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Article

Relationships among ß-hydroxybutyrate, calcium and non-esterified fatty acids in blood with milk yield losses at early lactation

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Abstract:

The objectives were to study the associations of concentrations of β-hydroxybutyrate acid (BHBA), calcium (Ca^{2+}), and non-esterified fatty acids (NEFA) in blood serum 7 d prepartum with losses in milk yield (MY) and metabolic dysfunctions at seven and 14 d of lactation. Three hundred and thirty-six (336) Holstein-Friesian (780 ± 36 kg BW; which had lactated more than twice) were sampled by coccygeal venipuncture, 7 d before, and 7 and 14 d after parturition. For each sample and metabolite serum concentrations were stratified in thresholds and related to MY. When BHBA levels were high 7 d before parturition and were related to MY at d-7 postpartum, it was observed that 11.00 % of the

cows lost 0.370 kg d^{-1} of milk. In contrast, no relationship was observed between BHBA prepartum and MY on d-14 of lactation. It was not observed any association between high NEFA and low Ca^{2+} levels prepartum and MY. NEFA concentrations ≥ 0.5 mmol L^{-1} on d-7 before calving were 7.6 more susceptible for lameness incidence ($P \leq 0.01$), and when BHBA ≥ 0.8 mmol L^{-1} cows were 2.4 times more likely to develop ketosis ($P \leq 0.05$) in the first 60 d in milk. In brief, data indicate that a high proportion of cows are above the thresholds of β-hydroxybutyrate and non-esterified fatty acids, and are also deficient in calcium, when determined one week before parturition. The risk thresholds for each metabolite were not associated with the amount of milk lost at d-14 after calving.

Key words: Negative energy balance, Biomarkers, Metabolites, Milk yield, Metabolic dysfunctions.

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Introduction

Animal health and herd productivity are the most difficult challenges that dairy producers confronting on a regular basis. The period around calving is critical due to the reduction in dry matter intake (DMI), increases in the demand of nutrients, energy and calcium (Ca^{2+}) for the maintenance and synthesis of milk. Due to the reduction in DMI, the requirements of the animals cannot be met, and the deficit allows the animal to fall into negative energy balance (NEB).

At early lactation, the concentration of glucose in blood serum is low, with a parallel increase in the concentration of non-esterified fatty acids (NEFA), and ketone bodies[(1)]. The most prominent circulating ketone body in ruminants is β-hydroxybutyrate (BHBA), which is used as an energy source in body tissues such as brain, heart[(2)], kidney and skeletal muscle[(3)]. However, the increase of BHBA above 1.2 mmol L^{-1} is an indicator of subclinical ketosis in dairy cows[(4)]. The increase in plasma BHBA reduces circulating glucose in blood[(5)] and increases the risk of ketosis, hypocalcemia, abomasal displacement and metritis with the consequent reduction in MY[(6)].

Measurements of BHBA, Ca^{2+}, and NEFA concentrations around calving may be potential indicators of the cow's ability to overcome metabolic challenges in the transition period,

and possibly allow predicting some disease risks and possible MY losses at the start of lactation. Calcium concentrations demonstrate the ability of the cow to replace extracellular Ca^{2+} loss as a result of the milk production process, and the balance between bone, and the efficiency of absorption of insulin and Ca^{2+}[7]. Non-esterified fatty acids serve as an indicator of mobilization of body fat and reflect the particularity of the cow to adapt to the NEB.

At the level of cow, reductions in serum Ca^{2+} concentrations, increases in NEFA and BHBA have been associated with an increased risk to contracting diseases[8] and milk loss[9]. The cow-level thresholds of these metabolites have been used to identify individuals at risk of damaging their health and productivity. However, individual interventions to minimize the undesirable effects of NEB on hypocalcemia, ketosis or other metabolic disorders around calving are difficult to achieve. Based on this premise, the objective of this study was to determine the serum concentrations of β-hydroxybutyrate, Ca^{2+}, and, non-esterified fatty acids, seven days before parturition, and the relationships between them and milk losses at seven and 14 d of lactation in Holstein-Friesian cows in confinement.

Material and methods

Study area

The study was carried out in a commercial dairy farm located in the Comarca Lagunera Region, Northern, Mexico. The dairy farm was selected based on the accessibility of the manager to participate in the study; the farm met the criteria of having approximately 2,000 milking cows and managing with two milking per day and complete sorghum-soybean diets. The dairy farm is located in San Pedro, Coahuila, at 1,100 m (25° 44' 36" N and 103° 10' 22" W). Temperatures at animal pens were from 4 to 20 °C during the study period. The climate of the region is desert. The precipitation is approximately 300 mm per year distributed mainly from July to September[10].

Animal feeding and management

Animals used in the study were 336 Holstein-Friesian pregnant cows, approximately 30 d before the probable date of calving. The body weight (BW) was approximately 780 ± 36

kg, with a body condition score (BCS) of 3.5 (scale 1, thin to 5, fat) and with more than two lactations. The possible dates of calving were obtained from the lists generated by the AfiFarm' (Ltd., Kibbutz Afikim, Israel) software. The cows were selected taking into account the milk production records of the previous lactation. The selection criterion was the average production indicated in kilograms of milk for 305 d of a previous year.

The number of cows considered in the study was obtained according to the criteria of Fox *et al*[11] for sample size, considering the total population of milking cows minus 16.0 % of cows in the dry period and 12.0 % of those with only one lactation. Those reported with mastitis, metabolic disorders or respiratory problems were also discarded. Finally, 336 cows were used in the sampling. Selected animals were sampled at 7 d before, and at 7 and 14 d after calving (-7d, +7d, and +14d).

Each dairy cow group (pre and post-partum) was fed with the respective diet. In each diet was used the same ingredients. Cows were provided *ad libitum* access to fresh water and were fed a total mixed ration (TMR) daily that was designed to meet NRC recommendations[12] for close-up and fresh cows. The close-up diet (prepartum) was based on oats hay, soybean meal, and cracked corn, and the diet for fresh cows (postpartum) was based on alfalfa and soybean meal (Table 1).

Table 1: Ingredients and chemical composition of the total mixed ration fed pre (close-up period), and post-partum (fresh period) to Holstein-Friesian cows in confinement conditions

Ingredients	Close-up diet, %[1]	Fresh diet, %[2]
Alfalfa hay	7.75	20.41
Soybean	13.78	25.36
Cotton seed		6.82
Destillery grain	4.68	1.47
Sorghum grain steam-flake		4.75
Corn steam-flake		3.43
Citrus pulp		12.76
Soy hulls	10.62	1.51
Corn silage		3.40
Oat hay	44.18	
Cracked corn	7.15	
Minerals	0.81	0.45
Molasses	9.24	12.76
Calcium soaps of fatty acids		5.08
Sodium bicarbonate	0.05	0.05
Dicalcium phosphate	1.38	1.45
Vitamin premix 1	0.36	0.30

Chemical composition[3]		
Metabolizable energy, Mcal kg^{-1}	1.68	3.08
Net energy of lactation, Mcal kg^{-1}	1.06	2.04
Crude protein, %	12.60	22.00
Acid detergent fiber, %	21.70	18.00
Neutral detergent fiber, %	25.60	18.70

[1]Close-up period was from wk 3 to 0 before calving.[2]Fresh diet period was from calving to d- 60 in milk.
[3]Chemical compositions were determined in lab following the procedures of AOAC(31); whereas NDF and ADF were analyzed according the methods of Goering and Van Soest(32).

Serum analysis

In each sampling day, approximately 10.0 mL of blood from the coccygeal vein/artery was collected in vacutainer tubes without anticoagulant (Beckton-Dickinson, Franklin Lakes, NJ) immediately following morning milking and before feeding. The samples were kept under refrigeration and their coagulation was allowed. The blood was centrifuged at 1,500 rpm for 25 min; serum was separated and stored at -20 °C within a period no longer than 6 h of collection.

The samples were analyzed for BHBA and NEFA in the Universidad Autónoma Nacional de México at the laboratories. NEFA concentrations in blood serum were determined with an enzymatic colorimetric assay Half-micro test number *11 383 175 001* distributed by Sigma Aldrich laboratories (Roche, Diagnostics, Mannheim, Germany); whereas the BHBA was analyzed with an enzymatic colorimetric kit via plate reader. This kit is distributed by Stanbio Laboratories (EKF Diagnostics-Stanbio, Boerne, TX, USA). An atomic absorption spectrophotometer (Analyst 700, Perkin Elmer) was used to analyze Ca^{2+} concentrations in blood serum. Calcium concentrations were determined following procedures of the manufacturer(13).

Recording diseases

The dairy herd was visited daily. Herd veterinarians recorded disease events nearly after morning milking with a standard sheet, which was handled in the systems area dairy farm by the AfiFarm software (Ltd., Kibbutz Afikim, Israel). Metabolic dysfunctions such as acidosis, hypocalcemia, ketosis, and lameness were classified as clinical events. The veterinarians followed the protocols established by the dairy for the detection of diseases and disorders to standardize the information collected. The definitions of the diseases have already been described previously by LeBlanc *et al*(14).

Statistical analysis

Statistical analyses were performed using SAS software[15] with cow as the experimental unit. Descriptive statistics were obtained with the UNIVARIATE procedure; they are shown in Table 2.

Table 2: Threshold, and descriptive statistics of samples from experiment used to evaluate the relation between blood serum metabolites concentrations and milk yield of Holstein-Friesian cows in confinement

Item	Threshold			Mean	Standard deviation
	Low	Medium	High		
Body condition score, (cows numbers)	≤ 2.25 (116)	2.25-3.50 (95)	≥ 3.50 (130)	3.55	0.18
Lactation, (cows numbers)	≤ 3.00 (104)	3.0-4.00 (108)	≥ 4.00 (124)	2.69	0.83
Milk yield, kg cow^{-1} d^{-1}, (cows numbers)	≤18.20 (122)	18.20-36.32 (123)	≥ 36.33 (91)	38.44	10.33

Differences among BHBA, Ca^{2+} and NEFA concentrations were analyzed using PROC MIXED in a completely randomized design for repeated measures. The final model is as indicated, after removing the covariables and the double or triple interactions that were not significant ($P \geq 0.05$).

$$\mathbf{Y_{ijk}} = \mu + COW_i + DAY\ OF\ SAMPLING_j + COW\ X\ DAY\ OF\ SAMPLING_{ij} + E_{ijk}$$

Were:

$\mathbf{Y_{ijk}}$ is an observation of the response variables; μ is the general mean;
$\mathbf{COW}_i$ is the random effect of the i-th cow (i= 1, 2,…336);
DAY OF SAMPLING is the effect of the j-th day of sampling (j= -7, 7, and 14);
$\mathbf{COW\ x\ DAY\ OF\ SAMPLING_{ij}}$ is the interaction Cow x Day of sampling;
$\mathbf{E_{ijk}}$ is the random error.

The covariance structure for BHBA, and NEFA concentrations was compound symmetry, whereas the more appropriate for Ca^{2+} was autoregressive of order one. Both of them were based on the lowest Akaike's information criterion.

BHBA, Ca^{2+}, and NEFA levels in blood serum were dichotomized and evaluated individually against MY on d 7 and 14 after calving. Dummy variables of MY and BCS were defined following procedures published by López-Ordaz *et al*[16]. For BCS, the cows were classified as moderate (2.25 to 3.25), and fat (≥ 3.5). Cows with BCS > 3.5, were categorized with number one, and were considered as risk factor. These values were performed using Proc Freq of SAS.

For each metabolite and date of sampling, at least three types of thresholds were made (low, medium and high). The thresholds were formed following the methodology proposed by Chapinal *et al*[9]. To predict the volume of milk lost or not harvested, the categorization of the cows was used in groups of low, medium and high risk. To study the difference between thresholds were created hierarchical Dummy variables, categorical values were 1.0 and 0.0, for cows considered in high and low risk, respectively, based on the serum concentrations of each metabolite in each sampling day thresholds used. In most of the thresholds the average level worked as a reference point.

With the high-risk thresholds defined for BHBA, Ca^{2+} and NEFA, the proportion of cows that were above and below the same thresholds for each metabolite and for each sampling date was determined with Chi-squared test (Table 3); whereas, blood concentrations of BHBA, Ca^{2+} and NEFA for animals that were above or below the high-risk thresholds were analyzed with Proc mixed from SAS. When the differences were significant between cows, the orthogonal contrasts test was used to establish the magnitude of the differences.

Table 3: Descriptive statistics of samples from experiment used to evaluate the relation between blood serum metabolites concentrations and milk yield of Holstein-Friesian cows in confinement

Item	Mean (n=336)	Standard Deviation	Minimum	Maximum
BHBA, mmol L^{-1}	0.73	0.45	0.20	3.84
Ca^{2+}, mmol L^{-1}	2.46	0.57	1.24	4.98
NEFA, mmol L^{-1}	0.59	0.46	0.12	2.95

To predict MY lost or not harvested, and the possible relationship between metabolites and metabolic disorders, the categorization of low, medium and high was used; whereas

MY was classified as low (≤ 18.20), medium (18.21 to 36.33), and high (≥ 36.33 kg d^{-1}) and where referred to as 0, -1 and 1, respectively, based on the discrete variables reported by López-Ordaz *et al*[16].

For each concentration threshold and day of sampling, multivariate conditional logistic regression models (Proc Glimmix) were developed, using a binary distribution and logit link function. The variables of interest were BHBA, Ca^{2+} and NEFA concentrations (the dichotomized proportions of animals in the low, moderate or high-risk group), date of interest sampling and the previous date (when -7 was the date of interest, 7 postpartum days was included in the model). It was also included CC, PL and date of births and the results are presented as odd ratio and the confidence intervals (CI = 95%) between animals above and below the reference threshold. The odd ratio expresses the advantage or probability of experiencing an event (for example lost or unharvest milk) for a high-risk group (above the threshold) when compared with a low risk group (below the threshold).

Results

Table 2 shows the thresholds and the number of the cows for BCS, lactation and MY of animals used in the study; whereas, Table 3 shows the number of repetitions, the mean and the standard deviations values of the blood serum metabolites concentrations of the animals considered in the study.

Table 4 shows the sampling dates, and the proportion of cows that were above and below the thresholds. The proportion of cows above the thresholds for BHBA at d-7 was less ($P \leq 0.05$) than those observed at d 7 and 14. In contrast, it was not observed differences between d 7 and 14.The proportion of cows above the thresholds for Ca^{2+} was greater ($P \leq 0.001$) for -7 and 7 d in comparison with 14 d. However, for NEFA, the proportion was greater ($P \leq 0.001$) in d 14 than the other days (224 *vs* 108 and 30, for 7 and – 7, respectively).

Table 4: Cows above or below the thresholds, and descriptive statistics of samples from experiment used to evaluate the relation between blood serum metabolites concentrations

Item	n	Cows above the thresholds			SEM	*P*
		-7	7	14		
BHBA ≤ 0.8 mmol L^{-1}	336	9^{a}	24^{b}	27^{b}	2.12	0.05
Calcium ≤ 2.3 mmol L^{-1}	336	108^{b}	108^{b}	84^{a}	11.34	0.001
NEFA ≥ 0.5 mmol L^{-1}	336	30^{a}	108^{b}	224^{c}	28.55	0.001
		Cows below the thresholds				
BHBA ≤ 0.8 mmol L^{-1}	336	327	312	309	3.00	0.05
Calcium ≤ 2.3 mmol L^{-1}	336	228^{a}	228^{a}	252^{b}	9.87	0.04
NEFA ≥ 0.5 mmol L^{-1}	336	306^{c}	228^{b}	122^{a}	17.99	0.001

[ab] Different literals in row mean statistical difference ($P<0.05$) between treatments.

The proportion of cows below the thresholds for BHBA at d 7 and 14 was less ($P\leq 0.05$) than those observed at d-7. In contrast, it was not observed differences between d 7 and 14 (Table 4). The proportion of cows below the thresholds for Ca^{2+} was greater ($P\leq 0.04$) than - 7 and 7 d in comparison with 14 d. However, for NEFA, the proportion was greater ($P\leq 0.001$) in d-7 than the other days (306 *vs* 228 and 122, for 7 and 14, respectively).

Table 5 shows the metabolite concentrations observed in cows that were above and below the thresholds obtained for BHBA, Ca^{2+} and NEFA, on d -7, 7 and 14. In cows above the thresholds, the concentrations of BHBA at d 7 and 14 were greater ($P\leq 0.02$) than those observed at d-7. On the contrary, the concentrations of Ca^{2+} and NEFA at d - 7, 7, and 14 prepartum were not different ($P\geq 0.05$).

Table 5: Metabolites concentrations, cows above and below the thresholds, and descriptive statistics of samples from experiment used to evaluate the relation between blood serum metabolites concentrations

Item	n	Cows above the thresholds			SEM	*P*
		-7	7	14		
BHBA ≤ 0.8 mmol L^{-1}	336	1.04[a]	1.65[b]	1.68[b]	0.12	0.05
Calcium ≤ 2.3 mmol L^{-1}	336	2.82	2.78	2.87	0.03	0.12
NEFA ≥ 0.5 mmol L^{-1}	336	1.08	1.14	1.16	0.04	0.23
		Cows below the thresholds				
BHBA ≤ 0.8 mmol L^{-1}	336	0.49[a]	0.71[b]	0.66[b]	0.06	0.02
Calcium ≤ 2.3 mmol L^{-1}	336	1.93	1.85	1.88	0.05	0.02
NEFA ≥ 0.5 mmol L^{-1}	336	0.20[a]	0.42[b]	0.41[b]	0.05	0.01

[ab] Different literals in row mean statistical difference (P<0.05) between treatments.

With respect to the cows below the thresholds, the concentrations of BHBA at d 7 and 14 were different (P≤0.02) compared with -7 d; on the contrary, no difference (P≥0.02) were observed between d 7 and 14. While for the same date, the Ca^{2+} concentrations were not different (P≥0.12) compared to the other two dates. The NEFA concentrations of d-7 were different (P≤0.01) to the concentration on d 7 and 14 postpartum. On the contrary, no difference was observed between d 7 and 14.

Cows on day seven after parturition showed high-risk with blood concentrations of NEFA. The relationship of blood concentrations determined on d-7 before calving and metabolic dysfunctions incidence in the first 7 d of lactation had a significant effect for the incidence of lameness (P≤0.01), with an OR estimate of 7.6 [1.50 – 38.74; CI = 95%; P=0.01] times more likely to present this clinical disorder when NEFA ≥0.5 mmol L^{-1}. It was also observed that 11 % of the cows with high concentrations of BHBH were lost approximately 0.37 kg cow^{-1} d^{-1} of milk [Odd ratio = 0.37; (0.14 to 1.01; CI = 95%; P=0.05)].

Discussion

The ketone bodies, with the BHBA as the main one, are known to be involved in the expression of ketosis, as depressants of feed intake and that affect negatively the fertility of dairy cows[17]. It was determined the serum concentrations of BHBA, Ca^{2+}, and NEFA 7 d before parturition, and 7 and 14 after parturition, in order to establish the relationships between those concentrations and milk losses in Holstein-Friesian cows in confinement.

In the current study, the low proportion of cows above the BHBA thresholds on d - 7 in comparison with d 7 and14 of lactation was explained by the metabolic and endocrine adaptations of the cow during the dry period in the absence of the requirement of nutrients for MY; while in lactation; the metabolism of nutrients is related to negative energy balance (NEB) caused by MY and inadequate feed intake.

The necessary adaptation is reflected by changes in several blood parameters. As noted in other studies, the concentration of glucose decreases, whereas the concentrations of BHBA and NEFA increase, concomitantly with related changes in the endocrine system mainly insulin and glucagon[18].

The difference in BHBA concentrations before and after parturition was also observed in other studies. Chapinal *et al*[9] in a large multiregional study conducted in 55 stables, to validate the relationships between concentrations of BHBA with diseases at the beginning of lactation, observed that cows sampled -7 d were four times below the risk threshold for BHBA compared to those sampled seven days after parturition. The difference was explained by the metabolism of energy in different physiological states.

In the Chapinal *et al*[9] study BHBA concentrations was similar to those observed here. In the present study, the serum concentrations of BHBA found were on average 0.73 with a variation of 0.20 to 3.84 mmol L^{-1}. The highest concentrations were obtained on d 7 and 14 of lactation in comparison with those obtained on d -7. The cow-level threshold of <0.8 mmol L^{-1} used in the present study is similar to those suggested by Chapinal *et al*[9] and Ospina *et al*[19]. Conceivably, subclinical ketosis may start at levels of BHBA greater than 1.0 mmol L^{-1}; however, the decision to set an appropriate subclinical threshold using serum or plasma BHBA appears to be somewhat arbitrary.

Kelly[20] suggested that 1.0 mmol L^{-1} be used to separate cows with low and high BHBA concentrations; whereas, Suthar *et al*[21] selected a subclinical ketosis threshold of 1.2 mmol L^{-1} of BHBA. Because increased ketone bodies post calving is considered to be part of a normal metabolic response to increased energy demand, it seems that a cut point to

define high ketone body concentrations should be based on both production and health impairment.

Other factors such as age of the cow are also part of the explanation of the high levels of BHBA in early lactation. In the current study, cows with three or more (2.5 *vs* 3.6 ± 1.5) lactations were more productive than young cows as a consequence a greater proportion were above the risk threshold (approximately 19 %). Older cows (> 4 lactations) consume more feed than younger cows, mobilize more glucose and respond with greater increases in BHBA due to the needs of lactation[13].

According to these criteria, in the present study approximately 3, 7 and 8 % cows would be considered with subclinical ketosis; because their BHBA concentrations were higher than 1.0 mmol L^{-1} for d -7, 7 and 14, respectively. It can be a call for attention to health and the possible requests for milk in the herd under study. Particularly, the herd in study has the most productive and reproductive records of the area, which means that other herds with lower parameters could present subclinical ketosis in greater proportions in the study area. Calcium plays a fundamental role in MY, because it is involved in the transmission of nerve impulses, contraction of muscles, blood coagulation, secretory activity of cells, cell differentiation, immune response, and enzymatic activation[22].

In the current study, the proportion of cows above the Ca^{2+} thresholds were lower for d 14 compared to 7 and -7; this may be due to the mobility of Ca^{2+} during the end of gestation only has very slight fluctuations because the changes in the requirements for the mineral are not very variable. On the contrary, in the days after parturition, the needs for the metabolite increase dramatically as the synthesis of the milk produced increases. According to the NRC[11] at the start of lactation, approximately all cows are on a negative Ca^{2+} balance, so as lactation progresses and MY increases in volume, as a consequence the need for mineral increases.

Van't Klooster[23] indicated that the absorption of Ca^{2+} increased 22 % towards the end of pregnancy to 36 % by d 8 of lactation in cows that consumed total mixed ration (TMR). What represented approximately 1.6 times increase in Ca^{2+} absorption. After that period, the changes were small and in response to increases in MY.

Chapinal *et al*[9] indicated that Ca^{2+} effectively tends to increase in order to cover the demand of the mineral for milk synthesis. This study included 48 herds, of which 33 % were above the risk threshold when Ca^{2+} was determined on d -7, compared to 40 % of them that were above the same threshold when determined Ca^{2+} on d 7 postpartum.

In the present study, serum Ca^{2+} concentrations were approximately 2.46 mmol L^{-1}, with a wide range of variation from 1.24 to 4.98 mmol L^{-1}. As indicated, previously, the Ca^{2+}

threshold was <2.3 mmol L^{-1}, suggesting that a high proportion of cows were at risk of presenting subclinical hypocalcemia (serum Ca^{2+} concentration <1.8 mmol L^{-1}[24]; although the signs of milk fever were imperceptible.

As indicated above, the herd under study has the highest productive indicators of the dairy basin, and still presented a high number of cows prone to subclinical hypocalcemia. The inference could be true for herds with similar management conditions, where, possibly that condition can be a problem that limits MY. Another part of the explanation is also related to the age of the cow. Adult cows with more than four lactations (approximately 19 %) were more prone to hypocalcemia and were above the proposed threshold for Ca^{2+}. In support of the above, Venjakob *et al*[25] observed that primiparous cows with serum concentrations of Ca^{2+} < 2.0 mmol L^{-1} had no effect on MY, while adult cows not only showed hypocalcemia, but also produced less milk (- 2.19 kg $animal^{-1}$ d^{-1}) than cows free of the disease.

Neves *et al*[26] observed that prepartum cows with concentrations of $Ca^{2+} \leq 2.4$ mmol L^{-1}, had higher risks of being classified as subclinical hypocalcemia one week before parturition. The authors indicated that the Ca^{2+} threshold (≤ 2.4 mmol L^{-1}) is required for the identification of animals in prepartum with a higher probability of presenting subclinical hypocalcemia. Herd-level studies using this cut-off point could establish objectives to measure the success of preventive strategies.

Additional studies are needed to determine the sampling times of blood around parturition, and to evaluate the relationship between fatty acids and Ca^{2+} in the prepartum. In modern dairy farms, the periparturient period (approximately 3 wk before and after parturition) presents the highest risks for developing diseases and mortality in cows. Ferguson[27] suggested that approximately one of two cows suffer adverse effects on their health, with approximately 75 % of the diseases occurring during the peripartal period[13]. The most common metabolic diseases, such as hypocalcemia and hyperketonemia, have been shown to alter the cow's immune system, increase risks for other diseases, and reduce productive behavior[17].

In the current study, approximately 30 cows were above that threshold for NEFA on d -7, while for days 7 and 14 of lactation, number of cows increased from 108 to 224, respectively. The postpartum increase in NEFA levels could be explained by the increase in nutritional requirements due to the particular needs of lactation. This effect is related to the fact that most cows are in NEB, and they need to mobilize their body reserves of lipids in order to cover their energy needs[11,28].

Chapinal *et al*[9] from a larger study in cows and locations than the present study, but based on same BHBA, Ca^{2+}, and NEFA thresholds concluded that approximately 11 % of

the herds studied were above the risk threshold on d -7 compared with 23 % of herds on day seven of lactation. The authors concluded that 5 to 50 % of the animals sampled with NEFA levels above the thresholds both a week before and after were associated with risks of abomasal displacement, milk loss and pregnancy reduction at the first insemination.

In the present study, NEFA levels averaged 0.59 mmol L^{-1} with a range between 0.12 and 2.95 mmol L^{-1}. This suggests that the cows in the study would be at risk of losing milk at the beginning of lactation, mainly due to some association with hypocalcemia and hyperketonemia due to the circulating levels of NEFA.

The results obtained in the current study do agree with those from other studies. Ospina *et al*[19] found that NEFA concentrations > 0.3 mEq L^{-1} from d 14 to 2 prepartum, and NEFA > 0.6, and BHBA > 10 mg dL^{-1} from 3 to 14 d postpartum, both prepartum and postpartum values above these thresholds were associated with increases in ketosis, metritis, abomasal displacement, and placental retention.

In the current study, it was search through conditional logistic regression to relate the concentrations of metabolites with metabolic dysfunctions around parturition. However, the associations obtained were not significantly important and due to this they were not reported. One significant unique observation was as the blood NEFA increased (≥ 0.5 mmol L^{-1}) there was an increase of up to 7.6 [Odd ratio = 7.61; (1.50 to 38.74; CI = 95%; *P*=0.01)] times the chances of laminitis occurrence; this may be due to the fact that lameness produces fever and pain in the hooves, which alter resting and feeding behavior of the animals[29,30].

Fever reduces the cow's appetite and pain in the hooves impair the ability to walk and look for feed. The deficiency of food increases the possibility of the removal of body reserves, increasing the free fatty acids.

Results from the current study were observed by others. Collard *et al*[29] indicated that plasma samples with NEFA concentrations of 0.6 to 0.8 mmol L^{-1} were associated with lameness presence around parturition in Holstein-Friesian dairy cows fed TMR.

The results obtained in the present study in relation with the levels of NEFA and laminitis are contradictory with other reports in literature. Calderon and Cook[30] reported no relationship between lameness and NEFA in Holstein-Friesian cows around parturition in a mattress-bedded commercial free stall facility. They also observed that lame cows had longer lying times throughout the transition period and notably for 3 d before and after parturition. Lameness was also associated with a greater risk for ketosis in the study farm, as evidenced by the elevated BHBA concentration found in cows.

The results obtained in the present study offer the opportunity to examine the effects of pre and postpartum concentrations of NEFA and BHBA on MY at the cow level. The identification of these prepartum levels will allow the dairy producers to improve their strategies of nutritional management, in order to avoid losses in MY or to avoid the presence of metabolic disorders such as laminitis. The results obtained also show how the metabolism state influence health cow during the transition period suggesting that nutritional management has to be carefully reviewed due to its impact on subsequent lactation.

Conclusions and implications

High proportion of cows are above the thresholds of β-hydroxybutyrate and non-esterified fatty acids, and most of them were also deficient in calcium, when determined one week before parturition. High β-hydroxybutyrate concentrations could promote losses in MY up to 0.37 kg $cow^{-1}d^{-1}$ seven days after calving in Holstein-Friesian cows. High non-esterified fatty levels were associated with 7.6 times more risk of laminitis. The risk thresholds for each metabolite were not associated with the amount of milk lost at d 14 after calving in Holstein-Friesian cows.

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