1. Introduction

Genetic variations at candidate genes touching economic traits (like growth, milk yield, meat production and reproductive traits) have stimulated research interest because they stayed well-considered as an aid to genetic selection and to mark evolutionary relationships in different livestock animal breeds [1]. In this aspect, myostatin (MSTN) as well as prolactin (PRL) are important potential genes due to their positive effect on growth enactment and meat quality traits.

Myostatin (MSTN) gene, also named as growing differentiation factor-8 (GDF-8) gene, is coding for converting growth factor-beta (TGF-β) super-family (one of the largest protein groups). This gene was physically mapped to goat chromosome 2q11-q12 [2,3], and consists of two introns and three exons [4]. Additionally, it is a powerful candidate gene, needful for growth and development of domestic animals due to its key function in muscularity, and its potential applications in animal farming [5]. Mutations in MSTN gene can quiet its expression or create a non-functional protein, which causes undesired muscularity (i.e. dramatic rise in both muscle fiber quantity [hyperplasia] and mass [hypertrophy]) or the “double-muscling” phenomenon in various species [6], such as dogs [7], pig [8], goat [Boer goat] [9] and, sheep [10].

Prolactin (PRL) gene plays a key role in changeable growth, variation and lactation, the hair growth cycle [11]. Many researchers recorded that PRL polymorphisms are related to wool or cashmere traits in goat and sheep [12-14]. Others studied the association polymorphism of PRL with dairy traits like benefit yield besides...
the yield of protein milk in cattle breeds [15–17]. Furthermore, many researcher suggested the significance of PRL gene in improving prolificacy in different sheep breeds [18,19].

Goat remains one of main important livestock kinds and affords a variation of products, such as fiber, milk, meat, and hides. Furthermore, goats are used as a model for biomedical studies [20,21]. Goats in Egypt are almost 3.13 million goats; they are extended essentially in three regions: Upper Egypt, Nile Delta besides in the desert rangelands [22]. There are five indigenous goat breeds: Baladi (local breed in Delta), Barki or Sahrawi (local breed in Desert), Sinaoy (Bedouin), Saidi and Zaraibi (or Egyptian Nubian). They are duple – purpose animals, with does breed intended for milk and bucks bred for meat [23].

The aim of the present study is to screen the genetic polymorphism of two functional genes (MSTN and PRL) in three goat breeds (Barki, Damascus and Zaraibi) via nucleotide sequence and PCR-RFLP methods in order to differentiate between these breeds.

2. Materials and methods

2.1. Animals

A total of 60 healthy goats, belonging to the three breeds under-study: Barki, Damascus and Zaraibi; 20 samples from each breed. All animals were born and reared in the Agriculture Research Station, belonging to Faculty of Agriculture, Cairo University.

2.2. DNA extraction

Blood samples were collected in tubes containing 0.5 M EDTA as anticoagulant and transported to the laboratory under cooled conditions. Genomic DNA was extracted and purified from whole blood collected samples using the salting out technique described by [24]. The DNA concentration was measured using the U.V spectrophotometer at wavelength 260 nm.

2.3. Polymerase Chain Reaction (PCR)

Two pairs of primers were used for amplifying each of MSTN and PRL loci using primers suggested by [25,26], respectively. The primer sequences are represented in Table 1.

2.4. Restriction Fragment Length Polymorphism (RFLP)

It was carried out in 15 μl of reaction mixture of each sample containing 5 μl of PCR product, 9.5 μl of 10 X buffer and 0.5 μl of fast digest restriction enzyme (MBI Fermentas, Germany) specific for each gene (Table 1). The reaction mixture was incubated at 37°C in water bath for a certain time as demonstrated in Table 1. Digestion products were separated by electrophoresis on 2.5% agarose gel, stained with ethidium bromide. The bands were visualized under UV light and the gels images were captured using digital gel documentation system (Bio-Rad, USA).

2.5. DNA sequencing

The PCR products representing different patterns and alleles of tested genes were purified and sequenced by Macrogen Incorporation (Seoul, South Korea) to identify the SNPs between different patterns and alleles. Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite.

2.6. Statistical analysis

The genotypic and allelic frequencies, the observed and expected heterozygosity and the $\chi^2$ test for Hardy-Weinberg equilibrium (HWE) were calculated using Pop Gene 32.1 package [27].

| Gene | Primer sequence (5’ → 3’), length of PCR product, region and specific restriction enzyme of MSTN and PRL genes. |
|------|---------------------------------------------------------------------------------------------------------|
| MSTN | F: CCG GAG AGA CTT TGG GCT TGA 337 bp Exon 3 HaellI 37°C for 10 minutes Azari et al. [25] |
| PRL  | F: ATTCCTGGAGCCAAAGAG 655 bp Exon 5 Eco24I 37°C overnight Lan et al. [26] |

F: forward R: reverse.

| Gene | Primary denaturation in 1st cycle | Denaturation | Annealing | Elongation | Final extension | Number of cycles |
|------|----------------------------------|-------------|-----------|-----------|----------------|-----------------|
| MSTN | 94/240                           | 94          | 60        | 55.5      | 72             | 120             | 35              |
| PRL  | 95/300                           | 94          | 30        | 5635      | 72             | 600             | 35              |

Figs. 1. Agarose gel electrophoresis of MSTN-PCR fragment (337 bp). Lane M, 25 bp DNA ladder. Lanes (1, 2, 4, 5, 6), Barki (7, 8, 9, 11), Damascus and Lanes (12, 13, 14, 16) Zaraibi breed.
Fig. 2. Agarose gel electrophoresis of MSTN-HaeIII/PCR-RFLP fragments. Lane M, 25 bp DNA ladder, lanes (1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15) Genotype BB (131, 123 and 83 bp).

CCGGAGAGACTTTGGGCTTGA
TTGTGATGAGCACTCCACAGAATCTCGATGCTGCTCGTT
ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGATAT
AAGGCCAATTACTGTCCCGGAGAATGTGAATTTTTATTTTGGCAAAGTAGATCCTCATACCCA
TCTTGTGCACCAAGCAACAACCCAAAAGTTGTCAGCGGACCCTTGGCTGTACCTCATAAAAGATGT
CTCCAAATATTGCTATTTTTATGGAACAAACATAATATATGGAAATGATCAAGGA
ACCGCCCTAGACCGCTGTGGGTGCTCATGA

Fig. 3a. The sequence analysis of Barki goat MSTN amplified fragment. Forward and backward primer with red color.

Query  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGCTCGTT  60
Sbjct  1    CCGGAGAGACTTTGGGCTTGATTGTGATGAGCACTCCACAGAATCTCGATGCTGCTCGTT  59

Query  61   ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGAT  120
Sbjct  60   ACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATTGCACCCAAAAGAT  119

Query  121  ATAAGGCCAATTACTGTCCCGGAGAATGTGAATTTTTATTTTGGCAAAGTAGATCCTCATA  180
Sbjct  120  ATAAGGCCAATTACTGTCCCGGAGAATGTGAATTTTTATTTTGGCAAAGTAGATCCTCATA  179

Query  181  CCCATCTTGTGCACCAAGCAACAACCCAAAAGTTGTCAGCGGACCCTTGGCTGTACCTCATA  240
Sbjct  180  CCCATCTTGTGCACCAAGCAACAACCCAAAAGTTGTCAGCGGACCCTTGGCTGTACCTCATA  239

Query  241  AGATGTCCTCCAAATATTGCTATTTTTATGGAACAAACATAATATATGGAAATGATCAAGGA
Sbjct  240  AGATGTCCTCCAAATATTGCTATTTTTATGGAACAAACATAATATATGGAAATGATCAAGGA

Query  301  TCAAGGCACTCCATGACCCTGTGGGTGCTCATGA  337
Sbjct  300  TCAAGGCACTCCATGACCCTGTGGGTGCTCATGA  337

Fig. 3b. Sequence alignment of Barki goat MSTN amplified fragment with published sequence.
3. Results and discussion

- MSTN-PCR produced a DNA fragment of 337 bp as shown in Fig. 1. Digestion of this fragment with the restriction enzyme HaeIII flanked only one cut B allele (131, 123 and 83 bp). The allele B frequency was 1.00 in the three breeds, meaning that only genotype BB was found in all breeds under study (Fig. 2). BB genotypic frequency was 1.00 for all the breeds.
- Sequence examination of the MSTN amplicon (amplified PCR product, 337 bp) was conducted, by using each of forward and backward primer. The nucleotide sequence of PCR product for MSTN gene in Barki breeds is shown in Fig. 3a
- The sequence alignment of Barki MSTN with published sequence (Accession number: KP120861.1, Capra hircus) showed 97% identities with one gap between positions 54 and 56, and eight SNPs; (A/G) transition at position 296, (T/A) transversion at position 298, two (A/C) transitions at positions 303 and 307, two (C/T) transitions at positions 309 and 312, (C/A) transitions at position 313 and (C/G) transversion at position 314 (Fig. 3b).

The nucleotide sequence of PCR product for MSTN gene in Damascus breed is presented in Fig. 4a.
- The sequence alignment of Damascus MSTN with published sequence (Accession number: KP120861.1, Capra hircus) showed 99% identities with three SNPs; one (G/A) transition at position 297 and two (A/G) transitions at positions 295 and 298 (Fig. 4b).
- The nucleotide sequence of PCR product for MSTN gene in Zarzai breed is shown in Fig. 5a.

![Fig. 4a](image-url)  
Sequence examination of Damascus goat MSTN amplified fragment. Forward plus reverse primers with red color.

![Fig. 4b](image-url)  
Sequence alignment of Damascus goat MSTN amplified fragment with published sequence.
The sequence alignment of Zaraibi MSTN with published sequence (Accession number: KP120861.1, Capra hircus) showed 96% identities with eleven gaps at positions 122, 123, 124, 125, 126, 151, 152, 167, 168, 169 and 220 (Fig. 5b).

The current results are parallel to those found by [25] in native Iranian Dalagh sheep. A 337 bp part for exon three of MSTN locus was amplified and digested via HaeIII enzyme. This enzyme digested the m allele, nevertheless M allele. Digestion of m allele created three pieces of 83, 123, and 131 bp. Furthermore, all test samples were monomorphs and showed only mm genotype. Similarly, [28] did not find any polymorphism around exon three of MSTN gene in Iranian Zel sheep breed.

Inversely, [29] showed the deletion of TTTTA in 5'UTR of caprine myostain gene in different Chinese goat populations via the meth-
ods of DNA sequencing and PCR-RFLP. They also inferred that the deletion of TTTTA in 5'-UTR of caprine MSTN gene significantly (P < .05 or P < .01) influenced the animal body bulk and weightiness from birth to four-month old. They observed that this SNP was conserved in various species and might be a unique mutation in goats. Likewise, [30] indicated that this deletion is found also in different Egyptian sheep breeds (Barki, Rahmani and Osseimi) and not limited to goats.

Contrariwise, [29] showed the deletion of TTTTA in 5'-UTR of caprine myostatin gene in different Chinese goat populations via the methods of DNA sequencing and PCR-RFLP. They also inferred that the deletion of TTTTA in 5'-UTR of caprine MSTN gene significantly (P < .05 or P < .01) influenced the animal body size and weight from birth to four-month old. They observed that this SNP was conserved in various species and might be a unique mutation in goats. Likewise, [30] indicated that this deletion is found also in different Egyptian sheep classes (Barki, Rahmani and Osseimi) and not limited to goats.

Subsequently, [31] tested MSTN gene polymorphism as a candidate marker trustworthy for growth in both Boer and Anhu white goats. Three pairs of primers (P1, P2 and P3) were designed to amplify the 5'-UTR and exon one fragments in the MSTN gene. The results exhibited two SNPs: DQ167575 g.197G > A and 345A > T, respectively (Table 3).

The PCR amplification of PRL gene a DNA fragment of 196 bp as viewed in Fig. 6. Digestion of PCR product (196 bp) of PRL gene with Eco24I revealed two different alleles, the uncut A allele (196 bp) and the cut B allele (169 and 27 bp). The allele A frequency was (0.53, 0.62 and 0.45) and the allele B frequency was 0.47, 0.38 and 0.55 for Barki, Damascus and Zaraibi, respectively (Table 3).

Three Genotypes were screened in the three studied goat breeds, AA (196 bp), AB (196, 169 and 27 bp) and BB (169 and 27 bp) (Fig. 7). AA genotypic frequency was 0.10, 0.25 and 0.00, AB genotypic frequency was 0.85, 0.75, and 0.90 and BB genotypic frequency was 0.05, 0.00 and 0.10 for Barki, Damascus and Zaraibi goats, respectively (Table 3).

Sequence study of the PRL amplicon (amplified PCR product, 196 bp) was conducted with forward and inverse primers. The nucleotide sequence of PCR product for PRL gene in Barki breeds is presented in Fig. 8a.

- The sequence alignment of Barki PRL with published sequence (Accession number: NM_001285547.1, Capra hircus) showed 97% identities (Fig. 8b).
- The nucleotide sequence of PCR product for PRL gene in Damascus breeds is shown in Fig. 9a.

The sequence alignment of Damascus PRL with published sequence (Accession number: EU256170.1, Capra hircus) showed 99% identities with two SNPs; one (T/C) transition at position 177 and other (G/A) transition at position 178 (Fig. 9b).

The nucleotide sequence of PCR product for PRL gene in Zaraibi class is publicized in Fig. 10a.

The sequence alignment of Zaraibi PRL with published sequence (Accession number: NM_001285547.1, Capra hircus) showed 99% identities with two SNPs; one (T/A) transversion at position 177 (Fig. 10b).

Regarding to polymorphism in PRL gene, the present results are in agreement with those reported by [26] in different Chinese indigenous goat breeds by PCR-SSCP and gene sequencing. They detected the X76049: g.576C > A (Pro176Thr) mutation which was established through Eco24I PCR-RFLP analysis. In exon five of the caprine PRL gene, three different SSCP banding patterns

| Gene/restriction enzyme | Breed | Genotype frequency | Allele frequency | Observed Het. (Ho) | Expected Het. (He) | $\chi^2$ |
|-------------------------|-------|-------------------|-----------------|-------------------|-------------------|---------|
|                         |       | CC                | CD              | DD                | C                | D       |
| PRL                    | Barki | 0.10             | 0.85            | 0.05              | 0.53             | 0.47    | 0.85    | 0.51    | 9.22*   |
|                         | Damascus | 0.25            | 0.75            | 0.00              | 0.62             | 0.38    | 0.75    | 0.48    | 6.65*   |
|                         | Zaraibi | 0.00             | 0.90            | 0.10              | 0.45             | 0.55    | 0.90    | 0.51    | 12.6*   |

* Means (P ≤ 0.05).

Fig. 7. Agarose gel electrophoresis of PRL-Eco24I/PCR-RFLP fragments. Lane M, 25 bp DNA ladder, lanes (5, 6) Genotype AA (196 bp), lanes (3, 4) Genotype AB (196, 169 and 27 bp) and lanes (1, 2, 7, 8, 9) Genotype BB (169 and 27 bp).

Fig. 8a. The sequence examination of Barki goat PRL amplified fragment. Forward, inverse primers with red color.

ATTTCTGGAGCCAAAGACACTGAGCCCTTACCTCTGTGGTACGGACCTCCCACTCCCTGCAAATACTAGGATGCAGAGCCACGT
CATCTGCTTTTCACCATCGCTCCACGCTGGCCACGGATTCAAGCAAGATTCGACACCTTACCTTAAGCTCCTGAATTG
CGAAATCATCTACAACAAAACAATGCTTAAGCCACAG

Fig. 9a. The sequence examination of Damascus goat PRL amplified fragment. Forward, reverse primers with red color.
(CC, CA and AA) were observed. Moreover, a novel SNP (X76049: g. 576C > A) was recognized, which resulted in an amino acid exchange from Pro (CCC) to Thr (ACC) at position 176 of the protein sequence. As the g.576C > A transversion destroys an Eco24I (GRGCY^C) restriction site, the Eco24I PCR-RFLP can be used for cost-effective genotyping of the goat PRL gene SNP.

Fig. 8b. Sequence alignment of Damascus goat PRL amplified fragment with published sequence.

Fig. 9a. The sequence investigation of Damascus goat PRL amplified fragment. Forward and backward primers with red color.

Fig. 9b. Sequence alignment of Damascus goat PRL amplified fragment with published sequence.
Conversely, [32] examined the genetic variant of the PRL gene of Indian goats, but no polymorphism was detected. The DNA sequence variations at PRL gene were examined in Malabari, Attapaddy black, Jamnapari and Salem black goat breeds by PCR-RFLP and DNA sequencing techniques. The digestion of PCR product from PRL locus with RsaI enzyme revealed a lone allele, namely, the allele A (156 bp) in all tested breeds. Subsequent sequencing, product of PCR was initiate to be of 156 bp in length and the BLAST examine at the NCBI site revealed 100; sequence homology with equivalent sequence of ovine mammary gland prolactin mRNA (Ass. X76050.1), caprine mRNA of pituitary prolactin (Accession number X76049.1) and mRNA of caprine mammary gland prolactin (Assent number X76048.1).

Due to productivity data lack, in the present study we did not perform an association analysis between the productivity and allele polymorphisms. We expect the productivity on some previous articles which identified the alleles linked with high productivity in other sheep breeds.

4. Conclusion

In the end, this study is considered to be a step advancing for further studies that may add to give additional information about the genetic polymorphism of meat and growth characters of Egyptian goat breeds and the improvement of these economically important traits.

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