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Original Article

HORMONAL, ANTIOXIDANT AND ENZYMATIC PROFILING IN CROSS-BRED CATTLE (*Bos tauraus*)

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***Abstract:** The present experiment was undertaken to study the effect of summer and winter season on the hormone and enzymatic profile of cross-bred (HF X J) cattle in Assam. The different hormones such as Cortisol, T_3 and T_4 are estimated by RIA technique and the different enzyme activities such as SOD (Superoxide Dismutase), GSH-Px (Glutathione Peroxidase) and CAT (Catalase) are analysed by different standard methods while LDH (Lactate Dehydrogenase) activity was estimated by using commercially available kit. It has been observed that all the stress related hormones and the erythrocytic enzyme activities were increased in summer compared to winter season and the difference was highly significant ($P<0.01$). The increase in hormone concentration and enzyme activity might be attributed to the thermal stress in the summer.*

Key words: Biochemical profile, Cross-bred cattle, Seasonal changes.

INTRODUCTION

Heat stress is one of the major influencing factors to reduce animal reproduction and productivity. With thermal discomfort, the animal seeks ways to lose heat by means of a concatenation of adaptation devices involving enzymatic and endocrine systems. Compositely, the adaptation characteristics can determine the tolerance of each animal to their environment, which can be estimated from the enzymatic traits [1]. Recent economic studies have suggested severe losses if the current management systems are not changed as a result of climate change [2]. Therefore, there is great need of attention in understanding how domestic animals respond to climate stressors. The cross-bred cattle of Assam used to experience on slot of summer heat stress because this region of our country suffer from some detrimental effect of thermal stress because most of the time, the state remains under tropical high heat and humid dominance. As the state depends, primarily, on the cross-bred cattle for milk, therefore, it is imperative to study the effect of thermal stress due to change in season on the enzymatic and hormonal profile of the cross-bred cattle.

MATERIALS AND METHODS

The protocol of this experiment was approved by Institutional Animal Ethics Committee of Assam Agricultural University, Khanapara, Guwahati, Assam. The experiment was conducted in the two seasons i.e. summer (June, July and August of 2015) and winter (December 2015, January and February of 2016). A total twelve numbers of female Cross-bred (Holstein Friesian X Jersey) cattle of between 2-3 years of age, free from any anatomical and reproductive disabilities and diseases were taken as the experimental animals. They were reared under semi-intensive system in the Instructional Livestock Farm (Cattle), C.V.Sc. Khanapara, A.A.U., Guwahati-22 with latitude and longitude position being 26o 10' N and 91o 44' E respectively. The animals were supplied with both green fodder and concentrate as per the standard feeding practices of the farm.

The venous blood was collected aseptically from the jugular vein of each of the experimental animals fortnightly for the whole experimental period. Serum was separated by centrifugation at 2000 g for 15 minutes for the estimation of the different hormones and lactate dehydrogenase enzyme. Further, 1% haemolysate was prepared for the estimation of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) respectively. The level of thermal stress related serum hormones such as triiodothyronine (T3), thyroxine (T4) and cortisol were estimated by Radio immuno-assay (RIA) technique using RIA kits supplied by Immunotech, Czech Republic. The tracer, I-125 was used in the estimation technique, which involved competition between free and isotope tagged hormones for binding to the limited antibody sites and subsequently quantification was made through calibration curve. The estimation used 6 well automatic gamma counter procured from Stratec W. Germany. The intra and inter assay co-efficient of variation were found to be 6.3 percent and 7.7 percent for triiodothyronine, 6.2 percent and 8.6 percent for thyroxine and 5.8 percent and 9.2 percent for cortisol. The results obtained in the assay were expressed in nmol/L. The erythrocytic superoxide dismutase activity was estimated following the NBT reduction method [3]. The erythrocytic glutathione peroxidase enzyme was assayed as per Rotruck and co-workers method [4] and the activity of erythrocytic catalase was assayed by Sinha method [5]. Lactate dehydrogenase enzyme activity was estimated by using the commercial kit manufactured by Greiner Diagnostic GmbH, Unter Gereuth 10-D-79353, Bahlingen-Germany. The data obtained were statistically analyzed [6].

RESULTS AND DISCUSSIONS

Serum Hormones: The mean serum cortisol of cross-bred cattle was recorded as 28.46±0.35 and 42.83± 0.29 nmol/L for the winter and summer season respectively (Table 1). There was a highly significant ($P<0.01$) difference in serum cortisol concentration between winter and summer season. Cortisol plays an important role in the different types of stress in animals and therefore, its concentration has been used as physiological markers of stress in animals [7,8]. The various stressors are responsible to activate hypothalamo-pituitary-adrenal axis in domestic farm animals resulting increase cortisol level. Activation of the hypothalamo-pituitary-adrenal axis and consequent increase in plasma glucocorticoides concentration are perhaps two of the most important responses of the animals to climatic stressful conditions [9,10]. The adrenal corticosteroids, mainly, cortisol elicite physiological adjustments which enable the animals to tolerate stressful condition and it have been observed that the plasma cortisol level increases during acute heat stress and decreases during the chronic phase [11]. Also, previous reporters observed that glucocorticoids function as vasodilators and facilitate heat loss, which have stimulatory effect on proteolysis and lipolysis and thereby, providing energy to the animal to replace the effect of reduction of feed intake [12].

Similarly, the mean serum tri-iodothyronine (T3) concentration in cross-bred cattle was recorded as 1.41±0.05 and 0.95± 0.04 nmol/L and the mean serum thyroxine (T4) concentration was recorded as 43.13±0.21 and 37.54± 0.28 nmol/L for the winter and summer season respectively (Table 1). The blood thyroid hormones are considered to be good indicators of metabolic status of an animal [13]. The appropriate thyroid gland function and activity of thyroid hormones are considered crucial to sustain productive performance in domestic animals [14]. Based on the metabolic or physiological status of the animals, the concentration of thyroid hormones varies. As such, season, breed and age of the animals has significant effect on plasma concentration of 3-3'-5-triiodothyronine (T3) and thyroxine (T4) [15-17]. In the present study, the serum T3 and T4 concentration significantly decrease ($P<0.01$) in summer season compared to the winter season. This decrease in thyroid hormone concentration in the summer might be due to the high ambient temperature that can markedly suppressed thyroid hormone levels due to lower feed intake. Heat production and body temperature regulation are effectively controlled by thyroid. A decline in the plasma concentration of T3 from 2.2 to 1.16 ng/

Table 1: Mean ± SE of the different hormones and enzymes in cross-bred cattle in summer and winter season in Assam. **Values differ significantly in respective rows (p<0.01). Means bearing the same superscript within a parameter in a row don't differ significantly and means bearing the same subscripts within a parameter in a column don't differ significantly

Attributes	Winter season	Summer season
Cortisol (nmol/L)	$28.46^{a}\pm0.35$	$42.83^{b}\pm0.29^{**}$
T_3 (nmol/L)	$1.41^{a}\pm0.05$	$0.95^{b}\pm0.04^{**}$
T_4 (nmol/L)	$43.13^{a}\pm0.21$	$37.54^{b}\pm0.28^{**}$
SOD (unit/mg protein)	$3.97^{a}\pm0.45$	$7.81^{b}\pm0.12^{**}$
GSH-Px (unit/mg protein)	$45.51^{a}\pm0.48$	$75.76^{b}\pm0.90^{**}$
CAT (unit/mg protein)	$27.43^{a}\pm0.58$	$50.35^{b}\pm0.47^{**}$
LDH (U/L)	$109.31^{a}\pm0.24$	$199.95^{b}\pm 0.44^{**}$

ml in thermal exposed lactating cows [18]. Also, acute heat exposure induced decrease in plasma T3 concentration in young and old buffalo calves [19] and Friesian calves [20]. Increase secretion of thyroid hormone increases the metabolism and hence heat production. Therefore, decreased thyroid hormone levels during heat stress are an adaptive response and also might be an attempt to reduce the metabolic rate and heat production [21,22]. Moreover, when the animals start to suffer due to heat in summer season, their food ingestion is reduced and thereby, metabolism slows down, causing a hypo-function of the thyroid gland [1]. Blood thyroxine (T4) levels are considered to be good indicators of the nutritional status of the animal [22] and correlated with feed intake in ruminant species including those that exhibit very marked seasonal cyclicity in feed intake, body weight and reproductive activity [23,24]. The production of more H2O2 under stress conditions during the summer might have reduced the levels of thyroid hormone [25]. Hence, the decrease in thyroid activity may be due to the production of H2O2 radicals and there exists a seasonal variation in thyroid gland activity, which is related to the ambient temperature and air humidity [26].

Erythrocytic antioxidant enzymes (SOD, GSH-Px, CAT): The mean erythrocytic superoxide dismutase (SOD), glutathione peroxides (GSH-Px) and catalase (CAT) concentration of cross-bred cattle was recorded as 3.97±0.45 and 7.81± 0.12, 45.51±0.48 and 75.76± 0.90 and 27.43±0.58 and 50.35± 0.47 unit/mg of protein for the winter and summer season respectively (Table 1). There was a highly significant (P<0.01) difference in all the erythrocytic antioxidant enzymes between the seasons. The thermal stress stimulates excessive production of reactive oxygen species (ROS), such as superoxide anion (O2-), hydroxyl ion (OH) and hydrogen peroxide (H2O2), which are continuously produced in the course of normal aerobic metabolism and these free radicals can damage healthy cells if they are not being eliminated and can cause some disturbances which are reflected as disturbed physiology and altered biochemical profile of the animal [27]. The major defense in detoxification of superoxide anion and hydrogen peroxide are superoxide dismutase, catalase and glutathione peroxidase [28]. There might be an increase in the production of hydrogen peroxide in the body during summer season which resulted in increase SOD activity. In order to neutralize that hydrogen peroxide, the glutathione peroxidase and catalase activity are also increased [29-31]. Thus, a positive and significant correlation exists between GSH-Px, SOD and CAT activities. Therefore, the increase in the antioxidant enzymes during the summer season might be due to the thermal stress. These antioxidant enzyme activities are the sensitive markers of oxidative stress as their level may increase or decrease in response to reactive oxygen species. SOD that catalizes dismutation of superoxide becomes important in the defense mechanisms against oxidative stress [32]. The role of intracellular SOD is to scavenge the superoxide (•O2–) that is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions [33]. Our results are corroborated with other workers [34-38].

Serum lactate dehydrogenase (LDH): The mean lactate dehydrogenase enzyme activity of cross-bred cattle was recorded as 109.31±0.24 and

199.95± 0.44 U/L for the winter and summer season (Table 1). There was a significant ($P<0.01$) increase in the LDH activity in summer compared to winter season. The mean plasma values of LDH ranged from 202.43±18.97 to 505.27±24.70 IU/L in adult Karan Fries cattle during the different season [39]. Similar significantly higher lactate dehydrogenase level in native Patanwadi sheep and its crosses with Merino and Rambouillet [40] was reported by when the sheep were exposed to direct sunlight from 32.3°C to 38.7°C for 3 consecutive days. The increase in the activity of LDH in plasma is due to the leakage of the enzymes from the liver cytosol into the blood stream, which reflects disruption of the liver function [41]. Our findings are similar to those of the previous researchers who reported a significantly different LDH concentration among the season, stage, time interval and season x stage. The LDH concentration is positively correlated to the physiological parameters such as respiration rate and pulse rate [39]. Therefore, increase in LDH enzyme activity during the summer season might be due to an increase in the respiration rate. Moreover, LDH plays an important role in cellular respiration through catalization by which pyruvate from glucose is converted into usable energy as lactate in the cells. When animals or animal tissues are subjected to heat stress that causes damage or injury to them, more LDH is released into the bloodstream. Moreover, the conditions that can cause increase LDH in the blood include liver disease, heart attack, anaemia, muscle trauma, bone fractures, etc [42].

CONCLUSION

During summer season in Assam, there is an increase in the production of stress related variables like antioxidants, serum enzymes and serum hormones in cross-bred cattle. All the erythrocytic and serum enzyme along with the serum hormones in cross-bred cattle were found to be significantly ($P<0.01$) higher in the summer compared to the winter season. This increase concentration might help the cross-bred cattle to adapt and acclimatize themselves to the changing environmental condition of Assam.

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