



Evaluation of Cortisol (Stress Hormone) Levels in Madgyal Lambs under Various Housing Systems in Different Seasons

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ABSTRACT

Background: The study highlights the impact of temperature and humidity fluctuations on cortisol and glucose concentration in small ruminants, emphasizing the significance of various housing systems in livestock performance. The study aims to investigate the impact of multiple housing systems during different seasons on the stress levels of sheep.

Methods: Eighteen (18) healthy Madgyal sheep of either sex were equally divided into three different experimental groups: conventional housing system (T1), slatted housing system (T2) and simple housing system (T3). Blood samples were collected during rainy, winter and summer seasons and analyzed for serum cortisol and blood glucose levels. This study recorded environmental data, including Dry Bulb Temperature (DBT) and Wet Bulb Temperature (WBT), to calculate the temperature humidity index throughout the year.

Result: In this study, the average mean THI was recorded in rainy, winter and summer seasons in T1(81.86±0.37, 78.83±0.83, 79.11±0.82); T2 (81.68±0.43, 77.37±0.82, 77.86±0.84) and T3 78.87±0.43, 75.51±0.92, 76.39±1.18), respectively, which was statistically significant. The average serum glucose levels in the T1, T2 and T3 study groups during rainy, winter and summer seasons were 70.17±2.61, 53.70±1.35, 63.25±2.20; 56.86±2.12, 42.68±2.49, 59.58±2.20 and 67.28±0.77, 49.99±1.33, 59.33±1.03, respectively and were found statistically significant within the housing system during different seasons. The study revealed that serum glucose levels were higher during the rainy season compared to the summer and winter seasons. The average serum cortisol concentration in the experimental groups T1, T2 and T3 during rainy, winter and summer seasons was 77.67±5.04, 20.25±2.92, 41.17 ±6.09, 29.00±2.45, 14.58±1.46, 25.00±2.68 and 47.83±4.35, 26.67±1.61 and 34.50±2.39 nmol/L, respectively and was found significantly higher within all the treatment groups. The blood glucose levels (mg/dl) and serum cortisol levels (nmol/L) were highest in the Madgyal lambs housed in a conventional housing system (T1) and lowest in the raised wooden slatted floor housing system (T2).

Key words: Housing systems, Madgyal lambs, Seasons, Serum glucose, Serum cortisol.

INTRODUCTION

Sheep with its multi-facet utility for wool, meat, milk, skins and manure, form an important component of the rural economy, particularly in the arid, semi-arid and mountainous areas of the country. The variation in climatic variables like temperature, humidity and radiation is well recognized because of the potential hazards to the growth and production of all domestic placental species. The high close temperature in the middle of high air wetness and the low close temperature causes further discomfort and enhances the stress level, which successively results in depression of the physiological and metabolic activities of those animals. The change of environmental temperature has a significant impact on physiological processes (Behera *et al.*, 2018). The physiological adaptations are essential to maintain traditional vital signs and stop hyperthermia. Moreover, beneath these conditions, the animal's productivity gets severely affected, which ends in an immense economic loss for the sheep business (DAHD and F 2019-20). The productivity of livestock is substantially increased through shelter management by mitigating environmental stress (Nienaber and Hahn, 2007). Environmental enrichment affects the physiology and behavior of animals of different species (Young, 2013) and

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can be particularly effective in the research setting to reduce the incidence or severity of undesirable or abnormal behaviors. The productivity of domestic animals may be determined to some extent by the activity of the adrenal cortex (Bassett *et al.*, 1968; Carroll *et al.*, 1963; Gavora and Kondra, 1970), it is appropriate that means of manipulating this activity be investigated. Stress in animals can be assessed by direct measurement of serum cortisol levels. (Roussel *et al.*, 2006). The blood glucose and hormonal profile of sheep were significantly affected by the housing system (Singh *et al.*, 2008).

Radioimmunoassay (RIA) is a sensitive in vitro method for the assessment of antigens, *i.e.*, hormones, minerals, vitamins, *etc.*, from biological fluids. ¹²⁵I is a commonly used radioisotope among others for RIA due to its long half-life ($t_{1/2} = 60$ days). This method offers a convenient assay of large numbers of samples with excellent precision. The practice of RIA in the measurement of hormones for veterinary clinical use has been extensively studied in India (Dadke, 2018, Roopali *et al.*, 2020, Galdhar *et al.*, 2021, Jayabhaye *et al.*, 2021, Salutgi *et al.*, 2023, Galdhar *et al.*, 2024 and Alone *et al.*, 2025).

Animals' homeostasis can be affected by environmental changes, leading to heat or thermal stress. This affects physiological processes like endocrine, neuroendocrine and behavioral responses, affecting hormone and neurotransmitter secretion. (Yadav *et al.*, 2024). The association between heat stress and increased cortisol secretion is sparsely documented in ruminants. Thermal stress causes the decrease in feed intake, disturbance in the metabolism of water, protein, energy and mineral balance, hormonal secretions and blood metabolites (Shahar *et al.*, 2017). High temperatures cause an elevation of blood cortisol concentration (Sejian and Srivastava 2010). Cortisol is the principal stress reliever in ruminant species and hence its higher level is beneficial to cope up with heat stress condition (Shaji *et al.* 2017).

This study aims to record the impact of different housing systems on the performance of Madgyal lambs in a hot and humid region, considering the significant impact of shelter on growth, stress and health of livestock, as well as the climatic conditions. The results of the present experiment would help to understand stress levels in Madgyal sheep under various housing types and improve their management in hot and humid climates with a suitable housing system.

MATERIALS AND METHODS

Selection of animals

The study was conducted at the sheep unit of the Instructional Livestock Farm Complex of Mumbai Veterinary College, Mumbai, situated at Aarey Colony, Unit No.3 Goregaon (East). The laboratory analysis was carried out in the laboratories at Mumbai Veterinary College. The Livestock Instructional Farm Complex is situated 100 meters above sea level at 29.420 N latitude and 79.540 E

longitude. A climate with a high level of humidity can be characterized as moderately hot. The study involved eighteen healthy and sound Madgyal lambs, aged around three to four months, of either sex. The lambs were divided into three treatment groups, with six lambs assigned to each group (lodging). The experiment was conducted on an institutional livestock farm complex for one year.

Study design and assessment of farm management

Eighteen (18) healthy Madgyal sheep of either sex were equally divided into three different experimental groups: conventional housing system (T1), slatted housing system (T2) and simple housing system (T3). Conventional housing systems which had concrete flooring with an asbestos roof (T1), Slatted housing systems having raised wooden platform flooring 5 feet above the ground with an asbestos roof (T2) and Simple housing systems which had kaccha mudroom flooring with asbestos roof, covered from all sides with chain link (T3).

Meteorological parameters and temperature humidity index (THI)

Dry and wet bulb temperature inside each shed was recorded twice in a day *viz.*, morning and evening, throughout the experiment. For this, the Dry and Wet bulb thermometer was fixed inside each shed. The Temperature Humidity Index (THI) was assessed as per the method described by McDowell (1972).

$$THI = 0.8 \times T^{\circ}C + (RH \%) \times (T^{\circ}C - 14.4) + 46.4.$$

T= Dry bulb temperature.

RH= Relative humidity.

Relative humidity was calculated with the psychrometric chart.

Estimation of blood cortisol and glucose

Blood samples from the experimental animals were collected early in the morning between 8 and 10 am to avoid the influence of circadian rhythms. The serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20°C in a deep freeze until analyzed.

Serum cortisol was estimated using commercial RIA kits (Cortisol - IM1841) manufactured by Beckman Coulter Company, for human purposes and the analysis was carried out at the Radio Isotope Laboratory, Department of Veterinary Nuclear Medicine, Mumbai Veterinary College, Mumbai (India). The study involved incubating serum samples, controls and calibrators in monoclonal antibody-coated tubes with ¹²⁵I-labeled cortisol tracer. The tubes were aspirated, bound radioactivity measured and a calibration curve established. Unknown values were determined by interpolating from the standard curve. Results were calculated using a spline curve fit with B/T or B/B0 on the logit vertical axis and analytic concentration of calibrators on the log horizontal axis. Cortisol hormone was assayed in each sample, with paired observations. Quality control parameters, *viz.*, magnitude of control samples and recovery

percentage, were studied to validate every assay. The blood serum glucose concentration from experimental animals was analyzed by the use of biochemistry analyzer.

Statistical analysis

The mean and standard error for each parameter of the collected data were calculated and analyzed statistically for comparison as per the methods suggested by Snedecor and Cochran (2004).

RESULTS AND DISCUSSION

Seasonal average temperature humidity index (THI)

The THI was recorded season-wise inside all three housing systems. The result showed the average THI inside T1, T2 and T3 housing systems in Table 1. The average THI was statistically significant ($p < 0.05$) during different seasons. T1 housing system showed significantly higher ($p < 0.05$) THI during the rainy season than the summer and winter seasons, respectively. The T2 housing system showed a significantly higher ($p < 0.05$) average THI during the rainy season than the summer and winter seasons, respectively. T3 housing system showed significantly higher average THI during the rainy season than winter, where THI during the summer season remains at par.

Table 1: Season-wise average temperature humidity index (THI) inside different type of housing systems.

Treatment groups	Seasons		
	Rainy	Winter	Summer
T ₁	81.86±0.37 ^a	78.83±0.83 ^b	79.11±0.82 ^b
T ₂	81.68±0.43 ^a	77.37±0.82 ^b	77.86±0.84 ^b
T ₃	78.87±0.43 ^a	75.51±0.92 ^b	76.39±1.18 ^{ab}

Means bearing different superscripts differ significantly ($p < 0.05$).

Table 2: Serum cortisol (nmol/L) level of experimental Madgyal lambs from different housing systems (seasonal).

Seasons	Cortisol (nmol/L)		
	T ₁ (6)	T ₂ (6)	T ₃ (6)
Rainy	77.67±5.04 ^c	20.25±2.92 ^a	41.17±6.09 ^b
Winter	29.00±2.45 ^b	14.58±1.46 ^a	25.00±2.68 ^b
Summer	47.83±4.35 ^b	26.67±1.61 ^a	34.50±2.39 ^a

Means bearing different superscripts differ significantly ($P < 0.05$).

Table 3: Glucose (mg/dl) of experimental Madgyal lambs from different housing systems in different seasons.

Seasons	Glucose (mg/dl)		
	T ₁ (6)	T ₂ (6)	T ₃ (6)
Rainy	70.17±2.61 ^c	53.7±1.35 ^a	63.25±2.20 ^b
Winter	56.86±2.12 ^b	42.68±2.49 ^a	59.58±2.20 ^b
Summer	67.28±0.77 ^c	49.99±1.33 ^a	59.33±1.03 ^b

Means bearing different superscripts (a, b, c) differ significantly ($P < 0.05$) among housing systems.

The average Serum Cortisol (nmol/L) concentration in the experimental groups T1, T2 and T3 during rainy, winter and summer seasons is shown in (Table 2).

The average serum cortisol concentration in the experimental animals from various groups was statistically significant ($P < 0.05$) in all the seasons viz. rainy, winter and summer. In rainy season the average serum cortisol concentration of T1 was significantly higher ($P < 0.05$) than T2 and T3 group whereas between T2 and T3 group the average serum cortisol concentration was significantly higher ($P < 0.05$) in T3 group than T2. The serum cortisol concentration in Madgyal lambs of T1 and T3 groups was significantly ($P < 0.05$) higher than T2 group during winter season. During summer season the serum cortisol concentration in Madgyal lambs of T1 group was significantly ($P < 0.05$) higher than the T3 and T2, respectively, however, the serum cortisol concentration of group T2 and T3 remains at par.

The average serum glucose level in the experimental groups T1, T2 and T3 during rainy, winter and summer seasons showed in Table 3. The average serum glucose in T1 was significantly higher ($P < 0.05$) than T3 and T2 whereas the serum glucose level was significantly higher ($P < 0.05$) in T3 group than T2 during rainy season. During winter season the average serum glucose in T2 group was significantly lower ($p < 0.05$) than T1 and T3 group whereas the T1 and T3 remained at par. The average serum glucose level in the experimental animals was found statistically significant within the housing system during different seasons.

The serum glucose level of T1 was significantly higher ($P < 0.05$) than T3 and T2 group, during the summer season whereas between T2 and T3 group was significantly higher ($P < 0.05$) than T2 group.

The results showed that the serum cortisol level in the experimental Madgyal lambs during the rainy season was significantly higher in all the treatment groups. The higher levels of relative humidity and THI during the rainy season may cause stress and in response to this, the level of cortisol might have increased. The result of the serum cortisol level in the experimental Madgyal lambs significantly differed within housing systems. Overall, the cortisol levels were highest in the rainy season compared to the summer and winter seasons. The concentration of cortisol in the blood increased significantly in animals exposed to high temperatures and humidity, indicating thermal stress. However, cortisol levels in the blood can vary due to several factors, including ambient temperature, humidity, management practices and physiological conditions (Priyanka *et al.*, 2013). This result is in accordance with Sales *et al.* (2020), who studied the effect of different seasons throughout the year on the cortisol level of Sannen bucks and found higher levels of cortisol during the rainy season. The results of the present study showed higher cortisol levels in lambs during periods of elevated THI and humidity, which is in agreement with the findings of Autukait *et al.* (2021), who confirmed that

increases in cortisol concentration are directly associated with corresponding rises in ambient temperature and humidity. They observed that cortisol levels increased significantly with rising THI values. The cortisol levels were higher in summer than winter season which may be because of the stressful conditions due to high humidity and THI during summer. The findings of this study are consistent with Rathwa *et al.* (2017), who observed significantly higher cortisol levels in indigenous sheep during the summer compared to the winter season. Similar results found by Ribeiro *et al.* (2016) on investigated the adaptive profile of the Garfagnina goat breed in two different seasons (spring and summer). Al-Samawi *et al.* (2014) studied the effect of seasonal variation in Aardi goats and reported significantly higher ($P < 0.0002$) serum cortisol concentrations during the summer season (26.29 ± 1.04 ng/ml) compared to the winter season (20.27 ± 1.15 ng/ml), which is in agreement with the findings of the present study. They found the cortisol concentration was positively correlated with air temperature (0.91 $P < 0.05$) means Cortisol concentration resulted positively associated with thermal load. Similar results were found by Chergui *et al.* (2017) while studied exploration of cortisol secretion in the Bedouin goat to understand the mechanisms of adaptation to extreme hot climates. The plasma concentrations of cortisol showed large variation across seasons summer and winter when the environmental conditions are at their extreme levels. The results of the present study were consistent with the findings of Pampori *et al.* (2022), who investigated the effect of different housing systems on crossbred sheep and reported a significant variation in the stress hormone cortisol among sheep housed under different conditions.

The present study is in agreement of Kochewad *et al.* (2017) and De *et al.* (2018) who found that the cortisol level in Deccani sheep and Malpura lambs respectively differed significantly within different rearing systems. However, results of present study are in contrast with Antil (2019) who studied the cortisol levels in kids within different housing systems and found no significant difference in cortisol levels. In the present work the cortisol levels were highest in rainy season compared to summer and winter are in contrast with the findings of Sathisha *et al.* (2021) stated that the level cortisol was significantly higher at summer and winter seasons compared to rainy season while studying on Influence of different seasons on serum cortisol of Mandya sheep. 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.

The mean values of glucose level of all the experimental Madgyal lambs from this experiment are in

the range of the reference values from Merk Veterinary Manual and Radostits *et al.* (2000) and Desco *et al.* (1989) who studied the blood biochemistry values of sheep. During winter season the average serum glucose in T2 group was significantly lower ($p < 0.05$) than T1 and T3 group whereas the T1 and T3 remained at par. The average serum glucose level in the experimental animals was found statistically significant within the housing system during different seasons. Deori *et al.* (2024) observed similar results with Assam Hill Goats exposed to environmental heat stress shows increased cortisol activity in compared with another group maintained under shade with no exposure to sunlight.

The serum glucose level of T1 was significantly higher ($P < 0.05$) than T3 and T2 group, during summer season whereas between T2 and T3, group T3 was significantly higher ($P < 0.05$) than T2 group. The serum glucose levels may be differed because of difference in stress level and comfort within housing system due to higher level of relative humidity, temperature humidity index and other microclimatic conditions.

The mean serum glucose values in this study showed higher level during the rainy season compared to summer and winter season, respectively, which indicates the stress on the lambs particularly in rainy and summer season may be due to high level of humidity and THI. The serum glucose levels were higher during all three seasons in the lambs of T1 group (conventional housing system) than T3 (simple open housing) and T2 (raised slatted wooden floor housing system) respectively. This may be the effect of different microclimatic environment in various housing systems. The results from present study are in accordance with the Kulkarni *et al.* (2010), Kochewad *et al.* (2017) who found the significant difference in glucose levels in Deccani ewes within different housing systems. Khalil *et al.* (2023) investigated the effect of three different housing systems and reported a significant variation in blood glucose levels of Osimi lambs across the housing conditions, which was in accordance with the present study. Similar findings were reported by Chaudhary *et al.* (2003), who observed that blood glucose levels in sheep were significantly affected by seasonal variations. Whereas, the findings of current study do not align with those of Boraei *et al.* (2017) who studied Farafra lambs and found no significant difference in the serum glucose level within housing systems. The results of the present study were in agreement with the Pampori *et al.* (2022), who investigated and found the level of glucose was significantly higher in sheep placed in different housing types.

CONCLUSION

The serum cortisol levels (nmol/L) and the blood glucose levels (mg/dl) were highest in the Madgyal lambs housed in the conventional housing system and lowest in the raised wooden slatted floor housing system shows more comfort and having hygienically clean environment with less stress than the conventional and open type housing systems.

The variations in cortisol concentration observed in the lambs during different seasons depict their thermotolerance. The blood glucose (mg/dl) and serum cortisol level (nmol/L) in the Madgyal lambs were highest during the rainy season and lowest during the winter season within all the housing systems. The Madgyal lambs housed in a slatted floor type of housing system have significantly lower blood glucose (mg/dl) and serum cortisol level (nmol/L) than conventional and open type of housing systems during the rainy season.

Conflict of interest

All authors declare that they have no conflict of interest.

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