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Effect of supplementation of niacin on physiological and blood biochemical parameters in crossbred cows during heat stress

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ABSTRACT

In order to investigate the effect of different levels of niacin supplementation on physiological and blood biochemical parameters during heat stress period (April to August; 120 days), eighteen crossbred early lactating cows (2nd to 4th lactation; 11.56±1.74 days in milk) were divided into three groups of six animals each. The basal ration was fed same to all cows (Green fodder, straw and concentrate was fed as per NRC, 2001), except the addition of niacin @ 600 and 800 mg/kg dry matter intake (DMI) in T1 and T2 groups, respectively. The temperature humidity index (THI) was calculated at 07:30 am and 02:30 pm daily. Fortnightly physiological parameters were recorded and blood was collected from the jugular vein at day 0 and subsequently at 15–day interval from all the experimental animals to study blood biochemical parameters. The results revealed that animals were in either moderate or severe stress at morning, whereas during afternoon the animals were in very severe stress during the entire trial. Supplementation of niacin @ 800 ppm niacin decreased significantly skin temperature, respiration rate, cortisol, super oxide dismutase (SOD) and catalase ($P<0.05$). Plasma NEFA was significantly low in both niacin supplemented groups ($P<0.05$). Plasma urea was found significantly ($P<0.05$) high in T2 (26.59) in comparison to control (24.90) and T1 (25.37). It is concluded that 800 ppm niacin supplementation to lactating crossbred cows resulted in better stress alleviation as indicated by the improved biomarker values *viz.,* SOD, catalase, cortisol and skin temperature.

Key words: Niacin, Crossbred Cows, Heat stress, Physiological and Blood Biochemical Parameters)

Heat stress is a serious problem in dairy farming which results in lower milk yield, disturbances in reproduction, health problems and economic loss (Wrinkle *et al.* 2012). Dairy cows suffering from heat stress during the summer exhibit decreased feed intake and activity, as well as increased respiratory rates, peripheral blood flow and sweating (West, 2003). It can decrease dry matter intake and milk yield of dairy cows by 6 to 30% and 15 to 20%, respectively (St. Pierre *et al.* 2003). Also, the estimated annual milk loss due to heat stress among cattle and buffaloes at all India level is 1.8 million tonnes, which is nearly 2% of the total milk production in the country (Upadhyay *et al.* 2007).

Certain vitamins (A, C, E, B_3) and minerals (Cr, Se, K) can play an important role in reducing heat stress. Out of this, nicotinic acid (NA), a B vitamin, is an important vitamin that elicits vasodilator reactions which is beneficial for cows under heat stress. Peripheral and internal vasodilatation, caused by therapeutic concentration of NA may enhance heat transfer from core to skin sites and generate the temperature gradient favoring heat loss from skin to environment (Khan *et al.* 2012). Several workers have reported a significant decrease in skin and vaginal temperatures during periods of mild or severe heat stress in niacin supplemented group in comparison to control (Di Costanzo *et al.* 1997; Zimbelman *et al.* 2008; Zimbelman *et al.* 2010; Zimbelman *et al.* 2013).

Another possible mechanism of niacin to counteract heat stress is at cellular level by stabilizing cellular protein structure by increasing Heat shock protein (Hsp) production. These proteins protect cells against heat stress by refolding proteins in the cytoplasm which have been denatured by high temperatures. It has been observed that expression of Hsp 70 is increased up to 20 fold when subjected to chronic thermal stress (Collier *et al.* 2006).

Earlier it was assumed that niacin is synthesized in adequate amounts in the rumen to meet the needs of the dairy cow (Khan *et al.* 2012). However, due to improvement in the genetic potential of dairy cows in recent years, the requirements tend to exceed the capacity of rumen microorganisms to synthesize sufficient amount of this

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vitamin (Weiss and Gonzalo 2006). Considering above facts, the study was undertaken to evaluate the effect of niacin supplementation on physiological and blood biochemical parameters in lactating crossbred cows under heat stress conditions.

MATERIALS AND METHODS

The present study was conducted in the cattle yard of National Dairy Research Institute (NDRI), Karnal, Haryana. The city Karnal is situated at an altitude of 250 meters above mean sea level, latitude and longitude position being 29°42" N and 79°54" E respectively. The maximum ambient temperature in summer goes up to 45ºC and minimum temperature in winter comes down to 0 ºC with a diurnal variation of 15–20 ºC.

Experimental design, animals and management

Eighteen healthy lactating crossbred cows (Karan Fries) in their early lactation (2^{nd} to 4^{th} lactation; 11.56±1.74 days in milk) were selected randomly from the herd of National Dairy Research Institute (NDRI), Karnal. The selected animals were divided into three groups of six animals each on the basis of milk production (17.30±1.80 kg) and body weight (414.33±8.35 kg). The experiment was conducted during 25^{th} April to 22^{nd} August. The design of the experiment was Randomized block design (RBD). There was separate manger for all cows. The cows were milked twice daily. When cows returned to their respective pens after the morning milking, head-locks were set for breeding. The animals were provided with fresh and clean drinking water free choice thrice daily at 06:00 am, 11:30 am and 06:30 pm.

Diet

All the cows were fed same basal ration consisted of wheat straw, chaffed green maize fodder and compounded concentrate mixture as per NRC (2001). But the ration of T1 and T2 groups were supplemented with niacin @ 600 and 800 ppm/animal/day, respectively. The quantity of niacin to be supplemented was mixed in a small quantity of concentrate mixture to ensure the full consumption. The total concentrate mixture offered to each animal was divided into three portions and fed at 04:30 am, 09:30 am and 04:30 pm, whereas green fodder was fed twice daily at 11:00 am and 06:00 pm. The dry fodder was given *ad lib*. The chemical composition of green fodder, straw and concentrate mixture used in the present study is presented in Table 1.

Recording of climatic variables and temperature humidity index

Microclimatic data viz., dry bulb temperature and wet bulb temperature and minimum and maximum temperature and relative humidity was recorded at 07:30 am and 02:30 pm using Zeal (UK) thermometer every day during experimental period. Temperature humidity index (THI) was calculated by using the formula of Johnson *et al.* 1963.

$$THI = 0.72 (Tdb + Twb) + 40.6$$

Where, Tdb = dry bulb temperature (ºC)
Twb = wet bulb temperature (ºC)

Blood sampling and observation

Blood samples (10 ml) were collected in sterile heparinised vacutainer (BD Vacutainer™, UK) tubes at fortnight intervals and plasma was separated by centrifugation at 3000 rpm for 30 minutes and stored at –20 °C in different aliquots for the analysis of various biochemical constituents. Different blood biochemical parameters viz. Superoxide Dismutase (SOD), Catalase (CAT), glucose and cortisol was estimated by using standard kits (Cayman Chemical Company, 1180 East Ellsworth Road Ann Arbour, MI 48108, USA). Plasma urea was also estimated by using kit (Span Diagnosis Ltd). Plasma Non-Esterified Fatty Acids (NEFA) was estimated by copper soap solvent extraction method modified by Shipe *et al.* 1980.

Physiological responses measurements

Respiration rate (RR**;** breaths per minute), pulse rate (PR; pulse rate per minute) of the animals was recorded by visual observation of abdomen and pulsation of middle coccygeal artery at the base of the tail, respectively. Rectal temperature (RT) was recorded by using digital thermometer by keeping the thermometer in contact of rectal mucosa for about two minutes. The peripheral skin temperature of the experimental animals at flank region were recorded using non-contact tele-thermometer (Raytek, Model Raynger ST2L, M/s. Surrey Scientific, Surrey, U.K.) by keeping it 2–3 inches away from the desired surface site.

Statistical Analysis

The data obtained was analyzed by using two way ANOVA (Snedecor and Cochran 1989). The test of significance among the different treatments was also analyzed (SPSS 1999).

Table 1. Chemical composition of maize fodder, wheat straw and concentrate mixture (on % dry matter basis)

Feed ingredients	DM	CP	OM	EE	ASH	NDF	ADF	Niacin content (ppm)
Maize fodder	15.42	8.45	90.33	1.95	9.67	51.20	28.44	273.92
Wheat straw	89.33	3.23	86.02	0.95	13.98	66.30	37.30	355.38
Concentrate mixture	90.56	21.22	92.82	4.10	7.18	32.19	16.33	538.16

RESULTS AND DISCUSSION

Environmental variables recorded during the experiment

The maximum and minimum temperature (ºC) at morning time ranged from 32.2 to 41.5 and 19.4 to 27.5 respectively throughout the experimental period (25th April - 22th August) being higher on 45 (represents the mean temperature of 24th May - 9th June) and 60th day (represents the mean temperature of 10th June – 24th June, Table 2). However, at afternoon the corresponding maximum and minimum temperature ranged from 32.6–42.2 and 20.4–28.9 respectively. The relative humidity in morning and afternoon session ranged between 52–90 and 19–75 respectively. The high temperature and low humidity represented the hot dry condition up to 25th June whereas afterwards there was hot humid condition (low temperature and high humidity). Temperature humidity index (THI) varied from 69.7–80.6 and 81.4–87.3 in morning and afternoon, respectively. The THI was increased in a linear fashion up to 75th day of experimental period afterwards it showed a descending trend. The THI values of morning depicted that animals were in moderate (25th April - 9th May) and severe stress (10th May onwards) whereas in afternoon they were in very severe heat stress condition throughout the experimental period of 120 days.

Plasma cortisol

The plasma cortisol (ng/ml) averaged higher at the beginning of trial (5.03, 4.95 and 5.09 in C, T1 and T2) and was lower at the end of trial (3.97, 3.35 and 3.15 in C, T1 and T2, respectively; Table 3), which may be due to comparatively lower THI at the end of trial. The highest value of plasma cortisol were observed at 60 days of experimental period (5.68, 5.15 and 4.68 for C, T1 and T2) with the corresponding highest THI (79.8, 87.3 for morning and afternoon) but the values were statistically similar. The mean plasma cortisol of entire experimental period was significantly higher (P<0.05) in C (4.83) than T2 (4.34), with intermediate value for T1 (4.59). In the present experiment reduced cortisol value in T1 and T2 groups indicated that these animals had comparatively less stress as compared to control. Marai and Habeeb(2010 also reported significant rise in plasma cortisol levels due to increase in ambient temperature from 17.5 ºC to 37.1 ºC. Similarly, Kadzere *et al.* (2002) stated that cortisol levels increased in chronically heat-stressed cows, if heat stress intensiûes.

Plasma Superoxide dismutase (SOD)

The mean plasma SOD (U/ml) in C, T1 and T2 was 3.28±0.05, 3.08±0.05 and 3.05±0.06, respectively (Table 3). The values were found to be significantly higher in control in comparison to T1 and T2 (P<0.05), but there was no difference between the T1 and T2 groups. Similar trend of plasma SOD values were observed during 5th fortnight. At 60 days of experimental period (when THI approached maximum) the plasma SOD averaged 3.39, 3.20 and 3.25, however its concentration reduced to 2.87, 2.60 and 2.50 at 120 days in control, T1 and T2 groups, respectively. Manish *et al*. (2011) reported significantly (P<0.05) higher erythrocyte SOD levels in heat stressed goats during the summer compared to pre-summer months. The lower SOD activity observed in T1 and T2 compared to control in this study indicated a reduced oxidative stress in animals supplemented with niacin which might be because of comparatively lower stress experienced by the treatment groups. Ganie (2012) also reported that supplementation of Vitamin C resulted in significantly lower SOD in buffaloes during summer season. Kumar *et al.* (2010 and 2011) also reported that there was decrease in SOD activity of buffaloes supplemented with vitamin C during thermal stress. To best of our knowledge, no literature was available regarding effect of niacin on superoxide dismutase.

Plasma catalase activity

The plasma catalase activity (mmol/min/ml) ranged from 39.94 to 60.58, 38.97 to 56.35 and 38.21 to 54.91 in C, T1 and T2 groups, respectively showing no significant difference between the periods (Table 3). The mean catalase activity during entire trial was found to be significantly higher (P<0.05) in control group (51.27) in comparison to

Table 2. Environmental parameters recorded during the experimental period (25th April-22nd August)

Days	Maximum temp! (°C)		Minimum temp! (°C)		Relative Humidity! (%)		THI!^	
	M*	A+	M*	A+	M*	A+	M*	A+
0#	35.4	37.8	19.6	20.4	69	26	69.7	81.4
15	36.5	38.7	19.4	20.8	57	20	70.2	81.8
30	39.7	39.9	23.2	25.2	52	19	74.1	84.7
45	41.5	42.2	25.1	27.4	56	25	76.7	86.5
60	41.2	41.9	27.0	28.8	63	35	79.8	87.3
75	37.4	37.7	27.5	28.9	74	55	80.6	87.0
90	34.6	34.8	26.8	28.6	84	60	80.4	85.6
105	32.3	32.8	26.2	28.5	90	75	79.3	83.0
120	32.3	32.6	25.9	28.8	90	73	78.8	83.4

*M= observation recorded at 7:30 am, +A= observation recorded at 2:30 pm

^THI= Temperature humidity index, !represents the mean of 15 days observation

0#represents the mean of the adaptation period

Table 3. Effect of niacin supplementation on levels of plasma cortisol, SOD and catalase in lactating crossbred cows

Fortnights	Cortisol (ng/ml)			SOD (U/ml)			Catalase (nmol/min/ml)		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0*	$5.03^{ab}\pm$ 0.58	$4.95^{ab}\pm$ 0.56	$5.09^{d}\pm$ 0.39	$3.65^{d}\pm$ 0.17	$3.51^{d}\pm$ 0.20	$3.39^{c}\pm$ 0.15	$60.58^{e}\pm$ 2.80	$56.35^{f}\pm$ 2.00	$54.91^{e}\pm$ 1.84
1	$4.21^{ab}\pm$ 0.66	$4.64^{ab}\pm$ 0.44	$4.21^{bcd}\pm$ 0.26	$3.46^{cd}\pm$ 0.18	$3.28^{cd}\pm$ 0.14	$3.27^{c}\pm$ 0.11	$56.35^{de}\pm$ 2.48	$51.78^{de}\pm$ 1.55	$51.98^{de}\pm$ 1.95
2	$4.27^{ab}\pm$ 0.38	$4.07^{ab}\pm$ 0.64	$4.00^{abc}\pm$ 0.26	$3.39^{cd}\pm$ 0.14	$3.21^{bcd}\pm$ 0.16	$3.25^{c}\pm$ 0.12	$51.88^{cd}\pm$ 2.45	$48.46^{cd}\pm$ 1.05	$49.06^{cd}\pm$ 2.03
3	$5.62^{b}\pm$ 0.48	$5.47^{b}\pm$ 0.47	$4.91^{cd}\pm$ 0.46	$3.29^{bcd}\pm$ 0.13	$3.13^{bcd}\pm$ 0.14	$3.34^{c}\pm$ 0.10	$55.26^{de}\pm$ 2.20	$52.30^{def}\pm$ 0.91	$52.28^{de}\pm$ 1.83
4	$5.68^{b}\pm$ 0.65	$5.15^{b}\pm$ 0.84	$4.68^{cd}\pm$ 0.27	$3.39^{cd}\pm$ 0.05	$3.20^{bcd}\pm$ 0.12	$3.25^{c}\pm$ 0.04	$57.05^{de}\pm$ 2.33	$54.96^{ef}\pm$ 0.74	$54.31^{de}\pm$ 1.61
5	$5.30^{ab}\pm$ 0.37	$4.77^{ab}\pm$ 0.45	$4.86^{cd}\pm$ 0.37	$3.33^{bcdB}\pm$ 0.06	$3.03^{bcA}\pm$ 0.10	$3.02^{bcA}\pm$ 0.05	$50.52^{cd}\pm$ 2.91	$49.03^{cd}\pm$ 1.50	$50.14^{cde}\pm$ 1.51
6	$4.96^{ab}\pm$ 0.34	$4.93^{ab}\pm$ 0.44	$4.55^{bcd}\pm$ 0.32	$3.11^{abc}\pm$ 0.09	$2.93^{abc}\pm$ 0.10	$2.82^{ab}\pm$ 0.13	$46.97^{bc}\pm$ 2.10	$46.30^{bc}\pm$ 1.54	$45.90^{bc}\pm$ 1.71
7	$4.43^{abA}\pm$ 0.28	$3.96^{abAB}\pm$ 0.25	$3.62^{abB}\pm$ 0.16	$3.00^{ab}\pm$ 0.05	$2.82^{ab}\pm$ 0.09	$2.64^{ab}\pm$ 0.20	$42.83^{ab}\pm$ 1.55	$42.59^{ab}\pm$ 1.72	$43.51^{b}\pm$ 1.58
8	$3.97^{a}\pm$ 0.34	$3.35^{a}\pm$ 0.41	$3.15^{a}\pm$ 0.09	$2.87^{a}\pm$ 0.08	$2.60^{a}\pm$ 0.08	$2.50^{a}\pm$ 0.21	$39.94^{a}\pm$ 1.86	$38.97^{a}\pm$ 1.03	$38.21^{a}\pm$ 1.37
Overall mean±SEM	$4.83^{x}\pm$ 0.17	$4.59^{xy}\pm$ 0.18	$4.34^{y}\pm$ 0.13	$3.28^{y}\pm$ 0.05	$3.08^{x}\pm$ 0.05	$3.05^{x}\pm$ 0.06	$51.27^{y}\pm$ 1.15	$48.97^{x}\pm$ 0.85	$48.93^{x}\pm$ 0.89

Observations with different superscripts (x and y) differ significantly (P<0.05) between the groups; Observations with different superscripts (a, b, c, d and e) differ significantly (P<0.05) within the group; Observations with different superscripts (A and B) differ significantly (P<0.05) within the period of groups

0* denotes values recorded on last day of adaptation period

T1 (48.97) and T2 (48.93). Moreover, the catalase activity in the control group remained elevated throughout the experimental period in comparison to T1 and T2. Since SOD activity increases H_2O_2 production, protection from reactive oxygen species would only be conferred by a coordinated increase of catalase and glutathione peroxidase activities (Sharma *et al.* 2011). In support of this conjecture, catalase activity was found to be increased in control cows whereas lower catalase activity in treatment as compared to control group indicated less oxidative stress in treatment cows as compared to control cows. Kumar *et al.* (2010 and 2011) also reported significantly lower catalase activity in buffaloes supplemented with vitamin C compared to non-supplemented group subjected to thermal stress. Ganie (2012) too gave the similar description. No previous work has been reported yet regarding the effect of niacin on plasma catalase activity.

Total plasma glucose

The plasma glucose level in lactating cows was analyzed fortnightly and the results are presented in Table 4. The mean levels of glucose in blood plasma in C, T1 and T2 were 43.37, 41.93and 42.61mg/dl on day 0 of experiment and the values increased to 51.89, 53.14 and 54.71mg/dl respectively at the end of trial. Animals were in early lactation (negative energy balance), thus had lower plasma glucose at the start of experiment. On an average, glucose levels (mg/dl) were 46.74, 47.0and 48.07 in C, T1 and T2 groups, respectively exhibiting no significant difference (P>0.05). The blood glucose levels during the present study are in the reference range (40.00 to 80.00 mg/dl) as reported earlier (More *et al.* 2008; Bhooshan *et al.* 2010). Similar trend in glucose level during summer season has been reported by Kumar and Das (2006).

Plasma Urea

The mean plasma urea concentration of whole trial (mg/dl) was significantly (P<0.05) higher in T2 (26.59), but there was no difference between control (24.90) and T1 (25.37) (Table 4). However, it lies within the normal range in all the experimental groups as reported earlier (Dhali *et al.* 2005; Shingu *et al.* 2009). Contrary to our findings, Kumar and Das (2006) reported that supplementation of 100 ppm and 200 ppm niacin had no significant effect on serum urea-N in male buffalo calves. Similarly, Di Constanzo *et al.* (1997) observed no significant effect of niacin supplementation on plasma urea in lactating Holstein cows. But Belibasakis and Tsirogianni (1996) observed significantly lower blood serum urea concentration while supplementing 10 g/day/cow niacin to Friesian cows in hot weather.

Plasma NEFA

The plasma NEFA (µmol/litre) ranged from 279.22 to 417.06, 250.88 to 402.94 and 248.53 to 415.10 in C, T1 and T2 groups, respectively with a decreasing trend from 60 days onwards in all the three experimental groups (Table 4). Overall, the plasma NEFA was found to be significantly

Table 4. Effect of niacin supplementation on levels of plasma glucose, urea and NEFA in lactating crossbred cows

Fortnights	Glucose (mg/dl)			Urea (mg/dl)			NEFA (μmol/litre)		
	C	T_1	T_2	C	T_1	T_2	C	T_1	T_2
0*	$43.37^{a}\pm$ 2.16	$41.93^{a}\pm$ 1.76	$42.61^{a}\pm$ 1.38	$22.25^{a}\pm$ 1.01	$23.01^{a}\pm$ 1.71	$23.70^{a}\pm$ 1.29	$417.06^{c}\pm$ 28.12	$402.94^{e}\pm$ 18.40	$415.10^{e}\pm$ 22.84
1	$45.39^{ab}\pm$ 1.99	$43.73^{a}\pm$ 1.64	$44.74^{ab}\pm$ 1.03	$22.31^{a}\pm$ 0.56	$23.07^{a}\pm$ 1.60	$24.04^{a}\pm$ 1.19	$392.06^{ab}\pm$ 21.78	$371.47^{cde}\pm$ 17.46	$380.49^{de}\pm$ 20.38
2	$46.18^{abc}\pm$ 1.55	$45.20^{a}\pm$ 0.97	$46.11^{abc}\pm$ 0.95	$23.01^{abA}\pm$ 0.58	$24.88^{abAB}\pm$ 1.32	$25.97^{abB}\pm$ 0.68	$372.55^{ab}\pm$ 21.83	$348.63^{cd}\pm$ 17.70	$357.55^{cde}\pm$ 18.64
3	$43.99^{a}\pm$ 1.59	$44.44^{a}\pm$ 0.92	$46.18^{abc}\pm$ 1.75	$26.15^{bc}\pm$ 1.02	$26.54^{ab}\pm$ 1.71	$28.11^{bc}\pm$ 0.97	$398.14^{ab}\pm$ 20.88	$377.55^{de}\pm$ 17.68	$373.92^{de}\pm$ 19.18
4	$43.66^{a}\pm$ 2.05	$45.16^{a}\pm$ 1.83	$45.52^{ab}\pm$ 1.45	$28.65^{c}\pm$ 1.48	$29.20^{b}\pm$ 1.68	$30.31^{c}\pm$ 0.90	$416.18^{c}\pm$ 20.96	$396.18^{de}\pm$ 17.60	$396.77^{de}\pm$ 18.58
5	$46.73^{abc}\pm$ 1.87	$46.80^{ab}\pm$ 1.76	$48.99^{bc}\pm$ 1.64	$26.30^{bc}\pm$ 1.45	$26.18^{ab}\pm$ 1.24	$28.02^{bc}\pm$ 1.03	$379.51^{ab}\pm$ 22.62	$353.92^{cde}\pm$ 15.13	$346.47^{cd}\pm$ 16.81
6	$48.66^{abc}\pm$ 1.73	$50.49^{bc}\pm$ 1.89	$50.33^{cd}\pm$ 1.00	$25.40^{abc}\pm$ 1.01	$25.30^{ab}\pm$ 0.98	$27.26^{b}\pm$ 0.58	$332.16^{ab}\pm$ 20.20	$324.21^{bc}\pm$ 18.48	$306.67^{bc}\pm$ 20.44
7	$50.79^{bc}\pm$ 1.95	$52.16^{c}\pm$ 1.54	$53.43^{de}\pm$ 1.42	$25.24^{ab}\pm$ 1.09	$25.09^{ab}\pm$ 0.65	$26.33^{ab}\pm$ 1.04	$306.96^{a}\pm$ 16.25	$284.90^{ab}\pm$ 16.31	$281.08^{ab}\pm$ 17.62
8	$51.89^{c}\pm$ 1.63	$53.14^{c}\pm$ 2.11	$54.71^{e}\pm$ 1.87	$24.76^{ab}\pm$ 0.89	$25.03^{ab}\pm$ 1.37	$25.54^{ab}\pm$ 0.77	$279.22^{a}\pm$ 13.99	$250.88^{a}\pm$ 11.65	$248.53^{a}\pm$ 12.09
Overall mean±SEM	46.74± 0.70	47.00± 0.72	48.07± 0.69	$24.90^{x}\pm$ 0.42	$25.37^{x}\pm$ 0.49	$26.59^{y}\pm$ 0.40	$365.98^{y}\pm$ 9.07	$345.63^{x}\pm$ 8.38	$345.17^{x}\pm$ 9.21

Observations with different superscripts (x and y) differ significantly (P<0.05) between the groups; Observations with different superscripts (a, b, c, d and e) differ significantly (P<0.05) within the group; Observations with different superscripts (A and B) differ significantly (P<0.05) within the period of groups

0* denotes values recorded on last day of adaptation period

lower (P<0.05) in T1 (345.63) and T2 (345.17) in comparison to control group (365.98). The decreased NEFA on niacin supplementation is due to anti-lipolytic nature of niacin (Nichoff *et al.* 2009). There is activation of HM74A receptor in adipose tissue to which NA is having high affinity legend that starts an inhibitory G-protein signal which reduces adipocyte cAMP concentrations by repressing adenylcyclase activity, which inhibits lipolysis (Wise *et al.* 2003). Contrary to our findings, Di Constanzo *et al.* (1997) and Small (2010) observed no effect of RPN supplementation on plasma NEFA. But in their studies, cow received treatments in late lactation. However, reduced NEFA concentration was also observed by researchers in response to niacin supplementation under normal environment (Erickson *et al.* 1992; Campbell *et al.* 1994; Minor *et al.* 1998).

Physiological responses

Rectal temperature (RT)

The mean values of RT (ºC) in C, T1 and T2 during morning and afternoon were 38.3, 38.3, 38.2 and 39.0, 39.0, 38.9, respectively. The statistical analysis revealed no significant difference between the treatment groups (Table 5). Di Constanzo *et al.* (1997) also reported no effect of niacin supplementation (12, 24, 36 g/d RPN) on RT in lactating cows under heat stress conditions. In contrast to our findings, Zimbelman *et al.* (2010) reported that feeding of 12 g/d of RPN resulted in decreased rectal temperature during heat stress compared with the control animals (38.17 vs. 38.34 ºC).The differences found might be due to protected niacin used in the latter study. The mean rectal temperature (ºC) at 7 days of experimental trial was significantly (P<0.05) low in control (38.1, 38.3) as compared to T1 (38.3, 38.6) and T2 (38.4, 38.6) during morning and afternoon respectively which is in contrast to the observations of overall average. This may be explained in light of the fact that vaso-dilatory role of niacin is active at high ambient temperature. With the increased THI, there is an increase in RT in all the experimental groups. Soly and Singh (2001) reported an increase in rectal temperature as the ambient temperature increased in both the group of animals maintained inside and outside the shelter. Silanikove (2000) stated that RT is an indicator of thermal balance and may be used an effective quantify tool to the harshness of the thermal environment. The mean RT (ºC) recorded at 2:30 pm in all the groups had higher values than the morning recordings (7:30 am). Banerjee and Ashutosh (2010) also found that mean rectal temperatures were significantly low at 7:00 am (38.39 ºC) than 3:00 pm (39.19 ºC) in Karan Fries cows in heat exposed conditions.

Skin temperature (ST)

The mean values of ST (ºC) were significantly (P<0.05) lower in T2 (36.7, 40.2) than control (37.2, 40.5) and T1 (37.0, 40.5) during morning and afternoon, respectively (Table 6). The above results corroborate the findings of Di Constanzo *et al.* (1997) who reported that supplementation of 12 g RPN/cow/day resulted in significantly lower rump

Table 5. Effect of niacin supplementation on rectal temperature (ºC) in lactating cross bred cows

Days	Control		Treatment 1		Treatment 2	
	M*	A+	M*	A+	M*	A+
0~	$38.10^{a}\pm0.05$	$38.72^{ab}\pm0.09$	$38.30^{bcd}\pm0.07$	$38.78^{ab}\pm0.09$	$38.20^{abc}\pm0.07$	$38.78^{a}\pm0.23$
7	$38.10^{aA}\pm0.01$	$38.30^{aP}\pm0.01$	$38.30^{bcdB}\pm0.06$	$38.60^{aQ}\pm0.08$	$38.35^{bcB}\pm0.08$	$38.63^{aQ}\pm0.10$
15	$38.28^{abB}\pm0.08$	$38.77^{abc}\pm0.03$	$38.17^{abcAB}\pm0.06$	$39.20^{b}\pm0.13$	$37.88^{abA}\pm0.15$	$38.82^{a}\pm0.31$
30	$38.28^{ab}\pm0.12$	$38.88^{bc}\pm0.11$	$38.20^{abc}\pm0.15$	$39.18^{b}\pm0.10$	$38.28^{bc}\pm0.14$	$38.80^{a}\pm0.29$
45	$38.02^{a}\pm0.10$	$39.67^{eQ}\pm0.17$	$38.30^{bcd}\pm0.12$	$39.58^{cQ}\pm0.12$	$37.87^{ab}\pm0.25$	$38.82^{aP}\pm0.08$
60	$38.58^{bc}\pm0.19$	$39.07^{bcd}\pm0.26$	$38.67^{d}\pm0.26$	$39.10^{b}\pm0.25$	$38.08^{ab}\pm0.23$	$38.90^{ab}\pm0.30$
75	$38.23^{ab}\pm0.16$	$38.73^{ab}\pm0.22$	$38.02^{ab}\pm0.12$	$38.80^{ab}\pm0.08$	$38.28^{bc}\pm0.11$	$38.92^{ab}\pm0.11$
90	$38.63^{bc}\pm0.22$	$39.23^{cde}\pm0.19$	$38.58^{cd}\pm0.20$	$38.93^{ab}\pm0.17$	$38.32^{bc}\pm0.07$	$38.93^{ab}\pm0.14$
105	$38.72^{cB}\pm0.16$	$39.42^{deQ}\pm0.18$	$38.17^{abcA}\pm0.03$	$38.88^{abP}\pm0.06$	$38.60^{cB}\pm0.15$	$39.53^{bQ}\pm0.21$
120	$37.90^{a}\pm0.14$	$39.43^{deQ}\pm0.13$	$37.83^{a}\pm0.02$	$39.10^{bPQ}\pm0.09$	$37.77^{a}\pm0.14$	$39.00^{abP}\pm0.14$
Overall mean±SEM	38.29±0.05	39.02±0.07	38.25±0.05	39.02±0.05	38.16±0.05	38.91±0.07

Observations with different superscripts (x and y) differ significantly (P<0.01) between the groups
Observations with different superscripts (a and b) differ significantly (P<0.05) within the group
Observations with different superscripts (A and B) differ significantly (P<0.01) within the period of groups
0* denotes values recorded on last day of adaptation period

Table 6. Effect of niacin supplementation on skin temperature (ºC) in lactating cross bred cows

Days	Control		Treatment 1		Treatment 2	
	M*	A+	M*	A+	M*	A+
0~	$36.2^{bA}\pm0.2$	$38.5^{aA}\pm0.2$	$36.0^{bA}\pm0.3$	$39.0^{aB}\pm0.2$	$36.9^{bB}\pm0.1$	$39.5^{bcC}\pm0.1$
7	$34.4^{aA}\pm0.1$	$38.4^{a}\pm0.1$	$35.4^{bB}\pm0.1$	$39.0^{a}\pm0.2$	$35.2^{aB}\pm0.1$	$38.9^{b}\pm0.3$
15	$37.8^{cA}\pm0.2$	$40.9^{d}\pm0.2$	$38.7^{eB}\pm0.2$	$40.6^{b}\pm0.2$	$38.7^{cB}\pm0.2$	$40.9^{e}\pm0.2$
30	$38.9^{d}\pm0.2$	$42.3^{eQ*}\pm0.2$	$38.6^{e}\pm0.2$	$42.2^{cAQ*}\pm0.4$	$38.8^{c}\pm0.2$	$41.4^{efP*}\pm0.2$
45	$37.6^{c}\pm0.3$	$42.0^{e}\pm0.2$	$37.7^{cd}\pm0.3$	$42.1^{c}\pm0.4$	$38.0^{c}\pm0.4$	$41.9^{f}\pm0.5$
60	$38.3^{cd}\pm0.3$	$41.7^{e}\pm0.5$	$38.4^{de}\pm0.3$	$42.1^{c}\pm0.2$	$38.2^{c}\pm0.7$	$41.0^{e}\pm0.5$
75	$34.3^{a}\pm0.4$	$39.3^{bQ*}\pm0.3$	$34.6^{a}\pm0.4$	$38.2^{aQ*}\pm0.7$	$34.4^{a}\pm0.2$	$36.2^{aP*}\pm0.1$
90	$36.2^{bA}\pm0.2$	$40.9^{d}\pm0.1$	$37.2^{cB}\pm0.3$	$41.1^{bc}\pm0.1$	$37.9^{cB}\pm0.4$	$41.0^{e}\pm0.2$
105	$37.9^{c}\pm0.3$	$40.9^{d}\pm0.2$	$37.7^{cd}\pm0.3$	$40.8^{b}\pm0.3$	$38.0^{c}\pm0.2$	$40.7^{de}\pm0.1$
120	$35.9^{b}\pm0.4$	$40.1^{c}\pm0.2$	$35.7^{b}\pm0.2$	$40.3^{b}\pm0.2$	$36.3^{b}\pm0.3$	$40.1^{cd}\pm0.1$
Overall mean±SEM	$36.7^{X}\pm0.2$	$40.5^{Q}\pm0.2$	$37.0^{Y}\pm0.2$	$40.5^{Q}\pm0.2$	$37.2^{Y}\pm0.2$	$40.2^{P}\pm0.2$

Observations with different superscripts (a, b, c, d and e) differ significantly (P<0.05) within the column
Observations with different superscripts (A and B) differ significantly (P<0.05) within the period of morning groups and observations with different superscripts (P and Q) differ significantly (P<0.05) within the period of evening groups; 0~ denotes observations recorded on last day of adaptation period; *M= observation recorded at 7:30 am, +A= observation recorded at 2:30 pm

temperature (33.7 vs. 34.7 ºC in control and treatment respectively). But, previous reports signified no effect of niacin supplementation on skin temperature in lactating cows during heat stress conditions (Small, 2010; Zimbelman *et al.* 2010; Yuan *et al.* 2011). The contradictions found might be due to breed difference and different ambient temperatures. Two possible explanations are available for the reduction in skin temperature but without a change in rectal temperature for cows fed diets supplemented with niacin. The likely explanation is that heat transfer might have been reduced, in which case a comparable reduction in heat gain would be expected if the core temperature were to remain constant. Another explanation would be increased evaporative heat loss, which would cool the skin, lower temperature and increase the thermal gradient for heat loss. The mean ST (ºC) recorded in the afternoon in all the groups had higher values than the morning recordings throughout the trial. It might be due to high ambient temperature during day time in comparison to morning time.

Respiration rate (RR)

The mean values of RR (breaths/min) were 46.1, 42.5 and 42.2 during morning and 68.7, 67.4 and 66.1 at afternoon in C, T1 and T2, respectively exhibiting no significant difference between treatments during afternoon. However, during morning it was significantly (P<0.05) lower in T1 and T2 as compared to control (Table 7). It might be because of higher ambient temperature at day time. Wrinkle *et al.* (2012) also reported that supplementing RPN at a consumption level of 19 g RPN/d results in lowering

Table 8. Effect of niacin supplementation on pulse rate (beats per minute) in lactating cross bred cows

Days	Control		Treatment 1		Treatment 2	
	M*	A+	M*	A+	M*	A+
0~	$76.5^{b}\pm2.2$	$85.0^{bc}\pm1.8$	$75.3^{abc}\pm0.1$	$84.3^{abc}\pm0.8$	$75.0^{a}\pm0.4$	$85.3^{ab}\pm0.8$
7	$68.5^{aA}\pm0.9$	$78.0^{aA}\pm1.0$	$71.7^{abB}\pm0.4$	$80.7^{abB}\pm0.4$	$72.0^{aB}\pm0.3$	$80.0^{aAB}\pm0.5$
15	$72.0^{abA}\pm0.5$	$77.5^{a}\pm0.6$	$74.5^{abcB}\pm0.4$	$79.8^{a}\pm0.5$	$72.4^{aA}\pm0.7$	$80.4^{a}\pm1.8$
30	$71.3^{ab}\pm1.1$	$82.3^{ab}\pm1.7$	$73.8^{abc}\pm1.3$	$81.8^{ab}\pm1.2$	$72.6^{a}\pm2.3$	$80.6^{a}\pm1.3$
45	$73.8^{ab}\pm1.3$	$83.3^{ab}\pm1.3$	$74.0^{abc}\pm1.1$	$80.4^{ab}\pm1.6$	$73.6^{a}\pm0.6$	$80.0^{a}\pm1.3$
60	$76.8^{b}\pm1.8$	$89.2^{bcd}\pm3.0$	$78.8^{c}\pm2.6$	$87.6^{cd}\pm1.7$	$76.0^{a}\pm3.0$	$89.7^{b}\pm3.2$
75	$72.0^{ab}\pm1.8$	$86.0^{bc}\pm1.0$	$68.8^{a}\pm3.1$	$82.8^{abc}\pm1.4$	$75.2^{a}\pm3.0$	$85.0^{ab}\pm2.4$
90	$76.0^{b}\pm2.9$	$93.7^{d}\pm3.0$	$76.7^{bc}\pm3.5$	$91.3^{d}\pm3.0$	$76.0^{a}\pm2.0$	$91.8^{b}\pm2.5$
105	$74.7^{ab}\pm3.6$	$91.0^{cd}\pm3.5$	$70.3^{ab}\pm2.4$	$85.3^{bc}\pm2.4$	$72.0^{a}\pm3.5$	$89.3^{b}\pm3.1$
120	$75.0^{ab}\pm2.0$	$91.3^{cd}\pm3.1$	$71.3^{ab}\pm2.8$	$86.3^{abc}\pm2.6$	$79.3^{a}\pm3.7$	$90.3^{b}\pm3.0$
Overall mean±SEM	73.7±0.7	85.7±0.9	73.5±0.7	84.0±0.7	74.4±0.7	85.2±0.9

Observations with different superscripts (a, b, c and d) differ significantly (P<0.05) within the group

Observations with different superscripts (A and B) differ significantly (P<0.05) within the period of morning groups; 0~ denotes observations recorded on last day of adaptation period; *M= observation recorded at 7:30 am, +A= observation recorded at 2:30 pm

(P=0.02) of RR at 09:00 h, but not impacted at other times of the day. Di Constanzo *et al.* (1997) and Zimbelman *et al.* (2010) found that supplementation of RPN has no significant effect on respiration rate in lactating cows under heat stress conditions; however the values were numerically lower. But there was no difference among the periods of treatments during morning and afternoon. However, at 60 days, in present trial (peak stress period) the RR (breaths/min) of afternoon had numerically lower values in T1 (73.4) and T2 (64.8) as compared to control (82.0), which signifies the stress relieving flushing action of niacin. The respiration rate (bpm) recorded at 07:30 am was lower than 02:30 pm in all the experimental groups. It might be due to higher ambient temperature at afternoon. Banerjee and Ashutosh (2010) and Soly and Singh (2001) also reported high RR during the afternoon hours than the morning hours in crossbred cows and calves respectively in the summer season. Silanikove (2000) indicated respiration rate as most accessible and easiest approach for evaluating the degree of heat stress in farm animals.

Pulse rate (PR)

The mean values for PR (beats/min) were higher at 02:30 pm (85.7, 84.0 and 85.2) than 07:30 am (73.7, 73.5 and 74.4) in C, T1 and T2 respectively (Table 8). Earlier reports also reported circadian changes in the pulse rate during the summer season (Das *et al.* 1999; Banerjee and Ashutosh, 2010). The mean values of PR (beats/min) during the entire experimental period in C, T1 and T2 showed no significant difference between the three experimental groups at morning and afternoon. No previous reports are available on this aspect so the results cannot be compared and discussed. However, at 60 days of experimental period, the PR (beats/min) was higher in all the three experimental groups in comparison to rest of the periods. It was attributed to higher THI recorded in this period.

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