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Impact various seasons on expression patterns HSP60 and physiological parameters

Abstract

The current research was conducted to understand how the impact of heat stress on some physiological parameters and study seasonal pattern HSP60 expressions associated with physiological response in goats (Alpine and Saanen and Boer breeds) subjected to various climatic conditions. This study was carried out in winter and summer periods on 160 goat types Saanen (n= 65), Alpine (n= 73) and Boer (n= 22) at the Dairy Goat Research Farm of Çukurova University located in the province of Adana, in eastern Mediterranean part of Turkey. Environmental data (ambient temperature, relative humidity and thermal humidity index) and physiological parameters including body temperature, surface temperature (head and skin), respiration rate and pulse rate were recorded. In addition serum concentration of HSP60 and HSP70 were measured though double-antibody sandwich ELISA test for each season. The thermal humidity index (84) showed that goats were subjected to thermal stress during summer. The skin, head and body temperature; respiration and pulse rate during this season were significantly ($P<0.05$) higher when compared to winter. On other hand, the results revealed that the HSP60 and HSP70 expression changed with season. This seasonal profile of HSP concentration demonstrated HSP60 (6.7±0.62 vs.11.1±0.85 ng.ml-1) and HSP70 (21.6±0.76 vs. 20.9±0.53 ng.ml-1) were significantly lower in winter when compared to summer. Furthermore, positive and significant ($P<0.05$) correlations were found between HSP concentration and physiological data. Goat increased their respiration rate, pulse rate and skin temperature to dissipate the extra-heat loaded. To further that physiological adaption, the body of goat set a cellular adaption mechanism which is associated with the high concentration of heat shock proteins.

Keywords: HSP60, goat, physiological, thermal stress, season

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Introduction

Thermal stress is most concerning now a day in the ever altering climatic scenario. In tropical and sub-tropical regions high ambient temperature is the major obstacle on animal production.[1] AS thermal stress is the perceived discomfort and physiological strain associated with an exposure to an extreme hot or cold environment.[2] Thermal stress includes both heat stress as well as cold stress during extreme summer season and during extreme winter season.[2] Domestic animals undergo various kinds of stress like physical, chemical, nutritional, psychological and thermal stress.[3] Heat shock protein (HSP), has strong role physiological endogens way for conserving cellular homeostatic against negatively external factors as and antiaptotic molecular.[4] Hsp60 is mitochondrial protein, during cells are exposed to stress factors, addition acts as a chaperone, its physiological roles under normal, non-stressful conditions. Also Hsp60: in the stressful conditions expressed by eukaryotic and prokaryotic is structurally highly conserved and abundantly,[5] the use of improved technologies in molecular analysis had allowed the improvement in the accuracy and intensity of use of genetic markers,[6,7] the current scenario was assess the impact of heat stress on some physiological parameters and study seasonal pattern HSP60 expressions associated with physiological response in goats (Alpine and Saanen and Boer breeds) subjected various climatic conditions.

Material and methods

The current study conducted in three seasons (summer and winter) and have carried out two stage in farm and in vitro.

Experiment in vitro: It have been collected serum involved 16 femal dairy goats included three breeds Alpine (n=73), Saanen (n=65) and Boer (n=22). For HSPs analysis Serum samples prepare 5-8ml of blood from jugular vein into heparinized vacutainers tubes (BD Biosciences) and immediately cooled at 4c then transported to laboratory for serum isolation. Samples were kept at room temperature for 20 min even clotting and centrifuged for 15min at rpm. Serums were taken into clen pipette 210ul labelled microtubes then stored -20c. Serum HSP60 level concentration was determined using the enzyme-linked immunosobent assay (ELISA) test.

Experiment In farm: estimation of physiological adaptation

From 160 goats as seen above it was conducted to study the impact change climate on physiological performance of goats. A total of 24 femal goats were involved in this phase: Alpine (n=8), Saanen (n=8) and Boer (n=8). All animals subjected to one week for adaptation before starting collection of physiological parameters and climate data. Physiological parameters including pulse rate (PR), respiration rate (RR), body temperature (BT), skin temperature (ST) and head

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temperature (HT) recorded in the morning at 07:00-08:00 and 13:00-14:00 ST and HT measured by using a infrared thermometer (Testo BP-960) at a distance of 8cm from the head and skin, RR and PR recorded by using a stethoscope, the BT was recorded by using a digital thermometer.

Collection environmental data

In the farm during performing experiment, the daily meteorological information i.e. air relative humidity and temperature in research site were recorded. A thermometer and a barometer were used for climatic data collection. To record were used to calculate the temperature humidity index (THI) abased on the following formula:

THI=db-(0.55-0.55 RH) (db – 58); db: the dry bulb temperature.[10]

Statistical analysis

HSPs concentrations and physiological data were statistically analysed separately following the GLM procedures in the Statistical Analysis System (SAS V. 2004). Differences were tested with Duncan's Multiple Range Test at a level of 5% or 1%. The following mathematical models were used for HSPs concentration and physiological parameters respectively: Pearson's correlations among milk and climate data were calculated using the CORR procedure of the

$$Y_{ijk} = \mu + \alpha_i + \beta_j + y_k + (\alpha\beta)_{ij} + (\alpha y)_{ik} + (\beta y)_{jk} + (\alpha y)_{ik} + (\alpha\beta y)_{ijk} + e_{ijkl}$$

Where, Y_{ijkl} : observed value.

μ : Mean of population.

α_i : Effect of breeds (1= Alpine, 2= Saanen and 3= Boer)

β_j : Effect of season (1= winter and 2= Summer)

y_k : Time (1= in morning 08:00-09:00 and 2= in afternoon 13:00-14:00) or HSPs concentration (HSP60)

$(\alpha\beta)_{ij}$: Interaction of breed and season

$(\alpha y)_{ik}$: Interaction of breed and HSPs/Times

$(\beta y)_{jk}$: Interaction of season and HSPs/Times

$(\alpha\beta y)_{ijk}$: Interaction of breed, season and HSPs/Times

Results

Climatic indices during experimental period

The climatic variables during experimental days are given in the Table 1. During trials, the average ambient temperature range between 9.4±0.2 and 28.7±0.1°C in the morning, while it varied between 16.2±0.3 and 34.9±0.1°C in the afternoon. The average relative humidity was approximately 59.8±0.6 in summer. Given that the THI ıs a variable depending on the AT and RH, it was found that the average THI were 55.09 and 80.81 in winter and summer respectively.

Physiological response

The Table 2 summarizes the daily and seasonal changes in physiological parameters. The results showed that the ST, BT, PR, RR and HT were statistically (P<0.05) different between seasons and times of recording. No significant differences were observed between the pulse rates and body temperatures of breeds groups when compared with each other (p>0.05). The body, skin and head temperatures in all breeds groups were significantly (P<0.05) lower in morning when compared to the recorded value in afternoon.

Seasonal and physiological response on HSP serum levels

The variation of HSP 60 due to goats' breeds and seasons and its correlation with physiological indicators are presented in the table 3 and table 4. The expressions of HSP60 were significantly higher (P <0.05) in all breed groups during summer season as compared with winter season. The overall average of HSP60 concentration during summer (11.1±0.85 ng/ml) was significantly higher (P<0.05) than winter (6.7±0.62 ng/ml). Regarding the breeds, the current results clear demonstrated that the Boer goats expressed more HSP protein under hot environment when compared to the others breeds. As seen in the Table 5, there was a strong and positive correlation between seasonal changes and heat shock protein (HSP60) concentration. Results also showed positive correlations between thermal humidity index and HSP concentration. Similar trends were observed between physiological parameters and HSP 60 level in experimental goats.

Table 1 Environmental parameters during experimental period

Seasons	Hours	AT (°C)	RH (%)	Average THI
Winter	08:00-09:00	9.4 ± 0.2	64.3 ± 1.6	55.09
	13:00-14:00	16.2 ± 0.3	52.6 ± 0.5	
Summer	08:00-09:00	28.7 ± 0.1	73.4 ± 0.6	80.81
	13:00-14:00	34.9 ± 0.1	46.3 ± 0.6	

Discussion

THI during summer according to THI's formula the index was stressfull. Avendaňo[8] realized that the homoeothermic capability of goats begins to come to terms when THI override 80%. Silanikove et al.[9] reported that the THI is a good indicator of stressful thermal climatic conditions and extreme distress and animal are unable to preserve thermoregulatory mechanisms and normal body temperature. THI at values more than 78 and THI values 75-78 stressful and THI values of 70 or less are considered comfortable. Thats was similar trends were found by Habibu.[10] Indeed, the AT and THI were lower than those found in the present study. The same authors demonstrated that the recorded AT (39.21°C) and THI (84.62) in the afternoon during the hot dry season were highest, while THI was lower in the morning during. The highest RH (77.36%) was obtained in the morning, while the lowest THI (56.76) was observed in the morning during dry season.

According to,[11,12] the thermal radiation increased at the peak of the summer and declined in the winter. As indicated that skin temperatures were 36.36 and 38.99°C for T1 and T2 respectively, which significantly (p<0.05) higher in T2 and lowest in T1.The obtained increasing in skin temperature for goats in T2 was attributed to exposure to heat stress. That might cause vasodilation of skin capillary bed and consequently increase the blood flow to the skin surface to facilitate heat dissipation. The skin temperature could also be elevated due to solar radiation as skin temperature has been shown to be directly related to solar radiation levels.[13] Shown that body temperature of goats was significantly (p<0.05) higher than that of the sheep in all the season.

Table 2 Physiological data of goats during experimental period

Breeds	Seasons	Times	BT (°C)	ST (°C)	HT (°C)	PR (bpm)	RR (breath/min)
Alpine	Winter	07:00-8:00	35.7 ± 0.05[a]	19.1 ± 0.3[e]	18.5 ± 0.2[e]	98.01± 3.2[c]	53.3 ± 2.4[b]
		13:00-14:00	36.3 ± 0.06[b]	26.8 ± 0.3[b]	25.1 ± 0.3[b]	106.2 ± 2.6[b]	56.1 ± 1.1[cb]
	Summer	07:00-8:00	38.0 ± 0.13[c]	35.3 ± 0.21[c]	34.9 ± 0.3[dc]	101.4± 1.5[c]	69.2 ± 3.1[c]
		13:00-14:00	40.9 ± 0.11[ba]	39.7 ± 0.3[a]	39.6 ± 0.3[a]	116.5 ± 1.9[a]	106.6 ± 3.3[a]
Saanen	Winter	07:00-8:00	34.7 ± 5.0[a]	18.8 ± 0.22[e]	18.9 ± 0.4[d]	109.3 ± 1.5[b]	52.6 ± 1.1[c]
		13:00-14:00	36.1 ± 0.1[b]	24.4 ± 0.33[c]	24.2 ± 0.2[b]	115.9 ± 0.8[a]	54.0 ± 1.3[cb]
	Summer	07:00-8:00	37.4 ± 0.12[b]	34.3 ± 0.2[d]	33.2 ± 0.60[d]	103.5±1.7[cb]	68.4 ± 2.6[c]
		13:00-14:00	40.2 ± 0.4b[a]	38.4 ± 0.5[b]	37.2 ± 0.1[b]	105.4 ± 2.0[cb]	106.2 ± 3.5[a]
Boer	Winter	07:00-8:00	34.8 ± 0.2[a]	20.1± 0.3[d]	21.2 ± 0.6[c]	107.5± 2.1[b]	58.4 ± 2.0[b]
		13:00-14:00	36.3 ± 0.1[a]	28.1 ± 0.3[a]	27.5 ± 0.42[a]	112.7 ± 1.6[ba]	67.4 ± 2.3[a]
	Summer	07:00-8:00	37.37 ± 0.1[c]	34.5 ± 0.2d[c]	35.1 ± 0.3[c]	107.9± 1.4[b]	63.9 ± 1.8[c]
		13:00-14:00	40.9 ± 0.4[a]	39.1 ± 0.2[ba]	40.8 ± 14.2[a]	113.7 ± 2.3[a]	89.3 ± 2.7[b]
Sig.	Breeds		NS	**	**	NS	**
	Seasons		*	**	**	*	**
	Breed × Season		NS	**	*	NS	**

Table 3 Seasonal variation of HSP60 concentration in experimental goats

Breeds	HSP60 (ng/ml)		Sig.
	Winter	Summer	
Saanen	4.3 ± 0.65[b]	8.7 ± 0.72[a]	
Alpine	4.4 ± 0.42[b]	6.5 ± 0.43[a]	**
Boer	9.1 ± 0.78[b]	20.4 ± 1.36[a]	
Overall Means	6.7 ± 0.62[b]	11.1 ± 0.85[a]	

**, a, b = significant at P< 0.05

Table 4 Correlations among HSP concentration, physiological and environmental parameters

	Seasons	THI	BT (°C)	ST (°C)	HT (°C)	PR (bpm)	RR (brths/min)
HSP60	0.982**	0.886**	0.996*	0.688*	0.617*	0.514*	0.540*

** Significant at P< 0.01; *Significant at P< 0.05

Mavrya et al.[14] reported that response respiration rate of lambs a significant increase ($p<0.05$) in respiration rate for groups, but the respiration rate values of increase was greater for G1 as compared to G2 lambs where respiration rate was in day 0 a.m. 35.3, 32.0 in open and p.m. was 62.4 in open and 57.8 in closed, at day 15 was 41.6, 37.6 a.m. in open and increased p.m. (60.in open, 56 in closed) in day 30 increased also a.m. 39.3 in open, 36.2 in closed and p.m. 64.3 in open and 61.8 in closed, the reason could be that the homoeothermic animals initially react to cold stress by enhancing the thermoregulatory mechanism, such as increase in respiration rate. These results are inconsistent with those of Okoruwa,[15] who stated that respiration rate was significantly ($p>0.05$) between T1 (16.04 breaths/min) and T2 (18.98 breath/min), but T3 (23.01 breaths/min) was significantly ($p<0.05$) higher than T1 and T2,[16] stated that of both HT and udder temperature increased consequently from morning to afternoon (+3.2 and +2.5). Then, it at midnight returned to normal

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level. However, the head and udder temperatures of both cooled goats were significantly lower than non-cooled ones ($p<0.05$). That may be attributed to surface radiation effects of the earth, because the goats are subjected to heat the whole day by way of both convection and radiation.

The relative difference between the present study and previous studies might be due to different breeds, place, ages of goats, climatic changes and trial conditions.in previous a study on the same city as the present study.

This impact climatic change may impact on performance of goats due to the assumption that seasonal variation led to different in the level of concentration of HSP to different extent in breeds adapted to temperature climatic conditions.[6] As added, Yilmaz et al[17] reported that mRNA gene expression levels for hsp60 in Saanen goats was 1.72 in the winter, whereas, mRNA expression level measured more than mRNA gene expression level measured during the spring,[18] stated that hsp60 gene expression was significantly higher ($p<0.01$) for Sirohi (7.8) and Jhakrana (5.3) as compared with Barbari (2.1) goats.

On the other hand, hsp60 gene expression was significantly ($p<0.05$) higher former than the later in the summer, hsp70 gene expression. It was change significantly higher ($p<0.01$) for Sirohi (3.2) and Jhakrana (2.8) goats as compared with Barbari (1.7) goats during the summer.

Conclusion

Based on the current findings, it could be concluded that the physiological parameters and behavioural response to heat shock protein, the respiration rate was most evidence as index for response heat stress and cold stress. Also hsp60 concentration could be utilized as a marker for thermal adaptation in goats, and information generated could be utilized for identification of specific breeds to cope up with the challenges of climate change.

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Conflicts of interest

No conflict of interest.

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