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Hafsa AL Yamani
Department of Animal Science, Çukurova University, Adana, Turkey

Nazan Koluman
Department of Animal Science, Çukurova University, Adana, Turkey

Corresponding Author:
Hafsa AL Yamani
Department of Animal Science, Çukurova University, Adana, Turkey

Concentration level of Hsp60 and Hsp70 in the dairy goat under variation seasons

Hafsa AL Yamani and Nazan Koluman

Abstract
Our work focuse on the relevance environmental data, physiological parameters and plasma comcentration of both heat shock proteins (HSP60 and HSP70) in the dairy goats. Data of study involved 65 Saanen and 73 Alpine crossbreds reared at the Dairy Goat Research Farm of Çukurova University, Adana (Turkey). Physiological parameters (rectal temperature, respiration and pulse rate) and climatic data (air temperature, relative humidity) were recorded. In addition, blood samples were collected from jugular vein to get the plasma samples which were used to assay the concentration of HSP 60 and 70.
The environmental conditions reflected through a high THI (THI= 82.6) showed that the goats were subjected to thermal stress. On the other hand, the average HSP 60 significantly lower in winter (4.4 ± 0.5 ng/ml) followed by spring (7.6 ± 0.6 ng/ml) and summer (9.05±0.82 ng/ml). Whilst, the HSP 70 was 22.6 ± 0.6, 14.4 ± 0.5 and 13.3 ± 0.4 ng/ml in summer, spring and winter respectively. We can concluded that under harsh conditions the dairy goats synthesised more heat shock protein which can be used as thermoregulatory indicator

Keywords: Heat shock protein, Goats, Physiological parameters

Introduction
Livestock are subjecte to many threats, which may turn it from leading source of income to dilemma for production forms such as decrease of diurnal and quarterly production, increased costs of breeding and production consequently heavy economic losses. Heat stress for prolonged periods of high temperature or low temperature limits animal productivity, which lead to economic losses.
Environmental change is associated with thermal stress resulting into various physiological and Biochemical reactions which are inter-correlated. The physiological adaptation to external environment has highly effect on productivity of dairy goat. Dairy goats have optimal temperature zones or thermo-neutral zones within which their body temperature remains relatively constant and favourable for optimal production.
However, when exposed to the ambient temperature below or above the thermo-neutral zone, goats alter their rectal temperature, respiration and heart rate to cope whether cold or heat stress. Further, thermal stress is genetically governed, and heat shock protein play key role influencing heat stress in animal (Sailo *et al*. 2015) [7].
Indeed, when exposed to harsh stimuli, genes activate and form heat shock proteıns (HSPs) to protect cells against stressors. HSPs are molecular chaperons that maintain native conformation of proteins and cell viability during stress period (Kishore *et al*., 2016) [6]. HSP70 is an essential molecular chaperone of primary importance to all mammalian cells. They protect cells, tissues, and organs from stress by helping protein folding (assembly and refolding) in endoplasmic reticulum (Gade *et al*., 2010; Jee, 2016) [4, 5]. As for the HSP60, it gathers protein by forming hetero-oligomeric protein complex (Jee, 2016) [5]. This paper reports the findings related to the investigation the relationship between physiological and serum concentration of heat shock proteins in Alpine and Saanen goats in three seasons i.e winter, spring and summer under subtropical Mediterranean conditions.

Materials and methods
Animal materials
The study was carried out on 65 Saanen and 73 Alpine crossbreds kept at the Dairy Goat Research Farm of Çukurova University, Adana (Turkey). The study area is characterized by subtropical climate conditions with cold dry winter (December to March) and hot humid summer (May to August). The lowest and highest ambient temperature which can reach -8.1°C and 45.6°C are recorded in January and August respectively. The average annual precipitation and relative humidity are 450 mm and 66% respectively. The trials was conducted during three seasons (winter, spring and summer).

Climatic parameters
The daily environmental data including air temperature and relative humidity (RH) on the research site were recorded using a thermometer and a barometer were used for climatic data collection. Then, temperature humidity index (THI) according to the formula developed by (Abdel-Samee, 1996):
THI = db - (0.55 - 0.55
RH) (db - 58); where db: the dry bulb temperature.

Physiological data
Rectal temperature (RT), respiration rate (RR) and pulse rate (PR) were recorded from experimental animals in the morning at (07:00-08:00) and afternoon (13:00-14:00). RR and PR were recorded by using a stethoscope. The RT was recorded using digital thermometer which inserted into the rectum: The stabilized rectal temperature was recorded after the two minutes. These parameters were recorded during every season over the experimentation period.

Heat shock proteins assay
To determine the serum concentration of HSP, serum samples were isolated from 5-8 ml of blood collected from jugular vein of each goats using heparinized vacutainers tubes. Blood samples transferred to the laboratory of the department of animal science, Çukurova University in cool box (approximately 4oC).
Then, blood samples were processed through centrifugation for 15 minutes at 1500 rpm. Using clean pipette, 210ul of serum were taken into labelled microtubes. These later were used to measure the HSP70 and 60 levels using the enzyme-linked immunosorbent assay (ELISA) test. The HSP assay procedure was described by the ELİSA kits supplier (SunRed Biotechnology Co., Catalogue No. 201-07-0733, Shanghai, China).

Statistical analysis
HSPs concentrations and physiological data were statistically analysed separately following the General Linear Model procedures in SPSS 20. Differences were tested with Duncan's Multiple Range Test at a level of 5% or 1%. Pearson's correlations among HSP and climate data were determined.

Results and discussion
Climatic and physiological parameters
The variation of environmental (AT, THI and RH) and physiological (RT, RR and PR) parameters are given in the table 1. The average THI were 54.5, 70.1 and 82.6 in winter, spring and summer respectively.
Given that the THI during summer was higher than the optimal THI for thermal comfort of goat (THI< 70) (Silanikove, 2000) [8], therefore the experimental goats were subjected to heat stress. The physiological parameters are great heat induced stress indicators in goats. In the current study, the highest records of RR, RT and PR were observed in summer, while the lowest in winter. In summer, the overall means of RT (39.8 ± 0.4oC) were significantly (P<0.05) higher than spring (37.6 ± 0.2oC) and winter (36.0 ± 0.08oC). Regarding the RR, it was significantly lower in winter (55.6 ± 1.4 breaths/min) than spring (62.1±1.9 breaths/min) and summer (97.7 ± 3.0 breaths/min). The PR was significantly higher in summer (108.8 ± 1.5 bpm) followed by spring 96.8 ± 3.1 and winter 94.3 ± 3.0 bpm. However, a significant difference was not observed between Winter and spring for the PR. Several evidences demonstrated the harmful effects of environmental change such high ambient temperature and solar radiation associated with heat stress become a major factor negatively affecting domestic animals productivity. To cope this thermal uncomforting period animals develop some physiological responses which are exhibited through an increase of RR, RT and PR. The findings in the present study are consistence with previous study in the same area (Darcan *et al*., 2007; Darcan and Güney, 2008; Darcan *et al*., 2009, Kaliber *et al*., 2016) [3, 2]

HSP 60 and 70 concentration in serum
The values of HSP 60 and HSP 70 were significantly (P< 0.05) higher in summer than spring and winter (Table 3). The average HSP 60 significantly lower in winter (4.4 ± 0.5 ng/ml) followed by spring (7.6 ± 0.6 ng/ml) and summer (9.05±0.82 ng/ml). Whilst, the HSP 70 was 22.6 ± 0.6, 14.4 ± 0.5 and 13.3 ± 0.4 ng/ml in summer, spring and winter respectively.

Table 1: Values (SEM) climatic data and THI of each of the experimental

Seasons	Hours	Air temperature (°C)	Relative humidity (%)	THI
Spring	8:00- 9:00	$20.7^{A} \pm 0.2$	$73.1^{A} \pm 1.3$	70.097
	1:00- 2:00	$26.8^{B} \pm 0.3$	$46.3^{B} \pm 1.4$	
Summer	8:00- 9:00	$28.7^{A} \pm 0.1$	$73.4^{A} \pm 0.6$	82.65
	1:00- 2:00	$34.9^{B} \pm 0.1$	$46.3^{B} \pm 0.6$	
Winter	8:00- 9:00	$9.4^{A} \pm 0.2$	$64.3^{A} \pm 1.6$	54.47
	1:00- 2:00	$16.2^{B} \pm 0.3$	$52.6^{B} \pm 0.5$	

*,a,b significant at *P*<0.05, NS: Non-Significant at *P*<0.05

Table 2: physiological parameters in experimental goats

Seasons	Breeds	Hours	RT (oC)	PR (bpm)	RR breaths/min
Spring	Alpin	07:00-8:00	37.3 ±0.1b	91.3 ± 2.7a	56.6 ± 1.9a
		13:00-14:00	38.4 ± 0.3a	97.6 ± 3.2b	67.2 ± 2.4b
	Saanen	07:00-8:00	36.5 ± 0.1a	98.4 ± 2.5a	58.7 ± 1.6a
		13:00-14:00	38.1 ± 0.3b	99.9 ± 3.9b	65.9 ± 1.8b
Summer	Alpin	07:00-8:00	39.0 ± 0.1a	105.7 ± 1.6a	89.3 ± 2.7a
		13:00-14:00	40.6 ± 0.8b	113.8 ± 2.3b	106.9 ± 2.9b
	Saanen	07:00-8:00	38.6 ± 0.4a	103.9 ± 0.9a	80.7 ± 2.9a
		13:00-14:00	40.9± 0.1b	108.5 ± 1.04b	113.8 ± 3.4b
Winter	Alpin	07:00-8:00	35.7 ± 0.05b	89.3 ± 2.7a	55.82 ± 2.2a
		13:00-14:00	36.3 ± 0.06b	93.5 ± 2.9	56.3 ± 1.1b
	Saanen	07:00-8:00	35.9 ± 0.1a	95.9 ± 3.5a	51.9 ± 1.1a
		13:00-14:00	36.1 ± 0.1b	98.6 ± 3.0b	58.2 ± 1.1b
Sig.	Seasons	-	*	*	*
	Breeds	-	NS	NS	NS

*AT: Ambient Temperature, RH: Relative Humidity, THI: Thermal Index Humidity; RT: Rectal Temperature, PR: Pulse Rate, RR: Respiration Rate; *,a,b significant at P<0.05, NS: Non-Significant at P<0.05*

Table 3: Levels of HSP 60 and 70 in experimental goats

HSP	HSP60 (ng/ml			HSP 70 (ng/ml)		
Seasons	Spring	Summer	Winter	Spring	Summer	Winter
Alpin	6.5±0.43b	8.7±0.52b	4.4±0.42a	14.1±0.41b	18.34±0.47b	12.7±0.5a
Saanen	8.7±0.72a	9.4±1.12a	4.3±0.65b	14.7±0.61a	26.9±0.66a	13.9±0.36b

**,a,b significant at P<0.05, NS: Non-Significant at P<0.05*

Table 4: The correlation between level of HSP60 and HSP70 with physiological parameters

	Season	THI	RT (oC)	PR (bpm)	RR (breaths/min)
HSP 60	0.982**	0.886**	0.996*	0.514*	0.540*
HSP 70	0.991**	0.880**	0.985*	0.538*	0.539*

**,a,b significant at P<0.05, NS: Non-Significant atP<0.05*

As seen in the table 2,3 and 4 a positive and significant correlation was observed between HSP concentration, seasons, THI and physiological parameters. 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 cattle (Kishore *et al*., 2016; Archana *et al*., 2017) [6, 1]. The HSP70 has been suggested to function as an indicator of thermo-tolerance in cells (Archana *et al*., 2017) [1].

Summary
Standing on the findings of the current study we can conclude that, in Saanen and Alpine goats exposed to heat stress, the thermoregulatory adaption is exhibited through an increase rectal temperature, respiration and pulse rate. Further the higher levels of HSP 60 and 70 recorded can be used as thermoregulation indicators.

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