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Influence of different seasons on serum hormonal and electrolytes profiles of Mandya sheep

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Abstract
The present study was designed to ascertain the effect of different seasons on serum hormonal and electrolytes profile in Mandya sheep. Study was conducted at Livestock Research and Information Centre, Nagamangala. Eight apparently healthy Mandya sheep with same physiological condition was selected and maintained in same managemental condition throughout the study period during each season. Temperature relative humidity index (THI) was calculated by utilizing the meteorological data on temperature and relative humidity for different seasons. Blood samples were collected once in each season during peak of the season. Hormonal parameters were analyzed by indirect ELISA method and serum electrolytes by reagents and kits. During different study periods, highest THI (%) of 79.17±0.31 was recorded in summer and intermediate value of 73.12±0.33 % was observed during rainy season and the lowest in winter. The serum T_3 and T_4 was significantly (P<0.05) higher at winter season compared to summer and rainy season. No significant difference was noticed between summer and rainy season. The level cortisol was significantly higher at summer and winter seasons compared to rainy season and also significantly higher levels was noticed in summer as compared to winter season. The mean value of serum insulin was significantly lower at summer season compared to winter and rainy season. The serum sodium, potassium and chloride level was significantly (P<0.05) lower during summer season compared to rainy and winter season. So, present findings confirms that both hot and cold ambient temperature is stressful to the sheep and negatively correlated growth and reproductive performance.

Keywords: electrolytes, hormones, seasons, sheep, stress

Introduction

The seasonal variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species. Low and High ambient temperature cause discomfort and enhance the stress level which in turn result in depression of the physiological and metabolic activities of the animal but, animals try to maintain normal core body temperature by changing the dry matter intake, pulse rate, respiration rate, blood metabolite concentration, peripheral blood flow rate, sweating and panting. Environmental stress also has measurable effects on the endocrine profile of animals. Metabolic hormones, such as thyroxin and triiodothyronine can be used to indicate metabolic changes in relation to altered feed intake during different seasons. Plasma cortisol concentrations have been used as physiological markers of stress [1]. Summer and winter stress leads to severe changes in the hormonal and electrolytes concentration there by decreases the production performance of the animals [2]. Small ruminants are useful livestock assets in that they depends a greater extent on plant materials that are of less value to humans as feed resources. Sheep and goats are very important due to their biological factors such as short generation interval, twinning, short growth periods, and requires lest managemental practices. Despite this, their low performance is a major challenge to small ruminant production due to seasonal variations of environmental conditions [3]. Mandya sheep is highly susuptible for slight variations in the managemental and environmental conditions as compare to other south Indian breeds and local non-descript sheep. Studies are needed to evaluate the physiological performance of Mandya sheep under local climatic conditions, so that the patterns of responses to climatic stress can be established. The information obtained can be utilized in adopting micro environmental control and nutritional strategies that can alleviate stress and improve reproductive and productive performance. The findings could also be used to monitor the health status of sheep.

Materials and Methods

Location and temperature humidity index (THI) of study period

This experimental trail conducted in the summer (May), rainy (August) and winter (December) months of 2019 at Livestock Research and Information Centre (Sheep), Nagamangala, Mandya District of Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India. Temperature relative humidity index of the different seasons was calculated by utilizing the meteorological data of temperature and relative humidity by utilizing the formula given [4] $THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$.

Selection of animals and grouping

Eight apparently healthy Mandya sheep which were recently separated from lambs were selected. All the selected animals were aged between 3 to 5 years and free from anatomical abnormalities. All the animals were dewormed in each season 20 days before serum sample collection. Selected animals were maintained in separate flock to maintain same physiological status and to provide uniform managemental condition during entire period of study

Blood sample collection and laboratory analysis

Blood sample will be collected during morning hours (8.00 to 9.00 A.M). Each time 4 ml of two blood samples, in the clot activator coated vial. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -80 ºC in different aliquots.

Quantitative determination of T_3, T_4, cortisol and insulin in serum was carried out using microplate based enzyme linked immunosorbent assay (ELISA) using a commercially available Accu Bind ELISA microwell kit manufactured by Monobind Inc. Lake Forest, USA. The serum lipid profile (triglycerides, total cholesterol, HDL- cholesterol, LDL-cholesterol and VLDL- cholesterol) and serum enzymes activity (LDL, GGT and ALP) was estimated by Microlab 300 semi-automated biochemical analyzer supplied by Merck *Pvt. Ltd*, Mumbai, with the help of commercially available reagent kits manufactured and supplied by Delta Lab, Mumbai-461510.

Statistical analysis

The data obtained were analysed statistically by ANOVA with the application of using 'GraphPad Prism' version 8.42 (2018) computerized software. The values were expressed as Mean ± Standard Error and the level of significance or non-significance was determined at P value of 0.05.

Results and Discussion

Environmental parameters

The maximum and minimum average ambient temperature was recorded during summer and winter season respectively. Relative humidity was maximum and minimum during rainy and winter season (Table No. 1). The average THI was significantly ($P<0.05$) higher during summer season followed by rainy months as compared to winter study period. Higher THI during summer might be due to the higher ambient temperature and higher relative humidity. Same pattern of THI was also recorded by many workers in different agro-climatic zones [5, 6, 7, 8 & 9]. The THI has been the widely used indicator to assess the thermal stress in livestock. It was suggested that THI can be classified into mild (72-80), severe (80-85), and deadly stress zones (>85) [10]. The THI value of 75 to 80 indicated moderate to high intensity of thermal stress and THI of 72 and below was considered as no heat stress, 73 to 77 as mild heat stress, 78 to 89 as moderate and above 90 as severe heat stress [11]. The THI value of 72 or less are considered comfortable, 75-78 stressful and values greater than 78 cause extreme distress [12]. THI value of less than 70 is normal, 70 to 78 will cause moderate stress, 78 to 82 is considered as severe stress and THI value of 82 or above is considered as emergency [13]. Therefore, in the present study, the THI of 68.10 ± 0.50 during the winter season indicated that the animals were under no heat stress. However, the THI of 73.12 ± 0.33 during rainy season indicates mild stress and 79.17 ± 0.31 during the summer seasons indicated that the animals were under moderate to high intensity of thermal stress.

Table 1: Mean ± SE values of average temperature (^{0}C), relative humidity (%) and temperature humidity index (THI) (%) during different seasons of study period

Parameter Seasons	Average Temperature (^{0}C)	Average Relative Humidity (%)	Temperature humidity index (THI) (%)
Summer	29.90 ± 0.21	55.25 ± 0.65	79.17 ± 0.31^C
Rainy	24.58 ± 0.21	67.58 ± 1.16	73.12 ± 0.33^B
Winter	22.03 ± 0.20	49.75 ± 0.92	68.10 ± 0.50^A

Hormonal profile

The hormonal profile of the Mandya sheep during different seasons was presented in Table No, 2. Significantly ($P<0.05$) higher levels of serum T_3 and T_4 were recorded during winter season than rainy and summer seasons. Seasonal variations in the concentration of triiodothyroxine (T_3) and thyroxin (T_4) in various species have been reported in Malpura ewes [14], Iraqi black female goats [15], Indian goats [16], indigenous sheep [9] and Hallikar cattle [17]. However, higher serum levels of thyroid hormones during summer was reported in crossbred dairy cows [18] and Iranian flat-tailed sheep [19]. Some studies found no significant differences in thyroid hormones levels between the seasons [8 & 20]. Thyroid gland is one of the most sensitive organs to the ambient temperature and cold environment may be a stimulus to increase the thyrotrophic hormone output thereby resulting in a higher concentration of T_3 and T_4 in serum. It is believed that in hot environments, adaptive response is usually associated with decrease in food intake and metabolic heat production. Therefore, during summer heat acclimation and physiological adjustment by thermoregulatory centre induce a decrease in endogenous heat production influenced mainly by thyroid hormones [21]. Hence the exposure of animals to the high environmental temperature during summer could have depressed the functional activity of thyroid gland and thereby caused a relatively lower concentration of thyroid hormones. Thermal exposure acts directly on the hypothalamic pituitary axis leading to reduction in thyroid stimulating hormone from anterior pituitary [22]. In the present study, the reduced T3 and T_4 hormone concentration in summer may be due to both direct effect of heat stress on thyroid gland activity as well as to reduced feed intake in these animals that are important for maintaining the thermal balance in animals during heat stress. The reduced feed intake and lower level of thyroid hormones could be the adaptive mechanism exhibited by these animals to prevent additional heat load since the increased action of these metabolic hormones might add to the heat load in these animals.

Among the three seasons under present study, the mean cortisol level was significantly *(P*<0.05) higher in summer followed by winter and the lowest was recorded during rainy season The rise in cortisol level during summer in the present study is in agreement with many researchers in buffaloes [23 & 24], Deccani than Nellore lamb [25], Polish Merino sheep [26], indigenous sheep [9] and Hallikar cattle [17] who reported higher plasma cortisol level during summer. The blood cortisol level is generally considered as a reliable physiological index for determining animal response to stress, as indicated by assessment of glucocorticoid levels under a variety of conditions. Certain environmental stressors have the potential to activate the hypothalamo-pituitary-adrenal cortical axis (HPA) and sympatho- adrenal medullary axis [27]. On the other hand researchers reported that glucocorticoid secretion was reduced when exposed to heat stress [28]. Significant increase in serum cortisol level was recorded during winter in Holstein dairy cows, sheep and goat [29, 30 & 16]. In contrary to present study non-significant difference in cortisol level at either heat stress or cold stress was observed in Iranian sheep observed [31]. Cortisol is the primary glucocorticoid and has a wide variety of physiological actions influencing metabolism, body water distribution, electrolyte balance and blood pressure. Cortisol secretion stimulates physiological modifications that enable the animal to tolerate the stress caused by a hot environment the initial reactions of the animal to acute heat stress are emotional rather than responding thermoregulation. The level of cortisol in the blood increased significantly in animals exposed to the high and low temperature, indicating the occurrence of thermal stress. Cortisol plays a role in gluconeogenesis for energy production to supply more energy to thermal stressed animals. Cortisol is the principal stress reliever in ruminant species and hence its level is significantly higher in the animals during summer in order to cope up against thermal stress conditions [14].

The plasma insulin concentration was significantly *(P*<0.05) lowest at summer season followed by rainy and highest at winter season. The lower levels of insulin in summer may be attributed to reduced hormone synthesis due to reduced food intake during summer. Significant reduction in plasma insulin level during summer season in lactating cows, Egyptian buffaloes and Hallikar cattle was recorded [17, 32, 33 & 34]. Heat stress, reduces DMI and leads to negative energy balance and a series of metabolic adaptations in animals, such as lowered lipid mobilization increased sensitivity to insulin, which results in reduced glucose concentration, decreased concentration of non-esterified fatty acids and insulin [35].

Serum electrolytes

Significantly (*P*<0.05) lower serum sodium level was noticed during summer season followed by rainy and highest at winter season. The mean serum levels of potassium and chloride was significantly lower during summer season and highest at winter and rainy season. The lower levels of in serum sodium level may be due to enhanced heat dissipation during heat stress may lead to electrolyte losses through sweat, saliva, polypnea and urine and lead to fall in plasma Na^+, K^+ and Cl^- level our findings are in agreement with [25 & 36]. In another study on Australian milking zebu, a lower serum K+ concentration was noticed during hot dry summer which was attributed to loss of K^+ in sweat during dry summer [37]. Summer and winter stress leads to an increased water intake, expanded blood volume and hence decreased cation (Na^+) levels [36]. The seasonal variation in potassium level in cattle was documented by [38]. During prolonged heat exposure plasma aldosterone level was reported to decline and concurrent with this, there were significant fall in serum and urinary K^+. The simultaneous relationship among thermal stress, plasma aldosterone level, and urine and serum electrolyte concentration in bovines was documented [39]. However some reported higher serum sodium values during summer in the Frieswal cattle of all age group, and they opined that dehydration that may occur as a result of increased breathing rate may be the reason [40, 41].

Table 2: Hormonal and Electrolyte profile in Mandya sheep during different seasons

Seasons Parameters	Summer	Rainy	Winter
T3 (ng/mL)	1.14 ± 0.05^X	1.18 ± 0.07^X	1.91 ± 0.10^Y
T4 (µg/mL)	6.11 ± 0.50^Z	12.30 ± 0.77^Y	23.91 ± 0.14^X
Cortisol (ng/mL)	13.98 ± 0.84^Z	4.17 ± 0.23^X	9.60 ± 0.71^Y
Insulin (ng/mL)	1.06 ± 0.05^Y	1.85 ± 0.01^X	1.92 ± 0.06^X
Serum sodium (mMol/L)	127.25 ± 1.43^Z	142.66 ± 0.84^Y	148.08 ± 1.52^X
Serum potassium (mEq/L)	3.94 ± 0.27^Y	4.86 ± 0.21^X	5.22 ± 0.03^X
Serum chloride (mEq/L)	97.63 ± 1.42^Y	107.96 ± 1.68^X	113.84 ± 2.15^X

The values with different superscripts within row (X, Y and Z) differ significantly (*P*<0.05).

Conclusion

Lower level of serum T_3, T_4 and insulin levels was observed during summer season indicating animals under heat stress. Higher level of serum cortisol was recorded in summer and winters seasons confirm both low and high ambient temperature are stressful conditions. Alterations in serum electrolytes level was observed during different seasons indicating disturbance in water and electrolyte balance in stressed animals. Need for the nutritional and managemental strategies to reduce the thermal stress and to enhance the productive and reproductive performance in sheep.

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