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Effect of Dietary Betaine on Oxidative Stress and Immune Response in Postpartum Lactating Murrah Buffaloes (*Bubalus bubalis*) during Hot-Humid Season

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Key words
Betaine, Buffalo, Immunoglobin G, Superoxide dismutase, HSP70, Total antioxidant capacity

ABSTRACT

Excessive heat and humidity causes reduction in appetite of postpartum buffaloes which directly leads negative energy balance (NEBAL) and increases oxidative stress in animals. Betaine is a growth promoting nutritional additive used in livestock. Betaine contains three methyl groups and thus acts as a methyl donor for metabolic reactions. Betaine acts as molecular chaperone, as it repairs heat shock proteins. The present study was aimed to investigate the effect of dietary betaine on heat and metabolic stress during lactation in Murrah buffaloes (*Bubalus bubalis*). For this purpose, a total of 18 postpartum Murrah buffaloes were randomly divided into control, low betaine and high betaine groups supplemented with betaine @ 50 g/animal/day and 100 g/animal/day respectively from day 5 postpartum and was continued up to 4 months postpartum. 5ml of blood sample was collected from animal for analysis of superoxide dismutase, total antioxidant capacity, HSP70, albumin/globulin ratio and immunoglobin G. The overall mean values of plasma superoxide dismutase, total antioxidant capacity and immunoglobin G of high betaine group was numerically higher than other groups which differed ($p<0.05$) significantly between groups. The overall mean value of plasma HSP70 of high betaine group was minimum followed by low betaine group and control group had maximum mean value which differed ($p<0.05$) significantly between groups. The overall mean values of albumin/globulin ratio of control group were higher than the betaine groups which differed ($p<0.05$) significantly between groups.

INTRODUCTION

India is the largest milk producing country in the world. Heat stress is one of the factor which cause reactive oxygen species mediated oxidative stress in farm animals. Production performance of buffaloes are mostly affected during summer stress due to change in physiological responses, feed intake, antioxidant system and decreased immunity. Heat stress results in lower milk production and poor fertility among buffaloes. Hyperthermia due to heat

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stress directly affects cellular functions of various tissues of reproductive system (Wolfenson *et al*., 2000). Buffaloes are mainly affected by oxidative stress, is one of major cause for inflammatory and immune dysfunction. Proper understanding the relationship among climatic factors and feeding regimens will provide a firm basis to improve the health and welfare of buffaloes.

Productivity loss during lactation period can be minimized by providing strategic feed supplement like betaine. Betaine is naturally present in wheat and sugar beets, also an oxidative product of choline. The main physiological role of betaine is a methyl donor and act as an osmolyte. Betaine acts as chaperone, as it repairs denatured proteins (Roth *et al*., 2012) and interacts with heat shock proteins. Betaine acts as an osmolyte to maintain the ionic balance of animal and improve the capacity against heat stress. Dietary supplementation of betaine positively affects nutrient digestibility. Its inclusion in the diet is providing essential amino acids like choline and methionine via metabolism of betaine. The present study was carried out to investigate the effect of betaine on antioxidant status and immunity in postpartum lactating Murrah buffaloes during hot-humid season.

MATERIALS AND METHODS

Experimental animals

The experiment was conducted at Livestock Farm Complex, Adhartal and Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.) from July 2019 to October 2019. Eighteen postpartum lactating Murrah buffaloes were randomly divided into three groups. T1 group was control. T2 and T3 group was 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) vide order no. 06/IAEC/Vety./2019 dated 09/08/2019.

Management of animals

The experimental animals were maintained under identical and optimal conditions of feeding and management. Experimental buffaloes were fed according to their body weight and production (ICAR Feeding Standard, 2013). All the animals were offered concentrate having 20%–22% crude protein (CP) and 70%–75% Total digestible nutrients (TDN). Betaine (Betaine HCl) supplementation was started day 5 postpartum and was continued up to 4 months postpartum. Blood sample was collected from buffaloes on 7th, 25th, 50th, 75th, 100th and 125th day postpartum.

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.

Determination of plasma superoxide dismutase (SOD), total antioxidant capacity (TAC), heat shock protein 70 (HSP70) and immunoglobin G (IgG)

SOD was estimated in plasma samples using SOD ELISA kit (KRISHGEN, Biosystems, India). TAC was estimated in plasma samples using TAC ELISA kit (KINESISDx, Los Angeles, USA). Bovine HSP70 was estimated in plasma samples using HSP70 ELISA kit (KINESISDx, Los Angeles, USA). IgG was estimated in plasma samples using bovine IgG ELISA kit (KRISHGEN, Biosystems, India). All these methods employ sandwich ELISA technique.

Albumin/ globulin ratio

Total protein and albumin concentration was estimated by using diagnostic kits procured from Erba Diagnostics, Mannheim GmbH, Germany. Star 21 automatic biochemistry auto-analyzer was used for this study. The concentration of globulin was calculated by the following formula:

Total globulin (g/dl) = Total protein (g/dl) – Total albumin (g/dl).

A/G ratio = Albumin/Globulin

Statistical analysis

The data obtained during experiment were analyzed by IBM SPSS-24 statistical software program using one way ANOVA. Various conditions and treatment groups were compared by using Duncan Multiple Range Test (DMRT). Correlation analysis was done for estimating the association between TAC and SOD, TAC and HSP70 as well as SOD and HSP70.

RESULTS AND DISCUSSION

Temperature humidity index (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. A substantial increase 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.

Oxidative stress related parameters

SOD activity

Table I shows the effect of dietary betaine on SOD, TAC and HSP70 in postpartum buffaloes at various interval for a total period of 125 days.

The overall mean value of plasma SOD was 1.83±0.07 ng/mL in control, 2.01±0.11 ng/mL in low betaine and 2.54±0.13 ng/mL in high betaine treated group. Significant deviations were found in high betaine groups on day 25, 75 and 100 postpartum.

Table I. Mean plasma superoxide dismutase (ng/mL), total antioxidant capacity (U/mL) and bovine heat shock protein 70kDa (ng/mL) in postpartum buffaloes at various intervals.

Treat-ment/ Days	Control (n=6)	Feed supplemented with betaine	
		50 g/animal/d (n=6)	100 g/animal/d (n=6)
Superoxide dismutase (ng/mL)			
day 7	1.94± 0.22	1.94± 0.44	1.99± 0.27
day 25	1.80± 0.06^{B}	1.91± 0.29^{B}	2.67± 0.19^{A}
day 50	2.12± 0.21	2.36± 0.23	2.40± 0.33
day 75	1.51± 0.07^{B}	1.92±0.28^{AB}	2.70± 0.3^{6A}
day 100	1.76± 0.10^{B}	2.05± 0.26^{B}	2.70± 0.16^{A}
day 125	1.85± 0.21	1.86± 0.16	2.78± 0.53
Average	1.83± 0.07^{B}	2.01± 0.11^{B}	**2.54± 0.13**^{A}
Total antioxidant capacity (U/mL)			
day 7	11.23± 1.21	11.21± 1.08	11.44± 1.76
day 25	9.73± 1.24	11.84± 1.23	11.92± 1.28
day 50	9.37± 1.01	11.24± 1.53	11.02± 1.43
day 75	9.72± 1.03	9.56± 1.59	11.45± 1.53
day 100	10.89± 1.10	10.93± 1.19	12.58± 1.22
day 125	11.66± 0.80	12.09± 1.06	12.94± 1.30
Average	10.43±0.43^{B}	11.14±0.51^{AB}	11.89±0.55^{A}
Bovine heat shock protein 70kDa (ng/mL)			
day 7	4.29± 0.62	4.44± 0.78	4.37± 0.62
day 25	4.99± 0.70	4.35± 0.75	3.66± 0.60
day 50	3.90± 1.08	3.67± 0.56	3.44± 0.68
day 75	4.22± 0.41	3.26± 0.53	3.28± 0.61
day 100	4.30± 0.66	3.47± 0.58	2.91± 0.39
day 125	4.46± 0.55	3.47± 0.46	3.14± 0.54
Average	4.36 ± 0.27^{A}	3.78± 0.25^{B}	3.47^{B}± 0.23

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

The present results were in agreement with Zhang *et al.* (2014), they reported that feeding betaine to cows increased SOD and malondialdehyde levels ($p<0.05$) during heat stress. Wang *et al.* (2019) also reported the greater plasma SOD concentrations 2 h after birth in calves of rumen-protected betaine (@ 20 g/day) fed cows as compared to control group. This investigation confirms amelioration of stress in betaine treatment group calves as compare to control group. Similarly, Shah *et al.* (2020) reported that the concentration of superoxide dismutase (SOD) was significantly higher ($p<0.05$) in Bet2 group than Bet1 and control groups in Holstein cows.

In contrast to present report, Sahraei *et al.* (2020) reported non-significant difference in blood concentrations of antioxidant enzymes in betaine supplemented and control groups of pregnant Sanjabi ewes. The correlation coefficient showed positive association between SOD and TAC in control and treatment groups. The SOD levels were more positively correlated with increase in overall mean of TAC in betaine supplemented groups as compare to control group (Table II). The ability of betaine to protect against oxidative stress is due to the fact that betaine is highly lipotropic and when administered exogenously, it can readily pass across the membrane lipid bilayer and diffuse into intracellular compartments (Kanabak *et al*., 2001).

Table II. Correlation coefficient between SOD, TAC and HSP70.

Parameters	Control group		Betaine supplement-ed groups	
	Value of r	Level of significance	Value of r	Level of significance
SOD and TAC	0.021	ns	0.102	ns
SOD and HSP70	-0.295	ns	-0.835	s
TAC and HSP70	0.109	ns	-0.028	ns

TAC capacity

Table I shows the mean TAC of buffaloes in all the groups during the experimental period.

The overall mean value of plasma TAC of high betaine group (11.89±0.55 U/mL) was maximum followed by low betaine (11.14±0.51 U/mL) group and control group (10.43±0.43 U/mL) had lowest TAC mean value which differed ($p<0.05$) significantly between groups.

Similar results were also reported by Zhang *et al.* (2014), they enunciated that feeding betaine to cows increased TAC ($p<0.05$) as compare to control cows during heat stress. Lakhani *et al.* (2019) performed an experiment on Karan Fries heifers supplemented with 0, 25 and 50 g/day/animal of betaine to 3 groups during three seasons and reported significant difference ($p<0.01$) in TAC concentrations between treatment groups. Shah *et al*. (2020) reported that the serum concentration of TAC in Bet1 (15 g/day per cow) and Bet2 (30 g/day per cow) was significantly increased ($p<0.05$) as compared to control.

The formation of endogenous free radicals and generation of lipid peroxides increases in dairy cows in lactational stress conditions and decreases the animal's antioxidant capacity. The antioxidant capacity of betaine enabled it to scavenge free radicals and protect cells from loss in rats (Lu *et al*., 2008). The present reports confirmed decrease in reactive oxygen species and free radicals along with an improvement in antioxidant capacity in betaine treated groups as compared to control group.

HSP70

The overall mean value of plasma HSP70 of high betaine group (3.47±0.23 ng/mL) was minimum followed by low betaine group (3.78±0.25 ng/mL) and control group (4.36±0.27 ng/mL) had maximum plasma HSP70 mean value which differed ($p<0.05$) significantly between groups (Table I).

Present findings are in agreement with Dangi *et al*. (2015), who reported lower HSPs expression in betaine supplemented group as compared to control group, suggesting a possible role of this chemical chaperone on heat stress amelioration in goats. Lakhani (2018) reported that there was decrease in HSP70.2 gene expression in treatment I and treatment II as compared to control (1.30 and 1.28 fold) and (2.08 and 1.92 fold) in hot-dry and hot-humid season respectively in Karan Fries heifers supplemented with betaine. Raheja (2017) concluded that betaine supplementation at the rate of 0, 50 g per cow per day during hot-humid season reduces the adverse effect of heat stress. Expression of HSP70 and HSP90 mRNA was significantly lower in betaine supplemented group as compared to control.

There was increase in SOD and TAC concentration with decrease in HSP70 concentration in betaine treated groups as compare to control groups. The correlation coefficient showed more negative association between TAC and HSP70, similarly between SOD and HSP70 in treatment groups as compare to control group (Table II). Betaine acts as chaperone, as it repairs denatured proteins and interacts with molecular chaperons, the heat shock proteins and protect against oxidative stress. Sheikh-Hamad *et al*. (1994) concluded that canine kidney cells treated *in vitro* to accumulate betaine have 3 times reduction in thermally induced heat shock protein expression and thus betaine attenuates the induction of intracellular heat shock proteins. Present reports confirm a decrease in plasma HSP70 concentration in betaine fed groups as compared to control group.

Total IgG

The overall mean value of plasma IgG of control group (3.06±0.17 mg/mL) was lowest followed by low betaine group (4.21±0.18 mg/mL) and highest value was found in high betaine group (4.41±0.19 mg/mL) which differed ($p<0.05$) significantly between groups (Table I).

The present results are in agreement with earlier workers (Pinotti *et al*., 2004; Sheikh *et al*., 2014; Acharya *et al*., 2019), who reported an increase in ($p<0.05$) total immunoglobulin concentration in rumen protected choline treated cows. Choline is oxidized to betaine that serves as an osmoregulator and is a substrate in the betaine–homocysteine methyltransferase reaction, which links choline and betaine to the folate-dependent one-carbon metabolism. Choline and betaine are important sources of one-carbon units, in particular, during folate deficiency. Choline or betaine supplementation improved vit. E and plasma folate status in transition dairy animals and improve immune system (Chatterjee *et al*., 2003).

Similarly, Hassan *et al*. (2011) reported that dietary betaine supplementation at all levels improved ($p<0.05$) both humoral immunity and cell-mediated immunity when compared with control in New Zealand White rabbits. Zhang *et al*. (1996) concluded that betaine is involved in regulating cytokines (e.g., tumor necrosis factor-α, TNF-α) production by liver macrophages (Kupffer cells) by inhibiting the prostaglandin synthesis, suggesting role of organic osmolytes (especially betaine) in modulating immune function in rats.

Albumin/Globulin (A/G) ratio

The mean albumin/globulin (A/G) ratio of buffaloes in all the groups during the experimental period has been presented in Table III.

The overall mean value of albumin/globulin ratio of control group (0.56±0.02) was maximum followed by low betaine group (0.46±0.03) and minimum value was found in high betaine group (0.42±0.03) which differed ($p<0.05$) significantly between groups.

On day 75 and 125 postpartum, the mean value of albumin/globulin ratio of control group (0.64±0.05 and 0.55±0.05) was maximum followed by low betaine group (0.46±0.08 and 0.42±0.05) and minimum value was found in high betaine group (0.40±0.05 and 0.38±0.04) respectively which differed ($p<0.05$) significantly between groups.

Present results were in agreement with Wang *et al*. (2019), they conducted a study on 24 multiparous Holstein dairy cows and divided into the control and rumen-protected betaine (@ 20 g/day) groups during transition period. The betaine group calves had lower A/G ratio (0.56) concentrations as compared to the control group calves (0.58).

In contrast to present report, Mishra *et al.* (2019) reported that there was non-significant difference ($p>0.05$) on albumin/globulin ratio following betaine supplementation as compare to control group in gestating sows.

Table III. Mean plasma immunoglobulin G (mg/mL) and albumin/globulin ratio (A/G) in postpartum buffaloes at various intervals.

Treat-ment/ Days	Control (n=6)	Feed supplemented with betaine	
		50 g/animal/d (n=6)	100 g/animal/d (n=6)
Immunoglobulin G (mg/mL)			
day 7	3.34± 0.49	3.42± 0.55	3.34± 0.26
day 25	3.93± 0.24	4.82± 0.40	5.01± 0.43
day 50	3.27± 0.15^{B}	4.53± 0.48^{A}	5.14± 0.51^{A}
day 75	2.08± 0.24^{B}	4.18± 0.40^{A}	5.08± 0.40^{A}
day 100	2.73± 0.40^{B}	4.15± 0.38^{A}	4.04± 0.39^{A}
day 125	2.99± 0.55	4.16± 0.35	3.88± 0.36
Average	3.06± 0.17^{B}	4.21± 0.18^{A}	4.41± 0.19^{A}
Albumin/ globulin ratio (A/G)			
day 7	0.52± 0.05	0.52± 0.08	0.52± 0.05
day 25	0.51± 0.07	0.50± 0.08	0.43± 0.07
day 50	0.58± 0.07	0.42± 0.05	0.40± 0.05
day 75	0.64± 0.05^{A}	0.46± 0.08^{AB}	0.40± 0.05^{B}
day 100	0.59± 0.04	0.42± 0.07	0.42± 0.10
day 125	0.55± 0.05^{A}	0.42± 0.05^{AB}	0.38± 0.04^{B}
Average	0.56± 0.02^{A}	0.46± 0.03^{B}	0.42± 0.03^{B}

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

CONCLUSION

Betaine acted as a chaperon and decrease the susceptibility of animals to stress by reducing the concentration of HSP70 in betaine supplemented buffaloes than control group. Supplementation of betaine during lactation period increased humoral immune response, antioxidant capacity and superoxide dismutase level in buffaloes thus alleviates the level of oxidative stress which reduces metabolic stress. It activates hypothalmo-pituitary and gonadal axis after parturition and lactation period and helps in early postpartum commencement of cyclicity in Murrah buffaloes during heat stress.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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