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Dr. Alok Khemraj Wankar
Assistant Professor, Department of Veterinary Physiology, College of Veterinary & Animal Sciences, (MAFSU), Parbhani, Maharashtra, India

Dr. Gyanendra Singh
Sr. Principle Scientist, Nuclear Research Laboratory Physiology & Climatology Division, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Parades, India

Dr. Brijesh Yadav
Assistant Professor, Department of Physiology, College of Veterinary Science & Animal Husbandry, Veterinary University, Mathura, Uttar Parades, India

Corresponding Author:
Dr. Alok Khemraj Wankar
Assistant Professor, Department of Veterinary Physiology, College of Veterinary & Animal Sciences, (MAFSU), Parbhani, Maharashtra, India

Physio-metabolic responses in buffaloes exposed to sustained thermal stress: Acclimatization dynamics

Dr. Alok Khemraj Wankar, Dr. Gyanendra Singh and Dr. Brijesh Yadav

Abstract
The study was conducted at Physiology & Climatology Division, IVRI on four dry adult *Murrah* buffaloes to observe the physio-metabolic alterations and acclimatization process during thermal stress [HS]. The animals were exposed in climatic chambers at 25, 30, 35 and 40 ºC; 21 days at each treatment. Daily exposure period was fixed from 10.00-15.00 hrs. Rectal temperature [RT], respiration rate [RR], pulse rate [PR] were measured daily in morning and afternoon. Blood was collected on day one and day 21. There was a significant increase in RR, RT & PR at 35 and 40 ºC, similarly enzymes AST, SOD, serum sodium, potassium also increased during heat stress period ($P<0.05$). No alteration was observed for other electrolytes, lipid and protein metabolites in the study. The results of the present study indicate that HS severely compromises thermoregulation, alters physio-metabolism and animals require more than 21 days to adapt to short/long term thermal stress.

Keywords: Physio-metabolic, acclimatization, thermal stress, thermoregulation, adapt

Introduction
The buffalo is mainly reared for its milk, meat and other agricultural purposes for centuries. Despite its natural selection to various geographical regions, still it is more adversely affected to high ambient temperatures and humidity [high THI]. Primary reasons for this low heat sustenance are its black colored skin and sparse sweat glands as compared to cattle [1]. There are immediate alterations in physiological responses, followed by short and long term metabolic adaptations during heat stress [HS]. These adaptations help in acclimatization to single/multiple stressors, essential for animal survival [2] but, at the cost of energy diversion leading to suboptimal production and reproductive performance. The negative impact of HS on animal husbandry will be further aggravated due to climate change and global warming in coming years. It is predicted that there will be a 1 to 6 °C increment in global temperature in 21 century [3].
There are several studies reporting decrease in feed intake, milk yield, reproduction, overall performance and alterations in physio-endocrinal axis during HS in livestock [4-7]. The impact of HS is more in tropical and sub-tropical regions where summer is characterized by high ambient temperatures and humidity for prolonged durations coupled with occasional heat waves. The actual acclimatization process and duration required are still not clearly recorded for different livestock species. In this scenario the present study was planned to observe the physio-metabolic responses and their duration in adult dry buffaloes during prolonged heat stress [low to high temperatures].

Materials and Methods
Experimental animals and treatments
The study was conducted on four adult, dry *Murrah* buffaloes (age: 5 > yrs; body weight: > 490 kg) at psychrometric chambers at Division of Physiology & Climatology, Nuclear Research Laboratory, Indian Veterinary Research Institute, Bareilly, India. All animals were kept under identical managemental and feeding conditions, with *ad libitum* water throughout the experiment [8]. The animals were exposed serially to increasing temperatures of 25, 30, 35 and 40 ºC in psychrometric chamber, 21 days at each temperature. Humidity was fixed at comfortable 60% in the chamber at all the treatments and daily exposure period was form 10.00 to 15.00 hrs.

Sampling

Physiological recordings were made twice daily i.e. at 10.10 and 15.10 hrs, respectively. Rectal temperature [RT: ºC] was recorded by inserting digital thermometer in rectum of animals and removed when the temperature reached maximum. Respiration rate [RR: breaths/min] were recorded by observing the flank movements for one minute. Pulse rate [PR:beats/min] was recorded by palpation of coccygeal artery at base of tail for one minute.

Blood samples were drawn by jugular venipuncture on day one and day 21 in morning (10.20 hrs) and afternoon (15.20 hrs) period at each treatment. Serum was harvested [3000 g x 20 min] and stored at -20 ºC, till further analysis.

Biochemical parameters

In all the serum samples sodium, potassium, calcium, chloride, total protein, albumin, globulin, urea, creatinine, cholesterol, triglyceride, aspartate aminotransferase [AST], alanine aminotransferase [ALT] were estimated by commercial kits (span diagnostics). While reactive oxygen molecules [ROS] and superoxide dismutase [SOD] were analysed by other protocol [9, 10].

Statistical analysis

Data were analyzed by the by one way analysis of variance model using SPSS 20.00 software [10] and indicated by their probability value (*P*). Differences among treatments were determined using Tukey's *b* test and indicated by the superscripts a, b, c, d. All data are presented as means ± SE and level of significance was declared at $P<0.05$.

Results

The means for physiological indices i.e., RT, RR & PR at different treatments are presented in Table. 1. There was significant increase in rectal temperature on all the days at 35 and 40 ºC as compared to 25 and 30 ºC ($P<0.05$) and highest RT was recorded on day 14 at 40 ºC (38.98±0.05). On other days at 25 and 30 ºC no significant variation was recorded for RT ($P>0.05$). Similar to RT, the respiration rates were also elevated during 35 and 40 ºC treatments as compared to lower exposure temperatures ($P<0.05$). Higher RR was observed on, day 14 at 35 ºC, days one and 21, respectively at 40 ºC [RR: breaths/min: 56.50±17.17; 82.00±3.46 & 58.00±6.00]. Pulse rate did not varied statistically at different days at 25, 30 or 35 ºC but a positive increase was noted at 40 ºC as compared to 25 and 30ºC ($P<0.05$).

Table 1: Physiological variables, RT, RR & PR at different treatments in buffaloes (Means±SE)

Parameter		Time	25ºC	30ºC	35ºC	40ºC
Rectal temperature (ºC)	Day 1	M	37.54±0.04	37.27±0.16 [a]	37.70±0.14	37.73±0.07
		A	37.58±0.17	37.58±0.22	38.05±0.08[b]	38.76±0.18 [b]
	Day 14	M	37.22±0.31 [a]	37.10±0.33[a]	37.59±0.12 [b]	37.87±0.06
		A	37.23±0.15 [a]	37.60±0.15	37.99±0.15	38.98±0.05 [b]
	Day 21	M	37.53±0.15	37.69±0.13	37.84±0.12	37.57±0.16
		A	37.63±0.07	37.87±0.17	38.04±0.08 [b]	38.22±0.06 [b]
Respiration Rate (breaths/min)	Day 1	M	11.00±1.00[a]	9.00±1.00 [a]	14.00±1.41 [a]	20.00±2.30[a]
		A	14.00±1.15 [a]	15.00±1.00 [a]	25.00±4.35	82.00±3.46[b]
	Day 14	M	10.50±0.95 [a]	12.00±1.63 [a]	18.00±2.58 [b]	13.50±1.50
		A	14.00±1.15 [a]	23.00±5.25	56.50±17.17[b]	37.50±16.64
	Day 21	M	13.00±1.91 [a]	20.00±2.82	20.50±1.70	19.00±4.12 [a]
		A	16.00±1.63 [a]	18.00±1.15 [a]	46.00±9.30[b]	58.00±6.00[b]
Pulse Rate (beats/min)	Day 1	M	45.00±3.41	46.00±2.00	48.00±4.89	51.00±3.00
		A	36.00±2.82[a]	39.00±1.91	44.00±4.32	46.00±2.58
	Day 14	M	49.50±1.50	51.00±3.00	50.50±3.94	53.50±2.50[b]
		A	36.00±1.63 [a]	36.00±1.63 [a]	48.00±5.88	41.00±1.91
	Day 21	M	44.50±3.09	52.00±2.82	48.00±0.00	57.00±3.00 [b]
		A	45.00±2.51	48.00±2.82	41.00±4.12	41.00±2.51

*Means with different superscript a, b vary significantly within row/column for respective parameter ($P<0.05$)

The results of biochemical estimation for electrolytes; enzymes, triglyceride and reactive oxygen molecules; protein metabolism are presented in Table 2, 3 and 4, respectively. Serum sodium did not varied significantly at 25 ºC ($P>0.05$), but was lower at 30 ºC as compared to 35 ºC and 40ºC ($P<0.05$). Similarly, higher values for potassium were obtained at 40 ºC than at 25 ºC ($P<0.05$), while no significant changes were noted at 30 and 35 ºC (Table 2). There was no notable variation for calcium and chloride during exposure at any of the treatments ($P>0.05$).

Table 2: Serum electrolyte profile at different treatments in buffaloes (Means±SE)

Parameter		Time	25 ºC	30 ºC	35 ºC	40 ºC
Sodium (mmol/L)	Day 1	M	143.47±6.68	146.26±3.04[a]	145.88±4.50[a]	134.37±4.32
		A	132.02±3.84	112.84±3.41[b]	126.01±11.80	117.71±13.39
	Day 21	M	133.09±5.13	147.35±4.64[a]	138.73±6.94	129.82±4.43
		A	124.50±1.78	123.51±8.83	115.34±3.43	145.94±2.94[a]
Potassium (mmol/L)	Day 1	M	4.46±0.20	5.23±0.26	4.66±0.34	5.30±0.29
		A	5.37±0.60	4.48±0.33	5.92±0.44	5.26±0.42
	Day 21	M	4.55±0.16	5.75±0.42	4.95±0.14	6.85±0.26 [a]
		A	4.21±0.17 [b]	4.88±0.38	4.74±0.09	4.90±0.21
Calcium (mmol/L)	Day1	M	1.94±0.25	2.36±0.28	2.13±0.14	2.25±0.16
		A	1.97±0.23	2.56±0.28	2.37±0.36	2.69±0.27
	Day 21	M	2.09±0.16	2.28±0.19	1.93±0.19	2.45±0.11

		A	2.10±0.14	2.75±0.29	2.38±0.25	2.06±0.18
Choride (mmol/L)	Day 1	M	73.41±6.11	101.35±3.19	92.82±3.60	78.74±8.52
		A	69.88±8.67	113.76±9.72	109.30±10.96	99.43±18.80
	Day 21	M	102.04±8.00	93.48±0.54	79.32±12.56	88.43±4.01
		A	96.49±13.88	115.03±10.93	103.00±2.90	107.11±3.80

*Means with different superscript a, b vary significantly within row/column for respective parameter ($P<0.05$)

Superoxide dismutase enzyme was highest on day 1 at 40 ºC but decreased again on day 21. Significant increase in SOD activity were observed at 40 ºC as compared to other temperatures ($P<0.05$). No concurrent significant increase was recorded for reactive oxygen species [ROS] during exposure at any days at different treatments (Table 3). Serum AST levels increased significantly on day 21 at 35 ºC as compared to 25, 30 and 40 ºC ($P<0.05$), but, no such variation was observed for serum ALT at any of the temperatures during exposure ($P>0.05$). Serum lipid derivatives like cholesterol and triglycerides did not varied amongst different days at any treatment during exposure ($P>0.05$), but numerically lower and higher values were obtained for triglyceride and cholesterol on day 21 at 35 and 40ºC during exposure than at 25 and 30 ºC, respectively [triglyceride: 0.69±0.08; 0.66±0.05 vs 0.74±0.12; 0.80±0.06 & cholesterol: 7.48±2.86; 6.79±0.94 vs 4.45±0.09; 5.26±0.46].

Table 3: Serum enzymes, ROS and lipid profile at different treatments in buffaloes (Means±SE)

Parameter		Time	25 ºC	30 ºC	35 ºC	40 ºC
SOD (U/mL)	Day 1	M	141.12±15.69^{b}	208.74±40.37	128.82±8.82^{b}	334.83±35.20^{a}
		A	219.77±10.84	262.71±21.80	212.42±12.43^{b}	222.31±25.46
	Day 21	M	178.55±37.84^{b}	159.28±6.75^{b}	146.99±33.82^{b}	167.68±6.71^{b}
		A	212.85±13.60	196.18±61.78b^{a}	190.11±10.76^{b}	188.27±37.09
ROS (mg H_2O_2 /dL)	Day 1	M	0.05±0.00	0.04±0.00	0.05±0.00	0.04±0.00
		A	0.05±0.00	0.04±0.00	0.04±0.00	0.06±0.00
	Day 21	M	0.05±0.00	0.04±0.00	0.04±0.00	0.06±0.00
		A	0.04±0.00	0.04±0.00	0.06±0.00	0.06±0.00
AST (U/L)	Day1	M	147.37±11.72^{a}	142.50±15.00^{a}	174.85±14.83	175.62±14.88
		A	119.85±20.45^{a}	121.88±20.59^{a}	147.34±18.99^{a}	138.78±9.76^{a}
	Day 21	M	162.01±2.85	191.28±9.49	224.14±15.22^{b}	160.21±26.24
		A	124.81±3.48^{a}	131.34±8.76^{a}	154.32±8.83^{a}	138.33±3.72^{a}
ALT (U/L)	Day 1	M	66.27±20.57	78.48±14.67	127.67±24.45	99.06±12.67
		A	46.65±13.31	52.18±14.90	109.56±31.71	112.32±11.83
	Day 21	M	92.79±25.33	105.69±15.87	129.06±28.41	113.72±22.24
		A	75.34±25.55	83.98±18.58	111.98±32.39	101.26±22.55
Cholesterol (mmol/L)	Day 1	M	3.60±0.44	4.70±0.75	4.36±0.40	4.87±0.45
		A	3.76±0.38	4.81±1.17	4.75±0.32	5.65±0.50
	Day 21	M	2.56±0.61	5.49±0.54	12.61±2.85	4.69±0.63
		A	4.45±0.09	5.26±0.46	7.48±2.86	6.79±0.94
Triglyceride (mmol/L)	Day 1	M	1.02±0.04	1.05±0.26	0.97±0.08	0.87±0.04
		A	0.73±0.04	0.86±0.11	0.56±0.05	0.66±0.02
	Day 21	M	0.94±0.09	1.01±0.14	0.91±0.01	0.94±0.08
		A	0.74±0.12	0.80±0.06	0.69±0.08	0.66±0.05

*Means with different superscript a, b vary significantly within row/column for respective parameter ($P<0.05$)

It was observed that serum total protein, albumin and globulin did not altered at any of the treatments during the study (Table 4). Similarly, no significant variation was recorded for serum urea and creatinine during exposure at different temperatures in buffaloes ($P>0.05$).

Table 4: Serum protein, albumin, globulin, urea and creatinine levels at different treatments in buffaloes (Means±SE)

Parameter		Time	25ºC	30 ºC	35ºC	40ºC
Total protein (gm/L)	Day 1	M	63.19±6.27	64.41±7.60	61.07±5.11	70.95±1.77
		A	60.27±7.36	56.51±3.84	56.21±4.72	58.80±4.05
	Day 21	M	61.50±6.77	60.44±4.94	70.99±5.03	64.18±7.38
		A	52.96±5.05	64.84±4.34	60.02±7.14	79.62±2.64
Albumin (gm/L)	Day 1	M	25.43±2.68	30.28±2.18	32.26±2.15	29.91±2.98
		A	19.35±1.72	23.44±1.12	30.66±4.00	34.71±3.72
	Day 21	M	29.11±1.85	30.34±2.75	29.53±1.78	31.27±4.26
		A	23.92±2.43	29.05±3.83	32.05±1.14	30.53±7.24
Globulin (gm/L)	Day1	M	37.76±8.19	34.13±7.10	28.81±3.69	41.03±2.03
		A	40.92±6.98	33.07±3.05	25.54±5.04	24.09±5.52
	Day 21	M	32.38±6.69	30.09±7.58	41.45±6.31	49.09±5.35
		A	29.03±4.23	35.79±4.73	27.96±7.55	28.64±9.17
Urea (mmol/L)	Day 1	M	14.97±0.84	10.54±0.55	11.01±2.05	12.43±1.39
		A	13.52±2.11	11.09±1.02	10.21±2.00	8.54±1.68
	Day 21	M	13.41±1.02	15.35±0.57	11.26±2.88	12.39±1.28

		A	13.82±0.92	8.48±1.82	8.61±1.44	9.89±2.84
Creatinine (mmol/L)	Day 1	M	1.24±0.22	0.89±0.27	0.95±0.06	1.05±0.24
		A	0.75±0.07	1.00±0.09	1.38±0.20	1.19±0.19
	Day 21	M	1.13±0.22	1.47±0.12	1.15±0.26	0.99±0.25
		A	1.02±0.10	1.34±0.36	1.22±0.13	1.19±0.07

Discussion

Immediate response to acute heat stressor is secretions of catecholamines and activation of thermoregulatory mechanisms. Respiration rate increased on all the days (P<0.05) at higher temperatures of 35 ºC and 40 ºC as compared to 25 ºC and 30 ºC, which indicated evaporative cooling was activated to dissipate the excess heat load. Similarly, other studies have confirmed increased RR in animals during heat stress which confirm present findings [12, 13]. There was concurrent increase in rectal temperature in buffaloes on all the days at 35 ºC and 40 ºC than lower exposure treatments (P<0.05). This increment in core body temperature confirms slow, accumulating heat load on the animals and that thermoregulation was altered. Although evaporative cooling was activated, but the raise in RT could not be prevented completely, thus increasing the core body temperature during HS [14, 15]. As compared to RR and RT, the cardiovascular systems response is variable during HS. It can be altered by changes in metabolism, catecholamines and autonomic nervous system [16]. The increased PR during HS than ambient temperatures in this study might be due to increased thermoregulatory activity or higher catecholamine secretions [17, 18]. The physiological responses are immediately activated during HS allowing essential biochemical modulations necessary for stress adaptation. As the RR, RT and PR were increased till day 21 in buffaloes, it can be concluded that more than 21 days of acclimatization is essential for systemic stress adaptation.

Serum sodium decreased on day one at 30 ºC during exposure, thereafter an increase was recorded at 35 and 40 ºC (P<0.05). This reflects active sodium conservation, probably under aldosterone action which is responsible for sodium conservation and potassium excretion in sweat, urine during HS. Another mechanism can be an isotonic expansion of extracellular fluid volume resulting in higher sodium levels in buffaloes [19]. Similarly, potassium levels increased (P<0.05) at 40 ºC than at 25 ºC, which might be due to higher exchange from intracellular compartment to extracellular fluids to replace and conserve sodium during thermal stress. This finding of higher potassium levels during HS in buffaloes is contradictory to previous reports and needs further exploration [20]. No alterations in chloride and calcium concentration at any treatments might be due to optimum retention for the minerals during HS period and our results are supported by similar findings in *Omani sheep* and *Holstein cattle* exposed to hot environmental conditions [21, 22].

Superoxide dismutase increase significantly on day 21 at 30 ºC, didn't varied at 35 ºC and again increased on day 1 at 40 ºC. This increase in SOD levels in buffaloes indicates elevated antioxidant activity during hyperthermia to prevent/reduce cellular damage [23, 24]. Contrastingly, the ROS levels did not altered (P>0.05) during HS in our study possibly due to optimum cellular antioxidant activity, which contradicts the previous results in cattle [25]. Although, serum aspartate aminotransferase [AST] decreased on day 21 during exposure at 35 ºC, but was higher than at 25, 30 and 40 ºC, respectively, probably due to increased gluconeogenesis during HS. This increase at 35 ºC might be due to immediate hepatic/cellular injury or stress, followed by gradual adaptation at 40 ºC as recorded in present study. Our findings are supported by other workers who also reported, an increased AST activity during heat stress period [26, 27]. However no such variation was recorded for serum ALT at any treatment during the study, probably indicating that ALT might not be an accurate biomarker of HS in cattle/buffaloes. The concentrations of serum cholesterol and triglyceride did not altered at any exposure temperature in buffaloes, particularly during HS, probably due to optimum energy balance, thus sparing the lipid metabolites. Similarly, no variation in serum triglyceride was noted in crossbred Holstein cattle exposed to different temperatures [28].

In present study, lack of variation for total protein, albumin, globulin, urea and creatinine (Table 4) in buffaloes, might be due to optimum hepatic protein metabolism during HS period. It also reflects balanced nitrogen metabolism as serum urea and creatinine levels did not increased. Our findings are corroborated by other similar animals studies reporting no significant variation for protein and other protein metabolites at optimum and heat stress conditions [29-31].

Conclusion

Due to our geographical location and semi modernized practices till date, the animals are definitely exposed to heat stress [moderate/severe; acute/chronic]. Immediate alteration is seen in physiological variables, followed by changes in enzymes, electrolytes, hormones and overall metabolism during HS. This enables the animals to sustain the acute or chronic heat stress conditions but at the cost of higher energy expenditure for thermoregulation. The present study confirmed the negative impact of HS on buffaloes and also established that a period of more than 21 days is essential for physio-metabolic acclimatization and homeostasis during thermal stress.

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