*Indian Journal of Animal Sciences* **92** (4): 460–464, April 2022/Article
https://doi.org/10.56093/ijans.v92i4.124158

# Impact of dietary betaine supplementation on appetite hormones and blood biochemical parameters in Karan Fries heifer during summer in tropics

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Received: 4 February 2021; Accepted: 27 December 2021

ABSTRACT

The present research ascertained variation in plasma levels of ghrelin and leptin, and blood biochemical parameters under summer conditions in Karan Fries (KF) heifers (n=18) supplemented with two levels of betaine. Present appraisal was conducted on Karan Fries heifers selected randomly. Experiment was divided into two phases: Experiment-I (thermoneutral season) and Experiment–II (summer season: hot-dry and hot-humid season). KF heifers were randomly divided into 3 groups (n=6) of Control, Treatment I (Betaine supplemented @ 25 g/d/animal) and Treatment II (Betaine supplemented @ 50 g/d/animal). Average maximum and minimum environmental temperatures were 23.5°C and 9.9°C and 35.3°C and 22.4°C, during Experiment I and Experiment II respectively. Blood samples were collected at fortnightly intervals. Betaine supplementation at 25 g/d and 50 g/d resulted in significant increase in plasma ghrelin, leptin, protein and cholesterol level and significant decrease in NEFA and triglycerides levels. However non-significant effect on plasma glucose levels were observed on increasing THI.

**Keywords**: Betaine, Biochemical, Hormones, Livestock, Nutrition

Heat stress has negative effect on biological and physiological functions of crossbred cattle through depressed production and reproduction performances (Albaa *et al*. 2019). Primarily, the affective state of animal is compromised instigating feelings of hunger and thirst (Polsky *et al*. 2017) during summers. Heat-stressed cows start mobilizing adipose lipids, preventing the initiation of glucose-sparing events because of a high energy demand (Wheelock *et al*. 2010). As a result, body starts utilizing its own body reserves leading to a decline in body weight. Betaine, a derivative of amino acid glycine and product of choline (de Zwart *et al*. 2003) was first isolated from sugar beets (*Beta vulgaris* subsp. *vulgaris*). Betaine maintains *p*H of stomach improving overall nutrient digestibility (Lakhani *et al.* 2020). On virtue of being methyl donor and having amino acid properties, betaine provides the essential amino acids required in the diet as feed stimulant to promote nutrient intake and ultimately growth. Mechanism by which feed intake is regulated are governed by appetite hormones ghrelin and leptin (Dar *et al*. 2019).

Leptin, polypeptide hormone, is primarily secreted by adipose tissue and plays a vital role in feed intake and energy metabolism (Friedman and Halaas 1998) by their action on adipocytes. Similarly, ghrelin termed as hunger hormone, stimulates appetite, maintains physiological process including sleep, insulin secretion and glucose update. The methyl donation property of betaine (Simon 1999) aids in improving the overall feed intake by animals and stimulates the appetite hormone secretion. The osmoprotective activity of betaine increase cellular enzyme activity and enhances tolerance of critical enzymes by cells and cellular membranes. With an increase in osmotic effect and enrichment in nutrient intake by animals, significant change in plasma glucose concentration could also be observed.

Knowledge on mechanism by which betaine acts on appetite hormones and the dose of betaine effective in controlling heat stress related blood biochemical changes is rare. Therefore need for research efforts to examine the potential relationship between heat stress, appetite hormone and metabolic state of the animal is utmost important. Keeping in mind the gaps, the present investigation was planned to assess the level of betaine supplementation effective in alleviation of negative effect of heat stress and improving energy status of Karan Fries heifers during summer conditions.

## MATERIALS AND METHODS

Experiment was approved and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India (Reg. No. 132/GO/ac/CPCSEA Dt. 3/7/2016).

The present study was conducted in National Innovation on Climate Resilient Agriculture (NICRA) of

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ICAR-National Dairy Research Institute (NDRI), Karnal (Haryana). Karnal is situated at an altitude of 250 meters above mean sea level, at 29º42'' N latitude and 79º54'' E longitudes. All KF were fed as per standard feeding (Maize Fodder:Concentrate :: 50:50). Calculation of THI was done according to the formula of National Research Council (1971): THI = (Tdb + Twb) × 0.72 + 40.6; where THI is Temperature Humidity Index, Tdb is dry bulb temperature (ºC) and Twb is wet-bulb temperature (ºC). All climatic variables were recorded twice daily at 8.30 AM and 2.30 PM the average value of each meteorological variable during hot dry was 35.33 to 22.33ºC and hot humid was 35.33 and 22.23ºC. Betaine as feed additive was procured from Genex Feed, Karnal (Haryana).

Eighteen Karan fries heifers (age 18±2.3 months) were selected from Livestock Research Centre (LRC) of National Dairy Research Institute, Karnal, Haryana. Study was conducted during Hot Dry (April-June), Hot Humid (July-September) and Thermo-Neutral (October-November) season. Experimental animals were randomly divided into three groups; Control (no betaine), Treatment I (25 g/d betaine supplementation) and Treatment II (50 g/d betaine supplementation) (n=6 animals) on basis of body weight of animals. Blood samples from experimental animals were collected from jugular vein in sterile EDTA vaccutainer (BD vacutainer TM, UK), at fortnightly interval. Glucose was estimated using GOD-PAP TRINDER'S kit purchased from Avecon Healthcare Pvt. Ltd. Triglycerides was estimated by Colorimetric Assay Kit (Recombigen laboratories Pvt. Ltd.). Total cholesterol was estimated by cholesterol Colorimetric Assay Kit by Recombigen Laboratories Pvt. Ltd. The absorbance were measured at 540 nm immediately after incubation using spectrophotometer (Model: Spectronics 118) against blank. The peptide bonds of proteins react with Cupric ions in alkaline solution to form a coloured chelate, the absorbance of which is measured at 578 nm. Leptin and ghrelin were analyzed using Bovine LEP ELISA Quantitation kit and "Bovine GHR ELISA kit" purchased from Cusabio Biotech Co., Ltd (China). OD was measured by Tecan Nano Quant ELISA reader (Infinite M 200 PRO, Bio Screen Instruments Pvt, Ltd).

*Statistical analysis:* Statistical analysis was performed using SPSS 16 and Prism 5 for windows. Statistical analysis of data was carried out to find the mean±SE. Two way ANOVA and one way ANOVA was done to find out the significant difference between treatments and seasons and their interaction. The pair-wise comparison of means was carried out using Tukey's multiple comparison test.

## RESULTS AND DISCUSSION

Total protein was significantly higher ($p<0.05$) in Control as compared to Treatment I and Treatment II during hot dry and hot humid season have been presented in Fig. 1. Mean values of total protein during thermo-neutral season was not significantly different between treatment groups. Significantly higher mean values of total protein (g/dl) during hot dry season was also reported by Shrikhande *et al.* (2008) and Al-Haidary *et al.* (2012) in Holstein heifers, lactating cows and Najdi rams, respectively. This may be due to loss of extracellular fluid and dehydration caused by enhanced respiratory rate during summer months (Al-Haidary *et al*. 2012). These findings are in agreement with Kumar *et al.* (2012) in goats and Raheja (2017) in cross-bred dairy cows. However, significant decrease in total protein was reported by Al-Saeed *et al.* (2009) in local breed of cattle, Omran *et al.* (2011) in Egyptian buffalo calves and Sharma and Puri (2013) in Marwari goats.

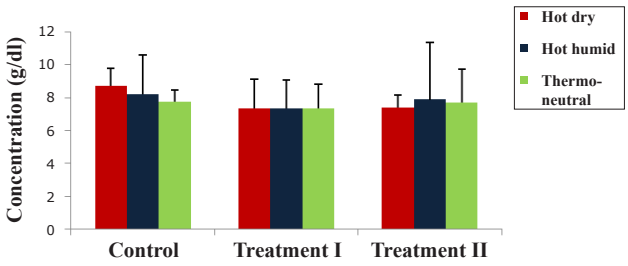


Fig. 1. Average values of total protein (g/dl) in control and treatment groups.

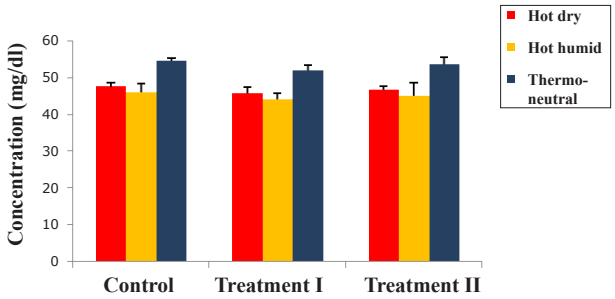


Fig. 2. Average values of plasma glucose (mg/dl) in control and treatment groups.

Plasma glucose (mg/dl) of Karan Fries heifers supplemented with different levels of betaine during three seasons is given in Fig. 2. Non significant ($p>0.05$) effect on glucose levels in the treatment groups was recorded. Seasonal impact was observed among groups, significantly ($p<0.05$) higher plasma glucose (mg/dl) was reported ($p<0·05$) in thermo-neutral season as compared to Hot dry, Hot humid season. Similar findings were reported by Cincovic *et al.* (2011) in Holstein cows, Ul-Haq *et al.* (2013) in crossbred dairy cows, Zhang *et al*. (2014) in Holstein Friesian cow and Tej *et al.* (2017) in crossbred female calves where decrease in plasma glucose was reported with increasing THI. However, Avendano-Reyes *et al.* (2010) reported a decline in glucose level in Holstein cows. This decline in level could be due to increased energy requirement by animal to combat the stress during hot humid condition as there was increase in THI values as well as relative humidity. This result is in agreement with Ronchi *et al.* (1999) in 6 months old heifers which may be due to augmentation of thyroid activity and metabolic rate accompanied with high levels of blood metabolites.

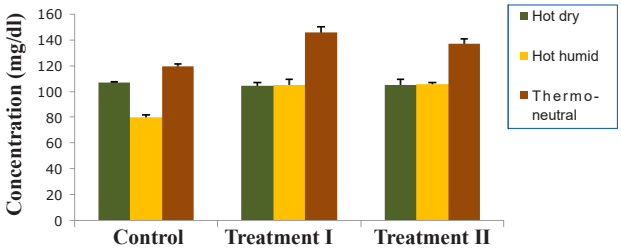


Fig. 3. Mean values of total cholesterol (mg/dl) in control and supplemented groups.

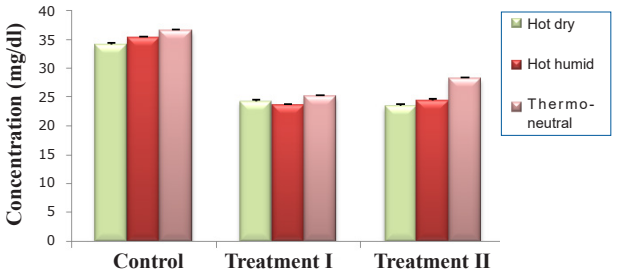


Fig. 4. Mean values of triglycerides (mg/dl) in control and supplemented groups.

Total cholesterol (mg/dl) of Karan Fries heifers supplemented with different levels of betaine during different seasons is given in Fig. 3. No significant ($p<0.05$) difference in total cholesterol (mg/dl) between control and supplemented groups during hot dry season was recorded whereas significantly higher value was observed ($p<0.05$) in hot humid season and thermo-neutral season in supplemented group as compared to control. Lowered cholesterol level in control group during the hot humid condition was attributed to lowered thyroid activity (Pandey 2012). Similar findings were reported by Rasooli *et al.* (2004) in Holstein heifer and Tej *et al*. (2017) in crossbred cows. Higher cholesterol levels in supplemented groups could be attributed to the lipotropic effects of betaine, enhancing fat absorption in the body through chylomicron formation. The findings were in agreement with Zhang *et al.* (2014) in Holstein cows who observed an increase in plasma cholesterol level on betaine supplementation during hotter period of the year. Similar findings were reported by Tej *et al.* (2017) in crossbred female calves during pre-monsoon, monsoon and post-monsoon seasons. Contrary to above result, Yang *et al*. (2009) reported decreased total cholesterol concentrations in blood and improved growth performance with dietary supplementation of betaine in pigs. High values of blood plasma cholesterol levels in the betaine supplemented group of Karan Fries heifers may be due to increase in feed intake. Thus, this suggests that betaine helps in improving the productive and growth performance owing to its role fat deposition and better uptake of fat by chylomicron formation in the body. The lipolytic glucocorticoids stimulate the fat mobilization from adipose tissue and increase the circulating concentrations of the free fatty acids. An increase in the concentration of cholesterol during thermo-neutral season in all groups would be expected after a stress episode (Abdel-Fattah 2014).

Non significant influence of seasons were ($p>0.05$) observed on mean values of triglyceride (mg/dl) between control and supplemented groups. Triglyceride levels were significantly lower ($p<0.05$) in hot dry and hot humid season compared to thermo-neutral season (Fig. 4). This reduction in triglycerides in supplemented groups could be attributed to the decreased TG-lipase activity as a result of enhanced secretion of the insulin on betaine supplementation. Also, insulin inactivates the hormone sensitive TG-lipase by decreasing the cAMP production (Satyanarayana and Chakrapani 2006). Significant decline ($p<0.05$) in triglycerides concentration in supplemented groups compared to control group could be the result of reduced lipolysis and fat sparer activity of increased insulin in the supplemented groups.

All the groups under study had significant ($p<0.05$) effect on average values of NEFA as presented in Table 1.

Table 1. Mean±SE values of NEFA (umol/L) in Karan Fries heifers

| Season | Control | Treatment I* | Treatment II** |
|---|---|---|---|
| Hot dry | $207.97^{ap} \pm 1.46$ | $164.89^{bp} \pm 3.98$ | $158.90^{bp} \pm 2.20$ |
| Hot humid | $196.89^{aq} \pm 1.96$ | $137.05^{bq} \pm 1.60$ | $134.79^{bq} \pm 2.00$ |
| Thermo-neutral | $163.03^{ar} \pm 2.98$ | $137.92^{bq} \pm 3.00$ | $131.68^{bq} \pm 2.98$ |

*Betaine @ 25 g/animal/day, **Betaine @ 50 g/animal/day. Mean values with different superscripts within a row (a, b and c) and within a column (p, q and r) differ significantly ($p<0.05$) (n=6).

Table 2. Mean±SE values of ghrelin (ng/ml) in Karan Fries heifers

| Season | Control | Treatment I* | Treatment II** |
|---|---|---|---|
| Hot dry | $2.11^{ap} \pm 0.14$ | $3.96^{bp} \pm 0.27$ | $3.01^{bp} \pm 0.12$ |
| Hot humid | $2.45^{ap} \pm 0.26$ | $3.03^{bp} \pm 0.16$ | $3.08^{bp} \pm 0.14$ |
| Thermo-neutral | $2.44^{aq} \pm 0.11$ | $3.72^{bq} \pm 0.16$ | $3.21^{bq} \pm 0.18$ |

*Betaine @ 25 g /animal / day, **Betaine @ 50 g /animal / day. Mean values with different superscripts within a row (a, b and c) and within a column (p, q and r) differ significantly ($p<0.05$) (n=6).

Table 3. Mean±SE values of leptin (ng/ml) in Karan Fries heifers

| Season | Control | Treatment I* | Treatment II** |
|---|---|---|---|
| Hot dry | $3.11\pm0.14^{ap}$ | $4.96\pm0.27^{bp}$ | $4.01\pm0.12^{bp}$ |
| Hot humid | $3.45\pm0.26^{ap}$ | $5.03\pm0.16^{bp}$ | $5.08\pm0.14^{bp}$ |
| Thermo-neutral | $4.64\pm0.11^{aq}$ | $5.22\pm0.16^{bq}$ | $5.21\pm0.18^{bq}$ |

*Betaine @ 25 g /animal / day, **Betaine @ 50 g /animal / day. Mean values with different superscripts within a row (a, b and c) and within a column (p, q and r) differ significantly ($p<0.05$) (n=6).

Significant increase in NEFA levels during hot dry season is because of release of catecholamine's and glucocorticoids hormones that typically promote adipocyte lipolysis and NEFA mobilization. NEFA concentration showed diurnal variations in dairy cows exposed to direct solar radiation and concentration increased in the hottest period of the day (Cincovic *et al.* 2011). The significant ($p<0.05$) decrease in plasma levels of NEFA in the supplemented groups indicated that effective checking of mobilization from adipose tissue. This reflects the role of betaine in reducing NEBAL by increasing feed intake in betaine supplemented group, lipogenic property of betaine; also because of positive correlation with triglycerides and negative correlation with cholesterol and plasma glucose.

Ghrelin is potent feed regulator and gut derived hormone that stimulates appetite, maintains physiological process including sleep, insulin secretion and glucose update. Mean values of ghrelin (ng/ml) of Karan Fries heifers supplemented with 25 g/d/animal and 50 g/d/animal of betaine during three seasons is given in Table 2. At high temperature, animal reduces internal heat productions to maintain their body temperature by decreasing basal metabolic rate leading to decrease in feed intake. Lower level of ghrelin in control group indicates affect of heat stress; this may be associated with decrease in dry matter (Rhoads *et al*. 2011). Significant increase in ghrelin level was observed in Treatment I and II indicating positive effect of betaine supplementation as betaine maintains constant energy balance and lower basal metabolic rate and increases dry matter intake in supplemented group.

Leptin plays a major role in regulating body weight and growth in mammals. It increases insulin sensitivity, glucose utilization and energy expenditure in muscles. Mean values of leptin (ng/ml) of Karan Fries heifers supplemented with different levels of betaine during three seasons is given in Table 3. Leptin was significantly ($p<0.05$) higher during thermo-neutral season and lower during hot dry season and there was no significant effect between hot dry and hot humid season within all the treatments under study. Wathes *et al.* (2007) observed increased leptin in well-fed heifers and decreased values were observed during early weeks of lactation when animal experience negative energy balance. Betaine supplementation is beneficial in elevating leptin level which has important role in body weight and body growth, increase in growth hormone release and also correlated with positive energy balance, decreased level of NEFA and better glucose utilization in muscles, this may be attributed to the fact that betaine is lipotropic and it causes fat deposition in the body and leptin is adipocyte hormone.

This study will help to develop suitable managemental strategies to improve health and productivity of crossbred under the recent scenario of climate change. Feeding of food stimulants such as betaine supplements to cattle during heat stress will avoid decline in growth performance and regulate activity of various hormones, enzymes and blood biochemical factors in improving performance of KF heifers under tropical summer environments.

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