

Physiological parameters and serum electrolyte changes after betaine supplementation in lactating Murrah buffaloes during hot-humid season

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ABSTRACT

Betaine acts as a methyl donor in metabolism and serves as an organic osmolyte, which is used by cells for protection against osmotic stress and high temperatures. The present study was carried out to investigate the effect of betaine on physiological parameters and serum electrolyte changes in postpartum lactating Murrah buffaloes during the hot-humid season. The study was performed at the Livestock Farm Complex, N.D.V.S.U., Jabalpur, in 2019. Eighteen postpartum Murrah buffaloes were randomly divided into three groups for the experiment. T1 was a control group. T2 and T3 groups were supplemented with betaine at 50 g/animal/day and 100 g/animal/day, respectively. Betaine was supplemented in the feed from day 5 postpartum and continued up to 4 months. The rectal temperature was found to be significantly lower in the T2 and T3 groups as compared to the T1 group on day 90 postpartum. The pulse rate was lower in the T2 and T3 groups as compared to the control group on day 105 postpartum. It was concluded that betaine improves serum sodium, potassium, and the chloride concentration as compared to the control group and reduces heat stress in lactating buffaloes.

Keywords: Betaine, Buffalo, Rectal temperature, Serum chloride, Serum sodium

Heat stress affects the fertility of buffaloes. High ambient temperatures cause a reduction in appetite, which directly leads to negative energy balance (NEBAL) in lactating animals. During the lactation period, buffaloes are in a state of great metabolic stress and their productivity is adversely affected by the extreme climatic conditions. Reduction in feed intake and milk production is a common sign of heat stress, which alters the physiological as well as serum electrolyte concentrations of animals.

Productivity loss during lactation period can be minimised by providing a strategic feed supplement like betaine which is an oxidative product of choline and methionine (Saeed *et al.* 2017). Betaine (N, N, N-trimethylglycine) molecule contains 3 methyl groups and so acts as a methyl donor in metabolism and helps in synthesis of cellular macromolecules (Craig 2004). Betaine acts as a methyl donor via S-adenosyl-methionine thereby sparing methionine, decreasing homocysteine concentrations, and increasing the available substrates for protein synthesis. The osmotic activity of betaine is due

to its dipolar zwitter ion, which has a net neutral charge, but a region of positive and negative charges, and very high solubility in water. This allows betaine to hold water molecules (intracellular) against a concentration gradient (Hall *et al.* 2016). The osmo-protective property of betaine has a beneficial role in the prevention of dehydration at high ambient temperature. It diminishes the increased vascular permeability and prevents the blood plasma water loss during hyperthermia. During hyperthermia, betaine improves the kidney function and increases electrolyte and water retention efficiency. Deshpande *et al.* (2020) concluded that physiological parameters of the betaine fed group were lower as compared to the control group in buffalo heifers during hot humid season.

Considering the above facts, the present study was planned to validate the beneficial effect of betaine supplementation on physiological parameters and serum electrolyte changes in postpartum lactating Murrah buffaloes during hot-humid season.

MATERIALS AND METHODS

The study was performed at Department of Veterinary Physiology and Biochemistry and Livestock Farm Complex, Adhartal, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur in 2019. The animals were selected on the basis of similarity in body weight, parity and age. Postpartum lactating Murrah

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buffaloes (18) of 3rd to 5th parity were randomly divided into three groups. T1 was a control group. T2 and T3 group were supplemented with betaine @ 50 g/animal/day and 100 g/animal/day, respectively. The experiment was conducted as per the guidelines of Institutional Animal Ethics Committee (IAEC).

Experimental animals were fed according to their body weight and production level (ICAR Feeding Standard 2013). Betaine (Betaine HCl, feed grade) supplementation was started on 5th day postpartum and continued up to 4 months. For the analysis of serum electrolyte 5 ml of blood sample was collected from the animals on 7, 25, 50, 75, 100 and 125 day postpartum from the external jugular vein. Temperature humidity index (THI) was calculated by using the following formula (NRC 1971):

$$\text{THI} = 0.72 (\text{Tdb} + \text{Twb}) + 40.6$$

Where, Tdb, dry bulb temperature; Twb, wet bulb temperature.

Rectal temperature, pulse rate and respiration rate: Physiological parameters were recorded at fortnightly intervals.

Body temperature was recorded as rectal temperature (°F) using digital thermometer. Pulse rate (beats per minute) was counted manually by palpating middle coccygeal artery. Respiration rate per minute was recorded by observing the outward and inward movement of flank which is considered as one respiration.

Electrolyte estimation: Serum sodium (Na⁺), potassium (K⁺), chloride (Cl⁻) were estimated by Acculyte-3P electrolyte analyzer.

Statistical analysis: The data obtained during experiment were analyzed by IBM SPSS-24 statistical software program using one way analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to compare various conditions and treatment groups.

RESULTS AND DISCUSSION

Temperature humidity index (THI): THI is the most common indicator of heat stress in dairy animals. According to NRC (1971), when the temperature humidity index value is below 72, the animals are in thermoneutral zone.

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

Physiological response

Rectal temperature: The overall mean value of rectal temperature in control group was higher than T2 and T3 groups which differed non-significantly ($p > 0.05$) between groups. On day 90, significant ($p < 0.05$) lower rectal temperature was found in T2 and T3 groups as compared to control group (Table 1). The probable reason for the above may be the osmoprotective function of betaine, which may have helped the supplemented animals to retain cellular water level and total body water content compared to control animals (Cronje 2005). Betaine supplementation during heat stress or transitional stress increases hepatic metabolism and a resultant upsurge in heat production by the liver may offset the decrease in heat production in the rest of the body due to the osmoprotective effects of betaine (Dunshea *et al.* 2019).

In agreement with present reports, Zhang *et al.* (2014) reported the lower rectal temperature in all betaine supplemented groups of Holstein Friesian dairy cows as compared to control groups during heat stress. Shankhpal *et al.* (2018) reported lower ($p < 0.05$) rectal temperature in cows supplemented with betaine as compared to control group during summer month. Shankhpal *et al.* (2019) reported that the average rectal temperature was significantly lower in betaine supplemented groups as compared to control group in buffaloes during heat stress. Similar findings were also reported by DiGiacomo *et al.* (2016), that dietary betaine supplementation significantly decreased rectal temperature and skin temperatures as compared to control in Merino sheep during heat load. Williams *et al.* (2021) concluded that the maximum vaginal temperature of cows fed with betaine was lower than that of cows not fed with betaine to an acute heat challenge.

Table 1. Mean rectal temperature and pulse rate in postpartum buffaloes at fortnightly intervals

Day	Mean pulse rate (beats/minute)			Mean rectal temperature (°F)		
	T1	T2	T3	T1	T2	T3
Initial	55.33±3.78	55.33±1.91	55.33±2.17	99.77±0.44	100.15±0.53	99.90±0.25
Day 15	56.67±2.81	57.33±3.21	53.33±3.21	99.77±0.45	99.62±0.45	99.70±0.25
Day 30	58.67±3.82	57.33±1.98	58.00±2.88	100.10±0.28	99.48±0.18	99.57±0.16
Day 45	62.00±2.25	58.67±4.89	61.33±2.67	99.62±0.11	99.63±0.23	100.15±0.28
Day 60	63.33±1.22	60.67±3.00	62.00±2.25	99.22±0.09	99.65±0.25	99.52±0.17
Day 75	59.33±1.91	58.67±0.80	59.33±3.00	100.03±0.45	99.52±0.14	99.18±0.18
Day 90	62.00±3.06	53.33±1.68	58.00±2.25	100.05 ^A ±0.17	99.33 ^B ±0.08	99.42 ^B ±0.30
Day 105	62.00 ^A ±2.48	54.00 ^B ±0.89	60.00 ^{AB} ±2.31	99.53±0.19	99.18±0.22	98.95±0.12
Day 120	59.33±1.61	56.00±0.80	58.00±3.06	99.57±0.21	99.13±0.25	98.88±0.25
Average	59.85±0.90	56.81±0.79	58.37±0.89	99.74±0.09	99.52±0.10	99.47±0.09

Means bearing different superscripts within row differ significantly ($p < 0.05$).

Pulse rate: The overall mean value of pulse rate of T1 group was higher than T2 and T3 group which differed non-significantly ($p>0.05$) between the groups. On day 105 postpartum significantly ($p<0.05$) lower pulse rate was found in T2 and T3 groups as compared to T1 group (Table 1). Pulse rate is an instant measure of sympathetic and parasympathetic nervous system activity in animal body. It is a non-invasive method to monitor the stress level of animals. Betaine reduces the concentration of stress markers in animals. The present results were in agreement with Shankhpal *et al.* (2018), who reported lower ($p<0.05$) pulse rate in cows supplemented with betaine as compared to control group during summer month. Shankhpal *et al.* (2019) also observed that the average pulse rate ($p=0.01$) was significantly lower in betaine fed groups as compared to control group in buffaloes during heat stress. Deshpande *et al.* (2020) reported the lower mean value of pulse rate in treatment group ($p<0.05$) than control during hot-humid season. Contrary to present reports, Zhang *et al.* (2014) recorded that betaine supplementation @ 0, 10, 15, 20 g/day in diet exhibited non-significant effect on pulse rate of the dairy cows.

Respiration rate: The overall mean value of respiration rate differed non-significantly between all the groups (Table 2). In agreement to the present results, Zhang *et al.* (2014) reported that the betaine supplementation @ 0, 10, 15, 20 g/day in diet exhibited non-significant effects on respiration rate of dairy cows. Similarly, Raheja (2017) recorded non-significant difference in respiration rate of betaine supplemented and control cows during hot-humid season. In disagreement to present reports, Deshpande *et al.* (2020) reported that the mean value of respiration rate in control group was significantly higher ($p<0.05$) than betaine treated group. Similarly, Mendoza *et al.* (2017) reported that betaine reduced respiration rate of pigs housed under heat-stressed conditions and the lowest value was observed when betaine was added at 0.15% inclusion level.

Serum electrolyte

Serum sodium (Na^+): The overall mean values of serum sodium of T1 group were numerically lower than T2 and T3 group and differed ($p<0.05$) significantly between the groups. On day 75 postpartum the mean value of serum sodium of T1 was lower than T2 and T3 group which differed ($p<0.05$) significantly between the groups

Table 2. Mean respiration rate (breaths/minute) in postpartum buffaloes at fortnightly intervals

Treatment days	T1	T2	T3
Initial	23.67±0.33	23.67±1.09	23.00±0.45
Day 15	24.67±0.67	24.67±0.67	23.33±0.67
Day 30	25.33±0.84	24.67±0.67	23.33±0.42
Day 45	23.67±1.50	22.67±1.23	23.00±0.68
Day 60	23.67±0.33	23.33±0.42	23.00±0.68
Day 75	23.67±0.61	23.33±0.67	23.00±0.68
Day 90	22.67±0.67	21.67±0.80	21.67±1.20
Day 105	22.33±0.80	20.67±1.23	20.00±1.46
Day 120	21.67±1.67	20.67±0.67	20.33±1.09
Average	23.48±0.38	22.81±0.51	22.30±0.44

(Table 3). Present results are in agreement with Mendoza *et al.* (2017), who concluded that the supplementation of betaine increased the serum sodium concentration on day 28 as compared to day 3 (0, 0.10, 0.15 and 0.20 %) in pigs during thermoneutral condition.

Cells adapt to external osmotic stress by accumulating low-molecular-weight inorganic ions (like sodium, potassium and chloride) and organic osmolytes (e.g. methylated amines, certain amino acids and sugar alcohols). However, the increase in intracellular concentrations of inorganic ions is limited because of their destabilizing effect on protein structure and enzyme function (Petronini *et al.* 1992). Blood electrolyte concentrations in the treatment groups were greater than those in the control group. Betaine is non-perturbing to cellular metabolism, highly compatible with enzyme function and stabilizes cellular metabolic function under different kinds of stress in various organisms and animal tissues (Chambers 1995). Osmotic adaptation to stress via betaine supplementation helps a variety of cells and organs to continue to function and protects against premature apoptosis.

Serum chloride (Cl^-): The overall mean values of serum chloride of T3 group were maximum followed by T2 group and minimum mean chloride concentration was found in control group which differed ($p<0.05$) significantly between the groups. On day 75 postpartum, the mean value of serum chloride of T3 group was maximum followed by T2 group and minimum mean chloride concentration was found in T1 group which differed ($p<0.05$) significantly between the groups (Table 3). The maximum mean

Table 3. Mean serum sodium and chloride in postpartum buffaloes at various intervals

Day	Mean serum sodium (mEq/L)			Mean serum chloride (mEq/L)		
	T1	T2	T3	T1	T2	T3
Day 7	135.71±0.24	135.99±0.85	135.84±0.27	99.65±0.23	100.27±0.77	100.08±1.08
Day 25	134.80±0.40	135.62±0.55	135.50±0.41	98.07±0.13	98.69±0.36	99.15±0.46
Day 50	134.89±0.38	135.17±0.28	135.86±0.53	98.31±0.41	98.66±0.28	99.40±0.50
Day 75	134.53 ^B ±0.25	135.55 ^{AB} ±0.52	136.64 ^A ±0.61	97.83 ^B ±0.13	98.28 ^B ±0.38	100.50 ^A ±0.64
Day 100	134.86±0.82	135.33±0.45	135.93±0.52	97.73±0.50	98.45±0.88	99.47±0.46
Day 125	134.66±1.68	135.46±0.79	135.43±1.07	98.55±0.86	99.47±0.76	99.18±0.52
Average	134.91 ^B ±0.17	135.52 ^A ±0.11	135.87 ^A ±0.17	98.36 ^B ±0.28	98.97 ^{AB} ±0.31	99.63 ^A ±0.22

Means bearing different superscripts within row differ significantly ($p<0.05$).

chloride concentration was found in betaine @ 100 g/day supplemented T3 group which is positively correlated with highest mean sodium concentration in T3 group on day 75 postpartum. Present results are in disagreement with Mendoza *et al.* (2017), who concluded that supplementation of betaine (0, 0.10, 0.15 and 0.20%) showed non-significant difference in the serum chloride concentration during thermoneutral and heat-stressed conditions on day 3 and day 28 in pigs.

Serum potassium (K^+): The overall mean values of serum potassium of T1 group were numerically lower than T2 and T3 group which differed ($p < 0.05$) significantly between the groups. However, on day 7, 25, 50, 75, 100 and 125 postpartum, the mean concentration of serum potassium showed ($p > 0.05$) non-significant difference between all the groups (Table 4). Mendoza *et al.* (2017) concluded that the supplementation of betaine (@ 0, 0.10, 0.15 and 0.20%) showed non-significant difference in the serum potassium on day 28 during thermoneutral condition in pigs which is in agreement with present findings.

Table 4. Mean serum potassium (mEq/L) in postpartum buffaloes at various intervals

Treatment days	T1	T2	T3
Day 7	4.23±0.11	4.43±0.09	4.18±0.14
Day 25	4.17±0.16	4.36±0.15	4.22±0.16
Day 50	4.03±0.18	4.26±0.11	4.28±0.09
Day 75	4.18±0.16	4.21±0.10	4.28±0.13
Day 100	4.19±0.15	4.27±0.10	4.27±0.05
Day 125	4.23±0.13	4.23±0.16	4.21±0.09
Average	4.17 ^B ±0.06	4.29 ^A ±0.05	4.24 ^{AB} ±0.04

Means bearing different superscripts within row differ significantly ($p < 0.05$).

It can be concluded that the betaine supplementation improves serum sodium, potassium and chloride concentration as compared to control group in buffaloes. Dietary betaine may assist in buffaloes to combat heat stress by reducing the values of the physiological parameters, i.e. rectal temperature and pulse rate. Therefore, betaine supplementation helps in improving productive and reproductive performance particularly during stressful situation of hot-humid condition in buffaloes. However, a large sample size is still needed to validate these results.

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