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Melatonin Experiment: Exposing its Effects on Goat Physiology in Warming Conditions

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Abstract

The welfare of cattle is threatened by climate change, especially in areas where temperatures are rising. The physiological traits of goats subjected to warmer temperatures are studied in this investigation to determine whether melatonin, a hormone renowned for regulating circadian cycles and stressful situations, can have any moderating impacts. Two goat groupings participated in a controlled study: Group 1 (G1) received melatonin nutrients, while Group 2 (G2) functioned as the untreated group. 50 female goats of the same weight and age were subjected to high heats (36°C and 42°C) for a total of five hours/day for five days in a row after being acclimated for four days at 26°C. During the course of the trial, 0.1 mg/kg of melatonin was injected at midday. Pre and post-melatonin treatment reactions are determined. The concentrations of hormones (T-4 and cortisol) and the proportion of each of genes associated with stress (ubiquitin and HSP-60) in mononuclear cells from the peripheral bloodstream have been evaluated in samples of blood drawn on different days. The findings demonstrated that as exposure temperatures rose in both G1 and G2, there was a substantial increase in rectal temperature and the rate of pulse. T-4 rates in the G1 decreased at 42°C, whereas cortisol levels in the G2 soared with exposure heat but kept decreasing in the G1. HSP-60 and other stress-associated genes were up-regulated in G1, especially at 42°C. In summary, reduced cortisol levels and increased expression of stress-associated genes suggested that melatonin had a cooling as well as cell-protective effect on goats in heated environments.

Keywords: Goats, melatonin, climate change, temperature, hormones, stress-associated genes, cell-protective effect

INTRODUCTION

Unprecedented changes in the global climate over the past several years have produced a wide range of effects on different ecosystems and the people who live in them. Amidst the wide range of impacted species, tamed creatures like goats are facing the physiological difficulties brought by increasing temperatures (1). Goats are among the most vulnerable animals to the stresses brought by climate change as global temperatures rise. Unlike some other livestock, goats are farmed in a range of challenging environments, including desert regions and mountainous terrain (2). There are numerous ways in the intricate relationships between increasing temperatures & goat physiology interact, affecting the immune system, metabolic processes, reproductive cycles and overall health (3). Temperature has a major impact on metabolism, one of the primary physiological processes. Goats' metabolism adjusts for elevated temperatures as a way to maintain equilibrium and expel excess heat (4). Warmer temperatures have an impact on goat physiology since the immune system acts as a protector of resilience and general health. Goats' immune systems can be weakened by high temperatures, making them more vulnerable to illnesses and infections. In order to maintain the health and well-being of goat populations, proactive veterinary procedures and management techniques are essential due to the complex web of interactions that exist among temperature, stress & the immune system (5).

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Study (6) presented the state of research on the effects of "physiological, haematological, biochemical and hormonal factors" on goat blood that has been grown in unfavourable conditions. Study (7) examined the effects of summer extremes of heat on the physiology and behaviour of Malabari, Osmanabadi and Salem black goats. Assessments included differences in regards to the "heat shock protein 70 (HSP70)" among these breeds. Research (8) examined that the heat stress affects the physiological parameters & milk yield of Baladi goats raised in subtropical Egypt. Research (9) provided a discussion of the physiological and anatomical reactions of mammals to heat stress, which can be used as a tool for adapting to biological environments. Highlighted the gaps in our understanding and provided some suggestions for reducing stress in the system used to produce animals. Research (10) Determined the physiological responses of adult male Sahel and Red Sokoto goats in Nigeria's Guinea Savannah during the height of "cold-dry (CDS), hot-dry (HDS) and rainy seasons."

Study (11) evaluated the effects of thermal stress & nutritional stress associated with the summer season separately and concurrently on goats' capacity for adaptation. Study (12) presented a revised version of the topic to the audience to emphasize the significance of animal physiological reactions and their function in enabling survival in a stressful environment. In stressed animals, the cardinal physiological factors that support thermal equilibrium and homeostasis are "respiration rate (RR), pulse rate (PR), skin temperature (ST) and rectal temperature (RT)." Study (13) investigated the "HSP90 & HSP70" gene polymorphism that affects thermo-tolerance and its relationship to "hemato-physio-biochemical" parameters, as demonstrated by the observed patterns of expression in "Chokla, Magra, Marwari & Madras red sheep breeds." Research (14) examined the scientific data regarding the impact of heat stress on the physiology and metabolism of cattle, as well as the safety and quality of the meat. Study (15) established the role of heat shock proteins in animal adaptability during heat stress. Livestock cellular & molecular responses are important because they can help to identify confirming indicators for thermal stress in animals.

Study (16) examined the ability of three local goat breeds to adapt to heat stress. The "Osmanabadi, Malabari and Salem Black breeds" of female goats, ages 10 months to 1 year, were included. Study (17) discussed the effect of thermal stress on glucose transporters that facilitate insulin, systemic glucose metabolism and the ensuing effects on health and milk production. Research (18) examined the connections between a few physiological, metabolic and oxidative reactions in sheep yet the Temperature-Humidity Index (THI) investigated the impact of heat-relieving techniques, physiological status as well as breed. Research (19) examined the corpus of research on how heat stress affects farm animals' immunological responses. Heat stress affects humoral as well as cell-mediated immune responses, noting an alteration in the immune system's reaction from cell-mediated to humoral. It was observed that shift weakened an animal's immune system. Study (20) employed label-free quantification (LFQ) to examine how heat affects the proteins in goat milk.

The objective of this study was to evaluate the effects of melatonin on the physiological reactions of goats to high temperatures. The results showed decreased cortisol levels and elevated expression of the stress gene, indicating a cooling & cell-protective effect.

METHODS

Study location

This study was conducted in the "Division of Physiology & Climatology, IVRI." The institute is situated at latitude of 29 degrees north and a longitude of 78 degrees east, experiencing a sub-tropical climate. In November, the experiment was carried out in an environment with a mean temperature of 20-26◦C and a relative humidity of 61–83%, respectively.

Pets

For the investigation, a hundred robust Barbari goats with consistent ages and weights were chosen, as indicated in Table (1). They were split into two groups (G1, G2), each consisting of fifteen animals (n = 50), one for

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treatment (G2) and one for group 1 (G1). The animals were kept in clean, well-ventilated sheds with unlimited access to food and water.

Table (1). Goats with consistent ages and weights (Source: Author)

Group	G1 (n=50)	G2 (n=50)
Age	25.5 ± 2.0	26.0 ± 1.5
weight	150.0 ± 10.0	148.5 ± 8.0
gender	Female	Female

Design experimentation

The animals in the study were housed in the adjacent shed and within the psychrometric chamber during their exposure to heat stress, which lasted for the duration of eighteen days. With separate feeders and waters, the room measured 7.6 x 7.6 m in size. From day 6 to day 18, both groups' rectal temperatures, heart rates and respiration rates were recorded every day 30 minutes before or after melatonin was administered. In order to adapt the animals to the climatic chamber, they were housed in a psychrometric chamber at a temperature of 26°C for five hours a day, from 9:00 a.m. to 02:00 p.m., for five days. The animals were exposed to 36 °C for 5 hours (9:00 a.m. to 02:00 p.m.) every day for the next six days and 42°C for the same amount of time (9:00 a.m. to 02:00 p.m.) every day for the next 6 days. Animals in the G2 received 0.1 mg/kg BW of dissolved melatonin injected every day from the fifth to the eighteenth day at the sixth hour (12:00 h). I/V normal saline sham injections were given to the animals in the G1 group. On alternate days, beginning on the sixth day, blood samples (12 ml) from the G1 and G2 groups were taken in serum vacutainers (6 ml) and then heparinized vacutainers (6 ml) following a one-hour melatonin dose. The serum was isolated and kept for later examination at −22 ◦C in micro centrifugation tubes. The blood utilized for mRNA isolation was heparinized.

Procedure for hormone assay

ERBA Thyrokit was used to assess “thyroxine (T4)”, with a sensitivity value of 11nmol/l and a coefficient of variance of 2.52% in the assay and 5.89% between assays.

Using a second antigens coating method on microtiterplates and cortisol-HRP to serve as a marker, a highly sensitive “EIA process” was used to measure the cortisol levels in the plasma of un-extracted goats. The “coefficient of variation (CV)” for the intra and inter-assay using pooled plasma was determined to be 7.35% and 10.44%, respectively. 21pg/ml was the assay's analytical sensitivity.

Beginnings or Primers

Primers were created using the Fast PCR program.

HSP 60 has two primer sequences: 5'-ACTGGCTCCTCATCTCACTC3' for the “forwardprimer”and5'CTGTTCAATAATCACTGTCCTTCC-3' for the “reverse”. There are 149base pairs in the fragment and 109% of the amplification was successful.

The primer sequences for ubiquitin are as follows: 5'-ATGCAGATCTTTGTGAAGAC-3' for the “forward primer” and 5'-CTTCTGGATGTTGTAGTC-3' for the reverse. 99.8% of the amplification was achieved at a fragment size of 192base pairs.

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Isolation of PBMCs

PBMCs were separated using the "Histopaque1077" density gradient centrifugation method. To create a smooth interface between the two layers, the blood was deposited onto the Histopaque. Additionally, it was centrifuged for 31 minutes at ambient temperature at 2005 rpm. The mononuclear fraction that was opaque and whitish at the interface was gathered. After three PBS (pH 7.4) washes, the cells were collected as a cell pellet leads to extraction of total RNA and assessment of its purity.

The entire RNA extraction & purity evaluation

Following a 501µl DEPC-PBS treatment, the PBMC pellets were again suspended and transferred to a 3ml micro-centrifuge tube that was DEPC-treated but not nuclease-free. RNA was extracted using Trizol reagent and normal procedure. After dissolving the RNA in nuclease-free water, the "optical density (OD)" absorbing ratio (270 nm/ 290 nm) between 1.9 and 2.1 measured with a "nano-drop spectrophotometer" to confirm the purity of the RNA. Making use of denaturing agarose gel and UV light visualization (2%) electrophoresis, the entire RNA's integrity and purity were evaluated. Good quality & intactness of RNA were shown by two intact bands of 29 s and 19 s with smearing.

Analysis of quantitative RT-PCR

Reverse transcription was performed on a constant 2 g of total RNA for 95 minutes at 43 ◦C using the "iScript™" Select "cDNA Synthesis Kit"& the "oligo-dT18" primer. The resultant "complementary DNAs (cDNAs)" were applied to RT-PCR reactions "quantitatively (qRT-PCR)." A typical real-time PCR procedure was applied to each gene, using PCR templates that contained 25 ng of total RNA that had been reverse-transcribed. A final volume of 20 l was obtained by adding "1.25 l forward primer (0.2 mM), 1.35 l reverse primer (0.3 mM), 4 l SsoFast™ Eva Green® Super mix." For every factor that was tested, the general real-time PCR methodology that followed was used: the denaturation for 31s in 96 °C, followed by 43 rounds of a three segment amplification & quantification program. A melting stage involves heating the material from 63 to 97 °C at a pace of 0.68 °C/s while monitoring the fluorescence and cooling it down to 41 °C. The "Eva Green (with dissociation curve)"real-time machine method was applied to acquire "cycle threshold (CT)"values as well as a plot of amplification for each of the parameters that were identified after the run was completed. By amplifying a series of standard dilutions, the efficiency levels of real-time PCR reactions were determined and slopes were generated. The precise molecular size of transcripts was verified by high-resolution gel electrophoresis and sequence analysis was used to further establish the specificity of the targeted products. Melting temperature analysis is a product-specific method of documenting specificity. Negative supervision was used to examine the development of primer dimers. PCR was performed on each sample using all the components except the template. Relative mRNA expression was calculated using the G1 group's mRNA expression at the thermo-neutral zone as a calibrator.

Analysis of data

With the use of SPSS 17.0 software, the collected data was analyzed using paired T-tests for within-group analysis & independent T-tests for between-group analysis.

RESULTS

The physiological responses of the G2 & G1 groups to heat stress are depicted in Fig. 1 as a function of rectal temperatures, rate of respiration and heart rate. As exposure temperatures increased, rectal temperature and respiration rate increased dramatically ($P < 0.06$) in both the G1 and G2 groups. The heart rate did not change as shown in Figure (1).

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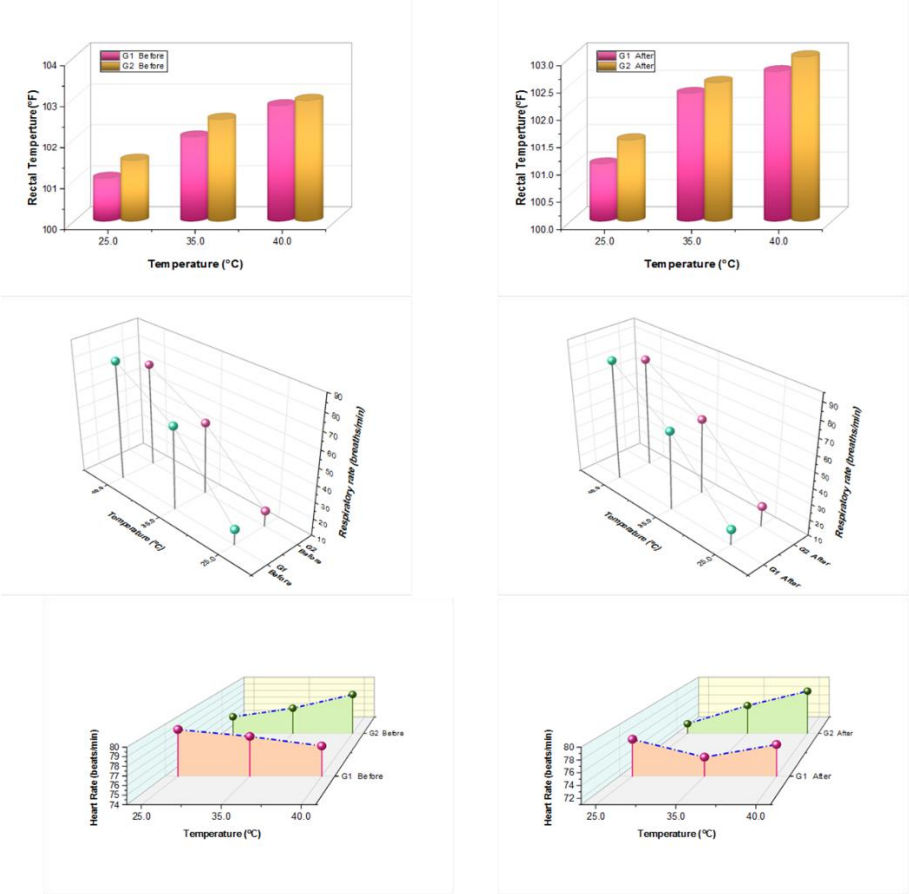

Figure (1). The physiological responses of the G2 & G1 groups to heat stress

(Source: Author)

Changes in serum cortisol and T4 levels are depicted in Figure (2). Temperature T4 displayed an ability to decrease as it was exposed. In contrast, In the G2 group, T4 concentration decreased ($P < 0.06$) during an exposure at a temperature of 42 ◦C as shown in Figure (2).

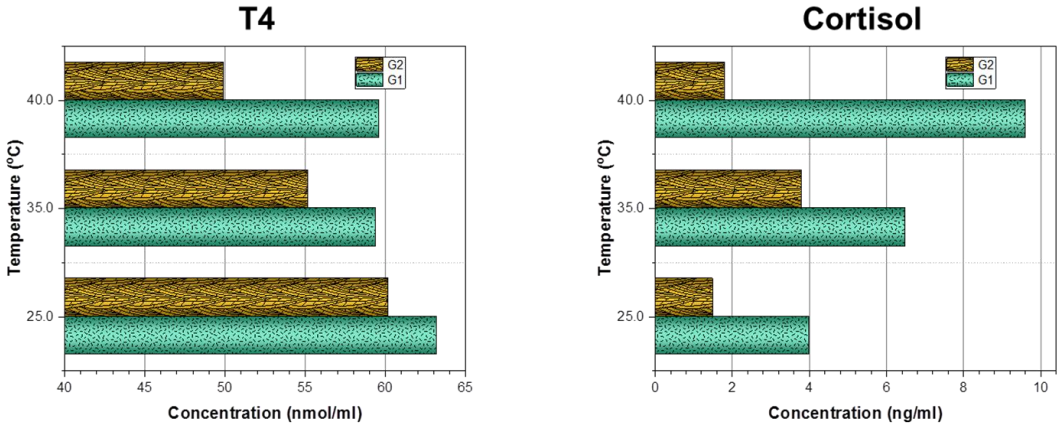

Figure (2). Changes in the Levels of Cortisol and T4 in the Serum

(Source: Author)

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The cortisol level of the G1 group increased (P < 0.06) with an increase in exposure temperature. However, in contrast to the temperatures of 26 ◦C and 42 ◦C, the group that received melatonin treatment exhibited a significant increase in levels of cortisol (P < 0.06) when exposed to a temperature of 36 ◦C. For every temperature of exposure, the cortisol level in the G1 and G2 groups changed (P < 0.06).

Heat stress-induced modifications to the ubiquitin and HSP 60's mRNA expression are illustrated in Figure (3), additionally with and without exogenous melatonin administration. In both groups, there was a significant (P < 0.06) increase in the proportional expression of HSP 60 and ubiquitin when exposure temperature increased. However, at a temperature of 42°C, the expression of HSP 60 was higher in the group treated with melatonin than in the G1.

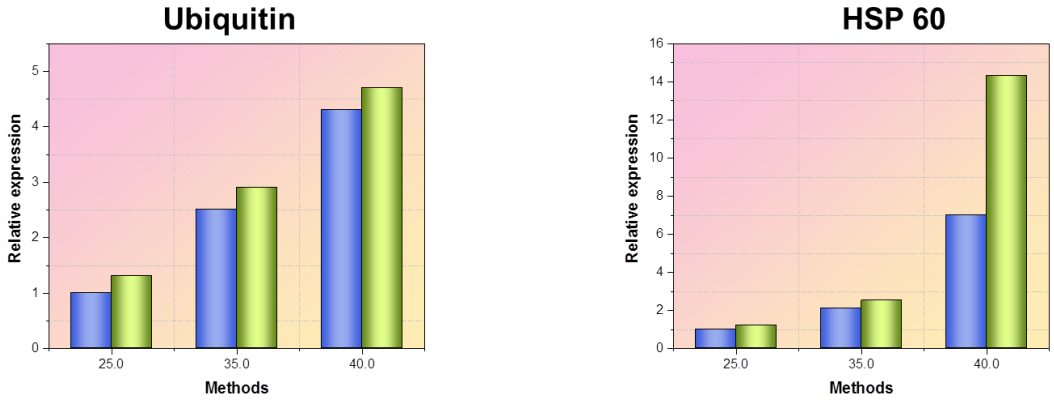

Figure (3). The changes in ubiquitin and HSP 60

(Source: Author)

DISCUSSION

Animals' degree of climate stress is measured by the “Temperature Humidity Index (THI)," with a score of 73 or smaller, suggesting “no heat stress (cool)" and 74–78 indicating mild “heat stress (HS)” as well as 79–90 indicating moderate heat stress. The current study demonstrated that animals experience excessive heat stress when exposed to 42°C, as evidenced by elevated respiratory rates, rectal temperatures and THI.

The animal experiences a coordinated series of physiological and behavioural reactions that support the preservation of physiological equilibrium and homeostasis (21). An animal under thermal stress will attempt to preserve thermal equilibrium by producing less heat and losing more heat. Heat generation is directly linked to bodily metabolism, which is regulated by the endocrine system and altered in response to heat stress (22). The main factor influencing the body's metabolic rate is thyroid hormones. It is known that they contribute to the creation of heat by promoting the development and function of uncoupling proteins, which decouple the phosphorylation of “adenosine diphosphate (ADP)” from the re-oxidation of reduced coenzymes. The body produces more heat when its metabolism is elevated due to increased thyroid hormone output. One way that the body adapts to heat stress is by reducing levels in T4 (23). Thyroid hormone levels have been found to be lower in tropical regions of the northern hemisphere (India) throughout the summer months of June-August. A non-significant declining trend in T4 concentrations was discovered when thermal exposure increased. It has been demonstrated that higher melatonin concentrations lessen the thyroid glands reaction to TSH (24). In our experiment, a decrease in the thyroid glands reactivity to TSH in the presence of melatonin can have contributed to the G2 lower T4 level at 42 ◦C.

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The current study's substantial increase in blood cortisol level proves that the goats were experiencing heat stress and the G2 comparatively large drops in comparison to the G1 indicate that melatonin can have a calming influence. The traditional reaction to stress is an increase in cortisol release. A quick mobilization of lipids and amino acids from cellular reserves brought by cortisol allows the body to employ these substances for energy production as well as the creation of other molecules, such as glucose, that are needed by various bodily tissues (25). Increased serum cortisol levels are related to heat stress's stimulatory effect on the hypothalamic-pituitary-adrenal axis.

Cells have built-in molecular systems that preserve cellular homeostasis; in addition to an animal's physiological and behavioural reactions to reduce heat stress. The molecular response to exposure when subjected to heat stress is acknowledged to be influenced by variations in gene expression (26). Changes in gene expression include the production and expression of fewer other proteins, the activation of "heat shock transcriptional factor 1 (HSF1)", as well as a rise in HSP expression. Expression of HSPs has been connected with an animal's ability to adapt to adverse environmental conditions and resilience to stress (27). HSPs are crucial for a number of processes, including the folding of freshly generated proteins, the dissolution of protein complexes and the transport of proteins inside cellular structures."Heat shock element (HSE)" Heat Stress Factor 1 (HSF1), which is connected to the promoter part of HSP genes, mediates the increase in HSP production under heat stress (28).

The control of gene expression that codes for HSP 60 under heat stress is a homeostatic mechanism that prevents cell damage caused by heat stress. The mitochondrial protein HSP 60 aids in protein refolding and keeps denatured proteins from clumping together. They have demonstrated that as people age, HSP 60 expresses itself more. The anti-apoptotic function of HSP 60, in addition to its function in folding protein & preventing the aggregation of denatured proteins, can be linked to the considerable increase in HSP 60 expression in the G1 and G2 groups after heating at 36 ◦C as well as 42 ◦C.

The bio-molecule most conserved throughout evolution is ubiquitin. At 8444 Da, its molecular weight is tiny, consisting of 77 amino acids. The protein in question is a thermo-stable globular complex that is stable at high temperatures and it is crucial for "immunological defence, transcription, apoptosis, DNA repair and protein degradation." Because ubiquitin aids in the degradation of proteins in heat-stressed cells, its higher expression following heat-stress exposure makes sense. G2 group showed elevated HSP 60 mRNA expression.

The way heat stress controls melatonin expression appears to be the cause of this. Since that HSP 60 prevents stressed cells from going through apoptosis, melatonin could reduce the impact of thermal stress by increasing the availability of HSP 60.

CONCLUSION

This study examined goats' physiological reactions to high temperatures in an effort to model how climate change can affect animal welfare. Melatonin treatment, which is well-known for balancing circadian rhythms and reducing stress, was investigated. Female goats were divided into two groups for the purposes of the study: Group 1 (G1), which received melatonin supplements and Group 2 (G2), which received no treatment. Both groups exhibited a substantial elevation in rectal temperature as well as pulse rate in response to warmer temperatures (36°C and 42°C) for duration of five consecutive days, equivalent to five hours per day. But at 42°C, G1 showed a significant drop in T-4 levels, whilst G2 showed an increase in cortisol levels with exposure to heat but a decrease in G1 levels. In G1, there was a notable up-regulation of stress-associated genes, specifically HSP-60, at 42°C. The results show that goats subjected to high temperatures benefited from melatonin's cooling and cell-protective effects, as evidenced by the up-regulation of stress-related genes and the increase in cortisol levels. Understanding possible methods to protect animals and their welfare especially in light of rising temperatures brought by climate change is made easier with the help of such details.

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