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Genetic variation of myostatin MSTN gene and protein polymorphism in three goat populations

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ABSTRACT

The aims of the present research are to analyze the polymorphism between three goat breeds Baladi, Hybrid (Baladi-Damascus), and Damascus breeds by Biochemical and advanced molecular assay. A total of 15 samples from three goat breeds; Baladi (n=5), Hybrid (n=5), and Damascus breeds (n=5) were used. The accession no. of MW654246, MW654247, and MW654248, with Baladi, Hybrid, and Damascus goat breed, respectively. The deposited sequences of objects are part of intron 3, and exon 3 is fully sequenced with three breeds, according to the findings. The frequency of the Myostatin gene was the same in all three breeds. Also, the deletion of C and A in two SNPs of 259 and 260, respectively with all breeds were showed. While the convert case with Baladi breed from A to T in the SNP of 236 is the case in Hybrid breed. Damascus breeds the convert of T to C in the SNP with 248. These results agree with the molecular genetics data output the higher similarity values between the three goat breeds were found. The value of similarity between samples Baladi and Hybrid goat breed was 0.92%. And between Damascus and Hybrid goat breeds were 0.74%. Finally, the value of similarity was lowest between Baladi and Damascus goat breed of 0.62% using analysis of plasma. The three goat breeds investigated exhibited low variation in the Myostatin gene; these results suggest that the Baladi breed has a higher amount of genetic material (DNA) that will be passed down to future generations than the Damascus breed.

Keywords: Molecular and biochemical assays, Myostatin (MSTN) gene, SNPs, Baladi, Damascus.

INTRODUCTION

Goats are one of Egypt's most important economic sources of meat. The majority of Egyptian goats are Baladi goats, which are found throughout the Nile Valley and Delta, as reported by El-Sayed *et al.,* (2016). The Damascus goat, also called as Shami, is an intuitive breed native to Syria and other Near Eastern countries, the Damascus goat has a rosy brown coat with long hair and long ears (Jnied *et al.,* 2012). Goat productivity enhancement serves as a global strategy for providing Egyptians with essential protein feed sources (El-Halawany *et al.,* 2017).

Molecular genetic techniques aid in the identification of genes or candidate genes that influence major economic characteristics; the use of these techniques for genetic enhancement relies on the ability to genotype individuals with greater potential for particular economic characteristics. The knowledge from candidate genes helps in the planning of breeding programs by increasing the selection precision (Rashed *et al.,* 2016). GDF-8, or myostatin, is a member of the transforming growth factor-b (TGF-b) superfamily that plays a key role in muscle growth and meat quality (Thomas *et al.,* 2000; Langley *et al.,* 2002). One of the primary regulators of myogenesis is myostatin (MSTN). Because of its role as a negative regulator of muscle mass deposition, MSTN gene regulation has received a lot of attention in recent years (Grade *et al.,* 2019). In both developing and mature skeletal muscles, the myostatin gene (MSTN) can be found. On the sheep second chromosome, it has three exons that code for proteins. Overall, nonsynonymous polymorphisms in the coding regions of genes are of major importance since they have a typically significant positive or negative effect on an organism's phenotype (or vice versa) (Grochowska *et al.,* 2020). A polyacrylamide gel electrophoresis is a valuable tool for establishing the quantitative distribution of proteins in goats (Shauket *et al.,* 1998; Simplício *et al.,* 2017) illustrated that the detection and quantification of acute-phase proteins by SDSP-AGE was useful and reliable as a complementary test in goats. The protein profile can be used as a marker depending on the presence or absence bands, band intensity, molecular weight and relative front values (El-Hamamsy *et al.*, 2018). In addition, the coding sequences of bovine myostatin in two double-muscled cattle's have been identified, as well as nine other vertebrate species' myostatin sequences (Belgian Blue and Piedmontese) (Mcpherron et al. 1997). Myostatin levels and/or function have been affected by natural mutations in a human subject (Schuelke et al., 2004) and some cows (Marchitelli *et al.,* 2003; Joulia Ekaza and Cabello, 2006) and sheep (Boman *et al.,* 2009) breeds. There have been increasing numbers of MSTN polymorphisms discovered since MSTN was discovered to play a critical role in skeletal muscle growth and development. Muscularity is affected by a single nucleotide polymorphism (SNP) DQ530260: g.6223G>A in intron 2 of the MSTN gene, which is situated on sheep's chromosome 2 (Clop *et al.,* 2006; Kijas *et al.,* 2007).

There were four different sheep breeds studied: Barki (MT361503), Ossimi (MT361504), Rahmani (MT361505) and Najdi (MT361506) (Osma *et al.,* 2021). Intron 1 and exon 1 polymorphism was found in the Myostatin gene in four indigenous Nigerian sheep breeds (Iroanya *et al.,* 2021). The purpose of this study was to explore polymorphisms in the

myostatin gene (MSTN), which is a possible gene for MAS, in three goat breeds: Baladi, Hybrid breed (Baladi-Damascus), and Damascus.

MATERIALS AND METHODS

Goat breeds:

Three goat breeds (Baladi, Damascus, and Hybrid breeds) were provided by the Faculty of Agriculture, Al-Azhar University, Egypt and analyzed by Animal Genetic Resources Department, National Gene Bank, Agricultural Research Center, Egypt.

1. **Blood samples collections:**

Five Baladi, five Damascene, and five hybrids (Baladi-Damasse) goat samples were used in this study. Samples of goat blood were collected in falcon tubes, which contained 0.2 ml of EDTA (0.5 M) as an anticoagulant.

2. **Extract of Blood plasma:**

In order to isolate the blood plasma, samples were centrifuged at 14000 rpm for 10 minutes at 4°C, then transferred to new tubes and stored at-20°C.

3. **Genomic DNA extraction:**

The complete genomic DNA extraction method was used according to Sambrook et al. (1989).

4. **Primer design:**

It was recommended by Boman et al. (2009) to use a set of primer sequences for the myostatin MSTN gene, which used RFLP analysis and restriction enzyme, but not used in our study, the sequences as indicated in Table (1).

Table (1): Primer sequence used for amplification of myostatin (MSTN) gene based on the database.

Gene	Primer Sequence ($5^- \rightarrow 3^-$)	Product size or length (bp)	Target Size (bp)
MSTN	**F:** CTCCTTGCGGTAGGAGAGTG **R:** GGTGCACAAGATGGGTATGAGGTTA	Less of 600bp	300bp

5. **Plasma proteins electrophoretic by SDS -PAGE**

Blood plasma protein samples were run on 12% polyacrylamide. Protein markers, prod range, premixed format, P770S, New England bio labs the protein marker Sid ranged from 2.3 TO 212 KD. Profile was carried out according to **Laemmli (1970)**.

6. **Statistical analysis of the results:**

The sequenced segments were aligned with published data in the NCBI databases using http://ncbi.nlm.nih.gov/BLAST/ (non-redundant nucleotide database) and submitted to the Gene Bank using Bank It tool: http://www.ncbi.nlm.nih.gov/BankIt/.FinchTV1.4.0, http://www.geospiza.com/finchtv/. CLC Sequence Viewer and MEGA-6 using in alignment with three breeds. Gel imaging was photographed using a gel documentation System and scored with Alphaimages 2200 software Version 4.0.1. Analyzer gel images were analyzed using Tree was constructed depending on Unweight Pair Group with Arithmetic Mean (UPGMA) method using diversity database software and phylogeny (Sneath and Sokal, 1973).

RESULTS

1. Myostatin (MSTN) gene polymorphism:

The best annealing temperature for three Egyptian goat breeds was 59.6°C, with one fragment in compliance with the targeted size of 300bp, according to DNA amplification utilizing Myostatin (MSTN). Myostatin is a protein that inhibits muscle development. Breeders are interested in growth features since they affect the profit from goat production, as shown in Figure (1). The results showed that the deposited sequences of the object are part of intron3 exon 3 and that the Baladi, Hybrid, and Damascus breeds are fully sequenced.

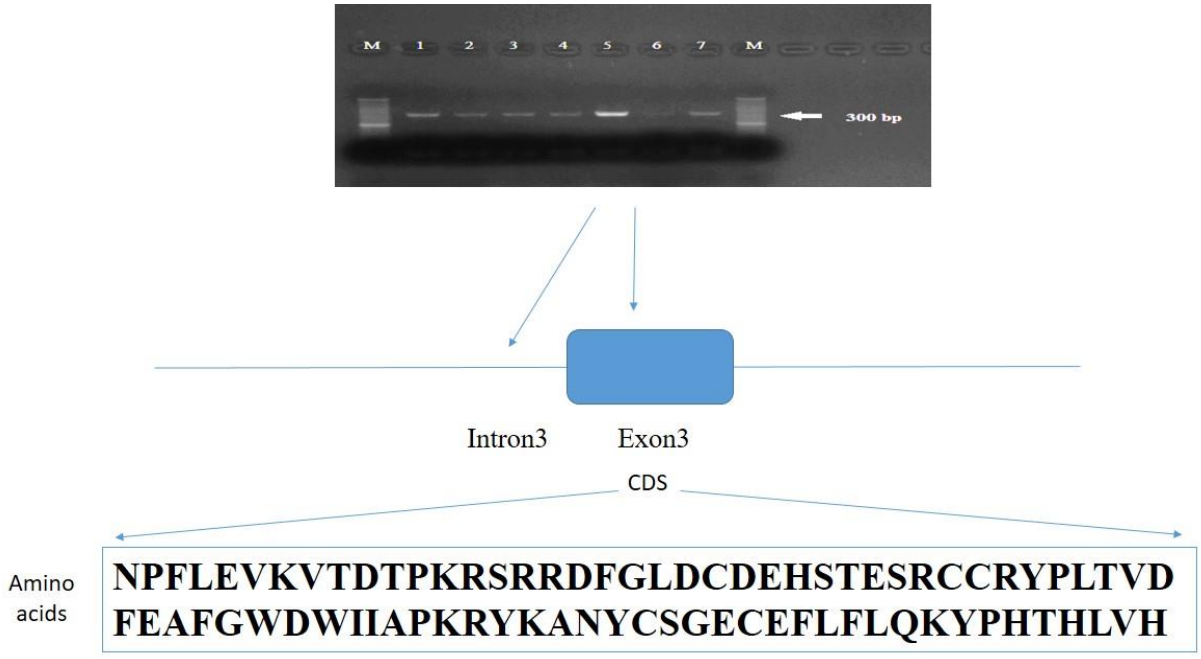

Figure (1): Profiling of the MSTN gene in three goat breeds. Electrophoresis analysis of PCR product; structure and SNPs position of MSTN gene; Amino acids based on sequencing results.

a) Baladi goat breed:

Baladi goat breed sequenced with GenBank accession no. of (MW654246) against nucleotide database. The results of Myostatin (MSTN) gene analysis showed the counts of the nucleotides for Baladi goat breeds listed in the Table (2) and Figures (1 and 2).

Data indicated that, in response to the sowing date, bread wheat genotypes showed variations in their growth and yield characters. Therefore, the best genotypes, lines 9 and 4 were the earliest genotypes for maturity date when sown on 30th Dec. On the other hand, lines 4 and 5 gave the highest grain yield when sown on 30th Nov. while, lines 5 and 7 recorded the highest grain yield when sown on 30th Dec. The result suggests that selection of lines 4, 5 and 7 that produced maximum grain yield. This study revealed that these genotypes can be used in future bread wheat breeding programs for the development of wheat cultivars for high temperature stress condition at the terminal growth stage (late sowing dates).

Table (2): The means of counts of nucleotides for three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) with Myostatin (MSTN) gene.

Goat Breeds	Means of Counts of nucleotides					
	MSTN gene					
	A	T	C	G	C+G	A+T
Baladi Breed	72.5	80.5	51	50	101	153
Hybrid (Baladi and Damascus) Breed	72.5	81.3	50.6	50	100.6	153.8
Damascus Breed	72.8	79.4	50.1	50	100.1	152.2
Average	**72.6**	**80.4**	**50.57**	**50**	**100.57**	**153**

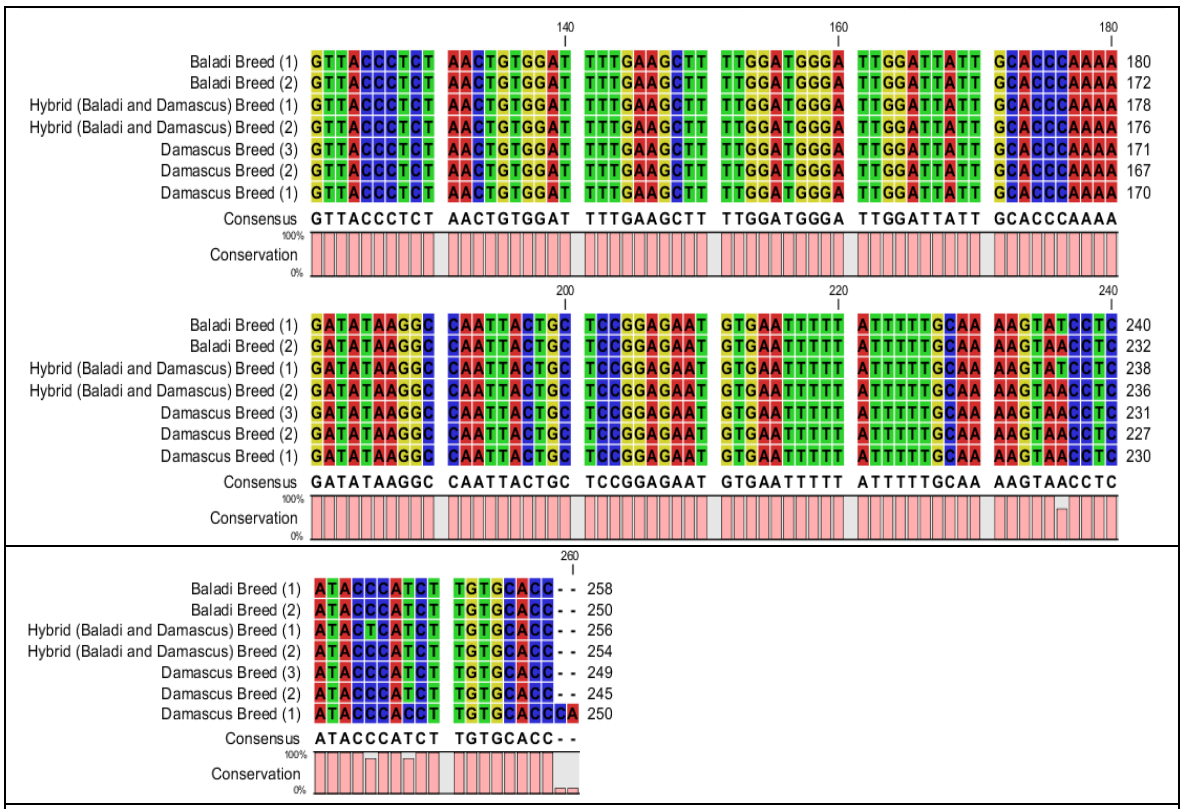

Figure (2): Sequences of DNA fragments that contain the MSTN polymorphism of three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus).

Means of counts of Adenine, Thiamine, Cytosine, and Guanine were 72.5, 80.5, 51, and 50 base nucleotides, respectively, with Baladi goat breed. Also, the means of total both Cytosine and Guanine (C+G) were 101 nucleotides in Baladi goat breed. Furthermore, the means of total both Adenine and Thiamine (A+T) were 153 nucleotides with Baladi goat breed. Frequencies of Guanine/Cytosine (G/C) nucleotides was 0.3975, while the frequencies of Adenine/Thiamine (A/T) nucleotides were 0.6025 with Baladi goat breed as scored in Table (3).

b) Hybrid goat breeds (Baladi and Damascus):

Hybrid goat breeds (Baladi and Damascus) sequenced with GenBank accession no. of (MW654247) against nucleotide database. The results of Myostatin (MSTN) gene analysis showed the counts of the nucleotides listed in Table (2) and Figures (1 and 2). The means of counts Adenine, Thiamine, Cytosine, and Guanine were 72.5, 81.3, 50.6, and 50 base nucleotides respectively in Hybrid goat breeds. While mean of both Cytosine and Guanine (C+G) was 100.6 nucleotides respectively with Hybrid goat breeds. Also, the means of both Adenine and Thiamine (A+T) were 153.8 nucleotides in Hybrid goat breeds. On the other hand, means of frequencies for Guanine/ Cytosine (G/C), nucleotides were 0.3955, while the frequencies of Adenine/Thiamine (A/T) nucleotides were 0.6045 as shown in Table (3).

Table (3): Frequencies for three breeds for three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) with Myostatin (MSTN) gene.

Goat Breeds	Frequencies of nucleotides		PIC
	G/C	A/T	
Baladi Breed	0.3975	0.6025	0.48
Hybrid (Baladi and Damascus) Breed	0.3955	0.6045	0.48
Damascus Breed	0.396778	0.603222	0.48

c) Damascus goat breed:

Damascus goat breed sequenced with the accession no. of (MW654248) against nucleotide database. The results of Myostatin (MSTN) gene analysis showed the counts of the nucleotides listed in Table (2) and Figures (1 and 2). The means of counts from Adenine, Thiamine, Cytosine, and Guanine were 72.8, 79.4, 50.1, and 50 base nucleotides respectively with Damascus goat breed. While, mean of both Cytosine and Guanine (C+G) was 100.1 nucleotides with Damascus goat breed. Furthermore, the means for both Adenine and Thiamine (A+T) was 152.2 nucleotides in the Damascus goat breed as showed in a Table 3.

In three Egyptian goat breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) with Myostatin (MSTN) gene the average means counts of nucleotides Adenine, Thiamine, Cytosine, and Guanine were 72.6, 80.4, 50.57, and 50 nucleotides respectively. Also, the average for both Cytosine and Guanine (C+G), Adenine, and Thiamine (A+T) were 100.57 and 153 nucleotides respectively. Finally, the polymorphic information content (PIC) was 0.48 in all goat samples as showed in tables (2 and 3). Also, the deletion of C and A in two SNPs of 259 and 260, respectively with all breeds were showed. While the convert case with Baladi breed from A to T in the SNP of 236 the case in Hybrid breed. While in Damascus breed the convert case of T to C in the SNP of 248. Also, that case in Hybrid breed as shown in Table 4.

Table (4): Deletion and variation in positions for three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) with Myostatin (MSTN) gene.

Baladi Breed (1)			**Baladi Breed (2)**		
Position (bp)	**Deletion**	**Variation**	**Position (bp)**	**Deletion**	**Variation**
236		A↔T	259	C	
259	C		260	A	
260	A				
Hybrid (Baladi and Damascus) Breed (1)			Hybrid (Baladi and Damascus) Breed (2)		
Position (bp)	Deletion	Variation	Position (bp)	Deletion	Variation
236		A↔T	259	C	
245		C↔T	260	A	
259	C				
260	A				
Damascus Breed (1)			Damascus Breed (2)		
Position (bp)	Deletion	Variation	Position (bp)	Deletion	Variation
248		T↔C	259	C	
			260	A	
Damascus Breed (3)					
259	C				
260	A				

These results agree with the molecular genetics data output of the higher similarity values between the three goats breeds were found as shown in Figure 3.

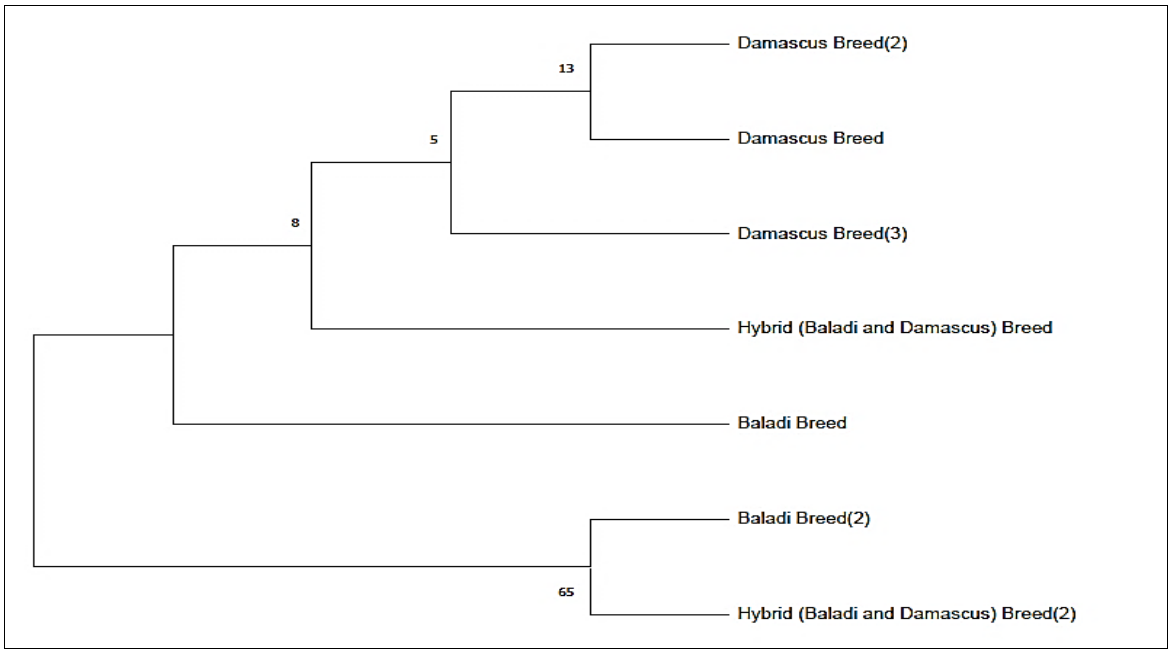

Figure (3): Diagram of genetic distance for three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) with Myostatin (MSTN) gene.

2. SDS-PAGE blood plasma protein:

Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) for the plasma protein was used to assess structure of Baladi, Damascus and Hybrid (Baladi-Damascus) goat breeds to compare with these groups by SDS-protein level. Fifteen blood plasma samples of all breeds were used to assess the genetic similarity within the studied Baladi, Damascus and Hybrid (Baladi-Damascus) goat breeds, Protein polymorphism has provided some useful information in animal breeding studies, such as the links between breeds and their evolution.

a) Baladi goat breeds:

Twenty-one bands form Baladi goat genotypes proteins were16.0, 18.0, 24.0, 34.0, 35.0, 46.0, 47.0, 49.0, 51.0, 52.0, 67.0, 71.0, 84.0, 90.0, 93.0, 104, 118, 129, 153, 172 and 235 KDa. Furthermore, a total of monomorphic 3 bands were observed on the gel. Also, a total polymorphic band was 18. Homogenesis and heterogenesis within Baladi goat genotypes protein samples were ranged from 14% to 86% respectively as shown in Tables (5 and 6).

Table (5): Monomorphic, Polymorphic, total bands, Homogenesis, Heterogenesis, Highest of molecular weight, and lowest of molecular weight to bands form goat genotypes proteins.

N	Genotypes	Monomorphic bands	Polymorphic bands	Total No. of bands	Homogenesis	Heterogenesis	Highest MW(KDa)	lowest MW(KDa)
1	Baladi breed	3	18	21	14%	86%	235	16.0
2	Damascus breed	5	13	18	28%	72%	235	19.0
3	Hybrid breed	4	15	19	21%	79%	235	16.0

Table (6): Molecular weights, Comparison between molecular weights and total bands of SDS-PAGE blood plasma proteins electrophoresis form goat breeds

Ref Band		Baladi 1	Baladi 2	Baladi 3	Baladi 4	Baladi 5	Hybrid 1	Hybrid 2	Hybrid 3	Hybrid 4	Hybrid 5	Damascus 1	Damascus 2	Damascus 3	Damascus 4	Damascus 5
no	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW
1	235	233	234	241	242	232	243	243	234	241	240	230	237	227	221	225
2	172	--	172	174	175	173	176	177	166	174	170	169	173	169	170	166
3	153	153	--	--	--	--	0	0	0	0	0	0	0	0	0	0
4	129	128	127	131	--	133	132	129	127	133	--	--	--	127	125	131
5	118	113	115	117	119	122	119	115	117	118	121	118	115	119	119	119
6	104	--	103	107	--	--	102	--	--	--	--	0	0	0	0	0
7	93.0	--	--	--	--	93.0	--	--	--	94.0	--	93.0	--	93.0	93.0	95.0
8	90.0	92.0	91.0	91.0	91.0	--	88.0	91.0	91.0	90.0	92.0	--	--	87.0	--	--
9	84.0	82.0	82.0	81.0	83.0	86.0	83.0	85.0	83.0	84.0	83.0	85.0	85.0	--	86.0	84.0
10	72.0	0	0	0	0	0	--	--	--	72.0	72.0	72.0	72.0	73.0	73.0	72.0
11	71.0	71.0	71.0	70. 0	71.0	70.0	70.0	71.0	71.0	--	--	0	0	0	0	0
12	67.0	67.0	67.0	67.0	67.0	68.0	67.0	68.0	68.0	67.0	--	0	0	0	0	0
13	52.0	52.0	53.0	--	--	52.0	0	0	0	0	0	--	--	--	--	52.0
14	51.0	50.0	--	--	51.0	--	51.0	51.0	--	50.0	51.0	--	50.0	50.0	51.0	--
15	49.0	--	48.0	--	48.0	49.0	48.0	49.0	48.0	--	49.0	49.0	--	--	--	--
16	47.0	47.0	--	--	--	--	--	--	--	47.0	--	47.0	47.0	47.0	48.0	47.0
17	46.0	--	--	--	46.0	46.0	--	--	--	--	46.0	0	0	0	0	0
18	36.0	0	0	0	0	0	0	0	0	0	0	35.0	35.0	35.0	--	36.0
19	35.0	35.0	35.0	--	--	--	--	--	34.0	--	35.0	--	--	--	35.0	--
20	34.0	--	--	34.0	34.0	34.0	34.0	34.0	--	34.0	--	0	0	0	0	0
21	29.0	0	0	0	0	0	0	0	0	0	0	28.0	29.0	29.0	28.0	--
22	24.0	24.0	24.0	24.0	24.0	25.0	24.0	24.0	24.0	24.0	24.0	24.0	25.0	25.0	25.0	24.0
23	20.0	0	0	0	0	0	0	0	0	0	0	--	20.0	20.0	21.0	21.0
24	19.0	0	0	0	0	0	0	0	0	0	0	19.0	--	--	--	--
25	18.0	18.0	17.0	--	--	--	0	0	0	0	0	0	0	0	0	0
26	16.0	--	--	16.0	17.0	16.0	16.0	16.0	16.0	17.0	16.0	0	0	0	0	0
Total bands within breed		14	14	12	13	14	14	13	12	14	12	12	11	13	13	12
Total bands all breeds		21					19					18				

Means presence (1), means absence (--) and no observed of bands (0)

The results showed the comparison between molecular weights and total bands of SDS-PAGE blood plasma proteins electrophoresis for Baladi breed, the size bands were ranged from 18.0 to 233 KDa sample 1 in Baladi, and ranged from 17.0 to 234 KDa in sample 2 with Baladi. And ranged from 16.0 to 241 KDa in sample 3 with Baladi, and ranged from 17.0 to 242 KDa in sample 4 with Baladi, and ranged from 16.0 to 232 KDa in sample 5 with Baladi. The total bands in Baladi breed ranged from 12 to 14 bands in all samples as showed in Table (6).

b) Damascus goat breeds:

SDS-protein banding pattern for Damascus goat breeds are shown in Tables (5 and 6). The total numbers of 18 bands were recorded with a range from 19.0 (lowest) to 235 KDa (Highest). There were only five monomorphic bands with molecular weights of 19.0, 35.0, 49.0, 52.0 and 90.0 KDa with percentage of 28% homogenysis. Moreover, there were thirteen polymorphic bands were only molecular weights of 20.0, 29.0, 36.0, 51.0, 84.0, 93.0 and KDa 129. Common bands of 24.0, 47.0, 72.0, 118, 172 and 235 KDa were observed in all samples, while all Damascus goat genotypes protein samples contained 72% heterogenesis.

Comparison between molecular weights (MW) and total bands of SDS-PAGE blood plasma proteins electrophoresis for Damascus breed was carried out. The total bands in Damascus breed were ranged from 11 to 13 bands in all samples. The size of bands ranged from 19.0 to 230 KDa for sample 1 in Damascus, 20.0 to 237 KDa in sample 2 with Damascus, 20.0 to 227 KDa in sample 3 with Damascus, 21.0 to 221 KDa in sample 4 with Damascus, and ranged from 20.0 to 225 KDa in sample 5 with Damascus as shown in Table (6).

c) Hybrid (Baladi - Damascus) goat breeds:

The banding pattern for Hybrid was shown in Tables (5, 6) and Figure (4 and 5) (Hybrid between Baladi and Damascus goat breeds).

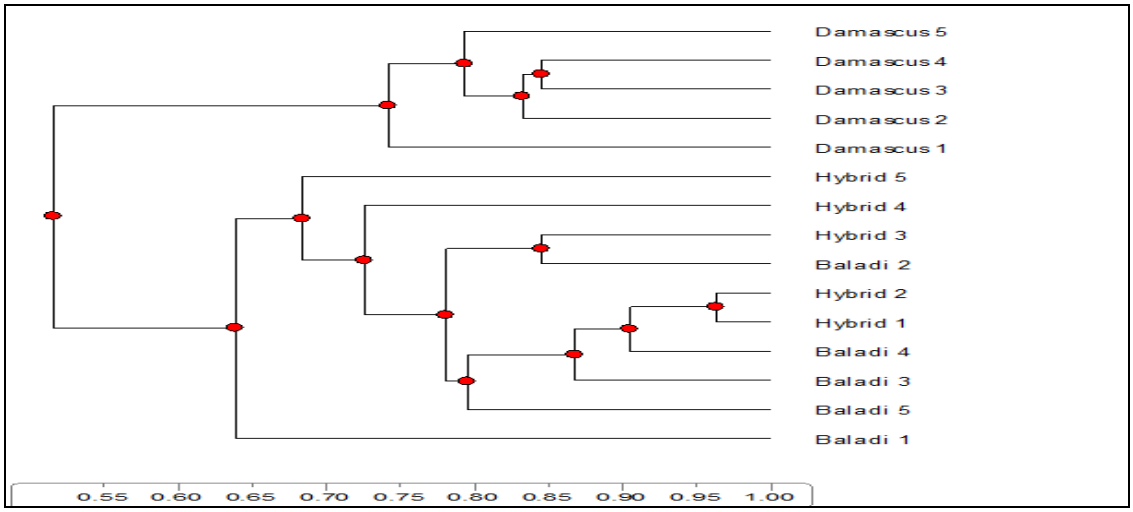

Figure (4): Genetic distances of the three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) breeds produced by protein profiling

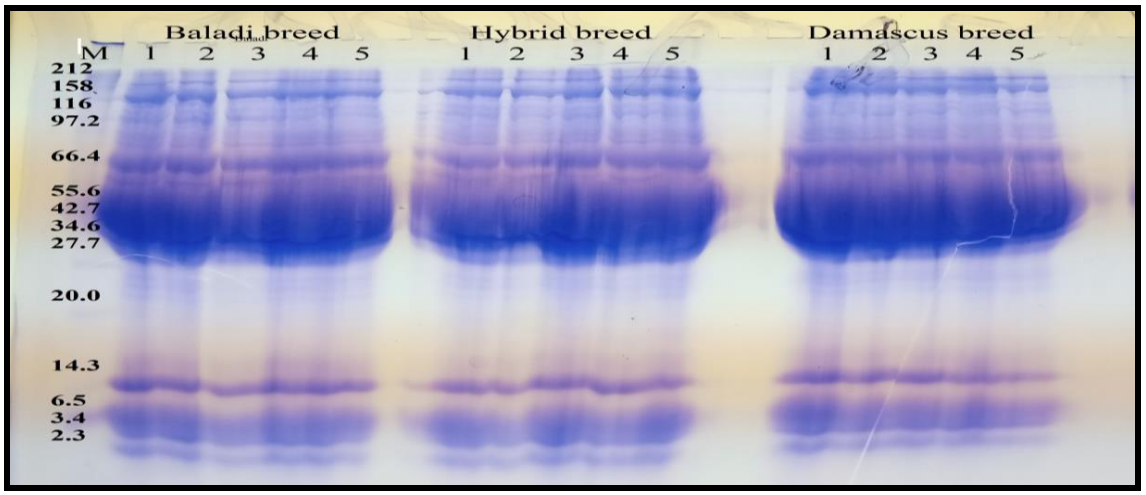

Figure (5): SDS-protein banding pattern for baladi, Damascus, and Hybrid (baladi and Damascus) goat breeds.

The total numbers of 19 bands were 16.0, 24.0, 34.0, 35.0, 46.0, 47.0, 49.0, 51.0, 67.0, 71.0, 72.0, 84.0, 90.0, 93.0, 104, 118, 129, 172 and 235 KDa. The results showed that the monomorphic bands were only with four molecular weights of 46.0, 47.0, 93.0 and 104 KDa with homogenesis of 21%. Also, polymorphic bands were only fifteen molecular weights of 34.0, 35.0, 49.0, 51.0, 67.0, 71.0, 72.0, and 129 KDa, with the heterogenesis percentage of 79%. While, the common bands of 16.0, 24.0, 84.0, 90.0, 118, 172 and 235 KDa were observed in all samples.

The Comparison between molecular weights (MW) and total bands of SDS-PAGE blood plasma proteins electrophoresis for Hybrid breed is shown in Table (6). The bands size were ranged from 16.0 to 243, 16.0 to 234, 17.0 to 241 and 16.0 to 240 KDa with the samples of (1, 2, 3, 4 and 5) respectively. The total bands in Baladi breed ranged from 12 to 14 bands in all samples.

In the three goat breeds revealed wide variations of different protein banding patterns of bands, Molecular weight of 153 KDa was only observed in Baladi breed with Baladi sample 1. Also, the molecular weight 18 KDa presence in samples 1 and 2 with Baladi goat breed. While absent in the other breeds. The Molecular weights of 20.0, 29.0 and 36.0 KDa were absents in samples 1, 4 and 5 respectively with Damascus goat breed. Molecular weight of 19.0 KDa was

present in sample 1 with Damascus goat breeds, these molecular weights as specific bands and observed only in Damascus goat breed. Furthermore, molecular weight of 52.0 KDa showed with samples 1, 2 and 5 in Baladi goat breed and sample 5 in Damascus goat breed. While, in Damascus and Hybrid goat breeds molecular weights of 72.0 KDa found in samples 1 and 5 with Damascus goat breed and scored in samples 4 and 5 with Hybrid goat breed. The molecular weights of 16.0, 34.0, 46.0, 67.0, 71.0 and 104 KDa observed in Baladi and hybrid goat breeds, while, not observed in Damascus goat breed.

2.1. Similarity matrices based SDS-PAGE:

The similarity of SDS-PAGE among the studied samples of all goat breeds investigation in Figure (4). The highest similarity was found between samples (1) and (2) with Hybrid goat breed were 0.96%. Also, the higher values were found between samples (3) and (4) in Baladi goat breed and samples (1) and (2) with Hybrid goat breed were 0.92%. While, the lowest value was found between sample (1) with Baladi goat breed and sample (1) with Damascus goat breeds were 0.38%. Also, the smallest value was found between samples (2, 4 and 5) in Baladi goat breed and samples (2 and 5) with Damascus goat breed were 0.40%.

DISCUSSION

This study aims is to analyze the polymorphism between three goat breeds Baladi, Hybrid (Baladi-Damascus), and Damascus breeds by Biochemical and advanced molecular assays. The accession no. of MW654246, MW654247, and MW654248, with Baladi, Hybrid, and Damascus goat breed, respectively. The deposited sequences of objects are part of intron 3, and exon 3 is fully sequenced with three breeds, according to the findings. Part of intron 1, exon 2 is fully sequenced with Zaraibi and Baladi breeds; intron 1, exon 1 with Baladi breed; and intron 2, part of exon 3 with Damascus breed are among the deposited sequences of the item according to Dowidar et al. (2018).

On the other hand, El-sayed et al. (2017) reported that the PIC values of TCRVB6 for Baladi, Zaraibi, and Farafra, BM6444 for Baladi, and ETH10 for Baladi and Farafra all exceeded 0.7, indicating that these loci could be used as genetic markers for genetic diversity analysis of Baladi, Zaraibi, and Farafra goat genotypes, as well as highly polymorphic information content, to improve the performance of Egyptian goat populations. Osma et al. (2021) investigated that sequence analysis of the first intron of MSTN gene identified six single-nucleotide polymorphisms (SNPs) in the studied breeds, four mutual SNPs were determined: c.18 G>T, c.241 T>C, c.243 G>A, and c.259 G>T. In addition, two SNPs c.159 A>T and c.173 T>G were monomorphic (AA and TT, respectively) in the Ossimi, Rahmani, and Najdi breeds and polymorphic in the Barki breed. Bi et al. (2020) results uncovered that the 5bp indel was significantly related to body height, height at hip cross, and chest width index ($p < 0.05$). In addition, individuals with DD genotype had a superior growing performance than those with the ID genotype. These findings suggested that the 5bp indel in MSTN gene are significantly associated with growth traits and the specific genotype might be promising for maker-assisted selection (MAS) of goats. Sharma et al. (2019) resulted that according to comparative research, goat and cow skim milks have very different protein compositions. Using SDS-PAGE, Gaddi goat skim milk had 19.01, 22.08, and 32.96 kDa proteins, but not non-Gaddi goat milk.

Jnied et al. (2012) indicated the total numbers of 15 bands were obtained. Their molecular weight ranged from 12 to 224 KDa. There were only five polymorphic bands with molecular weights of 12, 57, 68, 187 and 224 KDa, while the other bands were monomorphic. Marzouk *et al.,* (2019) illustrated that the SDS-PAGE analysis revealed 18 protein bands with different molecular weights, ranged from 115 to 14 kDa. These bands include 5 polymorphic, 9 monomorphic, and 4 unique (Gulabi breed has protein bands of 96.0 and 92.0 kDa, Shami breed has a band of 14 kDa. and Beetal breed has band of 36.5 kDa).

El-Hamamsy et al. (2018) reported that the Siwa had 20 protein bands that MW ranged from 17 to 269 KDa, the homogeneity of individual Siwa samples was 30%. El-Dakhla population had 23 protein bands that ranged from 17 to 283KDa. The heterogeneity's El -Dakhla population was 74%. While, Elfarfra populations varied in electrophoretic protein pattern, it had 21 bands that ranged from 17 to 252 KDa. Kayali *et al.,* (2012) concluded that the Ossimi, El-Adely (HORS), and El-Adely (HMYS) have genetic homogeneity percentages of 53%, 37%, and 55 %, respectively.

Montowska and Pospiech, (2007) abstracted that according to the literature, species-specific protein separations produce low-molecular-weight proteins composed of three light chains of myosin (14-23 kDa), troponin (19-30 kDa), and parvalbumin (14-23 kDa) (about 12 kDa), Cattle, sheep, lambs, goats, red deer, and rabbit meats may all be identified using the SDS-PAGE method, according to research. Hassanin, (2011) noted the presence of three protein types with molecular weights of 680, 524, and 431 KDa were noticed in lane number one which represents twin producing female sheep and the absence of two protein types with molecular weights 560.281 and 460.229 KDa, while found in all remaining lanes other animals groups.

Anous et al. (2008) noticed that Baladi goat breeds had an average similarity index of 0.65. Atta, *et al.,* (2009) concluded that in all the regions studied, the mean genetic similarity was 0.951, 0.890 and 0.921, respectively. Jnied *et al.,* (2012) obtained the average similarity value within Damascus breed was 0.94 which indicated a high homogeneity

value within this group. Kayali et al. (2012) reported the similarity values within Ossimi and El-Adely sheep breed ranged from 1 to 0.75 and 1 to 0.83 with an average of 0.91 and 0.94.

CONCLUSION

In conclusion, the means of nucleotides counts of (A, T, C, and G) for three breeds with Myostatin (MSTN) gene were higher than that of the means in Hybrid breed except for (A) Adenine with the Damascus Breed. While, the frequencies of three breeds Baladi, Damascus, and Hybrid breed (Baladi-Damascus) with Myostatin (MSTN) gene were equal values. Also, the deletion of C and A in two SNPs of 259 and 260, respectively with all breeds were showed. While the convert case with Baladi breed from A to T in the SNP of 236 that case in Hybrid breed. While in Damascus breed the convert case of T to C in the SNP of 248. Also, that case in Hybrid breed. These results agree with the molecular genetics data output the higher similarity values between the three goat breeds were found. The difference between samples of Baladi and Hybrid goat breed were 0.92%. Also, difference between Damascus and Hybrid goat breeds were 0.74%. Finally, the lowest value of similarity was found between Baladi and Damascus goat breed was 0.62% using analysis of plasma. Finally, the three goat breeds investigated exhibited variation in the Myostatin gene. These results suggest that the Baladi breed has a higher amount of genetic material (DNA) that will be passed down to future generations than the Damascus breed.

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التنوع الوراثي لجين الميوستاتين MSTN والبروتيني في ثلاث مجموعات من الماعز

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الملخص

تهدف هذة الدراسة إلى ايجاد التنوع ما بين سلالات ماعز ثلاثة البلدي والهجين (بلدي- دمشقي) وسلالة الدمشقي باستخدام طرق الكيمياء الحيوية والبيولوجيا الجزيئية المتقدمة. تم العمل باستخدام 15 عينة من الثلاث سلالات من الماعز حيث تم استخدام عدد من العينات لسلالة البلدي بواقع (عدد = 5) والهجين (عدد = 5) والدمشقي (عدد = 5). تم اخد ثلاث ارقام مرجعية من NCBI وهم كالتالي MW654246 و MW654247 وMW654248 لكلا من الثلاث سلالات (الماعز البلدي والهجين والدمشقي) على التوالي. وحيث وجد ان التتابع للاجزاء المستخدمة يقع كجزء من3 intron ، و exon 3 بالكامل مع الثلاث سلالات ، وفقًا للنتائج. ووجد ان جين الميوستاتين متشابة الي حد ما في جميع السلالات الثلاثة المستخدمة. أيضا وجد في النتائج حذف C و A عند اثنين من المواقع وهم 260 259 على التوالي مع جميع السلالات. بينما وجدت حالة تحول واحدة مع السلالة البلدي من Aإلى T في المواقع 236 هذة الحالة موجودة في السلالة الهجين ايضا. اما في سلالة الدمشقي وجدت حالة تحول من T إلى C في الموقع 248. اتفقت النتائج الوراثية مع تحليل البروتين حيث تم العثور على قيم تشابه أعلى بين سلالات الماعز الثلاثة. بلغت قيمة التشابه بين عينات سلالة الماعز البلدي والماعز الهجين 0.92٪. وبين الماعز الدمشقي والهجين 0.74٪. أخيرًا اعطت نسب التشابه نسبة أقل بين سلالة الماعز البلدي والدمشقي حيث بلغت 0.62٪ باستخدام تحليل البلازما.

أظهرت الثلاث سلالات من الماعز التي تم فحصها تباينًا طفيفا في جين الميوستاتين. تشير هذه النتائج إلى أن السلالة البلدي لديها كمية أكبر من المادة الوراثية (DNA) التي ستنقل إلى الأجيال القادمة أكثر من سلالة الدمشقي.

الكلمات المفتاحية: المقاييس الجزيئية والكيماوية الحيوية ، جين الميوستاتين (MSTN) ، SNPs، البلدي ، الدمشقي.