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Effect of Simulated Heat Stress on Digestibility, Methane Emission and Metabolic Adaptability in Crossbred Cattle

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ABSTRACT: The present experiment was conducted to evaluate the effect of simulated heat stress on digestibility and methane (CH_4) emission. Four non-lactating crossbred cattle were exposed to 25°C, 30°C, 35°C, and 40°C temperature with a relative humidity of 40% to 50% in a climatic chamber from 10:00 hours to 15:00 hours every day for 27 days. The physiological responses were recorded at 15:00 hours every day. The blood samples were collected at 15:00 hours on 1st, 6th, 11th, 16th, and 21st days and serum was collected for biochemical analysis. After 21 days, fecal and feed samples were collected continuously for six days for the estimation of digestibility. In the last 48 hours gas samples were collected continuously to estimate CH_4 emission. Heat stress in experimental animals at 35°C and 40°C was evident from an alteration ($p<0.05$) in rectal temperature, respiratory rate, pulse rate, water intake and serum thyroxin levels. The serum lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase activity and protein, urea, creatinine and triglyceride concentration changed ($p<0.05$), and body weight of the animals decreased ($p<0.05$) after temperature exposure at 40°C. The dry matter intake (DMI) was lower ($p<0.05$) at 40°C exposure. The dry matter and neutral detergent fibre digestibilities were higher ($p<0.05$) at 35°C compared to 25°C and 30°C exposure whereas, organic matter (OM) and acid detergent fibre digestibilities were higher ($p<0.05$) at 35°C than 40°C thermal exposure. The CH_4 emission/kg DMI and organic matter intake (OMI) declined ($p<0.05$) with increase in exposure temperature and reached its lowest levels at 40°C. It can be concluded from the present study that the digestibility and CH_4 emission were affected by intensity of heat stress. Further studies are necessary with respect to ruminal microbial changes to justify the variation in the digestibility and CH_4 emission during differential heat stress. (**Key Words:** Thermal Stress, Nutrient Utilization, Gas Emission, Adaptability, Cattle)

INTRODUCTION

Climate change projections suggest that globally environmental temperature is expected to increase between 2.3°C and 4.8°C by 2100 (IPCC, 2007). Global warming is estimated to reduce animal productivity by 25% in tropical and subtropical countries which account for more than half of the milk and meat production (Seguin, 2008).

Crossbreeding between the zebu and temperate breeds has been extensively practiced to enhance the milk productivity in tropical and subtropical countries (Thornton, 2010). Statistical data suggest that in spite of the higher performance of crossbred cattle the comparative health and production losses of these animals are more as compared to the zebu cattle due to increase in environmental temperature (Banerjee and Ashutosh, 2011).

Methane production from enteric fermentation is a function of the rate of organic matter (OM) fermentation, the types of volatile fatty acids produced, the efficiency of microbial biosynthesis (Monteny et al., 2001) and types of bacterial population (Kittelmann et al., 2014). Nkrumah et al. (2006) correlated the CH_4 production with dry matter intake (DMI) whereas the forage portion of the diet (Benchaar et al., 2001) and many other factors also have been used to predict the CH_4 production. Daily CH_4 emissions increased significantly with the activity of the cows but at the same time, it was negatively correlated to

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the indoor air temperature (Ngwabie et al., 2011). Reduced gut motility, rumination, ruminal contractions and passage rate of digesta during high ambient temperature are major factors which influences CH_4 by ruminants. CH_4 emission from sheep is positively correlated with mean retention time (MRT) of digesta, which is known to be influenced by the hormone triiodothyronine (Barnett et al., 2012; 2015).

The studies related to effect of different temperature exposures on digestibility had been conducted in temperate breeds simulating heat stress under temperate climate conditions (Nonaka et al., 2008; Yadav et al., 2013) but lacking in crossbreds in subtropical and tropical climatic conditions. Also, the study on the effect of heat stress on CH_4 emission in cattle is still less exploited. Therefore, in view of the global warming and the economical importance of the crossbred population in tropical and subtropical countries the present work has been designed to assess the effect of simulated heat stress on digestibility, CH_4 emission and metabolic adaptability in crossbred cattle.

MATERIALS AND METHODS

Animals, housing and management

The present experiment was conducted at the climatic chamber of Physiology and Climatology Division, Indian Veterinary Research Institute (IVRI), Izatnagar, India. It is located at 170 m above sea level (28°22′N and 79°24′E) in the northern upper Gangetic plain, having average annual rainfall of 90 to 120 cm. The meteorological variables during the experimental period are presented in Table 1. The experiment was conducted on four non-lactating Vrindavani crossbred cattle (Age, 3.78±0.22 years and body weight, 386.75±14.75 kg). Vrindavani cattle are recently developed synthetic crossbred cattle strain of India. It has the exotic inheritance of Holstein-Friesian, Brown Swiss, Jersey and indigenous inheritance of Hariana cattle. The animals were selected randomly from the herd of the Dairy Farm of IVRI, Izatnagar, India. All the experimental animals were vaccinated against foot and mouth disease and haemorrhagic septicaemia diseases. Deworming was done before the beginning of the experiment and subsequently at regular intervals. The animals were housed in well-ventilated shed under uniform managemental conditions having facilities for individual feeding and watering. The animals were offered basal diet of wheat straw *ad libitum* along with required amount of concentrate mixture to meet the maintenance requirement. The concentrate mixture consisted of 60% ground maize, 17% soybean meal, 20% wheat bran, 2% mineral mixture, and 1% common salt. Twenty gram of Vitablend AD_3 (Vitamin A, 50,000 IU/g and Vitamin D_3, 5,000 IU/g) was added in 100 kg concentrate mixture. The chemical composition of the wheat straw and concentrate mixture is presented in Table 2.

Table 2. Chemical composition (% dry matter basis) of the feeds offered to experimental animals

Attributes	Concentrate mixture	Wheat straw
Organic matter	90.01	91.68
Crude protein	21.97	3.27
Ether extract	2.23	1.09
Neutral detergent fiber	35.54	85.81
Acid detergent fiber	17.03	17.03
Hemicellulose	16.51	21.94
Total carbohydrates	55.77	87.33
Calcium	1.11	0.78
Phosphorus	1.06	0.23

All experimental procedures were 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.

The study was conducted in four phases. In the four phases of the study, the animals were exposed to 25°C, 30°C, 35°C, and 40°C temperature respectively, with a relative humidity (RH) of 40% to 50% in a climatic chamber for 5 h/d from 10:00 to 15:00 hour for 27 days. The time duration between two phases was at least 10 days in order to adapt the animal to prevailing environmental conditions. The animals were also acclimatized for a period of 15 days inside the chamber prior to the beginning of the experiment. The animals were kept in the shed attached to the climatic chamber before and after the thermal exposure every day. The physiological responses were recorded at 15:00 hours every day. The blood samples were collected at 15:00 hours on 1st, 6th, 11th, 16th, and 21st days and serum was collected for biochemical analysis. The body weight of the animals was recorded before and after completion of thermal exposure at each temperature. After 21 days, the fecal samples along with feed samples and residue lefts

Table 1. Climatic chamber and shed temperature, average relative humidity and temperature humidity index (THI)

Exposure temperature (°C)	Chamber THI	Shed temperature (°C)		Shed relative humidity %		Shed THI
		Minimum	Maximum	Minimum	Maximum	
25	71.25	9.24	19.89	55.42	80.16	56.90
30	77.53	14.28	25.87	58.57	81.97	62.83
35	83.81	19.08	31.06	53.29	84.35	68.92
40	90.09	24.98	34.21	59.27	81.43	74.57

were collected continuously for six days for estimating nutrients digestibility. In the last 48 hours gas samples were collected continuously and representative samples were taken four times at 12 hours interval to estimate CH_4 emission.

In the climatic chamber the environment can be varied as desired over a wide range and maintained constant within narrow limits (±0.5) of the desired temperature (°C) and RH (%). The climatic chamber has a dimension of 7.5 m×7.5 m×3.5 m with facilities of individual tie stall, feeders and waterers for eight cattle. It is airtight and made up of insulated material. For the air passage, it has one inlet and outlet valve each. The air is sucked out of the chamber with the help of an exhaustion pump. The outlet valve is fitted with flow meter to measure the volume of air going out of the chamber. The outgoing air is sampled with a special device attached to the outlet valve. The electric blowers are used to maintain the required temperature whereas the humidity is maintained with the help of boilers. The temperature, humidity and pressure of the chamber are continuously monitored.

The minimum and maximum temperature, and RH of the shed were recorded and temperature-humidity index (THI) was calculated according to formula given by Ravagnolo et al. (2000).

$$THI = (1.8T + 32) - [(0.0055RH)(1.8T - 26)]$$

Where, T = temperature (°C), RH = relative humidity.

Physiological observations

The rectal temperature (RT) was recorded by a clinical thermometer inserted about 5 cm deep into the rectum of animals so that it remained in contact with the mucous membrane for at least 1 to 2 minute. The observations were recorded in degrees celsius (°C). The respiration rate (RR) was recorded by observing the flank movement for one minute in which each inward and outward movement of the flank was counted as one complete respiration. The RR was expressed as breaths per minute. The pulse rate (PR) of the animals was counted by observing the pulsation of the middle coccygeal artery at the base of the tail and expressed as beats per minute.

Biochemical assays

The blood samples were collected from jugular vein by vein puncture with least stress to the animals, in sterile vials. Serum was collected after keeping the vial at slant for an hour and then centrifuging it at 2,000 rpm at 4°C for 10 minutes. Serum samples were stored at –80°C for further biochemical analysis.

Lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AKP) activity, and protein, urea, creatinine and triglyceride level in serum were estimated using commercially available kit (Cogent, clinical chemistry division of SPAN diagnostic Ltd., Mumbai, India).

Serum tri-iodothyronine (T_3) and thyroxin (T_4) concentrations were estimated by 125I-RIA kit supplied by Immunotech, Czech Republic using SR 300 Stratec gamma counter (Stratec Biomed systems AG, Brikenfeld, Germany). The coefficient of variation for intra and inter assays and sensitivity for T_3 and T_4 was 9.2% and 1.02 nM/L; 7.7% and 0.10 nM/L; 8.6% and 9.56 nM/L respectively.

Digestibility assays

A digestibility trial of six days duration was conducted at the end of every phase of the experiment. The animals were placed in climatic chamber with facilities for separate collection of feces. During the digestibility trial the feeding schedule of the animals remained same and they were fed their respective diet at 9:00 hour daily. During the digestibility trial weighed quantity of wheat straw and concentrate mixture was offered at about 9:00 hour. Water was offered *ad libitum* twice daily at 9:30 hour and 15:30 hour. Well-mixed representative samples of wheat straw and concentrate mixture and their left over residue were taken daily. The dried samples obtained during trial period were pooled item wise ground and stored in labeled airtight containers for further analyses of proximate principles and fibre fractions.

The feces voided in 24 hours by the individual animal was collected quantitatively at a fixed time (9:30 hour) daily, weighed and carried in polythene bags for further sampling in laboratory. A representative sample from each animal was taken separately in a labeled polythene bags. A suitable fecal aliquot (1/100 of fresh feces) was kept for drying at 100°C±1°C to a constant weight in a hot air oven for dry matter estimation. The dried samples obtained daily were pooled animal wise, ground to pass through 1 mm sieve and used for proximate analysis. A suitable fecal aliquot (1/200 of fresh feces) was mixed with 10 mL of 1:4 sulphuric acid and preserved for nitrogen (N) estimation in air-tight bottle.

In the last 48 hours of each phase of the experiment, after feeding, watering and collection of feed residues and feces, the doors of the climatic chamber were closed air tight. The chamber was maintained at respective experimental temperature with RH of about 40% to 50%. The flow rate, temperature of dry and wet bulb thermometer and atmospheric pressure were also recorded at hourly interval. A flow meter was also used to record the flow rate as well as the total volume of air coming out of climatic chamber. The incoming and outgoing air from climatic

chamber were collected continuously in Douglas bags by special sampling device with the help of two sampling air pumps and representative samples were analyzed four times (at 12, 24, 36, and 48th hour) in duplicate to estimate gas (methane, oxygen, and carbondioxide) composition.

Samples of the feed offered, residue lefts and feces voided were dried at 100°C in an oven for 24 hours and ground to pass through a 1-mm screen. Grounded samples were analyzed for DM (Method 930.15; AOAC, 1995), ash (Method 942.05; AOAC, 1995), crude protein (Method 984.13; AOAC, 1995), ether extract (Method 920.39; AOAC, 1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated as per procedure described by Van Soest et al. (1991). Calcium and phosphorous in the feed samples were estimated using commercially available kits. Methane in gas sample was analyzed by infra-red gas analyzer (Analytical Development Co. Ltd. Hoddesdon, England, model 300). The flow meter and infra-red analyzer were calibrated and CH_4 recovery (%) was calculated at standard temperature and pressure before beginning of the actual experiment. The CH_4 in the expired air was calculated by using following formula:

$$\text{Methane (L/d)} = V_{STP} \times X/1000000$$

Where,
V_{STP} = V(273/273+T) (P-Vp/760)
V = total volume of air
T = dry bulb temperature
P = borometric pressure in mm Hg
Vp = partial pressure of water vapor
X = methane in ppm

Statistical procedures

Data of physiological and biochemical parameters, DMI, digestibility and CH_4 emission were analyzed using repeated measures of one way analysis of variance (SAS, 9.4). Differences among treatments were determined using Tukey's test (SAS, 2014) and indicated by the superscripts ($p<0.05$). Least squares means and standard errors were reported. The body weight before and after the each phase of the experiment was analyzed using paired *t*-test. The level of significance was set at $p<0.05$.

RESULTS AND DISUSSON

Physiological responses

The means of RT, RR, and PR after thermal exposure at 25°C, 30°C, 35°C, and 40°C presented in Table 3 indicated that animals were in stress at 35°C exposure however intensity of stress was quit higher at 40°C temperature exposure. The increase in RT, RR, and PR after exposure at 35°C and 40°Cindicated that the animals were in stress at these temperature exposures and, 25°C and 30°Cexposure did not produce any stress in the animals. Similar findings have been also reported by many workers in crossbred cattle (Yadav et al., 2015). However, increase in physiological parameters in temperate cattle was exhibited at an ambient temperature between 27°C to 30°C (Wheelock et al., 2010).

In present experiment, a marked and consistent alteration in different serum enzyme activities and serum bio-chemicals was observed at 40°C while only AST activity altered at 35°C exposure. It indicated that at 35°C exposure the heat stress mediated changes in the crossbred animals began to exhibit and became prominent at 40°C exposure. However, in present study the RH was lower which did not attribute any stress effect and therefore increment in environmental temperature caused least stress to the animals. The increase in LDH, ALT, AST (Srikandakumar et al., 2003) and decrease in AKP activity (Marai et al., 2009) was reported under heat stress condition in different ruminant species which corroborated our findings. In our study, the body weight of animals decreased at 40°C (Table 5), which showed catabolic activity in the muscle as protein degradation and protein transport processes were enhanced during heat stress and this might have increased the LDH activity. The reduction of serum AKP activity during thermal stress at 40°C could be related to the endocrine acclimation response of cattle to the hot environment and to the related variations in energy metabolism, both in liver and gut activity reduction, as suggested by Ronchi et al. (1999).

Serum biochemical parameters and hormones

The LDH, AST, ALT, AP, and serum protein, urea, creatinine and triglyceride concentration is presented in Table 4. Invariably, significant changes ($p<0.05$) in the

Table 3. Effect of different temperature exposures on physiological parameters

Exposure temperature (°C)	25	30	35	40	SE	p value
RT	38.18[c]	38.16[c]	38.38[b]	39.14[a]	0.04	<0.0001
RR	21.64[c]	23.81[c]	35.04[b]	75.94[a]	0.99	<0.0001
PR	52.29[b]	53.04[b]	60.02[a]	61.64[a]	0.58	<0.0001

SE, standard error; RT, rectal temperature; RR, respiratory rate; PR, pulse rate.
Number of animals = 4, number of observation = 21×4 = 84.
Means bearing different superscripts in a row differ significantly ($p<0.05$).
The SE uses pooled estimate of error variance.

Table 4. Effect of different temperature exposures on serum enzymes and metabolites

Parameters	Treatment temperature (°C)				SE	p value		
	25	30	35	40		Temp	Day	Temp×day
LDH activity (IU/L)	220.97^{c}	269.94^{b}	293.51^{b}	515.22^{a}	11.52	<0.0001	0.07	0.56
ALT activity (IU/L)	34.04^{c}	35.70^{c}	35.39^{c}	79.27^{a}	1.63	<0.0001	0.17	0.004
AST activity (IU/L)	55.03^{d}	53.99^{d}	73.52^{c}	119.82^{a}	2.24	<0.0001	0.56	0.03
AKP activity (KA units)	13.99^{abc}	15.90^{ab}	15.17^{abc}	10.63^{c}	4.76	0.02	0.99	0.99
Protein (g/100 mL)	6.34^{b}	6.12^{b}	6.79^{a}	7.05^{a}	0.12	<0.0001	0.002	0.0004
Urea (mg/100 mL)	21.81^{b}	22.07^{b}	21.09^{b}	30.11^{a}	0.68	<0.0001	0.83	0.01
Creatinine (mg/100 mL)	1.92^{c}	1.95^{c}	2.37^{b}	2.50^{a}	0.04	<0.0001	0.73	0.022
Triglyceride (mg/100 mL)	88.93^{b}	95.26^{ab}	96.46^{ab}	102.21^{a}	2.03	<0.0001	0.008	<0.0001
Triiodothyronine (nM/mL)	1.64	1.42	1.48	1.76	0.34	0.056	0.37	0.65
Thyroxine (nM/mL)	69.38^{b}	61.17^{b}	55.63^{a}	56.94^{a}	3.29	<0.0001	0.08	0.04

SE, standard error; LDH, lactate dehydrogenase; ALT, alanine amino transferase; AST, aspartate amino transferase; AKP, alkaline phosphatase.
Number of animals = 4 Number of observation = 20.
Means bearing different superscripts in a row differ significantly ($p<0.05$).
The SE uses pooled estimate of error variance.

activity of the investigated serum enzymes and biochemicals was observed at 40°C exposure while only AST activity altered significantly ($p<0.05$) at 35°C exposure. The serum T_3 did not show significant ($p>0.05$) increase on different temperature exposures whereas T_4 decreased significantly ($p<0.05$) after 35°C and 40°C exposure. Serum protein and urea are the markers of nitrogen metabolism in all mammals whereas serum creatinine may be used to investigate the changes in muscle metabolism. Body weight of the animals decreased significantly ($p<0.05$) after thermal exposure at 40°C whereas there was no significant difference in weight of the animals after other three thermal exposures (Table 5). In present study, an increase in total protein concentration indicated a change in protein metabolism shifting towards catabolic side, which was evident with decrease in body weight of the animals, perhaps the need of energy for maintaining homeothermy was met by increase in tissue protein catabolism which resulted in increased serum protein, urea and creatinine concentration. The serum triglyceride levels were higher after exposure at 40°C as compared to 25°C which may be attributed to lipolysis to meet the excess requirement of energy for thermoregulation during high temperature exposure.

Table 5. Effect of different temperature exposures on body weight in crossbred cattle

Temperature exposure (°C)	Mean weight		SE	p value
	Before	After		
25	365.50	366.00	20.32	0.231
30	362.00	363.00	10.78	0.343
35	349.25	351.75	14.25	0.362
40	375.75^{a}	365.50^{b}	10.15	0.048

SE, standard error.
Number of animals = 4, number of observation = 8.
Means bearing different superscripts in a row differ significantly ($p<0.05$).

Heat stress in general is associated with significant depression in thyroid gland activity resulting in lowering of thyroid hormones level (Sejian et al., 2014). When the animals start suffering due to heat, food ingestion is reduced and metabolism slows down, causing a hypo-function of the thyroid gland (McManus et al., 2009) which leads to decrease in T_4 level. In our study, the serum T_4 level decreased which lead to reduction in the metabolic heat production, in an attempt to optimize the process of thermoregulation.

The means of water intake, DMI, nutrient digestibility and CH_4 emission at 25°C, 30°C, 35°C, and 40°C exposure during digestion trial are presented in Table 6. Water intake increased significantly ($p<0.05$) at exposure of 35°C and 40°C whereas the DMI declined at 40°C exposure only. The DM digestibility was significantly ($p<0.05$) higher at 35°C than at 25°C and 30°C exposure. However, OM and NDF digestibility were significantly ($p<0.05$) higher at 35°C than 40°C and 25°C exposure than 25°C and 30°C. The ADF digestibility was significantly ($p<0.05$) lower at 40°C thermal exposure than 35°C. Protein and ether extract digestibility were statistically similar at all the temperature exposures.

Increasing environmental temperature and rising RT above critical thresholds were related to decrease in DMI (West, 2003). Increase in heat load, reduced nutrient uptake in almost all species and in case of cattle, the nutrient uptake decreased up to about 30% of DMI (Wheelock et al., 2010). At 40°C, dietary intake might decline by as much as 40% (National Research Council, 1989). Similar findings have been also reported in Holstein heifers (Nonaka et al., 2008). In present study, the decline in DMI was only evident at 40°C exposure and further the decrease in DMI was owing to a decrease in roughage intake as the amount of concentrate mixture was fixed and the offered concentrate was completely consumed by the animals. It is

Table 6. Dry Matter Intake, nutrient digestibility and methane emission at different temperature exposures in crossbred cattle

Parameter	Treatment temperature (°C)				SE	p value
	25	30	35	40		
Dry matter intake						
Daily	5.94 ^{ab}	6.70 ^{b}	6.43 ^{b}	5.26 ^{a}	0.37	<0.0001
% body weight	1.62 ^{ab}	1.85 ^{b}	1.85 ^{b}	1.41 ^{a}	0.06	<0.0001
g/kg $W^{0.75}$	71.0 ^{ab}	80.8 ^{b}	79.7 ^{b}	62.0 ^{a}	2.4	<0.0001
Water intake liter (L)	16.48 ^{c}	16.65 ^{c}	20.96^{b}	25.01^{a}	0.61	<0.0001
Digestibility (%)						
Dry matter	59.68 ^{b}	60.62 ^{b}	66.34^{a}	62.53 ^{ab}	1.55	0.047
Organic matter	60.32^{b}	63.00^{ab}	67.65 ^{a}	62.73 ^{b}	1.57	0.027
Neutral detergent fiber	58.03 ^{b}	59.01^{b}	67.77 ^{a}	65.59 ^{a}	2.00	0.001
Acid detergent fiber	54.49 ^{ab}	58.42 ^{ab}	61.49 ^{a}	47.24 ^{b}	4.08	0.025
Crude protein	45.66	46.32	53.99	49.48	6.55	0.584
Ether extract	67.48	61.54	63.05	67.60	6.33	0.706
Methane emission						
L/d	249.09^{ab}	254.35^{a}	208.75 ^{c}	231.20 ^{b}	4.49	<0.0001
L/kg DMI	41.93^{a}	37.91^{b}	32.47^{c}	40.92^{ab}	0.72	<0.0001
L/kg OMI	49.82 ^{a}	42.25 ^{b}	37.14 ^{c}	51.05 ^{a}	0.85	<0.0001
CO_2emission (L/d)	3,607.25	3,468.50	3,456.75	3,739.50	100.21	0.207
O_2utilization (L/d)	4,073.10	4,020.61	4,023.48	4,691.83	170.94	0.044
Respiratory quotient	0.89 ^{a}	0.86 ^{ab}	0.86 ^{ab}	0.80 ^{b}	0.016	0.014

SE, standard error; OMI, organic matter intake.
Means bearing different superscripts in a row differ significantly ($p<0.05$).
The SE uses pooled estimate of error variance.

also revealed that the ratio of the intake of concentrate to roughage increased at 40°C exposure which consequently might affect the digestibility and CH_4 production.

In present study, the increase in digestibility at 35°C exposure may be attributed to increase in MRT of the digesta however MRT was not estimated in this study. Barnett et al. (2012; 2015) reported a positive correlation of ambient temperature and a negative correlation of triiodothyronine (T_3) with MRT of digesta. In present study, although the T_3 level did not alter but the decline in thyroxin levels at 35°C and 40°C exposure suggested that the MRT of digesta was comparatively higher at theses temperatures. The available literature also suggested that with increase in ambient temperature, MRT of digesta could increase which ultimately culminated in increased digestibility (Bernabucci et al., 1999; Nonaka et al., 2008). The similar DM and NDF digestibility at 40°C exposure as compared to 35°C could be explained based on the increase in MRT of the digesta however decrease in OM and ADF digestibility at 40°C could not be explained. Although in present study the alteration in the rumen environment has not been studied but based on established research, the decrease in OM and ADF digestibility at 40°C exposure could be attributed to change in rumen pH (Nonaka et al., 2008), temperature (Beatty et al., 2008) and microbial population (Kittelmann et al., 2014). Bernabucci et al. (1999) reported an improvement of diet digestibility during a short time exposure of Holstein heifers to hot conditions, and found that when the exposure was prolonged, the diet digestibility was reduced. Digestibility coefficients of DM, OM, NDF, and ADF in sheep were not affected by short exposure (10 days) but were lower ($p<0.01$) after prolonged exposure to heat (Bernabucci et al., 2009).Protein digestibility coefficients were similar at different temperatures in our study. Similar findings were also reported by Bernabucci et al. (2009).

The CH_4 emission per kg DMI and per kg organic matter intake (OMI) decreased significantly ($p<0.05$) at 30°C and 35°C exposure however it increased at 40°C exposures. Respiration quotient was significantly ($p<0.05$) lower at 40°C exposure as compared to 25°C, 30°C, and 35°C (Table 6). In present study CH_4 emission decreased progressively with increase in temperature up to 35°C and then increased to initial levels. Barnett et al. (2015) reported a positive correlation of ambient temperature and a negative correlation of triiodothyronine (T3) with CH_4 yield. In present experiment T3 level did not alter with increase in temperature hence, no correlation was established between T3 level and CH_4 emission in crossbred cattle. In our study the thyroxin level decreased with increase in exposure temperature and similarly CH_4 emission decreased above the thermoneutral temperature up to 35°C exposure. Ngwabie et al. (2011) also reported a negative correlation ($r = –0.84$) with the indoor air temperature and CH_4 emissions. The total DMI (Nkrumah et al., 2006) and the forage portion of the diet are reported to influence CH_4

emission (Benchaar et al., 2001). In spite of the lower DMI, and higher dietary concentrate to roughage ratio at 40°C exposure higher CH_4 production could not be justified. The highest CH_4 emission per kg DMI at 40°C exposure may be attributed to a change in rumen microbial population as suggested by Kittelmann et al. (2014). It may be speculated that a change in microbial population and fermentation pattern could result in higher CH_4 emission at 40°C exposure however the rumen fermentation pattern was not examined in the present experiment.

It can be concluded from the present study that 35°C temperature exposure began to induce heat stress and at 40°C exposure the heat stress was prominent in crossbred cattle as exhibited by physio-biochemical, enzymatic and hormonal changes and, alteration in water intake, DMI, and body weight. The DM, OM, ADF, and NDF digestibility increased at 35°C and the OM and ADF digestibility decreased at 40°C exposure as compared to 35°C. The CH_4 emission decreased at 30°C and 35°C exposure but increased at 40°C. It can also be concluded that heat stress influenced the digestibility and CH_4 emission; however to explore the alteration in digestibility and CH_4 emission at different temperature exposures the changes in microbial population need to thoroughly investigated.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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