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Effect of dietary supplementation of astaxanthin (potent antioxidant) on growth rate, DMI, FCR and metabolic changes in Karan Fries heifers during heat stress

SUNIL KUMAR[1],* S.V. SINGH[1] and S.C. BHAN[2]

[1]*Animal Physiology Division, ICAR- National Dairy Research Institute (NDRI), Karnal, 132001, Haryana, India*

[2] *India Meteorological Departments, New Delhi*

**Corresponding author Email- dr.sunil8507@gmail.com*

ABSTRACT

A study was conducted on twelve Karan Fries (Holstein Friesian X Tharparkar) heifers, (10-12 months), in LRC of NDRI, Karnal.Heifers were divided equally into two groups i.e. control, and treatment (supplemented with astaxanthin@ 0.25 mg per kgBW per day per animal) to assess the heat stress ameliorative action of astaxanthin during summer season. During experimental period, environmental variables were recorded and THI was calculated to assess levels of summer stress. Blood samples were collected from both the group of heifers at fortnightly interval for quantification of plasma leptin and ghrelin hormones.Body weights of heifers were recorded at monthly interval. The body weight gain and ADG were significantly ($P \leq 0.05$) higher in treatment group. Feed intake was higher ($P \leq 0.05$) and FCR was lower ($P \leq 0.05$) in astaxanthin supplemented group.Plasma leptin was higher($P \leq 0.05$), while, plasma ghrelinand surface skin temperature were numerically lower in treatment than control group of heifers.The study found that astaxanthin supplementation amelio rated the negative impact of summer stress and helped in enhancement of growth rate and ADG by improving the feed intake and by decreasing the FCR of heifers.

Key words : Growth, heifer, Karan Fries, mitigation, summer season, heat stress

Climate change, with a constant increase in the earth temperature, negatively affects livestock production (Nardone *et al*., 2010). Animal undergoes heat stress when the body temperature is higher than normal range. In this condition total heat load is greater than the capacity of animals to dissipate heat (Bernabucci *et al.,* 2010).The thermal comfort zone of heifers of tropical region lies in between15 to 25ºC.Significant changes in feed intake and physiological processes occur when temperatures exceeds 25ºC**.** However, the thermal comfort zone of animal depends upon several other factors such as environmental temperature, humidity, wind velocity, genotype, physiological state, susceptibility and their ability to acclimatize.Animals try to maintain the core body temperature through increasing heat loss and reducing heat production by physiological, metabolic and behavioral responses. Animals reduce dry matter intake to decrease the heat production,which in turn alters animal performance, i.e., growth rate, feed conversion ratio etc (Belsare and Pandey, 2008).Three management strategies have been identified to minimize the effects of heat stress:(1) physical modification of the environment (2) genetic development of heat tolerant breeds and (3) nutritional management practices. Nutritional management strategies may include the supplementation of antioxidant, which combats the adverse effect of heat stress. Abdul Niyas *et al.* (2017) reported that osmanabadi goats with nutritional supplements were able to withstand the heat stress.

Astaxanthin is a (3, 3'-dihydroxy-â, â-carotene-4, 4'-dione) xanthophyll carotenoid of predominantly marine origin. Its potent antioxidant and anti-inflammatory effects have been demonstrated in both animal and human studies. Astaxanthin can also be bio synthesized by plants, bacteria, fungi and green algae. Astaxanthin is a powerful free radical scavenger and protects cells from oxidative damage (Lee *et al.,* 2011). It has unique chemical properties due to its molecular structure. It possesses hydroxyl and keto moieties on each inone rings, which is responsible for its higher antioxidant activity (Kishimoto *et al.,* 2010). Its powerful antioxidant properties, without showing pro-oxidation due to the oxo function are also responsible for resonance stabilization of carbon centered radicals (Martin *et al.,* 1999). It catches free radicals not only at the conjugate polyene chain but also in the terminal ring moiety. The unsaturated polyene chain of astaxanthin traps free radicals on the membrane while the terminal ring of astaxanthin scavenges the free radicals both at the surface as well as in the interior of the phospholipids membrane. The unique properties of astaxanthin are associated with its potent anti peroxidation activity. Astaxanthin possess several functions such as protection against oxidation of PUFA, protection against UV light, immune responses and improved production and reproduction. Antioxidant activities of astaxanthin are found to be 10 times higher than β-carotene and 100 times higher than α-tocopherol against reactive oxygen species (Miki, 1991). However, Kristinson and Miyashita (2014) reported antioxidant activity of astaxanthin, 65 times more potent than vitamin E and ten times more powerful than leutin, canthaxanthin and zeaxanthin. Little information is available on the beneficial effects of astaxanthin supplementation on growth performance and metabolic changes in Karan Fries heifers through mitigating the impact of heat stress during harsh summer season. This study was carried out to examine whether astaxanthin ameliorates the adverse effects of heat stress, which might be helpful in improving the growth rate, feed intake, feed conversion efficiency, blood metabolites and body surface temperature of heifers.

MATERIALS AND METHODS

Geographical location of the study area

The experiment was conducted in the cattle yard of ICAR-National Dairy Research Institute, Karnal, Haryana, (India). Karnal (29°422 N latitude, 79°592 E longitudes) is situated at an altitude of 250 m above mean sea level. The maximum temperature goes up to 45°C during summer and minimum temperature to 3.5-4°C during winter. The average rainfall is about 700 mm.

Selection of animals and their management

The study was conducted on twelve Karan Fries heifers (Holstein Friesian X Tharparkar) of 10-12 months' age during summer season of the year 2017(01 April to 01 October). The heifers were randomly and equally divided into two groups - control and treatment. The heifers of both the groups werefed as per ICAR feeding standards. The heifers of treatment group were provided additional supplement of astaxanthin @ 0.25 mgkgBW/day/animal). All the animals were kept under standard management conditions followed at NDRI livestock farm.Mass deworming of the animals was done before commencement of the experimental. The experiment was approved by the institutional animal ethics committee constituted as per the article no. 13 of the CPCSEA rules lay down by Government of India.

Recording the attributes

The meteorological variables like maximum and minimum temperature, dry and wet bulb temperature,wind speed, sunshine hours were recorded at 0722 and 1422 hrs IST. Based on the above meteorological variables THI was calculated using following formula:

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

Body weight of experimental animals was recorded at monthly interval by using electronic weighing machine having capacity measurement range of 4 to 1500 kg. The weight gained by the animals during the month was taken as body weight gains for that month whereas the average daily gain (ADG) was calculated by subtracting the initial body weight from the final body weight and divided by the number of days.

ADG = (Final weight –Initial weight)/Number of days on feed.

Dry matter intake**s** (DMI) of individual animals were calculated by recording the feed offered and feed left at fortnightly basis. The dry matter content of different feed ingredients were calculated by keeping the feed samples in ahot oven at 100ºC for overnight and average of three consecutive days DMI was considered.Feed conversion ratio (FCR) was calculated as follows

FCR = (Dry matter intake)/Live weight gain.

Skin surface temperature was recorded at fortnightly interval by using non contact telethermometer (Raytek, model Rayngér ST2L, M/s. Surrey Scientific, Surrey, UK) by keeping it 2-3 inches away from the surface.Blood samples were collected at fortnightly intervals in heparinised vacutainer tube from all the experimental animals. Plasma was separated through centrifugation of blood tubes @ 3000 rpm for 25 minutes. The blood plasma was stored in aliquots in eppendorff tube at -20 °C for further analysis of plasma leptin and ghrelin.

ELISA for plasma leptin and ghrelin

Plasma leptin was estimated using "Bovine Leptin ELISA Kit" (Catalog no. E0014Bo) with specificity between 0.1 $ngml^{-1}$ to 40 $ngml^{-1}$, sensitivity of 0.05 $ngml^{-1}$ and the intra-assay and inter-assay were CV<8 per cent and CV<10 per cent, respectively. Plasma ghrelin was estimated using

"Bovine Ghrelin ELISA kit" (Catalog no. E0262Bo). The specificity of kit varied from 30 ngL^{-1} to 9000 ngL^{-1} and sensitivity of the kit was 14.86 ngL^{-1}, while, the intra-assay and inter-assay was CV<8 per cent and CV<10 per cent, respectively.

Statistical analysis

Analysis of variance was used to analyze the data by SAS software, version (9.1). Results were expressed as the means ± SEM in which a value with differences of $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The meteorological conditions of the Livestock Research Centre (LRC) of ICAR-NDRI, Karnal,where the experimental animals were kept during the period of experimentation are presented in Table 1. These meteorological variables were employed as an index to evaluate the summer stress during the different months of study.

Body weight gain and average daily gain

The influence of astaxanthin supplementation on monthly body weights gain (BWG) and average daily gain (ADG) of control and treatment groups of Karan Fries heifers are presented in Table 2. The BWG and hence ADG increased as the age and feed intake of animals increases. The overall monthly mean values of BWG and ADG of treatment group heifers(14.96±0.68 kg and 498.70±22.70 gm day^{-1}, respectively) were higher ($P \leq 0.05$) than those of the control group of heifers (12.02±0.49 kg and 400.92±16.37 gm day^{-1}, respectively). The magnitude of increase in weight and ADG of heifers was higher in first and last months of experiment mainly due to faster growth at early stage and due to influence of hormonal factors towards end of the experiment when the heifers approach puberty.

Cattle exposed to heat stress reduces feed intake and increases water intake and there are changes in the endocrine status, which in turn increase the maintenance requirements leading to reduced body weight, average daily gain and body conditions of livestock (Gaughan and Cawsell-Smith, 2015).The decline in feed intake and weight gain was found during late summer, when digestibility was poor in Mongolian steers. During heat stress the DMI of ruminants decreases in order to suppress the rate of rumination, fermentation and metabolism, which aid extra heat production. This negative effect of summer stress on weight gain of steers was reversed by supplementation of antioxidants in feed. In the present study, the BWG and ADG were higher in the astaxanthin supplemented group of animals. This can mainly be attributed to higher DMI and conservation of energy in treatment group compared to control group of heifers. Growth enhancing action of astaxanthin was also found in pigs (Bergstrom *et al.,*2009). The finding of present study are also in accordance to those of O'Brien *et al.* (2010) who reported reduced average daily gain in heat stressed group of Holstein bull calves as compared to thermoneutral group. During our experiment astaxanthin supplementation increased ADG by 24.44 per cent in Karan Fries heifers compared to control group. The Higher ADG of KF may be due astaxanthin supplementation which played major role in ameliorating the negative impact of environmental stressors.

Dry matter intake

The results of dry matter intake (DMI) of control and treatment groups of Karan Fries heifers are presented in Table 3. The overall mean values of DMI for treatment group (5.05±0.02 kg) were higher ($P \leq 0.05$) than the control (4.91±0.02 kg. The magnitude of increase in DMI with advancement of age was also found to be higher in treatment group than the control group. The DMI in treatment group was significantly ($P \leq 0.05$) higher due to higher intake facilitated by the heat amelioration effect of astaxanthin. DMI showed positive correlation with ADG, leptin and negative correlation with environmental stressors like ambient temperature and relative humidity and ghrelin. Reduced DMI was reported partly due to the significant thermal inertia associated with homeotherms and slower gut motility during heat challenge (Gordon *et al.,* 2008).The results of the present study are in accordance with those of Chandra *et al.* (2013) who reported higher DMI (11.29 kg) in supplemented group (Vit.E and Zn) than control group (10.92 kg) of Sahiwal cows. In present study, higher ($P \leq 0.05$) DMI was found in treatment group that could be due to supplementation of astaxanthin (antioxidant agents) which reduces the oxidative stress and prevented the animals from heat stress during summer season. The lower DMI in control group during heat stress may be an attempt to lower the heat production through reduced rumination, fermentation and metabolism by these animals to maintain the homeothermy.

Feed conversion ratio (FCR)

The results FCR in control and treatment groups of Karan Fries heifers are depicted in Fig. 1. The FCR varied from 11.59±0.97 to 14.57±0.96 and 9.63±0.37 to 12.65±1.72 in control and treatment group of Karan Fries

Table 1: Average climatic condition and environmental variables during summer season 2017 recorded at 0722 and 1422 hrs IST; Lat. 29°43' N; Long. 76°58'E and height 245 mtrs. ASL

Months	T max	T min	Db max	Db min	Wb max	Wb min	RH max	RH min	THI max	THI min	THI Avg	WS	SS
April	37.6 ±0.5	18.9 ±0.7	36.3 ±0.6	23.3 ±0.6	21.0 ±0.4	18.0 ±0.5	57.0 ±2.3	21.0 ±1.6	82.0 ±0.5	70.3 ±0.7	76.2 ±0.6	04.0 ±0.4	10.4 ±0.3
May	38.9 ±0.5	23.4 ±0.4	37.5 ±0.6	27.3 ±0.4	23.4 ±0.3	21.1 ±0.3	55.0 ±2.2	28.0 ±2.9	84.5 ±0.4	75.5 ±0.4	80.0 ±0.4	5.1 ±0.6	10.1 ±0.4
June	36.5 ±0.8	25.1 ±0.5	34.9 ±0.9	27.9 ±0.5	25.0 ±0.22	23.7 ±0.3	70.0 ±3.1	46.0 ±3.9	83.7 ±0.6	77.8 ±0.5	80.7 ±0.5	05.0 ±0.4	9.20 ±0.6
July	33.6 ±0.3	26.4 ±0.1	32.6 ±0.3	28.4 ±0.2	27.6 ±0.1	26.1 ±0.1	82.0 ±1.0	67.0 ±1.5	83.9 ±0.3	79.9 ±0.2	81.9 ±0.2	03.5 ±0.3	8.80 ±0.4
Aug.	33.0 ±0.3	25.9 ±0.1	31.7 ±0.2	27.4 ±0.1	27.8 ±0.1	26.0 ±0.1	86.0 ±2.1	72.0 ±1.8	83.4 ±0.3	79.0 ±0.2	81.2 ±0.2	01.4 ±0.3	6.30 ±0.7
Sept.	32.3 ±0.3	23.1 ±0.2	31.4 ±0.4	25.3 ±0.2	26.5 ±0.2	24.3 ±0.2	92.0 ±0.8	67.0 ±2.4	82.3 ±0.4	76.3 ±0.3	79.3 ±0.3	01.6 ±0.2	7.30 ±0.7

Tmax – Maximum temperature, Tmin – Minimum temperature, Dbmax – Maximum Dry bulb, Wbmax – Maximum Wet bulb, RHmax – Maximum Relative humidity, RHmin – Minimum Relative humidity, THImax – Maximun Temperature Humidity Index, Dbmin – Minimum Dry bulb, Wbmin – Minimum Wet bulb, THImin – Minimum Temperature Humidity Index, WS – Wind speed, SS – Sunshine hours.

Table 2: Influence of astaxanthin supplementation on body weight gain (kg) and ADG (g day^{-1}) of Karan Fries heifers during summer season

Months	Body weight gain		ADG	
	Control	Treatment	Control	Treatment
April	-	-	-	-
May	12.83^{X}±1.09	15.10^{X}±0.52	427.77^{X}±36.43	503.33^{X}±17.40
June	12.71^{X}±0.89	15.50^{X}±1.68	423.88^{X}±29.67	516.66^{X}±56.25
July	11.05^{X}±0.73	13.83^{X}±1.86	368.33^{X}±24.38	461.11^{X}±62.24
August	14.00^{X}±2.25	13.13^{X}±1.74	466.66^{X}±75.10	437.77^{X}±58.08
September	10.45^{X}±0.67	14.81^{Y}±1.84	348.33^{X}±22.38	493.88^{Y}±61.46
October	11.11^{X}±0.48	17.38^{Y}±2.06	370.55^{X}±16.04	579.44^{Y}±68.96
Mean± S.E.	12.02^{X}±0.49	14.96^{Y}±0.68	400.92^{X}±16.37	498.70^{Y}±22.70

The values are mean ± SE of six observations on six animals. The values with different superscripts a,b,c… within a column and X, Y within a row differed significantly ($P \leq 0.05$) within the parameter.

heifers, respectively. The overall mean values of FCR was significantly ($P \leq 0.05$) lower in treatment (10.81±0.50) compared to control (12.86±0.49) group. However, lowest value of FCR (9.63±0.37) was recorded for Karan Fries heifers during the month of May due to higher ADG. FCR is negatively correlated with ADG. FCR is a gross measure of feed conversion into desired output like growth, milk, meat production etc. and most often used as a tool to evaluate costs of production and it has been found to be negatively correlated with growth rate and mature size (Datt *et al.,* 2017). Cattle that will convert the feed at a high rate (lower FCR) are highly desirable for cattle owners. Improving the feed efficiency is important to the dairy industry for both economic and environmental sustainability point of view. During heat stress, the FCR of ruminants increased due to inefficient metabolism of the nutrients of feed. However, individual variation was found due to differences in genetic makeup. Selecting for improved FCR would result in an increase in genetic merit for growth, which leads to increased mature cow size and reduced feed costs for the herd. The

Table 3: Influence of astaxanthin supplementation on DMI (kg) and skin temperature(ºC) of Karan Fries heifers during summer season

	Fortnights	DMI		Skin temperature	
		Control	Treatment	Control	Treatment
April	I	4.78^{X}±0.02	4.81^{aX}±0.02	34.76^{a}±0.12	34.77^{a}±0.27
	II	4.80^{X}±0.009	4.90^{adX}±0.009	34.84^{a}±0.21	34.67^{a}±0.21
May	III	4.82^{X}±0.04	4.87^{adX}±0.01	36.35^{b}±0.52	36.27^{b}±0.16
	IV	4.86^{X}±0.01	4.95^{acdX}±0.02	36.03^{bc}±0.29	35.92^{b}±0.16
June	V	4.87^{X}±0.14	5.05^{adeY}±0.04	39.75^{d}±0.16	39.52^{cg}±0.34
	VI	4.90^{X}±0.11	5.12^{bdeY}±0.08	40.80^{efg}±0.18	40.60^{dhi}±0.25
July	VII	4.92^{X}±0.06	5.06^{adeX}±0.03	41.03^{e}±0.21	40.85^{dh}±0.31
	VIII	4.92^{X}±0.07	5.07^{adeX}±0.07	41.12^{ef}±0.23	41.04^{dfh}±0.09
August	IX	4.97^{X}±0.05	5.08^{adeX}±0.08	40.76^{defg}±0.15	40.51^{dgh}±0.28
	X	5.02^{X}±0.06	5.11^{adeX}±0.02	40.22^{df}±0.21	40.05^{efgi}±0.13
September	XI	5.02^{X}±0.07	5.25^{bceY}±0.06	40.02^{dg}±0.15	39.89^{cegi}±0.09
	XII	5.03^{X}±0.03	5.31^{beY}±0.04	39.67^{d}±0.24	39.34^{ce}±0.05
Mean± S.E.		4.91^{X}±0.02	5.05^{Y}±0.02	38.80±0.29	38.64±0.28

The values are mean ± SE of six observations on six animals. The values with different superscripts a,b,c… within a column and X, Y within a row differed significantly ($P \leq 0.05$) within the parameter

Table 4: Influence of astaxanthin supplementation on leptin (ng ml^{-1}) and ghrelin (pg ml^{-1}) of Karan Fries heifers during summer season

	Fortnights	Leptin		Ghrelin	
		Control	Treatment	Control	Treatment
April	I	16.34^{acX}±0.86	16.47^{aX}±0.23	35.70±1.60	35.69±0.94
	II	16.17^{acX}±0.81	16.80^{aX}±0.16	35.95±0.66	35.69±1.54
May	III	15.81^{acY}±0.26	17.98^{acX}±0.34	36.06±1.38	35.66±0.84
	IV	15.83^{acY}±0.26	17.81^{acX}±0.41	36.47±1.25	35.78±1.51
June	V	16.00^{acY}±0.36	18.51^{adX}±0.81	36.28±3.01	35.72±1.56
	VI	13.70^{bcY}±0.29	18.18^{adX}±0.75	36.33±1.70	35.93±2.08
July	VII	14.17^{acY}±0.96	19.01^{adX}±0.36	37.32±0.85	35.98±3.23
	VIII	14.34^{acY}±0.79	19.84^{bcdX}±0.76	38.74±3.78	36.15±3.07
August	IX	16.34^{acY}±0.56	19.90^{bcdX}±0.06	38.37±1.48	36.24±2.92
	X	16.51^{aY}±0.14	20.12^{bcdX}±0.50	35.49±0.83	35.32±1.32
September	XI	16.59^{aY}±0.78	20.29^{bcdX}±0.71	33.66±2.90	33.20±1.54
	XII	16.63^{aY}±0.43	20.84^{bdX}±0.49	33.37±3.65	32.76±3.13
Mean± S.E.		15.70^{Y}±0.19	18.81^{X}±0.21	36.15±0.61	35.34±0.58

The values are mean ± SE of six observations on six animals. The values with different superscripts a,b,c… within a column and X, Y within a row differed significantly ($P \leq 0.05$) within the parameter

results of present study are in accordance with the previous reports indicating that ratios or amount of feed intake per unit of gain were greater during HS treatment of Holstein calves indicating less weight gain per unit of daily feed

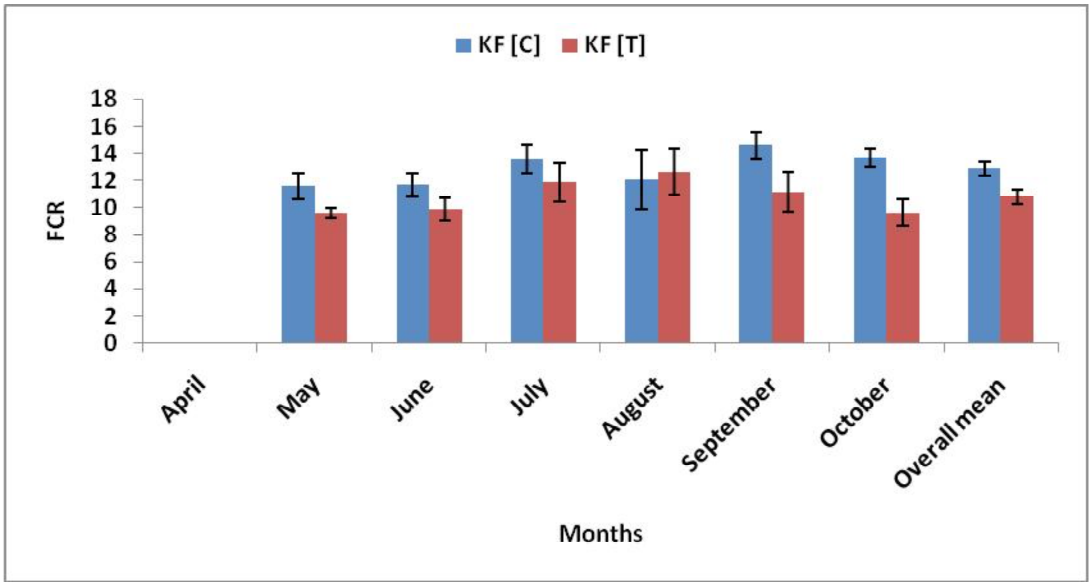

Fig. 1: Influence of astaxanthin supplementation on FCR of Karan Fries heifers during summer season

The values are mean ± SE of six observations on six animals

Table 5: Correlation among parameters studied with meteorological variables in Karan Fries heifers

	THI	T max	T min	Db temp	Wb temp	RH	DMI	ST	GHR	LEP
THI	1									
T max	-0.18*	1								
T min	0.97**	-0.33**	1							
Db temp	0.43**	0.79**	0.27**	1						
Wb temp	0.83**	-0.70**	0.89**	-0.14*	1					
RH	0.49**	-0.93**	0.61**	-0.56**	0.89**	1				
DMI	0.24**	-0.44**	0.29**	-0.27**	0.43**	0.49**	1			
ST	0.69**	-0.71**	0.78**	-0.22**	0.90**	0.84**	0.39**	1		
GHR	0.08	0.05	0.08	0.10	0.02	-0.03	-0.19*	0.03	1	
LEP	0.24**	-0.09	0.27**	0.07	0.22**	0.14*	0.22**	0.22**	0.06	1

** (P<0.01) Significant at 1% * (P<0.05) Significant at 5%

intake (Baccari *et al.,* 1983). This could be explained by the fact that heat-stressed heifers spend more energy for maintenance and to acclimatize to the stress. As a result, less energy would be used for growth, which leads to the decreased growth performance. Supplementation of antioxidant (astaxanthin) restores the gut motility normal which helped in absorption of nutrients leading to improved growth performance. Lower values of FCR in treatment group of present study might be due to supplementation of astaxanthin which minimized the adverse effects of harsh tropical climatic environment and resulted into improved growth performance.

Skin temperature

The average values of skin temperature (ST) of control and treatment group of Karan Fries heifers during summer season are presented in Table 3. The values of skin temperature of Karan Fries heifers in control and treatment group varied from 34.7±0.12^{0}C to 41.12±0.23^{0}C and 34.67±0.21^{0}C to 41.04±0.09^{0}C, respectively. The values of ST in control and treatment group of heifers increased as the ambient environmental temperature increases and found to vary significantly (P ≤ 0.05) among different months. Highest values of ST were recorded for the month of July, when the environmental temperature and relative humidity was comparatively higher and animals were under maximum discomfort. The overall mean value of skin temperature of heifers was found higher in control than treatment group. The skin temperature of heifers is positively correlated with environmental variables like maximum, minimum, dry andwet bulb temperature, relative humidity and THI. It is negatively correlated with weight gain, ADG and DMI.

The skin temperature rises in relation to the length of radiation exposure (Singh and Singh, 2006). The rise in ambient temperature causes increase of ST and then would evoke reflex sweating mechanism through thermo sensory impulses from receptors located in the skin. However, the number of sweat gland and hence sweating mechanism is lower in crossbred animals and hence, they experience greater discomfort and heat stress under tropical climatic conditions compared to zebu cattle. The results of present study are in accordance with the findings of Schutz *et al.* (2011) who reported elevated skin surface temperature due to solar radiation and it is directly related to ambient solar radiation levels which attributed to the exposure of heat stress.

Plasma leptin

The mean values of leptin ($ngml^{-1}$) in control and treatment group of Karan Fries heifers are given in Table4. The overall mean values of leptin in control (15.70±0.19 $ngml^{-1}$) groups were significantly ($P \leq 0.05$) lower than that of the treatment (18.81±0.21 $ngml^{-1}$) groups of heifers. The levels of leptin ($ng\ ml^{-1}$) in Karan Fries heifers varied from 13.70±0.29 to 16.63±0.43 and 16.47±0.23 to 20.84±0.49 in control and treatment group, respectively and remained in physiological range. The analysis of variance of data showed significant effects of treatment, fortnights and interaction between treatments and fortnight. Leptin was positively correlated with DMI while negatively correlated with THI, ST and ghrelin.

The circulating leptin concentrations showed positive correlation with DMI and ADG in heifers. The results of present study are in agreement with Foote *et al.* (2015) who showed a positive association of leptin concentrations with feed intake and ADG. Balland and Cowley (2015) also reported positive association of plasma leptin concentrations with DMI in rodent models of diet-induced obesity and leptin resistance. However, Leptin is thought to act as a long term regulator of DMI and, therefore, it would be expected to decrease DMI, when circulating concentrations was elevated in heifers. Leptin has been shown to have multifaceted action in addition to regulate feed intake and energy expenditure. It also plays a major role in the immune system by enhancing phagocytic activity of phagocytes and reproduction. Adequate leptin levels permit energy expenditure on the processes of growth, production and reproduction. DMI increased in the treatment group of heifers due to heat stress ameliorative action of astaxanthin which resulted into deposition of adipose tissue. This adipose tissue enhanced the secretion of leptin in heifers during prepubertal development coincident with increases in serum IGF-I concentrations and body weight. However, in the prepubertal heat stressed control group of heifers DMI was lower, leads to decrease in leptin secretion due to reduction in adiposity associated with decreased serum insulin, IGF-I and LH pulse frequency (Amstalden *et al.,* 2002). Also, the body weight gain was higher in treatment group and hence puberty in these animals was attained earlier than control, which leads to more secretion of leptin because it is the signal that informs the brain that metabolic store are adequate for the initiation of puberty.

Plasma ghrelin

The mean values of ghrelin ($pgml^{-1}$) in control and treatment groups of Karan Fries heifers are presented in Table 4.The overall mean values of ghrelin in control (36.15±0.61 $pg\ ml^{-1}$) were higher than treatment (35.34±0.58 $pgml^{-1}$) groups of Karan Fries heifers. Ghrelin showed positive correlation with environmental variables, ST,FCR, while negative correlation with body weight gain, ADG, DMI and leptin.

During the entire period of the study the blood ghrelin level did not fluctuated much and remained in the physiological limit. However, the control group of heifers had higher ghrelin levels compared to antioxidant (astaxanthin) supplemented groups. Heat stress has been shown to decrease feed intake in order to decrease the metabolic heat production (Kumar *et al.,* 2016); and fasting (36 h) as well as long-term energy restriction increased mean plasma ghrelin concentration by more than ûve-fold. The causes of higher ghrelin secretion during periods of energy deficiency include decreased plasma concentrations of insulin and glucose (Briatore *et al*., 2003). Plasma ghrelin concentration showed positive correlation with the serum BHB and NEFA ($p \leq 0.01$) and the levels of blood ghrelin, leptin, BHB and NEFA are considered as the sensitive indicators of the energy balance in dairy cows (Nowroozi-Asl *et al*., 2016). The higher levels of ghrelin in control group of animals during present study are in accordance with Rhoads *et al*. (2008) who reported higher mean plasma ghrelin concentration in dairy cattle during periods of heat stress. The higher levels of ghrelin in control group compared to astaxanthin supplemented animals might be due to decrease in DMI during heat stress. This might be due to greater sensitivity of KF heifers towards the harsh tropical climatic conditions.

CONCLUSION

Heat stress has significant negative effects on growth performance of heifers.Heat-stressed animal changes their metabolism and physiology in response to change in ambient environmental conditions. Heat stress directly and indirectly influence the efficiency of feed utilization leading to decrease in growth performance**.**Study showed that supplementation of antioxidant like astaxanthin in diets of Karan Fries heifers minimized the negative impact of heat stress, which, resulted in increased weight gain and ADG; and decreased FCR.Therefore, astaxanthin supplementation can be adopted as one of the strategies to ameliorate theeffects of heat stressin changing global warming scenario.

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