



Effect of Heat Stress on Physiological and Hemato-biochemical Profile of Cross Bred Dairy Cattle

Sindhu Berian^{1*}, S.K. Gupta¹, Sankalp Sharma², Imran Ganai³, Sourab Dua⁴ and Neelesh Sharma¹

¹Division of Veterinary Medicine, FV.Sc. & A.H., R.S. Pura, Jammu, INDIA

²Division of Veterinary Pathology, FV.Sc. & A.H., R.S. Pura, Jammu, INDIA

³Division of Animal Nutrition, FV.Sc. & A.H., R.S. Pura, Jammu, INDIA

⁴Division of Livestock Production and Technology, FV.Sc. & A.H., R.S. Pura, Jammu, INDIA

*Corresponding author: S Berian; Email: sberian8@gmail.com

Received: 10 Oct., 2018

Revised: 28 Jan., 2019

Accepted: 06 Feb., 2019

ABSTRACT

The present study was done to see the effect of heat stress on blood parameters in dairy cattle. Dairy cows of similar physiological status were selected. Blood samples were collected and analyzed based on the temperature-humidity index (THI). Blood samples were collected and analyzed for packed cell volume PCV(%), red blood cell count RBC count, white blood cell count (WBC) and hemoglobin (Hb). Significant rise in physiological parameters such as respiration rate (RR), pulse rate, was observed. Hematobiochemical parameters such as red blood cell count (RBC), (WBC), (PCV%), (Hb), Cholesterol, creatinine, ALT, AST, Cortisol and BUN were increased. From the present study it can be concluded that THI is a sensitive indicator of heat stress.

Keywords: Heat Stress, Physiological Parameters, Hemato-bochemical Parameters Dairy cattle, Temperature Humidity Index

Climate change, defined as the long-term imbalance of weather conditions like temperature, radiation, wind and rainfall characteristics of a particular region, is likely to be one of the main challenges for mankind during the present century. The earth's climate has warmed in the last century ($0.74 \pm 0.18^\circ\text{C}$) with the 1990s and 2000s being the warmest on instrumental record (Intergovernmental Panel on Climate Change (IPCC, 2007). Seasonal and environmental changes can effect hematological values in domestic animals (Feldman *et al.*, 2002). Rise in environment temperature decreases the milk production in high milk producing dairy cattle. With rise in population, Increased livestock productivity is associated with increased production diseases that reflect changes in blood profile (Hewett, 1974). Change in environmental variables such as ambient temperature, relative humidity, wind and rainfall were recognized as the potential hazards in livestock growth and production. Haematological parameters have been used to identify effect of heat stress on productivity in dairy cattle (Grünwaldt *et al.*, 2005). Blood parameter

profile as animal response indicators can serve as the basis for diagnosis, treatment, and prognosis of diseases (Otto *et al.*, 2000; Ndlovu *et al.*, 2007). Stress-induced changes in immune function have been recorded in dairy cattle, with alterations to cell-mediated and humoral immunity having a significant impact on immune competence which may render an animal more become more susceptible to infections (Carroll and Forsberg, 2007). Exposure of dairy cattle to high temperature environment stimulates thermoregulatory mechanisms and produces reduction in the rates of metabolism, feed intake and productivity (Abdelatif and Alameen, 2012). Sweating, high respiration rate, vasodilation with increased blood flow to skin surface, In order to maintain homeothermy, an animal must be in thermal equilibrium with its environment, which includes radiation, air temperature, air movement and humidity (Kadzere *et al.*, 2002). Animal suffer from heat stress when any combination of environmental conditions causes the effective temperature of the environment to be higher than the animal's "thermoneutral" zone (Armstrong,



1994). Productivity of dairy cattle depends on the use of specialized animals as well as on their reproductive health, nutritional characteristics, and environment in which they are raised. Selection for milk production reduces the ability of the cow to withstand the stress caused by heat, thereby increasing susceptibility to heat stress and decreasing production and reproductive efficiency during the hotter months of the year (Vasconcelos and Demetrio, 2011). Breeds of Exotic origin suffer more from heat stress, in part due to their higher productivity, reducing their threshold of thermal comfort (Silva *et al.*, 2002). Heat stress causes changes in homeostasis and has been quantified by the measurement of physiological variables such as body temperature, respiratory rate, and hormone concentrations. Despite the cited and well-known differences in breeds and reaction to heat stress (McManus *et al.*, 2009), there is still little information regarding the critical levels of these traits for crossbred cows. Temperature which is exaggerated by extensive production systems. In these cases, management interventions may be possible, but they are difficult and expensive to implement, particularly in poorly adapted cattle.

This study was done to investigate changes in the physiological parameters of dairy cows, determine critical threshold values, and identify the physiological parameters that show higher reliability for verification of heat stress in dairy cows. Thermal stress is a major cause of production losses in the dairy cattle. Dairy animals are more heat sensitive as average milk yield has increased. During thermal stress physiological and biochemical changes occur in the animal body which directly or indirectly affect the production. This review clearly describes about biochemical and physiological changes occur during thermal stress in bovines. High rectal temperature, reduced metabolic rate, decreased DM intake, efficiency of feed utilization and altered water metabolism are the physiologic responses that are associated with negative impacts of heat stress on production and reproduction in dairy animals. Heat stress causes changes in homeostasis and has been quantified by the measurement of physiological variables such as body temperature, respiratory rate, and hormone concentrations. Despite the cited and well-known differences in breeds and reaction to heat stress (McManus *et al.*, 2009), there is still little information regarding the critical levels of these traits for crossbred cows. Cattle in subtropical and

tropical environments are subjected to numerous stress factors, including parasites (tick and tick borne diseases, internal parasites, flies); seasonally poor nutrition; high temperatures or high daily temperature variation; and high and/or low humidity and temperature which is exaggerated by extensive production systems. In these cases, management interventions may be possible, but they are difficult and expensive to implement, particularly in poorly adapted cattle. The best method of ameliorating the effects of these environmental stress factors to improve productivity and animal welfare is to select and breed cattle that are adapted and productive, without the need for managerial interventions. Rosales (1994) defined stress as the cumulative detrimental effect of various factors on health and performance of animals. Stress represents the reaction of body to stimuli that disturb normal physiological equilibrium or homeostasis, often with detrimental effects as shown by Khansari *et al.* According to Stott 1981, stress is the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in new adaptations that can be detrimental or advantageous to the animal. Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, sub-tropical and arid areas. The degree to which an animal resists change in body temperature varies with different species because of differences in their heat regulating mechanisms. Under thermal stress, a number of physiological and behavioral responses vary in intensity and duration in relation to the animal genetic make-up and environmental factors through the integration of many organs and systems viz. behavioral, endocrine, cardio-respiratory and immune system.

India is currently losing nearly 2 per cent of the total milk production, amounting to over ₹ 2,661 crore due to rise in stress among lactating cattle and buffaloes because of the stress and global warming (Khan, 2013 and Das *et al.*, 2016). The estimated annual milk loss at present due to stress among cattle and buffaloes at all India level is 1.8 million tonnes, which is nearly 2% of the total milk production in the country (Upadhyay *et al.*, 2007).

MATERIALS AND METHODS

The clinico-physiological parameters like Respiration rate (breaths/min), Heart rate, Rectal temp (°F), Pulse rate (beats/min), Dehydration status (%) if any, Skin temperature

(°C) were recorded. The peripheral skin temperature was recorded using non-contact telethermometer in the flank region (Raytek, Model Raynger ST2L, M/s. Surrey Scientific, Surrey, U.K.) by keeping it 2-3 inches away from the desired surface site.

Physiological parameters

The physiological parameters like Respiration rate (breaths/min), Heart rate, Rectal temp (°F), Pulse rate (beats/min), Dehydration status (%) if any, Skin temperature (°C) were recorded. The peripheral skin temperature was recorded using non-contact telethermometer in the flank region (Raytek, Model Raynger ST2L, M/s. Surrey Scientific, Surrey, U.K.) by keeping it 2-3 inches away from the desired surface site.

Respiration rate (RR)

Respiration rate of the animals were recorded from visual observation of inward and outward abdominal movement. One outward movement was counted as one respiration and respiration rate are expressed as breaths per minute.

Pulse rate (PR)

Pulse rate (PR) of the animals was counted by observing the pulsation of middle coccygeal artery at the base of the tail and the results are expressed as pulse rate per minute.

Rectal temperature (RT)

Rectal temperature was recorded using digital thermometer by keeping the thermometer in contact of rectal mucosa for about 2 minutes.

Following observations were recorded, on the basis of which the animals were classified as thermally stressed as follows:

1. Climatic Variables
2. Temperature Humidity Index (THI) which was calculated using the formula of Thom (1959)

$$THI = 0.72 (Tdb + Twb) + 40.6$$

Tdb = dry bulb temperature (°C)

Twb = wet bulb temperature (°C)

Determination of thermal stress susceptibility in dairy cows

Based on the below given criteria animals will be categorized into thermally stressed and in comfort zone:

- ⊙ Thermal Humidity Index (THI)
- ⊙ THI 1 (<72): Temperature: 23±1°C and Relative Humidity: 40± 5 % (Zone of Comfort)
- ⊙ THI 2 (78): Temperature: 35±1°C and Relative Humidity: 50± 5 % (Mild Stress)
- ⊙ THI 3 (91): Temperature: 41±1°C and Relative Humidity: 45± 5 % (High Stress)

Hemato-biochemical parameters

The blood samples were collected from jugular vein after clipping of hair and disinfection by alcohol in K₃-EDTA, Heparinized and Serum vacutainers. The blood samples were brought immediately to the lab placed in an ice bath for estimation of hematological parameters. Estimated hematological parameters were Hemoglobin (Hb) using Drabkin's Method, Packed Cell Volume (PCV) by microhematocrit method, RBC, WBC count by haemocytometer, MCV, MCH, and MCHC by using standard formulas, and Blood pH by using pH meter. The following biochemical parameters were estimated (Table 1): Different Observations were recorded, were Cortisol(µg/dl), BUN (mg/dl), Total Protein (g/dl), Albumin (g/dl), Globulin (g/dl), A:G (g/dl), Aspartate aminotransferase (AST)(U/L), Alanine aminotransferase (ALT) (U/L), Creatinine (mg/dl), Cholesterol(mg/dl). Cortisol determination was done by DetectX Cortisol Enzyme Immunoassay Kit Method. It involves the immunological reaction between the limiting amounts of added anti-cortisol monoclonal antibody, the cortisol antigen in the sample or standard and the limiting amount of added cortisol-peroxidase conjugate. As the concentration of cortisol in the sample increases, the amount of cortisol-peroxidase conjugate bound decreases causing a decrease in signal, and vice versa. The signal is generated from the cortisol-peroxidase bound to the anti-cortisol antibody which itself is bound to the goat anti-mouse IgG coated plates. Excess cortisol peroxidase does not bind to the plates and is washed out of the well prior to the addition of substrate, After an hour incubation

the plate is washed and substrate is added. The substrate reacts with the bound cortisol-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of generated color is detected in a microtiter plate reader at 450 nm wavelength. Total protein (TP) was estimated by Biuret method (ERBA®), in which plasma forms a blue colored complex when treated with cupric ions in alkaline medium. The intensity of the blue color is proportional to the protein concentration. Sodium (Na) was estimated by Colorimetric method. Potassium (K) was estimated by Colorimetric method, which is based on principle that potassium reacts with sodium tetra phenol boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample. Chloride (Cl) was estimated using Ferric Thiocyanate Method, which is based on principle when chloride is mixed with a solution of undissociated mercuric thiocyanate, the chloride preferentially combines with mercury forming mercuric chloride. The thiocyanate released combines with ferric ions present in the solution to form strongly colored ferric thiocyanate with an absorption maximum at 480 nm. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by using standard kits from Transasia Bio-Medicals. The principle reaction of colorimetric determination of AST and ALT activity is based on the reaction of aspartate or alanine with α -ketoglutarate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazine formed with 2, 4-dinitrophenyl hydrazine. BUN by using Erba kits, Total plasma proteins, albumin (BCG Dye Method), cholesterol (CHOD PAP Method). Group 1 represents animals which were kept in thermocomfort zone Group 2 represents animals which were heat stressed

Statistical analysis

One-way repeated measure analysis of variance (ANOVA) was applied.

RESULTS AND DISCUSSION

Effect of temperature on environmental variables

Temperature Humidity Index (THI) at the start of work

was (1st week of April) averaged 85.16 ± 0.84 which decreased non-significantly during July, August month. Thereafter THI increased. Mean \pm SE values of THI from 0 day to 45th day were 85.16 ± 1.16 , 83.80 ± 0.80 , 84.28 ± 1.17 , 83.56 ± 1.21 , 83.56 ± 1.21 , and 85.96 ± 0.64 . THI was calculated on 0,7,15,30 and 45 day using regular daily metrological data as given below (Table 1)

Table 1: Mean values of THI

DAY	THI
0	85.16 ± 1.16^a
7	83.80 ± 0.80^a
15	84.28 ± 1.17^a
30	83.56 ± 1.21^a
45	85.96 ± 0.64^a

Values with superscript a ($P < 0.05$) differ non-significantly.

Clinico-Physiological parameters

Mean \pm SE value of physiological parameters of control group and heat stressed animals were given in Table 2.

Table 2: Effect of micronutrients supplementation on Physiological parameters in climatic stressed dairy cattle (Mean \pm S.E)

Parameters	Control (n=6)	Group 1 (n=6)
Respiration Rate (Breaths/min)	20.17 ± 0.09^{Aa}	60.07 ± 2.40^{Bc}
Heart Rate (beats/min)	57.50 ± 1.63^{Aa}	62.15 ± 0.01^{Ba}
Rectal Temperature (°F)	102.20 ± 0.09^{Ba}	103.12 ± 0.05^{Cd}
Pulse Rate (Pulse / min)	50.91 ± 1.42^{Aa}	78.83 ± 1.11^{BAb}
Dehydration (%)	3.46 ± 0.03^{Aa}	5.72 ± 0.21^{Ca}
Skin temperature (°F)	37.31 ± 0.04^{Aa}	38.69 ± 0.09^{Bb}

Values with superscript a, b, c, d ($P < 0.05$) differ significantly within the group whereas A, B ($P < 0.05$) differ significantly between the groups.

Mean \pm SE values of respiration rate (breaths/min) of control Group animals was 20.17 ± 0.09 , respectively. Mean \pm SE values of respiration rate (breaths/min) of Group 1 animals was 60.07 ± 2.40 . Heat stress resulted in significant ($P < 0.05$) increase in values of respiration rate (breaths/min) Mean \pm SE value of heart rate (beats/min) of control Group

animals was 57.50 ± 1.63 , respectively. Mean \pm SE value of heart rate (beats/min) of Group 1 animals was 62.15 ± 0.01 . Stress resulted in significant increase in values of heart rate (beats/min). Mean \pm SE value of rectal temperature ($^{\circ}$ F) of Control Group animals was 102.20 ± 0.09 . Mean \pm SE value of rectal temperature ($^{\circ}$ F) of Group 1 animals was 103.12 ± 0.05 . Mean \pm SE value of pulse rate of Control Group animals was 50.91 ± 1.42 . Mean \pm SE value of pulse rate of Group 1 animals was 78.83 ± 1.11 . Mean \pm SE value of dehydration of Control Group animals was 3.46 ± 0.03 . Mean \pm SE value of dehydration of Group 1 animals was 5.72 ± 0.21 , Mean \pm SE value of skin temperature of control Group animal was 37.31 ± 0.11 . The rectal temperature ($^{\circ}$ F) values of heat stressed animal was raised then control Group.

Effect on hematological parameters

Mean \pm SE values of hematological parameters are given in Table 3. Mean \pm SE values of Hb (g/dl) of control Group animals was 9.92 ± 0.20 , with significant decrease ($P < 0.05$) in Hb values due to stress. Mean \pm SE values of Hb (g/dl) of Group 1 animals was 11.12 ± 0.22 , Mean \pm SE values of PCV (%) of control Group animals was 35.41 ± 1.33 . There was rise in ($P < 0.05$) in PCV values due to stress. Mean \pm SE values of RBC ($\times 10^6 / \mu\text{l}$) of control Group animals was $5.40 \pm 0.05^{\text{Ca}}$, Mean \pm SE values of RBC ($\times 10^6 / \mu\text{l}$) of Group 1 animals was 33.17 ± 2.54 , respectively. Mean \pm SE values of RBC of Group 1 animals was, $3.93 \pm 0.22^{\text{Aa}}$ Mean \pm SE values of RBC decreases due to stress. Mean \pm SE values of WBC (g/dl) of control Group animals was 9.78 ± 0.25 . Mean \pm SE values of WBC (g/dl) of in stressed Group animals was declined. Mean \pm SE values of MCV (g/dl) of control Group animals was $44.85 \pm 0.64^{\text{Bab}}$ Mean \pm SE values of MCV (g/dl) of Group 1 animals was $91.86 \pm 6.32^{\text{Ba}}$, Mean \pm SE values of MCH (g/dl) of Control Group animals was $13.36 \pm 0.30^{\text{Ba}}$, Mean \pm SE values of MCH (g/dl) of Group 1 was raised, was $28.69 \pm 1.35^{\text{Cc}}$ Mean \pm SE values of MCHC (g/dl) of Control Group animals was $29.82 \pm 0.90^{\text{ABa}}$, Mean \pm SE values of MCHC (g/dl) of Group 1 animals was $31.47 \pm 1.35^{\text{ABb}}$, which showed rise due to heat stress. Mean \pm SE values of Blood pH of Control Group animals was $7.39 \pm 0.02^{\text{Aa}}$ there was non significant decrease in Blood pH values Mean \pm SE values of Blood pH of Group 1 animals was $7.33 \pm 0.01^{\text{Aa}}$.

Table 3: Effect of Heat stress on hematology in climatic stressed dairy cattle (Mean \pm S.E)

Parameter	Control (n=6)	Group 1 (n=6)
Hb(g/dl)	$9.92 \pm 0.20^{\text{Aa}}$	$11.12 \pm 0.22^{\text{Ba}}$
PCV(%)	$32.33 \pm 0.49^{\text{Aaa}}$	$35.41 \pm 0.88^{\text{Ba}}$
RBC($\times 10^6 / \mu\text{l}$)	$5.40 \pm 0.05^{\text{Ca}}$	$3.93 \pm 0.22^{\text{Aa}}$
WBC($\times 10^3 / \mu\text{l}$)	$9.78 \pm 0.25^{\text{Ca}}$	$5.68 \pm 0.16^{\text{Aa}}$
MCV(fl)	$44.85 \pm 0.64^{\text{Bab}}$	$91.86 \pm 6.32^{\text{Ba}}$
MCH(pg)	$13.36 \pm 0.30^{\text{Ba}}$	$28.69 \pm 1.35^{\text{Cc}}$
MCHC(g/dl)	$29.82 \pm 0.90^{\text{ABa}}$	$31.47 \pm 1.35^{\text{ABb}}$
Blood pH	$7.39 \pm 0.02^{\text{Aa}}$	$7.33 \pm 0.01^{\text{Aa}}$

Values with superscript a, b, c, d ($P < 0.05$) differ significantly within the group whereas A, B ($P < 0.05$) differ significantly between the groups.

Mean \pm SE values of biochemical parameters of stressed animals are given in Table 4. Mean \pm SE values of plasma cortisol ($\mu\text{g/dl}$) of Control Group animals was $0.85 \pm 0.01^{\text{ABa}}$ stress resulted in non-significant increase ($P < 0.05$) in plasma cortisol ($\mu\text{g/dl}$) values. Mean \pm SE values of plasma cortisol ($\mu\text{g/dl}$) of Group 1 animals was $0.88 \pm 0.02^{\text{Bc}}$. Stress resulted in significant increase ($P < 0.05$) in plasma cortisol ($\mu\text{g/dl}$) values. Mean \pm SE values of plasma BUN (mg/dl) of control Group animals was $14.26 \pm 0.22^{\text{Aa}}$. There was significant ($P < 0.05$) difference in plasma BUN (mg/dl) values from day 0 to day 45. Mean \pm SE values of plasma BUN (mg/dl) of Group 1 animals was $35.13 \pm 1.55^{\text{Aa}}$. Mean \pm SE values of total protein (g/dl) of Control Group animals was $6.44 \pm 0.16^{\text{Aa}}$. Mean \pm SE values of total protein (g/dl) of Group 1 was $6.72 \pm 0.27^{\text{Aa}}$. Stress resulted in non-significant ($P < 0.05$) decrease in total protein (g/dl) values. Mean \pm SE values of Albumin (g/dl) of Control Group animals was $3.30 \pm 0.33^{\text{Aa}}$, significant ($P < 0.05$) difference in Albumin (g/dl) values was observed due to heat stress. Mean \pm SE values of Albumin (g/dl) of Group 1 animals was $3.57 \pm 0.19^{\text{Ac}}$. Mean \pm SE values of Globulin (g/dl) of control Group animals was $2.74 \pm 0.21^{\text{Aa}}$ $2.46 \pm 0.22^{\text{Aa}}$ $2.52 \pm 0.21^{\text{Aa}}$ $2.52 \pm 0.24^{\text{Aa}}$ $2.45 \pm 0.47^{\text{Aa}}$, respectively. Mean \pm SE values of A.G (g/dl) of Group 1 animals on was $1.31 \pm 0.25^{\text{ABa}}$. Mean \pm SE values of AST (U/L) of Control Group animals was $82.95 \pm 0.73^{\text{Aa}}$. Significant increase in AST, (U/L) values was observed. Mean \pm SE values of AST (U/L) of Group 1 animals was $134.07 \pm 1.02^{\text{Bbc}}$. Stress results in significant increase in AST, level. Mean \pm SE values of ALT (U/L) of Control Group animals was $38.61 \pm 0.52^{\text{Aa}}$. Non-significant ($P < 0.05$) difference in

ALT values was observed. Mean \pm SE values ALT (U/L) of Group 1 animals was 54.97 ± 0.97^{Ca} . Stress results in non significant increase in ALT value. Mean \pm SE values of Creatinine (mg/dl) of Control Group animals was 1.31 ± 0.01^{Aa} respectively. Stress results in non significant increase in Creatinine value, Mean \pm SE values of Creatinine (mg/dl) of Group 1 animals was 1.78 ± 0.17^{Ba} . Stress results in significant increase in Creatinine value. Mean \pm SE values of Cholestorol (mg/dl) of Control Group animals was 127.64 ± 2.68^{Ab} . Significant ($P < 0.05$) increase in Cholesterol m (g/dl) values was seen.

Table 4: Effect of heat stress on biochemical parameters in climatic stressed dairy cattle (Mean \pm S.E)

Parameters	Control (n=6)	Group 1 (n=6)
Cortisol (μ g/dl)	0.85 ± 0.01^{ABa}	0.88 ± 0.02^{Bc}
BUN (mg/dl)	14.26 ± 0.22^{Aa}	35.13 ± 1.55^{Aa}
Total Protein (g/dl)	6.44 ± 0.16^{Aa}	6.72 ± 0.27^{Aa}
Albumin (g/dl)	3.30 ± 0.33^{Aa}	3.57 ± 0.19^{Ac}
Globulin (g/dl)	2.74 ± 0.21^{Aa}	3.15 ± 0.45^{Aa}
A:G (g/dl)	1.71 ± 0.19^{Aa}	1.31 ± 0.25^{ABa}
AST (U/L)	82.95 ± 0.73^{Aa}	134.07 ± 1.02^{Bbc}
ALT (U/L)	38.61 ± 0.52^{Aa}	54.97 ± 0.97^{Ca}
Creatinine (mg/dl)	1.31 ± 0.01^{Aa}	1.78 ± 0.17^{Ba}
Cholestorol (mg/dl)	127.64 ± 2.68^{Ab}	129.31 ± 2.71^{Ac}

Values with superscript a, b, c, d ($P < 0.05$) differ significantly within the group whereas A, B ($P < 0.05$) differ significantly between the groups.

CONCLUSION

Present study results showed variations in haematobiochemical parameters related to changes in ambient temperature, and temperature-humidity index, although within the physiological range for cattle. Therefore, we can claim that the seasonal variations can influence the hematobiochemical profile of cattle. These results provide insights into physiological responses of dairy cattle to raised temprature, allowing to better evaluate its ability to adapt and cope with environmental stress.

REFERENCES

Abdelatif, A.M. and Alameen, A.O. 2012. Influence of season and pregnancy on thermal and haematological responses of

crossbred dairy cows in a tropical environment. *Global Vet.*, **9**: 334–340.

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci.*, **77**: 2044-2050.

Azevedo, M., Pires, M.F.A., Saturnino, H.M., Lana, A.M. Q., Sampaio, I.B.M., Monteiro, J.B.N. and Morato, L.E. 2005. Estimativa de níveiscríticossuperiores do índice de temperatura e umidade paravacastleiteiras 1/2, 3/4, 7/8 Holandês-Zebu em lactação. *Revista Brasileira de Zootecnia*, **34**: 2000-2008.

Buffington, D.E., Collazo-Arocho, A., Canton, G.H., Pitt, D., Thatcher, W.W. and Collier, R.J. 1981. Black globe-humidity index (BGHI) as comfort equation for dairy cows. *Trans. ASAE.*, **24**: 711-714.

Carroll, J.A. and Forsberg, N.E. 2007. Influence of stress and nutrition on cattle immunity. *Vet. Clin. North Am. Food Anim. Pract.*, **23**: 105–149.

Collier, R.J., Zimbelman, R.B., Rhoads, R.P., Rhoads, M.L. and Baumguard, L.H. 2009. A re-evaluation of the impact of temperature humidity index (THI) and black globe temperature humidity index (BGHI) on milk production in high producing dairy cows, pp. 158-168. In: Proceedings of the 24th Southwest Nutrition and Management Conference, Tempe, Arizona.

Das, G.K. and Khan, F.A. 2010. Summer anoestrus in buffalo-A review. *Reprod. Domest. Anim.*, **45**: e483-e494.

Feldman, B.F., Zinkl, J.G. and Jain, N.C. 2002. Schalm's Veterinary Hematology. Lippincott Williams and Wilkins, Philadelphia, Baltimore, New York, London, Buenos Aires, Hong Kong, Sydney, Tokyo.

Grünwaldt, E.G., Guevara, J.C., Estévez, O.R., Vicente, A., Rousselle, H., Alcu ten, N., Aguerregaray, D. and Stasi, C.R. 2005. Biochemical and haematological measurements in beef cattle in Mendoza plain rangelands (Argentina). *Trop. Anim. Health Prod.*, **37**: 527-540.

Hewett, C. 1974. On the causes and effects of variations in the blood profile of Swedish dairy cattle. *Acta Vet. Scand.*, **50**: 1–152.

IPCC (Intergovernmental Panel on Climate Change) (2007). Climate Change 2007: Synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core writing team: Pachauri, R.K. and Reisinger, A. (Eds.)]. IPCC, Geneva, Switzerland.

Jain N.C. 1986. Schalm's Veterinary Haematology. 4th edition Lea and Febiger, Philadelphia.

Johnson, H.D., Ragsdale, A.C., Berry, I.L. and Shanklin, M.D. 1962. Effect of various temperature-humidity combinations on milk production of Holstein cattle. Research. *Bulletin Missouri Agricultural Experimental Station*, pp. 791.

- Kadzere, C.T., Murphy, M.R., Silanikove, N. and Maltz, E. 2002. Heat stress in lactating dairy cows: a review. *Livest. Prod. Sci.*, **77**: 59–91.
- Khan, F.A., Prasad, S. and Gupta, H.P. 2013 Effect of heat stress on pregnancy rates of crossbred dairy cattle in Terai region of Uttarakhand, India. *Asian Pacific J. Reprod.*, **4**: 277-279.
- Khansari, D.N., Murgo, A.J. and Faith, R.E. 1990. Effect of stress on the immune system. *Immunol. Today*, **11**: 170-175.
- Martello, L.S., Savastano Jr., H., Silva, S.L. and Titto, E.A.L. 2004. Respostas fisiológicas produtivas de vacas holandesas em lactação submetidas a diferentes ambientes. *Revista Brasileira de Zootecnia*, **33**: 3-11.
- Ndlovu, T., Chimonyo, M., Okoh, A.I., Muchenje, V., Dzama, K. and Raats, J.G. 2007. Assessing the nutritional status of beef cattle: current practices and future prospects. *Afr. J. Biotechnol.*, **6**: 2727–2734.
- Otto, F., Baggasse, P., Bogin, E., Harun, M. and Vilela, F. 2000. Biochemical blood profile of Angoni cattle in Mozambique. *Israel Vet. Med. Assoc.*, **55**: 1–9.
- Rosales, A.G. 1994. Stress syndrome in birds. *J. Appl. Poult. Res.*, **3**: 199-203.
- Salah, M.S., Al-Shaikh, M.A., Al-Saiadi, M.Y. and Mogawer, H.H. 1995. Effect of prolactin inhibition on thermoregulation, water and food intake in heat stressed fat-tailed male lambs. *Anim. Sci.*, **60**: 87-91.
- Silanikove, N., Maltz, E., Halevi, A. and Shinder, D. 1997. Metabolism of water, sodium, potassium and chloride by high yielding dairy cows at the onset of lactation. *J. Dairy Sci.*, **80**: 949-956.
- Silanikove, N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod. Sci.*, **67**: 1-18.
- Stott, G.H. 1981. What is animal stress and how is it measured. *J. Anim. Sci.*, **52**: 150-153.
- Upadhay, R.C., Singh, S.V., Kumar, A., Gupta, S.K. and Ashutosh. 2007. Impact of climate change on milk production of Murrah buffaloes. *Indian J. Anim. Sci.*, **6**(2): 1329-1332.
- Zecchini, M.S., Barbieri, K. Boureima, and Crimella, C. 2003. Heat stress parameters in Azawak cattle (*Bos indicus*): Four seasons of data collection. *Ital. J. Anim. Sci.*, **2**(Suppl.1): 142–144.

