

Thermoregulatory and adaptive responses of adult buffaloes (*Bubalus bubalis*) during hyperthermia: Physiological, behavioral, and metabolic approach

Alok K. Wankar¹, Gyanendra Singh² and Brijesh Yadav³

1. Department of Physiology and Biochemistry, Khalsa College of Veterinary and Animal Sciences, Amritsar, Punjab, India; 2. Physiology and Climatology Division, Nuclear Research Laboratory, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India; 3. Division of Physiology, College of Veterinary and Animal Husbandry, Mathura, Uttar Pradesh, India.

Corresponding author: Alok K. Wankar, e-mail: wankaralok@gmail.com, GS: gyansidd@gmail.com, BY: drbrijvet@gmail.com

Received: 05-07-2014, **Revised:** 10-09-2014, **Accepted:** 16-09-2014, **Published online:** 18-10-2014

doi: 10.14202/vetworld.2014.825-830 **How to cite this article:** Wankar AK, Singh G, Yadav B (2014) Thermoregulatory and adaptive responses of adult buffaloes (*Bubalus bubalis*) during hyperthermia: Physiological, behavioral and metabolic approach, *Veterinary World* 7(10): 825-830.

Abstract

Aim: The study was planned to evaluate the indigenous animal adaptive capabilities during optimum temperature versus heat stress (HS).

Materials and Methods: Four adult buffaloes were exposed at 25°C, 30°C, 35°C, and 40°C for 21 days at every treatment in environmentally controlled chamber and physio-biochemical variation and animal behavior was observed.

Results: The study revealed significantly increased rectal temperature, respiration rate, water intake, sodium, reactive oxygen metabolites, cortisol, aspartate aminotransferase, and alanine aminotransferase while, pulse rate and thyroid hormones decreased during thermal stress. Panting, restlessness, salivation, and sweating were higher during HS while, rumination and urination contrastingly lowered.

Conclusion: The results reflect the impact of hyperthermia both acute and chronic, on the animals forcing various physio-biochemical, endocrinal, and behavioral changes for acclimatization during a stressful period aimed at maintaining homeothermy.

Keywords: acclimatization, behavior, endocrinal, heat stress, panting, physio-biochemical.

Introduction

The accumulation of greenhouse gases in the Earth's atmosphere has led to predictions that global surface temperatures will increase between 1 and 6°C during the 21st century (Intergovernmental Panel on Climate Change, 2007). A 25% loss in animal productivity in developing countries has been estimated due to global warming [1]. The global warming scenario coupled with the subtropical harsh environment in Indian subcontinent exposes the livestock naturally to thermal stress for a prominent part of the year affecting production and economy.

The buffalo which is mainly reared for its milk, meat, and drought purpose is an integral part of the Indian agricultural system. Buffaloes have poor heat tolerance than cattle [2] due to many physiological and genetic reasons (less sweat glands, black colored skin) and so is more pronounced to thermal stress. During HS, heat increment exceeds heat loss modifying the homeostatic functions. As per Gudev *et al.* [3] heat stress (HS) elicits an integrative physiological and endocrinal modulation altering overall metabolism and helping the animal sustain during the stressful

period. Various in-depth studies on HS ruminants have made observations indicating severely compromised thermoregulatory functions and an overall negative effect of high temperature [4-6].

Hence, the study was planned to observe the indigenous buffalo's adaptive capabilities during HS versus optimum environmental conditions and to correlate with other studies to quantify the impact of stress.

Materials and Methods

Ethical approval

The experimental design and procedure were carefully assessed and approved by the Institute's Ethical Committee ensuring that no malpractice or potential harm toward animal welfare was done, and no subject was jeopardized during the entire exploratory period.

Animal management

The study was conducted at the Division of Physiology and Climatology during 2011 to 2012 on four adult dry buffaloes (9.00±0.40 years, 492.14±9.58 kg). Animals were randomly selected and exposed at 25°C, 30°C, 35°C and 40°C for 21 days at each treatment in the environmentally controlled chamber (7.5 m × 7.5 m × 2.5 m) equipped with individual tie stall, feeders, and waterers. Daily exposure period was from 10:00 to 15:00 h. Basal diet of wheat

Copyright: The authors. This article is an open access article licensed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>) which permits unrestricted use, distribution and reproduction in any medium, provided the work is properly cited.

straw *ad libitum* along with the required amount of concentrate mixture to meet the maintenance requirement [7] was offered daily at 08:30 and 15:15 h. Water was provided in graduated containers and intake was recorded accordingly. All the physiological readings were made daily at 15:05 h, immediately after exposure. Rectal temperature (RT, °C) was measured by inserting a digital thermometer 5 cm deep per rectum and reading was only made after 1 min. For recording respiration rate (RR, breaths/min) the flank movement was observed for 1 min, heart rate (HR) (pulse rate [PR], beats/min) was monitored by palpating the coccygeal artery for 1 min. Behavioral signs like restlessness, panting, rumination, etc., were monitored for the entire experimental period (during and after exposure). For biochemical studies, blood samples were collected on every 5th day starting from day one from jugular veni-puncture and serum was harvested within 1 h by centrifuging the sample at 3000 g for 15 min. The samples were frozen (-80°C) until the end of the trial and analyzed together. All experimental procedures were reviewed and approved by the animal Ethics Committee at the Institute.

Parameter investigation

All the samples were analyzed at Nuclear Research Laboratory of the division. In each serum sample, sodium, potassium, chloride, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), were measured on double beam UV-vis spectrophotometer (uv5704ss, Electronics Corporation of India, Ltd.) using commercial kits (Crest Biosystems; SPAN Diagnostic). All measurements were made according to the manufacturer's instructions. Serum

superoxide dismutase (SOD) and reactive oxygen metabolites were measured using established protocols [8,9]. Hormones assay for thyroxine (T₄), triiodothyronine (T₃), cortisol, and aldosterone, were determined by radioimmunoassay procedure on Sr-3000 Stratec Counter (Stratec Biomed Systems, Birkenfeld, Germany).

Statistical analyses

Data were analyzed by the one-way analysis of variance model using SPSS 16.0 software [10] and indicated by their probability value (p). The general model used was $Y_{ij} = \mu + A_i + B_j + e_{ij}$, where Y_{ij} is the dependent variable, μ is the least square mean, A_i is the effect of temperature, B_j is the animal effect and e_{ij} is the residual error. Differences among treatments were determined using Tukey's b test [10] and indicated by the superscripts a, b, c, d. All data were presented as means \pm standard error of the mean and level of significance was set at $p < 0.05$ for all the parameters.

Results

The changes in water intake (WI), RT, RRs, PR and biochemical variables at different treatments are presented in Tables-1 and 2, respectively. RT differed with the increasing exposure temperature and was higher ($p < 0.001$) at 35°C and 40°C as compared to 25°C and 30°C, respectively. Likewise, the RRs also increased during thermal stress period and were significantly high ($p < 0.001$) at 35°C and 40°C than at lower treatments. Contrastingly, the PR decreased significantly ($p < 0.001$) during heat exposition, and highest values were recorded at 25°C and 30°C, respectively. WI was significantly higher ($p < 0.01$) at 35°C than at

Table-1: Mean \pm SEM of water intake and physiological parameters at 25°C, 30°C, 35°C, and 40°C in buffaloes.

Parameter	25°C	30°C	35°C	40°C	SEM	p value
RT (°C)	37.54 ^a	37.64 ^a	38.01 ^b	38.77 ^c	0.031	<0.001
RR (breaths/min)	13.95 ^a	15.66 ^a	28.66 ^b	72.02 ^c	1.361	<0.001
PR (beats/min)	49.35 ^c	52.88 ^d	37.35 ^a	43.92 ^b	0.458	<0.001
WI (L/day)	25.90 ^a	24.9 ^a	28.72 ^b	27.07	0.361	0.001

RT=Rectal temperature; PR=Pulse rate; WI=Water intake; RR=Respiration rate. Means bearing different superscript a, b, c, d within a row differ significantly at ($p < 0.05$), SEM=Standard error of the mean

Table-2: Mean \pm SEM of different biochemical and endocrinal variants at 25°C, 30°C, 35°C, and 40°C in buffaloes.

Parameter	25°C	30°C	35°C	40°C	SEM	p value
Sodium (mmol/L)	131.17	120.78 ^a	127.94	139.51 ^b	1.859	0.003
Potassium (mmol/L)	4.97	4.63	5.08	4.81	0.090	0.326
Chloride (mmol/L)	99.81	101.65	102.84	98.52	2.710	0.948
Calcium (mmol/L)	9.70	10.16	9.70	9.41	0.241	0.754
ROS (mg H ₂ O ₂ /dL)	0.04 ^a	0.04 ^a	0.05 ^a	0.06 ^b	0.150	<0.001
SOD (U/mL)	226.67	211.98	209.52	192.77	5.673	0.214
AST (U/L)	131.57 ^a	137.21	158.47 ^b	139.46	3.511	0.037
ALT (U/L)	61.52 ^a	74.44	105.14 ^b	105.21 ^b	5.375	0.004
T ₃ (nmol/L)	1.50 ^a	1.23 ^b	1.18 ^b	1.12 ^b	0.033	<0.001
T ₄ (nmol/L)	43.80 ^b	36.16 ^a	38.18	32.20 ^a	1.027	<0.001
Cortisol (nmol/L)	0.71 ^a	1.96	4.56 ^b	3.51	0.424	0.006
Aldosterone (nmol/L)	0.017	0.014	0.014	0.013	0.004	0.163

SEM=Standard error of the mean; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; T₄=Thyroxine; T₃=Triiodothyronine; ROS=Reactive oxygen species, SOD=Superoxide dismutase. Means bearing different superscript a, b within a row differ significantly at ($p < 0.05$)

optimum conditions and 30°C, but no variation was observed at 40°C. During the thermally stressful period at 35°C and 40°C, sweating, panting, restlessness, and nervousness was more pronounced, while rumination and urination were reduced.

Significant rise ($p < 0.05$) was noted for sodium at 40°C than at 30°C but no variation ($p > 0.05$) was observed for potassium, calcium, and chloride at any treatment during entire experimental period. The reactive oxygen radicals levels were significantly high at 40°C as compared to any other treatment. SOD did not vary significantly ($p > 0.05$) at 25°C, 30°C, and 35°C, neither was any difference observed for the enzyme at peak temperature of 40°C. Gradual rise was observed for the enzymes AST and ALT with the heat increment and significant differences ($p < 0.05$) were observed at 35°C for AST and both 35°C and 40°C for ALT as compared to optimum treatments.

The highest concentration for thyroid hormones was seen at optimum conditions which dropped significantly ($p < 0.001$) with the increasing thermal stress. T_4 decreased at 30°C and 40°C while, T_3 was significantly low at all the treatments as compared to that at 25°C, respectively. Glucocorticoid activity was significantly higher ($p < 0.05$) at 35°C as compared to 25°C but we observed no difference for cortisol at 30°C and 40°C treatment. For aldosterone no significant variation ($p > 0.05$) was recorded for the entire experimental period.

Discussion

The significant rise in RT during HS (37.54°C vs. 38.01, 38.77°C) indicate increasing heat load on the buffaloes and inability to normally dissipate the accumulating heat, consequently raising the body temperature. Significant increase in RT ($p < 0.001$) was also seen in heat-exposed buffalo calves as compared to cool comfortable conditions [11]. Similarly, higher RTs (°C) were also seen in heat-exposed Tharparkar (38.74±0.11 vs. 38.96±0.05) and Karan Fries heifers (38.84±0.06 vs. 39.19±0.24) than at thermoneutral treatments validating our findings [12]. To increase the evaporative cooling panting is commonly seen in HS animals in an attempt to maintain normal body temperature. Banerjee and Ashutosh [13] observed significant rise in respiratory frequencies in Tharparkar and Karan Fries heifers at higher environmental temperatures. During thermally stressful period, the respiratory frequencies and RT in Alentejana, Frisian, Limousine and Mertolenga cattle breeds increased significantly by 2.7, 2.8, 2.5, 2.9% and 1.1, 2.0, 1.8 and 0.2%, respectively, in accordance to our findings [14]. Present findings completely agree with previous works [13,14] and higher RR in buffaloes was probably adopted to increase the evaporative cooling. The cardiovascular systems response during HS is varied, so the decrease in HR in buffaloes during thermal stress could be mediated by low epinephrine and nor-epinephrine levels [15], circulatory adjustments

like increase in peripheral resistance, increased resistance to venous return as an attempt to reduce metabolic heat generation [16]. Similar decrease in HRs was also observed in buffalo calves as essential cardiovascular adjustments during HS, which support present results [12]. The HR of 2-3 months old male *Bos taurus* calves showed a decline when the temperatures were raised from 18 to 40.5°C at 50% RH. The mean HR of 114.5/min at 18°C fell to 110.5/min and continued to fall to 95/min at 40.5°C in the climate chamber [17]. The cardiovascular and respiratory adjustments in accordance to increasing stress indicate the importance of physiological adaptations acting as the first line of defense in maintaining thermoregulation to prevent drastic metabolic alterations.

Water is directly involved in cooling of reticulo-rumen and also serves as the primary vehicle for heat transfer. Increased sweating and panting might had resulted into osmoconcentration of the extracellular fluid activating hypothalamic thirst center leading to higher WI in the acclimating buffaloes to HS. Similarly, Beatty *et al.* [18] observed significant increase ($p < 0.001$) in daily WI during thermally stressful period in *B. taurus* and *Bos indicus* cattle. Significant ($p < 0.01$) increase in total WI (28.5 and 48.3%) and free WI (25.2 and 56.4%) was also noted in 12-month-old buffalo and cattle calves due to chronic heat exposure further validating present findings [19]. Although no difference in WI was observed at 40°C still the WI was higher signifying the important role of water in heat dissipation. Animal behavior changed during HS, and they were more nervous, restless, and profuse salivation accompanied with panting was observed. Panting is seen when the normal heat dissipation mechanism is compromised, culminating into evaporative cooling as means of heat loss. We also observed lower frequency of rumination and urination at the higher treatments which possibly indicated changes in rumino-reticular environment, changing fermentation patterns and water conservation mechanism, respectively. Nardone *et al.* [20] also reported increase panting, lowered saliva production and decreased rumination during thermal stress in livestock. Furthermore, other workers [21] have reported reduction in average rumination time in cattle exposed to higher temperatures temperature (24±4 vs. 17±4 min) supporting present findings. The behavioral changes help in the adaptation to a stressful period and can be used to assess the impact of thermal stress in ruminants.

HS results in negative mineral balance and also reduces electrolyte absorption, so the significant rise ($p < 0.05$) in sodium at 40°C than at 30°C is unique finding of the present study. Aboul-Naga *et al.* [22] recorded a significant 3% decrease in serum sodium in Friesian calves exposed at 36°C. During HS sodium concentrations (mEq/L) decreased in Tharparkar (136.25±1.75 vs. 132.88±2.09) and Karan Fries heifers (133.33±2.44 vs. 128.39±2.00) [13] but, present

observations in buffaloes contradict them. An isotonic expansion of extracellular fluid volume or enhanced renal reabsorption or conservation might have resulted in higher sodium levels [16] in present study, but further exploration in ruminants will be needed to confirm the present finding observed during HS. Potassium concentrations did not varied at any treatment ($p>0.05$) although there are many studies reporting significant reduction in potassium levels in HS buffaloes which they attributed to losses in sweat and decreased retention [23,24]. The low levels of aldosterone combined with increased renal conservation might had resulted optimum potassium concentrations in present study, similar to that observed in heat-tolerant Romosinuano (4.27 vs. 4.22 mEq/L) and heat-susceptible Angus (4.31 vs. 4.50 mEq/L) cattle during thermoneutral against HS conditions [25]. Lack of variation for chloride and calcium in buffaloes was probably due to optimum retention for the minerals during HS period. Similar nonsignificant variation for chloride (109.50 vs. 116.50 mmol/l) was also noted in Omani sheep [26] and Holstein cattle [27] during hot environment than in thermoneutral conditions supporting present findings.

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of normal metabolic processes but hyperthermia has been reported to enhance numerous forms of ROS in different cells and tissues [28]. This increased ROS activity during HS (0.06 ± 0.150 mg H_2O_2 /dL at $40^\circ C$) can be attributed to changing cellular metabolism, leading to faster free radical production than that could be neutralized by the cellular antioxidant system. Present findings totally agree with observations made in dairy cows with similar increased free radicals (thiobarbituric acid reactive substances during summer (8.8 ± 0.4 nmol/ml) than in spring season (7.6 ± 0.4 nmol/ml) [29]. SOD and catalase (CAT) provide the first line of defense against ROS-induced damage [30]. Kumar *et al.* [31] observed significant ($p<0.05$) increased erythrocyte SOD activity with days of exposure in hot dry ($40^\circ C$) and hot humid ($37^\circ C$) stressful conditions in buffaloes. Our findings contradict other works reporting increased SOD activity in HS cattle and buffaloes [29,32] as no variation was seen for SOD during thermal stress which might be due to collaborative contributions by other antioxidant systems (CAT, glutathione peroxidase, vitamins A, E, and C and flavonoids) in neutralizing the free radicals [33]. The enzymes AST and ALT have a key role in gluconeogenesis hence are essential for stress adaptations [34]. The significantly higher enzyme levels during thermal stress were probably due to stimulation of gluconeogenesis by glucocorticoids to fulfill the increasing energy demands [35]. Similarly, Marai *et al.* reported significant increase in enzymes AST (613 vs. 758 units/L) and ALT (179 vs. 228 units/L) in the summer as compared to winter in Friesian calves [36]. A positive effect (increase) of THI on AST

activity was also observed in lactating dairy cows in other study [37] which validate the present observations of high enzyme activity during thermal stress.

Thyroid hormones are known to play an important role in animal's adaptation to environmental changes, and T_3 is more concerned with thermogenesis in farm animals. In the present study, T_3 and T_4 declined consistently with increasing temperatures probably mediated by higher glucocorticoid levels and lowered thyroid gland function. The T_3 levels were significantly lower ($p<0.05$) at $33^\circ C$ (0.95 ± 0.05 ng/mL) than at $20^\circ C$ and $28^\circ C$ (1.45 ± 0.05 and 1.32 ± 0.05 ng/mL), respectively, in prepubertal Holstein heifers [38]. The decrease in thyroid hormones as an adaptive stress response is established by many workers in cattle confirming present observations [14,39]. Cortisol also known as the "stress hormone" was significantly higher at $35^\circ C$ than at optimum treatments possibly due to the activation of hypothalamo-pituitary-adrenal axis increasing the cortisol concentration enabling the animals for necessary adaptation during stressful period [40]. In Friesian calves exposed to direct solar radiation of the hot summer, cortisol concentration increased from 11 to 29 ng/ml [41]. Similar increase in cortisol hormone during summer months as compared to the winter period was also reported in Tuli, Senepol, and Brahman-sired Angus heifers [42]. No notable difference for cortisol at $40^\circ C$ reflected gradual adaptation by the buffaloes, which make the corticoid hormone pertinent for both short- and long-term adaptation to HS [43]. The decrease in thyroid hormones with a concurrent increase in the glucocorticoid indicates indispensable role of the endocrine system essential to maintain energy and normal cellular homeostasis during a stressful period.

Acute thermal stress results in increasing concentrations of aldosterone in non-ruminants and decreasing concentrations during chronic thermal stress in ruminants [44]. Lack of variation for aldosterone is quiet surprising and unique finding of the study as higher sodium levels were noted during HS. These low hormone levels might have helped in essential potassium ion conservation which is known to be lost both in urine and sweat [17]. Further extensive explorations on aldosterone action and sodium conservation are essential to confirm these surprising observations made in HS buffaloes.

Conclusion

Native animals have some genetic and physiological advantages due to evolution and selection strategies but they still are vulnerable to both acute and prolonged thermal stress and hyperthermia has drastic impact on the behavior, normal physiology, and metabolism as was observed in present study. Sustenance during thermal stress is a synergistic function of different body systems, diverting all resources toward maintaining thermoregulation.

Authors' Contributions

GS planned the whole trial and revised the manuscript. AKW executed the trial and drafted the manuscript while, BY helped with the execution of trial and discussion of the results. All authors read and approved the final manuscript.

Acknowledgments

The authors sincerely thank to Institute and also to Indian Council of Agricultural Research for providing necessary facilities and funding. The authors also sincerely acknowledge all the technical staff, colleagues, and the animal shed and feed unit for their advice and timely help.

Competing Interests

The authors declare that they have no competing interests.

References

- Seguin, B. (2008) The consequences of global warming for agriculture and food production. In: Rowlinson, P., Steele, M. and Nefzaoui, A. editors. Proceedings International Conference "Livestock and Global Climate Change" Hammamet, Tunisia, 17-20 May, p9-11.
- Moran, J.B. (1973) Heat tolerance of Brahman cross, buffalo, bauteng and shorthorn steers during exposure to sun and as a result of exercise. *Aust. J. Exp. Agric.*, 24: 775-782.
- Gudev, D., Popova Ralcheva, S., Moneva, P., Aleksiev, P., Peeva, T.Z., Penchev, P. and Ilieva, I. (2007) Physiological indices in buffaloes exposed to sun. *Arch. Zootec.*, (10): 127-132.
- Marai, I.F.M. and Haebe, A.A.M. (2010) Buffalo's biological functions as affected by heat stress - A review. *Livest. Sci.*, 127: 89-109.
- West, J.W. (2003) Effects of heat-stress on production in dairy cattle. *J Dairy Sci.*, 86: 2131-2144.
- Ganaie, A.H., Shanker, G., Nazir, A.B., Ghasura, R.S Mir, N.A., Wani, S.A. and Dudhatra, G.B. (2013) Biochemical and physiological changes during thermal stress in bovines. *J. Vet. Sci. Technol.*, 4(1): 1-6.
- NRC. (2001) Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, DC.
- Madesh, M., Balasubramanian, K.A. (1998) Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian J. Biochem. Biophys.*, 35: 184-188.
- Brambilla, G., Fiori, M., Archetti, L.I. (2001) Evaluation of the oxidative stress in growing pigs by microplate assays. *J. Vet. Med. Sci.*, 48: 33-38.
- SPSS, Inc. (1997). SPSS (Statistical Package for Social Sciences) for Windows© (1993) Version 160 SPSS Inc., Chicago, IL.
- Korde, J.P., Singh, G., Varshney, V.P. and Shukla, D.C. (2007) Effects of long-term heat exposure on adaptive mechanism of blood acid-base in Buffalo calves. *Asian. Aust. J. Anim. Sci.*, 13: 329-332.
- Banerjee, D. and Ashutosh. (2011a) Effect of thermal exposure on diurnal rhythms of physiological parameters and feed water intake in Tharparkar and Karan Fries heifers. *Biol. Rhythm. Res.*, 42: 39-51.
- Banerjee, D. and Ashutosh. (2011b) Circadian changes in physiological responses and blood ionized sodium and potassium concentrations under thermal exposure in Tharparkar and Karan Fries heifers. *Biol. Rhythm. Res.*, 42: 131-139.
- Pereira, A.M.F., Baccari, F., Titto, E.A.L. and Afonso, J.A. (2008) Effect of thermal stress on physiological parameters, feed intake and plasma thyroid hormones concentration in alentejana, mertolenga, Frisian and limousine cattle breeds. *Int. J. Biometeorol.*, 52(3): 199-208.
- Janzekovic, M., Mursec, B. and Janzekovic, I. (2006) Techniques of measuring heart rate in cattle. *Teh. Vjesn.*, 13: 31-37.
- Reece, O. (2005) Dukes Physiology of Domestic Animals. 12th ed. Cornell University Press, US.
- Singh, S.P. and Newton, W.M. (1978) Acclimation of young calves to high temperatures: Physiologic responses. *Am. J. Vet. Res.*, 39(5): 795-7.
- Beatty, D.T., Barnes, A., Taylor, E., Pethick, D., McCarthy, M. and Maloney, S.K. (2006) Physiological responses of *Bos taurus* and *Bos indicus* cattle to prolonged continuous heat and humidity. *J. Anim. Sci.*, 84: 972-985.
- Nessim, M.G. (2004) Heat-induced biological changes as heat tolerance indices related to growth performance in buffaloes. PhD Thesis. Faculty of Agriculture Animal Shams University, Cairo, Egypt.
- Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S. and Bernabucci, U. (2010) Effects of climate changes on animal production and sustainability of livestock systems. *Livest. Sci.*, 130: 57-69.
- Miaron, J.O.O. and Christopherson, R.J. (1992) Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and particulate passage-rate constants, and apparent digestibility in steers. *Can. J. Anim. Sci.*, 72: 809-819.
- Aboul-Naga, A.I. (1983) The role of heat induced physiological changes of minerals metabolism in the heat stress syndrome in cattle. M.Sc. Thesis. Faculty of Agriculture, Mansoura University, Egypt.
- Hooda, O.K. and Singh, G. (2009) Changes in serum electrolytes, minerals and physiological responses in buffalo heifers exposed to high temperature in a psychrometric chamber. *Indian J. Anim. Nutr.*, 26(4): 327-332.
- Singh, P., Dixit, V.P., Singh, B. and Gerogie, G.C. (2001) Plasma aldosterone and major circulatory and urinary electrolytes in buffalo calves as affected by peak summer and winter stress. *Int. J. Anim. Sci.*, 16(1): 161-166.
- Scharf, B., Carroll, J.A., Riley, D.G., Chase, C.C., Coleman, S.W. Jr., Keisler, D.H., Weaver, R.L. and Spiers D.E. (2010) Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heat-susceptible (Angus) *Bos taurus* cattle during controlled heat challenge. *J. Anim. Sci.*, 88: 2321-2336.
- Srikandakumar, A., Johnson, E.H. and Mahgoub, O. (2003) Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin. Res.*, 49(2): 193-198.
- Chaiyabutr, N., Chanpongsang, S. and Suadsong, S. (2008) Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. *Int. J. Biometeorol.*, 52: 575-585.
- Katschinski, D.M., Boos, K., Schindler, S.G. and Fandrey, J. (2000) Pivotal role of reactive oxygen species as intracellular mediators of hyperthermia-induced apoptosis. *J. Biol. Chem.*, 275: 21094-21098.
- Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A. (2002) Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.*, 85: 2173-2179.
- Kumaraguruparan, R., Balachandran, C., Murali, M.B. and Nagini, S. (2005) Altered oxidant - Antioxidant profile in canine mammary tumors. *Vet. Res. Commun.*, 29: 287-296.
- Kumar, A., Singh, G., Kumar, B.V.S. and Meur, S.K. (2011) Modulation of antioxidant status and lipid peroxidation in erythrocyte by dietary supplementation during heat stress in buffaloes. *Livest. Sci.*, 138: 299-303.
- Kumar, A., Kumar, P. and Singh, S.V. (2007) Oxidative stress markers profile in erythrocytes of natural and heat exposed cattle and buffalos. *Indian J. Dairy. Sci.*, 60: 114-118.

33. Cerutti, P.A. (1985) Pro-oxidant status and tumor promotion. *Science*, 227: 375-377.
34. Kaneco, J.J., Harvey, J.W. and Bruss, M.L. (2008) Clinical Biochemistry of Domestic Animals. 6th ed. Academic Press, Elsevier, London.
35. Boots, L.R., Crist, W.L., Davis, D.R., Bruin, E.W. and Ludwick, I.M. (1969) Effect of age, body weight, stage of gestation and sex on plasma glutamic oxaloacetic and glutamic pyruvic transaminase activity in immature cattle. *J. Dairy Sci.*, 52: 211-216.
36. Marai, I.F.M., Habeeb, A.A.M., Daader, A.H. and Yousef, H.M. (1997) Effects of diet supplementation and body cooling on Friesian calves reared in high ambient temperature in the Eastern Desert of Egypt. *Trop. Anim. Health Prod.*, 29(4): 201-208.
37. Calamari, L., Petrera, F., Abeni, F. and Bertin, G. (2011) Metabolic and hematological profiles in heat stressed lactating dairy cows fed diets supplemented with different selenium sources and doses. *Livest. Sci.*, 142: 128-137.
38. Nonaka, I., Takusari, N., Tajima, K., Suzuki, T., Higuchi, K. and Kurihara, M. (2008) Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. *Livest. Sci.*, 113: 14-23.
39. Rasooli, A., Nouri, M., Khadjeh, G.H. and Rasekh, A. (2004) The influence of seasonal variations on thyroid activity and some biochemical parameters of cattle. *Iran. J. Vet. Res.*, 5(2): 1383-1391.
40. Dantzer, R. and Mormede, P. (1983) Stress in farm animals: A need for reevaluation. *J. Anim. Sci.*, 51(1): 16-18.
41. Yousef, J.L.M., Habeeb A.A. and EL-Kousey, H. (1997) Body weight gain and some physiological changes in Friesian calves protected with wood or reinforced concrete sheds during hot summer season of Egypt. *Egypt. J. Anim. Prod.*, 34: 89-101.
42. Hammond, A.C., Chase, C.C., Bowers, E.J. Jr., Olson, T.A. and Randel R.D. (1998) Heat tolerance in Tuli-, Senepol-, and Brahman-sired F1 Angus heifers in Florida. *J. Anim. Sci.*, 76(6): 1568-1577.
43. Beraidinell, L.J.G., Gogfrey, R.W., Adair, R., Lunstra, D.D., Byerely, D.J., Gardenas, H. and Randel, R.D. (1992) Cortisol and prolactin concentrations during different season in relocated Brahma and Hereford bulls. *Theriogenology*, 37(3): 641-654.
44. Colleir, R.J., Breede, D.K., Thatcher, W.W., Israe, L.A. and Wilcox, C.J. (1982) Influence of environment and its modification on dairy animal health and production. *J. Dairy Sci.*, 65: 2213-2227.
