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Effect of betaine supplementation on haemato-biochemical profile of lactating Mehsana buffaloes during summer

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Abstract
The present study was conducted to assess the effect of betaine supplementation on haemato-biochemical profile of lactating Mehsana buffaloes during summer. Eighteen Mehsana buffaloes were assigned randomly into three equal groups. The three were as T_1: (control): Basal diet; T_2: Basal diet + 25 gram/animal/day of betaine powder supplementation and T_3: Basal diet + 50 gram/animal/day of betaine powder supplementation. The blood sample was collected at 60 days of experiment. The haemoglobin and haematocrit values were without significant (p>0.05) difference among the treatment groups. No significant (p>0.05) effect on red blood cells (erythrocytes, MCV, MCH and MCHC) and white blood cells (leucocytes, granulocyte, lymphocytes and monocytes) count were observed due to dietary addition of betaine in the diet. The serum concentrations of glucose, total protein, albumin, globulin, urea, creatinine, triglycerides, cholesterol, serum glutamic pyruvic transaminase, and serum glutamic oxaloacetic transaminase were not affected (p>0.05) by betaine supplementation. Based on the results, it may be concluded that supplementation of betaine at the dose rate of 25 and 50 gram in the diet of lactating Mehsana buffaloes during summer did not any adverse effect on haemato-biochemical parameters.

Keywords: Betaine, blood metabolites, haematology, buffalo, summer

1. Introduction
In developing countries such as India, livestock significantly contributes to ensuring nutritional security for the growing population (Reddy *et al.*, 2023) [23]. Buffaloes are integral to the dairy industry, contributing to food security and sustaining rural livelihoods through the provision of milk, meat, and draught power (Venu *et al*., 2024) [28]. Environmental variations, including ambient temperature, relative humidity and wind velocity are identified as critical factors influencing livestock growth and productivity. (Berian *et al*., 2019) [4]. By 2100, the global average surface temperature is projected to rise by 1.8 to 4°C (IPCC, 2007) [14]. India has recently experienced some of its intense summers, driven by substantial changes in environmental variables such as increased ambient temperature, relative humidity, solar radiation, and wind speed. As a result, livestock experienced moderate to severe heat stress, particularly during the hot summer days. (Reddy *et al*., 2023) [23]. Milk production of livestock during the summer is reduced by up to 50%. (Belsare and Pandey, 2008) [3]. Hematological parameters in domestic animals can be influenced by seasonal and environmental variations (Feldman *et al*., 2002) [10] and thus, hematological parameters have been utilized to assess the impact of heat stress on productivity in dairy animals (Grunwaldt *et al*., 2005) [11]. Betaine is the trimethyl derivative of the amino acid glycine.-Dietary betaine (trimethylglycine) is known to perform several critical biological functions that support livestock productivity during heat stress (Ratriyanto and Mosenthin, 2018; Dunshea *et al*., 2019) [22, 21]. Betaine serves two primary functions in the animal's body. Firstly, betaine acts as an organic osmolyte, aiding in the retention of water molecules within vital cells, thereby preventing dehydration under heat stress conditions (Deshpande *et al*., 2020) [6]. Additionally, betaine regulates osmotic pressure within intestinal epithelial cells, supporting water balance and promoting intestinal cell proliferation, which in turn improves nutrient digestibility and overall animal performance. (Eklund *et al*., 2005) [9].

Another key function of betaine is its role as a methyl donor, making it a crucial component of one-carbon metabolism. (Bertolo *et al*., 2013) [5]. Inclusion of betaine in the di*et al*so provides essential amino acids, such as choline and methionine, through its metabolic processes. (Saeed *et al*., 2017) [24]. However, the impact of betaine supplementation in the diet of lactating Mehsana buffaloes on hemato-biochemical parameters has not been investigated under Indian conditions. Therefore, this study was conducted to evaluate the effect of betaine supplementation on the hemato-biochemical profile of lactating Mehsana buffaloes during the summer.

2 Materials and Methods

2.1 Animals and experimental design

The experimental procedures involving animals were approved by the Institutional Animal Ethics Committee (Approval No. VETCOLL/IAEC/2022/20/PROTOCOL-01). Eighteen lactating Mehsana buffaloes, ranging from 2nd to 5th parity, were randomly assigned to three treatment groups. The three groups were as fallow: T_1 (control) - Basal diet; T_2 - Basal diet + 25 gram/animal/day of betaine powder supplementation and T_3 - Basal diet + 50 gram/animal/day of betaine powder supplementation. In treatment groups T_2 and T_3, betaine powder was thoroughly mixed into the concentrate and administered once daily throughout the experimental period. The experimental animals were fed based on the nutrient requirements outlined by ICAR (2013) [13].

2.2 Collection of blood samples

Blood samples were collected on the 60th day of the experimental feeding period. Samples were drawn from the external jugular vein of each animal into two sterile vacutainer tubes: one containing an anticoagulant (EDTA) for the analysis of hematological parameters, and the other without anticoagulant for the determination of serum biochemical parameters.

2.3 Analysis of haematological parameters

The fresh blood samples containing anticoagulant were analyzed for hematological parameters, including hemoglobin, hematocrit, erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes, granulocyte, lymphocytes, and monocytes, using an automatic analyzer, as described by Patil *et al*. (2024) [19].

2.4 Analysis of blood biochemical parameters

The serum was obtained from the blood samples without anticoagulant. The serum samples were subsequently analysed for glucose, total protein, albumin, urea, creatinine, triglycerides, cholesterol, Serum Glutamic Pyruvic Transaminase (SGPT), and Serum Glutamic-Oxaloacetic Transaminase (SGOT) concentrations using commercial diagnostic kits, as per the methodology described by Pawar *et al*. (2024) [19].

2.5 Statistical Analysis

All experimental data were statistically analyzed using SPSS v.20.0 (SPSS Inc., Chicago, IL) following standard statistical methods (Snedecor and Cochran, 1994) [26]. Significant differences between treatment means were evaluated using Duncan's multiple range test, with differences considered significant at $p<0.05$.

3 Results and Discussion

3.1 Haematological parameters

The effect of betaine supplementation on hematological parameters in lactating Mehsana buffaloes is presented in Table 1. No significant ($p>0.05$) differences were observed in the mean hemoglobin (Hb) and hematocrit values among the treatment groups during the experimental feeding period. The recorded values for hemoglobin and hematocrit fell within the normal ranges of 8-15 g/dL and 26-37%, respectively (Kaneko *et al*., 2008) [15]. Supplementation with 25 g and 50 g of betaine did not significantly affect red blood cell parameters, including erythrocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), or white blood cell count (leukocytes, granulocytes, lymphocytes, and monocytes) in lactating Mehsana buffaloes throughout the experimental period.

Hematological parameters are essential for understanding the interplay between environmental influences and blood characteristics (Ovuru and Ekweozor, 2004) [18]. These parameters serve as key indicators for assessing the impact of both environmental and physiological factors on the health and productivity of livestock. Elevated ambient temperatures, a significant environmental stressor for livestock, have been demonstrated to influence hematological parameters by increasing the levels of stress-related hormones (e.g., cortisol) and initiating physiological adaptations to regulate homeostatic balance. Heat stress, for instance, can elevate oxidative stress and leads to changes in blood cell counts, suggesting an increased physiological burden. Betaine supplementation contributed to haematological stability under heat-stress conditions, which has significant implications for maintaining productivity and health in dairy animals (Shakkarpude *et al*., 2021) [25]. Consistent with the current findings, Shakkarpude *et al*. (2021) [25] investigated the effects of betaine supplementation on haematological parameters in postpartum lactating Murrah buffaloes. The study reported no significant differences in the mean values of total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) among the treatment groups. Similarly, Hussein *et al*. (2021) evaluated the long-term effects of betaine supplementation in growing lambs and observed no significant differences in blood parameters such as hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLTs), white blood cells (WBCs), segmented basophils, monocytes, or lymphocytes between the treatment and control groups. In a related study, Al-Qaisi *et al*. (2022) [2] reported no significant differences in circulating leukocytes, erythrocytes, neutrophils, lymphocytes, eosinophils, monocytes, platelets, hemoglobin, and hematocrit between dietary treatments with betaine under heat stress conditions. However, hematocrit levels were observed to be lower in betaine-fed calves on day seven of the study compared to the control group. Furthermore, Kondiba *et al*. (2023) [17] observed no significant differences in total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), and total leucocyte count (TLC) between betaine-supplemented lactating Deoni cows and the control group.

Table 1: Effect of betaine supplementation on haematological parameters of experiment on lactating Mehsana buffaloes (N=18)

Attributes	Dietary Treatments*			SEM	P-Value
	T_1(Control)	T_2	T_3		
Haemoglobin (g/dL)	10.73	11.30	11.65	0.307	0.496
Haematocrit (%)	32.25	30.80	33.02	0.969	0.665
Erythrocytes (10^6/µL)	5.28	5.36	5.58	0.155	0.739
Leukocytes (10^3/ µL)	9.17	8.60	7.90	0.351	0.359
MCV (fL)	50.63	49.55	47.23	1.871	0.773
MCH (pg)	18.83	18.90	17.73	0.945	0.867
MCHC (g/dL)	35.72	34.48	36.13	0.825	0.723
Granulocyte (10^3/ µL)	3.83	3.63	3.72	0.266	0.959
Lymphocytes (10^3/ µL)	3.62	3.88	3.70	0.345	0.955
Monocytes (10^3/ µL)	0.62	0.65	0.58	0.047	0.863

***** T_1 (Control): Basal diet, T_2: Basal diet + 25 gram/animal/day of betaine powder supplementation; T_3: Basal diet + 50 gram/animal/day of betaine powder supplementation

In contrast to the current findings, Abdelsattar *et al*. (2019) [1] reported that betaine supplementation significantly (p<0.05) increased hemoglobin concentration, red blood cell (RBC) count, and hematocrit values in growing lambs. Additionally, betaine supplementation significantly (p<0.05) reduced blood neutrophils, band cells, and eosinophils. Similarly, Kondiba *et al*. (2023) [17] observed significantly higher lymphocyte levels and lower neutrophil levels in the betaine-supplemented treatment group compared to the control group. Soliman *et al*. (2023) [27] also reported a significant increase in hemoglobin concentration in betaine-treated cows.

3.2 Blood biochemical parameters

The effects of betaine supplementation on the blood biochemical parameters of lactating Mehsana buffaloes are presented in Table 2. Serum concentrations of glucose, total protein, albumin, globulin, urea, creatinine, triglycerides, cholesterol, SGPT, and SGOT showed no significant (p>0.05) differences among the treatment groups.

Blood metabolites play a vital role in assessing an animal's health, particularly under heat stress conditions that lead to increased oxidative and metabolic load. High temperatures typically elevate blood cortisol, thereby raising glucose levels and impacting protein metabolism as the animal responds to increased energy demands (Djurhuus *et al*., 2004) [7]. Blood urea nitrogen (BUN) and creatinine serve as indicators of kidney and muscle function, while liver enzymes, such as SGPT and SGOT, reveal liver strain often associated with heat-induced oxidative damage. Furthermore, heat stress disrupts electrolyte balance, which can hinder muscle contraction and nervous system function.

As an organic osmolyte, betaine plays a pivotal role in cellular osmotic balance by counteracting dehydration, preserving protein structure, and sustaining enzyme activity during osmotic stress. It contributes methyl groups via methionine to form S-adenosylmethionine, facilitating essential metabolic functions such as growth, liver function, and lactation (Ratriyanto *et al*., 2009) [21]. The observed rise in serum total protein with betaine supplementation may indicate improved humoral and cellular immunity, likely linked to its function as a methyl donor, thus promoting protein synthesis and metabolism (Kidd *et al*., 1997) [16]. The findings from this study suggest that betaine supplementation may decrease plasma urea nitrogen levels and thus potentially enhancing total nitrogen retention. Improved nitrogen retention could confer immunological advantages and promote nitrogen accumulation within buffalo, which is crucial for supporting physiological functions and productivity, particularly under conditions of nutritional or environmental stress (Zhang *et al*., 2014) [29].

Table 2: Effect of betaine supplementation on blood biochemical parameters of lactating Mehsana buffaloes (N=18)

Attributes	Dietary Treatments*			SEM	P-Value
	T_1(Control)	T_2	T_3		
Glucose (mg/dL)	61.65	63.65	59.30	2.447	0.789
Total Proteins (g/dL)	7.38	7.65	8.25	0.159	0.065
Albumin (g/dL)	3.67	3.68	3.73	0.097	0.963
Globulin (g/dL)	3.43	3.32	3.68	0.010	0.326
Urea (mg/dL)	47.62	44.32	45.03	0.880	0.289
Creatinine (mg/dL)	1.31	1.28	1.29	0.035	0.960
Triglycerides (mg/dL)	16.25	20.47	16.37	1.832	0.593
Cholesterol (mg/dL)	133.67	124.35	121.65	5.165	0.637
SGPT (U/L)	48.15	46.05	47.73	1.302	0.804
SGOT (U/L)	136.60	133.00	135.47	2.834	0.882

***** T_1 (Control): Basal diet, T_2: Basal diet + 25 gram/animal/day of betaine powder supplementation; T_3: Basal diet + 50 gram/animal/day of betaine powder supplementation

Consistent with the current findings, Al-Qaisi *et al*. (2022) [2] conducted a study on 14 Holstein heifer calves, reporting no significant differences in circulating glucose, albumin, and triglycerides concentrations across treatment groups. Similarly, Kondiba *et al*. (2023) [17] investigated betaine supplementation's effects on the haematological and biochemical profiles of lactating Deoni cows. They observed a significant (p<0.05) reduction in cholesterol, triglycerides, and blood urea nitrogen (BUN) starting from the fourth week, with a notable increase in total proteins from the second week in betaine-supplemented cows. Furthermore, Soliman *et al*. (2023) [27] demonstrated that betaine supplementation significantly reduced levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicating a potential protective effect on liver function. These findings collectively support the positive impact of betaine on

improving metabolic efficiency and biochemical stability under supplementation. The findings suggested that betaine supplementation did not affect significantly blood serum glucose and protein concentration urea, and creatinine levels, which remained within the normal range.

4 Conclusion

Based on the findings, it may be concluded that supplementation of betaine at the dose rate of 25 and 50 gram/animal/day in the diet of lactating Mehsana buffaloes during summer did not have any adverse effect on haemato-biochemical parameters.

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Conflict of Interest

Not available

Financial Support

Not available

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