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Effect of Dietary Betaine on Serum Metabolites in Postpartum Lactating Murrah Buffaloes

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All the authors contributed in this research. JS: main investigator. AM: designed the research. DSY: helped in data collection. DDJA, AKJ and SM: helped in laboratory work. RKV: statistical analysis of data. AJ, MKA, KR, AB and MS: preparing manuscript.

Key words
Betaine, Biochemical parameter, Buffalo, Triglycerides, Phosphorus, Calcium

ABSTRACT

Betaine is an organic osmolyte that cells employ to protect themselves from high temperatures and osmotic stress. It also functions as a methyl donor in metabolism. Betaine supplementation induced some changes in blood biochemical indices. The study was performed at Livestock Farm Complex, Adhartal, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.). For the trial, 18 postpartum Murrah buffaloes were split into three groups at random. The control group was T1. Betaine was added to the T2 and T3 groups at 50 and 100 g/animal/day, respectively. Betaine was given in feed from 5th day postpartum and was continued up to 4 months. The mean values of blood glucose differed non-significantly ($p>0.05$) between all the treatment groups. The overall mean value of total cholesterol of T1 was higher than T2 and T3 group which differed ($p<0.05$) significantly between groups. On day 50 postpartum the mean value of plasma triglycerides of T3 was lower than T2 and T1 group and differ ($p<0.05$) significantly between groups. On day 25 and 50 postpartum, the mean value of blood urea nitrogen of T1 group was higher than T2 and T3 group which differed ($p<0.05$) significantly between groups. On day 75 and 125 postpartum, the mean value of blood calcium of T3 group was maximum followed by T2 and minimum blood calcium was found in T1 group which differed ($p<0.05$) significantly between groups. On day 50, 75, 100 and 125 postpartum, the mean value of blood phosphorus of T1 group was higher than T2 and T3 group which differed ($p<0.05$) significantly between groups. Dietary betaine supplementation improved fat metabolism and serum calcium concentration in postpartum lactating buffaloes. It can be concluded that betaine enhances the lactation performance of Murrah buffaloes by inducing alterations in their blood biochemical parameters.

INTRODUCTION

Dairy animals are constantly exposed to various stresses, including calving, lactation, housing, handling and extreme weather conditions. The degree of stress is particularly high under the hot climatic condition of India. For livestock, the persistently unfavorable climatic variations have become a significant obstacle. Genetic selection and nutritional strategies are two key approaches to reduce the impact of these stressful situations. Whole grains, spinach, and beets are the most popular food sources of betaine. Betaine is a very beneficial feed additive that has been shown to have potent antioxidant qualities and alters the distribution of fat and protein in ruminant bodies. It has been documented that betaine, a primary lipotropic drug, plays a part in fat metabolism (Huang *et al*., 2008; Lipinski *et al*., 2012) and contributes methyl groups to animal metabolism (Craig, 2004). Betaine acts as the

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methyl donor required for transmethylation reactions for the synthesis of numerous substances such as carnitine, creatine, phosphatidylcholine, and methyl purines, as well as methylated amino acids (Brougham *et al*., 2020). Betaine methylation plays a crucial role in the methionine–homocysteine cycle and is essential for the biosynthesis of carnitine and phospholipids. This process is prevalent across various animals and is associated with antioxidant, anti-inflammatory and hepatoprotective properties (Bingul *et al*., 2016). Additionally, betaine possesses osmoprotective qualities, which are critical for the functioning of the neurological, cardiovascular, immunological, and renal systems (Alirezaei *et al*., 2012). It has been shown that choline oxidation in the cell mitochondria produces betaine via biosynthesis. Nevertheless, it is still unclear how dietary supplementation with betaine would affect the blood's biochemical characteristics. The objective of this research was to examine how dietary supplementation with betaine affects blood metabolite levels.

MATERIALS AND METHODS

Study location

The experiment was carried out in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.), and the Livestock Farm Complex, Adhartal.

Selection of experimental animals

The animals were selected on the basis of similarity in body weight, parity, age and free from any anatomical, physiological and infectious disorders. 18 postpartum lactating Murrah buffaloes of 3rd to 5th parity were randomly divided into three groups having six animal in each group. Betaine supplementation was administered to the T2 and T3 groups at rates of 50 and 100 g/animal/day, respectively. Betaine (feed-grade betaine HCl 98% from Vetline, Simfa Labs Pvt. Ltd.) supplementation was initiated day 5 postpartum and continued for four months. The experiment was carried out in accordance with Institutional Animal Ethics Committee (IAEC) guidelines with order no. 06/IAEC/Vety./2019 on 09/08/2019. All techniques concerning animal care, management and blood collection from animals were extremely safe, and no harm was done to the animals.

Management of animals

Experimental buffaloes were stall fed and managed in an asbestos roof shelter with concrete floor and maintained in intensive system of housing. The experimental buffalo shed was thoroughly swept, then washed and rinsed with pressurized water. The walls and floor of the house were disinfected by using phenyl solution. Manger, waterer and other necessary equipment's were properly washed and cleaned. Experimental animals were fed according to their body weight and production (ICAR Feeding Standard, 2013). All the animals were offered identical basal ration consisting of green fodder, wheat straw and concentrate. The water was accessible to the animals at all times.

Temperature humidity index (THI)

It was calculated by recording the dry and wet bulb temperature throughout the experimental period daily in morning and evening (NRC, 1971).

$$THI=0.72\ (Tdb + Twb) + 40.6$$

where; Tdb is dry bulb temperature, and Twb is wet bulb temperature.

Analysis of parameters

For analysis of parameters 5mL of blood sample was collected from animal on 7th, 25th, 50th, 75th, 100th and 125th day postpartum from the external jugular vein. Plasma glucose, total cholesterol triglycerides, calcium and blood urea nitrogen were estimated by using diagnostic kits procured from Erba Diagnostics, Mannheim GmbH, Germany and Star 21 automatic biochemistry auto-analyzer. The plasma phosphorus was estimated by using diagnostic kits procured from Accucare and Star 21 automatic biochemistry auto-analyzer.

Statistical analysis

The data gathered during the experiment was examined using the IBM SPSS-24 statistical software through one-way analysis of variance. Various conditions and treatment groups were compared by using duncan multiple range test (DMRT). DMRT is a post hoc test to measure specific differences between pairs of means.

RESULTS AND DISCUSSION

THI

The average THI values recorded during the month of July, August, September and October were 79.41±0.42, 77.80±0.28, 77.65±0.29 and 74.98±0.32 respectively. Elevation in THI was observed during July, August, September and October months which lead to heat stress in experimental buffaloes, as it exceeded the upper critical limit (72 THI units) for buffaloes.

Blood glucose

The mean values of blood glucose differed non-significantly ($p>0.05$) between all the treatment groups

(Table I). The overall mean values of blood glucose of T1, T2 and T3 group were differed non-significantly between treatment. Higher feed intake may not significantly impact blood glucose levels due to effective regulation by insulin, nutrient composition (e.g., more protein or fat than carbohydrates), and the body's ability to maintain glucose homeostasis through metabolic adjustments.

The present results were in agreement with Lakhani (2018), who reported that there were non-significant ($p>0.05$) differences in the mean values of plasma glucose concentration between the groups in Karan Fries heifers supplemented with betaine. Also, non-significant difference in blood concentrations of glucose in betaine fed and control groups in pregnant Sanjabi ewes was reported by Sahraei *et al*. (2020) which is in agreement to present findings. DiGiacomo *et al*. (2016) also found that there was no influence of environmental temperature ($p=0.49$) or dietary betaine ($p=0.95$) on plasma glucose concentrations in sheep. Kondiba *et al*. (2023) also reported non-significant effect on plasma glucose levels as compared to that of control group in Deoni cows. In contrast to present reports, Shah *et al*. (2020) concluded that in Bet1 group (15 g/day per cow), there was significant ($p<0.05$) increase in the serum concentrations of glucose as compared to control and Bet2 group (30 g/day per cow) during heat stress in cows. The overall highest blood glucose was found in betaine @ 100 g/day supplemented T3 group. It might be due to increased feed intake and digestibility in the gastrointestinal tract.

Total cholesterol

The overall mean value of total cholesterol of T1 group was higher than T2 and T3 group which differed ($p<0.05$) significantly between groups. On day 50 and 125 postpartum the mean value of total cholesterol of T1 was higher than T2 and T3 group respectively and differed ($p<0.05$) significantly between groups (Table I). The probable reason as suggested by Huang *et al*. (2008) that there may be the reduction in adipose tissue observed in pigs fed additional betaine might result from diminished rates of lipogenesis that is a consequence of decreased activities and gene expression of lipogenic enzymes, as evidenced by the decrease in fatty acid synthase (FAS) mRNA expression. Glycine betaine diminishes both lipogenesis and the synthesis of fat (Figueroa-Soto and Valenzuela-Soto, 2018).

Similar findings were also reported by Mendoza *et al*. (2017), they concluded that supplementation of betaine decreased the serum cholesterol concentration on day 28 as compared to day 3 when fed betaine @ 0, 0.10, 0.15 and 0.20%, respectively in pigs during thermoneutral condition.

Table I. Blood glucose and urea nitrogen, and plasma total cholesterol, triglycerides, calcium and phosphorus in postpartum buffaloes at various intervals.

Treatment/ Days	Control	Group supplemented with betaine	
	T1 (n=6)	T2 (n=6)	T3 (n=6)
Blood glucose (mg/dL)			
day 7	52.00± 2.84	52.33± 2.11	53.50± 2.70
day 25	58.50± 3.89	57.17± 2.07	60.83± 3.17
day 50	55.33± 3.48	53.67± 1.52	58.83± 1.60
day 75	55.67± 0.99	57.00± 3.92	62.33± 4.96
day 100	57.67± 2.04	59.50± 3.80	60.83± 2.74
day 125	59.33± 1.58	60.67± 3.82	59.67± 2.84
Average	56.42± 1.09	56.72± 1.25	59.33± 1.29
Total cholesterol (mg/dL)			
day 7	87.33± 8.07	87.90± 7.59	87.19± 0.88
day 25	121.54± 11.44	124.69± 14.02	93.46± 7.43
day 50	147.07^{A}±11.77	119.72^{AB}± 19.03	98.81^{B}± 7.00
day 75	136.67± 16.03	116.60± 16.31	109.99± 7.03
day 100	141.58± 12.37	126.23± 15.44	110.39± 6.10
day 125	161.46^{A}± 9.14	120.15^{B}± 13.39	118.09^{B}± 2.32
Average	132.61^{A}± 5.96	115.88^{AB}± 5.98	102.99^{B}± 2.82
Plasma triglycerides (mg/dL)			
day 7	20.99± 0.60	21.83± 1.03	21.03± 1.01
day 25	27.38± 2.86	24.97± 0.99	23.17± 1.87
day 50	25.52^{A}± 1.09	25.36^{A}± 0.58	21.38^{B}± 1.48
day 75	25.90± 1.18	25.48± 0.95	24.75± 1.08
day 100	26.01± 1.58	26.21± 1.25	25.47± 1.58
day 125	28.97± 2.75	25.80± 1.78	24.19± 1.91
Average	25.80± 0.82	24.94± 0.50	23.33± 0.64
Plasma calcium (mg/dL)			
day 7	7.46± 0.30	7.53± 0.38	7.59± 0.18
day 25	7.69± 0.13	7.37± 0.36	8.69± 0.53
day 50	7.36± 0.27	7.55± 0.19	8.01± 0.35
day 75	7.21^{B}± 0.18	7.47^{B} ± 0.25	8.90^{A}± 0.67
day 100	7.24± 0.25	7.50± 0.38	8.08± 0.25
day 125	7.16^{B}± 0.27	7.32^{B}± 0.26	8.88^{A}± 0.57
Average	7.35^{B} ± 0.08	7.45^{B} ± 0.04	8.35^{A}± 0.22
Plasma phosphorus (mg/dL)			
day 7	7.74± 0.27	6.87± 0.40	6.73± 0.44
day 25	7.09± 0.45	7.13± 0.61	6.79± 0.24
day 50	8.32^{A}± 0.36	7.09^{B}± 0.34	6.39^{B}± 0.17

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Treatment /Days	Control	Group supplemented with betaine	
	T1 (n=6)	T2 (n=6)	T3 (n=6)
day 75	$8.49^{A}\pm 0.40$	$7.21^{B}\pm 0.37$	$7.12^{B}\pm 0.18$
day 100	$9.32^{A}\pm 0.47$	$7.71^{B}\pm 0.27$	$7.22^{B}\pm 0.01$
day 125	$9.14^{A}\pm 0.17$	$8.20^{B}\pm 0.23$	$7.19^{C}\pm 0.14$
Average	$8.35^{A}\pm 0.19$	$7.37^{B}\pm 0.17$	$6.91^{B}\pm 0.10$
Blood urea nitrogen (mg/dL)			
day 7	10.16 ± 1.15	10.15 ± 1.64	10.08 ± 0.94
day 25	$13.38^{A}\pm 0.54$	$10.01^{B}\pm 1.32$	$9.47^{B}\pm 1.19$
day 50	$14.50^{A}\pm 1.34$	$13.15^{AB}\pm 0.75$	$11.48^{B}\pm 0.30$
day 75	16.47 ± 1.65	13.84 ± 1.35	16.50 ± 1.37
day 100	14.41 ± 0.97	14.25 ± 1.14	14.74 ± 1.65
day 125	17.63 ± 2.39	12.71 ± 1.35	16.79 ± 1.28
Average	14.43 ± 1.06	12.35 ± 0.75	13.18 ± 1.32

Means bearing different superscripts (A, B, C) within row differ significantly ($p<0.05$) between groups.

According to Abu-Hafsa *et al.* (2024), supplementing heat-stressed rabbits with vitamin C, selenium, betaine, and pomegranate peels significantly reduced serum cholesterol levels ($p<0.05$). Mishra *et al.* (2019) reported that the serum cholesterol levels were significantly ($p<0.05$) lower in betaine fed group as compared to control group in sows. Li *et al.* (2017) recorded that the levels of serum cholesterol and high-density lipoprotein cholesterol were decreased ($p<0.05$) and total cholesterol content was increased in muscle ($p<0.05$) of betaine fed pigs. The underlying probable mechanism regulating fatty acid metabolism in pigs supplemented with betaine is associated with the up-regulation of genes involved in fatty acid transport and fatty acid oxidation. The present results were in disagreement with Raheja (2017), who reported that betaine supplementation @ of 0, 50 g per cow per day during hot-humid season increased plasma cholesterol significantly ($p<0.05$) in treatment group as compared to control on day 21 postpartum in Karan Fries cows. The serum levels of triglycerides and cholesterol in Hu sheep that were given diets enriched with betaine showed no significant differences (Cai *et al*., 2021).

Triglycerides

On day 7 postpartum, the mean values of plasma triglycerides were lower than day 125 in all groups. The overall mean value of plasma triglycerides of control group was higher than T2 and T3 group which differed ($p>0.05$) non-significantly between groups. On day 50 postpartum the mean value of plasma triglycerides of T3 was lower than T2 and T1 group, respectively and differed ($p<0.05$) significantly between groups (Table I).

The present results were in agreement with Fernandez *et al*. (2009), they reported goats fed with betaine had lower triglycerides level as compared to control which differed significantly ($p<0.05$) during heat stress. Lakhani (2018) reported that there were significantly lower ($p<0.05$) differences in the mean values of plasma triglycerides concentration between control and betaine supplemented groups in Karan Fries heifers. Mendoza *et al.* (2017) concluded that supplementation of betaine decreased the serum triglycerides concentration when fed betaine in pigs during thermoneutral condition. According to Abu Hafsa *et al.* (2024), supplementing heat-stressed rabbits with vitamin C, selenium, betaine, and pomegranate peels significantly reduced serum triglyceride levels ($p<0.05$). This reduction in triglycerides in betaine supplemented groups as compared to control could be due to combinations of a decrease in fatty acid synthesis and an increase in triglyceride lipase activity and β-oxidation of fatty acids. Betaine impacts lipid metabolism by serving as a methyl donor, enhancing S-adenosylmethionine (SAM) synthesis, which regulates lipogenic enzyme activity via epigenetic modifications. It activates AMPK, suppressing lipogenesis by downregulating enzymes like acetyl-CoA carboxylase (ACC) and sterol regulatory element-binding protein 1c (SREBP-1c). Betaine modulates PPAR-α and PPAR-γ pathways, balancing fatty acid oxidation and lipid storage. It influences hormone-sensitive lipase (HSL), affecting lipolysis and triglyceride breakdown. As a choline derivative, it aids phospholipid synthesis for lipid transport, reducing tissue lipid accumulation. These mechanisms collectively improve lipid metabolism and energy balance. The addition of betaine to the diet enhanced carnitine levels, which are involved in the beta oxidation of fatty acids in the mitochondria, thus preventing fatty acid deposition in animals (Fan *et al*., 2022).

Blood urea nitrogen (BUN)

The overall mean value of BUN of T1 group (14.43±1.06 mg/dL) was higher followed by T3 group (13.18±1.32 mg/dL) and lowest value was found in T2 group (12.35±0.75 mg/dL) which differed ($p>0.05$) non-significantly between groups (Table I). Similar results were reported by Zhang *et al.* (2014), they concluded that feeding betaine to cows had non-significant effect on concentration of blood urea nitrogen ($p>0.05$) during heat stress. The mean values of BUN were numerically higher in control as compared to betaine treated groups.

On 25 and 50 days postpartum, the mean values of BUN of T1 group was higher than T2 and T3 group which differed ($p<0.05$) significantly between groups (Table I). Present results were in agreement with Huang *et al*. (2007) who, observed that serum urea nitrogen concentration in pigs fed betaine was 21.58% less than that of control group ($p<0.01$). Similar results were also reported by Ghoneem and El-Tanany (2023) in lactating Damascus goats after betaine supplementation. Abu-Hafsa *et al*. (2024) suggested that the supplementation of Vitamin C, selenium, betaine and pomegranate peels were significantly ($p<0.05$) decrease the concentrations of urea in heat stressed rabbits. This might be due to dietary betaine, lowers blood urea nitrogen levels, enhances nitrogen retention, and reduces the need for metabolizable energy. Blood urea nitrogen levels correlate with the protein turnover rate. According to Coma *et al*. (1995), nitrogen retention is maximized when urea-nitrogen is minimized. Ramadhan and Khudair (2019), reported that the renal protective effect of betaine was clarified in groups (G2 and G4) manifested by significant decrease in serum creatinine, urea and uric acid concentration comparing to acrylamide (G3) treated group.

Blood calcium

On 75 and 125 days postpartum, the mean values of blood calcium of T3 group was maximum followed by T2 and minimum blood calcium was found in control group which differed significantly ($p<0.05$) between groups (Table I). The levels of blood calcium obtained in the present investigation were within the normal physiological values during lactation reported by Jacob *et al*. (2002), Jain *et al*. (2007) and Hagawane *et al*. (2009).

The overall mean value of blood calcium of T3 group was highest followed by T2 group and lowest value was found in T1 group which differed significantly ($p<0.05$) between groups (Table I). Present results were in agreement with Hassan *et al*. (2011), they reported that the mean concentration of calcium differed significantly ($p<0.05$) between treatments during high ambient temperature in New Zealand White rabbits. In contrast to present finding Mendoza *et al*. (2017) concluded that supplementation of betaine did not affect the serum calcium concentration on day 3 and day 28 when fed betaine during thermoneutral condition in pigs.

Blood phosphorus

The overall mean value of blood phosphorus of T1 group was higher followed by T2 group and lowest value was found in T3 group which differed ($p<0.05$) significantly between groups. On day 50, 75, 100 and 125 postpartum, the mean value of blood phosphorus of T1 group was higher than T2 and T3 group, respectively which differed ($p<0.05$) significantly between groups (Table I). It is a proven fact that dietary betaine reduces serum phosphorus levels and increases nitrogen and phosphorus retention. Similar findings were reported by Mendoza *et al*. (2017), they concluded that supplementation of betaine decreased the serum phosphorus concentration on day 3 and day 28, when fed betaine @ 0, 0.10, 0.15, 0.20 % respectively during thermoneutral condition in pigs. The observed reduction in BUN and serum phosphorus in betaine-supplemented groups can be attributed to improved nitrogen metabolism and protein synthesis (lowering urea production), enhanced phosphorus utilization in cellular processes, and improved renal clearance of both urea and phosphorus, supported by betaine's role as a methyl donor and osmoprotectant.

CONCLUSION

Betaine is a natural feed additive with immense potential to support and enhance animal health and production performance under various stressful conditions. Betaine supplementation significantly decreased the concentration of cholesterol, triglycerides, blood urea nitrogen and serum phosphorus. There was no effect of betaine supplementation on plasma glucose concentration in buffaloes. It may be concluded that betaine can improve the lactation performance of lactating Murrah buffaloes via inducing some changes in blood biochemical indices. The reduction in cholesterol, triglycerides, and blood urea nitrogen suggests better metabolic efficiency, which could translate to more efficient feed utilization and reduced metabolic waste. Improved feed efficiency and lactation performance could lower feed costs per unit of milk produced, making it a cost-effective strategy for farmers in resource-constrained or stress-affected regions. Incorporating betaine into feed formulations for dairy buffaloes could help optimize productivity during summer season.

DECLARATIONS

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IRB approval

The experiment was carried out in accordance with Institutional Animal Ethics Committee (IAEC) guidelines with order no. 06/IAEC/Vety./2019 on 09/08/2019.

Ethics statement

All techniques concerning animal care, management and blood collection from animals were extremely safe, and no harm was done to the animals.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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