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The Change Level of Concentration of Hsp70 in the Different Age in the Goats in Various Seasons

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Abstract

Difference of ability of breed/animal at tolerance of heat stress, the HSP70 effective is one of the major as a cellular thermometer in response to heat stress. Even estimate the ability of a specifically breed/ animal for heat stress, it is pressing to determine its thermo tolerance ability. Heat shock proteins (HSPs) are molecular chaperons that preserve domestic conformation of proteins and cell viability in the stress condition. The peripheral blood mononuclear cells (PBMCs) ubiquitous have been used as a cellular model for explained the differential heat shock reactive toward livestock species. The current research was aimed with the pretension that seasonal difference cause the alterations in the level of concentration HSP70, on other hand investigation in change concentration level of HSP70 in the different (1year and 2 years) in the goats for Alpine and Saanen breeds. The average concentration of HSP70 was higher (26.9, 18.34 and 14.7) for Saanen breed in the spring, summer and the winter respectively as respect to Alpine breed. In compassion to goats in the age of 1year and goats in the age of 2years relative concentration level of HSP70 showed significant increase HSP70 in the age of 1 year and during different seasons (spring, summer and winter) with significantly ($p<0.01$).generally the study concluded, Saanen goat in the different age shown difference in their ability to ameliorate the mischievous effect of heat stress therefore as to preserve cellular integrity.

Keywords: HSP70; Saanen; Alpine; Season; Age; Stress

Introduction

Climate change is real issue of the major threats for survival of species, ecosystems and the sustainability of livestock species production systems across the world and Stress is an integral component of the forces that drive the course of evolution as changes in the physical environment generate stress, which in turn affects homeostasis. It was rapid environmental change that led to the formidable diversification of life on earth; also heat stress occurs in animals when there is an imbalance between heat production within the body and its dissipation Kumar *et al.*, (2010) [1]. Additional to that heat stress effects health of dairy animals by imposing direct or indirect or affects in normal physiology, metabolism, hormonal, and immunity system Das *et al.*, (2016) [2]. As well as that HSP70 has closely related with what occurring to cell during increased or decreased temperature Hsp70 has a significant role in cell thermo tolerance (Barbe *et al.* 1998) [3] and animal survival (Barbe *et al.* 1998; King *et al.* 2002) [3,4]. HSP transcription is increased by heat shock and other stress stimuli in bovine; Increase in HSP70 levels from cell cultures in presence of different stressors often does not reflect the physiological adaptability of animals governing thermal regulation. This study investigated of change concentration level of HSP70 in the female goats according to different age (1 year and 2 year) during variation seasons (spring, summer and winter) for Alpine breed, Saanen breed in tropical climate.

Material and Methods

120 cross-bred female goats (75% German Fawn+ 25% Hair) have been studied in the goats Research farm of Cukurova University, in Adana Province in the east Mediterranean region of Turkey. The characteristic of climate in Adana is altitude air temperature and humidity during the summer season. The average daily ambient temperature 34.9 °C, whereas the highest and lowest temperature were 42 °C and 28 °C in July respectively, while the average daily ambient temperature 9 °C, whereas the highest and lowest were temperature 15 °C and -1c in January. The samples of blood (15 ml) were collected from jugular vein into the EDTA coated vacationers in the spring, summer and winter seasons (months of May, July and January). The sampling execution was conducted as per the institute animal ethics commit guidelines in place.

Statistical analysis

HSPs concentration data were statistically analyzed separately following GLM procedures in the statistical analysis system (SAS V.2004). Differences were tested with Duncan s Multiple Range test at level of 5% or 1% the following mathematical models were used for HSPs concentration

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

Where, Y_{ijkl} : observed value.

μ: mean of population.

α_i: effect of breeds (1= Alpine, 2= Saanen)

β_j: effect of season (1= spring, 2= Summer and 3=winter)

y_k: age (1= 1 year and 2= 2year) or HSPs concentration (HSP70)

$(\alpha\beta)_{ij}$: interaction of breed and age

Elisa kit

Sensitivity: 0.47ng/ml for HSP70 (the sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by sub-tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration. CV<10%, Inter-Assay: CV<12% for HSP70.Assay range:0.5ng/ml-150ng/ml.

Assay Procedure

Standard dilution: this test kit was supplied one original standard reagent. It has been diluted it according to the instruction. Every sample was made according to required quantity. Only chromogenic solution A and B, and stop solution were allowed; other operation are the same).Final measurement: taken blank well as zero, measure the optical density (OD) under 450 nm wavelength which should be carried out within 15 min after adding the stop solution. According to standards, concentration and the corresponding OD values, calculate out the standard curve linear regression equation to calculate the corresponding samples concentration. It was acceptable to use kinds of software (Gen 5) to make calculations.

Results

To explain the relative among concentration level of HSP, breeds types and seasonal diversity, Concentration level of HSP data was produced in PBMs samples across Alpine goats and Saanen goats. The essential objective this scenario was to determine whether seasonal diversity effect concentration of HSP70 .Melt curve analysis of concentration of HSP70 with single peak for each breed indicated effective PCR amplification (R^2 ranged 0.9901 in the spring, 1 in the summer and 0.9998 in the winter, Figures 1,2,3 and 4).

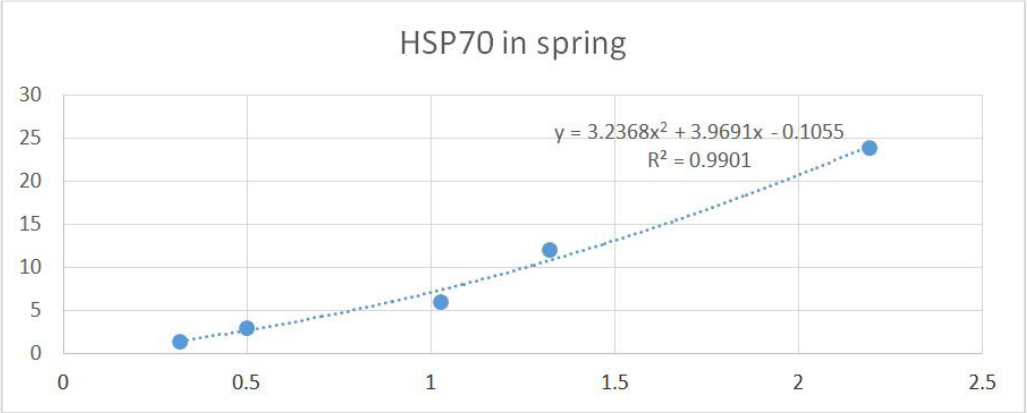

Figure 1: Level of concentration of HSP70 in the spring

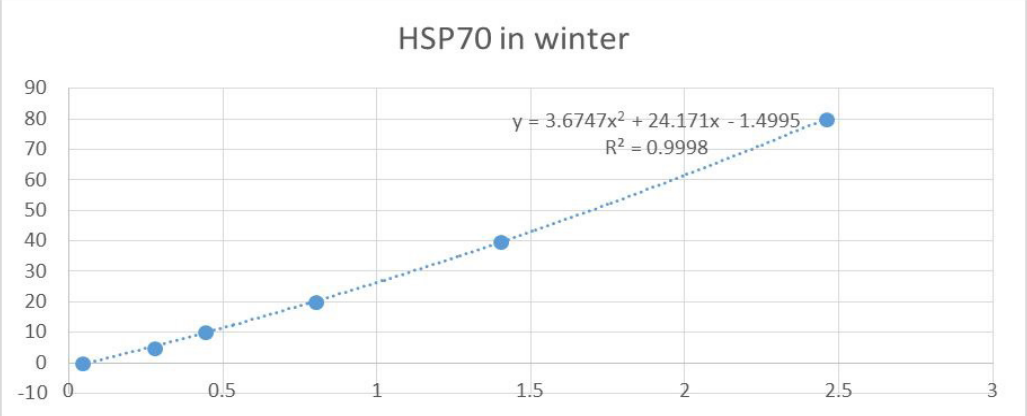

Figure 2: Level of concentration of HSP70 in the winter

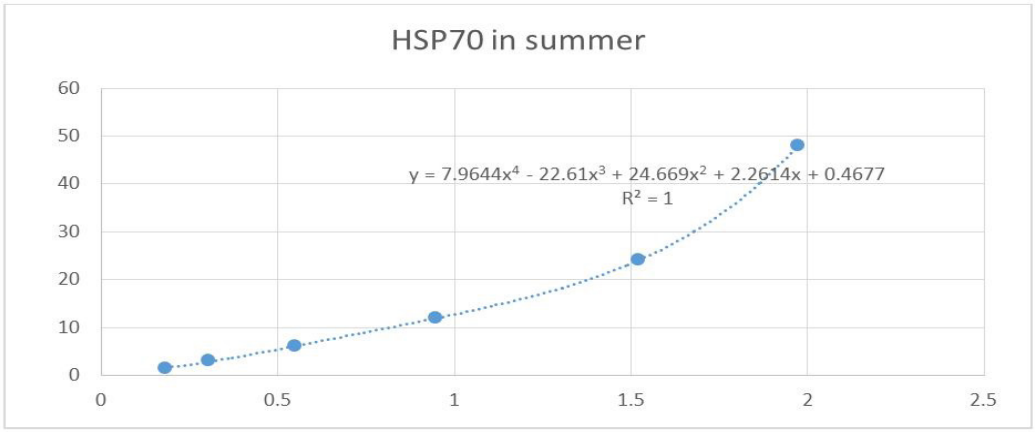

Figure 3: Level of concentration of HSP70 in the summer

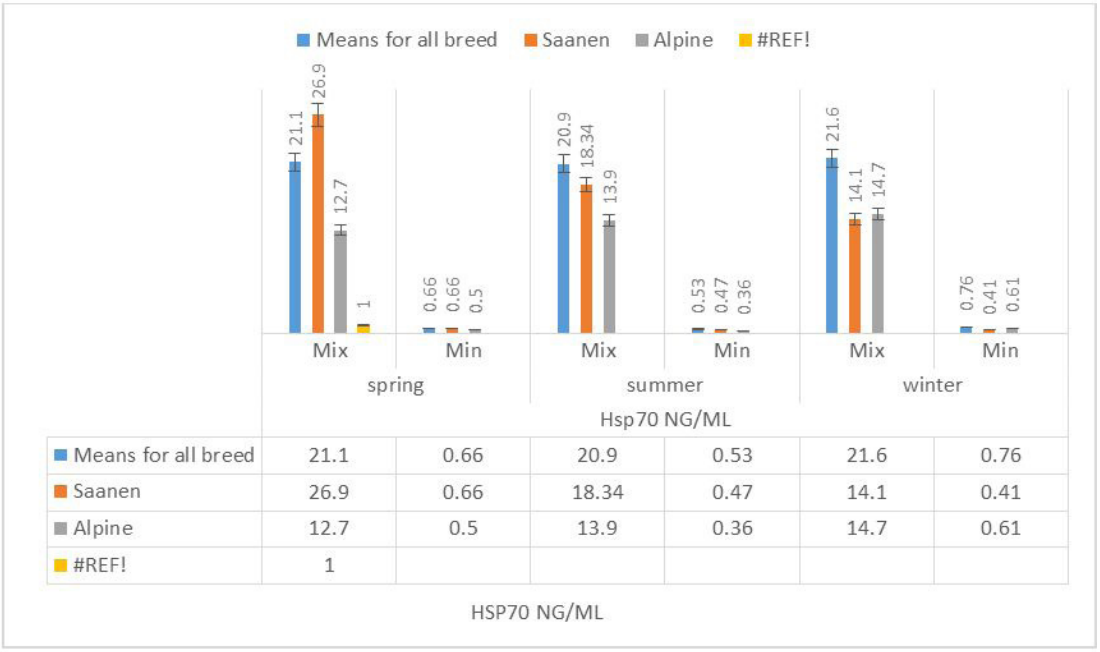

Figure 4: Level of concentration of HSP70 in the various seasons for Alpine and Saanen breed

The average concentration level of HSP70 with fold change of 21.1 in of both breed (Saanen and Alpine) (p=0.66) and fold change 26.9 in Saanen (p=0.66) in the spring and fold change 12.7 in Alpine (p=0.50) in the spring. its fold change was high in Alpine breed though the increase was significant (p<0.01) and among different dairy goat analyzed in the current study, Saanen goat displayed lower concentration level of HSP70with significant variance for of both age (1year and 2 years) of female goat (39.3(1year), 27.9(2years) in the spring) and 6.67 (1year), 6.35(2years) in the summer with respect to the winter (4.8 (1year), 2.9(2year)) in Saanen breed with respect to Alpine breed. Generally, as depicted Table 1. That the concentration level of HSP70 in the age of 2 years was higher as compared to concentration level of HSP70 in the age of 1 year. This result was agreement with the high degree of exposure of the Alpine breed to heat stress can be explained by the Alpine hair dye, which has a role in gaining summer temperatures and resistance in the winter. 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 Kishore *et al,* (2016) [5] while that Sodhi *et al.* (2013) [6] stated that the lower expression for HSP70 in cattle provided an evidence for its better cellular.

Breed	Age	Hsp70
Alpine	1	54.37±9.46[a]
Alpine	2	19.93±3.21[b]
Saanen	1	39.31±9.5[a]
Saanen	2	27.89±4.89[b]
Sig breed Age Breed x age	** * N.S	

Mean with the same latter are not significantly different p<0.01,**, high significant,* significant N.S, no significant

Table 1: Altering concentration of hsp70 according to age for Alpine and Saanen goats in the age of one and two in spring

Discussion

Lately much assertion has been given even understand the mechanism in molecular adaptation in goat breeds across seasonal variations particularly heat stress. It is now in fact well-setup fact that there is variability at breeds for tolerate the environmental heat burden. In our hand study investigation various gauge in dairy goat breeds acclimated to tropical and temperate climatic condition. So the present study proposed to estimate relative concentration level of hsp70 in circulating peripheral blood mononuclear cell small (PBMCs) of Alpine and Saanen goats during variation seasons and in the different of the age (1 year and 2years).

The PBMCs of peculiar Alpine reported higher in hsp concentration level as compared to acclimated Saanen breed during spring, summer and winter. Among seasons hsp70 gene investigated in the present study, hsp70 was found to be more concentration level in Alpine (Table 2).

Breed	Age	Hsp70
Alpine	1	22.04±8.34[a]
Alpine	2	19.13±10.43[b]
Saanen	1	6.67±1.29[a]
Saanen	2	6.35±1.19[b]
Sig breed Age Breed x age	N.S * N.S	

Mean with the same latter are not significantly different p<0.0001,*, significant, N.S, no significant
Table 2: Altering concentration of hsp70 according to age for Alpine and Saanen goats in the age of one and two in summer

The result was predicted as hsp70nconcentration level is considered to be more motivated in thermal stress due to stabilize the native conformation of portions and conservation of cell survivability (Beckham *et al.*, 2004) [7]. The results increase in concentration level according to different age hp70 was in accordance with Coa *et al* (2009) [8] added that increasing heat stress level led to increasing concentration of Hsp70 in the testis and epididymis of mice, and it was in accordance with our findings as seen in table 3. Above Asea AAA *et al.* (2018) [9] reported that amount of stress the expression of hsp70 varies with species, breed, age and type of tissue indicating the variations in the thermo tolerance.

Breed	Age	Hsp70
Alpine	1	5.59±0.75[a]
Alpine	2	3.25±0.86[a]
Saanen	1	4.8±0.7[b]
Saanen	2	2.99±0.74[b]
Sig breed Age Breed x age	* N.S N.S	

Mean with the same latter are not significantly different p<0.0001,*, significant, N.S, no significant
Table 3: Altering concentration of hsp70 according to age for Alpine and Saanen goats in the age of one and two in winter

Paul *et al.* (2015) [10] demonstrated across study of expression of TLRI-10 gene and hsp60 and hsp70 in PBMCs in variations seasons for three different age groups (group I, II and III in the age of 0-2, 2-5, and >5yrs respectively) for black Bengal goats, whereas stated that the mRNA expression of almost all the II TLR1-10, the mRNA expression of hsp60 in the winter 1.28, 0.8 and 1.02 times comparison with calibrator in the three age groups (0-2, 2-5 and more than 5 yrs) and the mRNA expression of hsp60 was higher (2.90, 4,6 and 6.6 times, respectively) than calibrator in the summer, and reported also that the mRNA expression in the winter was 1.5, 0.27 and 0.35 times compression with calibrator in three age groups (0-2, 2-5 and more than 5yrs respectively), as respect to the mRNA expression hsp70 was 2.6, 1.41 and 1.38 times more increased than calibrator in the summer and attributed that to short day length in the winter which causes decreased of immunity even reduced food availability and excessed thermoregulatory demands.

Dangi *et al.* (2012) [11] demonstrated that in tropical goats, the mRNA expression within the all age groups in winter and summer seasons was detected statistically non-significant (P> 0.05). In spite of that, during summer season, the mRNA expression within all age groups was detected importantly higher (P<0.05) I (1.06), II (1.32) and III (1.48) respectively than the corresponding values during winter season I (0.331), II (0.412) and III (0.436) respectively, In temperate goats, the mRNA expression between I (0.945), (0.998) and III (0.943), (1.33) age groups both in winter and summer season was detected statistically non-significant (P>0.05) while mRNA expression in I (0.998), (0.945) and III (1.33), (0.943) age groups during summer and winter seasons was found to be significantly higher (P<0.05) than II (0.137), (0.325) age group. Nevertheless, no important difference was found in the mRNA expression between summer and winter seasons in all the three age groups.

Conclusion

Concluded that the extent of HSPs concentration level and cell proliferation rate of PBMCs of Alpine goat, Saanen goat suggested difference in their ability to ameliorate the mischievous effect of heat stress therefore as to preserve cellular integrity. Causes: In addition Alpine goats with higher multitude of main hsp70 concentration during different seasons shown their lower tolerance to condition heat burden particularly in environment heat. despite heat shock response is an evolutionary maintained mechanism, various breeds as seen in the present scenario may be vary in their inherent ability to motivate hsp70 synthesis in the heat shock. Higher concentration level of HSPs and low cell proliferation rate in Alpin goats well be correlated to the higher stress condition of goats, needing more production of heat shock proteins to acclimate with the stressed condition.

Acknowledgement

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