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Effect of heat stress amelioration during dry period on biological responses, proinflammatory cytokines and milk production in subsequent lactation of Surti buffaloes

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ABSTRACT

Present study was conducted on 12 dry Surti buffaloes to assess the effect of heat stress amelioration during dry period on their biological responses and milk production in subsequent lactation. Buffaloes were divided into control (n=6) and treatment (n=6) groups. Treatment buffaloes were housed only during their dry period in shed having wall-mounted fans, roof whitewashed with microfine lime powder and open paddock covered with 75% green net. At -8, -3, +1 and +3 weeks of calving, blood collection and recording of observations (physiological, skin thermography and BCS) were done. Milk composition and daily milk yield were recorded at every 2 weeks up to 11 weeks of lactation, respectively. Shed modification was successful in lowereing air temperature and THI indicating thermal comfort for treatment buffaloes as also revealed by lower temperatures of rectum, tympanic membrane, surface like forehead, eye, udder, coronet and lower respiration rate. Treatment group maintained significantly higher BCS, glucose, total protein, GSH, TAS, *in vitro* lymphocyte proliferation, neutrophil phagocytic activity and lower levels of urea, creatinine, NEFA, BHBA, SOD, MDA, cortisol, TNF-α, IL-1β, IL-6 and HSP70. These results are indicative of minimum negative energy balance, stress and higher immune response. Milk yield and fat were higher in treatment group. Providing thermal comfort to buffaloes in dry period helped in abating the negative effects of heat stress not only during dry period but also during subsequent lactation along with improved milk yield.

Keywords: Biological responses, Dry period, Heat stress, Lactation, Surti buffaloes

Solar radiations are of paramount importance for heat stress and curtailing it in open paddock with green net and roof modification can reduce animals' heat load. Surti buffaloes are found in region of climate typical for tropical-coastal region. Temperature is high in summer and monsoon with summer being dry and monsoon humid. Hence animals in this area experience heat stress during summer as well as to some extent in monsoon.

Change in rectal temperature (RT) and respiration rate (RR) occurs during heat stress but measurement of RT is invasive. Alternatively, tympanic temperature (TT) and skin temperature by infrared thermography can be measured. TT reflects RT. Infrared thermography non-invasively measures skin surface temperature that depends on ambient surrounding (Yadav *et al.* 2017), cutaneous blood circulation and thermal gradient between core and periphery of body. Feed intake reduces to lower metabolic heat during thermal load but high metabolic demands creates negative energy balance elevating NEFA and BHBA. Heat induced oxidative stress increases proinflammatory cytokines (TNF-α), interleukins and proteins like HSP70. Variation also occurs in serum T_3, T_4, cortisol and metabolites of energy as well as protein metabolism.

Simultaneous to dry period, maximum growth of fetus and involution as well as proliferation of mammary gland occurs. Dry period is neglected for active management and is crucial when it occurs during summer months. Negative and deleterious effects of heat stress during dry period accumulate and compromises animals' ability to rejuvenate from previous lactation, diminishes its capability to tackle stress of calving and early lactation. Heat stress during the dry period reduces milk yield in the next lactation (Fabris *et al.* 2020). Hence ameliorating heat stress during dry period can be beneficial.

Thus, present study was planned with the objective to study effect of ameliorating heat stress during dry period on physiological, skin surface temperature, biochemical, immune responses and milk production during subsequent lactation in Surti buffaloes.

MATERIALS AND METHODS

The study was approved by Institutional Animal Ethics Committee vide letter no. NAU/NVC/IAEC/13/2019 Dt 01/06/2019.

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Selection of animals: Twelve dry Surti buffaloes (approx 7 months pregnant) with average body weight 426±4.04 kg, body condition score (BCS) of 3.88±0.09 and milk yield of 938.62±40.16 litres of previous lactation were randomly selected from LRS, NAU, Navsari and divided into control (n=6) and treatment (n=6) groups.

Housing modifications: Modified treatment shed was ventilated with fans, had one-third of the open paddock covered with 75% green net at a height of 10 feet and roof of covered area whitewashed with microfine lime powder. Control shed was kept without any modification and it comprised of maximum area under open paddock with covered standing area.

Collection of meteorological data and sample: Temperature and relative humidity of the sheds were recorded and temperature humidity index (THI) was calculated (Tucker *et al*. 2008). Measurement of physiological parameters (RT, RR and TT), infrared thermography of body surface, collection of whole blood, body condition score (Edmonson *et al.* 1989) were performed at -8 week (wk) -3wk, +1wk and +3wk. Milk samples for composition was collected at 1, 3, 5, 7, 9 and 11 wk of subsequent lactation. Daily milk yield was also recorded up to week 11.

Sample analysis: Whole blood was used for oxidative stress parameters such as lipid peroxidation (LPO; Rehman 1984), reduced glutathione (GSH; Moron *et al*. 1979) and superoxide dismutase (SOD; Madesh and Balasubramanian 1998). Lymphocyte proliferation assay stimulation index (LPASI) by MTT assay (Mosmann 1983) and neutrophil phagocytic activity (NPA) by NBT reductive method (Chai *et al*. 2005) were done. Plasma was used for analysis of total antioxidant status (TAS; Benzie and Strain 1999) and non-esterified fatty acid (Shipe *et al*. 1980). Serum was used for analysis of biochemical parameters (Randox kits), hormones and cytokines. Standard kits for hormones (Labor Diagnostica Nord GmbH & Co. KG, Nordhorn), proinflammatory markers (MyBioSource) and TNF-α (Cloud-Clone Corp. CCC, USA) were used. Milk composition was analysed using fully automated milkoscan FT1 milk analyser (FOSS, Slangerupgade 69, Denmark).

Statistical analysis: Data analysis was done using one way analysis of variance and differences between means were compared using Duncan multiple range test at significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Results are mentioned for meteorological conditions of sheds; physiological responses and skin surface temperatures of different body regions; blood biochemical parameters; oxidative stress parameters, stress hormones and cytokines in Tables 1 to 4; for immune responses, body condition scoring and milk composition in Supplementary Tables 1 to 3; for average daily milk yield and total milk yield in Supplementary Fig. 1 and 2 respectively.

Meteorological parameters: Reduction ($p \leq 0.05$) in daily average, minimum and maximum ambient temperature, relative humidity and THI in treatment shed indicated that modification of shed (white washing of roof with microfine lime powder and ventilation by fans) adopted for heat amelioration was successful. There was reduction of mean temperature, relative humidity and THI by approximately 2°C, 4.5% and 4 units in 24 h and about 4°C, 7.5% and 7 units during of only day time respectively (Table 1). Similar observations have been made in other studies. THI more than 75 is associated with severe heat stress that decreases production performance (De Rensis *et al*. 2015).

THI of 75-78 indicates mild heat stress, 79-83 indicates medium and >84 indicates severe heat stress for HF cows (Collier *et al.* 2011). THI interpretation varies with breed and adaptability to local climatic conditions so references specific for Indian conditions is lacking.

Physiological responses: Significantly ($p \leq 0.05$) reduced RT at +1wk, RR and TT at -3 wk, +1wk and +3wk in treatment buffaloes indicated thermal comfort (Table 2). Change in RT is gradual and whereas change in RR and TT may be comparatively immediate. In non-lactating phase, metabolic rate is at basal level and does not fluctuate much so any variation observed during this period is mainly due to ambient meteorological condition. Similarly, in Murrah buffaloes, increased RT and RR was observed by Lakhani *et al.* (2018). This indicated that during heat stress there is coherent rise of RT, RR and TT with increase in air temperature and THI.

Surface temperature of different body regions: Higher ($p \leq 0.05$) temperature of forehead and udder at +1wk and eye as well as coronet at +3wk in control buffaloes may be due to their inability to regulate core body temperature thereby establishing steeper thermal gradient between core body and periphery (Table 2). Similar results have reported in other study (Gómez *et al.* 2018).

Biochemical metabolites: In control group, serum metabolites associated with energy metabolism such as glucose and total cholesterol were significantly ($p \leq 0.05$) lower at - 3wk, +1wk and +3wk while NEFA and BHBA that serve as indicator of increased lipolysis (due to energy deficit) were significantly ($p \leq 0.05$) higher at +1wk and +3wk in blood of control buffaloes (Table 3).

Increased metabolic demand due to heat stress in control buffaloes might have overutilized glucose and total cholesterol, thereby diminishing their levels during prepartum that was further intensified at parturition and early lactation. Prior exposure to heat stress followed by parturition stress might have led to NEB.

NEFA being precursor for BHBA in ruminants showed concomitant rise. Excess protein catabolism due to increased metabolic demand depleted protein reserves to significantly ($p \leq 0.05$) lower levels at -3wk, +1wk and +3wk in control buffaloes with simultaneous significant ($p \leq 0.05$) increase in urea and creatinine (Table 3). Higher metabolic rate in control group also led to significantly ($p \leq 0.05$) increased liver enzyme GGT at +1wk and +3wk. Similar effects due to heat stress have been reported for glucose, cholesterol, BUN, creatinine, NEFA (Chaudhary

Table 1. Meteorological conditions (Mean±SE) of sheds during dry period of Surti buffaloes

	Daily avg		Daily minimum		Daily maximum		Day avg		Night avg	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Temp (°C)	31.52^{b}±0.16	29.24^{a}±0.02	22.65^{a}±0.14	23.30^{b}±0.03	41.41^{b}±0.17	36.92^{a}±0.05	36.62^{b}±0.18	32.71^{a}±0.09	26.42^{b}±0.15	25.77^{a}±0.08
RH (%)	74.94^{b}±1.05	70.16^{a}±1.38	53.26±1.26	43.77±1.53	92.43^{b}±0.66	88.96^{a}±1.03	64.57^{b}±1.12	57.07^{a}±1.57	85.30±1.02	83.25±1.20
THI	84.53^{b}±0.40	80.29^{a}±0.23	69.04±0.25	69.01±0.17	104.53^{b}±0.41	96.06^{a}±0.22	90.19^{b}±0.46	83.22^{a}±0.42	77.83^{b}±0.35	76.49^{a}±0.10

Mean bearing different superscript across row (a, b) differ significantly ($p \leq 0.05$) between groups.

Table 2. Physiological parameters and skin surface temperature by infrared thermography (Mean±SE) during dry period and subsequent lactation of Surti buffaloes

Parameter	-8 wk		- 3 wk		+1 wk		+3 wk	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
RT(°F)	101.52±0.17	101.47±0.28	101.77±0.21	101.78±0.13	102.37^{b}±0.10	101.68^{a}±0.16	102.16±0.16	101.73±0.21
RR (/min)	30.00±1.71	32.67±1.91	40.00^{b}±1.03	36.00^{a}±1.46	44.00^{b}±1.46	36.00^{a}±1.46	41.33^{b}±0.84	30.67^{a}±0.84
TT (°F)	102.10±0.28	102.78±0.19	102.87^{b}±0.17	102.13^{a}±0.12	103.20^{b}±0.16	102.13^{a}±0.12	103.03^{b}±0.11	102.35^{a}±0.27
Forehead (°C)	32.65±0.40	33.23±0.54	34.95±0.23	34.73±0.38	34.92^{b}±0.20	34.07^{a}±0.29	34.50±0.44	34.40±0.14
Eye (°C)	35.50±0.58	35.88±0.25	36.83±0.20	37.13±0.28	36.45±0.18	36.35±0.12	36.67^{b}±0.15	35.78^{a}±0.27
Left side of body (°C)	33.58±0.31	33.30±0.31	35.35±0.40	34.95±0.44	36.00±0.39	34.83±0.40	36.07±0.51	34.85±0.50
Right side of body (°C)	33.43±0.26	33.40±0.24	35.47±0.42	35.40±0.48	35.78±0.34	34.97±0.55	35.82±0.65	34.98±0.51
Udder (°C)	34.58±0.45	34.92±0.31	35.72±0.39	35.82±0.35	36.85^{b}±0.29	35.83^{a}±0.28	36.38±0.43	35.73±0.38
Teat (°C)	30.95±0.50	31.65±0.42	34.70±0.27	34.27±0.57	35.10±0.51	34.28±0.75	34.28±0.62	34.55±0.66
Coronet (°C)	30.43±0.45	30.78±0.17	31.30±0.53	30.85±0.54	32.12±0.63	30.47±0.44	32.22^{b}±0.45	30.57^{a}±0.55

Mean bearing different superscript across row (a, b) differ significantly ($p \leq 0.05$) between groups. '-' prepartum; '+' postpartum.

Table 3. Blood biochemical parameters, stress hormones and cytokines (Mean±SE) during dry period and subsequent lactation of Surti buffaloes

Parameter	-8wk		- 3wk		+1wk		+3wk	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Glucose (g/dl)	38.17±0.98	38.02±0.89	42.98^{a}±0.54	50.34^{b}±0.71	43.77^{a}±0.19	49.79^{b}±0.64	37.36^{a}±0.77	41.95^{b}±0.63
Total Protei (mg/dl)	6.23±0.09	6.31±0.22	6.21^{a}±0.09	7.35^{b}±0.10	5.84^{a}±0.11	6.97^{b}±0.03	6.09^{a}±0.09	6.94^{b}±0.04
Creatinine (mg/dl)	0.92±0.02	0.87±0.02	0.94^{b}±0.01	0.78^{a}±0.01	1.23^{b}±0.04	0.91^{a}±0.04	1.17^{b}±0.02	0.95^{a}±0.02
Urea (mg/dl)	29.20±0.58	31.19±0.56	30.20^{b}±0.50	25.59^{a}±0.33	40.58^{b}±0.73	31.87^{a}±0.78	36.22^{b}±1.17	30.21^{a}±0.86
Total Cholesterol (TC) (mg/dl)	98.40±1.70	98.56±1.76	98.61^{a}±1.13	107.02^{b}±2.18	84.96^{a}±1.44	96.79^{b}±0.55	97.08^{a}±1.82	103.77^{b}±1.07
GGT (U/L)	15.86±0.45	16.76±0.74	17.19±0.78	16.23±0.45	22.77^{b}±0.82	19.88^{a}±0.47	19.71^{b}±0.14	18.66^{a}±0.35
Calcium (mg/dl)	8.35±0.14	8.42±0.21	8.45^{b}±0.05	7.95^{a}±0.05	6.75±0.13	7.07±0.07	6.32^{a}±0.15	7.04^{b}±0.05
Phosphorus (mg/dl)	5.08±0.21	4.92±0.19	5.56^{b}±0.03	5.28^{a}±0.03	4.44^{a}±0.08	4.67^{b}±0.04	4.71±0.18	5.04±0.09
Mg (mg/dl)	2.35±0.15	2.40±0.17	2.11^{b}±0.01	2.03^{a}±0.01	2.51^{a}±0.03	2.65^{b}±0.01	2.35±0.01	2.35±0.02
BHBA (mmol/L)	0.50±0.02	0.47±0.02	0.25±0.01	0.25±0.03	0.63^{b}±0.02	0.53^{a}±0.00	0.56^{b}±0.00	0.50^{a}±0.02
NEFA (mmol/L)	0.28±0.03	0.31±0.02	0.25±0.01	0.28±0.02	0.60^{b}±0.00	0.54^{a}±0.00	0.49^{b}±0.00	0.44^{a}±0.00

Mean bearing different superscript across row (a, b) differ significantly ($p \leq 0.05$) between groups.'-' prepartum; '+' postpartum.

Table 4. Oxidative stress parameters, stress hormones and cytokines (Mean±SE) during dry period and subsequent lactation of Surti buffaloes

Parameter	-8wk		- 3wk		+1wk		+3wk	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
TAS (µmol/L)	230.82±8.73	229.16±4.36	253.37^{a}±3.41	279.91^{b}±8.84	235.92^{a}±2.49	276.29^{b}±3.27	248.50^{a}±4.15	301.40^{b}±4.19
SOD (U/mg of Hb)	3.02±0.05	3.06±0.12	4.74^{a}±0.05	4.17^{b}±0.02	6.24^{b}±0.08	4.66^{a}±0.07	5.47^{b}±0.13	4.31^{a}±0.11
LPO (nm MDA)	7.06±0.20	6.85±0.15	6.99^{a}±0.07	5.43^{b}±0.28	9.78^{b}±0.11	7.84^{a}±0.11	8.68^{b}±0.06	7.01^{b}±0.05
GSH (mg/dl)	7.79±0.23	7.84±0.33	6.07^{a}±0.07	6.70^{b}±0.08	5.03^{a}±0.03	6.11^{b}±0.04	5.44^{a}±0.06	6.16^{b}±0.05
Cortisol (ng/ml)	17.07±0.85	17.10±0.77	15.24±0.95	14.82±1.38	26.16^{b}0.99	17.67^{a}±0.74	19.33^{b}±0.82	16.40^{a}±0.54
T_3 (ng/L)	265.65±9.59	273.04±12.69	257.73±13.27	231.48±16.97	460.87^{b}±20.37	324.79^{a}±14.27	405.64^{b}±19.53	347.32^{a}±6.70
T_4 (ng/ml)	42.24±1.03	43.89±1.79	41.12±1.80	41.88±2.61	48.01^{b}±1.93	41.09^{a}±0.82	54.84^{b}±2.63	39.39^{a}±0.85
TNF-α (pg/ml)	97.67±4.64	100.48±2.97	88.28±5.08	84.32±7.23	186.99^{b}±5.04	135.64^{a}±4.50	143.39^{b}±4.46	127.05^{a}±3.49
IL-1β (pg/ml)	860.06±22.72	832.79±45.14	881.13±35.49	907.64±46.56	877.46^{b}±33.24	658.79^{a}±19.10	816.47^{b}±10.32	740.40^{a}±20.07
IL-6 (pg/ml)	163.49±12.90	143.60±13.58	186.10±15.08	162.75±10.18	241.32^{b}±19.91	166.48^{a}±13.77	202.25±7.51	188.24±18.45
HSP70 (pg/ml)	452.58±17.11	447.96±17.71	357.29±39.06	339.02±28.17	291.71^{b}±29.34	152.90^{a}±11.39	198.31±18.02	163.99±14.20

Mean bearing different superscript across row (a, b) differ significantly ($p \leq 0.05$) between groups. '-' prepartum; '+' postpartum.

et al. 2015); for plasma proteins (Hooda and Upadhyay 2014); BHBA (Fan *et al.* 2018) and GGT (Moreira *et al.* 2015).

Oxidative stress parameters: Buffaloes kept in control shed had significantly ($p \leq 0.05$) higher levels of SOD and MDA (lipid peroxidation) and significantly ($p \leq 0.05$) lower levels of TAS and GSH at -3wk, +1wk and +3wk (Table 4). SOD being an inducible enzyme increased due to heat stress. Detrimental effects of heat stress inflict cell membrane damage by lipid peroxidation causing MDA levels to rise. It can further be reasoned that to counter balance increased oxidative stress, antioxidants such as reduced GSH and TAS were depleted in control group. Similar findings of increase in SOD and LPO (Chaudhary *et al.* 2015) during heat stress have been reported. Lowered TAS and GSH levels (Chaudhary *et al.* 2019) have been reported in Surti buffaloes.

Stress hormones and proinflammatory cytokines: Stress related markers like cortisol, T_3, T_4 and IL-1β at +1wk and +3wk as well as IL-6 and HSP70 at +3wk were significantly ($p \leq 0.05$) higher in control buffaloes (Table 4). Heat stress causes activation of hypothalamo-hypohyseal-adrenal axis leading to increase in cortisol secretion. Chaudhary *et al.* (2015) have observed increased cortisol in Surti buffaloes with increase in THI. HSP70 rise has been observed during summers (Manjari *et al.* 2015) in Tarai buffaloes. During stress, metabolism occurs at a higher rate with concurrent higher levels of serum T_3 and T_4. Chaudhary *et al.* (2015) have also reported a significant rise in T_3 levels due to heat stress at higher THI but they found non-significant difference for T_4.

Proinflammatory cytokines IL-1β, IL-6 and TNF-α were higher during postpartum and early lactation in control that had prior exposure to heat stress in dry period. Presence of these biomolecules in their protein forms results from their respective mRNA expression. Relative mRNA expression of IL-6, IL-1β and TNF-α has been reported to be higher in the summer season throughout the study period (Grewal *et al.* 2019). Increase in plasma TNF-α and IL-6 were reported in dairy cows under long term heat stress (Min *et al.* 2016).

Immune response: Higher ($p \leq 0.05$) LPASI and NPA in treatment buffaloes at -3wk, +1wk and +3wk implies higher proliferation capability of lymphocytes on antigenic stimulation and higher potential of primary non-specific immune response respectively indicating robust immune health (Supplementary Table 1). Enhancement of proliferation of B and T cell as well as phagocytic activity of neutrophils by heat ameliorative strategies (Kumar *et al.* 2015) implies the adverse effect of heat stress on neutrophils and lymphocytes.

Body condition score: Utilization of body reserves during previous lactation was most likely the cause of low BCS at -8wk (Supplementary Table 2). Postpartum loss of BCS occurs due to increased metabolic demands at calving and early lactation. Lower ($p \leq 0.05$) BCS in control buffaloes at +1wk and +3wk could have occurred as heat stress reduces appetite and diminishes nutrient availability causing loss of body weight and occurrence of negative energy balance (Rhoads *et al*. 2009) along with fall in BCS (Collard *et al*. 2000, Rhoads *et al*. 2011).

Milk composition: Higher ($p \leq 0.05$) fat and lactose at wk7, 9 and 11, SNF at wk9 and 11 and lower ($p \leq 0.05$) free fatty acids and urea at wk9 and 11 of lactation in treatment buffaloes (Supplementary Table 3) corroborates with the results of Ahmad *et al.* (2019) in Nili-Ravi buffaloes.

Milk yield: At wk 8, average daily milk yield in treatment was 28.08% higher ($p<0.05$) as well as at peak that was earlier than peak of control buffaloes at wk 11 (Supplementary Fig. 1 and 2). Total milk by treatment buffaloes (321.93 kg) was 17.83% higher than control (273.22 kg). Dahl *et al*. (2017) have reviewed earlier studies that reported 5 to 7.5 kg/d higher milk yield during subsequent lactation when cows were cooled during dry period.

It was concluded from the present study that heat stress ameliorative strategies of shed modification by using wall mounted fans, whitewashing roof with microfine lime powder and using green net in open paddock successfully reduced ambient temperature and THI in animal shed. Providing thermal comfort to buffaloes in their dry period helps in abatement of negative effects of heat stress not only during dry period but also during subsequent lactation. It also helps to improve milk yield during subsequent lactation. Further studies are required in buffaloes to observe the effects of heat stress ameliorative strategies of shed modification on full lactation length during subsequent cycle.

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