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

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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-biochemical 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 ($0.74 \pm 0.18^\circ$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 diary 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,
Productivity of dairy cattle's 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 the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in 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 millliontonnes, 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...
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Physiological parameters

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)

\[
\text{THI} = 0.72 (T_{\text{db}} + T_{\text{wb}}) + 40.6
\]

\[
T_{\text{db}} = \text{dry bulb temperature (°C)}
\]

\[
T_{\text{wb}} = \text{wet bulb temperature (°C)}
\]

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:

- 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 K$_3$-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 ±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)

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

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

Clinico-Physiological parameters

Mean ±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 ± S.E)

| Parameters                      | Control (n=6)     | Group 1 (n=6)     |
|---------------------------------|-------------------|-------------------|
| Respiration Rate (Breaths/min)  | 20.17±0.09<sup>a</sup> | 60.07±2.40<sup>b</sup> |
| Heart Rate (beats/min)         | 57.50±1.63<sup>a</sup> | 62.15±0.01<sup>b</sup> |
| Rectal Temperature (°F)        | 102.20±0.09<sup>a</sup> | 103.12±0.05<sup>c</sup> |
| Pulse Rate (Pulse / min)       | 50.91±1.42<sup>a</sup> | 78.83±1.11<sup>Ab</sup> |
| Dehydration (%)                | 3.46±0.03<sup>a</sup> | 5.72±0.21<sup>c</sup> |
| Skin temperature (°F)          | 37.31±0.04<sup>a</sup> | 38.69±0.09<sup>b</sup> |

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 ±SE values of respiration rate (breaths/min) of control Group animals was 20.17±0.09, respectively. Mean ±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 ±SE value of heart rate (beats/min) of control Group
animals was 57.50±1.63, respectively. Mean ±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 ±SE value of rectal temperature (°F) of Control Group animals was 102.20±0.09. Mean ±SE value of rectal temperature (°F) of Group 1 animals was 103.12±0.05. Mean ±SE value of pulse rate of Control Group animals was 50.91±1.42. Mean ±SE value of pulse rate of Group 1 animals was 78.83±1.11. Mean ±SE value of dehydration of Control Group animals was 3.46±0.03. Mean ±SE value of dehydration of Group 1 animals was 5.72±0.21. Mean ±SE value of skin temperature of control Group animal was 37.31±0.11. The rectal temperature (°F) values of heat stressed animal was raised then control Group.

### Effect on hematological parameters

Mean ±SE values of hematological parameters are given in Table 3. Mean ±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 ±SE values of Hb (g/dl) of Group 1 animals was 11.12±0.22, Mean ±SE values of PCV (%) of control Group animals was 5.40±0.05. There was rise in (P<0.05) in PCV values due to stress. Mean ±SE values of RBC (×10⁶ /µl) of control Group animals was 5.40±0.05, Mean ±SE values of RBC (×10⁶ /µl) of Group 1 animals was 3.31±2.54, respectively. Mean ±SE values of RBC of Group 1 animals was 3.93±0.22. Mean ±SE values of RBC decreases due to stress. Mean ±SE values of WBC (g/dl) of control Group animals was 29.82±0.90. Mean ±SE values of WBC (g/dl) of Group 1 animals was 33.17±2.54, respectively. Mean ±SE value of dehydration of Group 1 animals was 78.83±1.11. Mean ±SE value of skin temperature of control Group animals was 37.31±0.11. The rectal temperature (°F) values of heat stressed animal was raised then control Group.

Mean ±SE values of biochemical parameters of stressed animals are given in Table 4. Mean ±SE values of plasma cortisol (µg/dl) of Control Group animals was 0.85±0.01. Stress resulted in non-significant increase (P<0.05) in plasma cortisol (µg/dl) values. Mean ±SE values of plasma cortisol (µg/dl) of Group 1 animals was 0.88±0.02. Stress resulted in significant increase (P<0.05) in plasma cortisol (µg/dl) values. Mean ±SE values of plasma BUN (mg/dl) of control Group animals was 14.26±0.22. There was significant (P<0.05) difference in plasma BUN (mg/dl) values from day 0 to day 45. Mean ±SE values of plasma BUN (mg/dl) of Group 1 animals was 35.13±1.55. Mean ±SE values of total protein (g/dl) of Control Group animals was 6.44±0.16. Mean ±SE values of total protein (g/dl) of Group 1 was 6.72±0.27. Stress resulted in non-significant (P<0.05) decrease in total protein (g/dl) values. Mean ±SE values of Albumin (g/dl) of Control Group animals was 3.30±0.33. Significant (P<0.05) difference in Albumin (g/dl) values was observed due to heat stress. Mean ±SE values of Albumin (g/dl) of Group 1 animals was 3.57±0.19. Mean ±SE values of Globulin (g/dl) of control Group animals was 2.74±0.21. Mean ±SE values of Globulin (g/dl) of Group 1 animals was 2.52±0.20. 2.52±0.20 2.52±0.20 2.52±0.20 2.52±0.20 2.52±0.20 2.52±0.20.

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

| Parameter     | Control (n=6)       | Group 1 (n=6)     |
|---------------|---------------------|-------------------|
| Hb (g/dl)     | 9.92±0.20           | 11.12±0.22        |
| PCV (%)       | 5.40±0.05           | 3.31±2.54         |
| RBC (×10⁶ /µl)| 3.93±0.22           | 33.17±2.54        |
| WBC (g/dl)    | 29.82±0.90          | 33.17±2.54        |
| MCV (fl)      | 44.85±0.64          | 91.86±6.32        |
| MCH (pg)      | 13.36±0.30          | 28.69±1.35        |
| MCHC (g/dl)   | 29.82±0.90          | 31.47±1.35        |
| Blood pH      | 7.39±0.02           | 7.33±0.01         |

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.
ALT values was observed. Mean ±SE values ALT (U/L) of Group 1 animals was 54.97±0.97Ca. Stress results in non significant increase in ALT value. Mean ±SE values of Creatinine (mg/dl) of Control Group animals was 1.31±0.01Ac respectively. Stress results in non significant increase in Creatinine value. Mean ±SE values of Cholesterol (mg/dl) of Group 1 animals was 17.78±1.55Ca. Stress results in significant increase in Creatinine value. Mean ±SE values of Cholesterol (mg/dl) of Control Group animals was 127.64±2.68Ab. 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 ± S.E)

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

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 temperature, allowing to better evaluate its ability to adapt and cope with environmental stress.

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