Effects of the season on physiological and endocrine traits and on HSP70 in Saanen goats under Mediterranean climate conditions¹⁾

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Summary

This study was conducted to determine the impact of heat stress on some physiological and endocrine traits in Saanen goats raised under Mediterranean climate conditions. The effects of thermal stress on heart rate (HR), respiration rate (RR) and rectal temperature (RT) on plasma total trii-odothyronine (T3), thyroxine (T4), Cortisol (C), and HSP70 concentrations were evaluated on twenty two Saanen goats of different ages in the second week of April 2013, July 2013, October 2013 and January 2014. Climatic data such as temperature (°C) and relative humidity (%) were recorded from Spring 2013 to Winter 2014. Live body and BCS values were also recorded during this period. The physiological parameters above were measured twice on each experiment day (morning and afternoon) in all seasons. Blood samples were collected in each afternoon of the experiment day to analyze T3, T4 C, and HSP70. All data were analyzed. According to the values of rectal temperature (RT), it was estimated that the goats were under extreme heat stress only in the summer season. The heart rate (HR) values in the winter season for morning and noon periods were found statistically significant ($p < 0.05$). The average respiratory rate (RR) in the spring season was found significantly lower. On the other hand, the RR for the noon period in the summer was higher than in the fall and winter seasons ($p < 0.05$). There was a significant difference ($p < 0.05$) between summer and fall seasons for C values. The highest value (96.62 ng/ml) was obtained in spring, whereas the lowest (60.58 ng/ml) in the fall. T3 levels in the fall and winter were found to be statistically significant ($p < 0.05$). They were the highest in the winter and spring, and the lowest in fall. T4 and T4/T3 levels in the winter were found to be statistically higher than in other seasons ($p < 0.05$). The lowest value for T4 was found in the fall and for T4/T3 in summer. Mean HSP70 value in spring was found to be statistically low ($p < 0.05$). The changes in THI values in different seasons, particularly between mornings and afternoons, indicated that thermal stress was evident, and that the animals became resistant to it eventually. The fluctuations of the C, T3, T4 and HSP70 values were indicators of the animals' reaction to thermal stress. The THI values in spring, which were between 16 and 18 THI, may be considered within the ideal comfort zone for goats. It was observed that Saanen goats were able to adapt to seasonal weather changes in the environmental conditions of the region.

Keywords: thermal stress, trii-odothyronine, thyroxine, cortisol, heat shock protein 70, Saanen goats

Animal species reared in different production systems are exposed to many environmental stress factors such as temperature, humidity and radiations. The

changes in environmental stress factors are recognized as potential hazards to the growth and production of all domestic livestock species (7). Among the stressors, heat stress has been of major concern in reducing an animal's productivity in tropical, sub-tropical and arid areas (32). The degree to which an animal can resist

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changes in body temperature varies for different species because of the distinctions in their heat regulating mechanisms (28).

Changes in temperature may lead to “thermal stress” on animals. Thermal stress is the perceived discomfort and physiological strain associated with the exposure to an extremely hot or cold environment (8). It affects every system of the body, thus physiological but also hormonal, cellular and some genetic parameters should be evaluated to determine influences of thermal stress. Animals show various responses to thermal stress at physiological, cellular, biochemical and molecular levels (8).

The respiration rate (RR), heart rate (HR) and rectal temperature (RT) of animals could be used as indicators of physiological adaptation to external environments. Several researchers studied physiological adaptation mechanisms by using these parameters in small ruminants (13, 18, 20, 31). The rectal temperature of goats was found to be elevated with high environmental temperature in several studies (13).

Thyroid hormones, trii-odothyronine (T3) and thyroxine (T4), play an important role in animal adaptation to environmental changes (16). They stimulate oxygen consumption and heat production by cells, which increase the basal metabolic rate, enhance glucose utilization, modify lipid metabolism, and stimulate cardiac and neural functions (35). T3 and T4 are also important in reproduction and as indicators of the nutritional and metabolic status of animals (30). Trii-odothyronine (T3) and thyroxine (T4) could be used as response markers to thermal stress at a biochemical level.

Heat shock proteins (HSP) could be used as response markers to thermal stress at a cellular level. HSPs consist of a group of highly conserved proteins that are induced in both prokaryotes and eukaryotes by elevated temperatures (26). The cellular response to heat stress involves the synthesis of a family of proteins of diverse molecular weights (HSP-100, -90, -70, -60 kDa), among which HSP70 is the most abundant. HSP70 synthesis increases when the cell is exposed to high temperatures, preventing damage to proteins from occurring (3). This response comprises part of the thermal acclimation mechanism of cells to high temperatures and for this reason it may be used as an indicator for the adaptation capacity of animals to heat stress (14).

The aim of the study was to determine the impact of heat stress on some physiological and endocrine traits in Saanen goats raised under Mediterranean conditions.

Material and methods

All experimental procedures were reviewed and approved by the animal ethics committee at Adnan Menderes University, Aydin, Turkey (Approval No.B.30.2.ADU/050.04/2012/075). The study was carried out in the goat research unit of the Faculty of Agriculture, Adnan Menderes

University (37° 45' 03.31" N and 27° 45' 27.16" E) from Spring 2013 to Winter 2014.

Climatic measurements. Mediterranean climate prevails in the region. Meteorological measurements such as temperature (°C) and relative humidity (%) were recorded throughout every day of the experiment from Spring 2013 to Winter 2014. Temperature Humidity Index (THI) was calculated to determine the effect of environmental conditions on animals. The following equation was used to calculate THI:

$$THI = T - (0.31 - 0.31 \cdot RH) \cdot (T - 14.4)$$

where T is the dry bulb temperature (°C) and RH is the relative humidity (%) (6, 16).

In order to determine heat stress in the environment, the temperature humidity index (THI) was categorized according to Silanikove, (32) and Marai et al. (13) as follows:

$$\text{Heat stress} = \begin{cases} \text{Absence} & \text{THI} < 22.3, \\ \text{Moderate} & 22.3 \leq \text{THI} < 23.3, \\ \text{Severe} & 23.3 \leq \text{THI} < 25.6, \\ \text{Extreme} & 25.6 \leq \text{THI}. \end{cases}$$

Animals. Twenty-two healthy Saanen goats (2-3 years old) from the goat research unit of the Faculty of Agriculture, Adnan Menderes University, were used in this study. The animals were housed in a well-ventilated and hygienic shed with ad libitum access to food and drinking water. On each day of the experiment, goats were observed to evaluate pathological alterations and all findings were recorded. The same management (care and feeding) program was applied to the animals from Spring 2013 to Winter 2014.

All the parameters were measured each season, namely in April for the spring, in July for the summer, in October for the fall and in January for the winter, in the second week of each month mentioned above twice at an interval of 7 days and the averages were recorded.

Live body weight and body condition score. The live body weight of the goats was measured by electronic scales. The body condition score of the goats was determined as the average value of the body condition score given by three researchers in each season. Body condition score was defined from 1 (very poor condition) to 5 (very good condition) with half-unit increments (27).

Physiological traits. The physiological variables evaluated consisted of heart rate (HR), respiration rate (RR) and rectal temperature (RT). These were determined twice on every day of the experiment (morning at 8:00-9:00 and afternoon 14:00-15:00). Heart rate (HR) was measured based on the pulsations noted in the femoral artery per unit of time and represented by number of heart beats per minute. Respiratory rate (RR) was recorded counting the number of breaths per minute through the auscultation of the thorax using a stethoscope. Rectal temperature (RT) of the goats was measured with a digital clinical thermometer inserted in the rectum of each animal and represented in °C/min.

Blood collection. Blood samples were collected from the goats in the afternoon, using gauge sterile needles and plastic syringes, from the external jugular vein and placed in tubes with heparin anticoagulant (10 IU/ml). Plasma was separated from blood by centrifugation at 3,000 rpm at room temperature for 15 min and divided into aliquots in micro centrifuge tubes where it was kept frozen at -20°C until

further analysis. Plasma samples were used to determine endocrine variables.

Endocrine traits. The endocrine variables evaluated were plasma trii-iodothyronine (T3), thyroxine (T4), cortisol (C) and heat shock protein 70 (HSP70). T3 and T4 were measured using a commercially available ELISA kit (goat trii-iodothyronine T3 Elisa kit catalog number CSB-E13385G and goat thyroxine T4 Elisa kit catalog number CSB-E13384G). The T3 and T4 standard curves were plotted between 0.5 and 8 ng/ml of protein and 20 and 320 ng/ml of protein in the solution, respectively. The sensitivities of the T3 and T4 assays were 0.25 ng/ml and 10.0 ng/ml, respectively. The concentrations of the T3 and T4 in the plasma were determined using the standard curve.

Cortisol was determined using a commercially available quantitative sandwich ELISA kit (goat cortisol Elisa kit catalog number CSB-E18048G) according to the manufacturer's protocol. The standard curve had a range of 0.05 to 200 ng/mL and the sensitivity of the assay was 0.05 ng/mL.

Heat shock protein 70 (HSP70) in plasma was detected by a commercially available quantitative sandwich ELISA kit (Elisa kit catalog number CSB-E13381G) according to the manufacturer's protocol. The concentrations of HSP70 protein were determined by comparison with a standard curve according to manufacturer's direction. The standard curve had a range of 23.44 to 1.500 ng/mL, and the sensitivity of the assay was 5.86 ng/mL.

The biochemical analyses of T3, T4, cortisol and HSP70 were conducted in the Biochemistry Laboratory of the Faculty of Veterinary Medicine, in Adnan Menderes University.

Statistical analysis. Animals having four-season data during morning and noon were included in the analysis. The linear mixed-effects model in Equation 1 was applied for the statistical analysis of live body weight and body condition score:

$$y_{ij} = \mu + \alpha_i + \beta_1 (X_{1ij} - \bar{X}_1) + e_{ij} \quad [1]$$

the linear mixed-effects model in Equation 2 was applied for the statistical analysis of trii-iodothyronine (T3), thyroxine (T4), trii-iodothyronine/thyroxine (T3/T4), cortisol and heat shock protein 70 (HSP70):

$$y_{ij} = \mu + \alpha_i + \beta_1 (X_{1ij} - \bar{X}_1) + \beta_2 (X_{2ij} - \bar{X}_2) + \beta_3 (X_{3ij} - \bar{X}_3) + e_{ij} \quad [2]$$

The linear mixed-effects model in Equation 3 was applied for the statistical analysis of rectal temperature, heart rate and respiration rate:

$$y_{ijk} = \mu + \alpha_i + \tau_j + (\alpha\tau)_{ij} + \beta_1 (X_{1ijk} - \bar{X}_1) + \beta_2 (X_{2ijk} - \bar{X}_2) + \beta_3 (X_{3ijk} - \bar{X}_3) + e_{ijk} \quad [3]$$

where $y_{ij(k)}$ is the response variable for live body weight, body condition score, triiodothyronine (T3), thyroxine (T4), cortisol, heat shock protein 70 (HSP70), rectal temperature, heart rate or respiration rate; μ is the overall mean; α_i is the i^{th} season effect (i = spring, summer, fall, winter); τ_j is the j^{th} time effect (j = morning, noon); $(\alpha\tau)_{ij}$ is the interaction between season and time; β_1 , β_2 and β_3 are the regression coefficients of age (X_1), live body weight (X_2) and body score condition (X_3); $\bar{X}_1$, $\bar{X}_2$ and $\bar{X}_3$ are the averages of age (X_1), live body weight (X_2) and body score condition (X_3) and $e_{ij(k)}$ is the residual random error.

The nlm package (21) for R (25) was used to fit the linear mixed-effects models (LME) shown in Equation 1, 2 and 3 with corresponding Σ matrix which is a block diagonal with blocks corresponding to the individuals and with each block having the compound-symmetry (CS) structure. The form of the Σ matrix was as follows:

$$\Sigma = \begin{bmatrix} \Sigma_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \Sigma_n \end{bmatrix} \text{ where } \Sigma_i = \begin{bmatrix} \sigma^2 + \sigma_1 & \sigma_1 & \cdots & \sigma_1 \\ \sigma_1 & \sigma^2 + \sigma_1 & \cdots & \sigma_1 \\ \vdots & \vdots & \ddots & \vdots \\ \sigma_1 & \sigma_1 & \cdots & \sigma^2 + \sigma_1 \end{bmatrix}$$

and $i = 1, 2, \dots, n$, where n is the number of animals.

The optimal compound-symmetry covariance structure for the data set was determined using Schwarz's Bayesian Criterion (12). Two unknown parameters, one modeling a common covariance (σ_1) and the other a residual variance (σ^2) of Σ matrix, were estimated by maximum likelihood which was not affected by unbalanced or missing data. The significance of the fixed terms was determined with conditional F-tests. The final, best fitting, model was then refitted using REML as suggested by Pinheiro and Bates (22) to obtain unbiased coefficients and confidence intervals. After significant effects of fixed factors were identified, the differences between least square means of fixed factor levels were considered significant at $p < 0.05$ (2-tailed) based on the Tukey adjustment type I error rate.

Results and discussion

Descriptive statistics of temperature, relative humidity and THI are given in Table 1. As indicated there, the summer is hot and dry, and the winter is mild in Aydin. The annual rainfall was between 580-1000 mm. The average relative humidity was 60.03% in the Spring 2013, 44.41% in the Summer 2013, 60.57% in the Fall 2013 and 75.66% in the Winter 2014. The relative humidity in the afternoon was about two-fold lower than that of the morning in spring, summer and fall; however, it was similar for morning and afternoon in the winter. Mean, minimum and maximum temperatures are given in Table 1, and they were 17.07°C, 5.15°C, 35.26°C in spring, 29.03°C, 17.32°C, 39.80°C in the summer, 16.48°C, 4.97°C, 31.25°C in the fall and 10.86°C, 1.11°C, 20.04°C in the winter, respectively.

Live body weight and body condition score. Analysis of live body weight using Eq. 1 showed that season and age had significant effects on the live body weight of the goats. The significant estimate of the regression coefficients of age ($\beta_1 = 8.1$) indicated positive linear relationship between age and live body weight.

The season effect on live body weight is described in Tab. 2. It differed significantly ($p < 0.05$) among seasons. The highest value was observed in winter. The values obtained in spring and summer were significantly lower than those obtained in fall and winter.

Analysis of body condition scores of goats using Eq. 1 indicated that there were no significant effects of season and age on body condition score of goats

Tab. 1. Descriptive statistics of temperature means of relative humidity, and temperature/humidity index (THI) values by season and year in Aydın

Season	Temperature (°C)			Relative Humidity (%)	THI
	Mean	Minimum	Maximum	Mean	Mean
Spring 2013					
April	17.07	5.15	35.26	60.03	16.74
The average of the experiment days	13.19	5.15	20.36	54.71	13.36
Morning measurement	12.38	–	–	57.26	12.96
Afternoon measurement	20.03	–	–	38.80	18.96
Summer 2013					
July	29.03	17.32	39.80	44.41	26.51
The average of the experiment days	29.60	21.89	35.63	51.08	27.30
Morning measurement	27.71	–	–	57.41	25.95
Afternoon measurement	35.59	–	–	38.41	31.54
Fall 2013					
October	16.48	4.97	31.25	60.57	13.59
The average of the experiment days	16.32	8.84	26.00	29.19	15.90
Morning measurement	12.71	–	–	69.43	12.87
Afternoon measurement	26.24	–	–	28.28	23.61
Winter 2014					
January	10.86	1.11	20.04	75.66	11.13
The average of the experiment days	11.96	9.96	15.47	81.50	12.10
Morning measurement	12.98	–	–	78.50	13.08
Afternoon measurement	13.46	–	–	78.45	13.52

Tab. 2. Mean of live body weight and body condition score of goats by season

Season	Live body weight	Body condition score
Spring 2013	42.80 ± 1.48 ^a	2.10 ± 0.06
Summer 2013	47.80 ± 1.48 ^a	2.04 ± 0.06
Fall 2013	53.60 ± 1.48 ^b	2.22 ± 0.07
Winter 2014	61.93 ± 1.48 ^c	1.96 ± 0.07

Explanation: a, b, c – means with different superscript letters differ significantly at $p < 0.05$

(Tab. 2). The lowest body condition score and highest live body weight of goats were obtained in the winter due to the latest period of the gestation on the animals.

Endocrine traits and HSP70. Analysis of triiodothyronine (T3), thyroxine (T4), triiodothyronine/thyroxine (T3/T4), cortisol (C) and heat shock protein 70 (HSP70) using Eq. 2 showed that season had a statistically significant effect ($p < 0.01$) on T3, T4, T3/T4, C and HSP70 of the goats. However, age, live body weight and body condition score of the goats did not show statistically significant effects on T3, T4, T3/T4, C and HSP70 of the goats (Fig. 1, 2, 3).

The effects of season on T3, T4, T3/T4 are given in Fig. 1. The level of T3 differed significantly ($p < 0.01$) among seasons (Fig. 1a). The highest level of T3 was obtained in the winter and the lowest value was observed in the fall.

Cortisol values (ng/ml) showed a significant difference ($p < 0.05$) between summer and fall measurements. The highest value of cortisol (96.62) was obtained in the spring, whereas the lowest value (60.58) was observed in the fall (Fig. 2). The normal range of plasma cortisol in goats are 57-63 nmol/L (11).

Mean HSP70 in the spring was found to be statistically lower than in the summer and fall ($p < 0.05$). There was a significant difference ($p < 0.05$) between spring and other seasons, and the lowest value of HSP70 was also observed in the spring. The highest

Fig. 1. Season effect on triiodothyronine (T3), thyroxine (T4), triiodothyronine/thyroxine (T3/T4). Upper-case letters indicate significant differences in T3, T4 and T3/T4 among seasons, respectively

value of the HSP70 was observed in the summer. Values of HSP70 in the fall and winter showed a slight decrease (Fig. 3).

Physiological traits. Rectal temperature, heart rate and respiration rate are the parameters which illustrate the mechanism of physiological adaptation. In this study, mean rectal temperature of the goats of the winter morning period was found to be higher than in the summer and fall ($p < 0.05$), whereas there were no differences for noon periods in any season (Fig. 4). The rectal temperature alterations were found to be only significant between morning and noon periods for the spring ($p < 0.05$).

Average heart rate (HR) values of the goats for the different seasons are given in Fig. 5. The lowest average of HR was obtained in the morning measurements of the summer and the highest value was obtained in the winter. When morning and afternoon values were compared, the HR values of the afternoon period were especially increased in the summer season ($p < 0.05$); nevertheless, there was a decrease of the HR of the afternoon period in the fall ($p < 0.05$). The HR values in the winter for morning and noon periods were found to be statistically significant ($p < 0.05$). A statistically significant difference was also found between noon periods of the summer and the fall ($p < 0.05$).

While the minimum of the respiratory rate (RR) was obtained in the spring, the maximum RR was obtained in the summer (Fig. 6). When RR values of the morning and afternoon periods were compared with each other, RR of the afternoon was observed to have increased in the summer ($p < 0.05$). Except for spring, all seasonal respiratory rates were increased at noon times ($P > 0.05$). RR in the spring morning period was found statistically lower than that of the summer and winter ($p < 0.05$).

The correlations related with LW, BCS, HR, RR, RT, T3, T4, C,

Fig. 2. Cortisol levels according to seasons (ng/ml)

Fig. 3. Differences in the values of the HSP70 (ng/ml) according to seasons

Fig. 4. Rectal temperature alterations according to seasons and time (°C)

Fig. 5. Heart rate alterations according to seasons and time (pulse/min)

Fig. 6. Respiration rate alterations according to seasons and time (breath/min)

Fig. 7. The correlations related with the following properties: Live Weight (LW) and Body Condition Score (BCS), Heart Rate (HR), Respiration Rate (RR), Rectal Temperature (RT), Trii-iodothyronine (T3), Thyroxine (T4), Cortisol (C), Heat Shock Protein 70 (HSP70)

HSP70 are given in Fig. 7. When there was a positive high correlation coefficient among the properties, it is shown in blue color, and the larger and darker circles indicate a more important correlation between the two properties. When there was a negative high correlation coefficient among the properties it is shown in red color, in the same manner.

According to Fig. 7, positive high correlation coefficients were found between T4 and RT, T4 and BCS, T4 and T3, T4 and T4/T3, while there was a significant negative correlation between T4/T3 and HSP70 for the morning period of spring. Positive and high correlation coefficients were observed between RT and RR, T3 and RR, T3 and RT, T4 and BCS, T4 and T3, T4/T3 and T4. On the other hand, a significant negative correlation was found between HR and BCS, T4/T3 and HSP70 for the noon period of spring (Fig. 7). With regards to the summer measurements, a positive high correlation coefficient was found between BW and BCS, between C and BW, between T4 and T3, and between T4/T3 and T4 for morning and noon periods.

Positive high correlation coefficients between RR and BCS and between T4/T3 and T4, and a negative high correlation coefficient between T4/T3 and T3 for the morning period of the fall were found (Fig. 7). With regards to the noon period of the fall, a high positive correlation coefficient between RR and BCS, C and HR, T4 and RR, T4 and RT, and T4/T3 and T4 was found significant. In addition, there was a high negative correlation between HSP and HR and between T4/T3 and T4 (Fig. 7). Moreover, the positive high correlation between C and HR in the noon of the fall season, and the negative high correlation between HR and BCS in the noon of the spring season, as well as between RT and HR in the morning of the winter should also be estimated as striking indications.

THI in Tab. 1 shows that there are some differences regarding heat stress among seasons. THIs observed in the morning were lower than those observed in the afternoon in all seasons. THIs in the spring, fall and winter were lower than 22.3 and no heat stress ($\text{THI} \leq 22.3$) was observed for these seasons. The highest value of THI (31.54) was obtained in the summer, with an average THI value of 26.51. Climatic data revealed that extreme heat stress ($25.95 \leq \text{THI}$) was observed in the summer in the study area. Relative humidity values in this study were higher than those (9.20-33.93%) reported by Al-Haidary et al. (1) but were similar to those (40.1-79.0%) reported by Okoruwa (17) and Helal et al. (9).

The observed temperatures for the afternoon period were higher than those for the morning period in spring, summer and fall; however, they were similar in the winter (Tab. 1). Temperature values for this study were found higher than or closer to the critical temperature of 24 to 27°C for most species as indicated by Helal et al. (9).

In this study, live body weights measured in spring and summer were significantly lower than those of fall and winter. Helal et al. (9) studied the effects of short- and long-term exposure to solar radiation during the summer on goats and found that exposure to solar radiation for 12 hours increased the loss in live body weight and the packed cell volume in goats. The effect of heat stress on the live body weight in goats was investigated and it was found that the exposure of goats to solar radiation in different times daily decreased the final live body weight (17). The loss of live body weight during exposure to solar radiation was attributed to the increase in the energy expended for heat dissipation through respiratory evaporation and subsequently to a reduction in the amount of water available for storage.

The lowest body condition score and highest live body weight of the goats observed in the winter could have resulted from their advanced stages of pregnancy. However, the body condition score of the goats was similar in all seasons and these findings indicated that there was no heat stress on the goats resulted from feeding and management.

In this study we found that seasons had a statistically significant effect ($p < 0.01$) on triiodothyronine (T3), thyroxine (T4), triiodothyronine/thyroxine (T3/T4), cortisol (C) and heat shock protein 70 (HSP70). While the activity of the thyroid gland increased in cold regions, it was reduced in warm environments (36). It was reported that, in the case of heat stress, levels of serum T3 and T4 would decrease (6, 32, 35). The plasma thyroxine (T4) and triiodothyronine (T3) levels were observed to decline under heat stress as compared to thermoneutral conditions (2). Moreover, the level of T4 also differed significantly among seasons (Fig. 1b) and showed a different significant pattern from that of T3. Measurements in the winter resulted in the highest level of T4 which was different from the levels observed in the other seasons. Although the lowest level of T4 was obtained in the fall, there were no statistically significant differences among the T4 values in spring, summer and fall.

It was reported that low ambient temperatures increased thyroid activity, whereas high temperatures depressed it (5). There were decreases in T3 and T4 levels from 4.55 and 21.27 to 3.21 and 16.70 pmol/L respectively (33). Also, previous values reported by Helal et al. (9) showed that T3 and T4 levels declined in goats during short and long term exposures to solar radiation. As an acclimation response to alleviate heat stress, a decline in thyroid functioning could be due to the effect of heat on hypothalamo-pituitary-adrenal cortical axis to decrease in thyrotropin releasing hormone which enables the animal to reduce its basal metabolism. T3 and T4 levels also increased during cold stress resulting in increase in oxygen consumption and heat production by cells to basal metabolism rate (4, 16, 35).

Rectal temperature is considered as a good index of body temperature even though there is a considerable variation in different parts of the body core at different times of the day (34). According to the values of this study, in spring afternoons the temperature was within the comfort temperature ranges for goats, and their RT decreased slightly when compared to the early morning hours. The mean temperature of the noon period was found to be lower than that of the morning (Fig. 4). Popoola et al. (19) reported that rectal temperature and respiration rate were significantly affected ($p < 0.001$) by the temperature humidity index (THI). The temperature in the afternoon was much higher than in the morning in the summer and fall (Tab. 1). It was also observed that the rectal temperature of the animals in the afternoon were higher when compared with the values of the morning. The rectal temperature of the goats was found to be elevated with high environmental temperatures in several studies (13).

The average RR in the spring was found to be significantly lower than in the summer and winter, while RR for noon period in the summer was higher than in the fall and winter ($p < 0.05$). It was reported that respiration rate was significantly affected ($p < 0.001$) by the temperature humidity index (THI) (19). A goat's normal respiratory rate is between 15 and 30 per minute (19). The respiratory rates in the morning and noon periods in the spring were estimated to be closer to the normal respiratory rates for goats in this study.

For the morning period in the winter, a high positive correlation coefficient was found between RT and HR, HSP and RT, and T4/T3 and T4. The high negative correlation coefficient between HSP and T4 and T4/T3 and T3 for the morning period in the winter was a significant result. While there was only a high positive correlation coefficient between T4/T3 and T4, it was found that there was a high negative correlation coefficient between HSP and T4, T4/T3 and T4 for the noon period in the winter (Fig. 7). In order to combat cold stress, the animals increase their metabolic activities to supply more body heat (19). This explains the increase of the heart rate observed in the winter in our study, probably in order to balance the body temperature in cold weather (Fig. 7).

T3 and T4 levels increase during cold stress, resulting in an increase in oxygen consumption and heat production by cells to increase the basal metabolism rate (4, 34). It was found that the environmental temperature had a predominant effect on the thyroid gland activity of the White Goats in different physiological periods (23). It was stated that the plasma T3 and T4 levels in goats around the middle of the gestation period were higher in respect to the oestrus period, probably due to the negative energy balance (35). In this study the plasma T3 and T4 levels in the winter season were found to be much higher than in other seasons. Especially in the second half of January, the goats were in an advanced pregnancy period, so the levels of T3

and T4 could be very high for either of those reasons: namely pregnancy and cold weather. While there was a positive high correlation coefficient between T4 and T3 in the spring and summer, a negative correlation coefficient was found between them in the fall and winter (Fig. 7).

In addition, thyroid hormones play a significant role in the fat metabolism. They increase the breakdown of fat in adipose tissue, causing fat formation or destruction to balance the body heat (37). Various factors like breed, age, sex and physiological condition affect blood thyroid hormone concentrations by modulating the hypothalamus-pituitary-thyroid axis in small ruminants (36). However, effects of the live weight, BCS and age of the goats on the T3, T4 and T4/T3 levels in this study were found insignificant, but there was a positive high correlation coefficient between T4 and BCS, and T3 and RT in the spring. The average BCS of the goats was found to be the lowest in the winter.

In this study, the THI values were over the comfort zone for the goats in the summer and fall and under the comfort zone in the winter. In the light of these findings, it could be claimed that the animals were under thermal stress in the summer, in the autumn and in the winter. It was observed that the combination of relative humidity and air temperature caused variations in the body temperature and the respiration rates of the animals.

Heat shock protein 70 (HSP70) is one of the most abundant proteins within the heat shock protein family that consists of highly conserved stress proteins, expressed in response to stress, which play crucial roles in environmental stress tolerance and adaptation (35). In this study, the highest HSP70 values were observed in the summer, whereas the lowest values were observed in the spring. There was an insignificant difference in HSP70 values in the goats between the summer and the winter or fall. It was found that the values of HSP70 in the spring were lower than in the other seasons, since the weather conditions in the spring were more favorable to the goats. The average THI values in the noon period in the summer (31.54) and fall (23.60) were higher than in the winter and spring. Blood samples were taken in the afternoon in this study, so it could be said that animals were under high temperature stress in the summer and fall. Similarly, animals were under low temperature stress in winter. According to the THI values in spring, it could be stated that optimum weather conditions (Tab. 1) for the goats were observed in spring. Some studies show that cells in better heat-adapted bovine breeds produce lower HSP70 concentrations than in less adapted breeds (10). Similarly, in goats adapted to the desert climate, HSP70 levels were higher than in less adapted goats (14).

Physiological parameters RT, RR and HR presented with fluctuations according to the changes in THI values in different seasons and particularly between

mornings and afternoons. This may indicate that thermal stress was the cause of these fluctuations, and the animals achieved resistance against this stress, as well as managed to adapt to seasonal climatic changes. Between seasons, the rises and falls in the cortisol level, T3, T4 and the HSP70 values were the indicators of the animals' reaction to thermal stress. Regarding the comfort zone, the best environmental temperature was found to be 13-27°C for Indian goats (15). In this study, the optimal values of all the parameters evaluated were obtained in the spring for these goats in the area. The THI values in the spring, which were between 16 and 18 THI, could be considered to be the ideal comfort zone for goats in the area. The effect of the heat increased when it was accompanied by high relative humidity. Physiological traits and growth performance of the goats varied at different thermal comfort levels determined by THI. High THI values were associated with poor growth performance as well as adverse effects on physiological characteristics of the animals. However, Saanen goats tolerated higher levels of THI than the values proposed for sheep and goats as their comfort level (13). Management strategies are needed to improve goat production by minimizing thermal stress effects on the goats in order to attain optimal animal comfort. However, further studies are needed to elucidate the tolerance level exhibited by Saanen goats in thermal stress.

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