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Research Article

Effect of heat stress on physiological responses and concentration of cortisol of Nellore brown lambs in conventional and elevated housing system

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Abstract

The study was conducted to investigate physiological responses and cortisol of Nellore brown lambs housed under conventional and elevated housing systems in summer without climate control. Twenty lambs (age 3-4 months) were divided randomly into two groups in a completely randomized design and ten lambs were maintained under conventional housing system with moorum floor and ten lambs were housed in an elevated housing system with wooden slatted floor for a period of 90 days. All the physiological parameters, rectal temperature, respiration rate and pulse rate were recorded at weekly intervals both morning and evening. Temperature humidity index was recorded daily at 8.30 AM and 2.30 PM. The study revealed that the evening THI was significantly ($P<0.05$) lower in elevated (85.73±0.31) housing system compared to conventional (86.67±0.32) housing system. Significantly ($P<0.05$) higher RT (°C), RR (breaths/min), PR (beats/min) were recorded in lambs maintained under conventional housing system compared to elevated housing system in the morning and evening. Overall cortisol concentration was significantly ($P<0.05$) high in conventional housing system. Type of housing had significantly influenced physiological responses and cortisol. All the physiological responses were increased from morning to evening with increase in THI in both housing systems. Comparison of housing systems revealed that the animals under conventional housing system were under more heat stress than elevated housing system. It can be concluded that elevated housing system was more beneficial to the lambs in summer season than conventional housing system under intensive system of rearing.

Keywords: Conventional housing system, Cortisol, Elevated housing system, Nellore brown lambs, Temperature humidity index.

Highlights

- Evening THI was higher in conventional housing; morning & daily THI were similar.
- Physiological responses were higher in conventional than in elevated housing system.
- Cortisol levels were lower in elevated housing, indicating less stress on the lambs.

INTRODUCTION

Sheep farming is an important agricultural activity in rural areas and has proven to be more beneficial to man because of their adaptability to various environmental conditions under which the breeds and strains have evolved and maintained. Housing is an important production component and plays a key role in lamb management. An ideal housing enables in moderating the range of microclimate to which the animals are exposed and the degree of comfort depends upon the type of housing and floor. Shelter for profitable sheep rearing plays an inevitable role in large scale commercial sheep enterprise especially in intensive rearing system. In specialized dairy sheep flocks, housing structures play a crucial role for enhancing livestock welfare and health. These structures offer protection from extreme temperatures, shield the animals from both heat and cold stress and ensure appropriate nutrition. Poor building designs and improper microclimates could lead to thermal stress and welfare issues (Clark and McArthur, 1994). Protecting farm animals from adverse climatic conditions is crucial for gaining maximum productivity. High temperatures and humidity during the summer season have detrimental effects on the physiological and productive performances of sheep. Design of housing significantly influences both the physiological responses and the productive performance of sheep (Bhatta *et al.*, 2005). The impact of heat or cold stress on thermal balance is reflected in

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changes in heat storage (RT), heat production, and/ or heat loss (respiratory evaporation). The physiological responses such as body temperature, pulse rate, heart rate and respiration rate serve as indicators of health.

Therefore, the present study was designed to identify a suitable housing system for economic rearing of lambs under intensive management and to evaluate the impact of two different housing systems on THI and physiological responses and cortisol during summer.

MATERIALS AND METHODS

Experimental site and thermal environment data: This study was carried out at sheep unit, Livestock Farm Complex (LFC), NTR College of Veterinary Science, Gannavaram, Krishna district. Gannavaram town is situated at an elevation of 12 meters above sea level on 81^0 longitude and 16.5^0 latitude. The experiment was conducted during hot summer season with an average low temperature of 31.35°C (88.43°F) and high temperature of 37.26°C (99.06°F). The average humidity is 55 per cent during the period of study.

Selection of animals: For the present study, twenty Nellore brown lambs of either sex with average body weight of 9.535±0.015 kg and age 3-4 months were selected from sheep unit of Livestock Farm Complex. These lambs were randomly divided into two groups of each ten animals on the basis of body weight in a completely randomised design. The study was carried out for a period of 90 days. All the lambs were kept for 15 days of pre-experimental period prior to start of the experiment for acclimatization of lambs to their respective housing.

Housing and management: All the experimental lambs are maintained under similar managemental conditions except housing system.

Group 1: The lambs were kept under conventional housing system with moorum floor in covered and open area served as control group. The average initial body weight (mean ± SE) of 10 animals (5 ewe lambs, 5 ram lambs) was 9.52±0.63 kg.

Group 2: The treatment group lambs were kept on elevated housing system with slatted wooden floor with a gap of 1.6 cm between slats raised at a height of 5 ft above ground level to facilitate easy cleaning and collection of dung and urine. The average initial body weight (mean ± SE) of 10 animals (5 ewe lambs, 5 ram lambs) was 9.55±0.52 kg.

All the lambs were dewormed with Albendazole @ 10 mg /kg body weight before start of the experiment and once in the middle of the trial. All the lambs were kept under hygienic conditions throughout the experimental period.

Feeding and watering: All the experimental lambs were fed as per the standard nutrient requirements given by ICAR. All the lambs were fed with concentrate mixture once in a day according to their body weights and *adlibitum* green fodder twice in a day, i.e., 9.00 AM and 3.00 PM. Potable drinking water was made available to each lamb throughout the experimental period.

Measuring environmental variables

The macroclimatic data for dry and wet bulb temperatures and relative humidity was obtained from the Meteorological office of Vijayawada International Airport located at about 0.5 km from the Livestock Farm complex. In addition, micro climatic data of the experimental animal sheds were recorded during the summer season with the help of digital hygrometer.

Temperature: Ambient in- house temperature (IT) (°C) was recorded twice a day i.e., at 8.30 AM and 2.30 PM by using electronic digital hygrometer (HTC-1).

Relative humidity: In house relative humidity (IRH) (%) was recorded twice a day i.e., at 8.30 AM and 2.30 PM by using electronic digital hygrometer (HTC- 1).

Temperature Humidity Index (THI): To estimate the heat stress of the lambs, Temperature-Humidity Index was calculated as per livestock and poultry heat stress indices (LPHSI, 1990).

$$THI = db°F - \{(0.55 - 0.55\ RH)\ (db°F - 58)\}$$

where,
T(db): Dry bulb temperature (°F)
RH: Relative humidity (RH %)/100.
THI was calculated twice per day morning and evening and the average value of the two readings was taken as THI of the day.

Measurement of physiological responses

Stress in animals, if any due to rearing on different housing systems was recorded by observing and recording different physiological responses *viz.* rectal temperature, respiration rate and pulse rate at weekly intervals at 8.00 AM in the morning and 4.00 PM at evening for all the experimental lambs.

Rectal temperature (RT): The rectal temperature was recorded at weekly intervals for a period of 90 days in each group accurately by inserting digital thermometer into rectum approximately 3" deep ensuring that the thermometer bulb was left in contact with the rectal mucosa. The observations were recorded in degree Fahrenheit (ºF). Normal temperature of sheep is 100.9 -103.8ºF (Sharma *et al*., 2021).

Respiration rate (RR): The respiration rate was recorded by observing flank movement of the lamb for one minute in which each inward and outward movement of the flank was counted as one complete respiration. The respiration rate was expressed as breaths per minute. Normal respiration rate of sheep is 12-25 breaths/min (Sharma *et al*., 2021).

Pulse rate (PR): Pulse rate (PR) of each lamb was measured using the femoral artery on the inner side of the rear leg approximately 1/3 of the way down with minimum disturbance to the animals. The pulse rate was expressed as beats per minute. Normal pulse rate of sheep is 70-80 beats/min.(Sharma *et al*., 2021)

Blood collection

Blood was collected from the jugular vein of the lambs twice during the experiment once at the beginning and once at the end of the experiment. The animals were restrained and then the area was first sterilized by using surgical spirit and 2 mL blood was collected from all the lambs by using hypodermic needles in the morning. Blood was kept in separate test tube without adding the anticoagulant and centrifuged at 3000 rpm for 15 min to separate serum, and then transferred into a sterilized small plastic Eppendorf tube (2 mL) and stored at -20ºC for further analysis. Sterilized glassware and analytical grade chemicals were used in the study.

The collected samples of serum from each group were examined for cortisol by commercial test kit, CALBIOTECH (Cortisol ELISA) at 505 nm wave length using spectrophotometer (Chishti *et al*., 2020).

Statistical analysis: The data was subjected to independent two samples T-test using SPSS IBM, version 26.0, SPSS Chicago (US).

RESULTS

Temperature humidity index (THI): Temperature-humidity index is an important indicator for estimating severity of thermal load to animals. Values of THI over 86 are considered as severe to extreme heat stress, 84-86 indicates severe heat stress, 82-84 indicates moderate heat stress, while less than 82 indicates absence of heat stress (LPHSI, 1990) in animals.

The results presented in Table (1) showed a significant ($P<0.05$) difference during evening THI in both conventional and elevated housing systems whereas, THI differed non-significantly during

Table 1. Temperature Humidity Index of Nellore brown lambs under conventional and elevated housing systems

Week	Morning		Evening		Daily average	
	CHS	EHS	CHS	EHS	CHS	EHS
1	83.41±0.57	83.25±0.31	85.40±0.52	84.46±0.23	84.41±0.41	83.86±0.22
2*	83.08±0.76	83.03±0.53	86.79±0.43[a]	85.29±0.26[b]	84.94±0.45	84.16±0.19
3	84.86±0.55	83.92±0.31	87.72±0.58	87.29±0.45	86.29±0.24	85.61±0.30
4	81.99±1.02	81.69±0.76	83.20±0.39	83.06±0.41	82.60±0.71	82.38±0.54
5	82.85±0.51	83.56±0.41	84.69±0.49	83.73±0.32	83.77±0.32	83.65±0.35
6	83.96±0.19	83.71±0.22	84.96±1.58	83.16±0.46	84.46±0.84	83.44±0.30
7	79.86±1.15	80.18±1.13	82.00±1.90	80.68±1.70	80.94±1.37	80.43±1.28
8	83.00±0.32	83.16±0.73	88.58±0.36	87.89±0.75	85.80±0.22	85.69±0.41
9	84.22±0.62	83.69±0.17	89.72±0.70	87.83±0.54	86.97±0.62	85.76±0.26
10	83.20±0.82	83.22±0.95	89.14±0.38	87.94±0.86	86.17±0.54	85.58±0.87
11	83.83±0.31	83.85±0.31	87.73±0.59	87.34±0.60	85.78±0.33	85.59±0.44
12	83.28±0.73	82.91±0.27	88.17±0.43	88.22±0.35	85.73±0.39	85.39±0.42
13	83.67±0.39	83.68±0.33	88.53±0.32	87.61±0.25	86.10±0.23	85.65±0.26
Overall mean*	83.17±0.21	83.07±0.18	86.67±0.32[a]	85.73±0.31[b]	84.92±0.23	84.40±0.21

Means with different superscripts within a row differ significantly* ($P<0.05$) CHS- Conventional housing system, EHS- Elevated housing system

Table 2. **Rectal temperature (ºF) of Nellore brown lambs under conventional and elevated housing systems**

Week	Morning		Evening		Daily average	
	CHS	EHS	CHS	EHS	CHS	EHS
1	102.94±0.11	102.83±0.11	104.10±0.11	103.81±0.14	103.52±0.10	103.32±0.12
2*	103.09±0.22	102.73±0.12	104.84±0.16 a	104.03±0.16 b	103.96±0.16 a	103.38±0.10 b
3*	103.05±0.87	102.99±0.14	105.04±0.18 a	104.12±0.24 b	103.99±0.10 a	103.55±0.11 b
4*	102.77±0.14	102.22±0.14	103.81±0.11 a	103.47±0.89 b	103.29±0.11 a	102.84±0.10 b
5	102.94±0.20	102.50±0.16	104.03±0.14	103.92±0.99	103.54±0.07	103.54±0.07
6	102.69±0.25	102.45±0.14	104.08±0.23	103.79±0.19	103.38±0.22	103.12±0.14
7*	102.19±0.21	102.36±0.16	103.27±0.18 a	102.76±0.10 b	102.73±0.17	102.56±0.10
8*	102.61±0.12	102.09±0.15	104.13±0.14	104.03±0.12	103.06±0.08 a	103.06±0.08 b
9*	102.99±0.25	102.23±0.17	104.26±0.18	103.79±0.16	103.62±0.20 a	103.01±0.15 b
10*	102.10±0.93	102.12±0.13	104.32±0.2 a	103.53±0.94 b	103.21±0.13 a	102.82±0.09 b
11*	102.82±0.11	102.19±0.16	103.93±0.18 a	103.40±0.11 b	103.37±0.11 a	102.79±0.12 b
12	102.86±0.72	102.84±0.19	104.54±0.15	104.31±0.17	103.70±0.09	103.57±0.16
13	102.65±0.13	102.66±0.11	103.87±0.19	103.88±0.15	103.26±0.12	103.27±0.12
Overall mean*	102.75±0.05	102.48±0.47	104.17±0.59 a	103.76±0.05 b	103.46±0.05 a	103.14±0.04 b

Means with different superscripts within a row differ significantly * (P<0.05)
CHS- Conventional housing system, EHS- Elevated housing system

Table 3. **Respiration rate (Breaths/min) of Nellore brown lambs under conventional and elevated housing systems**

Week	Morning		Evening		Daily average	
	CHS	EHS	CHS	EHS	CHS	EHS
1	51.20±1.84	50.40±1.22	79.40±1.61	75.40±1.68	65.30±1.25	62.90±0.91
2	41.40±2.00	41.80±1.44	83.40±4.00	83.20±4.08	62.40±1.83	62.50±2.14
3*	58.30±1.22	54.60±1.61	90.00±1.57 a	72.80±5.16 b	74.15±1.09 a	63.70±2.70 b
4*	50.60±2.25	50.40±1.99	77.80±2.14	74.40±1.54	64.20±1.77	62.40±1.11
5*	53.40±1.61 a	48.40±1.45 b	79.80±2.05	75.00±1.69	66.60±1.27 a	61.70±1.18 b
6	50.60±3.65	43.60±1.83	81.60± 1.65	70.60± 1.68	61.85±3.08	57.10±1.46
7*	53.40±1.43 a	47.60±1.86 b	73.10±2.79 a	70.60±2.99 b	67.50±0.64 a	59.10±1.38 b
8*	50.20±4.14	46.40±2.54	89.60±1.51 a	73.80±2.46 b	69.90±2.43 a	60.10±2.01 b
9*	53.40±6.52	53.10±5.19	90.20±1.8 a	82.20±1.75 b	71.80±3.21	67.65±2.96
10*	55.40±1.81 a	49.90±1.76 b	82.00±2.33 a	72.80±2.11 b	68.70±1.78 a	61.35±1.56 b
11	54.70±1.86	50.50±2.79	77.20±2.23	73.00±2.00	65.95±1.46	61.75±2.16
12*	52.40±1.88 a	46.80±1.84 b	87.60±4.23 a	71.40±3.54 b	70.00±2.45 a	59.10±1.68 b
13*	54.90±1.62	51.50±1.93	90.10±2.27	81.20±3.70	72.50±1.59 a	66.35±2.41 b
Overall mean*	51.41±0.84 a	47.88±0.72 b	83.21±0.81 a	75.11±0.84 b	67.76±0.61 a	61.98±0.56 b

Means with different superscripts within a row differ significantly * (P<0.05).
CHS- Conventional housing system, EHS- Elevated housing system

morning and daily average. Morning THI was less than evening THI in both housing systems.

Physiological parameters: The results presented in Table (2, 3 and 4) on rectal temperature, respiration rate and pulse rate showed highly significant (P<0.05) differences in the morning, evening and daily average in between conventional and elevated housing systems.

Cortisol: The cortisol concentration was high in conventional (14.92±2.4) than that of elevated

Table 4. **Pulse rate (Beats/min) of Nellore brown lambs under conventional and elevated housing systems**

Week	Morning		Evening		Daily average	
	CHS	EHS	CHS	EHS	CHS	EHS
1	72.80±1.24	70.00±1.39	84.20±2.05	82.40±1.22	78.50±1.17	76.20±0.63
2	72.20±1.50	71.80±1.20	81.80±2.59	81.40±2.35	80.20±1.78	77.90±1.78
3	75.80±4.85	73.20±3.25	88.20±2.93	85.40±3.39	78.80±2.64	77.30±2.32
4*	70.00±1.55	67.20±1.04	87.10±0.91[a]	82.90±0.61[b]	78.50±1.00[a]	75.05±0.54[b]
5*	71.20±0.68	72.80±1.58	90.10±0.81[a]	84.20±1.53[b]	80.65±0.52	78.50±0.93
6	75.10±2.29	70.20±2.45	90.60±0.73	87.40±2.29	82.85±1.20	78.80±1.55
7	69.40±1.71	66.40±1.18	81.90±2.12	80.10±1.88	75.65±1.41	73.20±0.99
8*	74.40±2.45	71.60±2.27	96.00±1.04[a]	92.60±1.12[b]	85.20±1.47	82.10±1.27
9*	75.30±0.94	70.90±3.18	99.30±1.37[a]	94.10±0.86[b]	86.55±0.77[a]	82.50±1.48[b]
10*	73.90±1.75	71.70±1.43	97.80±1.83 [a]	92.30±0.75[b]	85.85±0.7[a]	82.00±0.91[b]
11*	74.70±1.03	72.70±2.57	98.10±1.71[a]	93.30±0.98[b]	86.40±1.07	83.00±1.59
12*	73.90±1.09	71.20±1.66	99.90±1.30[a]	94.10±1.14[b]	86.90±0.94[a]	82.60±0.96[b]
13*	72.10±2.08	70.30±1.8	97.80±1.13[a]	95.00±1.29[b]	85.70±1.39	82.65±0.46
Overall mean*	73.14±0.57[a]	70.77±0.57[b]	91.75±0.73[a]	88.08±0.65[b]	82.45±0.48[a]	79.37±0.44[b]

Means with different superscripts within a row differ significantly * (P<0.05).
CHS- Conventional housing system, EHS- Elevated housing system

Table 5. **Cortisol (ng/mL) concentration of Nellore brown lambs under conventional and elevated housing system**

	Housing systems	
	Conventional housing system	Elevated housing system
Initial	12.52 ± 1.62	11.89 ± 2.16
Final	17.33 ± 5.04	14.10 ± 3.53
Overall mean*	14.92 ± 2.40 [a]	12.99 ± 1.10 [b]

Means with different superscripts within a row differ significantly * (P<0.05).
CHS- Conventional housing system, EHS- Elevated housing system

(12.99±1.1) housing system (Table 5). The overall mean cortisol of lambs was significantly (P<0.05) different in both housing systems.

DISCUSSION

Temperature humidity index (THI): Lambs were under moderate heat stress during morning as THI was 83.17 ± 0.21 in conventional and 83.07 ± 0.18 in elevated housing system. In the afternoon, lambs kept in conventional housing system were observed to be under extreme heat stress as the recorded THI was 86.67 ± 0.32, while in elevated housing system, lambs were under severe stress as THI was 85.73 ± 0.31. The THI was less in elevated housing system when compared to conventional housing system in morning and afternoon. Similar findings were reported by Rahman *et al*. (2013), Yasotha and Sivakumar (2013) and Singh *et al*. (2017) who found less THI in elevated housing system.

Morning THI was less than evening THI in both housing systems. Similar findings were reported by Singh *et al*. (2017) and Yamin *et al*. (2022) where the mean THI was lower in the morning when temperature was low and humidity was high, but higher in the evening when temperature was high and humidity was less.

In both housing systems, lambs were found to be more comfortable in the morning hours, whereas, in the hot daytime hours, they experienced severe to extreme heat stress depending upon the THI level, leading to significant alterations in their thermoregulatory mechanisms.

The increased THI in conventional housing system in the afternoon might be due to higher thermal conductivity of moorum floor where it absorbs more radiant heat. However, in elevated housing system there was no direct contact with the floor, thereby, reducing the absorption of radiant heat from the ground.

Physiological parameters: The rectal temperature in the evening was observed to be higher than morning. This might be due to the increase in THI values in the evening. These findings were in accordance with the reports of Rathwa (2017) and Yamin *et al*. (2022). The rectal temperature in the morning were within the normal physiological range in conventional (102.75 ± 0.05) and elevated (102.48 ± 0.47) housing systems indicating that the lambs were comfortable, whereas, in evening, rectal temperature was higher than normal range in both conventional (104.17 ± 0.59) and elevated (103.76 ± 0.05) housing systems indicating stressful condition in the afternoon. The probable reason for increased rectal temperature in the evening might be due to insufficient airflow which contributes for the building up of heat leading to heat stress in the lambs. Significantly ($P<0.05$) higher rectal temperature was recorded in conventional housing system than that of elevated housing system in both morning and evening. This was in agreement with the results reported by Singh *et al.* (2017) on day 21 of experiment.

Respiration rate exhibited a diurnal rhythm reaching maximum at afternoon and minimum during morning in both housing systems. The results were in accordance with the findings of Rathwa (2017), Li *et al.* (2018) and Yamin *et al.* (2022). Respiration rate increased with the increasing levels of THI for lambs and was higher in extreme severe heat stress. This might be due to excessive heat load on the animals leading to increase in body temperature which in turn activates the hypothalamus to stimulate the respiratory centre and induce an increase in respiration rate to enhance the evaporative heat dissipation (Zaahkouk *et al.*, 2004).

The respiration rate was highly significantly ($P<0.05$) different in the lambs under conventional housing system as compared to the elevated housing system in the morning and evening. The present findings were in line with the results of Rahman *et al.* (2013) and Divate *et al.* (2014). This might be due to improved ventilation which allows hot air to rise and escape, thereby, minimizing the accumulation of heat inside the elevated housing system. The increased respiration rate due to heat stress enables the animal to dissipate the excess body heat by vaporizing more moisture through expired air.

The daily pulse rate was minimal in early hours and a peak pulse rate was observed in the late afternoon. This might be due to increased THI in the evening. These findings were in agreement with the results of Rathwa (2017), Li *et al.* (2018) and Yamin *et al.* (2022).

Significantly increased pulse rate with increase in THI during summer season indicates that animals were in heat stressed condition in evenings than mornings. The overall mean pulse rate recorded in conventional housing system was significantly ($P<0.05$) higher than that of elevated housing systems in the morning and evening. These results were in agreement with those reported by Rahman *et al.* (2013).

Cortisol: As the temperature increased, the concentration of cortisol increased in both housing systems, especially with a higher level in conventional housing system making the lambs vulnerable to stress. This might be due to decreased feed intake in heat stress conditions, however more energy is required by these animals to disperse more heat to regulate their body temperature at a normal level during summer (Bhatta *et al.*, 2014). Thus, to meet the increased energy requirement despite low dry matter intake during heat stress, the animal body responds by producing energy through hepatic gluconeogenesis (Sejian *et al.*, 2008) through activation of hypothalamic-hypophyseal-adrenal axis and the release of cortisol (Marai *et al.*, 2007). The results obtained are in concordance with the findings of Sejian *et al.* (2010) and Hooper *et al.* (2018) where the level of cortisol was reported to be increased in heat stressed animals as the temperature increases.

The study suggested that sheep farmers of coastal region could rear their lambs under elevated housing system with slatted floor owing to the beneficial effects such as decreasing the summer stress and improvement in the thermoregulatory responses of lambs as compared to conventional housing system with moorum floor.

Conflict of interest: The authors declare that they have no potential conflict of interest.

Author's contribution: SS, CVS, BS: Conception and design; GJ: Acquisition of data and performed statistical analysis of data; SS, GJ: Performed interpretation of the results.

Ethical statement: No experimentation was conducted on animals. The blood samples were collected aseptically from the sheep by a qualified veterinarian following all applicable guidelines for the care and use of animals.

Data availability statement: The data mentioned in the article will be available with the corresponding author.

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