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Physio-biochemical responses and growth performance of buffalo heifers to betaine supplementation during hot humid season under field conditions

ADITYA DESHPANDE*, S V SINGH, YALLAPPA M SOMAGOND, PARVENDER SHEORAN, SAURAV NASKAR and V P CHAHAL

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

In order to find out the effect of betaine supplementation on physiological (rectal temperature, skin temperature, respiration rate and pulse rate) responses, morphological (body length, heart girth and height at withers) parameters, stress markers (cortisol and NEFA levels), growth hormone, dry matter intake (DMI) and body weight of buffalo heifers during hot humid season under field conditions, 14 buffalo heifers were selected and equally divided into 2 groups, i.e. control and treatment (supplemented betaine @ 25 g/animal/day over the farmers practice). Blood samples were collected at fortnightly interval from both the groups and analyzed for stress markers. THI was calculated for measuring the stress levels on buffalo heifers, which remained >76 indicating severe stress. Physiological responses were significantly lower in treatment group than control. Morphological parameters, body weight and DMI were higher in treatment than control. Plasma cortisol and NEFA levels were significantly lower, whereas plasma growth hormone was significantly higher in treatment group compared to control. ADG was significantly higher in treatment group compared to control. Positive correlation was observed between THI and stress markers, physiological responses and negatively correlated with ADG, DMI and growth hormone. It can be concluded that betaine acted as a potent growth promoter by lowering the levels of stress markers and enhancing the ADG and DMI of buffalo heifers.

Keywords: Betaine, Buffalo heifers, Growth, Hot humid season, Stress markers

Indian farming system is a combination of crops and livestock. India ranks first in the world buffalo population contributing more than 49% of the total milk production (BAHFS 2019). The day-by-day increasing population will result into the increased demand of milk and milk products. Health awareness and nutritious values of milk and milk product are also supporting the demand. To fulfill the increasing demand of milk and milk products of increasing population, the growth rate in dairy production and dairy processing must be raised to achieve regular demand and supply of milk and milk products.

In recent years, farmers have shifted dairy herd towards buffaloes. The buffalo population has increased by 3.19% in 2014–15 as compared to that in 2010–11 (DADF 2011, 2015). In tropical countries like India, buffaloes are preferred over cattle because of their better nutrient conversion efficiency of poor quality feeds and fodders, better disease resistance and adaptability to changing tropical climate (Paul *et al.* 2003).

Buffaloes exhibit heat stress when exposed to direct solar radiation due to dark skin, sparse hair coat and less number of sweat glands per unit skin area (Marai and Haeeb 2010). When heat stress is severe, farmers can introduce management practices such as providing shade or additional cold water or implementation of nutritional strategies. One of the nutritional strategies includes betaine supplementation (Dunshea *et al*. 2013).

Betaine is found ubiquitously in plants, animals and microorganisms, also in seafood, spinach, and wheat bran. The main physiological role of betaine is a methyl donor and an osmolyte. Betaine acts as an osmolyte, to maintain the cellular water and ion balance to improve the capacity against heat stress by preventing dehydration. Betaine helps various intestinal microbes against osmotic variations and thus improves microbial fermentation activity. Dietary supplementation of betaine positively affect nutrients' digestibility. Its inclusion in the diet provides essential amino acids like choline and methionine via metabolism of betaine (Saeed *et al*. 2017).

Keeping in view the above facts, the present study was planned to validate the beneficial effect of betaine supplementation to buffalo heifers under field conditions.

MATERIALS AND METHODS

Selection of experimental animals: Healthy buffalo heifers (14; 8–10 months) were selected from Kathwar

*Corresponding author e-mail: adityaddeshpande4444@gmail.com

village of district Kaithal (Haryana) adopted under Farmer FIRST (Farm, Innovations, Resources, Science and Technology) project for the study during hot humid season. These animals were further divided equally (7 each) into 2 groups, i.e. control and treatment on the basis of their body weight. The control group of heifers was maintained as per farmer's practice, whereas treatment group was supplemented additionally 25 g betaine/animal/day.

Experimental protocol: All the experimental animals were dewormed a week before initiation of experiment with *Fenbendazole* to minimize the error in growth trial. Experiment was carried out during July to October 2018. Betaine was supplemented to treatment group @ 25 g betaine/animal/day (BETAFIN® S1; purchased from Genex Feeds, Karnal, Haryana) during August and September. Blood samples were collected from both the groups of buffalo heifers on day 0, 15, 30, 45, 60, 75 and 90 in sterile heparinized vacutainers tubes. Immediately after collection plasma was separated by centrifuging the samples @ 2500 rpm for 25 min to separate the plasma. The plasma samples were stored at –20°C for plasma constituents' estimation (growth hormone, cortisol and NEFA levels). The body measurements of buffalo heifers were recorded by using flexible measuring tape to calculate approximate body weight of an animal at field conditions by Schaeffer's formula. The physiological responses, viz. rectal temperature, skin temperature, respiration rate and pulse rate were recorded using standard methods on the day of blood sampling. Environmental parameters, viz. dry and wet bulb temperature, relative humidity, maximum and minimum temperature were recorded fortnightly and temperature humidity index was calculated as per Johnson *et al.* (1963).

Estimation of biochemical parameters: Levels of plasma cortisol, growth hormone and NEFA were analyzed using ELISA kit as per the manufacturer's protocol (purchased from Bioassay Technology).

Ethical approval: Before starting of the experiment, Institutional Animals Ethics Committee Approval (No. 42-IAEC-18-7) was obtained.

Statistical analysis: The data were analyzed using SPSS (version 22) and Prism 5 for Windows and expressed as mean±SE Independent t-test, one way ANOVA and two way ANOVA were done to find out the significant difference between treatments, fortnight intervals and their interaction respectively. The pair wise comparison of means was carried out using post-hoc Duncan multiple comparison test. Evaluation of the correlation between all the factors in respective set of animals was made using a correlation coefficient at the level of probability ($P<0.01$ and $P<0.05$).

RESULTS AND DISCUSSION

Temperature humidity index (*THI*)*:* THI is most common magnitude measurement of heat stress in dairy animals (Armstrong 1994). According to NRC (1971), when THI value is below 72, animals are in thermoneutral zone. Value between 72 to 76 indicate moderate heat stress and 76 to 80 indicate severe heat stress. THI value >80 indicate fatal heat stress to animals. THI showed positive correlation with rectal temperature, skin temperature, respiration rate, pulse rate, cortisol and NEFA, and negative correlation with GH, BW, DMI and ADG (Table 6). THI showed positive correlation with cardinal signs, heat production, heat storage and negative correlation with heat loss (Vaidya *et al*. 2010).

Rectal temperature (RT): The overall mean value of control group (40.42±0.14°C) was higher ($P<0.05$) than treatment group (39.54±0.14°C). The mean values of RT of treatment group were 41.10±0.34°C during I fortnight, 39.78±0.42°C during III fortnight and 38.52±0.22°C during residual period, and differed significantly ($P<0.05$) with each other. During residual period on 6th fortnight, RT differed numerically in both groups and on 7th fortnight, RT of treatment group was significantly ($P<0.05$) lower compared to control (Table 1). Rectal temperature in betaine supplemented animals significantly ($P<0.05$) lower compared to control. This difference might be due to osmo-protective function of betaine, which helped the supplemented animals to retain cellular water level and total body water content compared to control animals (Cronje 2005). Betaine supplementation showed beneficial effect

Table 1. Effect of dietary betaine supplementation on rectal temperature and skin temperature of buffalo heifers during hot humid season

	Fortnight	Rectal temperature (°C)		Skin temperature (°C)	
		Control	Treatment	Control	Treatment
0th Day	I	40.47^{Xab}±0.20	41.10^{Xd}±0.34	36.08^{Xb}±0.31	36.07^{Xc}±0.34
During feeding	II	40.71^{Xb}±0.34	40.81^{Xcd}±0.30	35.00^{Xab}±0.31	36.05^{Xc}±0.73
	III	40.42^{Xab}±0.33	39.78^{Xbc}±0.42	35.37^{Xb}±0.54	34.92^{Xbc}±0.67
	IV	40.60^{Xab}±0.39	39.64^{Xb}±0.56	35.22^{Xb}±0.75	34.88^{Xbc}±0.82
	V	40.97^{Xb}±0.20	38.95^{Yab}±0.36	35.54^{Xb}±0.19	33.65^{Yab}±0.58
Residual period	VI	39.54^{Xa}±0.49	38.52^{Xa}±0.22	33.77^{Xa}±0.58	32.38^{Xa}±0.43
	VII	40.21^{Xab}±0.28	38.01^{Ya}±0.26	35.45^{Xb}±0.33	32.54^{Ya}±0.37
	Mean±SE	40.42^{X}±0.14	39.54^{Y}±0.14	35.00^{X}±0.21	34.36^{Y}±0.21

The values are mean±SE of 7 observations on 7 animals. The values with different superscripts within a column (a, b, c) and within a row (X,Y) differed significantly ($P<0.05$) within the treatment and among treatment groups respectively.

even after withdrawal of the betaine supplementation up to 1 month post treatment. Similar to results of the present study, betaine lowered BMR, reduced the maintenance requirement and metabolic heat production of growing pigs, and metabolic heat production was decreased significantly over time (Schrama *et al*. 2003). Lakhani (2018) also showed significantly (P<0.05) lower RT in betaine supplemented KF heifers compared to control during hot humid season. RT showed positive correlation with THI, ST, RR, PR, Cortisol and NEFA, whereas negative correlation with GH, body measurements, BW, DMI and ADG (Table 6).

Skin temperature (ST): The overall mean of control group was higher (35.00±0.21°C) compared to treatment (34.36±0.21°C) group. ST was higher in control compared to treatment group on 6th fortnight and significantly (P<0.05) higher in control on 7th fortnight during residual period (Table 1). Haque et al. (2012) reported that exposure to 40°C, 42°C and 45°C compared to 22°C showed great changes in ST which were induced in buffaloes by a marked increase in blood flow from the body core to the surface, which accelerates dissipation of heat from the skin surface. In the present study, skin temperature also showed similar trend in both the groups of buffalo heifers as RT during different fortnights of experiment and during residual period.

The results of the present study are in accordance to the findings of Kumar *et al*. (2019) who reported higher ST in control compared to astaxanthin supplemented group of Karan Fries heifers during summer conditions. The peak values of ST were recorded in July. The skin temperature of buffalo heifers was raised in relation to the length of radiation exposure of animals (Singh and Singh 2006). The rise in ambient temperature causes increase of ST and reflex sweating mechanism through thermo sensory impulses from receptors located in the skin. However, the number of sweat glands and sweating rate is lower in buffaloes (Marai and Haeeb, 2010). ST showed positive correlation with THI, RT, RR, PR, cortisol and NEFA, whereas negative correlation with GH, body measurements, BW, DMI and ADG (Table 6).

Respiration rate (RR): The overall mean value of control group (28.65±0.51/min) was higher (P<0.05) than treatment group (25.69±0.51/min). After the end of the supplementation period, the RR remained significantly (P<0.05) lower in treatment group than control on 6th fortnight (Table 2). Respiration rate is an indicator of respiratory evaporative heat loss. The increase in respiration rate with the increasing body temperature is efficient respiratory evaporative heat loss mechanism from highly vascular nasal passage mucosa for exchange of body heat with expiratory air and more demand of oxygen by the tissues in stressful condition (Haque *et al*. 2012). Lower (P<0.05) RR was observed in betaine supplemented KF heifers compared to control group during hot humid season (Lakhani 2018). Higher (P<0.05) respiration rate was observed in young buffalo heifers exposed in climatic chamber at 45°C (Hooda and Singh 2010). The RR showed positive correlation with THI, RT, ST, PR, cortisol and NEFA, whereas negative correlation with GH, body measurements, BW, DMI and ADG (Table 6).

Pulse rate (PR): The overall mean value of treatment group was lower (P<0.05) (64.44±0.78/min) than control (70.79±0.78/min). During 6th and 7th fortnight, PR remained lower (P<0.05) in betaine supplemented group than control (Table 2). Pulse rate is instant measure of sympathetic and parasympathetic nervous system activity in animal body. It is non-invasive method to check stress level of animals. The change in PR arises due to direct heat stimulation of peripheral thermal receptors, which transmit stimulus to thermal centre at hypothalamus. It triggers tachycardia for effective peripheral blood perfusion for heat dissipation through skin and sweating. Also, it helps to maintain efficient blood supply to vital organs during stressed conditions (Marai *et al*. 2007). Pulse rate increased (P<0.01) during spring and summer season compared to winter in both growing and adult Murrah buffaloes. This was due to increased peripheral circulation to enhance heat loss from skin surface (Vaidya *et al*. 2010). PR was significantly higher in heat stressed buffalo heifers compared to control group (Hooda and Singh 2010). The PR showed positive correlation with THI, RT, ST, RR, cortisol and NEFA, whereas negative correlation with GH, body measurements, BW, DMI and ADG (Table 6).

Table 2. Effect of dietary betaine supplementation on respiration rate and pulse rate of buffalo heifers during hot humid season

	Fortnight	Respiration rate (breaths/min)		Pulse rate (beats/min)	
		Control	Treatment	Control	Treatment
0th Day	I	31.14^{Xb}±1.18	31.00^{Xb}±1.15	72.00^{Xab}±2.42	71.71^{Xc}±2.94
During feeding	II	30.71^{Xb}±1.15	31.00^{Xb}±1.11	75.86^{Xb}±2.11	67.29^{Ybc}±1.32
	III	30.29^{Xb}±1.49	25.29^{Ya}±1.36	72.71^{Xab}±2.29	64.29^{Yab}±1.38
	IV	28.00^{Xab}±1.07	24.00^{Xa}±1.53	69.43^{Xab}±1.93	63.14^{Yab}±1.35
	V	29.00^{Xb}±1.41	22.00^{Ya}±0.85	68.71^{Xa}±2.69	63.43^{Xab}±2.00
Residual period	VI	26.86^{Xab}±1.88	22.29^{Ya}±0.68	68.29^{Xa}±1.21	60.86^{Ya}±2.29
	VII	24.57^{Xa}±1.31	24.29^{Xa}±1.51	68.57^{Xa}±2.22	60.43^{Ya}±1.85
	Mean±SE	28.65^{X}±0.51	25.69^{Y}±0.51	70.79^{X}±0.78	64.44^{Y}±0.78

The values are mean±SE of 7 observations on 7 animals. The values with different superscripts within a column (a, b, c) and within a row (X,Y) differed significantly (P<0.05) within the treatment and among treatment groups respectively.

Hormones

Cortisol levels: The overall mean value of control group (7.18±0.45 ng/ml) was higher ($P<0.05$) than treatment (04.92±0.48 ng/ml). Cortisol levels remained lower ($P<0.05$) in betaine supplemented group than control even 1 month after the discontinuation of supplementation (Table 3). Lakhani (2018) reported higher values of cortisol in control (10.36±0.16 ng/ml) than treatment group (9.82±0.25 ng/ml) of KF heifers during hot humid season. Cortisol elicits physiological adjustments, which enable animals to tolerate stressful conditions (Christison and Johnson 1972). Concentration of cortisol is altered by acute and chronic heat exposure (Alvarez and Johnson 1973). The initial rise in plasma glucocorticoids is due to activation of the adrenocorticotropic (ACTH) releasing mechanism in the hypothalamus by thermo receptors of the skin (Chowers *et al*. 1966). This glucocorticoid level later decline to normal, in spite of continuing heat stimulus, which indicates a negative glucocorticoid feedback and a decrease in the glucocorticoid binding transcortin (Lindner 1964). The glucocorticoids also work as vasodilators to help heat loss (Cunningham and Klein 2007). The cortisol showed positive correlation with THI, RT, ST, PR and NEFA and negative correlation with GH, BW, DMI and ADG (Table 6).

Growth hormone (GH): The overall mean value of control group (3.68±0.17 ng/ml) was lower ($P<0.05$) than treatment group (5.19±0.18 ng/ml). Levels of GH remained high ($P<0.05$) in betaine supplemented group compared to control upto one month of withdrawal of supplementation (Table 3). The GH showed positive correlation with BW, DMI and ADG and negative correlation with THI, RT, ST, PR, NEFA and cortisol (Table 6). Betaine promotes growth hormone releasing hormone (GHRH) gene transcription and GHRH secretion by the hypothalamus which results in increased plasma GH concentrations (Yan *et al.* 2001). Corticotrophin-releasing hormone is known to stimulate somatostatin release from the hypothalamus which in turn inhibits growth hormone secretion (Riedel *et al*. 1998). The improved growth performance by betaine supplementation is attributed to donation of methyl groups (Kidd *et al*. 1997), increasing growth hormone secretion (Huang *et al.* 2006) and improving gut health (Metzler-Zebeli *et al*. 2009). Also, betaine has been shown to stabilize cell membranes via membrane phospholipids interaction and reduce fecal water loss and increase the digestibility of nutrients (Klasing *et al*. 2002). Lakhani (2018) reported higher ($P<0.05$) values of GH (8.13±0.42 ng/ml) in betaine supplemented group of Karan Fries heifers compared to control (5.57±0.39 ng/ml) during hot humid conditions.

Blood metabolites

Non-esterified fatty acids (*NEFA*)*:* The overall mean value of control group was higher ($P<0.05$) (278.48±10.15 µmol/L) than treatment group (211.89±13.27 µmol/L) (Table 4). In summer, the energy supply is generally insufficient for cows due to reduced dry matter intake; therefore the body is in a state of negative energy balance. The energy supply is mainly dependent on the oxidation of free fatty acids (O'Brien *et al*. 2010). The liver activity of cows' declines during summer heat stress compared to cows inhabiting thermally optimal environments. In addition, the cholesterol and triglyceride level in blood plasma also declines. These phenomena may be caused by an increase in the mobilization of fat in the peripheral tissue (Abeni *et al*. 2000). Plasma NEFA concentrations in betaine supplemented Holstein Friesian primiparous cows was numerically lower (0.298 mEq/L) than control group (0.388 mEq/L). As a methyl donor, betaine has the role of promoting fatty acid oxidation (Davidson *et al*. 2008). The NEFA showed positive correlation with THI, RT, ST, PR and cortisol and negative correlation with GH, DMI, ADG, BW and body measurements (Table 6).

Body measurements

The overall mean value of control group (97.28±1.64 cm) was lower than treatment group (99.28±1.64 cm) (Fig. 1). Same trend was observed for overall mean value of height at withers (Fig. 2). This non-significant change in body measurements might be due to post-natal allometric growth during pre-pubertal period and different growth rate in different body parts.

Overall differences in the heart girth, body length and height at withers among the groups were non-significant (Movaliya *et al*. 2013). Non-significant changes in heart

Table 3. Effect of dietary betaine supplementation on cortisol and growth hormone of buffalo heifers during hot humid season

	Fortnight	Cortisol (ng/ml)		Growth hormone (ng/ml)	
		Control	Treatment	Control	Treatment
0th Day	I	8.08^{ab}±1.29	7.57^{c}±1.19	3.96^{Xab}±0.34	4.17^{Xa}±0.30
During feeding	II	9.59^{b}±1.06	7.93^{c}±1.44	3.97^{Xab}±0.62	4.76^{Xab}±0.23
	III	7.73^{ab}±1.07	5.57^{bc}±1.28	3.26^{Xab}±0.41	4.99^{Yabc}±0.25
	IV	6.72^{ab}±1.38	5.62^{bc}±1.20	3.28^{Xab}±0.57	4.82^{Xab}±0.53
	V	7.95^{Xab}±1.00	2.81^{Yab}±0.59	4.64^{Xb}±0.26	5.45^{Xabc}±0.37
Residual period	VI	4.90^{Xa}±1.19	1.81^{Ya}±0.44	3.57^{Xab}±0.52	6.25^{Yc}±0.36
	VII	5.26^{Xa}±0.82	3.14^{Yab}±0.49	3.08^{Xa}±0.24	5.93^{Ybc}±0.78
	Mean±SE	7.18^{X}±0.45	4.92^{Y}±0.48	3.68^{X}±0.17	5.19^{Y}±0.18

The values are mean±SE of 7 observations on 7 animals. The values with different superscripts within a column (a, b, c) and within a row (X,Y) differed significantly ($P<0.05$) within the treatment and among treatment groups respectively.

Table 4. Effect of dietary betaine supplementation on NEFA and body weight of buffalo heifers during hot humid season

	Fortnight	NEFA (μmol/L)		Body weight (kg)	
		Control	Treatment	Control	Treatment
0^{th} Day	I	$336.10^{d}\pm26.28$	$369.26^{c}\pm28.83$	$158.40^{Xa}\pm8.11$	$150.21^{Xa}\pm23.08$
During feeding	II	$328.71^{Xd}\pm24.83$	$239.72^{Yb}\pm16.48$	$166.33^{Xab}\pm8.66$	$161.16^{Xa}\pm24.29$
	III	$314.20^{Xcd}\pm16.29$	$240.92^{Yb}\pm14.59$	$174.61^{Xab}\pm9.23$	$173.72^{Xa}\pm25.45$
	IV	$279.81^{Xbcd}\pm18.63$	$214.93^{Yb}\pm21.22$	$183.22^{Xabc}\pm9.55$	$186.15^{Xa}\pm27.15$
	V	$258.30^{Xbc}\pm16.02$	$140.32^{Ya}\pm12.08$	$194.19^{Xbcd}\pm10.22$	$200.79^{Xa}\pm29.76$
Residual period	VI	$249.84^{Xb}\pm18.90$	$138.24^{Ya}\pm10.67$	$205.97^{Xcd}\pm11.06$	$218.83^{Xa}\pm31.82$
	VII	$182.15^{Xa}\pm17.54$	$139.81^{Ya}\pm28.56$	$218.88^{Xd}\pm11.68$	$237.18^{Xa}\pm33.44$
	Mean±SE	$278.48^{X}\pm10.15$	$211.89^{Y}\pm13.27$	$185.94^{X}\pm7.71$	$189.72^{X}\pm7.71$

The values are mean±SE of 7 observations on 7 animals. The values with different superscripts within a column (a, b, c) and within a row (X,Y) differed significantly (P<0.05) within the treatment and among treatment groups respectively.

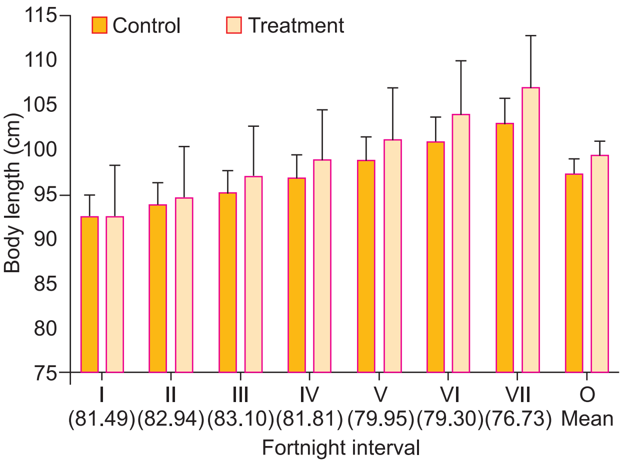

Fig. 1. Effect of dietary betaine supplementation on body length (cm) of buffalo heifers during summer season (THI mentioned in parenthesis).

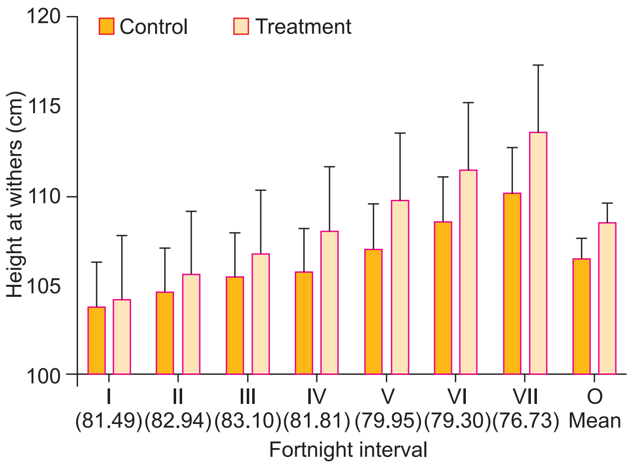

Fig. 2. Effect of dietary betaine supplementation on height at withers (cm) of buffalo heifers during summer season (THI mentioned in parenthesis).

girth during different phases of the experiment had also been reported by Luchini and Veth (2006) and Qiang *et al*. (2011). Differences in respect of initial biometry as well as gain in biometry in heifers of different groups during the experimental period were non-significant (Gajera *et al*. 2013).

Body weight (BW): The overall mean value of control group was lower (185.94±7.71 kg) than treatment group (189.72±7.71 kg) of buffalo heifers during hot humid season. Betaine had significantly higher residual effect on BW gain. Higher BW was observed in treatment group than control during residual period (Table 4). Increase in BW due to betaine supplementation is attributed to increased cellular water content by osmolyte property of betaine and increased nutrients bioavailability and efficiency. In ruminants, betaine also has a positive influence on fermentation, it increases total volatile fatty acid (VFA) production as well as the ratio of acetate to propionate (A: P). Betaine serves as a source of ruminal available nitrogen, increasing microbial fermentation rate and altering fermentation patterns (Wang *et al.* 2010). Brobeck (1960) also reported that direct effect of higher ambient temperature in reduction of feed intake and ultimately growth rate of animals. During thermal stress, reduction in feed intake helped in prevention of hyperthermia, decreased nutrients availability for productive processes and ultimately decreased the animal's growth rate (Lough *et al*. 1990). Glycine Betaine reduces lipogenesis as well as fat synthesis (Figueroa-Soto and Valenzuela-Soto 2018). The BW showed positive correlation with GH, DMI and ADG and negative correlation with THI, RT, ST, PR, NEFA and cortisol (Table 6).

Average daily gain (ADG)

The overall mean value of control group was significantly (P<0.05) lower (0.58±0.03 kg/day) than treatment group (0.83±0.03 kg/day). Betaine had significantly (P<0.05) higher residual effect on ADG, i.e. significantly (P<0.05) higher values were observed in treatment group than control during residual period (Table 5). Lakhani (2018) reported significantly (P<0.05) higher ADG in betaine supplemented group (445±2.13 g/day) than control (354±0.97 g/day) of KF heifers during hot humid condition. Similarly, Awad *et al*. (2014) also reported beneficial effect of dietary betaine supplementation at different levels under summer conditions in domyati ducklings as compared to control. Moreover, betaine has been shown to stabilize cell membranes through interaction

Table 5. Effect of dietary betaine supplementation on ADG and DMI of buffalo heifers during hot humid season

	Fortnight	ADG (kg)		DMI (kg)	
		Control	Treatment	Control	Treatment
0^{th} Day	I	00	00	$4.99^{Xa}\pm0.26$	$4.95^{Xa}\pm0.76$
During feeding	II	$0.52^{Xa}\pm0.05$	$0.73^{Xa}\pm0.08$	$5.31^{Xab}\pm0.29$	$5.27^{Xab}\pm0.87$
	III	$0.55^{Xa}\pm0.05$	$0.84^{Yab}\pm0.08$	$5.24^{Xab}\pm0.27$	$5.71^{Xab}\pm0.88$
	IV	$0.57^{Xab}\pm0.03$	$0.83^{Xab}\pm0.12$	$5.58^{Xab}\pm0.31$	$6.35^{Xab}\pm0.89$
	V	$0.73^{Xbc}\pm0.05$	$0.98^{Xab}\pm0.18$	$6.07^{Xbc}\pm0.42$	$6.80^{Xab}\pm0.89$
Residual period	VI	$0.79^{Xc}\pm0.07$	$1.20^{Yb}\pm0.13$	$6.81^{Xcd}\pm0.37$	$7.32^{Xab}\pm1.00$
	VII	$0.86^{Xc}\pm0.06$	$1.22^{Yb}\pm0.11$	$7.21^{Xd}\pm0.33$	$8.13^{Xb}\pm0.97$
	Mean±SE	$0.58^{X}\pm0.03$	$0.83^{Y}\pm0.03$	$5.89^{X}\pm0.24$	$6.36^{X}\pm0.24$

The values are mean±SE of 7 observations on 7 animals. The values with different superscripts within a column (a, b, c) and within a row (X,Y) differed significantly ($P<0.05$) within the treatment and among treatment groups respectively.

Table 6. Correlation coefficient among different parameters of buffalo heifers during hot humid season

	THI	RT	ST	RR	PR	Cortisol	GH	Length	Girth	Height	BW	DMI	ADG	NEFA
THI	1													
RT	0.413**	1												
ST	0.367**	0.865**	1											
RR	0.406**	0.493**	0.405**	1										
PR	0.308**	0.416**	0.312**	0.337**	1									
Cortisol	0.407**	0.368**	0.289**	0.417**	0.395**	1								
GH	-0.107	-0.306	-0.241	-0.182	-0.426	-.260**	1							
Length	-0.306	-0.279	-0.139	-0.339	-0.107	-.408**	0.129	1						
Girth	-0.415	-0.29	-0.206	-0.317	-0.049	-.379**	0.07	.839**	1					
Height	-0.28	-0.345	-0.238	-0.352	-0.051	-.369**	0.112	.938**	.817**	1				
BW	-0.377	-0.296	-0.168	-0.347	-0.112	-.402**	0.137	.932**	.962**	.888**	1			
DMI	-0.434	-0.356	-0.219	-0.372	-0.16	-.461**	0.172	.926**	.942**	.886**	.983**	1		
ADG	-0.426	-0.486	-0.384	-0.488	-.419**	-.494**	.299**	.635**	.688**	.605**	.714**	.742**	1	
NEFA	0.533**	0.491**	0.392**	0.513**	.449**	.490**	-0.195	-.216*	-.299**	-0.179	-.284**	-.353**	-.666**	1

Significance levels (*$P<0.05$, **$P<0.01$).

with membrane phospholipids and to reduce fecal water content and increase the digestibility of several nutrients (Klasing *et al*. 2002). The ADG showed positive correlation with GH, DMI and BW and negative correlation with THI, RT, ST, PR, NEFA and cortisol (Table 6).

Dry matter intake (DMI)

The overall mean value of control group was lower (5.89±0.24 kg) than treatment group (6.36±0.24 kg) of buffalo heifers during hot humid season. Betaine supplementation also showed residual effect on DMI of treatment group, i.e. higher DMI compared to control (Table 5). Similar results were observed in DMI by steers when rumen-unprotected betaine was top-dressed on the upper one-fourth of the ration, which increased palatability of the ration and stimulated intake (Loest *et al.* 2002). HF cows given betaine supplementation had higher DMI during heat stress compared to control (Hall *et al*. 2016). DMI was significantly ($P<0.05$) lower in heat stressed buffalo heifers group compared to control (Hooda and Singh 2010). Thermal stress decreases growth rate due to reduction in feed intake and decreased nutrients availability (Lough *et al*. 1990). Similarly, DMI in betaine supplemented crossbred lactating cows was higher ($P<0.01$) by 8.79% as compared to control (Shankhpal *et al*. 2018). The DMI showed positive correlation with GH, ADG, BW and body measurements and negative correlation with THI, RT, ST, PR, NEFA and cortisol (Table 6). Hence, it can be concluded that betaine acted as a potent growth promoter by reducing the concentration of stress markers in betaine supplemented buffalo heifers.

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