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COMPARISON OF HEAT SHOCK PROTEIN GENE (*HSP70-1*) SEQUENCE IN ARADI AND DAMASCUS GOAT BREEDS (*Capra hircus*) RAISED UNDER HEAT STRESS CONDITIONS

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KEYWORDS

Goat

HSP70-1

DNA – Sequence

Aradi

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ABSTRACT

Ten animals from two goat breeds (Aradi and Damascus) raised under heat stress conditions in Saudi Arabia were used in this study to compare the sequence of Heat Shock Proteins-70 gene (*HSP70-1*) between Aradi and Damascus goats with the reported one of Yunnan black goat (*Capra hircus)*. From the sequence of the above mentioned three breeds Aradi, Damascus and Yunnan, it could be identify that, Damascus goats are more mutant to *HSP70-1* gene sequence than other breeds. The results of study also showed an addition of A, T and G nucleotides in positions 935 (AGAAAGGCTC), 984 (GGTTCCTGGT) and 997 (GGGGGGGCTC) in Damascus Goats. However, Aradi goats did not differ in these positions from Yunnan black goats (AGAAAGGCTC, GGTTCCTGGT and GGGGGGGCTC). It could be concluded that HSP70-1 gene sequence varied between Aradi and Damascus breeds and Damascus breed showed more mutation in DNA sequence in 3 positions, Aradi breed did not show any variation in relation to that published for *Capra hircus.*

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1 Introduction

In response to environmental stressors, farm animals generate certain reactions at cellular level, like heat stress response, in which the cell produce a series of proteins called heat shock proteins (HSP).Heat shock proteins are family of proteins that are classified based on their molecular weight including the 70-kilodalton heat shock proteins (HSP70s). The HSP70s play a crucial role in the cell's machinery for protein folding and cells stress protection. Specifically, HSP70 assists the folding of newly formed polypeptide chains, acts as a molecular chaperone and mediate the repair and degradation of altered or denatured proteins. Heat stress in mammals induces cellular changes in gene expression and in the activity of expressed proteins, resulting in cell stress response (Lindquist, 1986; Jaattela, 1999). According to Luengrattana et al. (2000), one of the regions containing two tandem arrays HSP70 sequences was specified as HSP70-1 and HSP70-2. The other two regions containing a single HSP70 sequences were specified as HSP70-3 and HSP70-4. The unrestrained HSP70 expression is principally a result of the transcription of the HSP70-1 locus (Christians et al., 1997). Further, Ramunno et al. (2005) stated that characterization of HSP70-1 locus is playing an important role to pediment phylogenic relationship among specific species. Accordingly, to increase our knowledge about HSP70 gene and to provide some helpful database for goat breeding, sequence determination and characterization of goat HSP70-1 gene is important and targeted in the current study. Aradi goat breed is one of the most important goat breeds in Saudi Arabia, it is well-adapted to local environment and has been crossed with imported breeds like Damascus (from Syria) to improve its production and reproduction. It is well known that, genetic improvement of any livestock breed depends on the identification of animals that are capable of transmitting their desirable characteristics to their offspring. This study describes the results of comparison of the single HSP70 sequence (HSP 70-1) of Aradi and Damascus goat with reference to Yunnan black goat (Gade et al., 2010 and Dong et al., 2013) as a method of evaluation.

2 Material and Methods

This study carried out at biotechnology Laboratory, Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia. Five animals from each breed (Aradi and Damascus), raised in animal experimental farm of Qassim University used in this study. DNA of these two breeds was taken from the whole fresh blood samples using EDTA as anticoagulant. Blood samples were kept in ice box until reaching the laboratory within 2 hours and then the DNA extraction began. DNA from whole blood sample isolated using ILLUSTRA blood mini spin kit (GE Life Sciences, USA). Then DNA samples were checked for quantity and quality using Thermo Scientific™ NanoDropUSA). Primers used to amplify the selected gene (Table 1) taken according to Gade et al. (2010)

Table 1 Primers used in the present study

References	Name	Sequence
Gade et al., 2010	HSP70-1-F	5_ATGGCGAAAAACATGGCTATC-3
	HSP70-1-R	5_CTAATCCACCTCCTCAAT-3

2.1 Herd nutrition and management

Animal used in this study were housed in semi-shaded/open front barn. Goats were fed on a commercial pre-formulated total mixed ration (TMR, Alwafi-ARASCO-KSA). According to the manufacturer's specifications, the TMR consisted of alfalfa hay, barley, corn, wheat bran, soybean meal and crust, molasses, vitamins and minerals; and contained on DM basis 13% crude protein, 2% ether extract, 9% crude fiber, 8% ash, 1% calcium, 0.5% phosphorus, 0.7% sodium chloride, and 2.95 Mcal/kg digestible energy. The average high temperature through summer months when samples were collected was 46^{o}C and 43^{o}C, for August and September, respectively. The average low temperatures through these two months were 29^{o}C and 26^{o}C, for August and September, respectively.

2.2 Sequencing Protocol for required genes

The reaction mixtures were prepared according to Thermo Scientific protocol. Briefly, the terminator ready reaction consisted of (8.0 µL), template (20 µg), primer (3.2 pmol), deionized water to volume (20 µL) the mixture was then mixed well and spanned briefly, according to amplification conditions presented in table (2). Amplification through Veriti® Thermal Cycler Protocol used for Purification using sequential addition of

Table 2 PCR amplification conditions for used primers

PCR Product	Amplification conditions					
	Pre-denature	Cycles of reaction	Denature	Annealing	Extension	Final extension
HSP70 (HSP)	94^{o}C, 3min	35	94°C, 1min	49°C, 45sec	72°C, 2.20min	72°C, 10min

the BigDyeXTerminator Purification Kit reagents and ran in 3500 DNA Analyzer. Data were analyzed using Applied Biosystems DNA Sequencing Analysis Software Version 5.1.

3 Results

From the below sequence of the two breeds Aradi and Damascus the following differences could be specified, three nucleotides A, T and G were identified to the sequence of Damascus goat in positions 935, 984 and 996, respectively (Figure 1) but not appeared in Aradi sequence (Figure 2).

As presented in Figures 1 and 2 for Damascus and Aradi breeds, it can be deduced that the two breeds did not show any differences in either forward or reverse strand except in positions from 935 to 996. Moreover, no differences could be found between Aradi breed HSP70.1 sequence compared with that reported in Yunnan breed (Dong et al., 2013) and Yunnan black breed (Gade et al., 2010).

4 Discussion and Conclusions

The current study was aimed to compare the single *HSP*70 sequence of Aradi and Damascus goats with that published for Yunnan black and Yunnan goat. HSPs expression has been widely used to characterize the cellular response to heat stress. The sequences of HSP70-1 gene in small ruminants (Goat and sheep) are well maintained and are also matches with many other species. Goats, as other mammals, respond to heat stress at the cellular level by synthesizing HSPs, which help protect cells from the deleterious effects induced by heat stress (Welch, 1992; Morimoto

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ID   EMBOSS_001; SV 1; linear; unassigned DNA; STD; UNC; 1891 BP.
SQ   Sequence 1891 BP; 433 A; 551 C; 638 G; 269 T; 0 other;
     TTGCTTGAGG TACGTCTTCA CGTTGTGTGA TTGTTCCTGC GCGAGCATAG GATGTAAGAA       60
     TCATCGCCAA CGACCAGGGC AACCGCACCA CCCCCAGCTA CGTGGCTTTC ACCGATACCG      120
     AGCGGCTCAT CGGCGATGCA GCCAAGAACC AGGTGGCGCT GAACCCGCAG AACACCGTGT      180
     TCGACGCGAA GCGGCTGATC GGCCGCAAGT TCGGCGACCC GGTGGTGCAG TCGGACATGA      240
     AGCACTGGCC TTTCCGCGTG ATCAACGACG GAGACAAGCC TAAAGTGCAG GTGAGCTACA      300
     AGGGGGAGAC CAAGGCGTTC TACCCAGAGG AGATCTCGTC GATGGTGCTG ACCAAGATGA      360
     AAGAGATCGC CGAGGCGTAC CTGGGCCACC CGGTGACCAA CGCGGTGATC ACCGTGCCGG      420
     CCTACTTCAA CGACTCGCAG CGGCAGGCCA CCAAGGACGC GGGGGTGATC GCGGGGCTGA      480
     ACGTGCTGAG GATCATCAAC GAGCCCACGG CCGCCGCCAT CGCCTACGGC CTGGACCGGA      540
     CGGGCAAGGG GGAGCGCAAC GTGCTCATCT TTGACCTGGG CGGGGGCACG TTCGACGTGT      600
     CCATCCTGAC GATCGACGAC GGCATCTTCG AGGTGAAGGC CACGGCCGGG GACACGCACC      660
     TGGGCGGGGA GGACTTCGAC AACAGGCTGG TGAACCACTT CGTGGAGGAG TTCAAGAGGA      720
     AGCACAAGAA GGACATCAGC CAGAACAAGC GGGCCGTGAG GCGGCTGCGC ACGGCGTGCG      780
     AGCGGGCCAA GAGGACCTTG TCGTCCAGCA CCCAGGCCAG CCTGGAGATC GACTCCCTGT      840
     TCGAGGGCAT CGACTTCTAC ACGTCCATCA CCAGGGCACG GTTCGAGGAG CTGTGCTCCG      900
     ACCTGTTCCG GAGCACCCTG GAGCCCGTGG AGAAAGGCTC TACGCGACGC CAAGCTGGAC      960
     AAGGCCCAGA TCCACGACCT GGTTCCTGGT GGGGGGGCTC CACCCGCATC CCCAAAGTGC     1020
     AGAAGCTGCT GCAGGACTTC TTCAACGGGC GCGACCTCAA CAAGAGCATC AACCCGGACG     1080
     AGGCGGTGGC ATACGGGGCG GCGGTGCAGG CGGCCATCCT GATGGGGGAC AAGTCGGAGA     1140
     ACGTGCAGGA CCTGCTGCTG CTGGACGTGG CTCCCCTGTC GCTGGGACTG GAGACGGCCG     1200
     GAGGCGTGAT GACTGCCCTG ATCAAGCGCA ACTCCACCAT CCCCACGAAG CAGACGCAGA     1260
     TCTTCACCAC CTACTCGGAC AACCAGCCGG GCGTGCTGAT CCAGGTGTAC GAGGGCGAGA     1320
     GGGCCATGAC TCGGGACAAC AACCTGCTGG GGCGCTTCGA GCTGAGCGGC ATCCCGCCGG     1380
     CCCCGCGGGG GGTGCCCCAG ATCGAGGTGA CCTTCGACAT CGACGCCAAT GGCATCCTGA     1440
     ACGTCACGGC CACGGACAAG AGCACGGGCA AGGCCAACAA GATCACCATC ACCAACGACA     1500
     AGGGCCGGCT GAGCAAGGAG GAGATCGAGC GCATGGTGCA GGAGGCGGAG AAGTACAAGG     1560
     CAGAGGACGA GGTCCAGCGC GAGAGGGTGT CTGCCAAGAA CGCGCTGGAG TCGTACGCCT     1620
     TCAACATGAA GAGCGCCGTG GAGGATGAGG GGCTGAAGGG CAAGATCAGC GAGGCGGACA     1680
     AGAAGGTGGT GCTGGACAAG TGCCAGGAGG TGATTTCCTG GCTGGACGCC AACACCTTGG     1740
     CGGAGAAGGA CGAGTTTGAG CACAAGAGGA AGGAGCTGGA GCAGGTGTGT AACCCCATCA     1800
     TCAGCAGACT GTACCAGGGG GCGGGCGTAC CCCGGGGCTG CGACGGCTTA TGGGGCTACA     1860
     GCCCCAAACG AATCAACATA CGAACTAAAC C                                    1891
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Figure 1 Complete sequence of HSP1 gene, in Damascus Breed

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ID   EMBOSS_001; SV 1; linear; unassigned DNA; STD; UNC; 1887 BP.
SQ   Sequence 1887 BP; 440 A; 548 C; 637 G; 262 T; 0 other;
     GAGTGCCTCC TACGTCTTCA AGTTGGTCGG TGTTCCAGCG CGAGCAAGGA TGTAAGAATC    60
     ATCGCCAACG ACCAGGGCAA CCGCACCACC CCCAGCTACG TGGCTTTCAC CGATACCGAG   120
     CGGCTCATCG GCGATGCAGC CAAGAACCAG GTGGCGCTGA ACCCGCAGAA CACCGTGTTC   180
     GACGCGAAGC GGCTGATCGG CCGCAAGTTC GGCGACCCGG TGGTGCAGTC GGACATGAAG   240
     CACTGGCCTT TCCGCGTGAT CAACGACGGA GACAAGCCTA AAGTGCAGGT GAGCTACAAG   300
     GGGGAGACCA AGGCGTTCTA CCCAGAGGAG ATCTCGTCGA TGGTGCTGAC CAAGATGAAA   360
     GAGATCGCCG AGGCGTACCT GGGCCACCCG GTGACCAACG CGGTGATCAC CGTGCCGGCC   420
     TACTTCAACG ACTCGCAGCG GCAGGCCACC AAGGACGCGG GGGTGATCGC GGGGCTGAAC   480
     GTGCTGAGGA TCATCAACGA GCCCACGGCC GCCGCCATCG CCTACGGCCT GGACCGGACG   540
     GGCAAGGGGG AGCGCAACGT GCTCATCTTT GACCTGGGCG GGGGCACGTT CGACGTGTCC   600
     ATCCTGACGA TCGACGACGG CATCTTCGAG GTGAAGGCCA CGGCCGGGGA CACGCACCTG   660
     GGCGGGGAGG ACTTCGACAA CAGGCTGGTG AACCACTTCG TGGAGGAGTT CAAGAGGAAG   720
     CACAAGAAGG ACATCAGCCA GAACAAGCGG GCCGTGAGGC GGCTGCGCAC GGCGTGCGAG   780
     CGGGCCAAGA GGACCTTGTC GTCCAGCACC CAGGCCAGCC TGGAGATCGA CTCCCTGTTC   840
     GAGGGCATCG ACTTCTACAC GTCCATCACC AGGGCACGGT TCGAGGAGCT GTGCTCCGAC   900
     CTGTTCCGGA GCACCCTGGA GCCCGTGGAG AAGGCTCTAC GCGACGCCAA GCTGGACAAG   960
     GCCCAGATCC ACGACCTGGT CCTGGTGGGG GGCTCCACCC GCATCCCCAA AGTGCAGAAG  1020
     CTGCTGCAGG ACTTCTTCAA CGGGCGCGAC CTCAACAAGA GCATCAACCC GGACGAGGCG  1080
     GTGGCATACG GGGCGGCGGT GCAGGCGGCC ATCCTGATGG GGGACAAGTC GGAGAACGTG  1140
     CAGGACCTGC TGCTGCTGGA CGTGGCTCCC CTGTCGCTGG GACTGGAGAC GGCCGGAGGC  1200
     GTGATGACTG CCCTGATCAA GCGCAACTCC ACCATCCCCA CGAAGCAGAC GCAGATCTTC  1260
     ACCACCTACT CGGACAACCA GCCGGGCGTG CTGATCCAGG TGTACGAGGG CGAGAGGGCC  1320
     ATGACTCGGG ACAACAACCT GCTGGGGCGC TTCGAGCTGA GCGGCATCCC GCCGGCCCCG  1380
     CGGGGGGTGC CCCAGATCGA GGTGACCTTC GACATCGACG CCAATGGCAT CCTGAACGTC  1440
     ACGGCCACGG ACAAGAGCAC GGGCAAGGCC AACAAGATCA CCATCACCAA CGACAAGGGC  1500
     CGGCTGAGCA AGGAGGAGAT CGAGCGCATG GTGCAGGAGG CGGAGAAGTA CAAGGCAGAG  1560
     GACGAGGTCC AGCGCGAGAG GGTGTCTGCC AAGAACGCGC TGGAGTCGTA CGCCTTCAAC  1620
     ATGAAGAGCG CCGTGGAGGA TGAGGGGCTG AAGGGCAAGA TCAGCGAGGC GGACAAGAAG  1680
     GTGGTGCTGG ACAAGTGCCA GGAGGTGATT TCCTGGCTGG ACGCCAACAC CTTGGCGGAG  1740
     AAGGACGAGT TTGAGCACAA GAGGAAGGAG CTGGAGCAGG TGTGTAACCC CATCATCAGC  1800
     AGACTGTACC AGGGGGCGGG CGTGACTCGC CGAGAAGACA GCGACTGGCT ATGGAGCACA  1860
     GAACCTCTAA ACGATGACAA AAAAATA                                      1887
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Figure 2: Complete sequence of HSP1 gene, in Aradi Breed

et al., 1994).It has been proven that summer heat stress increases the mRNA expression of HSPs in goats either in tropical or temperate regions which might play a crucial role in resistance to hot environmental conditions (Dangi et al., 2012). However, the role of individual members of HSP70 family genes under heat stress conditions need more studies in different areas and breeds of goats. The current study revealed the expression pattern of individual member genes of Aradi goat (a local breed in Saudi Arabia) HSP70 family in comparison with Damascus goat (a Syrian breed that commonly crossed with Aradi breed to improve production and reproduction). A preliminary focus in this gene was a part of a big project to study the effect of exposure to heat stress on mRNA expression levels that encoded both HSP70 and HSP90 proteins. HSP70 Expression of genes can be utilized as a marker for heat tolerance in various species, and collected produced data will have valuable effect in improvement of technique to be used in Animal breeding systems for adapting to challenges of environmental changes.

As mentioned in results section, Aradi breed did not show any variation in HSP70-1sequence compare with that reported by Gade et al. (2009) in Yunnan black goat breed and Dong et al., (2013) in Yunnan breed. On the other hand, Damascus breed showed variation in HSP70-1 sequence compared with Yunnan or Yunnan black breeds (Gade et al., 2010; Dong et al., 2013).Three nucleotides A, T and G were identified in HSP70-1 sequence in

Damascus goat in positions 935, 984 and 996, respectively but did not identified in Aradi, Yunnan or Yunnan black goat breeds. It could be concluded that the Sequence data and typing results of *HSP70-1* gene varied between Aradi and Damascus breeds which can assist as a method for goat herd's evaluation. In addition to that, Damascus breed showed mutation in DNA sequence in 3 positions, Aradi breed did not show any variation in relation to that published for *C. hircus.*

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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