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Energetic status of crossbreed dairy cows during transition period in two different seasons

[*Perfil energético de vacas leiteiras mestiças no período de transição em duas estações do ano*]

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ABSTRACT

We used 31 crossbreed dairy cows to compare the energetic profile in summer and winter. Blood samples were taken weekly prepartum, at calving and on days 2, 5, 10, 15, 21 and 30 postpartum. All metabolic indicators analyzed were influenced by the physiological status. The glucose concentrations were higher during winter while the triglyceride concentrations and lactate dehydrogenase (LDH) were higher in the summer. The season influenced the concentrations of cholesterol, AST and GGT, showing a different pattern between summer and winter. Non-esterified fatty acids (NEFA) and beta-hidroxibutirate (BHB) were not influenced by the season. Cows that calved during winter had a greater body condition score (BCS) and lost more BCS until calving. During summer, 32.26% of the animals and 29.03% during winter had NEFA concentrations above the optimum level and 22.58% of the animals in summer and 19.35% in the winter had subclinical ketosis at some point during the transition period, making then more susceptible to diseases.

Keywords: NEFA, monitoring, herd health, heat stress

RESUMO

Foram utilizadas 31 vacas leiteiras mestiças para comparar o perfil energético em duas estações, verão e inverno. Amostras de sangue foram colhidas semanalmente durante o pré-parto, no dia do parto e nos dias dois, cinco, 10, 15, 21 e 30 do pós-parto. Todos os metabólitos analisados foram influenciados pelo estádio fisiológico. As concentrações de glicose foram maiores no inverno, enquanto as concentrações de triglicérides e lactato desidrogenase (LDH) foram maiores no verão. As concentrações de colesterol, AST e GGT mostraram um padrão de comportamento diferente entre o verão e o inverno. Ácidos graxos não esterificados (NEFA) e beta-hidroxibutirato (BHB) não foram influenciados pela estação do ano. As vacas que pariram durante o inverno tinham um escore de condição corporal (ECC) maior e perderam mais ECC até o parto. No verão, 32,26% e, no inverno, 29,03% dos animais apresentaram concentrações de NEFA acima do recomendado no pré-parto; 22,58% dos animais no verão e 19,35% no inverno tiveram cetose subclínica em algum momento do período de transição, tornando-os mais suscetíveis a outras doenças.

Palavras-chave: AGNE, monitoramento, saúde do rebanho, estresse por calor

INTRODUCTION

During the transition period of dairy cows, which is between three weeks pre-partum and three weeks postpartum, the body undergoes various physiological adaptations, causing large changes in the metabolic profile. All these adaptations occur in a short period of time and contribute to most of the health problems of dairy cows, including metabolic and infectious diseases.

The analysis of blood metabolites permits the evaluation of the incidence of metabolic disease in its clinical and subclinical forms, aiding in monitoring the energy, protein and mineral metabolism (Leblanc *et al.*, 2005; Mulligan *et al.*, 2006). This is part of the medicine production philosophy, which is based on prevention and monitoring of herd health in order to reconcile health, animal welfare and productivity (Leblanc, 2010; Stengärde, 2010).

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The intensification of milk production systems brings new challenges to maintain the health of each individual and the herd and thus the use of the metabolic profile of animals during the transition period has great potential to improve management and nutrition, making clear the deficient aspects and enabling faster, objective and efficient action. There is much information about the metabolic profile of Holstein cows, but little regarding crossbreed, which represent more than 70% of the milk production in Brazil (Facó *et al.*, 2002). Besides that, Brazil has a seasonal milk production due to differences in the availability of food and climate, with very different management and challenges faced in each season.

Therefore, we did the energy profile of crossbred Holstein x Gir dairy cows in a semi confinement system during two seasons for better understanding the metabolism of these animals during the transition period.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on Animal Experimentation (CETEA/UFMG) under protocol number 82/2011 and was done on a dairy farm in the municipality of Martinho Campos, Minas Gerais. The climate of the region is defined according to the Koppen classification as Aw, a tropical savanna climate with hot and rainy summers and dry and mild winters. The research consisted of two experimental periods, January to April 2011 (summer) and May to August 2011 (winter). The average temperatures were 25.5°C in the summer and 20.6°C in the winter. The maximum and minimum temperatures were respectively 36°C and 15.2°C in the summer and 34.3°C and 7.9ºC in the winter. The total precipitation was 707.5mm in the summer and 20.2mm in the winter.

The herd was composed of 250 lactating cows, producing 5,000 liters of milk per day. In each experimental period 31 pluriparous crossbred Holstein x Gir dairy cows were used, varying 5/8 to 31/32 Holstein with lactation order from second to seventh and average daily production of 20L of milk in the summer and 23L in the winter.

The animals remained in mixed paddocks of *Panicum maximum*, *Cynodon* sp. and *Brachiaria brizanta*. Furthermore, they received concentrate and corn silage mixed in the trough. During winter, the diet was formulated only with fodder and corn silage. All diets were formulated to meet nutritional requirements according to NRC (2001). In all paddocks there were drinkers and troughs for mineral supplementation.

Blood was collected weekly by puncture of the coccygeal vein during the prepartum, beginning in the fourth week before the calving expected date, followed by a harvest within 24 hours after calving, and in the second, fifth, 10th, 15th, 21th and 30th days postpartum. Blood collection was always done before feeding the animals. Blood samples were centrifuged and serum or plasma were separated and frozen for later analysis.

Concentrations of triglycerides, cholesterol, glucose, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), non-esterified fatty acids (NEFA) and beta-hidroxibutirate (BHB) were measured using the technique of spectrophotometry in an automatic device with commercial kits.

The body condition score (BCS) of each animal was assessed at all times of blood collection using a scale of 1 (one) to five (5) points, with minimal variation of 0.5 point, according to Dirksen (1993).

The design was a split plot arrangement, in which the seasons consisted of plots and the times of blood collection were the subplots. The metabolic averages between times of blood collection were compared by the Scott-Knott test and between seasons using the SNK test. For BCS analysis the Dunn Multiple Comparison was used for comparison between periods of the year and the Mann-Whitney test for comparison between assessments (Sampaio, 2002).

Using the BHB analysis, subclinical ketosis were diagnosed using as a criteria the concentrations above 1.2mmol/L according to LeBlanc (2010). The cutoffs NEFA above 0.4mmol/L in prepartum and 0.7mmol/L in the postpartum were used to assess the increased risk of disease according to LeBlanc *et al.* (2005) and Ospina *et al.* (2010).

RESULTS

The days of blood collection influenced all metabolic analyzed, showing the adaptation of animals throughout the transition period.

The glucose concentrations were higher in the winter while the triglyceride and LDH concentrations were higher in the summer (Table 1 and 2). Along the transition period, the season influenced the concentrations of cholesterol, AST and GGT, showing a different pattern between summer and winter. However, the concentrations of cholesterol, NEFA and BHB were similar in the two seasons (Table 1).

Cholesterol concentrations, despite not having varied, showed a different pattern during each season. It had a decline from the prepartum until calving, more marked in the summer, and increased after 10 days postpartum in the summer and 5 days postpartum in the winter, reaching the highest levels on day 30 postpartum in the summer and 21 days postpartum in the winter (Table 1 and Figure 1). Distinctly, the serum triglyceride concentration declined until calving and remained at the same level throughout the postpartum period in both seasons (Table 1).

The plasma glucose concentration in both seasons was lower postpartum when compared to prepartum and, at calving, it rose to higher levels in the whole experimental period (Table 1 and Figure 1).

The NEFA concentrations rose from one week prepartum until calving as can be seen in Figure 1. On the day of calving, the concentrations of BHB were also increased. Both were stable up to five days postpartum and subsequently the concentrations of BHB remained high, while NEFA declined (Table 1 and Figure 1).

Table 1. Mean concentrations of cholesterol, triglycerides, glucose (mg/dL), NEFA, BHB (mmol/L) in crossbred cows in the fourth (-4 wk.), third (-3 wk.), second (-2 wk.) and first (-1 wk.) weeks prepartum, on calving (Calv.) and in the second, fifth, 10th, 15th, 21th and 30th days postpartum in a semi-intensive system in summer and winter

	SEASON	TIME											Mean
		-4 wk	-3 wk	-2 wk	-1 wk	Calv.	2º d	5th d	10th d	15th d	21th d	30th d	
CHOLESTEROL	Summer	76.5aB	72.4aB	63.6aC	58.5aC	50.8aD	61.8aC	55.1aD	64.0aC	72.4aB	77.2aB	88.8aA	67.4
	SD	16.8	21.9	15.1	13.5	16.2	21.0	17.1	19.9	19.1	22.4	29.3	22.1
	Winter	72.5aD	67.0aD	59.7aE	51.2aE	52.7aE	53.4aE	66.6aD	84.4aC	102.7aB	115.9aA	124.3aA	77.3
	SD	20.5	20.1	18.1	15.1	27.1	13.1	13.1	17.5	30.4	29.1	30.8	33.3
	Average	74.5	69.7	61.7	54.9	51.8	57.6	60.8	74.2	87.6	96.6	106.5	72.3
	SD	18.8	21.0	16.6	14.7	21.9	17.9	16.1	21.2	29.5	32.4	34.8	28.8
TRIGLYCERIDES	Summer	43.9	41.2	42.1	35.0	28.7	26.5	29.6	27.2	32.2	26.0	25.2	32.50a
	SD	12.4	8.7	9.1	10.8	13.9	13.4	13.5	14.0	14.6	14.0	13.6	14.1
	Winter	37.3	33.0	31.8	29.1	18.0	20.6	20.2	23.8	23.5	21.3	22.4	25.55b
	SD	11.0	9.4	9.5	9.4	8.6	5.3	6.4	11.5	7.2	5.5	9.8	10.3
	Average	40.6A	37.1A	37.0A	32.0B	23.3C	23.5C	24.9C	25.5C	27.9C	23.7C	23.8C	29.0
	SD	11.9	9.9	10.6	10.4	12.8	10.5	11.5	12.8	12.2	10.7	11.8	12.7
GLUCOSE	Summer	45.4	44.9bB	42.7bB	44.3bB	50.7bA	41.5bC	38.7bD	34.6bE	37.6bD	38.8bD	40.9bC	41.8
	SD	4.7	4.8	3.3	4.2	16.4	7.3	8.2	7.1	7.3	7.4	7.3	8.8
	Winter	51.1	50.3aC	52.1aB	52.8aB	62.6aA	49.2aD	51.0aD	49.8aD	48.1aD	50.5aD	51.4aD	51.7
	SD	5.5	5.7	5.0	3.9	28.5	8.1	6.1	9.1	6.6	7.6	7.7	11.0
	Average	48.2	47.6	47.4	48.6	56.7	45.4	44.9	42.2	42.9	44.6	46.1	46.8
	SD	5.9	5.9	6.3	5.9	23.7	8.6	9.4	11.2	8.7	9.5	9.1	11.2
B H B	Summer	0.3	0.3	0.3	0.4	0.6	0.8	0.9	0.8	0.7	0.6	0.7	0.577a
	SD	0.1	0.1	0.2	0.2	0.5	1.2	1.3	0.9	0.7	0.5	0.6	0.7
	Winter	0.5	0.5	0.5	0.5	0.6	0.7	0.8	0.6	0.5	0.6	0.6	0.581a
	SD	0.2	0.2	0.2	0.2	0.5	0.4	0.8	0.4	0.2	0.3	0.2	0.4
	Average	0.39B	0.39B	0.38B	0.48B	0.60A	0.72A	0.84A	0.69A	0.59A	0.61A	0.65A	0.6
	SD	0.2	0.2	0.2	0.2	0.5	0.9	1.0	0.7	0.5	0.4	0.5	0.6
NEFA	Summer	0.0	0.1	0.1	0.3	0.3	0.3	0.4	0.2	0.2	0.2	0.1	0.21a
	SD	0.4	0.2	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.1	0.2
	Winter	0.2	0.1	0.2	0.2	0.3	0.2	0.3	0.3	0.2	0.2	0.2	0.21a
	SD	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.2
	Average	0.1	0.12D	0.16D	0.22B	0.29A	0.28A	0.32A	0.24B	0.19C	0.20C	0.15C	0.2
	SD	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2

Means followed by different capital letters in lines differ by the Scott-Knott test (P <0.05). Means followed by different lowercase letters in columns differ by the SNK test (P <0.05). SD = Standard deviation.

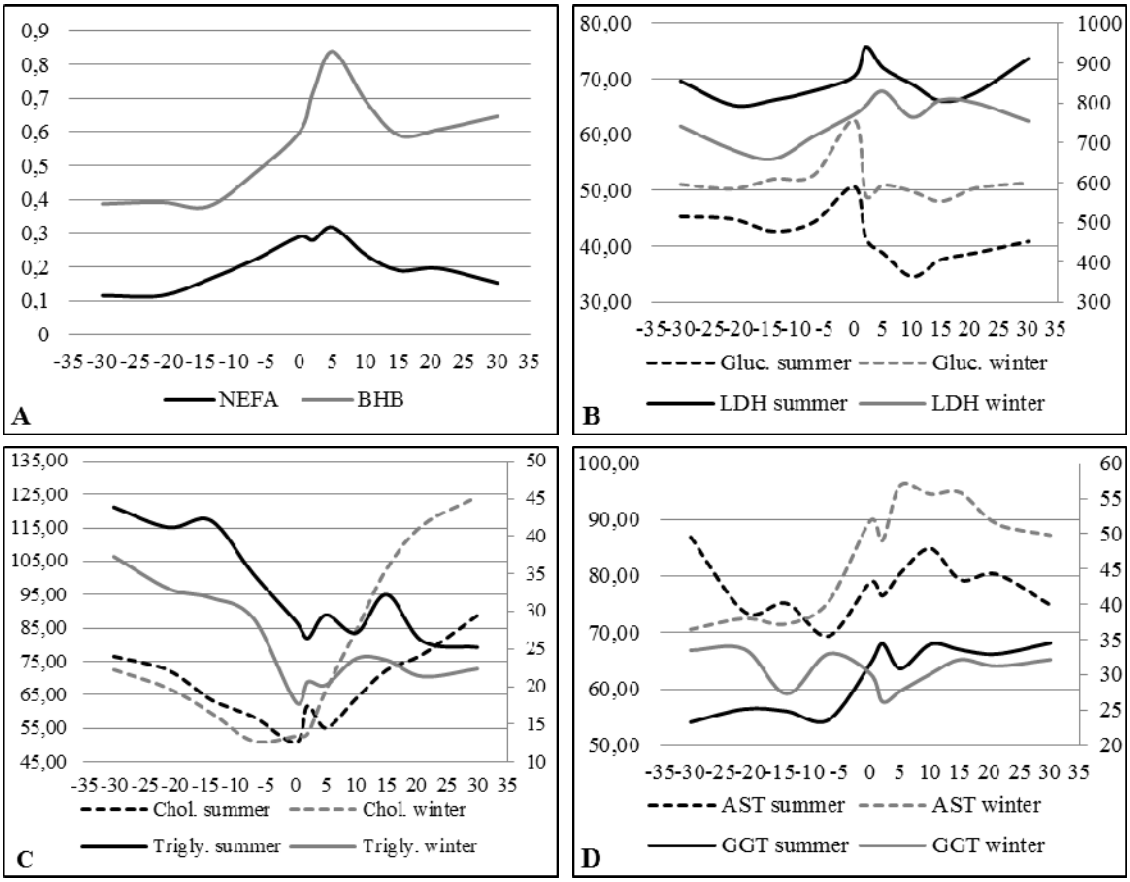

Figure 1. Effect of season during the transition period on NEFA and BHB (mmol/L) in Figure A, glucose (left axis; mg/dL) and LDH (right axis; U/L) in Figure B, triglycerides (left axis; mg/dL) and cholesterol (right axis; mg/dL) in Figure C, AST (left axis; U/L) and GGT (right axis; U/L) in Figure D.

The overall frequency of animals with NEFA values above 0.4mmol/L in prepartum was 32.26% in the summer and 29.03% in the winter. At calving and during the postpartum, no animal showed NEFA concentration above 0.7mmol/L (Figure 1).

Regarding the concentrations of BHB postpartum, 22.58% of the animals in summer and 19.35% in the winter had subclinical ketosis at some point. The maximum frequency of subclinical ketosis occurred on the fifth day postpartum, when 13.33% and 9.68% of cows had occurrences in the summer and winter, respectively.

The GGT activity in the summer and activities of LDH and AST increased postpartum (Table 2 and Figure 1). In the winter, the GGT activity varied more. Despite variation, the serum activity of GGT, AST and LDH always remained within the reference values for the species (Kaneko *et al.*, 2008; Smith, 2009).

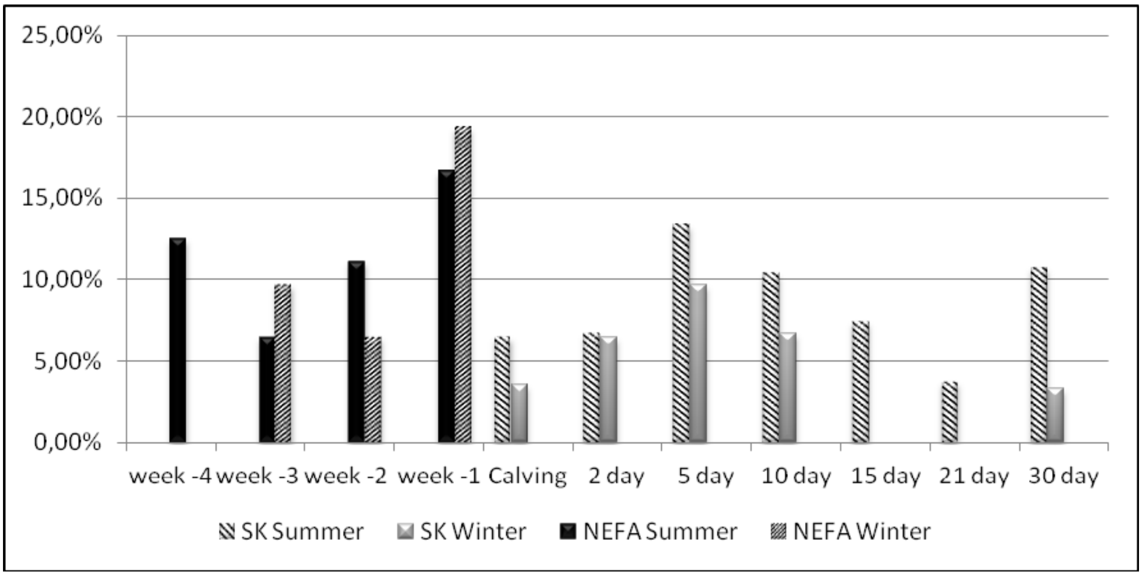

Figure 1: Incidence of subclinical ketosis (SK) and cows with serum concentrations of NEFA above 0.4mmol/L in prepartum and 0.7mmol/L in postpartum in a semi-intensive system in summer and winter.

Table 2. Average activity of GGT, AST and LDH (U / L) in pluriparous crossbred cows in the fourth (-4 wk.), third (-3 wk.), second (-2 wk.) and first (-1 wk.) weeks prepartum, on calving (Calv.) and in the second, fifth, 10th, 15th, 21th and 30th days postpartum in a semi-intensive system in summer and winter

	SEASON	TIME											Mean
		-4 wk	-3 wk	-2 wk	-1 wk	Calv.	2º d	5thd	10thd	15thd	21thd	30thd	
GGT	Summer	23.3bB	25.2bB	24.9aB	23.7bB	31.6aA	34.4aA	30.9aA	34.4aA	33.6aA	33.0aA	34.5aA	29.9
	SD	9.9	11.4	8.4	9.7	12.6	13.5	7.5	9.2	9.0	7.4	8.6	10.8
	Winter	33.5aA	33.7aA	27.4aB	33.0aA	30.2aA	26.2bB	27.7aB	30.1aA	32.2aA	31.3aA	32.2aA	30.7
	SD	19.6	16.4	11.1	11.8	13.3	12.4	12.9	10.4	12.2	12.9	13.3	13.4
	Average	28.4	29.4	26.1	28.4	30.9	30.4	29.3	32.3	32.9	32.1	33.4	30.3
	SD	16.6	14.7	9.9	11.7	12.9	13.5	10.7	10.0	10.6	10.5	11.3	12.2
AST	Summer	86.9aA	73.7aB	75.2aB	69.4aB	79.0aA	76.5aB	80.5aA	84.9aA	79.3aA	80.4aA	74.9aB	78.3
	SD	21.9	17.1	23.8	16.6	12.4	13.6	18.4	24.7	13.5	15.7	12.4	17.8
	Winter	70.6aB	72.5aB	71.6aB	75.5aB	89.8aA	86.3aA	96.2aA	94.5aA	94.8aA	89.3aA	87.3aA	84.3
	SD	11.8	14.1	11.0	15.8	15.7	14.9	22.0	24.3	20.7	20.1	19.0	19.9
	Average	78.8	73.1	73.4	72.5	84.4	81.4	88.3	89.7	87.1	84.8	81.1	81.3
	SD	18.4	15.5	18.3	16.0	14.9	14.6	21.6	24.7	19.1	18.5	17.2	19.1
LDH	Summer	855.4	795.0	807.4	830.7	867.9	941.0	887.7	847.4	805.2	826.2	912.0	852.3a
	SD	266.1	225.5	268.6	239.4	196.8	339.1	256.7	238.4	152.9	225.7	255.5	246.2
	Winter	742.2	683.0	659.4	716.6	771.3	793.9	829.3	764.3	808.0	800.3	754.9	756.6b
	SD	144.6	140.3	115.2	152.5	155.3	161.5	175.6	195.0	187.2	165.4	116.6	163.5
	Average	798.7B	738.9B	733.4B	773.6B	819.6A	867.4A	858.5A	805.8A	806.6A	813.2A	833.4A	804.5
	SD	215.0	195.9	222.6	207.2	183.4	272.3	219.4	221.0	169.7	195.4	210.0	214.0

Means followed by different capital letters in lines differ by the Scott-Knott test (P<0.05). Means followed by different lowercase letters in columns differ by the SNK test (P <0.05). SD = Standard deviation.

The cows that calved during winter had a greater BCS than cows that calved during summer (3.7 versus 3.4), but they lost more BCS until calving, when the BCS was similar between the two seasons. The mean of BCS lost was 0.7 for cows during the winter and 0.4 for cows in the summer (Table 3).

Table 3. Values of body condition score (BCS) of pluriparous crossbred cows in the fourth (-4 wk.), third (-3 wk.), second (-2 wk.) and first (-1 wk.) weeks prepartum, on calving (Calv.) and in the second, fifth, 10^{th}, 15^{th}, 21^{th} and 30^{th} days postpartum in semi-intensive system in summer and winter

		TIME										
		-4 wk	-3 wk	-2 wk	-1 wk	Calv.	2º d	5^{th}d	10^{th}d	15^{th}d	21^{th}d	30^{th}d
Summer	Median	3.4bABCD	3.5bAB	3.5bAB	3.5bA	3.5aAB	3.5aABC	3.5aABC	3.5aABC	3aCBD	3aD	3aCD
	Average	3.40	3.40	3.42	3.45	3.40	3.35	3.35	3.28	3.16	3.07	3.07
	Maxim	4.5	4.5	4.5	4.5	4.5	4	4	4	4	4	3.5
	Minimum	3	3	3	3	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Winter	Median	3.7aA	3.5aA	3.5aA	3.5aA	3.5aAB	3.5aABC	3.5aBCD	3aCD	3aCD	3aD	3aD
	Average	3.69	3.65	3.73	3.66	3.58	3.44	3.31	3.21	3.13	3.12	3.05
	Maxim	4.5	4.5	4.5	4.5	4.5	4	4	4	4	4	4
	Minimum	3	3	3	3	3	3	2.5	2.5	2.5	2.5	2.5

Means followed by different capital letters in lines differ by the Dunn's Multiple Comparison test ($P<0.05$). Means followed by different lowercase letters in columns differ by the Mann-Whitney test ($P<0.05$).

DISCUSSION

The rise in cholesterol levels after calving reflects an increase in the uptake of lipids in the liver, suggesting that the increase in cholesterol levels is associated with higher tissue mobilization, increase of food intake, greater synthesis of steroid hormones and lipoproteins, which are physiological processes of the postpartum (Stengärde, 2010; Kaneko *et al.*, 2008). The patters showed by cholesterol concentrations are very similar and related to food intake. Due to this relation, in the summer the variation of food intake was probably higher. A similar pattern was demonstrated by Godoy *et al.* (2004) in zebu and crossbreed cows.

The fall in triglycerides levels during postpartum occurs because of the use of triglycerides by mammary glands as precursors of milk fat (Bauman and Griinari, 2003). Aside from the mammary gland, the liver begins to remove more triglycerides from the bloodstream in lactating cows (Reynolds *et al.*, 2003).

The rise of glucose on calving day occurs very quickly, falling after few hours postpartum (Aquino Neto, 2012). This rise is a physiological process, a result of increased concentration of glucagon, catecholamines and glucocorticoids, mechanisms to prioritize the use of glucose by the mammary gland (Park *et al.*, 2010).

The results for plasma concentration of NEFA demonstrated that the mobilization of adipose tissue has intensified in the first week prepartum and remained higher until the fifth day postpartum as described also by Reynolds *et al.* (2003), LeBlanc (2005), Park *et al.* (2010) and Paula *et al.* (2011).

Increasing concentrations of BHB on calving day and postpartum occurred one week after the increase in NEFA, as this is the major cause of ketogenesis. This also demonstrates that the amount of NEFA that reached the liver was greater than the capacity of this organ to oxidize them completely (Ospina *et al.*, 2010).

The results show a situation in which although there were lower levels of glucose circulating in the summer there was no greater tissue mobilization demonstrated by the similarity of NEFA and BHB in the two seasons. By observing the BCS of animals, we can perceive an indication of the opposite (Table 3). Cows that calved in the winter began with higher BCS but lost more BCS until calving.

One factor that may explain these findings is heat stress. Heat stressed cows have increased insulin response, making them metabolically inflexible, decreasing the oxidation of NEFA and the use of ketone bodies as an energy source. Thus, cows under heat stress, even in negative energy balance, do not increase circulating concentrations of NEFA and BHB and have lower levels of glucose than cows on classical negative energy balance. This mechanism is a form of the cow producing less heat, since the oxidation of glucose releases a smaller amount of energy as heat oxidation NEFA (13% difference) (Wheelock, 2010; Baumgard and Rhoads, 2013).

The thermo neutral zone for dairy cattle is between 5ºC and 25°C and can vary depending on humidity (Ferreira *et al.*, 2009). Maximum temperatures during the two periods reached higher values than those considered ideal for dairy cattle, but the average and minimum temperatures and the precipitation are largely different between the two periods. With these variations, we observed that the animals were more likely to suffer from heat stress in the summer.

The frequency of animals with NEFA concentrations above 0.4mmol/L in the first week prepartum was 19.35% in the summer and 16.67% in the winter. These numbers are higher than the 10% suggested as optimum by Oetzel (2004). Animals that have concentrations of NEFA above 0.4mmol/L in prepartum are two to four times more likely to develop displaced abomasum, 1.8 times more likely to develop ketosis and retained placenta, 2.2 times more likely to have metritis and two times more likely to be discarded in the first 60 days in milk (LeBlanc, 2006; Ospina *et al.*, 2010).

The larger number of animals with subclinical ketosis (22.58% in the summer and 19.35% in the winter) is a warning, since these animals have 4 to 8 times more chance to have displaced abomasum, are less likely to become pregnant in the first service, produce less milk, have increased duration and severity of mastitis, are 2.3 times more likely to develop metritis and 4.9 times more likely to develop clinical ketosis (LeBlanc, 2006, Ospina *et al.*, 2010).

Regarding the increased activity of liver enzymes in the postpartum, the increase of the blood flow to the liver and the enhanced hepatic metabolism after calving may be the cause. In these circumstances there is a cellular adaptation (Reynolds *et al.*, 2003; Thall, 2012). Moreover, the large hepatic blood flow in postpartum may cause mild hypoxia, resulting in damage to hepatocytes. In these cases, often the values found were within normal (Thrall, 2012).

Particularly, the increasing concentration of LDH in the postpartum is a sign of the use of lactate for glucose formation near calving due to lack of other sources for gluconeogenesis, making the Cori cycle essential for this period (Reynolds *et al.*, 2003). Especially in the summer, when the activity of LDH was higher and serum glucose was lower, lactate may have played an important role to supply the energy needs.

The increased AST activity is related to the occurrence of catabolism of muscle tissue to use proteins for gluconeogenesis (Herdt, 2000). Similarly, Aquino Neto (2012) reported increased enzymatic activity of AST in the early postpartum due to muscular effort during calving, causing damage to muscle tissue and release of this enzyme (Fagliari *et al.*, 1998).

Despite the strong influence of seasons on the metabolic profile of animals, it is difficult to isolate the effect of different factors existing in summer and winter, such as temperature, rainfall and diet.

CONCLUSION

The metabolic profile of crossbred Holstein x Gir cows in a semi-confined system differ greatly between summer and winter, indicating that each season presents its own challenges, such as the heat stress that cows undergo during summer with decreased concentrations of glucose without incising NEFA and BHB levels. In both seasons cows went through great changes in metabolic profile, with increased hepatic metabolism in postpartum, with more cholesterol production increasing the use of triglycerides and lipolysis. With all these changes, some animals could not maintain a healthy metabolism and went through subclinical ketosis or had high NEFA, making them more susceptible to other diseases.

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