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Effect of cooling strategies on milk production, physiological variables and blood profile during hot-dry and hot-humid summer in Sahiwal cattle

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ABSTRACT

The present experiment was undertaken to study the effect of misting and splashing during hot-dry and hot-humid months in lactating Sahiwal cattle. The study was conducted during May, June (hot-dry) and July (hot-humid) with monthly average temperature-humidity index (THI) of 79.88, 80.57 and 85.36, respectively. Eighteen lactating cattle were selected on the basis of days in milk, milk yield, and parity and then divided into three groups: control (no cooling), cooling by misting and cooling by splashing. Milk production was not affected by hot-dry and hot-humid heat stress in Sahiwal cattle. RT and RR were significantly lower in misting and splashing group as compared to control during hot-dry period. The heat stress-induced leucocytosis, lymphocytopenia and neutrophilia were significantly ameliorated by both misting and splashing during the study period. Heat stress-induced alterations in serum creatinine, sodium and ROS concentration were significantly mitigated by misting in hot-humid climate. The prolactin level significantly decreased in animals by misting during the study period. The results suggested that misting was more efficient than splashing as a cooling strategy in hot dry condition while splashing was more effective in hot-humid condition and mitigated the heat stress-induced alterations in different welfare parameters but could not fully nullify it. However, the resultant stress was not sufficient enough to decrease the milk yield in Sahiwal cattle. It may also be concluded that the Sahiwal cattle have high heat tolerance and could sustain production even in a hot-dry and hot-humid environment.

Keywords: Heat stress, Milk production, Misting, Sahiwal cattle, Splashing

Alterations in environmental temperature and relative humidity are the important tenets of climate change and are globally jeopardizing animal production and health especially in subtropical and tropical countries. India has several thermo-tolerant indigenous breeds of cattle which have the ability to adapt in different agro-climatic zones in changing climate conditions (Kumar *et al*. 2018, 2020). Cross-breeding between zebu and exotic cattle breeds resulted in augmentation in milk productivity in India and rearing of cattle alien to an agro-climatic region has become common amongst the livestock farmers. Despite the higher productivity, susceptibility to diseases and production losses are more in exotic and crossbred cattle, than in zebu cattle especially under changing climate scenarios (Yadav *et al*. 2013, 2015, 2016a).

The temperature-humidity index (THI) is used to measure the level of environmental heat stress in livestock worldwide. THI value has been defined: no stress <72, moderate stress 72–78, heavy stress >79–83 (Akyuz *et al*. 2010) however, the stratification of THI for measuring the comfort levels in bovine breeds of Indian subcontinent varies (Kumar *et al*. 2020). In semi-arid regions of India, the THI during summer months ranges from 80 to 88 (Yadav *et al*. 2016b). However, the stress components in terms of meterological variables vary dramatically. In month of May and June, stress is mainly due to excess temperature whereas in July and August stress is mainly endowed to high humidity (Yadav *et al*. 2016b). The cumulative heat load culminates in a cascade of physiological reactions in the animal which hampers the production performance and animal welfare. For sustainable milk production and animal welfare during summer months, several cooling strategies have been attempted in cattle (Butt *et al*. 2020, Lees *et al*. 2020). Fan-assisted ventilation and cooling improved the milk production during hot-dry summer in Sahiwal cattle (Ahmed *et al*. 2018, Butt *et al*. 2020). Studies on cooling methodologies during both hot-dry and hot-humid summer are scarce. The present study was attempted to explicate the effect of misting and splashing during hot-dry and hot-humid summer months in lactating Sahiwal cattle.

MATERIALS AND METHODS

The experiment was carried out at the Livestock Farm Complex, Veterinary University (DUVASU), situated in the

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Table 1. Meteorological variables and THI during the study period

Environ-mental variable	Temperature			Relative humidity	THI
	Minimum	Maximum	Average		
May	25.60^{a}	46.46^{a}	36.03^{a}	21.67^{a}	79.88^{a}
June	28.02^{b}	41.08^{b}	34.55^{b}	33.53^{b}	80.57^{a}
July	26.91^{b}	36.83^{c}	31.88^{c}	77.32^{c}	85.36^{b}
SE	0.51	0.82	0.61	2.41	0.60
P value	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Standard error uses pooled estimate of error variance. Means with different superscripts in a column differ significantly (P<0.05).

semiarid region at longitude 78°E and latitude 27°N with elevation of 177 m from mean sea level. The average annual ambient temperature ranges from 5 to 45°C and relative humidity (RH) varies from 15 to 90%. The study was conducted during May to July when average temperature, RH and THI ranged from 32–36°C, 30–75% and 79–90, respectively. Meteorological variables during the study were recorded and THI was calculated (Mader *et al*. 2006).

$$THI = (0.8\ T_{db}) + [(RH/100)\ (T_{db} - 14.4)] + 46.4$$

where, T_{db}, Dry bulb temperature; RH, Relative humidity.

Experimental animals and management: For this study, 18 lactating Sahiwal cattle maintained at LFC were selected based on their parity, stage of lactation (2^{nd} to 4^{th} month), and milk yield. All the animals were offered a balanced ration throughout the experimental period along with *ad lib.* clean drinking water. Prophylactic measures against endo and ecto-parasitic infestations and infectious diseases were carried out to ascertain proper health of the animals. The animals were kept in well-ventilated sheds. Different sheds were used for the animals of different treatment groups in tail to tail system of housing. The shed was equipped with misting systems and ceiling fans. Mistings was applied in the area of 72 m^2 with the help of eight misting pumps, each having a capacity to use 1.5 litres of water per minute with a droplet size of 3–5 micron. Splashing was done outside the shade near the wallowing tank. The research protocol and the animal usage in this experiment were approved by the institutional animal ethics committee, Veterinary University Mathura, India.

Experimental design: The experiment was conducted during hot-dry and hot-humid summer from May to July. The average ambient temperature was higher and RH was lower during May and June months while average ambient temperature was lower and RH was higher during the month of July (Table 1). Eighteen lactating cattle were randomly allocated into three equal groups and were subjected to three different treatments: negative control (no cooling), T1 (misting) (for 5 min duration at an interval of every 30 min from 11:30 h to 15:30 h) and T2 (splashing) (thrice a day for 15 min each; at 11:30 h, and 13:30 and 15:30 h). Milk production was recorded daily and blood sampling was done once (n=18) in the last week of each month at 14:30 h. The blood was used for haematological examinations and serum was harvested for biochemical and hormonal estimations.

Physiological parameters: Clinical thermometer was used to measure rectal temperatures (RT) in °C. Recording of respiratory rates (RR) was done by observing costal movements per minute and expressed as breaths per minute. Pulsation of the middle coccygeal artery at the base of the tail was used to record pulse rate (PR) and was expressed as beats per minute.

Blood sampling and analysis: 8 ml of blood was sampled through jugular venipuncture in heparinised vacutainer tubes and also in tubes without anticoagulant. After complete blood clotting, serum was harvested by centrifugation at 3,500 rpm at room temperature for 20 min and then kept at –20°C till further analysis. The blood samples were analyzed for total packed cell volume (PCV), hemoglobin (Hb), total erythrocyte count (TEC) and total leukocyte count (TLC) by standard methods. For differential leukocyte count (DLC), blood films were prepared, stained with Leishman solution and counted later as per protocol.

Hormone assay: Serum alkaline phosphatase (AKP) and aspartate aminotransferase (AST) activity and urea, glucose, creatinine, chloride, calcium and phosphorous concentration were estimated using kits (SPAN Diagnostic Ltd, India). Flame photometry was used to estimate the levels of sodium and potassium in the serum. Serum reactive oxygen species (ROS) (Brambilla *et al*. 2001) level and superoxide dismutase (SOD) (Madesh and Balasubramanian 1998) activity were estimated using microtitre plate method. Serum cortisol, prolactin, tri-iodothyronine (T_3) and thyroxin (T_4) levels were measured by ELISA kits (Thermo Fisher Scientific India, Private Limited).

Statistical analysis: The effects of month, treatment, and interaction of month and treatment on different variables were analyzed using repeated measures of ANOVA model (SAS, 9.4). Differences among the period and treatment were determined using Tukey's test indicated by both P values and superscripts (P<0.05). Least squares means and pooled standard errors were reported. The level of significance was set at P<0.05.

RESULTS AND DISCUSSION

Temperature and humidity are the major components of the meteorological variables which determine the level of heat stress in animals. THI has been the preferred parameter of animal scientists to express level of heat stress throughout world, with variation in weightage to temperature and humidity in different climatic zones (Dikman and Hansen 2009). In temperate climate, temperature is the main driver of heat stress whereas; temperature and humidity both play a critical role in the tropical and sub-tropical climate in ascertaining the level of heat stress. In certain months of the year, like May and June, the stress is due to increase in heat load due to increased temperature, while during July, August and September, stress is due to inability of the animal to reduce heat load due to increased humidity. Hence, the cooling strategies also differ according to the source of

Table 2. Effect of misting and splashing on physiologic parameters and milk yield during summer in Sahiwal cattle

Parameter		RT	RR	PR	Milk yield/Month
Over-experimental period	Control	38.59^{bc}	37.67^{de}	51.17^{ab}	177.89
	Misting	38.27^{e}	35.5^{c}	48.28^{ab}	177.55
	Splashing	38.46^{cd}	34.56^{bc}	48.72^{ab}	174.41
May	Control	38.60^{ab}	35.00^{c}	52.67 ^{a}	180.80
	Misting	38.27^{e}	33.00 ^{ab}	50.83 ^{ab}	181.50
	Splashing	38.49^{cd}	32.00 ^{a}	51.50 ^{ab}	185.66
June	Control	38.75^{a}	40.33^{e}	47.67^{ab}	175.50
	Misting	38.27^{e}	37.17^{d}	47.50^{ab}	176.50
	Splashing	38.52^{bc}	37.17^{d}	47.50^{ab}	172.00
July	Control	38.42^{bcde}	37.67^{de}	53.17^{a}	176.67
	Misting	38.28^{de}	36.33^{cd}	46.50^{c}	170.50
	Splashing	38.37^{cde}	34.50^{bc}	47.17^{bc}	170.42
Pooled SE		0.05	0.61	0.46	23.16
Period (P value)		0.0010*	0.0010*	0.0010*	0.74
Treatment (P value)		0.0010*	0.0010*	0.0010*	0.90
Period* Treatment (P value)		0.015*	0.091	<0.0001*	0.99

Standard error uses pooled estimate of error variance. Effect of period, treatment and interaction of period and treatment are indicated with P values. Means within a column having different superscripts differ significantly (P<0.05). RT, Rectal temperature (°C); RR, Respiratory rate (breathes/minute); PR, Pulse rate (beats/minute).

meteorological variable causing heat stress during different summer months. The present study was aimed to ascertain the type of cooling method during hot-dry and hot-humid summer months in lactating cattle to optimize production and animal welfare.

The meteorological variables and THI during the study period is presented in Table 1. During the month of June and July, both average minimum and maximum temperature were found to be significantly (P<0.01) higher as compared to May. Average temperature decreased significantly (P<0.01) from May to July whereas, relative humidity followed reverse trend. THI was significantly (P<0.01) lower in May and June as compared to July. The meteorological data in the present study suggested that lower RH and higher ambient temperature during hot-dry period (May and June) facilitated better evaporative cooling, whereas, in July (hot-humid), higher RH diminished the rate of evaporative heat loss. Although a wide variation exists in upper critical THI, depending upon different climatic zones of the world (Pinto *et al*. 2020, Srivastava *et al.* 2021). THI data of the present study indicated that heat stress was higher during hot-humid period as compared to hot-dry period.

The effect of misting and splashing on milk production is presented in Table 2. For this experiment, each group of animals had similar milk production at the beginning of the experiment. Milk yield decreased non-significantly (P>0.05) during moderate and high THI periods. Different treatments did not induce any change in milk production during the experiment. The effect of heat stress in physiological, productive and reproductive terms has been well documented in indigenous Indian cattle (Kumar *et al.* 2018, 2020) and accordingly shed and nutritional management have been attempted to ameliorate heat stress. The present study revealed an apparent decrease in milk production during the experimental period in all groups. In the present study it was hypothesized that reduction in milk yield was due to heat stress, however, the results suggested that the decrease in milk production may be due to advancing lactation, from 2–4 month to 5–7 month, and negated the hypothesis. Absence of appreciable decrease in milk yield during summer months can be attributed to higher thermo-tolerance and low milk production of the Sahiwal cattle. In low milk producing animals less metabolic heat is generated which is dissipated easily using different efficient mechanisms of heat loss in Zebu cattle. Kohli *et al*. (2014) also reported that milk yield declined only in high producing crossbreed cattle (>18 litre/day) during high THI and it was not affected in low producing cattle (<8 litre/day) which was in concurrence with the findings of the present study. On the contrary, Kumar *et al*. (2020) reported that milk production began to decline at a THI more than 81 in Hariana cattle that was attributed to less thermo-tolerance of Hariana cattle breed.

A significant (P<0.05) effect of period (hot-dry and hot-humid), treatment, and interaction of period and treatment was found on different physiological parameters during the study. The RT and RR were significantly (P<0.05) lower in misting and splashing group as compared to control group during hot and dry period, while, RR decreased significantly (P<0.05) and RT decreased non-significantly (P>0.05) during hot-humid period. Pulse rate did not change significantly (P>0.05) during hot-dry period in control group whereas in hot-humid period increase in PR was significantly (P<0.05) ameliorated by both misting and splashing (Table 2).In the present experiment, the animals responded to increased heat load due to high THI by increasing physiological heat loss mechanism, viz. RR, PR and RT as reported in previous studies (Yadav *et al*. 2015, 2016a, 2017), although the quantum of increase was

Table 3. Effect of misting and splashing on hematological parameters during summer in lactating Sahiwal cattle

Parameter		PCV	Hemoglobin	TEC	TLC	Neutro	Lympho	Mono	Eosino	Baso
Over-experimental period	Control	30.27^{ab}	9.99^{a}	5.04^{a}	7.45^{a}	33.22^{a}	57.66^{b}	4.50	4.61	0
	Misting	30.28^{ab}	10.31^{b}	5.24^{b}	6.96^{c}	27.44^{b}	64.05^{a}	4.22	4.28	0
	Splashing	31.18^{a}	10.31^{b}	5.31^{b}	7.10^{bc}	27.22^{b}	64.06^{a}	3.89	4.83	0
May	Control	31.30^{a}	10.37^{b}	5.23^{b}	7.34^{ab}	32.50^{a}	58.83^{b}	4.50	4.17	0
	Misting	31.58^{a}	10.67^{b}	5.42^{b}	6.98^{c}	26.67^{b}	65.33^{a}	4.00	4.00	0
	Splashing	31.30^{a}	10.45^{b}	5.37^{b}	7.08^{c}	26.83^{b}	64.00^{a}	3.80	5.33	0
June	Control	30.30^{ab}	10.00^{ab}	5.00^{a}	7.53^{a}	32.50^{a}	58.83^{b}	4.50	4.17	0
	Misting	30.78^{ab}	10.60^{b}	5.36^{b}	6.97^{c}	27.50^{b}	64.00^{a}	4.33	4.17	0
	Splashing	31.7^{ab}	10.30^{b}	5.29^{b}	7.13^{bc}	28.00^{b}	64.00^{a}	3.83	4.17	0
July	Control	29.20^{b}	9.60^{a}	4.89^{a}	7.5^{a}	34.67^{a}	55.33^{b}	4.50	5.50	0
	Misting	28.50^{b}	9.67^{a}	4.96^{a}	6.94c	28.17^{b}	62.83^{a}	4.33	4.67	0
	Splashing	30.95^{ab}	10.18^{b}	5.28^{b}	7.10^{bc}	26.83^{b}	64.17^{a}	4.00	5.00	0
Pooled SE		0.79	0.26	0.13	0.06	0.63	0.81	0.29	0.66	0
Period (P value)		0.02*	0.01*	0.03*	0.24	0.07	0.02*	0.77	0.26	0
Treatment (P value)	0.29	0.22	0.04*	<0.0001*	<0.0001*	<0.0001*	0.06	0.59	0	
Period* Treatment (P value)		0.53	0.51	0.51	0.36	0.13	0.12	0.96	0.71	0

Effect of period, treatment and interaction of period and treatment are indicated with p values. Means within a column having different superscripts differ significantly (P<0.05). The standard error uses pooled estimate of error variance. PCV, Packed cell volume(%); Hb, Hemoglobin (g %); TEC, Total erythrocyte count (10^6/µl); TLC, Total leukocyte count (10^3/µl); Neutro, Neutrophil (%); Lympho, Lymphocyte (%); Mono, Monocyte (%); Eosino, Eosinophil (%); Baso, Basophil (%).

minimal. Misting and splashing were able to resist an increase in RR, PR and RT by enforcing additional physical and physiological heat loss and decreasing the heat load (Yadav *et al*. 2016b, Ahmed *et al*. 2018). Results of physiological responses also suggested that misting and splashing both were equally effective as a cooling strategy during hot-dry period; however, splashing was more efficient during hot-humid period in reducing the heat load. In the present study, splashing was done outside the shed, in open, where a lower RH facilitated better evaporative heat loss as compared to misting which resulted in increased humidity in the shed and decreased the evaporative heat loss.

A significant (P<0.05) change was observed in PCV, haemoglobin, TEC, TLC, neutrophil and lymphocyte percentage during different periods (Table 3). TEC was found to be significantly (P<0.05) lower in the control group as compared to both misting and splashing groups in second half of hot-dry period, whereas, it was higher only in the splashing group in hot-humid period. The heat stress-induced leucocytosis, neutrophilia and lymphocytopenia were significantly (P<0.05) ameliorated by both misting and splashing during the study period.The present experiment exhibited that misting and splashing were equally effective in preventing an alteration in various haematological parameters (TEC, TLC, Neutrophil % and lymphocyte %) during hot-dry and hot-humid periods in cattle. A decrease in TEC and PCV in the control group was attributed to increased water intake due to heat stress resulting in hemodilution (Parmer *et al*. 2013). Heat stress-induced increase in glucocorticoids mediates alterations in the distribution of lymphocytes and neutrophils which changes the neutrophil and lymphocyte count (Parmar *et al*. 2013). However, misting and splashing were able to ameliorate heat stress and no change could be observed in neutrophil and lymphocyte count in treatment groups in the present study.

The serum urea, creatinine, sodium, AST, AKP, calcium, phosphorus, SOD and ROS levels altered significantly (P<0.05) during study period (Table 4). Serum creatinine, sodium and ROS concentration were significantly (P<0.05) lower in the misting group as compared to the control and splashing group. Further the concentration of urea, creatinine, AST, SOD and ROS were significantly (P<0.05) lower during hot and dry period than during hot and humid period, while the concentration of AKP and phosphorus were significantly (P<0.05) higher during the hot and dry period.Plasma metabolite, electrolyte, liver enzyme and redox status reflects the general health status of the animal (Sreedhar *et al*. 2013). In the present study, heat stress-induced alterations were non-significant in plasma metabolite, electrolyte, liver enzyme and redox status. The decrease in feed intake and increase in water intake during heat stress is one of the probable factors responsible for an apparent alteration in plasma profile besides other direct impact of heat stress.

The serum prolactin and T_4 level changed significantly (P<0.05) during the study period. The prolactin concentration decreased significantly (P<0.05) in second half of hot-dry and hot-humid period by misting. However, other hormones did not change significantly (P<0.05) by misting and splashing during the study period (Table 5). For acclimatization to heat stress, endocrine alterations are of paramount importance (Lakhani *et al*. 2020, Yadav *et*

Table 4. Effect of misting and splashing on serum metabolites, electrolytes, enzyme activity and redox status during summer in lactating Sahiwal cattle

Parameter		Urea	Creatinine	Glucose	Sodium	Potassium	Chloride	AST	AKP	Calcium	Phosphorus	SOD	ROS
Over-experimental period	Control	20.16^{a}	1.85^{a}	52.44	140.50^{a}	6.39	88.83	39.81^{ab}	9.96^{ab}	10.62^{bc}	5.27^{a}	305.17^{b}	4.63^{a}
	Misting	19.85^{a}	1.79^{b}	53.58	139.50^{b}	6.55	88.80	38.98^{ab}	10.08^{ab}	10.79^{bc}	5.46^{ab}	300.67^{b}	4.43^{b}
	Splashing	19.98^{a}	1.85^{a}	52.98	141.66^{a}	6.28	88.74	39.94^{ab}	9.99^{ab}	10.65^{bc}	5.34^{ab}	302.67^{b}	4.49^{b}
May	Control	19.60^{a}	1.78^{bc}	52.62	139.50^{ab}	6.33	89.10	37.11^{b}	10.39^{a}	11.00^{ab}	5.70^{a}	295.00^{b}	4.50^{ab}
	Misting	19.37^{a}	1.72^{c}	53.43	140.50^{ab}	6.33	89.03	36.94^{b}	10.28^{ab}	11.03^{a}	5.70^{a}	289.33^{b}	4.41^{b}
	Splashing	19.50^{a}	1.73^{c}	53.67	140.83^{ab}	6.50	88.88	37.63^{b}	10.35^{ab}	10.90^{ab}	5.73^{a}	291.67^{b}	4.44^{b}
June	Control	19.90^{a}	1.74^{c}	53.07	141.17^{ab}	6.50	89.17	37.53^{b}	10.23^{ab}	11.13^{a}	5.33^{ab}	294.67^{b}	4.53^{ab}
	Misting	19.50^{a}	1.73^{c}	54.23	139.33^{b}	7.00	89.07	37.01^{b}	10.36^{ab}	11.00^{ab}	5.27^{a}	288.00^{b}	4.41^{b}
	Splashing	19.77^{a}	1.77^{c}	53.03	142.33^{a}	6.50	89.17	37.43^{b}	10.31^{ab}	11.07^{a}	5.33^{ab}	293.83^{b}	4.45^{b}
July	Control	20.97^{b}	2.04^{a}	51.63	140.83^{ab}	6.33	88.23	44.79^{a}	9.25^{c}	9.73^{c}	4.77^{ab}	325.83^{a}	4.87^{a}
	Misting	20.67^{b}	1.92^{ab}	53.07	138.67^{b}	6.33	88.30	43.00^{a}	9.59^{bc}	10.33^{abc}	5.40^{ab}	324.67^{a}	4.47^{ab}
	Splashing	20.67^{b}	2.04^{a}	52.23	141.83^{ab}	5.83	88.17	44.76^{a}	9.32^{c}	9.97^{bc}	4.97^{ab}	322.50^{a}	4.57^{ab}
Pooled SE		0.35	0.03	0.63	0.74	0.33	0.51	0.87	0.17	0.22	0.17	5.42	0.08
Period (P value)		0.0001*	<0.0001*	0.08	0.52	0.18	0.08	<0.000*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0146*
Treatment (P value)		0.56	0.03*	0.09	0.004*	0.58	0.97	0.36	0.69	0.62	0.42	0.59	0.02*
Period* Treatment (P value)		0.94	0.27	0.82	0.17	0.67	0.99	0.88	0.76	0.56	0.28	0.96	0.34

Effect of period, treatment and interaction of period and treatment are indicated with p values. Means within a column having different superscripts differ significantly ($P<0.05$). The standard error uses pooled estimate of error variance. Urea (mg %), Creatinine (mg/100 ml), Glucose (mg/100 ml), Sodium (meq/L), Potassium (meq/L), Chloride (mg/100 ml). AST, Aspartate aminotransferase (IU/L); AKP, Alkaline phosphatase (KA units); SOD, Superoxide dismutase (U/ml); ROS, Reactive oxygen species (mg H_2O_2 equivalents/ml).

Table 5. Effect of misting and splashing on endocrine parameters during summer in lactating Sahiwal cattle

Parameter		Cortisol	Prolactin	T_3	T_4
Over-experimental period	Control	3.03^{ab}	59.02^{b}	1.23^{ab}	32.86^{ab}
	Misting	2.83^{ab}	37.41^{a}	1.26^{ab}	33.24^{a}
	Splashing	3.00^{ab}	59.46^{b}	1.19^{ab}	29.84^{ab}
May	Control	2.70^{a}	33.22^{e}	1.42^{a}	43.34^{a}
	Misting	2.83^{a}	29.52^{e}	1.11^{ab}	33.08^{ab}
	Splashing	2.72^{a}	32.89^{e}	1.34^{ab}	31.52^{ab}
June	Control	3.19^{ab}	61.24^{cd}	1.11^{ab}	29.45^{ab}
	Misting	2.97^{a}	32.66^{e}	1.40^{a}	31.77^{ab}
	Splashing	3.01^{ab}	66.57^{bc}	1.12^{ab}	28.73^{ab}
July	Control	3.19^{ab}	82.59^{a}	1.17^{ab}	25.80^{b}
	Misting	2.69^{a}	50.06^{d}	1.26^{ab}	34.87^{ab}
	Splashing	3.26^{ab}	78.92^{ab}	1.10^{ab}	29.26^{ab}
Pooled SE		0.16	3.17	0.09	3.26
Period (P value)		0.04*	<0.0001*	0.55	0.04*
Treatment (P value)		0.274	<0.0001*	0.80	0.38
Period*Treatment (P value)		0.187	<0.0001*	0.0022*	0.052

Effect of period, treatment and interaction of period and treatment are indicated with P values. Means within a column having different superscripts differ significantly ($P<0.05$). The standard error uses pooled estimate of error variance. All concentrations are in ng/ml.

al. 2021). Acclamatory hormones mainly include thyroid hormones, prolactin (Kumar *et al*. 2018) and glucocorticoids (Lakhani *et al*. 2020, Yadav *et al*. 2021). In the present study, cortisol, prolactin and thyroxin level were altered during hot-dry and hot-humid periods however the alteration was more prominent in hot-humid period. Heat stress-mediated increase in cortisol (Yadav *et al*. 2015) and prolactin (Kumar *et al*. 2018) has been earlier confirmed in cattle during heat stress. Misting was observed to be efficient in preventing an increase in prolactin level in second half of dry-hot period and hot-humid period. Amaral *et al*. (2009) reported that the use of sprinklers during heat stress reduced the prolactin levels which corroborates with the results of our study. The endocrine response to heat stress and response after its abatement suggested that animals make hormonal adjustment after a certain threshold level of stress to support required metabolism and recover to its original level when the stress is reduced below threshold level.

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