Journal MVZ Cordoba
2020; 25(3):e1821.
https://doi.org/10.21897/rmvz.1821

Original

Serum Beta-hydroxybutyrate concentrations and its association with postpartum diseases in dairy cows

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Received: November 2019; Accepted: June 2020; Published: August 2020.

ABSTRACT

Objective. Establish the prevalence of bovine ketosis based on serum concentrations (mmol/L) of beta-hydroxybutyrate (βHB) and estimate its association with early postpartum diseases in dairy cows. **Materials and methods.** A cross-sectional epidemiological study with individual information on blood levels of βHB and clinical occurrence of puerperal diseases in 1,149 animals was carried out. Besides, the efficiency of the test was evaluated as a predictor of postpartum disease. Subsequently, univariate analysis and a final logistic regression model were performed to explore the factors associated with hyperketonemia. The association between blood βHB, the appearance of ketosis, and the occurrence of puerperal diseases were calculated by analyzing the receiver operating characteristic (ROC). **Results**. According to βHB levels, the prevalence of ketosis was 7.9%, clinical ketosis 0.6%, and subclinical ketosis 7.3%. The test was a predictor of puerperal diseases (LR+ of 13.6). A body condition score of ≥3.5, 2 and ≥3 number of births, the presence of retained fetal membranes, milk fever, and postpartum disease, are risk factors for ketosis. The analysis of the ROC curve showed that the measurement of βHB (≥1.2 mmol/L) in blood serves to diagnose ketosis ($p<0.0001$). **Conclusions**. The measurement of blood levels of βHB allowed establishing that the presence of ketosis is low; however, it is an alert not only for this disease but for early postpartum diseases. The results of this study confirm the risk factors observed in previous studies.

Keywords: Early lactation; hyperketonemia; ketosis; odds ratio; risk factors (*Sources: MeSH, National agricultural library*).

RESUMEN

Objetivo. Determinar la prevalencia de cetosis bovina según las concentraciones séricas (mmol/L) de beta-hidroxibutirato (βHB) y estimar su asociación con enfermedades del posparto temprano en vacas lecheras. **Materiales y métodos.** Se llevó a cabo un estudio epidemiológico de corte transversal con información

How to cite (Vancouver).
Huertas-Molina O, Guzman CV, Palacio BL, Zambrano-Varón J, Olivera-Angel M. Serum Beta-hydroxybutyrate concentrations and its association with postpartum diseases in dairy cows. Rev MVZ Cordoba. 2020; 25(3):e1821. https://doi.org/10.21897/rmvz.1821

ISSNe: 1909-0544

individual de los niveles sanguíneos de βHB y presentación clínica de enfermedades puerperales de 1.149 animales; además, se evaluó la eficiencia de la prueba como predictora de enfermedades posparto. Posteriormente, se realizó un análisis univariado y un modelo de regresión logística final para explorar los factores asociados con hipercetonemia. La relación entre βHB sanguíneo, la presentación de cetosis y la ocurrencia de enfermedades puerperales se calculó analizando la característica operativa del receptor (ROC, por sus siglas en inglés). **Resultados**. Según los niveles de βHB, la prevalencia de cetosis fue de 7.9%, cetosis clínica 0.6% y cetosis subclínica 7.3%. La prueba fue predictora de enfermedad puerperal (LR+ del 13.6). La condición corporal ≥3.5, número de partos igual a 2 y ≥3, y la presencia de retención de placenta, fiebre de leche y la enfermedad posparto, son factores de riesgo de cetosis. El análisis de la curva ROC mostró que la medición de βHB (≥1.2 mmol/L) en sangre sirve para diagnosticar cetosis (p<0.0001). **Conclusiones.** La medición de niveles sanguíneos de βHB permitió determinar que la presentación de cetosis es baja; sin embargo, es una alerta no solamente para esta enfermedad sino para las enfermedades del posparto temprano. Los resultados de este estudio confirman lo reportado en estudios previos sobre los factores de riesgo de cetosis.

Palabras clave: Cetosis; factores de riesgo; hipercetonemia; lactancia temprana; odds ratio (*Fuentes: MeSH, National agricultural library*).

INTRODUCTION

Ketosis is a metabolic disorder in which the circulating levels of ketone bodies (acetone, acetoacetate, and βHB) increase as a consequence of the rise in circulating NEFA (non-esterified fatty acids) or energy requirements; the latter, in turn, can exceed the carbohydrate intake capacity during the early postpartum and result in a failure of the adaptation mechanisms to the negative energy balance (NEB), which leads to hyperketonemia and the appearance of clinical and subclinical ketosis (1). Ketosis is a widely distributed disease throughout the world. In Europe, the prevalence varies between 4.9% and 72%; in North America, it has been reported between 12.1% and 61%, and in South America the registered values reach 21% (2). Two studies in Colombia reported a prevalence of 8.3 and 48.6% (3,4). As can be observed in many parts of the world and also in Colombia, the broad prevalence range reported may be due to the heterogeneous distribution of the population.

The most representative paraclinical findings are hypoglycemia and the high concentration of ketone bodies (hyperketonemia) in blood, urine, milk (5), and extra-hepatic tissues (6,7). Following the epidemiology review of ketosis (7), this disease has been classified into two types according to its origin. Type I ketosis occurs when the energy requirements of the animal exceeded the gluconeogenic capacity of hepatocytes due to lack of oxalacetate and propionate, and the increased activity of the Carinitil Palmitoyl Transferase 1 (CPT-1) enzyme (6). On the other hand, type II ketosis has been related to energetic over-conditioning during the dry season (8) and inappetence caused by the presence of early postpartum diseases, mainly metritis and mastitis (7). Animals with this type of ketosis have a compromised immune function due to the acute increase in circulating NEFA, cytokines, adipokines, and acute phase proteins (9).

The most convenient way to classify the disease is according to the presence of clinical signs (7,10). Clinical ketosis occurs with a progressive decrease in consumption, marked weight loss, dehydration, dry stools, itching, and decreased milk production (7). Severe clinical cases show nervous symptoms such as constant licking, seizures, and apparent blindness and ataxia (10). These signs have further been related to βHB levels in blood and milk of ≥3 mmol/L and ≥0.2 mmol/L, respectively (1).

The detection of hyperketonemia identifies subclinical ketosis in blood, milk, and urine without the presence of clinical symptoms. The plasma concentrations of βHB compatible with the adverse effects on production and health range between ≥1.2 and ≤2.9 mmol/L (1,11).

Recent studies have defined that an animal with ketosis shows serum concentrations of βHB ≥1.2 mmol/L (3,4)

Different factors associated with the risk of presenting ketosis in production systems under stable and grazing conditions have been described (2,4,5,12,13,14). Studies have reported as risk factors for the occurrence of ketosis, the race (OR: 1.46, 95% CI (1.23-

1.73) p<0.05) (12), the number of births (three or more births) (OR: 2.8, 95% CI (2.0-3.7), p<0.01) (2), a high body condition score (BCS) (OR: 5.25, 95% CI (1.32-21.11), p=0.0188) (15), the presence of puerperal diseases such as mastitis (OR: 1.9, 95% CI (1.3–2.7), p<0.01) (16), abomasum displacement (OR: 24.6, 95% CI (5.65–107.4), p<0.0001) (5), retention of fetal membranes (OR: 1.6, 95% CI (1.1-2.3), p=0.01) (16), lameness (OR: 2.1, 95% CI (1.5-2.7), p<0.0001) (13), and metritis (OR: 4.94, 95% CI (1.17–0.98), p= 0.0302) (15).

From a physiological point of view, hyperketonemia can also be considered as a predisposing factor for the occurrence of clinical ketosis (OR: 14.7 (95% CI (7.2–29.8) p<0.01)) and puerperal diseases (1), such as displacement of the abomasum triggered by clinical signs of ketosis including loss of appetite, and decrease or absence of rumen mobility (17). On the other hand, the pathological increase in circulating levels of βHB may favor the presence of metritis (18) and lameness (19), due to the correlation of this disease with oxidative stress and decreased immune function (19). In addition to concomitant diseases and clinical signs of ketosis, subclinical ketosis generates unnoticed milk production losses and decreased consumption (4), resulting in increased production costs (1). In Colombia, few studies have been carried out on the prevalence and consequences of ketosis on milk production.

Accordingly, this is a cross-sectional epidemiological study with the aim of establishing the prevalence of ketosis according to the levels of βHB in the departments of Antioquia, Boyacá, Caldas, and Cundinamarca, and its association with the occurrence of puerperal diseases in dairy cattle during the early postpartum period under grazing conditions.

MATERIALS AND METHODS

Database. In this study, the database provided by Bayer S.A. was used to carry out the analyzes according to the information recorded from samplings that include department, number of farms, number of cows per farm, and cows calved between 7 and 15 days postpartum. Besides, the serum βHB level and the presence of the diseases listed in Table 1 were recorded in the database.

The previous analysis showed that the number of farms was 52 in Antioquia, 5 in Boyacá, 3 in Caldas, and 50 in Cundinamarca, for a total of 1,149 animals distributed in 40 municipalities. All the owners of the animals stated their willingness to participate voluntarily in the study. The number of animals sampled per herd ranged from 2 to 43, and samplings were carried out throughout the year 2014. About 92.5% of the animals in this study were of the Holstein breed, and the rest included other dairy breeds.

Table 1. Definition of postpartum diseases, according to Brunner et al. (3).

Disease	Definition
Placenta retention	Failure to expel fetal membranes for more than 24 hours.
Metritis	Increased size of the uterus with/without foul-smelling, purulent uterine secretion associated with systemic signs (fever, inappetence/anorexia, inactivity, and decreased milk production).
Mastitis	Visibly abnormal milk with or without changes in the appearance of the udder (signs of inflammation, redness, or hard quarters).
Milk fever	A cow that, after 24-48 hours of postpartum, requires a calcium injection due to clinical signs of hypocalcemia, such as muscle weakness and pathological postpartum decubitus.
Abomasum displacement	Distension and displacement of the abomasum to the right or left. Presence of a "ping" sound by auscultation and abdominal percussion confirmed by surgery.
Lameness	Presence of claudication with a locomotion score ≥3 (scale of 1-5) and with abnormal findings during hoof examination (i.e., digital and interdigital dermatitis, white line disease, sole abscess, and trauma, among others).
Clinical ketosis	Decreased milk production, consumption, and inappetence, reduced activity, positive test (βHB ≥1.2 mmol/L) of ketone bodies in blood or milk, absence of abomasum displacement, or other primary causes, and smell of acetone in the breath or milk.

Ethical aspects. Regarding data processing, an agreement between the company Bayer S.A. and Universidad de Antioquia was signed, authorizing the use of the information to disseminate and discuss the results obtained from the study.

Blood sample collection and βHB measurement. The serum concentration of βHB was immediately established through a blood sample extracted from the coccygeal vein of the animal using a portable meter (Precision Xceed, Abbott Diabetes Care Inc., Alameda, CA, USA), which had previously been validated for use in cattle (20). When working with an upper limit of 1.2 mmol βHB/L, the sensitivity and specificity of the test ranged between 75-96.3% and 91.0-98.0%, respectively (4).

Definition of variables. The study unit was an animal. Ketosis was established according to Tatone et al. (20) when the concentration of βHB in a sample was ≥1.2 mmol/L. Categorical independent variables were classified as follows: a) number of calving (1, 2, and ≥3), b) body condition (≤2.75, >2.75-<3.5, and ≥3.5), c) the postpartum day (7-15) in which the sample was obtained, d) the presence of postpartum diseases (Yes/No), and e) postpartum disease defined as the set of the presence of one or more diseases.

Postpartum diseases were diagnosed through clinical examination by trained veterinarians and following the definitions established in Table 1.

Statistical analysis. The overall prevalence of subclinical ketosis was estimated when serum βHB concentrations were ≥1.2-<3 mmol/L) (13) and of clinical ketosis when the concentration was ≥3 mmol/L (2). Additionally, the prevalence of puerperal diseases was calculated on the day of the blood collection samples and considering the database record taken by veterinarians. Thus, its association with serum concentrations of βHB ≥1.2 mmol/L was estimated. The latter was the cut-off point for defining ketosis (13). Moreover, the efficiency of the test was evaluated as a predictor of each of the diseases and of these as a whole. The following variables were calculated to meet this objective.

a. True prevalence (TP): the proportion of animals that showed each of the puerperal diseases; the population at risk for this calculation are all the animals included in the study (21).
b. Apparent prevalence (AP): the proportion of animals positive for ketosis (population at risk) and each of the puerperal diseases (21).
c. Sensitivity: the conditional probability that an animal detected with ketosis by the test (βHB ≥1.2 mmol/L) corresponds to a diagnosed case of puerperal disease (21).
d. Specificity: the conditional probability that an animal is negative for bovine ketosis (βHB <1.2 mmol/L) in those animals that did not suffer from the puerperal disease (21).
e. Positive predictive value (PPV): the proportion of animals that were identified with ketosis by the test and that effectively presented a postpartum disease (21).
f. Negative predictive value (NPV): the proportion of animals that, being identified as negative for ketosis by the test, did not show a postpartum disease (21).
g. Positive likelihood ratio (LR): expresses the possibility of observing a positive result by contrasting animals with and without the disease in question (e.g., puerperal disease). In the case of this study, positive LR is the increase in the probability of presenting a puerperal disease when the animal showed concentrations of βHB >1.2 mmol/L compared to those positive for ketosis without a puerperal disease.

According to McGee (22), in the cases in which LR is 2-5, the concentrations of βHB ≥1.2 mmol/L do not imply that the test identifies animals with any postpartum pathological condition. Meanwhile, this probability improves its prediction moderately when the LR ≥5-<10. Furthermore, when the LR is >10, the test is very good in its ability to identify an animal with any postpartum pathological condition (22).

Regression models. A univariate analysis was initially performed with 2x2 contingency tables, where the odds ratio (OR) and the confidence interval (CI) of 95% were estimated to analyze the association between the prevalence of ketosis according to the concentrations of βHB and the presence of puerperal diseases. Subsequently, the X^2 test was performed following the Hosmer & Lemeshow criteria, and those variables whose *p*-value <0.2 were included in a final binary logistic regression model. The calculated OR and 95% CI allowed estimating the magnitude of the association of the statistically significant variables ($p<0.05$). The software SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used to perform the statistical analyses.

ROC curves were used to assess the relationship between the results of the concentration of βHB (mmol/L) with the presence of hyperketonemia and the occurrence of puerperal diseases. These allow differentiating the usefulness of the test as a diagnosis for hyperketonemia or any of the puerperal diseases under study. The results obtained from this test were interpreted from the measurement of the area under the curve (AUC) and the significance level of the asymptotic curve ($p<0.05$). To know if the measurement of βHB in blood could be related to hyperketonemia or any of the postpartum diseases under study, the following was considered: if the AUC =0.5, the test is considered non-informative; if 0.5 <AUC ≤0.7, it is accurate; if 0.7 <AUC ≤0.9, it is very accurate; if 0.9 <AUC <1, it is highly accurate; and if AUC = 1, it is considered as perfect (13).

RESULTS

By measuring the serum βHB concentration of animals from different milk production systems and under grazing conditions, the prevalence of bovine ketosis was estimated during the early postpartum period. According to the serum levels, the prevalence of bovine ketosis was 7.9% and ranged from 4.4 to 12.5% in a period of 7 to 15 postpartum days (daily variation). About 0.6% of the population showed clinical ketosis and 7.3% subclinical ketosis (Figure 1). The median βHB concentration was 0.6 mmol/L, and the observed range of variation was between 0.1 and 4.2 mmol/L. About 2.8% of the animals from the first calving, 8.7% from the second calving, and 10% of the third or further calving showed levels of βHB ≥1.2 mmol/L, just as 6.5% of the animals that had a BCS score lower than 2.75 (8.7% with BCS between 2.75-<3.5 and 17.1% with BCS higher than 3.5). The herd size was between 28 and 650 animals per farm, where 46% had less than 150 animals, and 54% had 150 or more animals, with a ketosis prevalence of 8.1 and 7.8%, respectively.

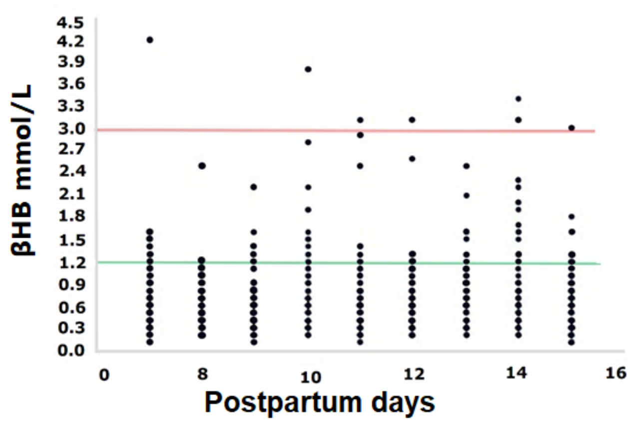

Figure 1. Distribution of serum βHB concentrations corresponding to the presence of ketosis in dairy cattle during the early postpartum period (n = 1.149). Clinical ketosis: βHB 3.0 mmol/L (red line); subclinical ketosis: βHB ≥1.2-<3.0 mmol/L (green line).

It is noteworthy that within the puerperal diseases evaluated, no cases of clinical ketosis were reported. In contrast, 11.1% of the animals had a diagnosis of at least one puerperal disease whose prevalence was distributed as follows: mastitis (4.5%), placenta retention (4.2%), lameness (1.3%), milk fever (1.2%), metritis (0.8%), and one case of abomasum displacement (0.08%).

Table 2 shows the efficiency evaluation of the cut-off point for ketosis (≥1.2 mmol/L) to define the prevalence of puerperal diseases.

The candidate variables to enter the final logistic regression model associated with the increase in serum βHB concentration (>1.2 mmol/L) and the presence of puerperal diseases in dairy cattle were body condition, the number of calving, placental retention, milk fever, and postpartum disease. The results of the final model are shown in Table 3.

The ROC curve and the AUC of the results of the βHB concentrations of ≥1.2 mmol/L for each of the observed puerperal diseases are summarized in figure 2 and table 4.

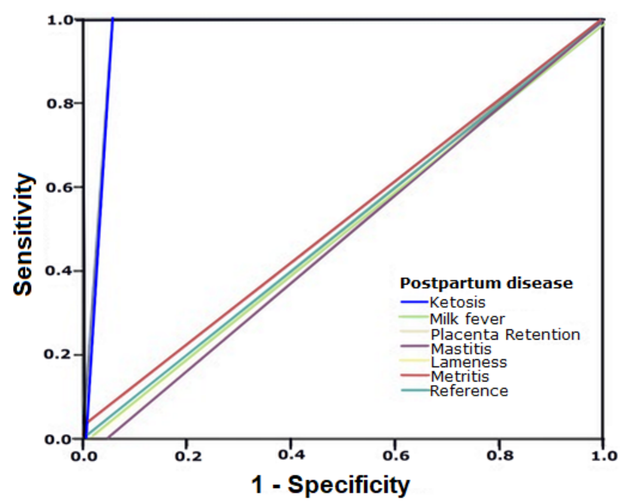

Figure 2. Receiver Operating Characteristics (ROC) curve to evaluate the relationship between the measurement of the concentration of βHB (mmol/L), the presence of hyperketonemia, and the occurrence of puerperal diseases in bovine milk.

Figure 2 and Table 4 show that the only entity associated with elevated serum concentrations (βHB ≥1.2 mmol/L) was hyperketonemia (AUC = 0.971; asymptotic significance = $p<0.001$). This means that βHB levels are not directly related to the presence of puerperal diseases.

Table 2. Prevalence of postpartum diseases in dairy cattle defined by the increase in serum levels of βHB (≥1.2 mmol/L) and evaluation of the efficiency of the diagnostic test.

	TP(%)	AP(%)	Sen(%)	Spe(%)	PPV(%)	NPV(%)	LR+	LR-
Milk fever	1.2	0.6	50	92.6	7.7	99.3	6.3	0.5
Placenta retention	4.2	0.3	8.3	92.1	4.4	95.8	10.5	0.2
Mastitis	4.5	0.1	1.9	91.8	1.1	95.2	2.3	0.9
Abomasum displacement	0.08	0	0	92.1	0	99.9	0.0	1.1
Lameness	1.3	0	0	91.9	0	98.6	0.0	1.1
Metritis	0.8	0.2	22.2	92.2	2.2	99.3	2.8	0.8
Postpartum disease	11.1	1.1	89.8	93.4	59.4	98.6	13.6	0.1

TP: true prevalence; AP: apparent prevalence; Se: sensitivity; Spe: specificity; PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; and LR-: negative likelihood ratio.

Table 3. Factors associated with the occurrence of an increase in serum βHB concentration (>1.2 mmol/L) and the presence of puerperal diseases in dairy cattle.

Variable	Category	n	OR*	CI 95%**		p-value ***
				Lower	Higher	
Body condition	< 2.5	260				
	>2.5 - <3.5	563	1.2	0.7	2.1	0.51
	≥3.5	76	2.7	1.3	5.9	0.011
Number of calvings	1	287				
	2	241	3.1	1.3	7.1	0.009
	≥3	621	3.7	1.7	7.8	0.001
Abomasum displacement	No	1148				
	Yes	1	0.000	0.000	0	0.9
Mastitis	No	1097				
	Yes	52	4.5	0.62	33.4	0.135
Lameness	No	1134				
	Yes	15	3.3	0.74	14.7	0.118
Placenta retention	No	1101				
	Yes	48	7.9	1.90	32.4	0.004
Milk fever	No	1135				
	Yes	14	11.6	3.79	35.40	0.0001
Postpartum disease ****	No	1022				
	Yes	127	8.9	3.336	24.244	0.0001

* OR = Odds ratio;
** 95% CI = 95% confidence interval
*** Values of p<0.05 are statistically significant;
**** Postpartum disease = presence of at least one disease.

Table 4. Area under the curve distribution results of the βHB measurement at a cut-off point ≥1.2 mmol/L concerning the identification of pathologies in the early postpartum in dairy cattle.

Area under the curve					
Test result	**Area**	**Standard error[a]**	**Significance asymptotic[b]**	**CI 95% asymptotic**	
				Lower	**Higher**
Hyperketonemia	0.971	0.005	0.001	0.961	0.982
Milk Fever	0.494	0.056	0.912	0.385	0.603
Placenta retention	0.479	0.054	0.704	0.373	0.584
Mastitis	0.477	0.054	0.680	0.371	0.582
Lameness	0.493	0.056	0.905	0.384	0.602
Metritis	0.515	0.058	0.790	0.402	0.628

[a] Under a nonparametric assumption
[b] Null hypothesis: true area = 0.5, p<0.05 is statistically significant
CI: confidence interval

DISCUSSION

In the current study, the prevalence of ketosis (7.9%) was lower compared to what was reported in Mexico (29.1%) (23) during the period of 7–15 days postpartum. The same behavior was observed when compared with studies that were carried out during the first 21 days of postpartum in Colombia (8.3% and 48.6%) (3,4), and others performed in the South American continent where the prevalence was around 10.7% (3). The prevalence of clinical ketosis (0.6%) was lower compared to what was reported by other studies in Colombia (6.0%) (4), the Netherlands (11.6%) (5), Canada (3.7%) (5), and Mexico (10.8%) (2,3). The prevalence of subclinical ketosis was also lower compared to what was found in the Cundiboyacense highlands of Colombia (42.6% and 8%) (3,4), England (17%) (1), and the Netherlands (47.2%) (2).

The explanation of these results may depend on the heterogeneous nature of the populations and the stability of the forage supply offered by the productive systems under grazing (4,14) and in stables (total mixed rations - TMR), which can significantly influence the milk production and ketogenic (butyrate and acetate) or glycogenic (propionate) volatile fatty acids (17) produced by ruminal bacteria (24).

The assessment of the body condition has been used mainly as an indicator of energy balance (25); these coincide with our results in that animals with higher BCS (>3-3.5) have a higher risk of presenting ketosis. According to the definition by Gordon et al (10), these cases can be classified according to their origin as Type 2, which occurs mainly during periods of decreased consumption during calving or peripartum, resulting in the increased mitochondrial activity of the Carnitil-Palmitoyl enzyme transferase 1 (CPT-1) (6). This, added to the high concentrations of circulating NEFA in obese females or those with a higher body condition of the herd, may increase the possibility of excessively producing ketone bodies, even in hyperglycemic states (17). This occurs because NEFA can also be re-esterified to triglycerides in the cytosol of the hepatocyte and accumulate inside and outside the cell due to lack of transport. This, in turn, depends on the synthesis and secretion of very-low-density lipoproteins (VLDL). In ruminants, the production of VLDL is low, indicating more production of ketone bodies and fatty infiltration of the liver, particularly in animals with a high body condition, as has been observed in other reports in dairy systems under grazing conditions where the risk of ketosis has been higher (OR = 5.25, p=0.018) both in Argentina (15), and in Colombia (RR = 3.35, p=0.02) (4).

The number of calvings has been an essential factor in the presence of hyperketonemia and bovine ketosis. The risk of hyperketonemia increased with the number of calvings, as follows: animals with two (OR: 3.1, 95% CI (1.3-7.1) p=0.009) and three or more calvings (OR: 3.7, 95 CI (1.7-7.8) p=0.001) had a higher risk, respectively, compared to first calving animals as reported by other studies (2). This may be associated with the fact that milk production tends to increase with the number of lactations (25), which increases energy requirements, generating primary ketosis (7,10). In this way, the induction of lipolysis caused by the decreasing glucose concentrations at the beginning of lactation or by the presence of concomitant diseases can lead to an excessive increase in the concentration of Acetyl-CoA in the mitochondria, generating the excessive formation of both βHB and acetoacetate in circulation (6). Therefore, it can easily be detected in blood, which can facilitate the diagnosis.

Similarly, the relationship between the presentation of puerperal diseases and bovine ketosis has been documented. However, the prevalence of milk fever observed in this study (1.2%) was similar to the values of 1.3 and 1.7% reported in Canada and Europe, respectively (5,16), and less than the value of 3.3% found in the Colombian Cundiboyacense highland region (4). Our findings coincide with other studies in establishing a higher risk of presenting ketosis in those animals with clinical cases of hypocalcemia (OR: 11.6, 95% CI (3.79-35.40) p=0.0001. The decrease in food consumption and anorexia that is observed during stage 2 of the clinical signs of hypocalcemia, partly explain the increase in ketone bodies during the development of the disease.

Other diseases of frequent occurrence in the puerperium, such as retention of fetal membranes, were associated with the occurrence of ketosis in our work (OR: 7.9, 95% CI (1.9-32.4) p=0.004), as has been reported in other studies as a risk factor for ketosis of 4.10. Further, this occurrence has been explained physiologically by the decrease in food consumption (2-6 kg/day) that occurs when clinical signs of metritis develop (26). In this study, none of the animals diagnosed with metritis had a history of placental retention.

It is worth clarifying that the relationship between ketosis and the presentation of a puerperal disease (OR= 8.9, CI (3,336-24,244) p=0.0001), can be bidirectional and have various causes. Therefore, it is difficult to attribute a relationship between them (1,4). However, the decrease in food consumption caused by the clinical presentation of puerperal diseases (17), as well as the typical negative energy balance of postpartum, affect blood glucose concentration and triggers severe lipolysis and primary ketosis in up to 30% of the hypoglycemic animals (27). Furthermore, ketosis has been reported to cause other postpartum diseases, such as metritis or lameness (18,19,28), due to the known compromise of the immune function of the cow in transition and insulin resistance generated by high circulating NEFA levels (9). IL-6, TNF-α, and acute phase proteins (e.g., haptoglobin and serum amyloid A) (18,19) favor the presence of these entities because they trigger inflammatory alterations in the uterus, favoring secondary infections (18). On the other hand, the induction of these factors facilitates the action mechanisms of lipoteichoic acid and lipopolysaccharides produced by ruminal bacteria, which can cause inflammation or necrosis of the peripheral microvasculature and lameness (19).

Although serum concentrations of βHB ≥1.2 (22) or ≥1.1 mmol/L (16) in blood and 0.10 mmol/L in milk have been observed to increase between 4.7 (CI% (2.06 - 10.91) p=0.0003) and 14.7 (95% CI (7.2 - 29.8) p<0.01) times the risk of showing clinical signs of ketosis (16), in the current study, animals with clinical signs of this disease were not identified.

Hyperketonemia was considered a good predictor of a puerperal disease, although the conditional probability that an animal with a positive test result is sick and the proportion of sick and positive animals are low (Sen: 10.2%; PPV: 59.4%). In this study, hyperketonemia, defined as the serum concentration of βHB at a cut-off point of 1.2 mmol/L, showed to be efficient in identifying individuals with a postpartum disease with a sensitivity of 89.8% and specificity of 93.4%. However, it is not accurate in being a good diagnostic method for the specific postpartum diseases evaluated in the current study since its sensitivity varied between 0-50%, but its specificity varied between 91.8-92.6%. In this context, finding concentrations of βHB ≥1.2 mmol/L allowed to accurately identify those animals that did not have any postpartum clinical condition (21). This was additionally confirmed with the low positive predictive values found (0–59.4%) and especially when observing the range of negative predictive values (95.6-99.9%), which clearly indicates that the test is useful to rule out hyperketonemia in animals with other clinical conditions (21).

Similarly, when observing the interpretation results of the test using PI+, the test could be efficient in identifying animals that had placenta retention (PI+ = 10.5) or a puerperal disease (PI+ = 13.6). In another study, serum βHB concentrations higher than 1.0 and up to 1.7

mmol/L were associated, but they were not predictors in cases of abomasum displacement (PI+ = 1.87-3.1), clinical ketosis (PI+ = 2.17-3.8), or metritis (PI+ = 2.01-2.13) (5). Moreover, in an additional study, the PI was less than 5 when the prevalence of clinical ketosis, metritis, abomasum displacement, and lameness was defined (13). This post-test interpretation implies that the definition of the cut-off point for the interpretation of the test result may be particularly valid in the identification of cases of puerperal diseases.

An additional way to see the efficiency of the cut-off point used in this study (βHB ≥1.2 mmol/L) and its relationship with the identification of early postpartum pathologies, based on the use of ROC curves, revealed that in the case of hyperketonemia, the AUC = 0.971, $p<0.05$) was highly accurate in detecting cases of ketosis at the mentioned cut-off point. This is different from what has been reported by other studies in which a much less accurate relationship was observed (AUC = 0.6-0.84) when the cut-off points varied from 1.1-1.4 mmol/L (13,14).

The difference between studies shows the importance of defining an adequate cut-off point when validating the tests and highlights the importance of considering the differences inherent in the management of milk production systems (3,20), the type of diet and its effect on the production of volatile fatty acids at the ruminal level (24), in association with the factors that determine the level of production, body condition at the peripartum (25), and risk factors that favor the presence of puerperal diseases and the risk of ketosis (3,4,14,15).

In conclusion, serum concentrations of βHB ≥1.2 mmol/L serve to alert not only the presence of ketosis but also other clinical conditions during the puerperium. Although it is a specific predictor of a particular disease, it is useful in detecting conditions that affect health during postpartum at the herd level. Additionally, under grazing conditions, a high body condition, the number of calvings, the presence of a puerperal disease, milk fever, or placental retention are risk factors for hyperketonemia, which highlights the need to implement monitoring and prevention strategies for these factors to control this entity.

Conflicts of interest

The authors declare that there are no conflicts of interest in the information presented in this manuscript.

Acknowledgments

The authors wish to thank COLCIENCIAS [call 751 of 2016] and the research group BIOGÉNESIS for financing this study.

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