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Evaluation of β-hydroxybutyrate, calcium, and non-esterified fatty acids at day one postpartum in predicting the risk of diseases in dairy cows

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Abstract

In this study, the usability of calving type, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and calcium (Ca) concentrations in predicting postpartum diseases was examined. For this purpose, 120 Holstein cows were used in the study. These cows were divided into two subgroups according to the calving type: unassisted parturition (eutocic, n = 58) and assisted parturition (dystocia, n = 62). Cows were followed for 15 days postpartum. Diseases of retained foetal membranes (RFM), metritis, and clinical mastitis that developed in the postpartum period were recorded. Then, NEFA, BHB, and Ca concentrations were measured in blood samples collected at parturition from cows with RFM (n = 19), metritis (n = 34), clinical mastitis (n = 20) and cows that did not develop the disease (n = 47). Our results show the decreased Ca concentration to be positively associated with the risk of mastitis ($P < 0.001$, odds ratio [OR] 4.599), metritis ($P = 0.008$, OR 4.286), and RFM ($P < 0.001$, OR 5.016); and increased BHB concentration to be positively associated with the risk of metritis ($P = 0.002$, OR 4.069) and RFM ($P < 0.001$, OR 9.498). The results of the study indicated that low Ca and high BHB concentration at parturition could negatively affect the productive efficiency of dairy cows.

Cows, dystocia, mastitis, metritis, transition period

The majority of diseases develop in dairy cows during the transition period (from 3 weeks before to 3 weeks after calving), a critical phase characterized by notable alterations in their physiological processes, metabolic activities, and inflammatory reactions (Horst et al. 2021; Somagond et al. 2023). Endocrine, metabolic, and physiological changes occur in the peripartum period due to parturition, lactogenesis, and colostrogenesis (Sheldon et al. 2009; Braga-Paiano et al. 2019; Kizil et al. 2023). Fat mobilization is a frequent occurrence in that period, typically attributed to an energy deficit, resulting in the generation of non-esterified fatty acids (NEFA). Elevated levels of this particular metabolite during the antepartum and postpartum periods have been linked to a range of post-partum diseases, including hyperketonaemia, metritis, abomasal displacement, and mastitis (LeBlanc et al. 2005; Ospina et al. 2010a). In transition animals (Duffield et al. 2009; Ospina et al. 2010b; Todorović and Davidović 2012; McArt et al. 2013), the metabolites β-hydroxybutyrate (BHB) and/or NEFA are frequently used as indicators of negative energy balance.

After calving, there is a sudden increase in mineral requirements and energy needed to maintain the milk supply (Degaris and Lean 2008). Hypocalcaemia is a metabolic condition of significant economic importance in cows during the transition period

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(Seely and McArt 2023). The topic of subclinical hypocalcaemia receives growing interest as a result of its probable correlation with compromised welfare and efficiency (Caixeta et al. 2015). Low calcium (Ca) concentrations in the blood immediately after calving may be a predictor of developing diseases (Oetzel 2004). A decrease in blood Ca levels is associated with an increase in blood BHB and NEFA concentrations (Chapinal et al. 2011). Moreover, these indicators play a role in predicting diseases during the transition period (Van Winden et al. 2003). It is estimated that the incidence of diseases such as ketosis and displacement of the abomasum will be higher in cows with blood NEFA and BHB concentrations above the critical threshold. Also, it was determined that the decrease in serum Ca concentration after calving increases the risk of displacement of the abomasum (LeBlanc et al. 2005; Ospina et al. 2010a).

The aim of this study is to ascertain whether the concentrations of NEFA, BHB, and Ca at day one postpartum in dairy cows can be utilized as indicators of possible postpartum mastitis, metritis, and retained foetal membranes (RFM) diseases.

Materials and Methods

Animals and study design

This study was conducted following approval by the Kastamonu University Local Ethics Committee of Animal Experimentation (10.11.2023, 45/9).

This study was conducted on a dairy farm in where 800 Holstein cows were produced. Holstein cows (n = 120), multiparous (2nd and 3rd lactation, 4–6 years old), and with a body condition score ranging 3.5–4.0, were included in this study. We scored their body condition on a scale of 1 to 5. This scoring system was developed using guidelines established by Edmonson et al. (1989). Also, according to those with BCS 4 or higher were classified as obese and were excluded from the study. Cows were living in free-stall barns. The cows were fed a mixed ration and milked twice a day. Cows had access to water *ad libitum*.

According to information regarding calving, it was evaluated as Type 1 (n = 58): unassisted parturition (eutocic) and Type 2 (n = 62): assistance parturition (dystocia; veterinary-assisted delivery without caesarean section or foetotomy). The cows were monitored for 15 days postpartum, and the diseases developing in the cows were determined. In addition, a cohort of 47 cows who remained disease-free during the 15-day postpartum period was established. The cows were diagnosed with RFM (n = 19) according to the criteria of Beagley et al. (2010), metritis (n = 34) according to the criteria of Sheldon et al. (2006), and clinical mastitis (n = 20) according to the criteria of Cobirka et al. (2020). Placenta retention up to 48 h postpartum is known as RFM. In most study, RFM in cattle is defined within 12 to 24 h. Placenta retention up to 24 h postpartum is known as RFM in cows (Drillich et al. 2003; Beagley et al. 2010). The 24-h period following the calving was also considered in this study. An enlarged uterus with foul-smelling, watery-dark brown discharge was a sign of metritis. Rectal temperatures were measured in metritis-affected cows, and those that registered ≥ 39.5 °C were identified as having puerperal metritis. Cows that developed metritis and RFM together were excluded from the study. The people who worked with the farm checked all the cows for signs of clinical mastitis right before each milking. If there was abnormal milk or signs (e.g. redness, pain, and swelling) of inflammation in one or more quarters, it meant the cow had clinical mastitis.

Sample analysis

Blood samples were collected from the cows shortly after calving (within 2 h). The blood samples were collected from the coccygeal vein into plain tubes without anticoagulants. After collection, blood was kept at room temperature for 1 h and then centrifuged at 2,795 × *g* for 10 min. Serum samples were then transferred to the Eppendorf tubes and stored at −20 °C until biochemical analysis. After serum thawing, NEFA, BHB, and Ca concentrations in serum samples were measured with an automatic biochemistry analyser (910Vet, Diasys Respons, Holzheim, Germany), using a commercially available kit (Reagent, Diasys Respons). Biochemical analysis was performed at Animal Hospital Laboratory of the Faculty of Veterinary Medicine, Kastamonu University.

Statistical analysis

The information is displayed as mean ± standard deviation. P values < 0.05 were regarded as significant. Statistical analyses were carried out using the SPSS statistical programme (version 22.0, Chicago, IL, USA). To ascertain whether data distributions were normal, Shapiro-Wilk test was performed. Since the parameters showed normal distribution, parametric test was performed. Student's t-test was used to analyse the parameters according to calving type. Then, one-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons were used to analyse the parameters in the diseased groups and healthy cows.

In order to determine whether or not NEFA, BHB, Ca, and calving type are predictive of the development of metritis, mastitis, and RFM diseases, a multivariate logistic regression analysis was carried out. Calving type was classified into two types; Type 1: unassisted parturition (Eutocic) and Type 2: assistance parturition (Dystocia; without caesarean section or foetotomy). Statistical analyses were performed using the jamovi software (version 2.4) (The jamovi project 2023).

Results

Table 1 shows the blood NEFA, BHB, and Ca concentrations at parturition according to calving type. When calving type comparisons were performed, it was found that blood Ca, BHB, and NEFA concentrations at the time of parturition in cows with metritis, mastitis, and RFM within postpartum 15 days did not differ significantly from one another. In the healthy group, the BHB concentration was higher in animals calving type 2 (0.53 ± 0.06 mmol/l) than in calving type 1 (0.38 ± 0.03 mmol/l) ($P = 0.020$).

In healthy cows with calving type 1, the Ca concentration (2.37 ± 0.34 mmol/l) was higher at parturition, but the NEFA concentration (0.60 ± 0.04 mmol/l) was lower compared to diseases groups ($P < 0.05$). In addition, BHB concentrations were discovered to be higher in cows with metritis (1.51 ± 0.13 mmol/l) and RFM (1.56 ± 0.27 mmol/l) compared to healthy cows (0.38 ± 0.03 mmol/l) ($P < 0.05$) (Table 1).

The study found that the Ca concentration (2.27 ± 0.23 mmol/l) at parturition in healthy cows with calving type 2 was significantly greater than in cows that developed disease ($P < 0.05$). It was determined that the BHB concentration at parturition was higher in cows with calving type 2 that developed metritis (1.45 ± 0.15 mmol/l) and RFM (1.17 ± 0.23 mmol/l) ($P < 0.05$). It was found that cows with calving type 2 that developed metritis had higher NEFA concentrations (1.27 ± 0.11 mmol/l) at parturition ($P < 0.05$) (Table 1).

NEFA, BHB, Ca, and calving type were included in the initial multivariable model for the risk of metritis, mastitis, and RFM. The final results of the multivariable model are presented in Table 2. Decreased Ca concentration increases the risk of mastitis ($P < .001$, odds ratio (OR) 4.599), metritis ($P = 0.008$, OR 4.286), and RFM ($P < 0.001$, OR 5.016). Increased BHB concentration increases the risk of metritis ($P = 0.002$, OR 4.069) and RFM ($P < 0.001$, OR 9.498). On the other hand, calving type and NEFA concentration had no effect on the development of these diseases.

Table 1. Calving type and indicators in postpartum diseases.

Calving type	Indicator	Postpartum within 15 days				*P*
		Healthy	Metritis	Mastitis	RFM	
Type 1		(n = 17)	(n = 20)	(n = 8)	(n = 13)	
(n = 58)	Ca (mmol/l)	$2.37^{a} \pm 0.34$	$2.11^{ab} \pm 0.18$	$1.79^{c} \pm 0.23$	$1.92^{bc} \pm 0.13$	< 0.001
	BHB (mmol/l)	$0.38^{b} \pm 0.03$	$1.51^{a} \pm 0.13$	$0.80^{b} \pm 0.25$	$1.56^{a} \pm 0.27$	< 0.001
	NEFA (mmol/l)	$0.60^{c} \pm 0.04$	$1.31^{a} \pm 0.09$	$0.94^{bc} \pm 0.14$	$1.03^{ab} \pm 0.11$	< 0.001
Type 2		(n = 30)	(n = 14)	(n = 12)	(n = 6)	
(n = 62)	Ca (mmol/l)	$2.27^{a} \pm 0.23$	$2.04^{b} \pm 0.12$	$1.89^{b} \pm 0.17$	$1.98^{b} \pm 0.07$	< 0.001
	BHB (mmol/l)	$0.53^{b} \pm 0.06$	$1.45^{a} \pm 0.15$	$0.59^{b} \pm 0.11$	$1.17^{a} \pm 0.23$	< 0.001
	NEFA (mmol/l)	$0.60^{b} \pm 0.05$	$1.27^{a} \pm 0.11$	$0.75^{b} \pm 0.15$	$1.02^{ab} \pm 0.19$	< 0.001
P	Ca (mmol/l)	0.336	0.269	0.264	0.392	
	BHB (mmol/l)	0.020	0.760	0.403	0.391	
	NEFA (mmol/l)	0.929	0.772	0.398	0.964	

$^{a, b, c}$: Significant difference between groups with different superscripts in the same row ($P < 0.05$).
RFM: retained foetal membranes; Ca: calcium; BHB: β-hydroxybutyrate; NEFA: non-esterified fatty acids

Table 2. Results of the multivariable logistic regressions of calving type and metabolic responses measured calving time associated with the risk of mastitis, RFM, and metritis.

Variable	Predictor	95% Confidence interval					
		Estimate	Lower	Upper	SE	*P*	Odds ratio
Mastitis - Healthy	Intercept	39.25	21.92	56.58	8.84	< 0.001	1.11e + 17
	Ca (mmol/l)	−20.04	−28.51	−11.58	4.31	< 0.001	4.59903
	BHB (mmol/l)	1.57	−1.39	4.54	1.51	0.300	4.80896
	NEFA (mmol/l)	0.33	−3.23	3.90	1.81	0.855	1.39517
	Calving type;						
Type 1 - Type 2	0.49	−1.23	2.22	0.88	0.576	1.63611	
Metritis - Healthy	Intercept	16.95	1.42	32.46	7.91	0.032	2.29e + 7
	Ca (mmol/l)	−10.06	−17.50	−2.62	3.79	0.008	4.28585
	BHB (mmol/l)	3.78	1.34	6.22	1.24	0.002	4.06935
	NEFA (mmol/l)	0.64	−2.36	3.66	1.54	0.673	1.91169
	Calving type;						
Type 1- Type 2	1.16	−0.41	2.73	0.81	0.149	3.18912	
RFM - Healthy	Intercept	31.60	15.09	48.12	8.42	< 0.001	5.34e + 13
	Ca (mmol/l)	−16.73	−24.73	−8.42	4.08	< 0.001	5.01582
	BHB (mmol/l)	4.61	1.97	7.24	1.34	< 0.001	9.49834
	NEFA (mmol/l)	−2.14	−5.56	1.28	1.74	0.218	0.11721
	Calving type;						
Type 1 - Type 2	1.61	−0.08	3.31	0.87	0.063	5.01660	

RFM: retained foetal membranes; Ca: calcium; BHB: β-hydroxybutyrate; NEFA: non-esterified fatty acids

Discussion

In this study, it was found that blood NEFA, BHB, and Ca concentrations at the time of parturition had a significant impact on the development of diseases such as mastitis, metritis, and RFM in the postpartum period. The study's findings showed that low blood Ca concentrations at parturition were associated with increased risk of mastitis, metritis, and RFM (ORs were 4.599, 4.286, and 5.016, respectively). Moreover, it has been found that high blood BHB concentration at parturition is positively correlated with the development of metritis and RFM.

Previous research has identified elevated concentrations of NEFA before parturition as a potential risk factor for metritis (Ospina et al. 2010b), RFM (Chebel 2021), ketosis (Melendez et al. 2009), abomasum displacement (LeBlanc et al. 2005), dystocia, and milk fever (Kia et al. 2023).

Kia et al. (2023) reported on the correlation between NEFA concentrations one week before calving and the likelihood of milk fever occurring. Cows with NEFA concentrations above 0.3 mmol/l were found to be 1.9 times more likely to develop milk fever. Similar to the results of our study, when the median value of NEFA concentration (0.9 mEq/l) was used as the threshold value, no relationship was found between NEFA concentration at calving and the incidence of mastitis and milk fever. On the other hand, the incidence of clinical mastitis and milk fever was higher in cows with NEFA concentrations of 1.2 mEq/l than in cows with values < 1.2 mEq/l (Melendez et al. 2009). According to Duffield et al. (2009) cows with serum BHB concentrations ≥ 1.2 mmol/l in the first week after parturition had an increased risk of metritis and abomasal displacement in the weeks that followed. In a different study, Ospina et al. (2010a) reported that in cows with BHB concentration ≥ 1.0 mmol/l between 3 and 14 days postpartum, the probability of clinical ketosis, metritis, and abomasal displacement increased in the subsequent period. In our study, a positive correlation was found between high BHB concentration at parturition and the development of metritis and RFM in cows.

According to Rodríguez et al. (2017), there is a correlation between ketosis and subclinical hypocalcaemia, defined as serum Ca concentration ≤ 2.14 mmol/l 24 to 48 h after calving. The likelihood of ketosis in cows with subclinical hypocalcaemia was 5.5 times higher. Chamberlin et al. (2013) discovered that the livers of hypocalcaemic cows (defined as having an iCa of less than 1.0 mmol/l on the day of calving) had greater lipid content and NEFA concentration. Similar to the findings of this study, in the postpartum period, cows with low Ca concentrations were more susceptible to mastitis, metritis, and RFM than those with high Ca concentrations (Safak et al. 2023). Akar and Yıldız (2005) discovered that cows with low Ca concentration were more likely to develop RFM, which is consistent with our results. Ocal et al. (1999), on the other hand, discovered no difference in Ca concentration between cows with RFM and those without. It was found in this study that RFM development was impacted by a decrease in Ca concentration.

Dystocia has a negative impact on Ca balance after calving and on overall welfare. Both hypocalcaemia and dystocia are known to predispose to postpartum diseases such as RFM and metritis (Benzaquen et al. 2015). Erb et al. (1985) determined that the incidence of dystocia and RFM was 4.2 times and 2 times, respectively, in cows with hypocalcaemia. Risco et al. (1994) discovered that cows with RFM had lower plasma Ca concentrations at 24 h after parturition (6.27 ± 0.18 mg/dl) and for 7 days postpartum (7.40 ± 0.18 mg/dl) compared to those without RFM. On the other hand, in our study, no difference was found between the dystocia and eutocic groups in terms of the development of metritis, RFM, and mastitis.

This study has some limitations due to the low number of cows in the study groups. Future studies should include larger numbers of animals.

In conclusion, circulating concentrations of NEFA, BHB, and Ca were the most influential predictors of increased risk of postpartum disease. It was concluded that measuring these indicators at day one postpartum could be useful in the early diagnosis of diseases that will develop postpartum. Low Ca and high BHB concentrations at parturition could negatively affect the productive efficiency of dairy cows.

Conflict of interest

The authors declare that there is no conflict of interest.

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