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Metabolic imbalances, hoof injuries, and metabolic profile of high-producing Holstein × Gir cows showing lameness[1]

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ABSTRACT.- Cucunubo Santos L.G., Breda J.C.S., Cerri F.M., Flabian K.K.M.C., Facury Filho E.J. & Lisbôa J.A.N. 2022. **Metabolic imbalances, hoof injuries, and metabolic profile of high-producing Holstein × Gir cows showing lameness.** *Pesquisa Veterinária Brasileira 42:e07107, 2022*. Departamento de Clínicas Veterinárias, Centro de Ciências Agrárias, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid PR-445 Km 380, Cx. Postal 10011, Londrina, PR 86057-970, Brazil. E-mail: janlisboa@uel.br

This study attempted to determine the associations between metabolic imbalances and lameness or hoof injuries in high-producing Holstein × Gir cows, and to determine whether the metabolic profile affects the occurrence of lameness. Eighty cows were followed from -60 to 60 days relative to calving and hoof injuries were reported on days -60, 7 and 60. Locomotion score (LS), body condition score (BCS), the concentrations of non-esterified fatty acids, β-hydroxybutyrate, glucose, cholesterol, albumin, total protein, blood urea nitrogen (BUN), calcium, phosphorus, and magnesium, and the activity of aspartate aminotransferase were determined at days -42, -21, -7, 0, 7, 21 and 42. The McNemar and Chi-square tests were used to compare frequencies of lameness and hoof injuries over time and to verify the associations between lameness, BCS, hoof injuries, and metabolic disorders. Two-way repeated measures ANOVA was used considering groups (non-lame × lame cows) and variations of BCS and metabolites over time. Lameness and hoof injuries increased between days -60 (20% and 66.3%) and 60 (44.7% and 98.6%). Excessive postpartum loss of BCS (P=0.017) and subclinical hypocalcemia (P=0.012) were associated with lameness on day 60. In general, the metabolic profile did not differ between lame and non-lame cows but cholesterol, albumin, BUN and magnesium concentrations were higher in non-lame cows. The postpartum decrease in BCS can affect the occurrence of lameness, and the metabolic profile of lame cows shows little difference from that of non-lame cows.

INDEX TERMS: Dairy cow, transition period, body condition score, metabolic disorders, hoof lesions, lameness, bovine.

RESUMO.- [**Desequilíbrios metabólicos, lesões nos cascos e perfil metabólico de vacas Holandesa × Gir de alta produção apresentando claudicação.**] Este estudo objetivou verificar as associações entre desequilíbrios metabólicos e claudicação ou lesões nos cascos em vacas mestiças Holandesa × Gir de alta produção e determinar se o perfil metabólico afeta a ocorrência de claudicação. Oitenta vacas foram acompanhadas de -60 a 60 dias em relação ao parto e as lesões nos cascos foram avaliadas nos dias -60, 7 e 60. O escore de locomoção (EL), o escore de condição corporal (ECC), as concentrações de ácidos graxos não esterificados, β-hidroxibutirato, glicose, colesterol, albumina, proteína total, nitrogênio ureico no sangue (BUN), cálcio, fósforo e magnésio e a atividade da aspartato aminotransferase foram determinados nos dias -42, -21, -7, 0, 7, 21 e 42. Os testes de McNemar e de Qui-

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quadrado foram empregados para comparar as frequências de claudicação e de lesões nos cascos ao longo do tempo e para verificar as associações entre claudicação, ECC, lesões nos cascos e distúrbios metabólicos. A análise de variâncias de medidas repetidas bifatorial foi usada considerando-se grupos (vacas com claudicação × vacas sem claudicação) e variações de BCS e de metabólitos ao longo do tempo. A claudicação e as lesões nos cascos aumentaram entre os dias -60 (20% e 66,3%) e 60 (44,7% e 98,6%). A perda excessiva de ECC no pós-parto (*P*=0,017) e a hipocalcemia subclínica ao parto (*P*=0,012) foram associadas com claudicação no dia 60. Em geral, o perfil metabólico não diferiu entre vacas com e sem claudicação, mas as concentrações de colesterol, albumina, BUN e magnésio foram maiores em vacas sem claudicação. A redução do ECC no periodo pós-parto pode afetar a ocorrência de claudicação, e o perfil metabólico das vacas claudicantes apresenta pouca diferença em relação ao das vacas não claudicantes.

TERMOS DE INDEXAÇÃO: vaca leiteira, período de transição, escore de condição corporal, distúrbios metabólicos, lesões do casco, claudicação, bovinos.

INTRODUCTION

Lameness is a very important problem in cows, affecting animals welfare, interfering with natural behavior, ovarian activity, and estrus intensity (Whay & Shearer 2017), and causing economic losses due to decreased productivity of dairy herds, treatment costs (Liang et al. 2017), and compromised reproductive efficiency (Charfeddine & Pérez-Cabal 2017). The intensity of lameness is related to the presence and severity of hoof injuries, which increase the sensitivity to mechanical nociceptive stimuli (Whay et al. 1997). Changes in the locomotion score (LS) vary depending on the type of hoof injury, and lesions such as sole ulcer, double sole, interdigital dermatitis, and fissure of the white line markedly affect the LS (Tadich et al. 2010, Bran et al. 2018, Moreira et al. 2019).

Postpartum body condition score (BCS) is associated with the occurrence of lameness, and cows with BCS <3.0 have a higher risk (Hoedemaker et al. 2009, Bran et al 2018). This finding could be explained by the relationship between the BCS and the thickness of the digital cushion. The lower the BCS is, the lower the thickness is, increasing the presentation of hoof injuries, mainly sole ulcer (Bicalho et al. 2009, Newsome et al. 2017). This relationship between BCS and the digital cushion by itself does not explain the presentation of injuries, and other genetic and hormonal factors and physiological states are likely to be involved (Newsome et al. 2017). High-producing dairy cows have a negative energy balance (NEB) in the transition period, causing them to lose BCS. Large losses of BCS are associated with metabolic disorders (Allen & Piantoni 2013), which could influence the occurrence of lameness.

The prevalence of lameness in dairy herds in Brazil is between 16% and 50%, including observations in Holstein (Costa et al. 2018, Moreira et al. 2019) and Holstein × Gir cows (Moreira et al. 2019). Holstein × Gir (Zebu) cows are crosses that have high milk yield and high rusticity, which makes them important in tropical countries (Santana et al. 2014). In these cows a high presentation of hoof injuries such as heel erosion, white line fissure, and sole hemorrhages was reported (Moreira et al. 2018).

Few studies have investigated the relationship between energy metabolism or metabolic profile and the occurrence of lameness (Ristevski et al. 2017, Paiano et al. 2019) or hoof injury (Sepúlveda-Varas et al. 2018). All of these studies involved observations in Holstein cows. Only one study was carried out with Holstein × Gir cows, but investigated the physical characteristics of the hoof (Casagrande 2013). Our hypothesis was that the presentation of lameness or hoof injuries in high producing Holstein × Gir cows is associated with metabolic imbalances in the transition period and with the loss of BCS in early lactation and that the metabolic profile is different between lame and non-lame cows. The objective of this study was to determine the associations between metabolic imbalances and lameness or hoof injuries, as well as to determine whether the metabolic profile interferes with the occurrence of lameness in the pre- and postpartum periods.

MATERIALS AND METHODS

The study presented here was a longitudinal and prospective cohort study with convenient sample and was part of a PhD thesis (Cucunubo Santos 2020). The project was approved by the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Estadual de Londrina" (UEL), Brazil, under protocol number 24111.2017.23, and was carried out in accordance with Brazilian law and guidelines of National Council for Animal Experiment Control (CONCEA). Eighty Holstein × Gir cows with 7 months of gestation were selected in a dairy farm located in Minas Gerais, Brazil (19°38'09.5" S, 44°56'14.7" W, and 698m altitude). The average production was 13,600kg per day, with 620 lactating cows.

The study was performed from May 2018 to February 2019, and the average minimum temperature varied from 12 to 20°C, while the maximum varied from 28 to 31°C. The studied cows were in their second and third (n=60) or fourth to sixth (n=20) pregnancies.

Cows in the dry period (60 to 21 prepartum days) were kept on grass pasture (*Panicum maximum*) with free access to water and mineral salt, receiving corn silage eventually according to the pasture availability. During the transition period and lactation, the cows were kept in an open confinement system, being fed a total mixed ration (TMR) calculated for each stage (Table 1). In addition to the TMR, lactating cows had access to pastures during the rainy months (November to February). The open confinement system was characterized by roofless corrals with unpaved ground, except the feeding area to avoid the accumulation of feces and mud. The cows had good available individual space (10 to 20m^2).

Cows were milked twice a day using a mechanical milking system. The distance between the corral and the milking parlor was 100m with a regular paved floor. The cows waited up to 30 min in the waiting area. Once a week they passed through a footbath (3.0×1.5×0.2m) containing 5% formaldehyde located at the milking parlor exit. Preventive hoof trimming was routinely performed on all cows on the farm only once a year. This procedure occurred two months before the study began.

All procedures were performed by a single trained examiner. BCS was determined with the aid of BCS Cowdition app (Bayer Animal Health, Germany), available for smartphones, using the scale described by Ferguson et al. (1994), while the LS was determined using a scale from 1 to 5 described by Sprecher et al. (1997). BCS and LS were evaluated on days -60 (-55±5), -21 (-19±4), -7 (-6±2), 0, 7, 21, 42, and 60 relative to calving. The 4 limbs were examinated to determine the presentation of hoof injuries on days -60, 7 and 60 relative to calving. The hoofs were subjected to a simple and superficial cleaning, removing 2mm of sole with a hoof knife. The hoof injuries were defined according to the standardized classification (ICAR 2020, Borges et al. 2017). The severity of the injuries was also evaluated using the methodology of Tadich et al. (2010) and Miguel-Pacheco et al. (2017). Mild, moderate, and severe injuries were classified as degree 1, 2, and 3, respectively.

Blood samples were taken by coccygeal venipuncture on days -42 (-40±4), -21 (-19±4), -7 (-6±2), 0, 7, 21, and 42 relative to calving. Vacuum tubes without anticoagulant and with EDTA and sodium fluoride were used, and serum and plasma were obtained after centrifugation (2,000 × *g* for 10min) and kept frozen (-20°C) in 2mL aliquots until processing. Serum concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), cholesterol, albumin (Alb), total protein (TP), urea nitrogen (BUN), total calcium (Ca), magnesium (Mg), and phosphorus (P); plasma concentration of glucose; and serum activity of aspartate aminotransferase (AST) were measured in an automated spectrophotometer (Dimension Xpand Plus®; Siemens; São Paulo/SP, Brazil).

At days 7 and 42 of lactation, 200mL of ruminal fluid was taken by ororuminal tube 4 hours after the morning feeding. The first 180mL were discarded and the pH was measured using a portable digital pH meter (Akso Exact Instrument®; China).

The McNemar test was applied to compare proportions of cows with and without lameness and/or hoof injuries at different times relative to calving (-60 × 7 days, -60 × 60 days, and 7 × 60 days). Considering the specific days -60, 7 and 60 relative to calving, the associations between the presentation of lameness and the low BCS, the presence of hoof injury, and the presentation of pre- and postpartum metabolic imbalances were tested by Chi-square or by the exact Fisher test. On the same specific days, the associations between the loss of BCS in the postpartum period (0 to 60 days in milk; DIM) with lameness or the presence of some hoof injuries were also tested. For this, the BCS loss was classified as moderate (≤0.5) and excessive (≥0.75).

For analysis of the metabolic profile, the cows were grouped into lame and non-lame cows, in the prepartum and in the postpartum periods. Two-way repeated measures ANOVA was used to test the effect of time relative to calving (-42, -21 -7, 0, 7, 21, and 42 days), the effect of lameness (non-lame × lame cows), and the interaction between these two factors. All measured metabolites and the BCS were included in these analyses. Two-way ANOVA was used to compare the pH of rumen fluid in lame and non-lame cows in the postpartum period at the two times of sample collection (7 and 42 DIM). Tukey's test was used for all multiple comparisons. An error probability of 5% was admitted for all tests. The SigmaPlot package for Windows 13.1 (Systat Software Inc., San Jose, California) was used for statistical analysis. In the specific case of McNemar test, the OpenEpi program was used[6].

Table 1. Ingested diets in the prepartum transition period and in the first 100 days of lactation by high-producing Holstein × Gir cows

	Prepartum*		Postpartum	
	(kg DM/d)	% (DM)	(kg DM/d)	% (DM)
Ingredients				
Corn silage	8.3	82.2	10.5	52.71
Dried citrus pulp	-	-	1.5	7.53
Soybean meal	-	-	0.72	3.61
Lactating concentrate feed	-	-	5.40	27.52
Cotton seed	-	-	1.8	9.03
Prepartum concentrate feed	1.8	17.8	-	-
Chemical composition				
Dry matter (%)	48.8		48.4	
Net energy (mcal/kg)	2.12		2.27	
Crude protein (%)	15.2		15.6	
NDF (%)	32.3		36.1	
ADF (%)	16.7		12.9	
EE (%)	3.93		4.16	
Ca (%)	1.10		0.55	
P (%)	0.24		0.30	
Mg (%)	0.40		0.57	
K (%)	0.97		1.12	
Na (%)	0.43		0.62	
S (%)	0.44		0.25	
Cl (%)	1.10		0.52	
DCAD (mEq/100g DM)	-14.9		25.4	

* Period of intake: from 21 day before calving; Diets formulated according to the NRC (2001) for high-producing Holstein cows weighing approximately 650kg before calving and 580kg at the beginning of lactation; DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, EE = ethereal extract, DCAD = dietary cation-anion difference.

RESULTS

The studied cows yielded 7,461±2,096kg of milk in 291±65 DIM. The presentation of lameness and hoof lesions varied differently over time, increasing in the postpartum period (Fig.1, Table 2). Most affected cows had mild lameness. At 60 DIM, a total of 476 lesions were found in the examined hoofs, and 80.8% of these injuries were classified as mild. Severe injuries were infrequent (2.4%). The most frequent injuries were heel erosion and axial wall fissure, while other infectious injuries were rare (Table 2).

There was a trend of association between lameness and low BCS (Table 3). This condition was found in 47.6% (10/21) of lame cows and 23.7% (14/59) of non-lame cows at 7 DIM, and in 76.4% (26/34) of lame cows and 52.3% (22/42) of non-lame cows at 60 DIM. Lameness was associated with hoof

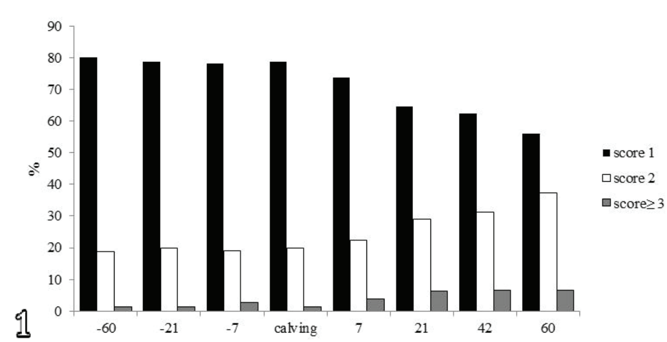

Fig.1. Percentage of lameness in high-producing Holstein × Gir cows from 60 days pre-calving to 60 days in milk. Locomotion score: 1 = healthy cow; 2 = mild lameness; 3 or 4 = moderate or severe lameness.

[6] Available at <http://www.OpenEpi.com> Accessed on May 30, 2019.

injuries, with SCH on days -60 and 60, and with prepartum Alb<30g/L at 7 DIM. Pre- and postpartum NEB was not related to lameness (Table 3).

Postpartum BCS loss was moderate in 50 cows (65.8%) and excessive in 26 (34.2%). The magnitude of BCS loss was not related to the occurrence of lameness, heel erosion, axial wall or white line fissures, and sole hemorrhage or erosion, on days -60 and 7. However, on day 60, lameness was more frequent (*P*=0.017) in cows that had excessive BCS loss (17/26, 65.4%) compared to those that had moderate BCS loss (17/50, 34.0%). There was a trend towards a higher frequency of axial wall fissure (20/26, 76.9%, *P*=0.062) in cows with excessive loss of BCS compared to cows with moderate loss (26/50, 52.0%).

Lame cows in the prepartum period had lower concentrations of BHB, cholesterol, Alb, BUN, and Mg (Table 4). Interactions were shown for cholesterol and Mg. Cholesterol was higher in non-lame cows at 21 and 42 DIM (4.00±0.86 and 5.19±1.30mmol/L, respectively) compared to lame cows (3.44±0.98 and 4.07±1.36mmol/L, respectively). Mg was higher, at 7 and 21 DIM, in non-lame cows (0.91±0.15 and

Table 2. Associations comparing the presentations of lameness and/or hoof injuries in high-producing Holstein × Gir cows at days -60, 7, and 60 relative to calving

Occurrence	Day -60 (n = 80) n (%)	Day 7 (n = 80) n (%)	Day 60 (n = 76) n (%)	*P* value: Days -60 × 7	Days 7 × 60	Days -60 × 60
Lameness*	16 (20.0)	21 (26.3)	34 (44.7)	0.332	0.002	<0.001
LS 2	15 (18.8)	18 (22.5)	29 (38.1)	0.646	0.013	0.007
LS 3 or 4	1 (1.3)	3 (3.8)	5 (6.6)	ND	ND	ND
Hoof injuries	53 (66.3)	72 (90.0)	74 (98.6)	0.001	0.121	<0.001
Heel erosion	16 (20.0)	31 (38.8)	51 (67.1)	0.014	0.002	<0.001
White line fissure	12 (15.0)	52 (65.0)	26 (34.2)	<0.001	<0.001	0.014
Axial wall fissure	25 (31.2)	40 (50.0)	45 (60.5)	0.024	0.336	0.003
Sole hemorrhage‡	11 (13.8)	13 (16.3)	18 (23.7)	0.789	0.211	0.052
Digital dermatitis	0	3 (3.7)	3 (3.7)	ND	ND	ND
Septic pododermatitis	1 (1.2)	1 (1.2)	4 (5.2)	ND	ND	ND
Interdigital dermatitis	0	0	2 (2.6)	ND	ND	ND
Interdigital hyperplasia	0	2 (2.5)	2 (2.6)	ND	ND	ND
Just infectious type injury†	7 (8.8)	0	0	ND	ND	ND
Just non-infectious type injury††	35 (43.8)	43 (53.8)	21 (28.9)	0.859	0.004	0.090
Mixed injuries†††	10 (12.5)	33 (41.3)	53 (69.7)	<0.001	<0.001	<0.001
Lameness with degree 1 injury§	12 (15.0)	8 (10.0)	9 (11.8)	0.386	0.772	1.000
Lameness with degree 2 injury[a]	4 (5.0)	13 (16.3)	25 (32.9)	0.026	0.009	<0.001
Non-lame without injury	25 (31.2)	4 (5.0)	1 (1.3)	ND	ND	ND
Non-lame with degree 1 injury	34 (42.5)	43 (53.7)	18 (25.0)	0.044	<0.001	<0.001
Non-lame with degree 2 injury[b]	5 (6.3)	12 (15.0)	23 (30.3)	0.145	0.021	<0.001

* Lameness: locomotion score ≥2; LS = locomotion score; ‡ sole hemorrage: circumscribed form (frequent) and diffused form (infrequent); ND = not determined; few positive cows; † Digital dermatitis, interdigital dermatitis, heel erosion, or septic pododermatitis without other concomitant injuries; †† any type of injury not concomitant to the infectious type; ††† concomitant non-infectious and infectious injuries; § Injury severity: 1 = mild degree; 2 = moderate degree; and 3 = severe degree; [a] Degree 3 injury were observed in 1 cow at days -60 and 7, and in 3 cows at day 60; [b] degree 3 injury were observed in two cows at day 7 and in three cows at day 60.

Table 3. Associations between the presentation of lameness at days -60, 7, and 60 relative to calving in high-producing Holstein × Gir cows and the presentation of pre- and postpartum metabolic disorders

Occurrence	n	Day -60 16 lame cows	Day 7 21 lame cows	Day 60 34 lame cows
		P	P	P
BCS ≤2.75	*	0.465	0.076	0.054
Hoof injuries	**	0.007	ND	ND
Prepartum NEB (NEFA >0.4mmol/L) †a	16	0.130	0.656	0.902
Postpartum NEB (NEFA >0.7mmol/L) †a	31	0.070	0.850	0.443
SCH at calving (Ca <2.125mmol/L) [b]	43	0.014	0.536	0.012
Prepartum albuminemia <30g/L †c	33	0.282	0.047	0.107

Total n: 80 cows on days -60 and 7, and 75 cows on day 60; BCS = body condition score, NEB = negative energy balance, NEFA = non-sterifyed fatty acids, SCH = subclinical hypocalcemia, ND = not determined; * 36 cows at day -60, 24 cows at day 7, and 47 cows at day 60; ** 53 cows at day -60, 76 cows at day 7, and 74 cows at day 60; All lame cows at days 7 and 60 presented hoof injuries; † at any sampling time; Cut-off points established by [a] Mcart et al. (2013), [b] Goff. (2014), and [c] Ruprechter et al. (2018).

0.99±0.14mmol/L, respectively) than in lame cows (0.77±0.16 and 0.91±0.15mmol/L, respectively).

Considering the presentation in the postpartum period, lame cows showed lower values of BCS, cholesterol, glucose, Alb, BUN, Ca and Mg (Table 4). Interactions were verified for cholesterol and glucose. Cholesterol was lower in lame cows on days -42 (2.80±1.10mmol/L), 7 (2.18±0.56mmol/L), 21 (3.36±0.82mmol/L) and 42 (4,168±1.38mmol/L), compared to non-lame cows (3.20±1.22mmol/L, 2.71±0.67mmol/L, 4.18±0.84mmol/L, and 5.40±1.17mmol/L, respectively). On calving day, glucose was also lower in lame cows (4.81±2.04mmol/L vs. 5.70 ±2.24mmol/L).

The pH of rumen fluid was lower (P<0.001) in lame (6.54±0.25) than non-lame (6.71±0.31) cows, as well as on day 7 (6.56±0.30) compared to day 42 (6.74±0.24).

DISCUSSION AND CONCLUSION

The occurrence of lameness increased after calving, reinforcing previous observations in Holstein cows (Whay et al. 1997), and the presentation of moderate and severe lameness was higher at 60 DIM (6.6%). This frequency was lower than that reported in Holstein × Gir (16%) (Moreira et al. 2018) and Holstein cows (42.5%) (Costa et al. 2018). The frequency of mild lameness at 60 DIM (38.2%) was, in its turn, higher than that reported by Moreira et al. (2018) (18%). The low prevalence of lameness indicates good farm management and can be the result of the hoof trimming performed 2 months before the start of the study: however, a single hoof trimming does not always have a positive effect on cows with moderate lameness (García-Muñoz et al. 2017). The foot baths containing formaldehyde, used routinely in the studied herd, are considered one of the effective preventive measures for lameness (Greenough 2007c).

Hoof injuries also increased in early lactation, affecting almost all cows, and heel erosion was the most frequent injury. These results are similar to previous observations in Holstein × Gir cows (Moreira et al. 2018). Axial wall fissure also presented high occurrence in our study, which contrasts with the claim that it has low prevalence in Brazil (Borges et al. 2017). This type of injury is common in cows kept in pasture systems and is associated with long-distance walking on trails with abundant gravel (Somers & O'Grady 2015). The studied

Table 4. Metabolic profile of high-producing Holstein × Gir cows grouped by presentation of lameness in the prepartum period and in early lactation. Effect of group (G), effect of days relative to calving (D), and interaction between the two factors

	Groups		SEM	P value		
	Non-lame*	Lame*		G	D†	G × D
Prepartum period (-60 days to calving)						
n	63	17				
BCS	3.11	2.912	0.029	0.054	<0.001	0.898
NEFA (mmol/L)	0.491	0.476	0.026	0.476	<0.001	0.980
BHB (mmol/L)	0.442	0.388	0.024	0.025	<0.001	0.091
Cholesterol (mmol/L)	3.003	2.654	0.090	0.043	<0.001	<0.001
Glucose (mmol/L)	3.627	3.524	0.126	0.302	<0.001	0.423
AST (U/L)	71.06	69.714	2.833	0.707	<0.001	0.243
Protein (g/L)	77.08	77.26	4.65	0.961	0.015	0.911
Albumin (g/L)	34.31	31.61	0.630	<0.001	<0.001	0.740
BUN (mmol/L)	8.410	7.380	0.380	0.016	<0.001	0.628
Ca (mmol/L)	2.204	2.180	0.022	0.369	<0.001	0.344
P (mmol/L)	1.937	1.898	0.041	0.397	<0.001	0.578
Mg (mmol/L)	0.970	0.912	0.015	0.019	<0.001	0.028
Postpartum period (calving to 60 days)						
n	51	29				
BCS	3.160	2.922	0.022	0.008	<0.001	0.155
NEFA (mmol/L)	0.488	0.488	0.022	0.997	<0.001	0.354
BHB (mmol/L)	0.430	0.432	0.021	0.929	<0.001	0.955
Cholesterol (mmol/L)	3.119	2.596	0.076	<0.001	<0.001	<0.001
Glucose (mmol/L)	3.673	3.484	0.106	0.025	<0.001	0.039
AST (U/L)	69.195	73.628	2.428	0.144	<0.001	0.486
Protein (g/L)	78.05	75.76	3.950	0.477	<0.001	0.746
Albumin (g/L)	34.74	31.98	0.530	<0.001	<0.001	0.790
BUN (mmol/L)	9.220	7.620	0.324	0.014	<0.001	0.080
Ca (mmol/L)	2.217	2.167	0.019	0.027	<0.001	0.125
P (mmol/L)	1.194	1.908	0.035	0.413	<0.001	0.807
Mg (mmol/L)	0.974	0.929	0.013	0.030	<0.001	0.218

* Healthy: locomotion score = 1, Lameness: locomotion score ≥2 in at least 2 consecutive times; The locomotion score and the BCS were evaluated at -60, -21, -7, 0, 7, 21, 42, and 60 days relative to calving; † Blood samples were taken at -42, -21, -7, 0, 7, 21, and 42 days relative to calving; SEM = standard error of mean, BCS = body condition score, NEFA = non-sterifyed fatty acids, BHB = beta-hydroxybutyrate, BUN = blood urea nitrogen, AST = aspartate aminotransferase.

cows were kept on a compacted dirt floor containing small stones, which could explain the high prevalence. However, the stones were present in relatively small quantities.

Hoof injuries were associated with presentation of lameness. However, some cows showed hoof injuries but no changes in the LS, reinforcing that the LS may not be a sensitive indicator of the presence of all types of injuries (Tadich et al. 2010, Moreira et al. 2018). The excessive postpartum loss of BCS was related to the occurrence of lameness at 60 DIM, tending to be associated with the occurrence of some hoof injuries. Low BCS in early lactation is a risk factor for the presentation of lameness in high-producing Holstein cows kept in confined (Hoedemaker et al. 2009, Ristevski et al. 2017) or in pasture (Alawneh et al. 2014) systems. This was also shown for Holstein × Gir cows (Moreira et al. 2019). Bicalho et al. (2009) found a direct relationship between BCS and the thickness of the digital cushion and demonstrated that the presentations of sole ulcer and white line disease were associated with decreased thickness of the digital cushion. However, the results of Newsome et al. (2017) indicate that despite this relationship, other predisposing factors, such as the integrity of the suspensory apparatus, calving, physiological and hormonal status, genetics, and the previous existence of hoof injuries, may be even more important.

No association was found between NEB and lameness, and the occurrence of subclinical ketosis was infrequent (8.7%), which could indicate that the cows presented a good energy balance. The concentrations of NEFA and BHB did not differ between lame and non-lame cows in the postpartum period, but BHB concentrations were higher in lame cows before calving. Since the concentration was lower than 1.2mmol/L, which is considered the critical value indicator of subclinical ketosis (McArt et al. 2013), it can be assumed that the difference does not have biological importance. To date, there is poor information on the relationship between metabolic status and the presentation of lameness and hoof injuries. It was shown that cows with postpartum hoof injuries (Sepúlveda-Varas et al. 2018) and cows with severe lameness (Paiano et al. 2019) had higher concentrations of NEFA and BHB. In Holstein cows, it was shown that high milk yield, low BCS and high concentration of BHB are important risk factors for the occurrence of chronic lameness (Ristevski et al. 2017). NEFA and BHB are energy metabolite indicators of lipomobilization and high concentrations in the transition period are associated with a higher risk for some diseases (Allen & Piantoni 2013, McArt et al. 2013). However, there are no cutoff points related to the occurrence of lameness or hoof injuries.

The contrast between our results and those of these other researchers can probably be explained by the fact that most of the studied cows had mild lameness and a mild degree of hoof injuries. In contrast, Ristevski et al. (2017) and Paiano et al. (2019) studied cows with moderate to severe lameness and Sepúlveda-Varas et al. (2018) studied cows that had severe hoof injuries. In these situations, the involvement of energy metabolism could be more pronounced. Zhang et al. (2015) also found no differences in NEFA and BHB concentrations in cows with severe lameness compared to healthy cows in early lactation. However, the number of cows per group was very small.

In relation to cholesterol, the lame cows showed lower concentrations mainly in the postpartum period, which agrees with previous observations (Paiano et al. 2019). Cholesterol is considered an indicator of energy balance, especially in early lactation, since its concentration is positively related to dry matter intake (Cavestany et al. 2005). On the other hand, cows with postpartum diseases have lower concentrations (Sepúlveda-Varas et al. 2015, Ruprechter et al. 2018), which could reflect lower food intake. The effects of lameness or hoof injuries on cholesterol metabolism should be clarified. Cows with clinical laminitis have been shown to have higher expression of ApoA-IV apoliprotein and HMGCR membrane glycoprotein, which are related to cholesterol transport and biosynthesis, respectively (Dong et al. 2015). It is uncertain whether this phenomenon also occurs in other digital diseases.

Holstein × Gir cows that showed lameness, before or after calving, showed slightly lower concentrations of Alb and BUN, but the TP did not differ from non-lame cows. Similar results for TP and Alb have already been reported in lame Holstein cows at the beginning of lactation (Paiano et al. 2019). In contrast, cows with chronic laminitis may have lower concentrations of TP (Belge et al. 2004). Alb is a negative acute phase protein that can predict postpartum diseases when decreased before calving (Bertoni et al. 2008, Ruprechter et al. 2018). The concentrations of BUN, in turn, vary according to dietary protein and energy levels (Cavestany et al. 2005), and the synthesis of urea in the liver may be decreased due to the presence of various inflammatory processes at the beginning of lactation, which compromise liver functions (Bertoni et al. 2008). Therefore, the inflammatory nature of the injuries seen in the hooves could explain the results for Alb and BUN. Impaired liver function, if present, can be considered mild in the studied cows and was not accompanied by increased AST activity.

Regarding minerals, SCH at calving was the main imbalance observed in the studied cows and was associated with lameness at 60 DIM, but not at 7 DIM. We are unaware of other studies on the subject that can be compared. The biological importance of the observed relationship can be considered doubtful, since hypocalcemia is a transient imbalance close to calving, while the frequencies of lameness and hoof injuries gradually increase up to 60 days after calving. The concentrations of Ca and Mg were slightly lower in cows with lameness. Normally serum calcium is linked to protein, especially albumin, which can influence the total calcium serum concentration (Goff 2014) and could explain our results since lame cows also had lower Alb concentrations, just as previously reported (Paiano et al. 2019). There are no other studies that have investigated Ca, Mg or P values in cows with lameness or hoof injuries. However, in Holstein × Gir cows, serum Ca and Mg showed a positive correlation with hoof hardness and growth at the beginning of lactation, while serum P showed a negative correlation with hoof hardness (Casagrande 2013). Ca and Mg participate in the synthesis of keratin, and the deficiencies of these minerals decrease the quality of the hoof (Greenough 2007b), which can result in a greater presentation of injuries and lameness. Despite the transient SCH, the studied cows maintained physiological serum values of Ca, P and Mg and ingested sufficient amounts of these minerals, indicating that they were balanced.

Based on the pH values of the rumen fluid, it was shown that the studied cows did not present subacute ruminal acidosis (SARA), considered a primary cause of laminitis (Greenough 2007a). A pH value ≤5.9 confirms the diagnosis of SARA when the ruminal fluid is obtained through an oro-ruminal tube (Abdela 2016). Although lame cows had a lower pH than non-lame cows, rumen fermentation disorder must be ruled out as a factor influencing the results.

This is one of the few studies that investigated the relationship between the metabolic profile and the occurrence of lameness or hoof injury, being the first with Holstein × Gir cows, a breed suitable for tropical countries with high milk yield capacity. The variations in the metabolites throughout the prepartum transition period and the beginning of lactation were compatible with those already reported in Holstein (Cavestany et al. 2005) and Holstein × Gir (Moreira et al. 2015) cows, which indicates that the studied cows remained reasonably balanced. Despite this, and although the majority of lameness cases were mild, the excessive loss of BCS after calving can contribute to the occurrence of lameness and hoof injuries at the beginning of lactation. Well-nourished cows with mild to moderate lameness may maintain a metabolic profile with little difference from cows without lameness. Further studies are needed to clarify whether there is a relationship between moderate to severe lameness and marked metabolic changes in high-yielding Holstein × Gir cows.

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