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The impact of chronic heat stress on thyroid hormone dynamics in Sahiwal and Tharparkar cattle

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Abstract
Thyroid hormones are regarded to be significant biomarkers for monitoring stress levels. The objective of this research was to determine the metabolic thyroid hormone profile of Tharparkar and Sahiwal calves during chronic heat stress. The concentrations of triiodothyronine (T_3) and thyroxine (T_4) in serum were determined. In the study, healthy male animals aged 1.5 to 2 years were divided into two distinct groups (five animals in each group). The study included seven days of acclimatization, 49 days of heat exposure at 38 °C (6 hours per day), and seven days of recovery. The collection of blood was done on day -7, 0, 7, 14, 21, 28, 35, 42, 49 and 56 day. During the experimental period, the levels of T_3, T_4 were found to be significantly (p<0.05) lower in both during the heat stress period. When both breeds are subjected to heat stress, serum T_3 and T_4 concentrations decrease. The serum T_4 levels were significantly (p<0.05) lower in Sahiwal than in Tharparkar, but the reduction in T_3 levels was not statistically significant. As a result, thyroid hormone profiles indicate that Tharparkar is more thermo-adaptable than Sahiwal.

Keywords: Heat stress, thyroxine, tri-iodothyronine, cattle
Abbreviation Key: T_3, Tri-iodothyronine; T_4, Thyroxine

Introduction
Adaptation to heat stress is accomplished by modifying an animal's endocrine status, which affects tissue responsiveness to environmental triggers (Bernabucci *et al.*, 2010) [3]. The thyroid gland synthesizes T_3 (Tri-iodothyronine) and T_4 (Thyroxine), which are more sensitive to changes in environmental temperature and serve as crucial indicators of heat stress. There is a transient stimulation of the Hypothalamus-pituitary-thyroid axis during both acute and chronic stress, which plays a critical role in energy regulation by altering BMR via thyroid hormone secretion (Mullur *et al.*, 2014) [14]. Thyroid status is a significant indicator of metabolic rate, and thyroid hormones are critical for the regulation of thermogenesis (Silva, 2006) [24], resulting in an important consideration in heat stress adaptation (Bernabucci *et al.*, 2002) [4]. The thyroid gland is extremely sensitive to temperature changes in the environment (Rasooli *et al.*, 2004) [18]. When animals begin to suffer from heat exhaustion, their dietary intake decreases and their metabolism slows, resulting in thyroid gland hypofunction (McManus *et al.*, 2009) [13]. The thyroid hormones are linked to the acclimation process and thus metabolic homeostasis (Perera *et al.*, 1985) [17]. When animals were exposed to heat stress, plasma levels of T_3 and T_4 decreased compared to thermoneutral conditions (Pereira *et al.*, 2008) [16]. The reduction in key metabolic hormones when the animal is exposed to high temperatures is due to the animals' attempt to reduce endogenous heat production (Sejian *et al.*, 2018) [22]. According to Bhimte *et al.* (2018) [5], the decrease in T_3 during chronic stress is caused by increased glucocorticoid levels, which inhibit the conversion of T_4 to T_3. Despite there is a brief rise in T_4 concentration during acute heat stress for about 10 minutes, this is most likely due to rapid metabolism in response to the flight or fight response. T_3 and T_4 react slowly to heat stress, and reaching steady-state can take several days or even weeks. It takes 72 hours after heat exposure to reach the minimum level of T_3 concentration (Jonklaas *et al*, 2015) [11]. According to Habeeb *et al.* (2001) [8], increased temperature causes a reduction in body weight gain. Aggarwal and Singh (2010) [1] observed that wallowing buffaloes had higher T_4 levels during hot, dry, and humid seasons. Acute heat exposure of old and young buffalo calves to temperatures ranging from 33 to 43 ºC and relative humidity of 40-60% reduced T_4 and T_3 levels (Nessim, 2004) [15].

The T_3 and T_4 concentrations are much lower in the summer, owing to the direct impact of heat stress on thyroid gland function, as well as reduced feed intake to avoid further metabolic heat load (Banerjee *et al.,* 2015; Indu *et al.,* 2014) [2, 10]. The free radical H_2O_2 is used as a substrate by the thyroperoxidase enzyme, which initiates the synthesis of thyroid hormones T_3 and T_4. Thyroid hormone levels may have dropped due to increased H_2O_2 output throughout stressful situations (Usha *et al.,* 2002) [26]. To improve their resistance to heat stress, the animals adjust their endocrine status with thyroid hormones. Exploring various conservation options and appropriate developmental research programmes for indigenous breeds such as Tharparkar and Sahiwal cattle is important. Therefore, there is an urgent need for the identification of climate-resilient livestock, as well as advanced research techniques and a fool proof extension programme for heat stress alleviation.

Materials and Methods

Site of experiment and animal

The research was carried out at the IVRI's Physiology and Climatology division in India. The institute is located at an elevation of 564 feet over mean sea level, in a subtropical climate at 28°22'N and 79°24'E, with peak summers from April to June and peak winters from November to January. The annual rainfall in the area is 90-120cm, with the most precipitation in July and August. The experiment was carried out on two groups of apparently healthy male cattle aged 2 to 2.5 years. Both groups consists of five animals each, the first group of Sahiwal (n=5) and the second group of Tharparkar (n=5), which were kept under uniform management conditions and given ad libidtum feed and water at the Psychrometric chamber. The minimum and maximum temperatures, dry and wet bulb temperatures, relative humidity (RH), and Temperature Humidity Index (THI) were all recorded daily.

Schedule for experimental studies

The experiment was carried out for 56 days, which included a seven-day pre-exposure period, 49 days of exposure at 38 °C, and a seven-day recovery period. The animals were exposed to heat stress at 38 °C for 6 hours a day, from 8 a.m. to 2 p.m.

Blood sample collection and serum isolation

Blood samples were collected at seven-day intervals (including pre-exposure and recovery) during each experimental period via jugular vein puncture in sterilized vacutainers containing clot activators for serum isolation. Syringes were used to collect the serum that oozed out of 3 ml blood-containing vacutainers that were left slanted for 4 hours and centrifuged for 5 minutes at 3000 rpm. The serum samples were stored until the enzyme assays at − 20 °C. The T_3 and T_4 profiles were computed using the serum samples. The serum hormonal profile was estimated using commercially available quantitative colorimetric assay kits for tri-iodothyronine and thyroxine.

Statistical analysis

The experimental data is expressed in terms of Mean ± SEM for every component. ANOVA (one-way analysis of variance) and Tukey's multiple range tests in SAS, Graph Pad Prism version 7.0 were the statistical methods used to analyze the data. Significant differences between Tharparkar and Sahiwal cattle were determined using one-way ANOVA.

Results and Discussion

Appropriate thyroid gland function and thyroid hormone activity are crucial for domestic animals to maintain reproductive and productive performance (Mullur *et al.,* 2014) [14]. Brzezinska-Slebodzinska (2001) [6] found that a 1 ºC rise in temperature was sufficient to lower serum T_3 levels. As illustrated in Fig. 1, both breeds showed a significantly (p<0.05) lower T_3 level on the 21st day of the heat exposure period (38 °C) compared to the control period (25 °C). Both the control and heat stress periods (38 °C) showed no discernible difference (p>0.05) between the two breeds when compared at the same temperature. When the two breeds were exposed to heat (38 °C) during the experiment, there was no statistically significant difference (p>0.05) in the levels of T_3 between and within groups. Both the breeds showed a decrease in T_4 level at heat stress period (38 °C) as compared to control period (25 °C) as shown in Fig 2. In case of Tharparkar cattle significant (p<0.05) decrease in level of Thyroxine at 21st and 28th day of heat stress (38°C). In both the breeds, no significant (p<0.05) difference was observed in control period (25 °C). In Sahiwal breed, T_4 level significantly (p<0.05) reduced at 7th, 14th and 21st day of heat exposure (38 °C). As an adaptive response to heat stress, reduced T_3 and T_4 levels are observed (Saber *et al.,* 2009) [20-21]. Heat stress also induces thyroid gland hypofunction, which modifies the animal's metabolism and minimizes metabolic heat output (Rebez *et al.,* 2023) [19]. When an animal is exposed to high temperatures, its concerted effort to reduce its endogenous heat production by lowering its metabolic rate and, consequently, its metabolic heat production, results in a decrease in important metabolic hormones (Bernabucci *et al.,* 2010) [3]. According to McManus *et al.* (2009) [13], during heat stress, there is a decrease in feed intake as well as cellular metabolism, which ultimately lowers the levels of thyroid hormones. Numerous studies on cattle have revealed that heat stress causes a drop in T_3 and T_4 levels (Tejaswi *et al.,* 2020) [25]. Increased glucocorticoid levels that prevent T_4 from converting to T_3 are another reason for the decrease in T_3 during chronic stress, according to Bhimte *et al.* (2018) [5]. Steers' T_3 and T_4 concentrations drop in response to heat stress, which also causes an instantaneous depression of the central pituitary–thyroid axis (Kahl *et al.,* 2015) [12]. The depressive effects of hyperthermia on the thyroid cause a decrease in the production of heat through metabolism. The body produces more heat as a result of increased thyroid hormone secretion and metabolism. As a result, a thermal stress adaptation mechanism is the decline in T_3 and T_4 (Saber *et al.* 2009) [20-21]. According to Collins *et al.* (2005) [7] and Sharma *et al.* (2013) [23], thyroid hormones are the main factor influencing the maintenance of the body's metabolic rate because they stimulate the phosphorylation and expression of mitochondrial uncoupling proteins, which in turn produces heat. Thyroid hormone secretion is lowered in order to reduce heat production as part of the physiological response to thermal stress (Hansen *et al.,* 2004) [9]. Thyroid hormones (T_3 and T_4) were found to gradually drop in both breeds in this study when they were exposed to heat. The reduced thyroid function from hyperthermia results in less heat being produced during metabolism. The body produces more heat when its metabolism is accelerated due to elevated thyroid hormone secretion. According to Saber *et al.* (2009) [20-21], thermal stress reduction in T_3 and T_4 is therefore an adaptive mechanism for thermal stress.

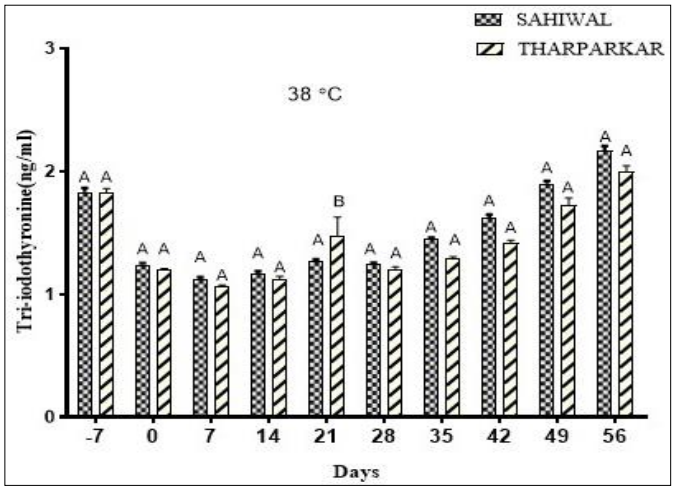

Fig 1: Tri-iodothyronine in Sahiwal and Tharparkar breeds compared before, during and after heat exposure (38 °C)

The bars bearing superscript (A, B) represents significant difference (<0.05) between the group

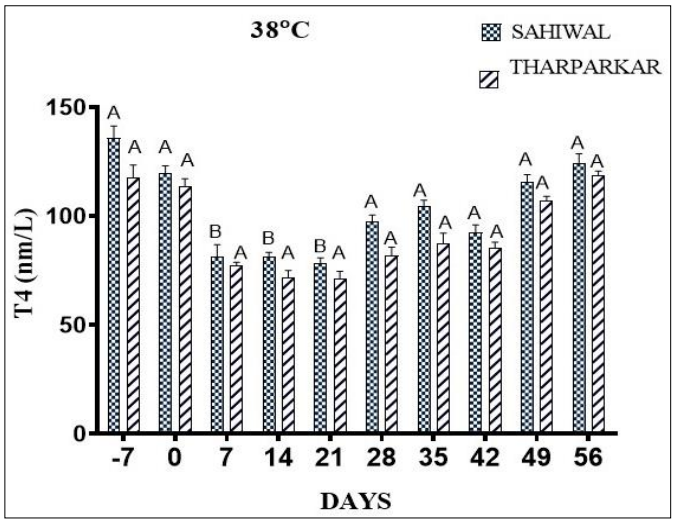

Fig 2: Thyroxine level in Sahiwal and Tharparkar breeds compared before, during and after heat exposure (38 °C)

The bars bearing superscript (A, B) represents significant difference (<0.05) between the group

Declaration

Conflict of interest: The authors declare no competing interests.

Data availability: The data will be made available and would be shared on reasonable request.

Ethics approval: All the animal experiments had prior approval from the animal ethics committee of the Indian Veterinary Research Institute (IVRI), Izatnagar, UP, India.

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Conclusion

According to the results of the experiment, heat stress in Tharparkar and Sahiwal cattle affects the thyroid's hormonal profile. Serum levels of thyroid hormones are dependent on exposure to a temperature of 38°C. The breeds' ability to handle stressful conditions is indicated by the steady reduction in T_4 and T_3 levels. The current study's findings also showed that Tharparkar is more thermo-adaptable than Sahiwal due to slight reduction in thyroid hormone levels.

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