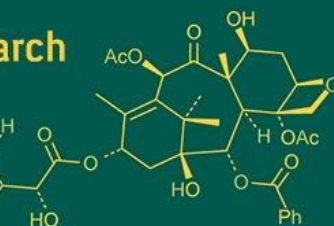


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## Endocrine response of crossbred calves to acute heat stress in a climate-controlled environment

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### Abstract

Heat stress in cattle due to global climatic change affects their overall health and production. Cattle are homeotherms and initiate thermoregulatory responses to heat stress. Hypothalamo-pituitary-adrenal axis is one of the principal endocrine regulators of stress responses in livestock. The activation of this axis as a response to heat stress might lead to enhanced production and release of cortisol into circulation. Several studies reported that the rise in serum cortisol concentration was temperature-dependent and is considered as a most reliable and primary physiological index for determining heat stress response in animals. Hence the present study was conducted to assess the endocrine response of crossbred calves to acute heat stress (Treatment) and compared it with thermo-neutral zone (Control) in a climate-controlled animal chamber. Venous blood samples without anticoagulant were collected on days 0, 1, 5 and 10 of the experiment in both control and treatment groups for serum cortisol estimation by radio immunoassay. It was evident from the findings that rise in serum cortisol was temperature-dependent. Furthermore, the results demonstrated cortisol acclimation pathway in acute heat-stressed crossbred calves under controlled climatic conditions. The study also thrown light on declined level of this stress hormone under thermo-neutral zone.

**Keywords:** Heat stress, thermo-neutral zone, serum cortisol, crossbred calves, climatic chamber

### 1. Introduction

Heat stress in cattle associated with global climatic change affects their overall health and production. Cattle are homeothermic animals and initiate thermoregulatory responses to heat stress (HS). Livestock maintains thermal homeostasis by adopting various thermo-regulatory mechanisms that significantly promote their welfare under stress (Indu and Pareek, 2015) [6]. When the endogenous heat production of cattle exceeds its capacity to lose heat, it results in HS (Bernabucci *et al.*, 2010) [2]. The physio-biochemical functions of cattle will remain normal in thermo-neutral zone (TNZ) or comfort zone. In their comfort zone, there won't be any energy expenditure for thermoregulation. When the ambient temperature exceeds the upper critical limit of TNZ, cattle experience HS (Bagath *et al.*, 2019) [1]. The hypothalamo-pituitary-adrenal axis (HPA axis) act as one of the principal endocrine regulators of stress responses. The activation of the HPA axis as a response to stress might lead to enhanced production and release of cortisol into circulation which is associated with immune suppression in cattle. Hence serum cortisol is considered a most reliable and primary physiological index for determining stress response in animals (Binsiya *et al.*, 2017) [4]. Cortisol secretion during acute stress acted as a stimulus for the immune system and its secretion during chronic stress was associated with immune suppression (Ju *et al.*, 2014) [7]. Generally, serum cortisol concentration will be elevated during short-term heat stress acclimation (STHSA) and it will be gradually decreased during long-term heat stress acclimation (LTHSA) due to negative glucocorticoid feedback to HPA axis as an adaptation to prolonged HS in cattle (Bharati *et al.*, 2017) [3]. In view of this, the present study was conducted with the objective of assessing the endocrine response of crossbred calves exposed to acute heat stress (Treatment) and thermo-neutral zone (Control) in a climate-controlled environment.

### 2. Materials and Methods

#### 2.1 Ethical approval

The research was conducted at the climatic chamber of Climate Controlled Research Complex (CCRC) of Centre for Animal Adaptation to Environment and Climate Change

Studies (CAADECCS), Kerala Veterinary and Animal Sciences University, following the approval of Committee for the Control and Supervision of Experimentation on Animals (CCSEA), New Delhi.

## 2.2 Layout of the experiment

Six healthy female crossbred calves of 10 months of age with identical body weight were selected randomly from University Livestock Farm and Fodder Research Development Scheme (ULF & FRDS), Mannuthy. The same were distributed as thermo-neutral zone (TNZ) or control group; acute heat stress or treatment group. The calves were given an acclimatisation period of 10 days each

in animal holding facility (Fig.1) and inside the climate-controlled chamber (Fig. 2). It was followed by experimental period of 10 days each under TNZ and acute HS in the chamber. In TNZ, the chamber was maintained at temperature of 27 °C. For acute HS, maximum temperature of 40 °C was simulated for three hours a day (11 A.M. to 2 P.M.) for period of 10 days. For both TNZ and HS, the relative humidity was maintained as 55-65 per cent. The temperature humidity index (THI) for the chamber was calculated based on the standard formula of Mader *et al.* (2006)<sup>[9]</sup>. After the experiment, the animals were re-shifted to the farm for rehabilitation and reuse, as per the recommendations of CCSEA.



**Fig 1:** Animals selected for experiment kept in animal holding facility for acclimatisation period.



**Fig.2:** Experimental animals housed in the climate-controlled animal chamber.

## 2.3 Collection of Venous Blood samples

Blood samples were collected in both the experimental groups on days 0, 1, 5 and 10 of the experiment into clot activator vacutainers between 1 P.M. and 2 P.M. through jugular venipuncture. Blood clotting was ascertained after one hour of incubation at a controlled temperature of 5 °C to ensure optimal serum preparation and were subjected for centrifugation at 2700×g at 4 °C for 15 minutes. Sera were separated and were stored at -20 °C for cortisol estimation.

## 2.4 Estimation of endocrine response

Cortisol levels were estimated in serum by using Cortisol C. T. Radio immuno-assay (RIA) kit (M/s Beckman Coulter, United States) using gamma counter (Perkin Elmer model Wizard, Fig. 3).

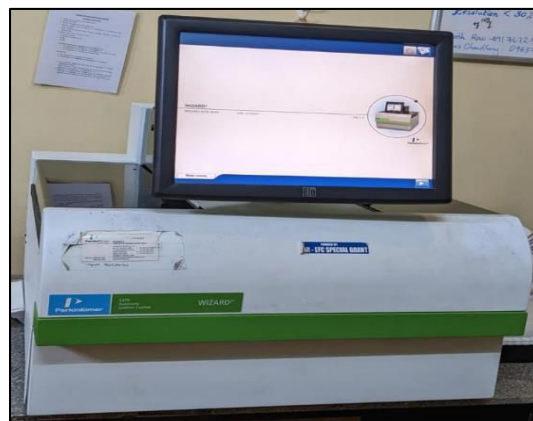


**Fig 3:** Gamma counter for the estimation of serum cortisol by radio-immunoassay.

### 2.4.1 Principle

Serum, the control and calibrators are incubated in monoclonal antibody-coated tubes with  $^{125}\text{I}$ -labeled (radioisotope) cortisol tracer. The liquid contents of the tube are aspirated and bound radioactivity is measured after incubation. A calibration curve is established and unknown values are determined by interpolation from the standard curve.

### 2.4.2 Reagents of assay

Anti-cortisol monoclonal antibody-coated tubes (ready to

use)

$^{125}\text{I}$ -labeled cortisol tracer (ready to use)

Calibrators (0, 20, 70, 200, 700 and 2000 nM/L) (ready to use)

### 2.4.3 Procedure

1. The stepwise assay procedure for serum cortisol estimation is as follows, summarized in Table 1.
2. The tubes were placed in the gamma counter (Fig.3) for counting radiations.

**Table 1:** Assay procedure for cortisol estimation

| Steps                                                                   | Calibrators | Serum            | Tubes for total count (T) |
|-------------------------------------------------------------------------|-------------|------------------|---------------------------|
| 1. Additions                                                            |             |                  |                           |
| To antibody-coated tubes, added successively                            | 50 L        | -                | -                         |
| - Calibrators                                                           | -           | 50 $\mu\text{L}$ | -                         |
| - Serum samples                                                         | 50          | 50               | 500                       |
| - Tracer                                                                |             |                  |                           |
| 1. Incubation                                                           |             |                  |                           |
| One hour at 18-25 °C with shaking (400 rpm)                             |             |                  |                           |
| 1. Count                                                                |             |                  |                           |
| Aspirated carefully contents of tubes except two tubes for total count. |             |                  |                           |

### 2.4.4 Calculation of results

Results were obtained from standard curve by interpolation. The results were calculated using a semi-logarithmic curve fit with B (bound)/T (total %) on vertical axis and the cortisol concentration of the calibrators on the horizontal axis (nM/L). The sample values were directly read from the standard curve as nM/L and converted into ng/mL (1 nM/L = 0.3627 ng/mL).

### 2.5 Statistical analysis

The results of the experiment were expressed as Mean  $\pm$  Standard Error. The statistical significance was assessed by linear mixed model analysis of variance (ANOVA) using SPSS version 24.0 statistical software.

## 3. Results and Discussion

The overall serum cortisol level of calves under acute heat stressed condition was significantly higher ( $p < 0.001$ ) when compared to TNZ (Table 2). The mean values differed significantly between different days of both the groups. In TNZ, the highest mean value of serum cortisol was noticed on day 0 (before exposure under TNZ). There was significant decline in serum cortisol levels throughout the experimental days in TNZ. The mean serum cortisol level was significantly higher ( $p < 0.01$ ) on day one of sudden heat exposure when compared to day zero. It was further

increased on day five of heat stress. There was a highly significant dip ( $p < 0.01$ ) in serum cortisol level on day 10 of HS when compared to all other days of HS (Fig. 4). Furthermore, high significant difference ( $p < 0.01$ ) was observed in serum cortisol values of day 0, 1 and 5 between the TNZ and HS groups. On day 10, there was no significant difference found between the groups.

The current findings of significantly higher ( $14.64 \pm 0.78$  ng/mL;  $p < 0.001$ ) serum cortisol level in acute heat stressed calves when compared to thermo-neutral condition ( $8.28 \pm 0.38$  ng/mL) was consistent with the results reported by Woo *et al.* (2024) [10]. They found a significantly increased ( $p < 0.05$ ) serum cortisol values with an increase in THI. In their study, serum cortisol value was lowest at comfort zone (11.96 ng/mL) and highest in HS (17.17 ng/mL) which further validating the present findings. Similar findings were also reported by Kim *et al.* (2018) [8] who found a higher serum cortisol level in calves exposed to THI of 87.72 (17.1 ng/mL) than those exposed to THI of 76.51 (4.6 ng/mL). Hence the rise in serum cortisol concentrations was temperature-dependent which is in accordance with the findings of Yadav *et al.* (2015) [11]. According to Ju *et al.* (2014) [7], the increase in cortisol level in calves during acute stress might have acted as a stimulus for the immune system.

**Table 2:** Serum cortisol (ng/mL) level in crossbred calves exposed to thermo-neutral zone and acute heat stress condition in climate-controlled environment (Mean  $\pm$  SE, n = 6).

| Days Group          | Day 0                          | Day 1                          | Day 5                          | Day 10                        | Overall                       |
|---------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|
| Thermo-Neutral Zone | 16.53 <sup>aA</sup> $\pm$ 0.83 | 9.008 <sup>bA</sup> $\pm$ 0.48 | 3.61 <sup>cA</sup> $\pm$ 0.77  | 3.98 <sup>cA</sup> $\pm$ 0.60 | 8.28 <sup>A</sup> $\pm$ 0.38  |
| Acute Heat Stress   | 5.89 <sup>aB</sup> $\pm$ 1.56  | 21.89 <sup>bB</sup> $\pm$ 1.93 | 23.28 <sup>bB</sup> $\pm$ 0.96 | 7.48 <sup>cA</sup> $\pm$ 1.61 | 14.64 <sup>B</sup> $\pm$ 0.78 |
| p-value             | <0.01**                        | <0.01**                        | <0.001***                      | 0.097 <sup>ns</sup>           | <0.001***                     |

Means bearing different superscripts within a row (a-c) and columns (A-B) differ significantly

\*\*\*Significant at 0.001 level, \*\*Significant at 0.001 level, ns-non-significant at 0.05 level

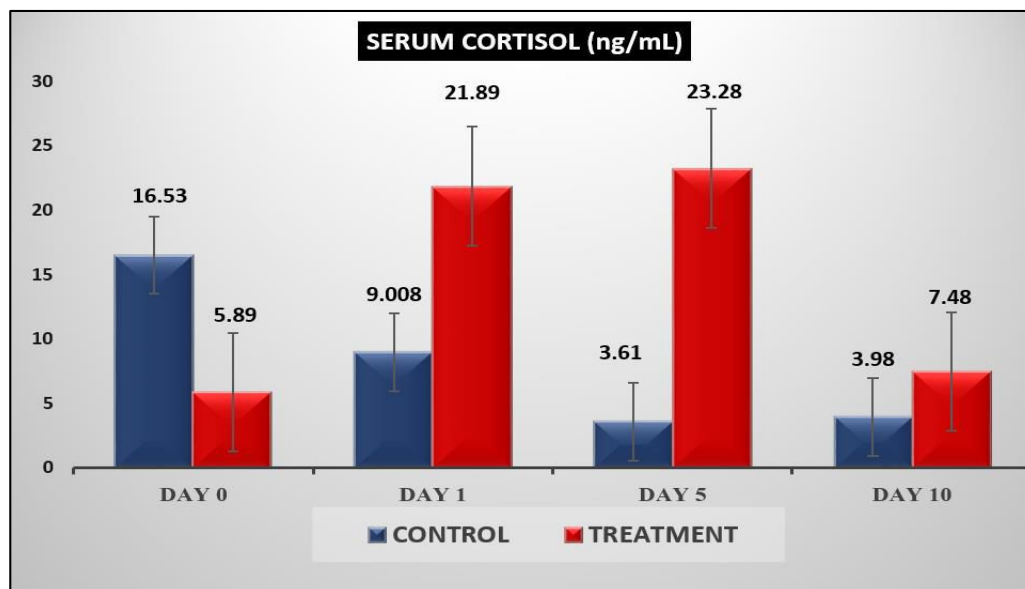


**Fig 4:** Day wise comparison of serum cortisol level (ng/mL) of crossbred calves exposed to thermo-neutral zone and acute heat stress in climate-controlled environment.

In the present study, the mean serum cortisol level in calves was significantly higher ( $p < 0.01$ ) on the first day of sudden heat exposure when compared to before exposure or day zero. It was further increased on day 5 of HS and there was a significant dip ( $p < 0.01$ ) on day 10 of HS when compared to all other days of treatment. This is consistent with Yadav *et al.* (2021) [12] who evidenced a significantly higher serum cortisol level in crossbred cattle on day 1 of heat exposure at 40 °C than under TNZ at 25 °C followed by a gradual dip from day 6 to day 21 with a significant dip on day 16 of heat exposure at 40 °C. The initial peak in serum cortisol level might be attributed to sudden activation of the HPA axis. The gradual reduction from the day 5 to significant reduction on the day 10 might be due to the cortisol acclimation pathway in heat-stressed crossbred calves. Similar to the current findings, Bharati *et al.* (2017) [3] found that serum cortisol concentration was significantly elevated ( $p < 0.05$ ) during short-term heat stress acclimation (STHSA) and it was gradually decreased during long-term heat stress acclimation (LTHSA). They opined that the decline in cortisol levels during LHTSA was attributed to negative glucocorticoid feedback to HPA axis, as an adaptation to prolonged HS in cattle.

Garner *et al.* (2017) [5] found a contradictory finding of no significant difference in mean cortisol values between thermoneutral (3.7 ng/mL) and temperature challenged (3.2 ng/mL) cows kept in a climate-controlled chamber for four days. Similarly, Zarina (2016) [13] also documented that there was no significant correlation between serum cortisol levels and THI.

#### 4. Conclusion

The present study elucidated the impact of acute heat stress on HPA axis of crossbred calves under climate-controlled environmental conditions. From the study, it was precise and evident that rise in serum cortisol was temperature-dependent. Furthermore, the results demonstrated cortisol acclimation pathway in heat-stressed crossbred calves (STHSA and LTHSA) under controlled climatic conditions. The decline in serum cortisol levels under TNZ in crossbred calves gave a focus on maintaining them in comfort zone for their welfare, production and productivity.

#### 5. Acknowledgement

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#### 6. Conflict of Interest

The authors declared no conflict of interest.

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