RESEARCH ARTICLE

# Effect of thermal stress on physiological, hormonal and haematological parameters in Tharparkar and Karan Fries calves

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**Abstract:** The present investigation was undertaken to study the effect of thermal stress on physiological, haematological and hormonal parameters in Tharparkar and Karan Fries calves. The animals of both breeds were divided into two groups. Group I of each breed was kept in the natural climatic conditions; whereas group II of each breed was exposed to temperatures 10, 25 & 44$^0$C in the climatic chamber for 4 hours. Blood samples were collected before exposure and at the end of 4 h exposure. The physiological responses; respiration rate (RR), heart rate (HR), rectal temperature (RT), skin temperature (ST); haematological profile, hormone levels in the plasma were estimated. The physiological responses (RR, HR, RT, and ST) increased significantly in both breeds at 44$^0$C compared to 10$^0$C and 25$^0$C. Significant increase was also recorded in RBC, haematocrit and haemoglobin, whereas no significant changes were observed in TLC and DLC (lymphocyte, monocyte, and granulocyte). At 44$^0$C, prolactin and cortisol levels increased significantly in both breeds whereas no significant differences were observed in GH concentration. The increase in physiological responses, RBC, haematocrit, haemoglobin was higher in Karan Fries than Tharparkar. The breed differences in hormone levels also signified that Tharparkar was more thermotolerant than Karan Fries cattle. The significantly higher deviations in physiological, haematological, hormonal levels reported in Karan Fries than Tharparkar in the present study may serve as indicators of distress level in Karan Fries calves during thermal stress.

**Keywords:** Tharparkar, Karan Fries, calves, thermal stress, physiological response, hormones, haematology

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## Introduction

Humped cattle of Indian origin (*Bos indicus* or zebu cattle) and humpless cattle of Europe and Africa (*Bos taurus*) emerged from a common ancestor. *B. indicus* diverged from *B. taurus* somewhere between 110,000 and 850,000 years ago (Bradley *et al*., 1996, MacHugh *et al*., 1997). *Bos indicus* breeds can regulate body heat better than *Bos taurus* breeds, due to inherent differences in physiological functions (sweating rate, coat characteristics, skin colour, metabolic rate, food and water consumption (Blackshaw and Blackshaw, 1994). Domestic animals are subjected to various kinds of stresses such as physical, nutritional, chemical, psychological and thermal (Marai *et al*., 2007; Nardone *et al*., 2006). Among these stressors, heat stress hampers significantly animal productivity in tropical countries. Thermal stress includes both heat stress during extreme summer and cold stress during extreme winter (Silanikove, 1992). Thermal stress affects the productivity of cattle as a reflection of homeokinetic changes taking place in the body of the animal in an attempt to regulate the body temperature. During thermal stress, breeds of Zebu cattle regulate the physiological and biochemical changes with lesser efforts compared to breeds of *B.taurus* of European origin. Among the genetic adaptations that have developed in Zebu cattle during evolution have been the acquisition of genes for thermal tolerance. Superior ability for regulation of body temperature in zebu cattle during heat stress is due to its lower metabolic rate as well as increased capacity for heat loss due to superior heat loss mechanisms. Blood performs a number of functions in the body of animals and any change in its constituents reflects the current functional status of the body system. Thus, the blood constituents can be used as an index for assessing the adaptation capacity of cattle to climate change (Manresa *et al*., 1940). Higher values of physiological parameters (RR, RT, and HR) have been reported under thermal stress compared to thermal neutral zone (Mc Dowell and Woodward, 1982; Al-Tamimi, 2007). The hormones associated with adaptation to heat stress are Prolactin (PRL), growth hormone (GH), glucocorticoids, mineralocorticoids (Farooq *et al*., 2010). The present study was undertaken to understand the differences in physiological responses and blood constituents of Zebu and crossbred cattle during thermal stress and how the whole body system influence maintenance of homeothermy.

## Materials and Methods

Tharparkar (10) and Karan Fries (10) calves of 6 to 12 months age were selected from the herd of NDRI, Karnal and divided into two groups. The animals were offered roughage *ad libitum* and concentrate at the rate of 1.5 kg (16% CP and 70% TDN) per animal per day. Water was made available all day round. Group I of each breed was maintained in a shed under natural climatic conditions viz $11^0$C-$12^0$C (pre exposure) and $14^0$C-$16.6^0$C (post exposure), RH of 51-53%, THI of 53.5-60.7 were concurrent for $10^0$C and $44^0$C exposures respectively; $25^0$C exposure was done when the ambient temperature was 25±$2^0$C, RH 68% and THI 73.6. Group II of each breed was exposed at 10, 25 and $44^0$C and RH 65% for 4 hours in a climatic chamber at different time interval (3 weeks time between different exposure temperatures). Blood samples were collected before and after 4 h of exposure from both groups by jugular venipuncture in lithium heparin-coated vacutainer® tubes (BD Biosciences, Franklin Lakes, NJ, USA). Physiological responses (RR, HR, RT, ST) were recorded before exposure and at the end of thermal exposure. The plasma separated by centrifuging the samples at 3000 rpm for 30 minutes was aliquoted in storage vials and stored at -$20^0$C for hormone assay.

Respiration rate of the animal was recorded by counting inward and outward flank movements. Rectal temperature was recorded with a digital thermometer by keeping the thermometer in contact with rectal mucosa 2 inches inside the rectum for 2 minutes. Heart rate was recorded by placing a stethoscope on the left side at the sixth intercostal space sufficiently forward between the upper foreleg and the chest wall. The skin temperature of the animal at the rump region was recorded using the infrared thermal imaging camera. Haematological parameters were recorded in a blood autoanalyzer. Plasma growth hormone (catalogue No. SEA044Bo), prolactin (catalogue No. SEA846Bo) and cortisol (catalogue No. CEA462Ge) were estimated with ELISA kits supplied by *Uscn Life Sciences Inc.* Export Processing Zone Building F, Wuhan, Hubei 430056, PRC

## Statistical analysis

The t test was used to determine significance of the data for comparison of the experimental period with the combined means during the pre- and post temperature exposure periods. Results were expressed as the means ± SEM. A difference with value $P<0.05$ was considered statistically significant otherwise is considered statistically non significant.

## Results and discussion

### Physiological responses

The physiological responses (RR, RT, HR, ST) increased significantly in Tharparkar and Karan fries calves on exposure to high temperature ($44^0$C). The increase was higher in Karan Fries than Tharparkar. The percentage increase of Tharparkar and Karan Fries calves at $44^0$C in RR, RT, HR and ST was 200.86, 306.30; 4.37, 4.62; 16.22, 20.11; 55.71, 68.88%, respectively. In this study both the breeds showed significant differences in all physiological parameters after exposure at $44^0$C. The findings are supported by the finding of other authors. The higher RT in Karan Fries than Tharparkar is in accordance with the findings of Weldy *et al.* (1964). The elevation in RR in this study is in agreement the results reported by Betty et al. (2006) who observed that RR increased linearly in *Bos taurus* with increase in environmental temperature from $26.0^0$C to $32.0^0$C. Das *et al.* (1999) reported that the surface temperature of Murrah buffalo calves increased from $30.66 \pm 0.31$ to $49.93 \pm 1.47^0$C when the calves were exposed at $42.6^0$C. Bhan *et al*. (2013) observed that the magnitude of increase in respiration rate and skin temperature and pulse rate in Sahiwal heifers were significantly higher in hot humid season than summer and spring season. Rectal temperature is an indicator of thermal stress and can be used as a marker to assess the severity of thermal environment which affects the physiological status of the animal (Johnson, 1980 and West, 1999). The increased heart rate (HR) with increase of exposure temperature in the present study is in agreement with the study of Chaiyabutr *et al*. (1987) who reported that during 5 hours of acute heat exposure ($41^0$C) in Swamp buffaloes, the heart rate increased from 43 ± 2 to 51± 1beats / minute. The increase in RR, RT, HR, and ST in the present study indicated that the animals were unable to balance the heat load and the increase was more in Karan Fries calves. Similar results were reported by Pereira et *al.* (2008) who observed that Frisian cattle subjected to heat stress, developed high thermal polypnoea (more than 105 breath per minute) and could not prevent an increase in the rectal temperature (from $38.7^0$C to $40.0^0$C). The authors attributed it to the lower heat tolerance of Frisian cattle.

### Hormonal responses

Prolactin, growth hormone, thyroxine and glucocorticoids are the main hormones involved in adaptation to thermal stress as these are essential for homeorhesis and homeostasis (Beede and Collier, 1986). In chronically heat stressed cattle the decrease in basal metabolism appears to be related to reduction in thyroxine, growth hormone, glucocorticoid concentrations and increase in plasma prolactin (Collier *et al*; 1982). The mean ± SEM of all hormonal parameters of this study in Tharparkar & Karan Fries calves are given in the table 2.

The GH values didn't differ significantly between breeds or within the breed when the calves were exposed to temperatures $10^0$C, $25^0$C or $44^0$C. Mitra *et al*. (1972), reported that decline in level of growth hormone is noticed only in chronic heat stress. The static concentration of GH observed in acute heat stress can be explained as GH act as a calorigenic hormone (Farooq *et al*., 2010). The rectal temperature, respiration rate and cortisol were

**Table 1** Effect of thermal stress on physiological parameters in Tharparkar and Karan Fries calves

| PARAMETER | | TP | | | | KF | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GROUP I | GROUP II (25$^0$C) | 10$^0$C | 44$^0$C | GROUP I | GROUP II (25$^0$C) | 10$^0$C | 44$^0$C |
| Respiration Rate (breaths /min) | Pre exposure | 23.33± 1.23 | 22.2±1.32 | 22±0.77 | 23.2$^a$±0.8 | 22.33 ±1.35 | 24.8±0.48 | 23±0.70 | 22.2$^a$±0.73 |
| | Post exposure | 22.40$^A$±1.05 | 22.2$^A$±1.32 | 21.8$^A$±0.8 | 69.8$^{XBb}$±1.39 | 22.60$^A$±0.98 | 24.8$^A$±0.48 | 22.4$^A$±1.32 | 90.2$^{YBb}$±2.15 |
| Rectal Temperature ($^0$F) | Pre exposure | 101.56 ± 0.16 | 101.28±0.26 | 101.64±0.42 | 101.06$^a$±0.26 | 102.28± 0.32 | 101.24±0.38 | 102.48±0.24 | 101.24$^a$±0.38 |
| | Post exposure | 101.24$^A$ ± 0.19 | 101.28$^A$±0.26 | 101.76$^A$±0.22 | 105.48$^{Bb}$±0.32 | 102.27$^A$± 0.33 | 101.4$^A$±0.42 | 102.32$^A$±0.44 | 105.92$^{Bb}$±0.47 |
| Heart Rate (beats /min) | Pre exposure | 67.2±0.71 | 67.2±0.55 | 67±0.61 | 67.2$^a$±0.96 | 68.6 ± 1.42 | 68.6±1.43 | 65.4±1.07 | 68.6$^a$±1.43 |
| | Post exposure | 65.2$^A$± 1.3 | 67.2$^A$±0.55 | 61.1$^A$±1.89 | 78.1$^{Bb}$±3.62 | 66.5$^A$ ± 1.14 | 68.6$^A$±1.43 | 62.9$^A$±1.51 | 82.4$^{Bb}$±2.53 |
| Skin Temperature ($^0$C) | Pre exposure | 21.66±1.50 | 21.66±1.50 | 24.56±0.29 | 23.8$^a$±2.2 | 23.80±2.20 | 23.8±2.2 | 26.42±0.82 | 21.66$^a$±1.50 |
| | Post exposure | 23.61$^A$±1.62 | 21.66$^A$±1.50 | 22.5$^A$±0.47 | 37.06$^{Bb}$±0.51 | 24.24$^A$±1.57 | 23.8$^A$±2.2 | 26.74$^A$±1.90 | 36.58$^{Bb}$±0.31 |

Means with different superscripts (a & b) in a column differ significantly (P< 0.001) for respective breeds; Means with different superscripts (A & B) in a row differ significantly for respective breed; (P< 0.001), Means with different superscripts (X& Y) in a row differ significantly (P< 0.001) for respective parameters

**Table 2** Effect of thermal stress on hormonal profile of Tharparkar and Karan Fries calves

| PARAMETER | | TP | | | | KF | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GROUP I | GROUP II (25$^0$C) | 10$^0$C | 44$^0$C | GROUP I | (25$^0$C) | GROUP II 10$^0$C | 44$^0$C |
| Growth Hormone (ng/ml) | Pre exposure | 5.48 ± 0.48 | 5.25±0.48 | 5.15±0.38 | 4.95±0.38 | 5.66 ± 1.11 | 5.79±1.13 | 5.08±0.34 | 5.42±0.30 |
| | Post exposure | 5.73 ± 0.61 | 5.25±0.48 | 5.40±0.36 | 4.58±0.62 | 6.58 ± 1.63 | 5.78±1.12 | 5.35±0.48 | 4.38±0.69 |
| Prolactin (ng/ml) | Pre exposure | 2.51 ± 0.39 | 3.23±0.27 | 2.27±0.53 | 2.45$^a$±0.53 | 2.73 ± 0.42 | 2.63±0.21 | 2.61±0.44 | 2.54$^a$±0.48 |
| | Post exposure | 2.77$^A$ ± 0.31 | 3.23$^A$±0.27 | 1.97$^A$±0.36 | 10.32$^{XBb}$±0.69 | 2.76$^A$ ± 0.41 | 2.63$^A$±0.21 | 2.37$^A$±0.29 | 14.03$^{YBb}$±0.32 |
| Cortisol (ng/ml) | Pre exposure | 4.56 ± 0.01 | 4.56±0.02 | 4.67±0.05 | 4.62$^a$±0.03 | 4.62 ± 0.46 | 4.55±0.02 | 4.67±0.04 | 4.69$^a$±0.05 |
| | Post exposure | 4.54$^A$ ± 0.02 | 4.56$^A$±0.02 | 4.60$^A$±0.04 | 6.98$^{Bb}$±0.04 | 4.54$^A$ ± 0.01 | 4.56$^A$±0.02 | 4.63$^A$±0.03 | 7.15$^{Bb}$±0.03 |

Means with different superscripts (a & b) in a column differ significantly (P< 0.01) for each breed; Means with different superscripts (A & B) in a row differ significantly (P< 0.01) for respective breed; Means with different superscripts (X & Y) in a row differ significantly (P< 0.01) for respective hormone.

**Table 3** Effect of thermal stress on RBC, TLC & DLC in Tharparkar and Karan Fries calves

| PARAMETER | | TP | | | | KF | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GROUP I | | GROUP II | | GROUP I | | GROUP II | |
| | | | ($25^0$C) | $10^0$C | $44^0$C | | ($25^0$C) | $10^0$C | $44^0$C |
| RBC | Pre exposure | 9.09 ± 0.49 | 10.33±0.63 | 9.58±0.36 | $9.14^{a}$±0.2 | 9.28 ± 0.44 | 9.52±0.32 | 9.51±0.24 | $9.06^{a}$±0.14 |
| ($\times10^6$/ µl) | Post exposure | $9.05^{A}$± 0.52 | $10.33^{A}$±0.63 | $9.76^{A}$±0.25 | $9.96^{Ab}$±0.32 | $9.34^{A}$± 0.35 | $9.52^{A}$±0.32 | $9.68^{A}$±0.30 | $10.62^{Bb}$±0.1 |
| WBC | Pre exposure | 13.44±0.38 | 12.92±0.57 | 14.16±0.63 | 13.88±0.73 | 12.97±0.58 | 12.94±0.35 | 12.82±0.51 | 13.14±0.58 |
| ($\times10^3$ / µl) | Post exposure | 13.72±0.54 | 12.92±0.57 | 13.74±0.72 | 13.34±0.61 | 12.76±0.62 | 12.94±0.35 | 12.62±0.29 | 13.2±0.40 |
| Lymphocyte (%) | Pre exposure | 63.32 ± 1.38 | 61.86±1.54 | 61.46±0.61 | 62.84±0.61 | 62.49±1.96 | 62.28±1.28 | 62.7±0.94 | 61.92±0.76 |
| | Post exposure | 63.32 ± 1.42 | 61.86±1.54 | 61.84±0.90 | 61.62±1.00 | 62.13±1.85 | 62.28±1.28 | 62.02±1.04 | 61.2±0.94 |
| Granulocyte | Pre exposure | 23.52 ± 0.87 | 22.26±1.59 | 25.06±1.10 | 23.74±1.39 | 22.78 ± 1.33 | 21.78±1.57 | 23.96±0.99 | 24.56±0.57 |
| (%) | Post exposure | 22.68 ± 0.39 | 23.46±1.10 | 24.68±1.12 | 25.82±1.13 | 22.89 ± 1.49 | 22.18±1.26 | 23.6±1.10 | 25.86±1.37 |
| Monocyte | Pre exposure | 13.16 ± 1.57 | 15.88±0.85 | 13.48±1.16 | 13.42±1.67 | 14.73± 1.25 | 15.94±0.75 | 13.34±0.65 | 13.52±0.80 |
| (%) | Post exposure | 13.99 ±1.60 | 14.68±1.18 | 13.48±1.16 | 12.56±1.76 | 14.98± 1.08 | 15.54±0.30 | 14.38±0.66 | 12.94±0.66 |

Means with different superscripts (a & b) in a column differ significantly (P< 0.05) for each breed; Means with different superscripts (A & B) in a row differ significantly (P< 0.05) for respective breed; Means with different superscripts (X & Y) in a row differ significantly (P< 0.01) between breeds for respective parameter.



**Table 4** Effect of thermal stress on haemoglobin & haematocrit in Tharparkar and Karan Fries calves

| PARAMETER | | TP | | | | KF | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GROUP I | | GROUP II | | GROUP I | | GROUP II | |
| | | | ($25^0$C) | $10^0$C | $44^0$C | | ($25^0$C) | $10^0$C | $44^0$C |
| Haemoglobin | Pre exposure | 10.33 ± 0.58 | 10.24±0.35 | 10.38±0.19 | 10.40±0.24 | 10.31±0.58 | 10.24±0.36 | 10.74±0.26 | $10.26^{a}$±0.42 |
| (g/dl) | Post exposure | 10.46 ± 0.62 | $10.24^{A}$±0.35 | $10.48^{A}$±0.30 | $11.12^{B}$±0.34 | $10.41^{A}$± 0.71 | $10.24^{A}$±0.35 | $10.64^{A}$±0.21 | $11.48^{Bb}$±0.52 |
| Haematocrit | Pre exposure | 32.65 ± 0.71 | 31.78±1.14 | 31.96±0.82 | 32.42±0.87 | 32.06 ± 0.74 | 31.93±0.87 | 31.32±1.27 | $31.04^{a}$±1.57 |
| (%) | Post exposure | 32.27 ± 0.55 | 31.78±1.14 | 32.5±0.70 | 35.12±1.30 | 31.5 ± 0.79 | 31.94±0.87 | 31.46±1.27 | $36.56^{b}$±2.00 |

Means with different superscripts (a & b) in a column differ significantly (P< 0.05) for each breed; Means with different superscripts (A & B) in a row differ significantly (P< 0.05) for respective breed; Means with different superscripts (X & Y) in a row differ significantly (P< 0.01) between breeds for respective parameter.

found to be more elevated in heat stressed cows given bovine somatotropin than cows treated with placebo (Elvinger *et al.,* 1992). Decreased concentration of growth hormone leads to less calorigenesis and less heat production (Baumann and Currie, 1980). In this study the heat treatment was acute (4 h), so the results are in agreement with the reports that growth hormone declines only during chronic heat stress. Even though the animal body was not able to reduce growth hormone concentration, an increase in its level is to be omitted to maintain homeostasis. This is in support by earlier study of Yousef and Johnson (1966) who observed that a decline in GH production is necessary to maintain homeostasis of the animal under heat stress.

Prolactin concentration of both the breeds differed significantly only when the calves were exposed at $44^0$C ($p<0.01$). Serum prolactin levels dropped quickly when ambient temperature gets lowered and vice versa (Wetteman & Tucker, 1974). In heat stressed steers there is increase in ($P<0.01$) the secretion and disappearance rates of serum prolactin concentrations but there is decrease in metabolic clearance rates (Smith *et al*; 1977). Lacroix *et al.* (1977) reported that prolactin levels of male calves were maximal during summer and minimal in winter. Beede *et al*. (1982) and Collier *et al*. (1982) substantiated the results with their reports that increasing dietary K from 0.64 or 1.08 to 1.64% markedly reduced plasma prolactin in heat-stressed cattle, suggesting that prolactin may be involved in increased K and Na turnover and conservation during thermal stress. Prolactin is known to produce substantial accumulation of water in the body. Prolactin has been shown to reduce sodium and water excretion in the perfused cat kidney (Lockett, 1965). Buckman *et al.* (1973) stated that plasma prolactin levels are suppressed in hydration. The significant elevation in plasma prolactin concentration recorded in this study after acute heat exposure at $44^0$C, might be an attempt to conserve water and sodium to prevent excessive dehydration. At $44^0$C, the prolactin level of Karan Fries calves were significantly higher compared to Tharparkar ($p<0.01$). This indicates Karan Fries as more susceptible breed to heat stress and dehydration.

The levels of cortisol increased significantly when the calves were exposed at $44^0$C. In Tharparkar it went up to $6.98 \pm 0.04$ ng/ml. The cortisol concentration were statistically non significant between the breeds at all the three temperatures. The results are corroborating with the findings that increased secretion of cortisol is a thermoregulatory response and enables an animal to tolerate heat stress (Christison and Johnson, 1972). It may increase within 20 minute of exposure to acute heat stress, and maintain a plateau between 2 - 4hours. Plasma cortisol shoots up markedly when cattle were acutely exposed to high ambient temperatures and decreases during the chronic phase (Habeeb *et al*., 1992). Christison and Johnson (1972) suggested that under severe acute heat stress the hyperglycemic action of cortisol is very much essential. They reported that the increase in plasma cortisol due to acute heat stress was due to increased adrenocortical activity because the metabolic clearance rate of cortisol remains constant. Ray (1968) explains the physiological significance of increased plasma cortisol as to mobilise amino acids by protein catabolism for gluconeogenesis.

Haematological parameters

The mean ± SEM of RBC count, WBC count, lymphocyte, granulocyte and monocyte percentage in Tharparkar & Karan Fries calves are given in the table 3. The WBC count, lymphocyte, granulocyte and monocyte percentage didn't differ significantly after any of the treatments in both the breeds. The results similar to the present study were observed by Broucek *et al.* (2009) who reported no significant difference in leukocyte, monocyte, neutrophil and basophil counts in Holstein calves under hot environments. Erythrocyte values of both breeds increased significantly compared to its pre exposure level $44^0$C. In Karan Fries the same had significantly higher values compared to $10^0$C & $25^0$C.

The mean ± SEM of haemoglobin and haematocrit values of Tharparkar & Karan Fries calves are given in the table 4. Haemoglobin values increased significantly in both the breeds post $44^0$C treatment in comparison to the other two temperatures, whereas haematocrit values increased significantly only in Karan Fries calves after $44^0$C exposure. The increase in haemoglobin and haematocrit observed in the present study is well supported by Dill and Costill (1974) who reported that under conditions of heat stress and dehydration the haemoglobin and haematocrit values increased as a result of decrease in plasma volume. Heat stress caused an increase in packed cell volume in cows and attributed it to the loss of water from body due to dehydration (Elvinger *et al;* 1992). All these haematological parameters didn't show any statistically significant breed differences.

## Conclusions

The statistically significant differences observed in physiological, haematological and hormonal responses between the two breeds indicated that Karan Fries calves were more sensitive to thermal stress.

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