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RESEARCH ARTICLE

Effect of ascorbic acid and amla powder (*embilica officinalis*) supplementation on physiological parameters in cross-bred dairy cattle during heat stress

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Abstract

To study and compare the effect of dietary supplementation of ascorbic acid and amla powder (*Embilica officinalis*) on physiological parameters during summer, twelve cross-bred dairy cattle were randomly divided into three groups of four animals 4 each. The T_1 group was control, T_2 and T_3 groups were supplemented with ascorbic acid and amla powder @ 50 mg/kg body weight and 200 mg/kg body, respectively. The overall mean maximum and minimum temperature recorded during the study were 38.68±2.43°C and 26.45±2.13°C, respectively, while the overall mean relative humidity and temperature humidity index (THI) recorded at 0800 hrs and 1400 hrs were 81.32±5.21%, 74.06±3.92, and 51.31±4.93%, 87.60±4.32, respectively. Rectal temperature (RT), respiratory rate (RR) and pulse rate (PR) were non-significant between different treatments during 0800 hrs, while corresponding values at 1400 hrs were significantly ($P<0.01$) higher in T_1 (39.91±0.36°C, 62.05±3.69 breaths/minute, and 90.25±5.22 beats/minute) than T_2 (39.1±0.45°C, 50.92±3.77 breaths/minute and 77.08±4.71 beats/minute) and T_3 (39.17°C±0.46°C, 52.97±3.85 breaths/minute and 78.68±4.36 beats/minute). There was no significant difference between T_2 and T_3 for all parameters. From this study, it is concluded that supplementation of either ascorbic acid or its equivalent in the form of amla powder @ 50 mg and 200mg/kg BW respectively, can ameliorate the heat stress in dairy cattle.

INTRODUCTION

Hot, humid environmental conditions prevail seasonally in many parts of Jammu region of Jammu & Kashmir state. In summer the atmospheric temperature may rise to as high as 46°C during day time and 30°C at night. The high temperature along with high humidity and increased day length (13-14 hrs) aggravate the heat stress in livestocks. The effects of heat stress can be minimized through nutritional management, changes in the microenvironment and genetic improvement of animals. Of these, nutritional management is most easy and cost effective approach to minimize heat stress in dairy cattle. It has been established that ascorbic acid ameliorates the adverse effects of heat stress in humans (Tauler et al., 2003), pigs (Fayomi et al., 2004), goats (Sivakumar et al., 2010; Khan and Konwar, 2010; Kumar et al., 2012), buffaloes (Gade et al., 2010;) and poultry (Ayo and Sinkalu, 2007). Although ruminants can synthesize vitamin C in the liver and it is not considered to be an essential nutrient for healthy cattle (McDowell, 1989), reduction in plasma vitamin C concentration was reported in calves stressed by housing condition (Cummin

and Brunner, 1991), in high producing dairy cows (Macleod et al., 1999), in lactating cow with artificially induced mastitis (Weiss et al., 2004) and in heat stressed cow (Padilla et al., 2006).

Amla, or Indian gooseberry (*Emblica officinalis or Phyllanthus emblica*), an edible fruit is well-known for its high content of vitamin C and its potent antioxidant activity, more potent than many other herbs. Amla has been reported to posses hepatoprotective, hypolipidemic (Thakur and Mandal, 1984), expectorant, purgative, spasmolytic, antibacterial and hypoglycemic (Jayshri and Jolly, 1993) activity. As amla is easily available in the market and is an economical source of vitamin C, it can be used as a source of supplemental vitamin C.

Physiological responses like rectal temperature (RT), pulse rate (PR) and respiration rate (RR) reflect the degree of stress imposed on animals by climatic parameters (Ganaie et al., 2013). In the present study ascorbic acid and amla powder was supplemented to cross-bred dairy cattle to observe their effect on amelioration of heat stress in terms of on physiological parameters as these parameters give an immediate response to the climatic stress and consequently the level of discomfort/comfort to the animal.

MATERIALS AND METHODS

The experiment was conducted at the Instructional Dairy Farm, Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry, R. S. Pura, SKUAST-J, Jammu, located 32°36'0"N latitude and 74°47'59"E longitude, during the months of June, July and August i.e., the hottest period of the year in this region. The duration of trial was 60 days.

Twelve cross-bred dairy cows of average body weight 430 kg were randomly divided into three groups (T_1, T_2 and T_3) of four animals each. The animals of T_2 and T_3 group were daily supplemented orally with ascorbic acid and amla powder @ 50 and 200mg/kg b.wt, respectively, while the T_1 group received no supplementation. Two hundred milligram of amla powder is equivalent to 50 mg of ascorbic acid in terms of activity. All animals were housed in well-ventilated byres. The adaptation period in the shed was fifteen days for all the treatments prior to the start of experiment. Animals were fed on concentrate feed @ 2 kg /h/day and allowed for four hours daily grazing in early morning. Wheat straw was provided adlib.

Different microclimatic observations viz. maximum and minimum temperature, dry and wet bulb temperature and Temperature humidity Index (THI) were recorded in the morning (0800 hrs) and afternoon (1400 hrs) on daily basis during the experimental period. Maximum and minimum temperature was measured by using maximum minimum thermometer. Relative humidity in percentage was calculated from the dry bulb and wet bulb readings (°C) on daily basis using the psychometric tables. Temperature Humidity Index (THI) values were calculated as per U.S. Weather Bureau, by the following equation: THI = 0.72 (Dry bulb temp. °C + Wet bulb temp. °C) + 40.6, where 0.72 and 40.6 are constants in the above equation.

Physiological variables like pulse rate, respiratory rate and rectal temperature of individual animals were recorded twice daily at 0800 hrs and 1400 hrs during the experimental period. In the morning the physiological parameters were recorded before feeding of the animals. Respiration rate was recorded by observing the flank movement from a distance without disturbing the animals. Pulse rate was recorded on the coccygeal artery. The rectal temperature was recorded by using a clinical thermometer. The body weight of the animals was estimated at the starting of the experiment before feeding and watering for calculation of dose of ascorbic acid and amla powder. The data recorded were analyzed as per the procedure described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSIONS

Microclimatic changes-

Air temperature The mean maximum and minimum temperature ranged from 37.89°C±2.12°C to 40.41°C±2.87°C and 25.21°C±2.31°C to 27.86°C±2.23°C, respectively, with an overall mean of 38.68°C±2.43°C maximum temperature and 26.45°C±2.13°C minimum temperature during the experimental period inside the cattle shed (Table 1).

High ambient temperature results in an increase in internal body temperature of cattle which decreases feed intake and changes eating patterns (De Dios and Hahn, 1993). Dikmen and Hansen (2009) reported a dry bulb temperature of 28.4°C to be the upper critical temperature of lactating dairy cows at which rectal temperature was 38.5°C.

Relative Humidity The percent relative humidity recorded ranged from 74.23±5.43 to 88.02±6.21 per cent at 0800 hrs and from 46.21±4.32 to 56.34±3.92 per cent at 1400 hrs, with an overall mean of 81.32±5.21 and 51.31±4.93 per

cent at 0800 hrs and at 1400 hrs, respectively (Table 1). The relative humidity recorded in the present experiments was higher than 40 per cent and even some time crossing 80 per cent. Starr (1981) reported that heat balance could become a problem at 20°C and above, when relative humidity was in excess of 60 per cent.

Temperature Humidity Index (THI): During the experimental period, the overall mean THI recorded in the cattle shed at 0800 hrs and 1400 hrs were 74.06±3.92 (range 71.12-77.12) and 87.60±4.32 (range 86.32-89.87), respectively (Table 1). The present findings indicate that all the experimental animals were under mild heat stress during morning hours as compared to afternoon hours, during which the animals were under moderate to extreme heat stress. Wiersme and Armstrong (1989) observed that, a THI value of 72-78 causes mild stress, 78-88 leads to moderate stress while above 88 result in extreme heat stress in cattle.

Table 1. Overall mean±S.E of weekly micro climatic variables recorded during the experimental period

Period	Temperature °C		Relative Humidity %		Temperature Humidity Index (THI)	
	Maximum	Minimum	Morning, 0800hrs	Afternoon 1400hrs	Morning 0800hrs	Afternoon 1400hrs
1st week	40.41±2.87	27.86±2.23	74.23±5.43	46.21±4.32	71.12±3.32	89.87±3.54
2nd week	39.42±2.18	26.32±2.43	78.45±4.23	47.12±4.23	71.34±3.45	88.12±3.65
3rd week	37.95±2.86	25.21±2.31	82.12±4.56	48.13±4.14	74.35±2.35	86.54±3.32
4th week	38.72±2.64	26.89±2.31	84.01±4.35	49.22±4.56	75.21±3.54	87.31±4.12
5th week	39.57±2.41	26.87±2.32	88.02±6.21	56.34±3.92	77.12±3.54	88.67±4.15
6th week	38.50±1.98	25.81±2.13	84.11±5.12	53.21±3.97	76.31±3.87	87.75±3.98
7th week	38.75±2.43	27.16±2.11	79.34±5.23	49.76±3.87	72.71±2.87	86.32±3.87
8th week	37.89±2.12	26.92±2.17	82.23±4.89	52.31±3.76	74.32±3.42	86.43±3.78
Overall mean	38.68±2.43	26.45±2.13	81.32±5.21	51.31±4.93	74.06±3.92	87.60±4.32

Table 2 Overall mean±S.E of physiological parameters of cross bred dairy cattle under different treatments at morning (0800 hrs) and afternoon (1400 hrs).

Parameters	T_1	T_2	T_3	F- Value	CD Value
Rectal temperature (0800 hrs)	38.85±0.31	38.78±0.29	38.81±0.36	1.19^{NS}	-
Rectal temperature (1400 hrs)	39.91^{a} ±0.36	39.10^{b} ±0.45	39.17^{b} ±0.46	131.19^{**}	1.31^{**}
Respiration rate (0800 hrs)	44.33±2.51	43.96±3.54	44.11±2.53	0.12^{NS}	-
Respiration rate (1400 hrs)	62.05^{a} ±3.69	50.92^{b} ±3.77	52.97^{b} ±3.85	58.17^{**}	6.31^{**}
Pulse rate (0800 hrs)	60.89±4.29	60.29±4.33	60.49±3.30	0.91NS	-
Pulse rate (1400 hrs)	90.25^{a}±5.22	77.08b±4.71	78.68b±4.36	32.03**	8.21**

Means bearing different superscript (a, b, c) within a row differ significantly. Level of significance; ** = P<0.01; NS = non significant

Physiological Parameters

Rectal Temperature The overall mean rectal temperature of cattle is presented in Table 2. The overall mean rectal temperature recorded at 0800 hrs in the T_1, T_2 and T_3 were 38.85°C±0.31°C, 38.78°C±0.29°C and 38.81°C±0.36°C, respectively, and their corresponding values at 1400 hrs were 39.91°C±0.36°C, 39.10°C±0.45°C, and 39.17°C±0.46°C, respectively. The overall mean rectal of all the groups at 0800 hrs were within the normal range (37.5-39.5^{0}C) for cattle. At 1400 hrs the rectal temperature recorded in the T_1 group was above the normal range while in T_2 and T_3 groups were within the normal range. The climatic data (Table I) showed that the THI values were above the comfort zone which indicated that the animals were under thermal stress. Khongdee et. al., (2006) and Berman (2008) also observed higher rectal temperature in cattle under thermal stress.

The present result showing a diurnal rise of rectal temperature in all the groups at 1400 hour is in accordance with that of Bhan et al., 2012; Vaidya et al., 2010. The increase in rectal temperature might be an attempt to decrease water losses by evaporative cooling (Silanikove, 1992). The rectal temperature increased by 1.06^{0}C in T_1 group, 0.32^{0}C in T_2 group and 0.36^{0}C in T_3 group at 1400 hrs which showed that dietary supplementation with ascorbic acid and amla reduced the diurnal rise in temperature. This might be due to the hypothermic effect of ascorbic acid as reported by Tauler et al., (2003).

Among the groups, supplementation of ascorbic acid and amla reduced the rectal temperature in T_2 and T_3 groups numerically at 0800 hrs and significantly (P<0.01) at 1400 hrs, while no significant difference was observed between T_2 and T_3. Similar results were reported in ascorbic acid supplemented Murrah buffaloes (Gade et al., 2010) and goats (Sivakumar et al., 2010; Khan and Konwar, 2010; Minka et al., 2009 and Kumar et al., 2012). The

hypothermic effect of amla powder reported in this study might be due its high concentration of ascorbic acid, alongwith active tannoid principles, and other polyphenols such as ellagic acid, gallic acid, tannins, etc., which markedly increase the free radical scavenging, generated during the stress. Firoza et al., (2000) also reported that the *Emblica officinalis* has a high superoxide-scavenging activity. Maini et al., (2007) reported that electrolyte mix of Emblica officinalis reduces the oxidative stress and lipid peroxidation in broilers during summer.

Respiration Rate The overall mean respiration rates (Table 2) of the cattle recorded at 0800 hours were 44.33±2.51, 43.96±3.54 and 44.11±2.53 breath/minute in T_1, T_2 and T_3, respectively, and their corresponding values at 1400 hours were 62.05±3.69, 50.92±3.77 and 52.97±3.85 breath/minute, respectively.

The values of respiration rate were within the normal range (26-50 breath/min) in all the group at 0800 hrs and in T_2 and T_3 groups at 1400 hrs. While in T_1 group the RR exceeded the normal range at 1400 hrs, indicating higher level of heat stress. Respiration is the most sensitive physiological character to the change of climate and physical environment in cattle (Sabuncuoglu, 2004). The increase or decrease in the respiration rates is an adaptive response of the animal to maintain homeothermy. In domestic animals respiration rate increases due to the activation of warm receptors in skin when exposed to higher ambient temperature. Activation of the receptors in turn sends neural signals to the hypothalamus that increases respiratory activity to accelerate heat loss from the body by respiratory evaporization (AL-Haidary and Ahmed, 2004). An evaporative heat loss from the respiratory tract is regarded as one of the primary mechanisms for maintenance of heat balance (Mc Dowell et al., 1976) to maintain the internal body temperature (Marai et al., 2007) which accounts for 30% of total heat dissipation (Bhan et al., 2012).

The present result showed a diurnal increase in the overall mean respiratory rates at 1400 hours in all the groups is similar to the report of Bhan et al., 2012; Vaidya et al., 2010. The respiration rates increased by 17.17 breath/min in T_1 group, 6.96 breath/min in T_2 group and 8.86 breath/min in T_3 group at 1400hrs which reveals that dietary supplementation of ascorbic acid and amla reduces the diurnal rise in respiration rate. This might be due to the effect of ascorbic acid and amla in maintaining the rectal temperature in T_2 and T_3 groups.

Among the groups, respiratory rates of the T_1 were numerically higher at 0800 hour and significantly ($P<0.01$) higher at 1400 hours compared to T_2 and T_3 groups. However, no significant difference was observed between T_2 and T_3 at both 0800 hours and 1400hrs recordings. Similar results were reported in ascorbic acid supplemented buffaloes (Gade et al., 2010; Sivakumar et al., 2010; Kumar et al., 2012 and Ganaie et al., 2012) and goats (Khan and Konwar, 2010). The decreased respiratory rates in AA supplemented group indicated that supplementation of ascorbic acid can ameliorate heat stress in cattle.

Amla powder supplementation in T_3 group provided ascorbic acid and other polyphenols having a strong antioxidant activity, thus alleviating the effect of heat stress. Lowered respiratory rate in T_3 group may be due to the presence of ascorbic acid and polyphenols in the amla powder (Bhattacharya et al., 1999).

Pulse Rate The overall mean pulse rates (Table 2) at 0800 hours were 60.89±4.29, 60.29±4.33 and 60.49±3.30 beats/minute in the T_1, T_2 and T_3 groups, respectively, and the corresponding values at 1400 hours were 90.25±5.22, 77.08±4.71 and 78.68±4.36 beats/minute.

The values of pulse rate recorded were within the normal range (48-84 bpm) in T_1, T_2 and T_3 groups at 0800 hrs and in T_2 and T_3 groups at 1400 hrs. Whereas the overall mean pulse rate of T_1 group was above the normal range at 1400 hrs. An increase in pulse rate in T_1 group is an adaptive response of the animal to maintain homeothermy. Increase in pulse rate increases blood flow to the surface and facilitate heat loss by sensible means (Marai et al., 2007).

Diurnal increase in pulse rate was observed in all the groups at 1400 hrs. Similar findings were reported in cattle (Vaidya et al., 2010; Bhan et al., 2012), ewes (Alexier et. al., 2004) and goat kids (Hosam, 2007). The pulse rate increased by 29.36 beats/min in T_1 group, 16.79 beats/min in T_2 group and 18.19 beats/min in T_3 group at 1400 hrs. The result showed that diurnal rise in pulse rate was reduced by supplementation of ascorbic acid and amla power which could be due to the hypothermic effect of ascorbic acid as reported by Tauler et al., (2003).

The overall mean pulse rate was numerically higher in the T_1 group at 0800 hrs and significantly higher ($P<0.01$) at 1400 hrs as compared to the T_2 and T_3 groups. While similar results was observed between T_2 and T_3 groups. Administration of ascorbic acid and amla powder significantly ($P<0.01$) reduced the pulse rate by 13.17 beats per minute in T_2 and 11.57 beats per minute in T_3 compared to T_1 at 1400 hour recording. Similar results were

also observed in Murrah buffaloes (Gade et al., 2010; Ganaie et al., 2012) and goats (Khan and Konwar, 2010; Kumar et al., (2012) due to the effect of ascorbic acid supplementation.

The lower values of pulse rate in T_3 groups is due to the presence of ascorbic acid and polyphenols in the amla powder (Bhattacharya et al., 1999).

Conclusion It is evident from the result that the cross bred dairy cattle are affected by heat stress during summers. Both ascorbic acid and amla powder can be supplemented during extreme summers to ameliorate the heat stress by keeping minimum changes in physiological variables. No significant differences in physiological parameters were observed between the groups of cattle treated with ascorbic acid and amla powder. Amla powder being cheaper can be effectively supplemented to alleviate heat stress in cross bred dairy cattle.

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