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Clinical vs. Subclinical mastitis in Sahiwal Cows: The role of season and pathogens in metabolic disruption

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Abstract

The present study investigated the metabolic alterations associated with intramammary infections (IMI) caused by *Staphylococcus aureus* and *Escherichia coli* in early-lactating Sahiwal cows, with emphasis on β -hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) in plasma and milk. A total of 24 mastitis-affected cows (12 clinical, 12 subclinical) were selected after screening 73 animals using mCMT and clinical examination. Results showed that cows infected with *E. coli* exhibited significantly higher ($P < 0.05$) plasma and milk BHBA and NEFA concentrations compared to *S. aureus*. Seasonal variations were also significant ($P < 0.05$), with both plasma and milk BHBA and NEFA reaching their highest levels during summer, indicating pronounced metabolic stress under heat load. Clinical mastitis cows demonstrated markedly elevated ($P < 0.05$) BHBA and NEFA values in both plasma and milk across all seasons compared to subclinical cases, revealing greater lipid mobilization and impaired energy balance in severe infections. The study overall underscores the joint impact of mastitogen type, the severity of the disease, and seasonal heat stress on metabolic dysregulation in Sahiwal cows. It highlights the importance of BHBA and NEFA as potential biomarkers for evaluating the severity of mastitis and metabolic stress.

Keywords: Sahiwal, Mastitis, Season, Metabolic parameters-NEFA, BHBA

Introduction

During the initial phase of lactation, dairy cows experience a negative energy balance to satisfy the substantial demands for milk production, resulting in the rapid mobilization of fat and protein (Macrae et al., 2019) [17]. During this period, the cow transitions from a non-lactating state to the beginning of abundant colostrum and milk synthesis, resulting in major nutritional, physiological, metabolic, and immunological changes (Sundrum, 2015) [27]. Cows must adapt metabolically to the significant increase in nutritional needs for high milk production and the subsequent lactation's return of reproductive function (Bisinotto et al., 2012) [1]. Inadequate intake of dry matter, at the beginning of lactation, results in insufficient adaptation and metabolism regulation (Fiore et al., 2018) [3]. High-yielding dairy cows face a significant metabolic challenge that causes drastic changes in their metabolic profiles. These changes can be used to assess the health of cows (Macrae et al., 2006) [18] and identify risk factors for metabolic and periparturient clinical diseases (Hammon et al., 2006; McArt et al., 2013) [7, 19], out of which mastitis is the costliest disease in the dairy industry. Certain periparturient illnesses disrupt metabolic processes, leading to changes that may be interpreted as metabolic markers. The concentration of these metabolites changes in a diseased state, revealing details about the pathophysiology of the illness (Shin et al., 2015) [26]. The majority of research has concentrated on the impact of metabolic status on the immune response in dairy cows (Moyes et al., 2009; Zarrin et al., 2014) [21, 34]. During mastitis, the immunometabolic responses mainly concentrate on the transcription-level reactions in both liver and mammary tissues (Lor et al., 2011; Moyes et al., 2009; Vels et al., 2009) [16, 21, 31]. Prior research suggests that the liver's capacity to metabolize fatty acids diminishes, and essential genes linked to metabolic functions are down-regulated following an intramammary *E. coli* challenge (Jorgensen et al., 2012) [11], as well as after an intramammary endotoxin challenge (Jiang et al., 2008) [9].

Additionally, alterations in circulating non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), and glucose have been observed before the reductions in feed intake and milk production during an intramammary infection (IMI) in dairy cows (Grauagnard et al., 2013; Waldron et al., 2006; Waldron et al., 2003)^[4, 32] The objective of this study was to characterize the changes in various metabolic parameters (NEFA and BHBA) in blood and milk during IMI with *E. coli* and *S. aureus* in Sahiwal cows during early lactation.

Materials and methods

At the Livestock Research Centre (LRC) of NDRI located in Karnal, Haryana, a total of 73 Sahiwal cows were initially assessed for either clinical or subclinical mastitis. The modified California Mastitis Test (mCMT) was employed to identify subclinical mastitis in the milk of these cows. Clinical symptoms were utilized to diagnose cows suffering from clinical mastitis. From the 73 animals, 24 Sahiwal cows were chosen, comprising 12 clinical and 12 subclinical cases. Blood samples (approximately 10ml) were collected in sterile heparinised vacutainers from each cow via jugular vein puncture. The plasma was isolated through centrifugation at 3000 rpm for 15 minutes. Composite milk samples (representing all four quarters) were gathered from cows affected by SCM and CM. A portion of whole milk and skimmed milk (obtained after centrifugation at 3000 rpm for 20 minutes at 4°C) was preserved at -20°C for the analysis of metabolic profiles. Bacteriological examinations were conducted following standard procedures. EMB agar was utilized to isolate *E. coli* for microbiological assessments, while MSA (Mannitol salt agar) was employed to isolate *Staphylococcus aureus*. These products were procured from Hi-Media and TM Media Company, respectively. The copper soap solvent extraction method modified by Shipe et al., (1980) was adopted for the H V W L P D W L R Q H B A lin 14 m S was estimated by $\pm$ -Hydroxy butyrate (Ketone Body) Assay Kit - (catalogue No:201-04-0182) obtained from Sun Red Laboratory. The GraphPad Prism software program was used to analyze the data using an unpaired t-test.

Result and Discussion

Microbiological analysis indicated that *Staphylococcus aureus* was the bacterium most commonly isolated from the milk samples that were examined (Surya et al., 2021)^[30].

Table 1: Variations in plasma and milk Acute Phase Proteins of Sahiwal cows infected with different mastitogens

Breed	Mastitogen	n	Plasma		Milk	
			BHBA (mM/L)	NEFA (μ M/L)	BHBA (mM/L)	NEFA (μ M/L)
Sahiwal	<i>S. aureus</i>	20	0.51 $\pm$ 0.05	413.32 $\pm$ 25.16	0.45 $\pm$ 0.02	223.60 $\pm$ 13.31
	<i>E. coli</i>	4	0.62 $\pm$ 0.08	478.59 $\pm$ 102.31	0.59 $\pm$ 0.03	277.25 $\pm$ 39.33

In Sahiwal cows, both *Staphylococcus aureus* and *Escherichia coli* mastitogens had an impact on circulating D Q G P L O N P H W D E R O L F L Q G L F D W B R U V Y S H F L L F D Q Q hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA). Cows that were infected with *E. coli* exhibited significantly higher plasma BHBA levels (0.62 $\pm$ 0.08 mM/L) and plasma NEFA levels (478.59 $\pm$ 102.31 μ M/L) compared to those infected with *S. aureus*, which showed

levels of 0.51 $\pm$ 0.05 mM/L and 413.32 $\pm$ 25.16 μ M/L, respectively. A similar pattern was noted in milk, where *E. coli* mastitis led to increased BHBA (0.59 $\pm$ 0.03 mM/L) and NEFA (277.25 $\pm$ 39.33 μ M/L) levels in comparison to *S. aureus* infections, which recorded 0.45 $\pm$ 0.02 mM/L and 223.60 $\pm$ 13.31 μ M/L, respectively.

Elevated BHBA levels in milk and serum increase the risk of mastitis in dairy cows (Suriyasathaporn et al., 2000)^[28]. Grinberg et al., (2008)^[5] reported that BHBA reduces neutrophil phagocytosis and NET formation, weakening bactericidal activity against *E. coli*. These negative effects on immune function help explain the higher susceptibility of ketotic cows to mastitis. Kornalijnslipje et al., (2003)^[13] further noted that severe *E. coli* mastitis was associated with -OH-butyrate levels above 1.4 mmol/L. Cows with *E. coli* mastitis show significantly higher serum NEFA levels (Jirillo et al., 2002; Katoh, 2002)^[10, 12], and Hagiwara et al., (2014) found that non-survivors of acute *E. coli* mastitis had the highest NEFA concentrations. This indicates that NEFA levels rise with infection severity. Increased NEFA and BHBA, along with reduced glucose, characterize severe negative energy balance. Such metabolic stress makes cows more susceptible to diseases like mastitis (Moyes et al., 2009; Perkins et al., 2002; Suriyasathaporn et al., 1999)^[21, 25, 29].

Table 2: Seasonal variations in plasma metabolic parameters in mastitis-infected Sahiwal cows

Season	Thermo neutral	Winter	Summer
BHBA (mM/L)	0.45 ^A $\pm$ 0.06	0.53 ^A $\pm$ 0.07	0.60 ^B $\pm$ 0.08
NEFA (μ M/L)	384.56 ^X $\pm$ 29.80	364.56 ^X $\pm$ 37.21	523.47 ^Y $\pm$ 48.77

Values with different superscripts for different seasons in a row differ at 5% level

Table 3: Seasonal variations in milk metabolic parameters in mastitis-infected Sahiwal cows

Season	Thermo neutral	Winter	Summer
BHBA (mM/L)	0.42 $\pm$ 0.03	0.49 $\pm$ 0.04	0.50 $\pm$ 0.03
NEFA (μ M/L)	200.94 ^X $\pm$ 15.39	218.80 ^X $\pm$ 15.92	277.89 ^Y $\pm$ 27.41

Values with different superscripts for different seasons in a row differ at 5% level. Significant seasonal variations were observed in plasma metabolic parameters of mastitis-infected Sahiwal cows. Plasma BHBA levels exhibited a progressive increase from the thermoneutral season (0.45 $\pm$ 0.06 mM/L) to winter (0.53 $\pm$ 0.07 mM/L), with the peak value observed in summer (0.60 $\pm$ 0.08 mM/L), indicating a notable rise during periods of heat stress. Similarly, plasma NEFA concentrations reached their highest levels in summer (523.47 $\pm$ 48.77 μ M/L), significantly surpassing those recorded during the thermoneutral (384.56 $\pm$ 29.80 μ M/L) and winter seasons (364.56 $\pm$ 37.21 μ M/L). The metabolic indicators of milk exhibited a similar pattern. The concentration of BHBA in milk experienced a slight seasonal rise, increasing from 0.42 $\pm$ 0.03 mM/L during the thermoneutral season to 0.49 $\pm$ 0.04 mM/L in winter and reaching 0.50 $\pm$ 0.03 mM/L in summer. Additionally, the levels of NEFA in milk peaked in summer at 277.89 $\pm$ 27.41 μ M/L, which was significantly greater than the values recorded during the thermoneutral period (200.94 $\pm$ 15.39 μ M/L) and winter (218.80 $\pm$ 15.92 μ M/L). Demonstrated

that the levels of BHBA and NEFA fluctuated based on breed, season, and lactation stage, with elevated values observed in winter. Conversely, Kumar et al., (2018) and Gulab Chandra et al., (2012) [14, 2] reported that NEFA and BHBA levels increased during the summer months in both indigenous and KF cows. These findings indicate that metabolic responses vary among different breeds and in response to environmental stressors. Our results are consistent with observations in indigenous cattle, revealing significantly higher levels of NEFA and BHBA during the summer in mastitis-affected Sahiwal cows.

Table 4: Plasma metabolic parameters in Sahiwal cows suffering from clinical and subclinical mastitis during different seasons

Season	Treatment	BHBA (mM/L)	NEFA(μM/L)
Thermoneutral	SCM	0.32 ^a ± 0.02	378.65 ± 48.20
	CM	0.57 ^{bA} ± 0.08	390.48 ± 42.40
Winter	SCM	0.36 ^c ± 0.05	324.28 ± 39.35
	CM	0.70 ^d ± 0.06	404.83 ± 61.89
Summer	SCM	0.39 ^e ± 0.03	458.87 ± 41.83
	CM	0.81 ^{fB} ± 0.05	588.07 ± 81.04

Values with different superscripts for mastitis type in each season in a column differ at 5% level

Table 5: Variations in milk metabolic parameters in Sahiwal cows suffering from Clinical and Subclinical Mastitis in different seasons

Season	Treatment	BHBA (mM/L)	NEFA(μM/L)
Thermoneutral	SCM	0.32 ^a ± 0.06	165.17 ^f ± 5.99
	CM	0.48 ^b ± 0.04	236.71 ^{sA} ± 14.71
Winter	SCM	0.40 ^c ± 0.03	179.38 ^u ± 4.25
	CM	0.58 ^d ± 0.03	258.21 ^v ± 11.35
Summer	SCM	0.39 ^e ± 0.03	208.12 ^x ± 8.85
	CM	0.47 ^f ± 0.03	347.67 ^{yB} ± 13.45

Values with different superscripts for mastitis type in each season in a column differ at 5% level. Notable variations in the plasma metabolic profile of Sahiwal cows affected by mastitis were identified based on season and severity. Plasma BHBA concentrations were consistently elevated in cases of clinical mastitis (CM) when compared to subclinical mastitis (SCM) throughout all seasons, rising from 0.57 ± 0.08 mM/L in thermoneutral CM to 0.70 ± 0.06 mM/L in winter CM, and reaching a peak of 0.81 ± 0.05 mM/L in summer CM. Conversely, SCM cows exhibited lower BHBA levels, ranging from 0.32 to 0.39 mM/L. Similarly, plasma NEFA levels displayed a comparable pattern, with the highest concentrations observed in summer CM at 588.07 ± 81.04 μM/L, suggesting increased lipid mobilization due to heat stress.

Milk metabolic indicators exhibited similar seasonal fluctuations. The concentration of Milk BHBA rose from SCM to CM across each season, with measurements varying from 0.32 ± 0.06 to 0.48 ± 0.04 mM/L under thermoneutral conditions, 0.40 ± 0.03 to 0.58 ± 0.03 mM/L in winter, and 0.39 ± 0.03 to 0.47 ± 0.03 mM/L in summer. Furthermore, Milk NEFA levels were significantly elevated in CM cows, especially during the summer months (347.67 ± 13.45 μM/L), in contrast to SCM cows (208.12 ± 8.85 μM/L).

Mohapatra et al., (2017) [20] reported significantly elevated (P < 0.05) BHBA and NEFA levels in cows with mastitis compared to healthy cows during the early postpartum period, which supports our findings. The

variations in BHBA levels were also observed in the milk of infected cows. Clinical cases of mastitis exhibited significantly higher (P < 0.01) BHBA levels when compared to subclinical mastitis cases in both Sahiwal cows. Oltenacu and Ekesbo (1994) [24] indicated that there was no variation in milk BHBA at the onset of mastitis; however, subsequent research revealed a more significant correlation. Moyes et al., (2009) [21] discovered that increased BHBA levels during the transition phase heightened the likelihood of mastitis occurring in early lactation. Comprehensive data indicated that cows with a greater probability of developing mastitis within a four-week period. Additionally, Moyes et al., (2009) [21] affirmed that elevated BHBA levels make cows more susceptible to clinical mastitis during calving and early lactation.

Conclusion

This study shows that NEFA and BHBA are useful metabolic indicators for evaluating the severity of mastitis, the impact of pathogens, and seasonal stress in Sahiwal cows. Metabolite levels were considerably higher in *E. coli* infections than in *S. aureus* infections, suggesting more metabolic disturbance. NEFA and BHBA concentrations in milk and plasma were significantly higher (P < 0.05) in clinical mastitis than in subclinical instances. The effects of heat stress and negative energy balance were highlighted by these metabolic changes, which were most noticeable in the summer. In general, monitoring these metabolites can help detect mastitis in dairy herds earlier and manage it better.

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