over time (repeated measures) considered statistically significant.

## RESULTS

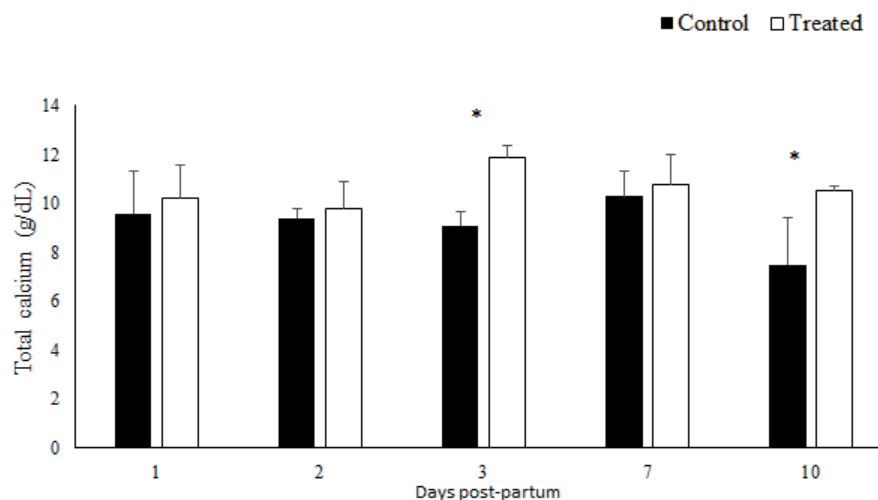
After parturition, no clinical signs linked to hypocalcemia, placenta retention and mastitis were observed in either group. Serum total calcium levels were higher in treated animals than in the control group on post-partum days 3 and 10 (Figure 1). Over time, a calcium increase from day 2 to 3 ( $P < 0.05$ ) was observed.

Serum levels of urea (post-partum day 3) and triglycerides (post-partum days 7 and 10), and CK activity (days 2 and 3 post-partum) were higher in the treated group than in the control group. Serum levels of glucose and cholesterol were lower in the treated group than in the control group on post-

partum day 2 (Tables II and III). No difference was observed between groups regarding ketone bodies, total proteins, albumin or globulin content (Tables II and III).

Over time, ketone body levels increased after delivery in both groups (Table II), and globulin levels were higher on post-partum day 3 than on other evaluated days (control group only) (Table II). CK activity increased soon after delivery in both groups, and decreased on day 10 (Table III). Glucose and triglyceride serum levels were higher shortly after calving, and then decreased in both groups (Table III). Cholesterol levels only differed over time in the control group, that is, on post-partum days 2, 3 and 7 compared to the others days (Table III). Other variables (total protein, urea and albumin) did not differ over time ( $P < 0.05$ ).

Regarding milk composition, we observed no difference between groups in terms of proteins or defatted dry extract. However, fat and lactose content were higher in the treated group than the control group on days 20 and 10 post-partum, respectively (Table IV). Over time, there were no differences in milk composition variables ( $P > 0.05$  – Table IV).



**Figure 1** - Serum total calcium levels of cows supplemented with calcium on days 1, 2, 3, 7 and 10 post-partum. Over time, a calcium increase from day 2 to 3 ( $P < 0.05$ ) was observed. Note: The asterisk (\*) illustrates the significant difference between groups at each time point of the experiment ( $P < 0.05$ ).

**TABLE II**  
**Serum levels of ketone bodies, total proteins, albumin, globulin and urea on post-partum of cows supplemented with calcium on the day of parturition.**

Variable	Day	Control	Supplemented	P value
Ketone bodies (mMol/L)	1	0.76 ± 0.24 <sup>C</sup>	0.54 ± 0.1 <sup>C</sup>	<b>0.341</b>
	2	0.84 ± 0.13 <sup>C</sup>	0.69 ± 0.13 <sup>C</sup>	<b>0.083</b>
	3	0.91 ± 0.20 <sup>BC</sup>	0.82 ± 0.21 <sup>BC</sup>	<b>0.434</b>
	7	1.5 ± 0.85 <sup>AB</sup>	1.42 ± 0.40 <sup>A</sup>	<b>0.744</b>
	10	1.55 ± 0.54 <sup>A</sup>	1.27 ± 0.51 <sup>AB</sup>	<b>0.131</b>
	<b>P value</b>		<b>0.001</b>	<b>0.001</b>
Total proteins (g/dL)	1	7.3 ± 1.07	8.02 ± 0.85	<b>0.673</b>
	2	9.85 ± 2.11	8.71 ± 2.10	<b>0.431</b>
	3	11.5 ± 2.9	10.0 ± 2.4	<b>0.709</b>
	7	7.91 ± 1.08	7.51 ± 1.53	<b>0.849</b>
	10	7.77 ± 1.15	7.77 ± 1.21	<b>0.978</b>
	<b>P value</b>		<b>0.092</b>	<b>0.145</b>
Albumin (g/dL)	1	2.86 ± 0.56	3.38 ± 0.85	<b>0.455</b>
	2	4.53 ± 1.05	4.11 ± 1.08	<b>0.675</b>
	3	3.50 ± 0.68	4.11 ± 0.93	<b>0.753</b>
	7	3.27 ± 0.79	3.46 ± 0.81	<b>0.871</b>
	10	3.01 ± 0.26	2.96 ± 0.39	<b>0.849</b>
	<b>P value</b>		<b>0.061</b>	<b>0.356</b>
Globulin (g/dL)	1	4.44 ± 1.00 <sup>B</sup>	4.64 ± 1.06	<b>0.905</b>
	2	5.32 ± 2.20 <sup>AB</sup>	5.03 ± 1.19	<b>0.844</b>
	3	8.00 ± 2.65 <sup>A</sup>	6.05 ± 1.94	<b>0.074</b>
	7	4.55 ± 1.20 <sup>B</sup>	4.05 ± 1.35	<b>0.678</b>
	10	4.76 ± 1.25 <sup>B</sup>	4.81 ± 1.07	<b>0.889</b>
	<b>P value</b>		<b>0.001</b>	<b>0.087</b>
Urea (mg/dL)	1	43 ± 9.6	38.7 ± 8.48	<b>0.686</b>
	2	34.3 ± 8.9	44.2 ± 7.02	<b>0.065</b>
	3	35.8 ± 5.2	47 ± 8.6	<b>0.040*</b>
	7	38.0 ± 7.8	39.3 ± 11.2	<b>0.845</b>
	10	42.4 ± 5.0	39.5 ± 7.8	<b>0.817</b>
	<b>P value</b>		<b>0.225</b>	<b>0.536</b>

Note:  $P < 0.05$  in the same line shows difference between groups.  $P < 0.05$  in the same column shows difference in each group over time (different letters in the same column shows difference between evaluated periods).

**TABLE III**  
**Serum creatine kinase (CK) activity, and glucose, triglycerides and cholesterol levels on post-partum of cows supplemented with calcium on the day of parturition.**

Variable	Day	Control	Supplemented	P value
CK (mg/dL)	1	282.1 ± 143.5 <sup>B</sup>	294.1 ± 100.8 <sup>B</sup>	<b>0.875</b>
	2	113.0 ± 50.8 <sup>C</sup>	250.3 ± 19.0 <sup>B</sup>	<b>0.001*</b>
	3	169.6 ± 48.5 <sup>C</sup>	514 ± 85.9 <sup>A</sup>	<b>0.001*</b>
	7	465.0 ± 32.3 <sup>A</sup>	485.5 ± 68.5 <sup>A</sup>	<b>0.905</b>
	10	258.6 ± 11.0 <sup>B</sup>	275 ± 125.6 <sup>B</sup>	<b>0.853</b>
<b>P value</b>		<b>0.001</b>	<b>0.001</b>	
Glucose (mg/dL)	1	53.6 ± 23.6 <sup>B</sup>	72 ± 23.5 <sup>A</sup>	<b>0.148</b>
	2	108.6 ± 27.2 <sup>A</sup>	85.5 ± 15.5 <sup>A</sup>	<b>0.047*</b>
	3	107.9 ± 31.4 <sup>A</sup>	79.2 ± 22.7 <sup>A</sup>	<b>0.096</b>
	7	48.6 ± 15.6 <sup>B</sup>	47.2 ± 9.4 <sup>B</sup>	<b>0.924</b>
	10	69.2 ± 27.7 <sup>B</sup>	49.6 ± 13.6 <sup>B</sup>	<b>0.175</b>
<b>P value</b>		<b>0.001</b>	<b>0.008</b>	
Triglycerides (mg/dL)	1	10.5 ± 6.6 <sup>B</sup>	8.2 ± 3.9 <sup>BC</sup>	<b>0.743</b>
	2	36.2 ± 11.8 <sup>A</sup>	33.1 ± 6.5 <sup>A</sup>	<b>0.834</b>
	3	6.69 ± 2.9 <sup>B</sup>	6.66 ± 2.7 <sup>C</sup>	<b>0.897</b>
	7	7.28 ± 1.49 <sup>B</sup>	13.1 ± 4.4 <sup>B</sup>	<b>0.001*</b>
	10	8.00 ± 2.8 <sup>B</sup>	12.0 ± 4.1 <sup>B</sup>	<b>0.047*</b>
<b>P value</b>		<b>0.001</b>	<b>0.001</b>	
Cholesterol (mg/dL)	1	62.5 ± 13.7 <sup>C</sup>	63.3 ± 24.0	<b>0.879</b>
	2	131.9 ± 35.0 <sup>A</sup>	85.6 ± 32.0	<b>0.001*</b>
	3	111.4 ± 32 <sup>A</sup>	87.1 ± 31.0	<b>0.067</b>
	7	99.1 ± 34.2 <sup>AB</sup>	64.8 ± 23.2	<b>0.033</b>
	10	73.5 ± 13.1 <sup>BC</sup>	59.12 ± 16.7	<b>0.082</b>
<b>P value</b>		<b>0.001</b>	<b>0.201</b>	

Note:  $P < 0.05$  in the same line shows difference between groups.  $P < 0.05$  in the same column shows difference in each group over time (different letters in the same column shows difference between evaluated periods).

## DISCUSSION

According to literature, fluctuations in blood Ca were observed after prophylactic intravenous Ca supplementation in multiparous Jersey × Holstein crossbreed cows (Blanc et al. 2014). Similarly, we observed also fluctuations, but with significant higher serum total calcium levels in treated animals than in the control group. Researchers verified that the post-partum oral supplementation with

2 doses of 50 to 60 g of calcium increased this mineral concentration in serum and reduced the prevalence of subclinical hypocalcemia in Jersey cows; but this authors observed that the initial calcemic status highly influenced treatment effects, with subclinically hypocalcemic cows showing a greater serum calcium concentration increase after supplementation than normocalcemic cows (Valldecabres et al. 2018). Based on these data,

**TABLE IV**  
**Milk composition on days 5, 10 and 20 post-partum of cows that received calcium injectable on parturition day.**

Variable (%)	Day	Control	Supplemented	<i>P</i> value
Fat	5	3.35 ± 0.9	4.18 ± 1.20	<b>0.340</b>
	10	3.15 ± 1.2	3.61 ± 1.17	<b>0.635</b>
	20	3.34 ± 1.38	5.44 ± 2.12	0.027*
<b><i>P</i> value</b>		<b>0.356</b>	<b>0.095</b>	
Protein	5	3.53 ± 0.15	3.47 ± 0.10	<b>0.137</b>
	10	3.35 ± 0.14	3.50 ± 0.12	<b>0.080</b>
	20	3.15 ± 0.11	3.14 ± 0.10	0.897
<b><i>P</i> value</b>		<b>0.368</b>	<b>0.436</b>	
Lactose	5	5.17 ± 0.22	5.09 ± 0.16	<b>0.834</b>
	10	4.75 ± 0.20	5.14 ± 0.17	<b>0.012*</b>
	20	4.61 ± 0.17	4.58 ± 0.68	0.817
<b><i>P</i> value</b>		<b>0.265</b>	<b>0.652</b>	
Defatted dry extract	5	9.54 ± 0.30	9.53 ± 0.26	<b>0.967</b>
	10	9.18 ± 0.33	9.58 ± 0.36	<b>0.535</b>
	20	8.67 ± 0.32	8.81 ± 1.2	0.605
<b><i>P</i> value</b>		<b>0.351</b>	<b>0.476</b>	
Minerals	5	0.85 ± 0.09	0.86 ± 0.12	<b>0.950</b>
	10	0.89 ± 0.11	0.88 ± 0.12	<b>0.912</b>
	20	0.87 ± 0.08	0.89 ± 0.09	<b>0.844</b>
<b><i>P</i> value</b>		<b>0.865</b>	<b>0.896</b>	

Note:  $P < 0.05$  in the same line shows difference between groups.  $P < 0.05$  in the same column shows difference in each group over time (different letters in the same column shows difference between evaluated periods).

the researchers conclude that the oral calcium supplementation seemed to disrupt Ca homeorhetic mechanisms in normocalcemic cows, and future studies should evaluate the long-term implications on production and reproduction (Valdecabres et al. 2018). A study conducted by Jahani-Moghadam et al. (2018) reported that oral Calcium bolus administration increased concentrations of calcium in serum on Day 2 post-partum in Holstein dairy cows, and increased first service conception rates, in cows fed a diet with a positive DCAD prior to calving compared to cows that received no oral Ca bolus supplementation. It is important emphasize that use of a single dose of calcium 24 h following parturition was reduced several risks, including

retention placenta risk, mastitis, heart disease risk and displaced abomasum in multiparous cows (Leno et al. 2018). Thus, treatment with oral calcium can be considered an approach to prevent not only hypocalcemia but also other disorders of dairy cows during the post-partum period.

After parturition, a negative energy balance favors the occurrence of subclinical or clinical ketosis, as observed by Ceciliani et al. (2018) in dairy cows during the transition period. Although no clinical signs associated with ketosis were observed in this study, it is important to highlight a tendency ( $P=0.08$ ) toward increased ketone bodies in the control group, with a trend toward fewer ketone bodies in the treated group than in the control



group. Valdecabres et al. (2018) reported that oral calcium supplementation reduced the incidence of subclinical ketosis in multiparous cows during the post-partum period. The transitory increase in serum urea levels in the treated group can be considered a direct consequence of increased serum total calcium levels, since calcium is principally metabolized via the kidney (Massey and Whiting 1993). In addition, it is important emphasize that increased serum urea levels can indicate substantial catabolism of amino acids, because urea is a major nitrogenous end-product of protein and amino acid catabolism (Gowda et al. 2010).

In cow calving and post-partum, there is an increase in the inflammatory response (LeBlanc 2012), as it is known that inflammatory activity is one factor associated with reduced CK (Lee et al. 2000), and the study indicates that inflammation may initiate a process leading to reduction in CK levels, i.e. the CK may have an anti-inflammatory effect (Bekkelund and Johnsen 2018). However, in our study have increase in serum CK activity observed in calcium-treated animals can be considered an attempt on the part of the organism to provide energy in a period of negative energy balance, such as the post-partum period. This occurs because CK catalyzes the reversible conversion of creatine and ATP to phosphocreatine and ADP (Wallimann et al. 1992), and phosphocreatine serves as an energy reservoir that rapidly produces ATP. Thus, CK is a crucial enzyme for generation of phosphocreatine, functioning as an energy buffer and as an energy carrier (Schlattner et al. 2006). However, it is important emphasize that the augmentation of CK activity can be considered an adverse effect of calcium supplementation, since CK is released into the circulation from damaged cells (e.g., damaged muscles) (Kim et al. 2016). This can be corroborated by the positive correlation between calcium and CK serum levels during myocardial infarction (Speich et al. 1988). In the present study, we observed that animals treated

with calcium had lower serum glucose levels on the second day post-partum than did the control group, demonstrating that calcium can influence carbohydrate metabolism. Glucose reduction may be explained by the fact that glucose is a substrate for lactose synthesis; this phenomenon was observed in cows receiving calcium that produced a higher percentage of lactose in their milk. In agreement with our observations, Valdecabres et al. (2018) demonstrated that Jersey cows prophylactically treated with oral calcium in the post-partum period had lower serum glucose levels than did the control group, while no difference was observed in this parameter in Holstein dairy cows treated orally with calcium boluses (Jahani-Moghadam et al. 2018). Finally, we observed that calcium also altered lipid metabolism, demonstrated by increased triglycerides and decreased cholesterol levels, change that we do not find an explanation in the literature.

We observed that fat and lactose content was higher in animals treated with calcium than in the control group, contrary to observations by Jahani-Moghadam et al. (2018), who no observed difference in milk composition in Holstein dairy cows treated with oral calcium, and contrary as well to observations by Amanlou et al. (2016) in Holstein cows treated subcutaneously with calcium. More recently, Domino et al. (2017) demonstrated that two sources of calcium (calcium gluconate or calcium bolus) did not affect the milk composition of multiparous Holstein cows. It is important emphasize that increases in these parameters occurred only in one moment of sample collection, and then returned to the same values as the control group. The increase on milk fat content can be directly associated with augmentation of calcium via diet, since several studies have demonstrated the effects of calcium on lipid metabolism (Fu et al. 2011). However, this alteration should not raise concern, since the results demonstrated that it was a transitory effect.



### CONCLUSION

The use of a single dose of calcium subcutaneously maintained calcium levels above 8 mg/dL, and this avoided the subclinical hypocalcemia observed in five cows in the control group. In addition, calcium application produced few alterations in milk composition at the evaluated time points, all of which can be considered positive effects. Thus, this prophylactic treatment can be considered an approach to avoid hypocalcemia in dairy cows during the post-partum period. However, it is important to emphasize that the most appropriate method to prevent hypocalcemia is the use of anionic diet in pre-partum (Albani and Da Silva 2017).

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### AUTHOR CONTRIBUTIONS

Albani, Lopes and Da Silva planned the experiment and performed; Campigotto and Baldissera participated in the laboratory analysis. All authors participated in the discussion of the results and writing of the manuscript.

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