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Heat stress alleviation in lactating buffaloes: Effect on physiological response, metabolic hormone, milk production and composition

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ABSTRACT

To find out the effect of heat stress alleviation on physiological response, metabolic hormone profiles, milk production and composition in lactating buffaloes during hot-dry (HD) and hot-humid (HH) seasons, 42 lactating Nili-Ravi buffaloes were uniformly divided into 2 groups of 21 each. The treatment (T_1) group was supplemented with niacin, yeast, edible oil and provided with curtains, additional ceiling fans and mist fans in the shed. The feeding time, frequency and method for providing concentrate were altered. The control (T_0) group buffaloes were kept in separate shed without any nutrient supplementation, modification in microclimate and management. The rectal temperatures, recorded at 10 AM and 3 PM, were significantly lower in treatment group buffaloes than control group under both seasons. Similar trends in pulse rate and respiration rate were recorded among treatment and control group buffaloes in both seasons. Plasma cortisol, T_3 and T_4 values were almost similar in both control and treatment groups. The average daily milk production was significantly higher in treatment group than control group from starting to the end of the experiment. The average total fat and SNF production in treatment group were significantly higher compared to control group. The overall fat per cent in milk of control group buffaloes was recorded to be 7.71% which was significantly higher than treatment group. It was concluded that nutrient supplementations, microclimate modifications and management alterations together in the form of one package help reducing heat stress.It has beneficial effect on physiological responses, total milk production and composition in lactating buffaloes during hot dry and hot humid summer without affecting cortisol, T_3 and T_4 profile.

Key words: Cortisol, Heat stress, Milk composition, Milk production, Nili-Ravi, Physiological response, T_3, T_4

Summer stress results in increased respiration frequency, pulse rate and rectal temperature which in turn impose higher energy demand upon the animal body (Hooda and Singh 2010). Heat stress in lactating buffaloes affect their milk production (Singh *et al*. 2005) and milk composition (El-Khashab 2010). Plasma cortisol, used as physiological markers of stress, is reduced during heat acclimatizaion and helps the animal in reducing heat production (Scott and Wiersma 1971). It elicits physiological adjustments, which enable animals to tolerate stressful conditions. Thyroxine (T_4) and triiodothyronin (T_3) also play important role to maintain basal metabolic rate in the body. Haeeb *et al.* (2000) found lowest T_3 level in buffaloes during summer resulting in decline in milk components. During thermal stress physiological and biochemical changes occurring in the animal body directly or indirectly affect the production (Ganaie *et al.* 2013). However, the characterisation of physiological and metabolic responses to heat is imperative for developing ameliorative strategies involving different methods for the improvement of production in heat-stressed ruminants (DiGiacomo 2011). Muller *et al.* (1986) pointed out that niacin supplementation through feed in lactating cows during summer increased milk yield. A significant decrease of pulse and respiration rates due to yeast supplementation was also reported in buffaloes by Singh *et al.* (2011). But, information on the effects of heat stress alleviation strategies used in the form of one package in lactating buffaloes is very limited. Therefore, an attempt was made to find out the effect of heat stress alleviation using a combination of strategies in the form of one package on physiological response, metabolic hormone profiles, milk production and its composition in lactating Nili-Ravi buffaloes during summer.

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MATERIALS AND METHODS

The present study was conducted at Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha (latitude, 30° 22’ 28” N and 76° 8’ 54” E), Patiala, Punjab, India. The ambient temperature reaches lowest near 1°C in winter and highest 45°C in summer. To find out the effect of heat stress alleviation in lactating buffaloes during hot-dry (HD; April to mid June) and hot-humid (HH; mid

June to August) seasons, 42 lactating Nili-Ravi buffaloes were selected from the Institute's herd and were uniformly divided into 2 groups i.e. 21 in each based on their lactation number, stage of lactation, body weight, dam's highest milk yield and milk yield in current lactation. One group was considered as treatment (T_1) and another as control (T_0). From each group 10 buffaloes were uniformly selected for physiological responses and blood sampling. All buffaloes from each group were considered for milk production and composition. The feeding, housing and management of buffaloes are presented in Table 1. Curtain covering prevented entry of hot air in the shed. Fogger fans and other ceiling fans improved air circulation in the shed and quick dissipation of heat from animal's body through evaporative cooling. Increased frequency of feeding and that too in cooler part of the day and mixed ration stimulated more feed and fodder intake and distributed heat production throughout. Mustard oil supplementation increased nutrient density, whereas, niacin and yeast helped to reduce fermentative heat production within the gut.

Both experimental group animals were fed individually. The farm prepared balance concentrate mixture was offered to all experimental buffaloes. Windows of T_1 group buffalo shed were covered with curtains to prevent entry of hot air. On the other hand, windows of T_0 group buffalo shed remained open. All buffaloes of T_1 and T_0 group were tied individually within the shed throughout 24 h except at the time of wallowing.

The minimum and maximum temperature within shed of T_0 and T_1 group were 17°C and 42°C, and 15°C and 38°C, respectively during hot dry (HD) period. The respective values during hot humid (HH) period were 20°C, 40°C, 19°C and 36°C. The average temperature humidity indexes (THI) of control group animal shed were recorded to be 82.75 and 91.18, and 83.40 and 86.40 at 9AM and 2 PM in HD and HH period, respectively. The respective values in treatment group animal shed were 78.84, 86.52, 79.48 and 84.16.

Physiological responses: The rectal temperature was recorded with the help of digital thermometer. Pulse rate was taken from coccygeal artery. Respiration rate was noted by observing the movement of flank region of buffaloes. All these parameters were recorded at 10 AM and 3 PM during starting of experiment and then at every week up to the end of the experiment.

Blood collection and sampling: After proper restraining and humane handling, blood was collected from jugular vein. Blood was collected into 10 ml BD vacutainer tube containing sodium heparin as anticoagulant. Four samples during HD period particularly when ambient temperature exceeded 40°C and 4 samples during HH period when ambient temperature exceeded 30°C were collected for analysis of plasma cortisol, T_3 and T_4. Samples from both groups were collected between 6 to 7 AM. Average interval between 2 samplings was about 12 days from the beginning to the end of seasons. ELISA kits were used to estimate plasma cortisol and RIA kits from BARC were used to estimate T_3 and T_4.

Milk production and composition: Daily morning and evening milk production were recorded for all experimental buffaloes. Per day average milk production were calculated in control and treatment group buffaloes. Morning and evening milk fat %, SNF %, lactose %, protein %, density, salt %, freezing point, pH were estimated at the beginning of experiment and then, fortnightly intervals up to the end of experiment with the help of automatic milk analyser.

Table 1. Feeding, housing and management regime of lactating Nili-Ravi buffaloes under different groups

<table>
<tr><th>Parameters</th><th colspan="2">Control group (T_0)</th><th colspan="3">Treatment group (T_1)</th></tr>
<tr><td>Microclimate modifications</td><td colspan="2">No mist fan
4 Ceiling fans
Two times wallowing
Shed not covered with curtains</td><td colspan="3">Fogger/mist fan
Addl. (6) ceiling fan
Addl. (2) washing with water
Shed covered with curtains</td></tr>
<tr><td>Management alterations</td><td colspan="2">Two times feeding</td><td colspan="3">Three times feeding
Feeding during cool hours</td></tr>
<tr><td>Nutrient supplementation</td><td colspan="2">Nil</td><td colspan="3">Niacin (6 g/buffalo/day), yeast (10 g/buffalo/day) and mustard oil (150 g/buffalo/day)</td></tr>
<tr><td rowspan="2">Concentratefeed</td><td rowspan="2">½ part provided during morning milking around 4 AM</td><td rowspan="2">½ part provided during evening milking around 4 PM</td><td colspan="2">½ part mixed with nutrient supplements</td><td rowspan="2">½ part (without nutrient supplements) mixed with green fodder and straw and provided around 12 noon</td></tr>
<tr><td>½ part provided during morning milking around 4 AM</td><td>½ part provided during evening milking around 4 PM</td></tr>
<tr><td>Water</td><td colspan="2">Clean, fresh and ad lib.</td><td colspan="3">Clean, fresh and ad lib.</td></tr>
</table>

Statistical analysis: All data of control (T_0) and treatment (T_1) group during HD and HH seasons are reported as means±SEM. Data were analysed by the method of analysis of contrast variables using the GLM (generalized linear model) procedures on analysis of variance for repeated measures using the Greenhouse-Geisser adjusted univariate significance tests (Little *et al.* 1998). The differences between treatment means were considered to be significant when P<0.05.

RESULTS AND DISCUSSION

The physiological responses of lactating Nili-Ravi buffaloes under hot dry and hot humid seasons are presented in Table 2. During HD season, the rectal temperature at 10 AM was 99.55°F in control group buffaloes and 98.66°F in treatment group. At 3 PM, both seasons had comparatively lower temperature in treatment group than control. At both time, treatment group buffaloes showed significantly (P<0.0001) lower rectal temperature than control group. Similar trend of pulse rate and respiration rate were also recorded among treatment and control group buffaloes under HD and HH seasons at both times (Table 2). The positive effect might be due to the cumulative effect of using different microclimatic modifications, nutrient supplementations and management alterations together. Thus, internal heat production as well as external heat load were reduced favouring improved physiological parameters.

Singh *et al.* (2005) found that in lactating Nili-Ravi buffaloes there were significant decline in rectal temperature, pulse rate and respiration rate when the buffaloes were either wallowed or splashed with water than the buffaloes not provided cooling. In another experiment on Nili-Ravi buffalo calves, Das *et al.* (2011) concluded that rectal temperature, pulse rate, and respiration rate were lower with the increase in number of washing frequency in hot environments.

Joshi and Tripahi (1991) noted an increase in rectal temperature from 102.0 °F to 103.8 °F when buffalo calves were exposed to 40.5 °C for 8 h daily for 3 months. A rise in rectal temperature up to 2.6 °C was recorded when they were exposed to direct sun rays in June and July. They concluded that the high rectal temperature in the heat stressed animals was the indicator of disturbance in the homeothermic status of the animals which was not being effectively countered by the enhanced heat loss by physical and physiological process of thermolysis. Similar finding was also put forth by Gudev *et al.* (2007) in lactating Bulgarian Murrah buffaloes.

Joshi *et al.* (1982) reported moderate increase in pulse rate during exposure to hot environment in Murrah buffaloes. In review, Ganaie *et al.* (2013) described that pulse rate increased with the increase in environment temperature in swamp buffaloes and the increasing trend in pulse rate continued even when the ambient temperature declined. It indicated that the physiological response of animals returned to its normal levels only after a definite period when animals were brought to comfort zone. Radadia *et al*. (1980) observed positive correlation between ambient temperature and respiration as well as pulse rate in lactating Murrah buffaloes. In an experiment, Singh *et al.* (2011) indicated that yeast supplementation in heat stressed buffaloes decreased respiration and pulse rate significantly and rectal temperature nonsignificantly.

Salem (1980) recorded an increase in respiration rate of buffaloes during summer compared to other seasons. There was no significant difference in rectal temperature of buffaloes during hot humid, warm and cold seasons but pulse rates were significantly (P<0.01) higher in warm followed by cold and hot humid seasons. The respiration rates were also significantly (P<0.01) different and highest during hot humid followed by warm and cold seasons. Reports of Das et *al*. (1999) and Joshi and Tripathy (1991) in Murrah buffalo calves also supported this finding. Rectal temperature, pulse rate and respiration rate increased significantly (P<0.05) in buffalo heifers after exposure to the thermal stress (40°C) than those not exposed to that stress (Hooda and Singh 2010). Vijoykumar (2005) in buffalo heifers and Gudev *et al.* (2007) in buffaloes also reported increased respiration rate resulting from exposure of animals into hot environment.

Table 2. Physiological responses of lactating Nili-Ravi buffaloes under hot dry and hot humid seasons in different groups

Parameters	Time	Season	Control group	Treatment group	SEM	P value
Rectal temperature (°F)	10 AM	HD	99.55	98.66	0.239	<0.0001
		HH	99.84	98.86	0.196	<0.0001
	3PM	HD	100.91	99.71	0.213	<0.0001
		HH	101.39	99.98	0.172	<0.0001
Pulse rate (beats/ min)	10 AM	HD	52.08	45.60	0.782	<0.0001
		HH	59.51	50.46	0.653	<0.0001
	3PM	HD	60.20	51.72	0.905	<0.0001
		HH	68.12	57.82	0724	<0.0001
Respiration rate(times / min)	10 AM	HD	26.17	22.00	0.653	<0.0001
		HH	29.80	24.07	0.592	<0.0001
	3PM	HD	33.60	26.70	0.764	<0.0001
		HH	37.82	29.38	0.694	<0.0001

HD, Hot dry; HH, hot humid.

Table 3. Plasma hormones of lactating Nili-Ravi buffaloes under hot dry and hot humid seasons in different groups

Parameters	Time	Control group	Treatment group	SEM	P Value
Cortisol	HD	3.93	4.64	1.81	0.456
	HH	1.62	1.66	0.75	0.959
T_3	HD	0.45	0.53	0.101	0.106
	HH	0.57	0.55	0.058	0.595
T_4	HD	10.84	10.23	0.601	0.310
	HH	9.59	9.21	0.490	0.442

HD, Hot dry; HH, hot humid.

Plasma cortisol, T_3 and T_4 concentrations of control and treatment group under hot dry and hot humid seasons are presented in Table 3. Plasma cortisol concentrations were slightly higher in treatment group buffaloes than that in control under both seasons. No statistically significant difference between 2 groups was recorded. The temperature difference between control and treatment group buffaloes might be insufficient to evoke response for these hormones. In other way, the blood samples were taken for hormone estimation in the morning hours when the microclimate was comfortable and remained similar for both groups as no modifications were done at that time. Abily *et al.* (1975) observed that cortisol level decreased during prolonged heat exposure after a temporary increase at the beginning of heat stress. Decline in cortisol in heat stressed lactating buffaloes during July might be responsible for decline in milk components. Marai and Haeeb (2010) concluded that plasma cortisol level increases during acute heat stress and decreases during chronic phase. The former is attributed to the fact that glucocorticoid hormones have hyperglycaemic action through the gluconeogenesis process resulting in more glucose formation in heat stressed animals. Whereas, the later is attributed to the fact that cortisol is thermogenic in animals and the reduction of adrenocortical activity under thermal stress is a thermoregulatory protective mechanism preventing a rise in metabolic heat production in hot environment. Dwaraknath *et al.* (1984) pointed out that due to variation of cortisol concentration in calves, the effect of hot climate had conflicting results on cortisol. Contrary to these reports, significantly lower cortisol concentrations in buffalo heifers (Vijoykumar 2005) and in lactating buffaloes (El-Khashab 2010) were recorded when buffaloes were provided cooling than those not provided cooling.

In this experiment, plasma T_3 concentration did not show any particular trend for both groups. In lactating buffaloes, plasma T_3 concentration decreased significantly (P< 0.01) by 17.2% with the increased ambient temperature from 17.5 to 37.1°C (Haeeb *et al.* 2000). Similar finding was also reported in cows by Pereira *et al.* (2008).

Plasma T_4 concentration was little bit higher in control group buffaloes than treatment group during both seasons. But, the values did not differ significantly between 2 groups. Dwaraknath *et al.* (1984) estimated lower plasma T_4 concentration in buffalo bull under high ambient temperature.

The mean daily milk production was 6.9 kg / buffalo in control group whereas 8.1 kg in treatment group. Milk production was significantly (P<0.0001) higher in T_1 group than T_0 group during both seasons from the start to the end of experiment. Singh *et al.* (2005) obtained higher milk yield from Nili-Ravi buffaloes through providing cooling. The report of El-Khashab (2010) on milk yield also supported the present finding. Milk production during summer was higher in niacin supplemented group than non-supplemented (Muller *et al.* 1986).

The average total fat and SNF production in treatment group was higher in comparison to control group (15.40 kg and 19.99 kg). The production differences of both fat and SNF were also statistically (P<0.015 and P<0.0001) significant between 2 experimental groups (Tables 4, 5).

The overall fat per cent in milk (Table 6) of control group buffaloes was significantly (P<0.017) higher than treatment group. During HD season, average milk fat % was recorded as 7.60% in control group and 7.32% in treatment group. The respective values in HH season were 7.83% and 7.15% and the difference was statistically significant (P<0.007) between the groups. Considering the morning and evening session, under both seasons control group produced slightly higher milk fat per cent than treatment group.

Considering season, morning and evening milking sessions, treatment group buffaloes produced slightly higher lactose and density than control group buffaloes. Under HH season, differences between 2 experimental groups were statistically significant (Table 6). The results (Table 6)

Table 4. Total fat production (kg) of Nili-Ravi buffaloes under different groups

Month	Control gp	Treatment gp	SEM	P value		
				Group	Day	Group × day
1	16.85	18.24	0.840	0.015	0.045	0.469
2	15.96	16.67				
3	14.96	17.05				
4	14.22	16.90				
5	14.72	16.95			L 1 vs. Later	
Overall	15.40	17.16			(0.003)	

Table 5. Total SNF production (kg) of Nili-Ravi buffaloes under different groups

Month	Control gp	Treatment gp	SEM	P value		
				Group	Day	Group × day
1	22.50	25.19	1.225	<0.0001	0.042	0.138
2	20.33	22.02				
3	19.21	22.99				
4	18.86	25.14				
5	19.03	23.84			L 1 vs. Later	
Overall	19.99	23.84			(0.002)	

Table 6. Milk composition of Nili-Ravi buffaloes under hot dry and hot humid seasons under different groups

Parameters	Season	Control group	Treatment group	SEM	P Value
Av. milk fat %	HD	7.60	7.32	0.156	0.233
	HH	7.83	7.15	0.222	0.007
-Morning	HD	7.84	7.54	0.188	0.200
	HH	7.90	7.29	0.262	0.020
-Evening	HD	7.36	7.20	0.193	0.506
	HH	7.75	6.95	0.234	0.003
Av. Milk SNF %	HD	9.90	9.84	0.093	0.487
	HH	10.06	10.04	0.086	0.834
-Morning	HD	9.82	9.74	0.114	0.421
	HH	10.06	10.13	0.094	0.524
-Evening	HD	10.01	9.92	0.124	0.371
	HH	10.05	9.46	0.113	0.338
Milk lactose (%)	HD	4.37	4.43	0.055	0.261
	HH	4.41	4.61	0.057	<0.0001
-Morning	HD	4.25	4.29	0.066	0.398
	HH	4.39	4.60	0.077	0.003
-Evening	HD	4.50	4.57	0.074	0.221
	HH	4.44	4.62	0.086	0.002
Milk protein (%)	HD	3.63	3.61	0.031	0.548
	HH	3.68	3.69	0.032	0.849
-Morning	HD	3.61	3.58	0.037	0.444
	HH	3.69	3.72	0.033	0.446
-Evening	HD	3.66	3.65	0.047	0.736
	HH	3.69	3.66	0.041	0.345
Milk density	HD	29.92	31.08	3.655	0.219
	HH	30.21	31.05	0.347	0.011
-Morning	HD	29.25	29.39	0.377	0.647
	HH	30.05	31.05	0.633	0.020
-Evening	HD	30.60	32.76	7.368	0.256
	HH	30.37	31.07	0.397	0.054
Milk pH	HD	6.35	6.33	0.037	0.528
	HH	6.44	6.46	0.021	0.586
-Morning	HD	6.30	6.33	0.076	0.469
	HH	6.42	6.42	0.029	0.868
-Evening	HD	6.39	6.33	0.038	0.036
	HH	6.48	6.50	0.023	0.226
Milk salts (%)	HD	0.81	0.80	0.007	0.580
	HH	0.82	0.82	0.007	0.911
-Morning	HD	0.80	0.79	0.010	0.424
	HH	0.82	0.82	0.007	0.397
-Evening	HD	0.81	0.81	0.010	0.826
	HH	0.82	0.81	0.010	0.323
Milk freezing	HD	0.56	0.56	0.007	0.516
pt.(-°C)	HH	0.57	0.57	0.021	0.279
-Morning	HD	0.54	0.55	0.007	0.757
	HH	0.56	0.58	0.033	0.282
-Evening	HD	0.57	0.58	0.010	0.395
	HH	0.57	0.57	0.040	0.748

HD, Hot dry ; HH, hot humid.

envisaged that SNF, protein, freezing point, salts and pH showed no statistically significant differences between 2 experimental groups. Considering season, time of milking, treatment group buffaloes produced slightly higher lactose and dense milk than control group buffaloes. Whereas, the overall SNF, protein, salts and pH values were almost similar in both groups even when season and milking time were taken into account separately.

Singh *et al.* (2005) did not find any significant difference in milk composition (fat, SNF and total solids) between heat stressed and protected Nili-Ravi buffaloes. Fat and protein percentages were comparatively higher in cows provided cooling than those were not cooled (Dupreez 2000). Similar findings were also reported by El-Khashab (2010) in buffaloes. Dupreez (1994) recorded that heat stress reduced butter fat by 20–40%, non-fat solids by 10–20% and total milk protein by 10–20%.

From the experiment it was concluded that nutrient supplementation, microclimate modifications and management alterations together in the form of one package help reduce heat stress. It has beneficial effect on physiological responses, total milk production and compositions in lactating buffaloes during hot dry and hot humid summer months without affecting plasma cortisol, T_3 and T_4 profile.

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REFERENCES

Abilay T A, Johnson H D and Madan M. 1975. Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycle. *Journal of Dairy Science* **58**: 1836–40.

Das S K, Upadhyay R C and Madan M L. 1999. Heat stress in Murrah buffalo calves. *Livestock Production Science* **61**: 71–78.

Das K S, Singh G, Paul S S, Mailk R, Oberoi and Deb S M. 2011. Physiological responses and performance of Nili-Ravi buffalo calves under different washing frequency during hot summer months in tropics. *Tropical Animal Health and Production* **43**(1): 35–39.

DiGiacomo K. 2011. 'The physiological and metabolic responses to heat in ruminants.' Ph.D Thesis, Melbourne School of Land and Environment-Agriculture and Food Systems, University of Melbourne.

Dupreez J H. 1994. 'The role of heat stress in the performance of dairy cattle in southern Africa.' Ph.D. Thesis, University of Pretoria.

Dwaraknath P I C, Agarwal S P, Agarwal V K, Dixit N I C and Sharma I J. 1984. Hormonal profiles in buffalo bulls. I . The use of nuclear techniques to improve domestic buffalo production in Asia. *Proceedings of isotope and radiation* *applications of agricultural development*. Manila, Philippines.

El-Khashab Mona A. 2010. Physiological and productive responses to amelioration of heat stress in lactating buffaloes under hot summer conditions in Egypt. Available at: http: // www.spsa-egy.org/?p=835.

Ganaie A H, Shanker G, Bumla N A, hasura R S, Mir N A, Wani S A and Dudhatra G B. 2013. Biochemical and physiological changes during thermal stress in bovines. *Journal of Veterinary Science and Technology* **4**: 126.

Gudev D, Popova-Ralcheva S, Moneva P, Aleksiev Y, Peeva T, Ilieva and Penchev P. 2007. Effect of heat stress on some physiological and biochemical parameters in buffaloes. *Italian Journal of Animal Science* **6** (suppl. 2): 1325–28.

Habeeb A A M, Ibrahim M K and Yousef H M. 2000. Blood and milk contents of triiodothyronine (T3) and cortisol in lactating buffaloes and changes in milk yield and comosition as a function of lactation number and ambient temperature. *Arab. Journal of Nuclear Science and Applications* **33**: 313–22.

Hooda O K and Singh G. 2010. Effect of thermal stress on feed intake, plasma enzymes and blood biochemicals in buffalo heifers. *Indian journal of Animal Nutrition* **27**: 122–27.

Joshi B C and Tripathi K C. 1991. Heat stress effect on weight gain and related physiological responses of buffalo calves. *Journal of Veterinary Physiology and Allied Sciences* **9**: 33–43.

Joshi B C, Joshi H B, Guha S and Ahmad M S. 1982. Physiological responses of Murrah buffalo heifers to hot arid and hot humid microenvironment. *Journal of Veterinary Physilogy & Allied Sciences* **1**: 34–40.

Little R C, Henry P R and Ammerman C B. 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science* **76**: 1216–31.

Marai I F M, Daader A M, Abdel-Samee A M and Ibrahim H. 1997. Lactating Friesian and Holstein cows as affected by heat stress and combination of amelioration techniques under Egyptian conditions. *Proceedings of the International Conference on Animal, Poultry, Rabbits and Fish Production and Health*. Cairo, Egypt.

Marai I F F and Haeeb A A M. 2010. Buffalo's biological functions as affected by heat stress- A review. *Livestock Science* **127**: 89–109.

Muller L D, Heinrichs A J, Cooper J B and Atkin Y H. 1986. Supplemental niacin for lactating cows during summer feeding. *Journal of Dairy Science* **69**: 1416–20.

Pareira A M F, Baccari Jr. F., Titto E A L and Almeida J A A. 2008. Effect of thermal stress on physiological parameters, feed intake and plasma thyroid hormones concentration in Alentejana, Mertolenga, Frisian and Limousine cattle breeds. *International Journal of Biometeriology* **52**: 199–208.

Radadia N S, Sastry N S R, Pal R N and Juneja I J. 1980. Studies on the effect of certain summer managemental practices on lactating Murrah buffaloes. 3. Physiological reactions and some attributes of blood. *Haryana Agricultural University Journal of Research* **10**: 442–47.

Salem I A. 1980. Seasonal variations in some body reactions and blood constituents in lactating buffaloes and Friesian cows with reference to acclimatization. *Journal of the Egyptian Veterinary Medical Association* **40**: 63–72.

Singh G, Kamboj M L and Patil N V. 2005. Effect of thermal protective measures during hot humid season on productive and reproductive performance of Nili-Ravi buffaloes. *Indian Buffalo Journal* **3**: 101–04.

Singh J K. 1998. 'Seasonal effect on physiological and endocrine changes during estrous cycle in buffaloes.' Ph.D. Thesis, National Dairy Research Institute, Karnal, Haryana.

Singh S P, Hooda O K and Kumar P. 2011. Ffect of yeast supplementation on feed intake and thermal stress mitigation in buffaloes. *Indian Journal of Animal Sciences* **81**(9): 961–64.

Stott G H and Weiersma F. 1971. Plasma corticosteroids as indicators of gonadotrophin secretion and infertility in stressed bovine. *Journal of Dairy Science* **53**: 652 (Abstract).

Vijoykumar P. 2005. 'Effect of thermal stress management on nutritional, physiological and behavioural responses of buffalo heifers.' Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, India.