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Association HSP70with some physiological parameters in dairy goat under south Turkey conditions

Abstract

Background: It has been demonstrated that the composition of HSP70 heat shock protein 70 is temperature correlated HSP70 effects is one as cellular thermometer in response to heat stress and other stimuli. Goat also possess anatomical and physiological characteristics that qualify for their wide ability to ecological adaptability, nevertheless, the productivity of the individual declines during thermal stress .the study in the issuance of this article accomplished to analyze the level of concentration of heat shock protein variance heat stress phenotypes in response to chronic heat stress.

Ruslt: The investigation has been carried out in Alpin (brown colour), Saanen (Wightcolour), these breeds same age (1-2 year) and differ in size and production performance. The thermostress appreciation was at a temperature humidity index (THI) 80.8 during summer, 55.1 during winter and 70.1 during spring. Physiological parameters for thermal stress susceptibility was carried at by combining pulse rate (PR) (bpm), respiration rate (RR) (breath/min), body temperature (BT) °C, skin temperature (ST) °C and head temperature (HT). HSP70 level of concentrations were analyzed with ELISA, the gene expression pattern of HSP70 in different seasons indicated that the HSP70 for Saanen breed was 26.9, 18.3 and 14.1 during spring, summer and winter respectively. HSP70 for Alpine was 12.7, 13.7 and 14.7 in the spring, summer and winter respectively.

Conclusion: The seasonal variation associated with changes of environmental variable, especially ambient temperature had great effects on physiological and cellular function of goats. Under adverse climatic conditions the body and skin temperature of goats increased due to the heat loading into the animal body. To maintain the homeothermy, goats increased their respiratory frequency to promote heat dissipation. Moreover, goats' body develop a cellular pathway to cope thermal challenge. This later is expressed through the high synthesis of heat shock protein that was observed in this study. HSPs represents an important molecular indicator when assessing heat stress in goats.

Keywords: HSP70, goat, physiological data, seasons, thermal stress

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Introduction

Climate change is one of the challenges for containing of various species, sustainability and ecosystems of livestock production sector systems, across the subtropical and temperate countries. Stress is respond of the body to stimuli that occurred disturbs homeostasis, therefore detrimental effects.[1] Goat was the most adapted species to imposed HS in terms of production, reproduction and also to disease resistance.[2] The cellular mechanisms faced to the stress conditions. the altered expression of stress genes one of the cellular response.[3] During heat shock HSP70 has been described as conserved heat shock protein and strongly concentrating in the nucleolus, the necessary metabolic processes inside the nucleolus, processing Rdnatranscriotion, and assembling with ribosomal proteins, and the nuclear architecture are sensitive to temperature changes,[4] to surpass the thermal stress species implement adaptation mechanisms including behavioral, physiological , biochemical, hormonal, cellular and morphological adaptive responses,[5] molecular chaperons that maintain native conformation of proteins and cell viability during stress period.[5] Heat stress directly or indirectly effect to animals, heat stress lead to lowing feed intake to decrease metabolic heat production and dissipation mechanisms, increased both of perspiration and respiratory rate and therefore decrease preserve body temperature with negative influence on production and reproduction, and animal health.[6] The aim of the current study was investigated related heat shock protein HSP70 with some physiological parameters on the dairy goats.

Material and method

Study location

This study was conducted at the Dairy Goat Research Farm of Cukurova University located in the province of Adana (36°59' N 35°18'E) in eastern Mediterranean region of Turkey. The climate is subtropical type characterized by mild and wet winters and hot and dry summers. The lowest (-8.1°C) and highest (45.6°C) ambient temperature are recorded in January and August respectively. The average annual precipitation and relative humidity were 450 mm and 66% respectively.

Animal management

A total of 160 does were used during trials: Saanen (n= 65), Alpine (n= 73) and Boer (n= 22). Goats were averagely 18 months old. Goats were clinically healthy and free from any physical abnormalities.

Animals were housed in semi-opened pens. Goats were fed twice daily with forage (oats and alfalfa hay), corn silage and 500g concentrate feed containing 18% crude protein and 2500 kcal/kg metabolism energy in dry matter. Clean water was provided ad-libitum for all animals.

Experimental procedure

The experiments were carried out in two season: winter (January-February) and summer (July-August) when the ambient temperature were lowest and highest respectively. This study was conducted in two experimental phases:

Experiment 1: Serum isolation and HSP assay procedure

The trials relating to the determination of serum heat shock protein concentration involved 160 female goats: Saanen (n= 65), Alpine (n= 73) and Boer (n= 22). To prepare serum samples, 5-8 ml of blood were collected from jugular vein into heparinized vacutainers tubes (BD Biosciences). Blood samples were immediately cooled (approximately 4°C) and transported to laboratory for serum isolation. Samples were kept at room temperature for 20 min for clotting and centrifuged for 15 minutes at 1500 rpm. Using clean pipette, 210ul of serum were taken into labelled microtubes and stored at –20°C. The frozen serum samples were transferred to the laboratory for HSPs analysis. Serum HSP70 levels were determined using the enzyme-linked immunosorbent assay (ELISA) test.

Measurement of HSP70 levels

The HSP70 levels in the serum samples were measured by ELISA kit (SunRed Biotechnology Co., Catalogue No. 201-07-0728, Shanghai, China). The assay procedure was the double-antibody sandwich test performed according to the supplier's instructions.

After preparing reagents, samples and standards, the prepared serum specimens and standards were added to the wells of a microplate pre-coated with antibodies labelled with enzyme and incubated at room temperature (37°C) for 60 min. After reaction the plate were washed five times and the chromogen solution A and B were added and the components were incubated for 10 minutes at room temperature (37°C) in the dark. Reactions were stopped with the supplied stop solution. Within 15 min after adding the stop solution, the OD was read under 450 nm wave length. The obtained OD values were used to calculate the HSP70 concentration in each specimen from the standard curve.[9]

Experiment 2: Assessment of physiological adaption

Derived from experimentation 1, it was carried out to investigate the effect of temperature changes on physiological parameters of goats. A total of 24 goats were involved in this experiment: Saanen (n= 8), Alpine (n= 8) and Boer (n= 8). Before starting collection of experimental data, all animals were subjected to seven days of acclimation. Physiological data including body temperature (BT), respiration rate (RR), skin temperature (ST), pulse rate (PR) and head temperature (HT) were recorded in the morning at (07:00-08:00) and afternoon (13:00-14:00). RR and PR were recorded by using a stethoscope. ST and HT was measured by infrared thermometer (Testo BP-960) at a distance of 8 cm from the head and skin. The BT was recorded using digital thermometer (Generation Guard's Clinical Digital Thermometer) that was introduced into the rectum: The stabilized body temperature was recorded after the sound of the alarm signal was heard.

Environmental data

During the experimental days, the daily meteorological data i.e. air temperature and relative humidity on the research site were recorded. A thermometer and a barometer were used for climatic data collection. Recorded data were used to calculate the temperature humidity index (THI) according to 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 (HSP70)

$(\alpha\beta)_{ij}$: 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 and discussion

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 (Table 1).

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	

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 (Table 2).

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	**

Seasonal and physiological effects on HSP serum levels

The expressions of HSP70 were significantly higher (P < 0.05) in all breed groups during summer season as compared with winter season., 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 4, there was a strong and positive correlation between seasonal changes and heat shock protein (HSP70) concentration. Results also showed positive correlations between thermal humidity index and HSP concentration. Similar trends were observed between physiological parameters and HSP 70 level in experimental goats (Table 3&4).

Table 3 Seasonal variation of HSP70 concentration in experimental goats

Breeds	HSP70 (ng/ml)		Sig.
	Winter	Summer	
Saanen	14.1±0.41^{b}	18.34±0.47^{a}	
Alpine	13.9±0.36^{b}	14.7±0.61^{a}	**
Boer	30.6±0.77^{b}	36.8±1.26^{a}	
Overall Means	21.6±0.76^{a}	20.9±0.53^{b}	

$^{**, a, b}$ = 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)
HSP70	0.991**	0.880**	0.985*	0.680*	0.656*	0.538*	0.539*

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

According to Darcan et al,[7] and Silanikove et al,[8] goats are in thermal comfort when the THI is equal or less than 70. THI between 75 and 78 means goats are subjected to stressful conditions, while a THI higher than 78 induces distress.[11] Standing on this point, the results of the current study demonstrated that goats were subjected to severe thermal stress in summer. This is due to the variation climatic variables including ambient temperature, humidity and solar radiations which potential harmful effect on dairy goats. Heat stress is the result of an imbalance between metabolic heat production inside the animal body and its dissipation to the surrounding.[9] Thermal stress is a major factor that can negatively affect both production and reproduction in dairy animals, especially in animals of high genetic merit.[9] When subjected to heat stress, homoeothermic animals employ several thermoregulatory mechanisms to offset heat gain by an equivalent loss to maintain their core body temperature and attain thermal equilibrium.[10] This thermoregulatory responses is manifested by the alterations of physiological parameters. Similar differences between hourly ST, BT and HT temperatures were reported by various studies.[11–13] The increase of BT during afternoon may be attributed to thermal stress as AT and THI values increased markedly from morning to afternoon.[12] Among the seasons, there was a higher BT in summer (P < 0.05). According to Niyase et al,[14] and Erasmus et al,[15] the reference value of rectal temperature in goats ranges between 38.5 and 39.7°C. In this current study, the average value observed in afternoon during summer was slightly higher than the reference values for adult goats, 38.5 to 39.7°C[19]. Although there were seasonal effects and in the day shifts in BT, the goats were able to keep BT within normal range and thus maintain homeothermy.[15] The current findings showed that goats had significantly ($P<0.05$) lower RR in the morning. Furthermore, the daily magnitude of respiration rates was higher in summer when THI was high (80.81).

These results were in concordance with several previous studies investigating the effect of heat stress on physiological adaption in goats.[10,11] The initial response of an animal to heat stress is the increasing of its respiratory rate, causing loss of heat by evaporation. During heat exposure, animals access respiration as a mechanism to avoid increased rectal temperature, maintaining homeothermy.

The average heart rate for goats is 90 beats/min and can vary from 70 to 120 beats per minute.[13] In this study, the pulse rate was between 98.01±3.2 and 116.5±1.9 beats.min^{-1}. In addition, the PR was higher ($P<0.05$) in the afternoon during the summer. The mean observed in this study is above the average reported by 17. When subjected to thermal stress goats increase cardiovascular rhythmicity as the result associated to the increase of RR, which promote heat dissipation mechanisms.[15] The increase of PR is due to the increased muscle activity to control the simultaneous increase in respiratory rate and a reduction in peripheral vascular resistance promotes greater blood circulation to dissipate heat through the skin.[13,15] The high HSPs concentration observed in Boer groups and during summer may be justified by the origin and adaptability of this breed to hot-tropical climate. Indeed, Boer goat originally from tropical region of Africa are naturally adapted breed to sub-tropical environmental conditions and better survive to thermal shock in comparison to Alpine and Saanen whish are western European goat breeds.[16] Since elevated levels of HSP was reported during exposure to different environmental stresses and water deprivation, the high HSP concentration observed in this study during summer was in accordance with previous studies carried out in different livestock species.

Summary

Based on the current findings, it could be concluded that the seasonal variation associated with changes of environmental variable, especially ambient temperature had great effects on physiological and cellular function of goats. Under adverse climatic conditions the body and skin temperature of goats increased due to the heat loading into the animal body. To maintain the homeothermy, goats increased their respiratory frequency to promote heat dissipation. Moreover, goats' body develop a cellular pathway to cope thermal challenge. This later is expressed through the high synthesis of heat shock protein that was observed in this study. HSPs represents an important molecular indicator when assessing heat stress in goats.

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Conflicts of interest

Author declares that there are no conflicts of interest.

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