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VARIATIONS IN MILK PARAMETERS MAY BE AN EARLY INDICATOR OF SUBCLINICAL KETOSIS

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ABSTRACT

Ketosis (acetonemia) is a severe metabolic disorder in dairy cattle associated with negative energy balance (NEB), occurring either independently or in association with other clinical disorders. Subclinical ketosis, characterized by elevated ketone bodies without clinical symptoms, is challenging to detect due to the impracticality of regular body fluid analysis on farms. This study aimed to establish correlations between milk components and blood β-hydroxybutyrate (BHBA) concentration, offering a non-invasive approach to predicting subclinical ketosis. Twelve crossbred cattle in their second parity from two different farms in the Wayanad district of Kerala were observed. Milk parameters were assessed on alternate days, and blood BHBA levels were measured weekly from 6 to 60 days postpartum. Statistical analysis, conducted using SPSS software (version 24.0), applied Pearson's correlation coefficient method to ascertain the correlation between milk parameters and blood BHBA concentrations. The results demonstrated a significant negative correlation between milk parameters - specifically SNF, Protein, Lactose, and Salt- and blood BHBA levels. This relationship remained consistent in the observed population with BHBA values exceeding 1.0 mmol/L, suggesting that routine milk composition analysis may serve as a practical indicator for the early detection of subclinical ketosis in dairy cattle.

Keywords: Subclinical ketosis, Ketosis, BHBA, Negative energy balance

INTRODUCTION

Ketosis (acetonemia) in dairy cattle is a severe metabolic disorder associated with negative energy balance (NEB), that can occur alone or in association with other clinical disorders. Ketosis in dairy cows is

caused by excessive lipolysis associated with NEB leading to accumulation of ketone bodies such as acetone, acetoacetate and beta-hydroxybutyrate. This metabolic disorder is generally seen in high producing dairy cows. Subclinical ketosis is characterised by high amount of ketone bodies in blood without any clinical manifestations of ketoacidosis which can only be diagnosed by quantifying blood glucose, ketone bodies, and non-esterified fatty acids (NEFA) in bodily fluids like blood, milk, and urine.

Minimising the prevalence, severity and effects of negative energy balance during the early postpartum period has aroused as a major concern for the dairy industry. Subclinical ketosis was found to afflict 40 to 60 per cent of cows in herds that were examined regularly throughout early lactation (Simensen *et al*., 1990; Duffield *et al.,* 1998) which was significantly greater than the 2 to 15per cent incidence of clinical ketosis (Duffield, 2000). Also, 90 per cent of subclinical ketosis is reported within the first two months with a peak level of BHBA during the second and third weeks of the periparturient period (Andersson, 1988). Economic losses are significant because of decreased milk yield, treatment costs, and, on occasion, fatalities or disposal of affected cows (McSherry *et al.,*1960, Radostits *et al*., 2000, Ha *et al.,* 2022).

Studies on the relationship between milk composition and some nutritional parameters showed that estimated energy balance is negatively correlated with milk fat percentage and fat-to-protein ratio and is positively correlated with milk protein percentage (Grieve *et al*., 1986). It is also reported that the lactose percentage is negatively correlated with conditions like Mastitis, Ketosis, and milk fever (Costa *et al*., 2019). A moderate positive correlation between free glucose and lactose in milk was observed by Larsen and Moyes (2015), suggesting lactose percentage may be a potential health indicator.

Since BHBA is the major ketone body with a normal circulatory concentration <1.0mmol/L in ruminants and is more stable than acetone and acetoacetate, blood concentrations of BHBA have been accepted as the "gold standard" test for ketosis (Tyopponen and Kauppinen, 1980; Zhang *et al*., 2012; Constable *et al*., 2017). A threshold plasma BHBA concentration of >1.2 mmol/L indicates subclinical ketosis (Duffield *et al*., 2009), whereas >3.0 mmol/L is suggestive of clinical ketosis (Oetzel, 2004). Apart from the economic loss due to reduced milk production, subclinical ketosis increases the susceptibility of the animal to other conditions such as lameness, metritis, and displaced abomasum. This will result in

an increased culling rate and higher herd expenditures, resulting in a significant loss to the farmer.

Due to various financial and organisational limitations, continuous monitoring of blood ketone bodies is impractical in dairy farm management. Present study investigates the scope of adopting fluctuations in various milk parameters at early lactation which can be correlated with varying levels of BHBA, for early detection/ prediction of cows that are at high risk of clinical or subclinical ketosis.

MATERIALS AND METHODS

A total of 12 crossbred dairy cows maintained under uniform management conditions from two different farms in the Wayanad district of Kerala in their early lactation period were selected for the study.

Sample collection

Milk samples were collected on alternate days from the 6th day to the 60th day after calving and their compositions were assessed. Blood samples were subjected to blood BHBA estimation weekly using a ketometer. Blood BHBA levels between the ranges of 1.2 mmol/L to 2.9 mmol/L were considered as positive for subclinical ketosis and those cows with blood BHBA $\geq$ 3.0 mmol/L were diagnosed as clinical ketosis (Oetzel, 2004).

Analysis of milk parameters

Milk samples were collected on alternate days and analysed for milk fat, non-fat solids (SNF), proteins, lactose, water content percentages, temperature, freezing point, salts and density by Lactoscan Milk Analyzer (Milktronic Ltd., Bulgaria).

Estimation of BHBA

Blood BHBA was estimated weekly by using the Freestyle Libre blood glucose and ketone monitoring system (Abbott laboratories, USA) and Freestyle Optium blood beta ketone test strips (Abbott Laboratories, USA).

Statistical analysis

Statistical analysis was done to ascertain the correlation between serum BHBA and milk components using the statistical software SPSS (IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp.).

RESULTS AND DISCUSSION

Milk and blood samples were collected between day 6 and day 60 from calving from a population of 12 cattle. Milk samples collected on alternate days were analysed, and weekly averages were taken for each milk parameter. The

relationship between week-wise blood BHBA concentrations and milk parameters (fat, SNF, density, protein, lactose, freezing point, and salts) was determined using Karl Pearson's correlation coefficient method. This method evaluates whether milk parameters change in response to BHBA levels in a consistent or opposite trend across samples.

The results were expressed as means ± standard deviation and analysed by SPSS software (IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp.). p values of <0.05 were considered statistically significant, and those of <0.01 were considered highly significant. An increased level of one parameter is likely to be reflected by higher values of the other according to a positive correlation coefficient. It goes from -1, which means the two parameters respond in entirely opposing ways, to +1, which means the two parameters react uniformly in all samples. A rise in one parameter's level is therefore likely to be reflected by an increase in the other parameter's level, according to a positive correlation coefficient. When the coefficient is zero, there is no association that can be found.

During the peak lactation period, a correlation was observed between various milk constituents and blood BHBA values. The week-wise analysis showed significant correlations from the sixth week to the ninth week ($p<0.05$). Among the parameters selected, SNF, protein, lactose, freezing point, and salts showed a negative correlation with blood BHBA levels ($p<0.05$) and (r) values between -0.5 and -0.8. This suggests a strong inverse relationship between these variables. However, the fat and fat/protein ratio did not show any correlation with blood BHBA levels (Table 1).

The analysis of the correlation between milk components and blood BHBA in cows during their early lactation period

Table 1. Results of correlation analysis

BHBA Milk parameters	Week 6	Week 7	Week 8	Week 9	From day 6 - 60
SNF	-0.730**	-0.614*	-	-0.697*	-0.304**
Density	-	-	-	-0.683*	-
Protein	-0.689*	-0.591*	-	-0.733**	-0.287**
Lactose	-0.735**	-0.647*	-0.614*	-0.670*	-0.299**
Freezing point	-0.790**	-0.696*	-	-0.584*	-0.274*
Salts	-0.691*	-0.579*	-	-0.701*	-0.320**
Weakly values of blood BHBA and milk parameters were analysed by Karl Pearson's correlation coefficient method. ** $p\leq 0.0$, * $p\leq 0.05$					

(from day 6 to 60 after calving) revealed that milk SNF, protein, lactose and salts showed a moderate negative correlation with blood BHBA values ($p \leq 0.01$), and the freezing point showed a small negative correlation with blood BHBA ($p \leq 0.05$) (Table 1).

In the study population, five out of twelve cattle had blood BHBA values of 0.9 mmol/L or above and were considered at high risk for subclinical ketosis. Two of these cows had BHBA values exceeding 1.2 mmol/L, which is the threshold value for subclinical ketosis. The statistical analysis showed that during the period of peak lactation, the population under study showed a correlation between various milk parameters with blood BHBA. The milk parameters, SNF, protein, lactose, freezing point, and salts showed a negative correlation with blood BHBA levels whereas fat and fat/protein ratio did not show any correlation with blood BHBA levels. These correlations were observable in the high-risk group and highlighted in bold (Table 2). This data indicates that the milk parameters showed a significant decline when the BHBA levels exceeded 0.9 mmol/L, which is considered as the threshold for subclinical ketosis. The mean values of the milk parameters in cows

Table 2

ANIMAL NO	DATE	DAY	FAT (per cent)	SNF (per cent)	DENSITY (kg/m3) *	PROTEIN (%)	LACTOSE (%)	FREEZING POINT (°C)	SALTS (%)	F/P RATIO	BHBA (mmol/L)
074360	18/8	27	1.9	8.6	1032.35	3.1	4.67	0.52	0.7	0.61	**0.9**
	24/8	33	9.7	**7.8**	**1023.11**	**2.78**	**4.44**	0.55	**0.63**	3.49	**1.6**
079685	26/10	8	5.00	9.80	1034.42	3.57	5.43	0.65	0.80	1.40	**1.30**
	28/10	10	3.40	**7.80**	**1027.94**	**2.86**	**4.33**	**0.50**	**0.64**	1.19	**0.90**
	3/11	16	4.40	**7.80**	**1027.02**	**2.83**	**4.32**	**0.50**	**0.63**	1.55	**0.90**
	17/11	30	3.40	**7.90**	**1028.29**	**2.89**	**4.38**	**0.50**	**0.65**	1.18	**1.00**
	23/11	36	3.50	**7.10**	**1024.91**	**2.67**	**3.90**	**0.44**	**0.51**	1.31	**1.00**
	1/12	44	5.20	**7.90**	**1026.73**	**2.95**	**4.38**	**0.51**	**0.64**	1.76	**1.10**
	7/12	50	4.20	**7.40**	**1025.63**	**2.78**	**4.10**	**0.47**	**0.60**	1.51	**1.20**
164458	26/10	23	3.20	8.60	1030.93	3.13	4.72	0.55	0.70	1.02	**1.10**
	3/11	31	3.40	**7.90**	**1028.29**	**2.89**	**4.38**	**0.50**	**0.65**	1.18	**1.00**
	9/11	37	3.40	**7.10**	**1024.91**	**2.57**	**3.90**	**0.44**	**0.57**	1.32	**1.00**
	17/11	45	5.10	**7.90**	**1026.73**	**2.85**	**4.38**	**0.51**	**0.64**	1.79	**1.10**
	23/11	51	4.10	**7.40**	**1025.63**	**2.68**	**4.10**	**0.47**	**0.60**	1.53	**1.20**
	1/12	59	5.10	**7.50**	**1026.60**	**2.85**	**4.20**	**0.51**	**0.59**	1.79	**1.10**
225364	18/8	32	2.3	8.40	1030.79	3.06	4.58	0.52	0.68	0.75	**0.90**
405561	18/8	28	3.00	8.30	1030.14	3.04	4.58	0.53	0.68	0.99	**0.90**
537100	12/10	19	3.40	9.60	1034.81	3.51	5.28	0.62	0.78	0.97	**0.90**
	20/10	27	3.50	8.70	1031.32	3.19	4.81	0.56	0.71	1.10	**0.90**
	26/10	33	3.30	9.10	1033.05	3.34	5.03	0.59	0.75	0.99	**1.00**
918344	27/9	22	5.70	8.80	1029.49	3.18	4.90	0.60	0.73	1.79	**0.90**
	2/10	58	6.20	8.30	**1027.84**	**3.02**	4.66	0.55	0.68	2.05	**1.00**

with BHBA levels above 0.9 mmol/L were lower than the average normal readings of the observed population. These findings suggest that elevated BHBA levels impair the quality and composition of milk in dairy cows.

The results obtained in the present study is in agreement with the previous reports. The negative correlation of Protein and lactose with BHBA in ketotic animals is in agreement with studies made by Duffield *et al*. (1997) and Yang *et al*. (2019). Grieve *et al*. (1986) and Koeck *et al*. (2014), also reported protein percentage in milk is positively correlated to the energy balance of the cow and negatively correlated to milk fat percentage and fat/protein ratio.

Although milk protein levels are lowered in the high-risk categories while blood BHBA levels are increased, in contrast to the previous reports (Duffield *et al*., 2009), the present study could not find any correlation between fat/protein ratio with blood BHBA levels. However, two cows (079685 & 164458) in the population with high BHBA showed high-fat content in their milk and a strong positive correlation (r) 0.849 and 0.864 with blood BHBA ($p \leq 0.01$), which agrees with the previous reports. Miettinen *et al*. (1993) also determined the metabolic balance of cattle by performing acetone and urea assay of milk and found that acetone concentration in milk is positively correlated with milk fat and negatively correlated with milk protein, fat-protein ratio, and milk production during the seventh to eighth weeks postpartum. Larsen and Moyes (2015) reported a moderate positive correlation of lactose with BHBA levels and can be used to monitor the condition of farm animals. However, the present study's finding is contradictory to the above report. A recent report by Costa *et al*. (2019) states that lactose is negatively correlated with the conditions like mastitis, ketosis and milk fever. Together with our results and the previous report, It is suggested that lactose can be a candidate molecule for early prediction of subclinical ketosis in dairy cattle.

SUMMARY

To prevent clinical ketosis and reduce the economic losses associated with subclinical ketosis, early diagnosis of the condition may be crucial. According to the study findings, a negative correlation was observed between blood BHBA and milk parameters such as SNF, protein, lactose, freezing point, and salts. Over the study period, there was a significant reduction in these milk parameters as blood BHBA increased. The correlation between blood BHBA and milk parameters, specifically SNF, protein, salts, and most significantly lactose, was consistently present. This

suggests that these milk parameters could potentially serve as early markers for subclinical ketosis, which would aid farmers in predicting its onset by routine milk analysis and Dairy Herd Improvement (DHI) test data.

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