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Association of Genetic Polymorphisms in *HSF1* and *HSPA6* Genes with Growth and Adaptation Traits in Barki Lambs

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ABSTRACT

Barki is a sheep breed that is well-adapted to the harsh environmental conditions in Egypt without compromising its reproduction and productivity. Further, it remarkably contributes to the livelihood of many breeders, particularly in the desert regions of Egypt. This study investigated potential polymorphisms in the *HSF1* and *HSPA6* genes and their impacts on growth, adaptation traits, and blood parameters in 194 Barki lambs. Growth traits included birth weight, weaning weight (WW), and weights at 6 (W6) and 9 (W9) months of age. Adaptation traits included respiration rate, heart rate, body temperature, rectal temperature, and hormonal and biochemical blood profiles. The PCR-Restricted Fragment Length Polymorphism technique was applied to detect the allelic and genotypic frequencies of *HSF1* and *HSPA6* genes using *SmaI* and *Cfr42I (SacII)* restriction enzymes, respectively. The *HSF1* exhibited two alleles (A and B) with only two genotypes, AB (0.46) and BB (0.52). However, the *HSPA6* gene showed a monomorphic pattern. Association analysis revealed a significant effect of the *HSF1* polymorphism on WW, W6, and W9. However, neither *HSPA6* nor *HSF1* polymorphisms significantly influenced adaptation traits or blood parameters. Lambs carrying AB genotypes had a relatively higher WW (16.14 kg) than BB lambs (13.7 kg). The fixed effect of lamb sex was significant on the weight at 9 months, with values of 34.43 kg for males and 28.17 kg for females. Male lambs were significantly heavier than females. Furthermore, lamb sex had a significant effect on respiratory rate (69.35 rpm for males and 60.48 for females), heart rate (87.48 rpm for males and 95.1 for females), urea (74 mg/dl for males and 41.21 mg/dl for females), aspartate aminotransferase (92.6 IU/L for males and 87.92 IU/L for females) and T_3 hormone (2.75 ng/ml for males and 2.29 ng/ml for females). These findings elucidated that the *HSF1* gene can be considered a functional candidate for improving growth performance in Barki lambs.

Keywords: Barki lamb, Genetic variation, Growth performance, Heat shock protein, Physiological parameter

INTRODUCTION

Global climate change has threatened the survival and lifestyles of various living organisms worldwide, particularly in arid and semi-arid regions (Das et al., 2016). Heat stress, a remarkable aspect of global climate change, adversely affects different biological processes in livestock, including growth performance, milk production, blood parameters, reproductive efficiency, and immunity, and can ultimately lead to mortality (Salles et al., 2016; Pragna et al., 2018).

Egypt, with approximately 94% of its land classified as desert, faces extreme challenges in food supply and water resources for both humans and livestock. The Barki sheep, predominantly found in the Egyptian desert, is one of the three major sheep breeds in Egypt (Sallam et al., 2019a). Research has shown that this breed is well adapted to such challenging environmental conditions without compromising its productivity (Abousoliman et al., 2020). The Egyptian Barki sheep breed has evolved to carry the genes of adaptation to harsh conditions, which may reinforce the efforts to mitigate the effects of heat stress on the animal's performance and survival. This can be achieved by implementing breeding programs that aim at selecting animals with greater resilience to heat stress (Baena et al., 2018).

Generally, livestock species can cope with heat stress through various mechanisms, including behavioral responses (Aleena et al., 2018), physiological responses (Kumar et al., 2018), alteration of blood biochemistry, and metabolic responses (Indu et al., 2015). Behavioral adaptation might include controlling feed and water intake, while physiological adaptation could include changes in some hormone levels, such as thyroid hormones. Fortunately, variations in adaptive capacity have been observed among individuals within different livestock species, suggesting that some animals possess a greater ability to adapt to extreme environmental conditions than others (Sejian et al., 2018).

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Marker-assisted selection in livestock has recently been made possible by the discovery of genetic markers and candidate genes linked to important economic traits, including growth performance and adaptation ability. Thus, selecting animals with high genetic potential for adaptation and high growth performance may help reduce the negative effects of heat stress. The selection of superior animals with higher heat stress tolerance requires exploring the genetic variability of the livestock exposed to hot environmental conditions to detect genes or genomic regions associated with adaptability traits (Guillermo et al., 2021).

Heat shock proteins (HSPs), also known as stress proteins, comprise a large group of proteins that are induced by various stressors, including heat stress, inflammation, diseases, and environmental stressors (Hoffmann et al., 2003; Roti Roti, 2008). Because they inhibit apoptosis, the increase of HSPs in damaged cells is crucial for maintaining viability and, thereby, influencing growth performance (Hecker and Mcgarvey, 2011). When activated, HSPs facilitate the production of new proteins to replace those affected by stress and assist in the refolding process of the damaged proteins (Deb et al., 2014). Three isoforms of HSPs, namely heat transcription factor 1 (*HSF1*), *HSF2*, and *HSF4*, have been identified in livestock species. *HSF1*, located on chromosome 9 of the ovine genome, is the main transcription factor that controls the response to heat shock (Westerheide and Morimoto, 2005). Additionally, *Heat Shock 70kDa Protein 6* (*HSPA6*), located on ovine chromosome 1, has been linked to heat tolerance in Angus cattle (Zimin et al., 2009; Baena et al., 2018). Furthermore, copy number variations in *HSF1* have been significantly associated with weight gain and body measurements in the Ashidan yaks (Ren et al., 2022). Moreover, *HSPA6* was found to be overexpressed in certain Indian goat breeds during the hot summer months (Banerjee et al., 2014).

The main objective of the present study was to investigate the genetic polymorphism of *HSF1* and *HSPA6* genes and their association with growth performance factors (birth weight, weaning weight, 6-month weight, and 9-month weight) as well as adaptation ability in Egyptian Barki lambs.

MATERIALS AND METHODS

Ethical approval

The experiment was performed according to all ethics and animal rights (Desert Research Center), considering all regulations in conformity with the European Union Directive for the Protection of Experimental Animals (2010/63/EU). All laboratory protocols were implemented, conforming to the ISO 9001:2015 quality regulations of DRC.

Animal resources and phenotypes

A population of 194 lambs (99 males and 95 females) from the Maryout Research Station (Latitude 31◦ 00’ N; Longitude 29◦ 47’ E), affiliated with the Desert Research Centre (DRC) in Egypt, were employed in this study. Lambs were housed in a semi-open yard with their dams from birth until weaning at 3 months of age. After weaning, all lambs were gradually separated from their dams. At 6 months of age, the male lambs were housed in a separate yard away from the females. All lambs were fed on their dams’ milk until weaning age, while they were fed a concentrate mixture and Egyptian clover hay according to the optimal feeding requirements thereafter. Fresh water was freely available to the lambs all day *ad libitum*.

Body weights at birth and weaning (3, 6, and 9 months of age) were recorded for each lamb using a digital balance. Adaptation measurements, including respiration rate (RR), heart rate (HR), body temperature (BT), and rectal temperature (RT), were measured in July between 13:00 and 15:00 under a temperature of 36°C and a humidity of 65%. Body and rectal temperatures (°C) were measured by an IR thermometer laser Class 2 (Cooper-ONDA 630-670 nm, USA). Respiration rate (rpm) was measured in respirations per minute (rpm) by counting flank movements per minute. Heart rate (bpm) was measured in beats per minute (bpm) by using a clinical stethoscope.

The Temperature-Humidity Index (THI) was calculated using the following equation (Amundson et al., 2006):

$$THI = 0.8\ T + RH\ (T-14.4) + 46.4$$

where T represents the ambient or dry-bulb temperature in °C, and RH is the relative humidity expressed as a proportion.

Blood biochemical and hormonal profile

Blood samples of all lambs were collected from the jugular vein in the plasma vacationer. Blood plasma was separated by centrifuging whole blood at 5000 g for 10 minutes and then was kept at −20°C until analyses. Plasma total proteins (TP, g/dL), albumin (ALB, g/dL), globulin (GLB, g/dL), glucose (GLU, mg/dL), cholesterol (CHO, mg/dL), triglycerides (TG, mg/dL), Urea (Urea, mg/dL), alanine aminotransferase (ALT, IU/L), and aspartate aminotransferase (AST, IU/L) were determined using colorimetric kits (Biomed, Egypt) as per the manufacturer’s instructions. Changes in plasma triiodothyronine (T_3, ng/mL) and thyroxine (T_4, ng/mL) profiles were analyzed using competitive solid phase enzyme immunoassay kits (Monobind, USA).

Blood samples, DNA extraction, and PCR conditions

Blood samples were collected from the jugular vein of each lamb using vacationer tubes containing EDTA-Na2 and stored immediately at -20°C until DNA extraction. DNA was extracted using a commercial kit (G-spin™ Total DNA Extraction kit; iNtRON Biotechnology, Korea) following the manufacturer's instructions. The samples were subjected to a polymerase chain reaction (PCR) to amplify the target regions of exon 9 on *HSF1* and exon 1 on *HSPA6* genes. The PCR assay was performed in a total volume of 12.5 µL containing 1 µL forward primer, 1 µL reverse primer, 1 µL DNA, 6.5 µL PCR red master mix, and 3 µL nuclease-free water using the following program: The denaturation step of 95ºC for 3 minutes was followed by 35 cycles at 94ºC for 40 seconds, annealing temperature according to each primer pair (Table 1) for 30 seconds, and extension of 72ºC for 50 seconds followed by a final extension of 72ºC for 5 minutes. PCR products were visualized on a 2% agarose gel. Gene-specific primers were designed using Primer3 software (v.0.4.0) according to the latest sheep genome information (Oar_V4.0).

Table 1. Primer sequences of *HSF1* and *HSPA6* genes*

Gene name	Primer sequencing	Product size (bp)	Annealing temperature	RFLP Enzyme
HSF1 exon 9	F: CCTGGTTCGTGTCAAGGAGG	229 bp	60.2	*SmaI*
	R: GCACTTCTCGGGAGCTGAG			
HSPA6 exon1	F: CCTTCTCAGACACGGAACGG	798 bp	60	*Cfr42I* (*SacII*)
	R: GGTCCGAGCACAGTTCTTCA			

*****Primer sequences were designed based on the targeted amplified gene regions from NCBI according to the reference genome *Ovis Aries* (Oar_V4.0)

Restriction fragment length polymorphism

The RFLP technique was applied to detect the genetic polymorphisms and the different genotypes of the studied genes. PCR products of *HSF1* and *HSPA6* genes were digested with *SmaI* and *Cfr42I* (*SacII*) fast digest restriction enzymes (Promega, Madison, USA), respectively. The PCR-RFLP reaction volume was 25 µl, consisting of 4 µl 10X digestion buffer, 1 µl restriction enzyme, and 12 µl H2O, in addition to 8 µl PCR product. All reactions were incubated at 37°C for 15-20 minutes. For genotype detection, 10 µl of each reaction was separated on a 2.5% agarose gel and visualized using a gel documentation system.

Statistical analysis

The statistical analysis was conducted using the general linear model (GLM) of the analysis of variance (ANOVA) in SPSS V20 (IBM, New York, NY, USA) to assess the association between *HSF1* genotypes and the studied traits. The model included the phenotypes, including growth and adaptation traits as the response variables, the fixed effects of genotype (2 levels), and the sex of the lamb (2 levels). A T-test was used to determine the significance between the two levels of each variable. A p-value < 0.05 was considered statistically significant.

RESULTS

Descriptive statistics of the phenotypic data

An overview of descriptive statistics for growth traits, including birth weight, weaning weight at 3 months, and weights at 6 and 9 months, is presented in Table 2.

Adaptation traits, including RR, HR, BT, RT, blood biochemical parameters (glucose, cholesterol, total proteins, albumin, globulin, triglycerides, Urea, alanine aminotransferase, and aspartate aminotransferase), hormonal profile of triiodothyronine and thyroxine are summarized in Table 3. The Temperature-Humidity Index (THI) was calculated to be 86.34, indicating that the lambs were exposed to moderate heat stress (THI: 80-89), which is below the threshold for severe heat stress (THI ≥ 90).

Correlation coefficients among growth and adaptation traits are presented in Table 4. A significant positive correlation was observed among growth performance traits. Highly significant positive correlation coefficients were noticed among body weight at different ages. Birth weight showed a significant positive correlation with weaning weight, 6-month weight, 9-month weight, glucose, albumin, and urea, with values of 0.34, 0.33, 0.29, 0.25, 0.23, and 0.27, respectively. Likewise, weaning weight was positively correlated with 6-month weight (0.85), 9-month weight (0.65), urea (0.31), and body temperature (0.27). Furthermore, a positive significant correlation was found among some adaptation traits such as glucose, total protein, ALT, AST, and cholesterol. In contrast, significant negative correlations were detected between certain traits, such as RR and 9-month weight, urea, and ALT, with r^2 values of -0.22, -0.34, and -0.21, respectively.

Table 2. Descriptive statistics of lambs' body weights at different ages

Trait	Mean	SE	SD	Min	Max
B.W (kg)	3.88	0.06	0.55	3.00	5.50
WW 3 months (kg)	14.81	0.33	3.24	8.00	25.00
6 months Weight (kg)	23.72	0.50	4.82	10.00	39.50
9 months Weight(kg)	31.63	0.65	6.28	13.00	48.00

N: Number of animals, SE: Standard error, SD: Standard deviation, Min: Minimum, Max: Maximum.

Table 3. Adaptation measurements, blood biochemical parameters, and hormonal profile of lambs

Trait	Mean	SE	SD	Min	Max
Physiological parameters					
RR (rpm)	65.38	1.61	15.64	26.00	98.00
HR (bpm)	90.88	0.92	8.93	70.00	110.00
BT (^{o}C)	37.11	0.08	0.76	34.80	40.00
RT (^{o}C)	40.54	0.03	0.33	39.80	42.00
Biochemical parameters					
GLU (mg/dL)	78.52	1.73	16.77	43.99	121.03
CHO (mg/dL)	111.05	1.94	18.79	70.42	170.14
TP (g/dL)	5.81	0.05	0.49	4.99	7.15
ALB (g/dL)	2.60	0.02	0.19	2.06	3.46
GLB (g/dL)	3.21	0.05	0.45	2.07	4.34
TG (mg/dL)	114.43	2.33	22.58	84.40	200.61
Urea (mg/dL)	59.35	2.40	23.30	25.73	135.13
AST (IU/L)	236.00	2.12	20.57	198.94	294.75
ALT (IU/L)	90.51	0.71	6.87	72.00	109.23
Hormonal profile					
T_3 (ng/mL)	2.55	0.08	0.74	1.13	6.21
T_4 (ng/mL)	9.73	0.20	1.89	6.42	14.94

RR: Respiration rate, HR: Heart rate, BT: Body temperature, RT: Rectal temperature, T_3: Triiodothyronine, T_4: Tetraiodothyronine, GLU: Glucose, CHO: Cholesterol, TP: Total protein, ALB: Albumin, GLB: Globulin, TG: Triglycerides, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, N: Number of animals, SE: Standard error, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 4. Spearman correlation coefficients among growth and adaptation traits in Barki lambs

Trait	BW	WW	6M W	9M W	GLU	CHO	TP	ALB	GLB	TG	UR	ALT	AST	T3	T4	RR	HR	BT	RT
BW	1	0.34**	0.33**	0.29**	0.25*	-0.09	0.12	0.23*	0.03	0.16	0.27**	0.01	0.08	0.17	-0.03	0.09	-0.11	0.20	0.04
WW		1	0.85**	0.65**	0.07	-0.11	0.09	0.04	0.07	0.01	0.31**	-0.09	0.09	0.10	0.03	0.01	-0.02	0.27**	0.12
6 M W			1	0.87**	0.10	-0.05	0.07	0.05	0.05	0.06	0.42**	-0.06	0.11	0.14	0.01	0.07	-0.10	0.31**	0.15
9 M W				1	0.13	-0.01	0.04	-0.05	0.07	0.05	0.46**	0.04	0.20	0.16	-0.07	0.14	-0.22*	0.25*	0.05
GLU					1	0.19	0.34**	0.19	0.30**	0.29**	0.19	0.19	0.37**	0.21*	0.07	0.02	0.02	0.22*	0.04
CHO						1	0.36**	0.09	0.35**	0.34**	-0.08	0.26*	0.23*	0.12	-0.03	0.03	0.01	0.03	0.06
TP							1	0.41**	0.92**	0.42**	0.20	0.45**	0.40**	-0.01	0.09	0.06	0.01	0.13	0.08
ALB								1	0.02	0.32**	0.16	0.31**	0.27**	-0.08	0.15	0.02	-0.08	0.08	0.14
GLB									1	0.32**	0.15	0.36**	0.33**	0.02	0.03	0.06	0.04	0.11	0.03
TG										1	0.21*	0.43**	0.46**	0.15	-0.04	0.15	-0.09	0.05	0.19
UREA											1	0.28**	0.46**	0.19	-0.02	0.12	-0.34**	0.25*	0.09
ALT												1	0.50**	0.01	-0.11	0.12	-0.21*	0.21*	0.24*
AST													1	0.16	0.02	0.20	0.19	0.16	0.03
T_3														1	0.15	0.1	-0.04	0.15	0.04
T_4															1	0.01	-0.07	0.06	0.11
RR																1	-0.05	0.07	0.13
HR																	1	0.08	-0.01
BT																		1	0.03
RT																			1

BW: Birth weight, WW: Weaning weight, 6MW: Weight at 6 months, 9MW: Weight at 9 months, RR: Respiration rate, HR: Heart rate, BT: Body temperature, RT: Rectal temperature, T3: Triiodothyronine, T4: Tetraiodothyronine, GLU: Glucose, CHO: Cholesterol, TP: Total protein, ALB: Albumin, GLB: Globulin, TG: Triglycerides, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, * Significance level of P-value < 0.05, ** Significance level of P-value < 0.01.

Genetic characterization, RFLP analysis of *HSF1* and *HSPA6* genes

The Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RFLP) technique was used to detect the allelic and genotypic frequencies of *HSF1* and *HSPA6* genes. Two alleles (A and B) were determined for the *HSF1* gene, with values of 0.28 and 0.72, respectively (Table 5). Figure 1 shows the different genotypes of the *HSF1* gene using the *SmaI* enzyme. Only two genotypes of AB (at 229, 182, and 47 bp) and BB (at 182 and 47 bp) were detected with percentage frequencies of 0.46 and 0.52, respectively.

Table 5 shows only one allele C of the *HSPA6* gene with a frequency value of 1. Using *Cfr42I* (*SacII*) FastDigest restriction enzymes, Figure 2 shows only the CC (at 568 and 230 bp) genotype for the *HSPA6* gene without the presence of any genetic polymorphism at the selected amplicon.

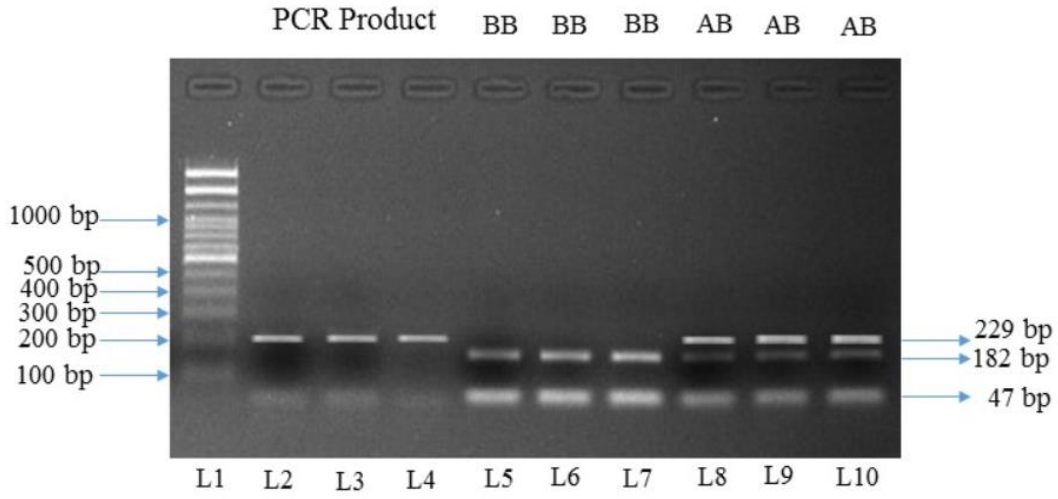

Figure 1. PCR-RFLP patterns of the Ovine *HSF1* gene in Barki lambs represent different genotypes. Lane 1 represents the DNA marker, Lanes 2-4 represent the PCR product, Lanes 5-7 represent the BB genotype, and Lanes 8-10 represent the AB genotype.

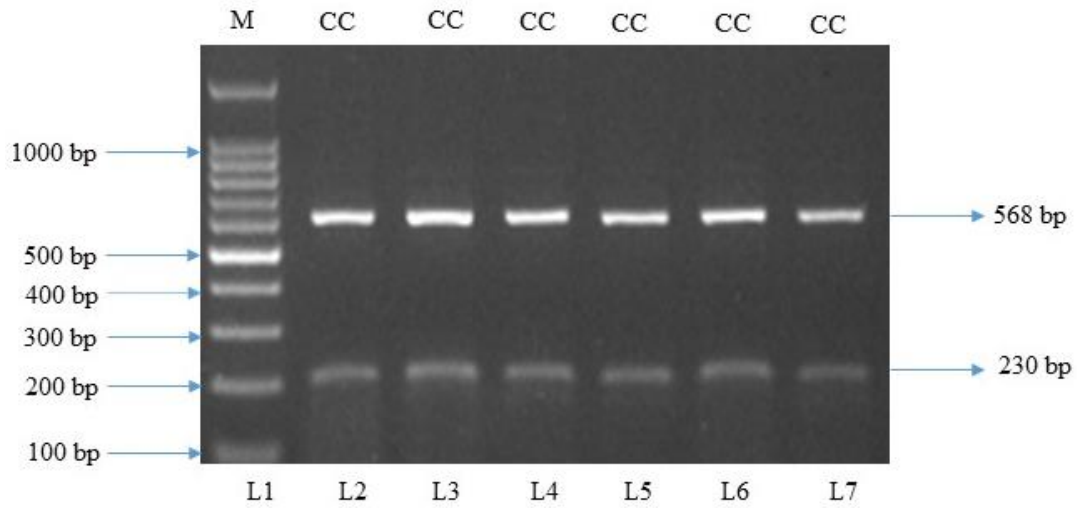

Figure 2. PCR-RFLP patterns of the Ovine *HSPA6* gene in Barki lambs. Lane 1 represents the DNA marker, and lanes 2-7 represent the CC genotype.

Effect of *HSF1* and *HSPA6* genotypes and lambs' sex on growth and adaptation traits

Table 6 presents the effects of *HSF1* genotypes and the lambs' sex on growth traits in terms of body weight at different ages. Association analyses among the phenotypes of growth traits and the different genotypes revealed a highly significant ($p < 0.05$) effect of the genetic polymorphism of the *HSF1* gene on weaning weight at 3, 6, and 9-month weights. Lambs carrying the AB genotypes had a relatively higher weaning weight (16.14 kg) than those with the BB genotype (13.7 kg). Additionally, lambs' sex was significantly associated with 9-month weight, with males (34.43 ± 5.32 kg) being heavier than females (28.17 ± 5.66 kg).

The effects of *HSF1* genotypes and the lambs' sex on adaptation traits and plasma blood parameters are presented in Table 7. Association analysis between the phenotypes of adaptation traits and the different genotypes did not show any significant ($p \geq 0.05$) effect. The sex of the lamb had a significant ($p < 0.05$) effect on RR, which was higher in

males (69.35 ± 18.12 rpm) than in females (60.48 ± 10.08 rpm). Conversely, HR was significantly higher in females (95.10 ± 7.19 bpm) than in males (87.48 ± 8.80 bpm, $p < 0.05$). Additionally, the plasma urea, AST, and T_3 concentrations were affected by the sex of the lamb, in which case higher concentrations were recorded in males (241.03 ± 21.39 mg/dL, 92.60 ± 6.68 IU/L and 2.75 ± 0.88 ng/mL, respectively) than in females (41.21 ± 12.21 mg/dL, 87.92 ± 6.25 IU/L and 2.29 ± 0.41 ng/mL, respectively).

Table 5. Allelic and genotypic frequencies of *HSF1* and *HSPA6* genes

Gene	Alleles	Allelic frequency	Genotypes	Genotypic frequency
HSF1	A	0.28	AA	--
	B	0.72	AB	0.46
			BB	0.52
HSPA6	C	1.00	CC	1.00

Table 6. Growth traits in relation to *HSF1* genotypes and lambs' sex

Trait	Genotype		P value	Sex		P value
	AB	BB		Males	Females	
BW	3.99 ± 0.60	3.79 ± 0.50	0.493 (N.S)	3.98 ± 0.56	3.76 ± 0.51	0.277 (N.S)
WW 3 months	16.14 ± 3.25	13.70 ± 2.80	0.000 (**)	15.10 ± 3.48	14.46 ± 2.91	0.107 (N.S)
6 months	25.97 ± 4.46	21.82 ± 4.30	0.000 (**)	24.77 ± 4.79	22.42 ± 4.57	0.745 (N.S)
9 months	35.24 ± 5.07	28.59 ± 5.59	0.001 (**)	34.43 ± 5.32	28.17 ± 5.66	0.019 (*)

BW: Birth weight, WW: Weaning weight, N.S: Not significant, * Significance level of P-value < 0.05, ** Significance level of P-value < 0.01.

Table 7. Adaptation traits in relation to *HSF1* genotypes and lambs' sex

Trait	Genotype		P value	Sex		P value
	AB	BB		Males	Females	
RR	67.51 ± 17.71	63.59 ± 13.57	0.566 (N.S)	69.35 ± 18.12	60.48 ± 10.08	0.012(*)
HR	89.33 ± 10.12	92.20 ± 7.64	0.163 (N.S)	87.48 ± 8.80	95.10 ± 7.19	0.000 (**)
BT	37.19 ± 0.83	37.05 ± 0.70	0.865 (N.S)	37.27 ± 0.78	36.92 ± 0.70	0.092 (N.S)
RT	40.57 ± 0.31	40.51 ± 0.35	0.562 (N.S)	40.54 ± 0.34	40.53 ± 0.34	0.934 (N.S)
GLU	80.30 ± 15.80	77.30 ± 17.59	0.679 (N.S)	80.39 ± 17.25	76.22 ± 16.05	0.527 (N.S)
CHO	112.54 ± 22.14	109.78 ± 15.53	0.892 (N.S)	111.70 ± 21.51	110.24 ± 14.96	0.699 (N.S)
TP	5.86 ± 0.49	5.77 ± 0.50	0.695 (N.S)	5.81 ± 0.50	5.81 ± 0.49	0.962 (N.S)
ALB	2.58 ± 0.18	2.62 ± 0.20	0.085 (N.S)	2.59 ± 0.19	2.61 ± 0.19	0.266 (N.S)
GLB	3.27 ± 0.44	3.15 ± 0.46	0.259 (N.S)	3.21 ± 0.47	3.20 ± 0.43	0.674 (N.S)
TG	115.44 ± 21.62	113.58 ± 23.53	0.797 (N.S)	115.61 ± 21.27	112.97 ± 24.28	0.472 (N.S)
Urea	71.11 ± 16.52	49.43 ± 23.68	0.180 (N.S)	74.00 ± 19.49	41.21 ± 12.21	0.000 (**)
ALT	241.81 ± 20.31	231.10 ± 19.67	0.423 (N.S)	241.03 ± 21.39	229.77 ± 17.84	0.071 (N.S)
AST	92.67 ± 6.38	88.69 ± 6.80	0.384 (N.S)	92.60 ± 6.68	87.92 ± 6.25	0.024 (*)
T_3	2.63 ± 0.86	2.48 ± 0.63	0.784 (N.S)	2.75 ± 0.88	2.29 ± 0.41	0.021 (*)
T_4	9.52 ± 2.04	9.91 ± 1.76	0.528 (N.S)	9.51 ± 2.00	10.02 ± 1.74	0.618 (N.S)

RR: Respiration rate, HR: Heart rate, BT: Body temperature, RT: Rectal temperature, T3: Triiodothyronine, T4: Tetraiodothyronine, GLU: Glucose, CHO: Cholesterol, TP: Total protein, ALB: Albumin, GLB: Globulin, TG: Triglycerides, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, N.S: Not significant, * Significance level of P-value < 0.05, ** Significance level of P-value < 0.01.

DISCUSSION

Recent climate changes have increased awareness of their impact on all living organisms, including livestock. The current study examined the impact of heat stress, as a representative of climate change, on growth and adaptive traits in Barki sheep, which are well-adapted to high temperatures. Results also explored variations in the *HSP1* and *HSPA6* genes. In general, the average estimates of BW (3.88 kg) and WW (14.8 kg) in this study were consistent with those previously reported on Barki lambs (Sallam et al., 2019b; Abousoliman et al., 2021), however, higher than those reviewed in earlier studies on the same breed (Elshazly and Youngs, 2019). Similarly, the average body weight at 6

months (23.7 kg) and 9 months (31.6 kg) aligned with previous findings on Barki sheep (Sallam et al., 2019a); however, were lower than those reported in another study (Ibrahim et al., 2023a).

The results for adaptation traits, comprising the physiological parameters, blood biochemical parameters, and hormonal profiles, showed higher values than those reported earlier under normal environmental conditions without any exposure to heat stress. This reflects the effect of higher temperatures on these traits (Marai et al., 2007). When animals are exposed to high ambient temperatures, they trigger some physiological responses such as increasing RR, BT, HR, and blood biochemical components to mitigate the effect of heat stress and to maintain the thermal equilibrium of their bodies (Silanikove, 2000; Al-Tamimi, 2007; Mcmanus et al., 2009).

Correlation coefficients among growth and adaptation traits showed some positive medium values between growth traits and some biochemical parameters, including glucose, albumin, and urea, as well as negative low values between growth traits and certain physiological parameters, such as body and rectal temperatures, respiratory rate, and heart rate. Likewise, a significant positive correlation was found among blood parameters such as glucose, total protein, ALT, and AST. These results confirm the negative effect of heat stress on the metabolism of the animal in terms of consuming more energy to mitigate that effect. In contrast, other traits showed significant negative correlations. For example, among RR and 9-month weight, urea and ALT, with r^2 values of -0.22. The findings of the present study align with previous studies indicating the adverse effects of heat stress on biological processes and the animals' metabolism to cope with the effect (Rawash et al., 2022). Furthermore, it may reflect the animal's potential to respond to heat stress in different ways (Sejian et al., 2018).

The transcription of heat shock proteins belonging to various families, including *HSP27*, *HSP60*, *HSP70*, *HSP90*, and *HSP110/104*, plays a critical role in regulating heat stress (Kregel, 2002). This study examined the various genotypes of the *HSF1* and *HSPA6* genes and their associations with growth and adaptation traits in a population of Barki sheep lambs using the PCR-RFLP technique.

The results of the present study showed a high phenotypic variation in the studied traits. Nonetheless, no genotypic variation was detected in the investigated region of the *HSPA6* gene, which was monomorphic in the studied animals. This may be attributed to high levels of inbreeding, leading to increased homozygosity in the population (Ibrahim et al., 2023a). Notably, livestock's ability to respond to environmental changes depends largely on genetic richness, diversity, and variations (Sallam et al., 2012). Therefore, examining additional gene regions with larger sample sizes may help identify *HSPA6* polymorphisms in Barki sheep (Osman et al., 2021). Interestingly, the *HSF1* gene exhibited the least variation (2 SNPs) among all investigated thermos-regulation genes in the Chinese Holstein cattle (Li et al., 2011). Moreover, one of these SNPs did not affect the thermal tolerance traits, which aligns with the results of the present study. On the contrary, a higher number of polymorphic variants was reported for *HSPA6* and *HSF1* genes in Barki and Aboudeleik sheep and Angus cattle (Baena et al., 2018; Ibrahim et al., 2023b). Association analysis revealed that the *HSF1* gene was significantly associated with WW, 6-month weight, and 9-month weight. This result may suggest that *HSF1* is a potential candidate gene for growth performance in sheep. These findings are in agreement with those previously reported in Barki lambs for the same studied traits (Ibrahim et al., 2023b). The HSF1 gene, highly conserved in eukaryotes, primarily mediates the transcription response to protein toxic stress (Vihervaara and Sistonen, 2014) and regulates protein metabolism (Douglas et al., 2015). Compared to other genes with lesser activity, the *HSF1* gene is more protective against heat stress (Sonna et al., 2002; Dai et al., 2007). The *HSF1* gene has been reported to control heat shock responses in farm animals, with significant associations between its genetic polymorphism and heat tolerance in Chinese cattle (Ellis and Van Der Vies, 1991; Rong et al., 2019). However, association analysis showed no significant effect of *HSF1* genotypes on any of the adaptation traits, contrasting with previous findings on Barki lambs that reported significant associations between genetic polymorphism of the *HSF1* gene and skin temperature, rectal temperature, and respiratory rate (Ibrahim et al., 2023b). Investigating the impact of the *HSF1* gene on different adaptation traits may require examining additional variations at different gene loci under various environmental conditions and with larger animal populations.

The current results revealed that heart rate and some biochemical blood parameters (e.g., urea, T_3, and AST) were significantly affected by the sex of the lamb. These results align with those noted previously in the Brazilian sheep (Mcmanus et al., 2009). The higher HR observed in males can be explained by their greater body weight at 9 months of age, as heavier animals require increased blood circulation to meet their nutritional and metabolic demands (Pesántez-Pacheco et al., 2019). Moreover, the elevated RR may serve as a primary mechanism for the maintenance of sheep body temperature under heat stress conditions. Generally, the heart rate values found in this investigation are within the range of recently published data on sheep (Sleiman and Saab, 1995).

The findings of the current study indicated higher urea concentrations in male lambs, consistent with previous findings that male lambs exhibit significantly higher urea levels than females. This is likely due to enhanced protein metabolism, which positively influences body weight in males (Teleb et al., 2009; Karaşahin et al., 2022). Overall, the

urea concentrations observed in this study were within the normal range for sheep, as previously reported (Abutarbush, 2010). Similarly, T3 concentrations were higher in male lambs, potentially due to their increased body weight (Table 6). Thyroid hormones are key regulators of growth, with their levels closely linked to body weight gain during the growing period (Karaşahin et al., 2022). Since they enhance the metabolic rate, glucose availability to cells, protein synthesis, and lipid metabolism, as well as stimulate cardiac functions (Capen and Martin, 1989; Todini, 2007). Additionally, the overall means of plasma aspartate aminotransferase (AST) concentrations were found to be higher in male lambs. AST and ALT enzyme activities are generally affected by various factors, such as feed intake, season of the year (Westerbacka et al., 2005), and heat stress (Čukić et al., 2023). Due to rapid gluconeogenesis and protein metabolism linked to growth, male lambs' elevated AST levels may be a sign of impairment in certain muscle and liver cells (Krebs et al., 1967). This study highlights, for the first time, a new putative candidate gene (*HSF1*) that may play a role in Barki sheep survival under heat-stress conditions.

CONCLUSION

Improving complex traits in sheep using genetics is being implemented in different sheep populations. In this study, genetic polymorphism within *HSF1* and *HSPA6* genes, as well as their effect on growth, blood parameters, and adaptation traits, were investigated in Barki lambs. The results revealed significant associations between *HSF1* gene genotypes and weaning weight, 6-month weight, and 9-month weight, while the *HSPA6* gene was found to be monomorphic. Moreover, the sex of lambs significantly influenced 9-month weight, respiratory rate, heart rate, urea, AST, and T_3 hormone in the studied Barki lambs. These results suggest that the *HSF1* gene could serve as a candidate marker for improving growth traits in the Barki sheep breed under tough environmental conditions. Investigating the genetic potential of the Egyptian Barki sheep breed is crucial for planning selection schemes and breeding programs in the future.

DECLARATIONS

Availability of data and materials

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

Author contributions

Ibrahim Abousoliman and Ibrahim Samir Abd El-hamid conducted the field experiment and the laboratory analyses. Ibrahim Abousoliman and Ahmed Mohamed Sallam analyzed the data. Ibrahim Abousoliman wrote the first draft of the manuscript. Ibrahim Abousoliman and Ahmed Mohamed Sallam secured funding for the study. All authors reviewed and approved the final version of the manuscript.

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Competing interests

The authors declare no conflicts of interest.

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Ethical considerations

Authors are responsible for ensuring originality, denial of publication permissions, maintaining high ethical standards, and avoiding fabrication of data, falsification, plagiarism, or improper publication.

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