Research Article

Effectiveness of β-Lactoglobulin and Leptin Genes Variants on Zaraiby and Damascus Goat Milk Traits

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Abstract | *β-lactoglobulin* (*β-LG*) and *Leptin* (*LEP*) genes are described as the functional genes for milk yield and milk composition. This study aims to identify different genotypes of *β-LG* and *LEP* genes associated with milk composition in Zaraiby and Damascus goats. 80 samples of blood and milk were collected from farm animals in Sakha, Egypt. The potential of hydrogen (pH), fat, protein, lactose, and solid not fat (SNF) were determined by biochemical methods in all milk samples. Different genotypes of *β-LG* and *LEP* genes were detected by single-strand conformation polymorphism (SSCP-PCR), and then validated by sequence analysis. The results of SSCP-PCR showed a monomorphic pattern for the *β-LG* gene and a polymorphic pattern for the *LEP* gene. The sequence analysis showed that the *β-LG* gene has one genotype without any single nucleotide polymorphism (SNP) while the *LEP* gene has two genotypes (CC and CT) with one SNP at position 194 C>T. The statistical analysis of milk and genotypes data showed that the *β-LG* gene has not any effect in the measurement of milk composition in Zaraiby and Damascus goats, while the CT genotype of the *LEP* gene causes an insignificant reduction in protein percentage and insignificant increase in fat percentage in Damascus goat. In conclusion, the fat percent of Damascus goat milk is affected by the T allele of the *Leptin* gene. Therefore, the T allele may help improve milk fat content through breeding programs.

Keywords | *β-lactoglobulin*, *Leptin* gene, Milk traits, Goat

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INTRODUCTION

Goat's milk owns the merit of being easier and faster to digest than the cow's milk, because the protein curds developed in the stomach are softer than those of cow's milk. Mean of daily milk yield in Zaraibi and Damascus goats were 576 ± 9.7 and 587 ± 9.2 g, respectively Alsheikh (2013). Keskin et al. (2004) found that the total solids, protein, fat, lactose and ash content were 12.2±0.16%, 3.5± 0.07%, 4.3±0.12%, 3.6±0.08%, and 0.77±0.02% for the Damascus goat. In Zaraiby goat, Fat 4.06±0.06, Protein 3.01±0.03, Lactose 4.62±0.03, Total solids 12.44±0.012, Solids non fat (SNF) 8.38±0.07 Ash 0.75±0.02 pH 6.63±0.03 (Abedo et al., 2013).

The genetic polymorphism of milk proteins was firstly illustrated by Aschaffenburg and Drewry (1955). Since then, numerous surveys have did to explore milk protein polymorphism, and a great innovation happened thanks to the advent of DNA and protein analysis methods.

Beta-Lactoglobulin (*BLG*) is the richest whey protein in the goat milk that outstandingly affects the technological properties of milk such as cheese making. It is secreted by mammary gland cells. Caprine *BLG* gene is located on chromosome 11, comprising seven exons and six introns (Işik et al., 2017). The association of *BLG* variants with milk yield and composition, in different caprine breeds, has been widely explored by researchers. It has been demonstrated that *BLG* polymorphisms influences milk-related traits (Kahilo et al., 2014; El-Hanafy et al., 2015; El-Shazly et al., 2017; Işik et al., 2017; Hedayat-Evrigh et al., 2020). In a survey carried out on three Saudi caprine breeds, El-Hanafy et al. (2015) viewed that goats having AA genotype of *BLG* gene produced more daily milk yield compared to those having AB and BB genotypes. Also, Hedayat-Evrigh et al. (2020) observed that the genetic polymorphism of exon 7 and 3'UTR region of *BLG* gene, for Iranian indigenous Khalkhali does, showed a significant effect on milk components, excluding lactose percentage ($P<0.05$). Besides, milk with AA and AC genotypes had higher protein and fat percentages, respectively.

Instead, some studies have stated no significant impact of *BLG* variants on both yield and components of milk (Mahmood et al., 2016; Ambarwati et al., 2019; Razmkabir et al., 2021). Here is the polymorphism of Senduro goat *β-LG* gene with a SNP (G4179A) in *BLG* exon 2, where classified as low polymorphic, with no linkage between the obtained genotypes with milk yield or constitution (Palayukan et al., 2020). Additionally, Razmkabir et al. (2021) indicated a small to medium correlation between *BLG* variants and daily milk yield in goats. The B allele of *BLG* gene positively, but slightly revealed the fat percentage of milk. More, no linkage pointed out between *BLG* variants and milk protein. Contrariwise, Gharedaghi et al. (2016) verified that exon 7 *β-LG* gene variation was significantly ($P < 0.05$) related to high milk protein proportion in Indian Mahabadi goats, but exhibited no significant effect on milk yield or milk fat percentage.

Concerning *Leptin*, it is a non-glycosylated protein, secreted by the white adipose tissue, and a little by gastrointestinal tract and placenta. It plays an essential role for controlling immunity, body weight, milk performance, feed intake, and reproduction (Abousoliman et al., 2020). Caprine *Leptin* (*LEP*) gene exists on chromosome 4, and has three exons and two introns, but only two of its exons are translated into protein (Vazir et al., 2019). *Leptin* gene affects various traits in goats, including growth, reproduction and dairy performance (Avondo et al., 2019; Abousoliman et al., 2020; Ibrahim et al., 2020). For example, Gregorio et al. (2014) compared goat, sheep, cattle and water buffalo *Leptin* genes and actions of the first intron microsatellite polymorphism in goats, and viewed *LEP* gene as a potential marker for metabolism and mammary gland health in dairy goats.

With regards to the dairy traits, Ibrahim et al. (2020) illustrated the interrelation of variation in a 356 bp region in caprine *LEP* gene exon 1 with different Barki milk traits. They realized that the detected *Leptin* variants exhibited significant ($P < 0.05$) additive effects on milk pH and high significant ($P < 0.01$) dominance, influencing the milk yield (MY) and protein content (PRO). Moreover, Avondo et al. (2019) declared that *Leptin* intron 1 genotype of the SNP (c.483T>A) effectively affected fatty acid composition (the levels of mono- and polyunsaturated fatty acids, desaturation index and favorable atherogenic index) in Girgentana lactating does. Nonetheless, no differences appeared in milk yield or gross composition between different shown *LEP* intron 1 genotypes.

At last, the positive effects of *BLG* and *Leptin* genes variants in enrichment of milk traits in goat are inadequate, and need more studies to be assured. Therefore, the aim of the present study is to investigate the correlation between *BLG* and *Leptin* genetic polymorphisms and the milk traits, including yield and composition, in both Damascus and Zaraibi goats, bred in Egypt.

MATERIALS AND METHODS

Sample collection

Sixty milk and blood samples were collected from Zaraibi goats (30 samples) and Damascus (30 samples) during the lactation period. Samples were collected from Sakha farm in Kafr El-Sheikh under the same feeding conditions.

Milk composition analysis

Milk composition analysis was carried out to measure the fat, SNF, and protein content and pH level. Milk samples (5 mL) were assessed by infrared spectroscopy (Milkoscan, Indifoss) at 37 °C to measure fat%, SNF% and protein%. Lactose concentration was also calculated (Bradley et al., 1992).

DNA extraction

Genomic DNA was extracted from blood samples using a Qiagen kit according to the manufacturer's pamphlet.

Polymerase chain reaction

PCR amplification reaction was performed in a total reaction volume of 25 µl containing 100 ng of template DNA and 12.5 µl of commercially available PCR master mix (Promega, Germany) containing specific primers with concentration 10 pmol/µl. The primer sequences were created in Macrogen Company, Korea (Table 1). The PCR amplifications were carried out in a SimpliAmp thermacycler (appliedbiosystems, thermos fisher scientific) with the following conditions: One cycle at 95°C for 3 min. 33 cycles at 95°C for 30 sec., 55°C (*β-lactoglobulin*) OR

65°C (*Leptin*) for 30 sec., 72°C for 30 sec. and finally 72°C for 5 min. The amplification was verified by electrophoresis on 2% agarose gel (w/v).

Genotyping

SSCP technique was utilized to illustrate the genetic polymorphism of *β-LG* and *LEP* genes. The SSCP analysis was carried out as follows: 6 µL PCR product was diluted in 12 µL denaturing solution (95% formamide, 0.025% xylene cyanol and 0.025% bromophenol blue, 25 mM EDTA), denatured at 96°C for 10 min, chilled on ice and resolved on 12% acrylamide: Bisacrylamide gels (29: 1) according to Tahira et al. (2009).

Sequence analysis

PCR product of two genotype of *LEP* gene was purified using purification kit (Sigma), and then sent to Macrogen Company for sequencing. The obtained data was alignment in GenBank, and analyzed by Bioedite software.

Statistical analysis

Obtained data were analyzed by T. test independent using SPSS program version 19. The results were significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

β-lactoglobulin and Leptin genes detection

β lactoglobulin of zaraiby and damascus goats was detected and amplified by specific primer at 177 bp, while *Leptin* gene was detected and amplified by specific primer at 290 bp (Figure 1).

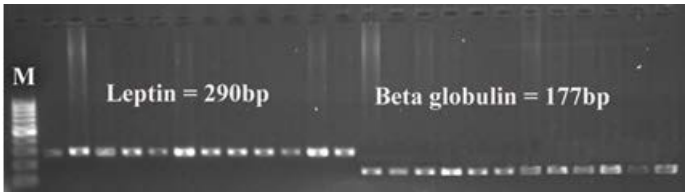

Figure 1: Detection of β lactoglobulin and *Leptin* genes in zaraiby and Damascus goats at 177 and 290bp, respectively.

Genotyping and sequence analysis

Single strand confirmation polymorphism polymerase chain reaction (SSCP-PCR) was used to detect the different genotypes of β lactoglobulin and *Leptin* genes in zaraiby and Damascus goats. *β* lactoglobulin gene was monomorphic in all zaraiby (Figure 2a) and all Damascus goats (Figure 2b), while *Leptin* gene was monomorphic in all zarabiy goats (Figure 3a), and polymorphic in Damascus goat where recorded two patterns as shown in (Figure 3b, c). Sequence analysis showed presence one SNP in Pattern II of Damascus goat at position 194 C>T (Figure 4).

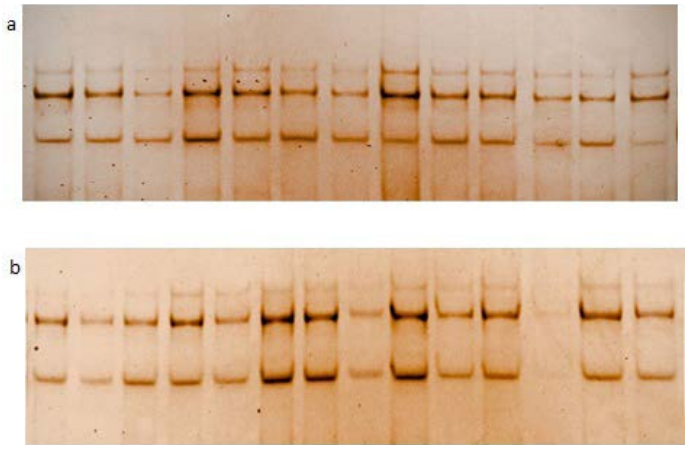

Figure 2: (a) show monomorphic patterns of beta globulin in all zarabiy, and (b) show monomorphic patterns of beta globulin in Damascus goats SSCP-PCR.

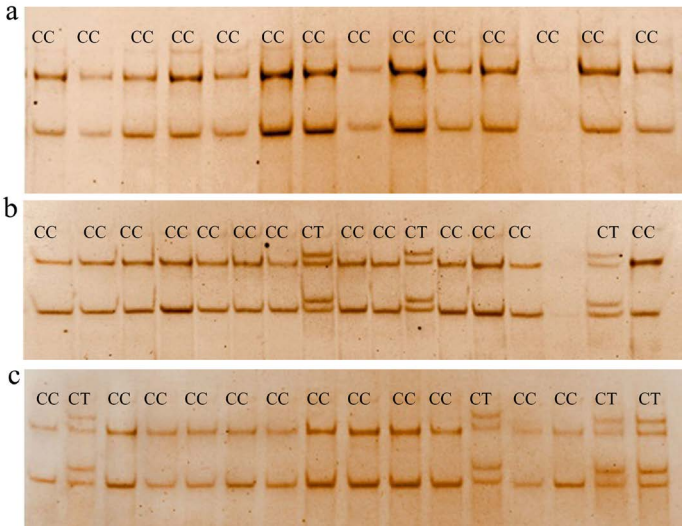

Figure 3: (a) show monomorphic patterns of *Leptin* gene in all zaraiby goats by SSCP-PCR, **(b, c)** show polymorphic patterns of *Leptin* gene in Damascus goats by SSCP-PCR; black arrow refers to CT genotype of *Leptin* gene

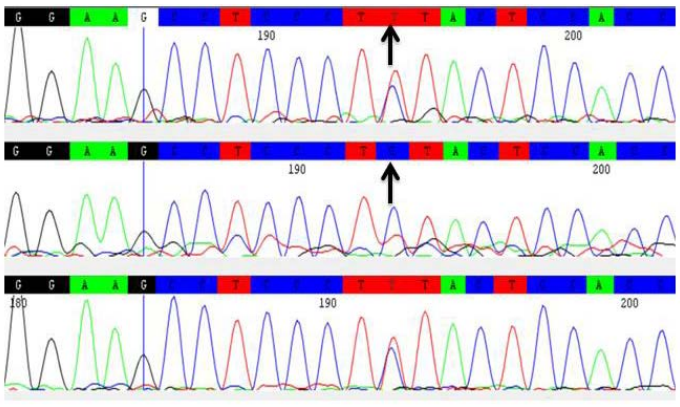

Figure 4: (a) show SNP of *Leptin* gene in Damascus goats .

Table 1: Primer sequences.

Gene name	Primer sequence	Band size
β-lactoglobulin	F 5-CCTCTGGGGACAGACGACG-3 R 5-GACTCAGAAGGGAGAGCACAGG-3	177 bp
Leptin	F 5'-GCTCCACCCTCTCCTGAGTTTGTCC-3' R 5'-TGTCCTGTAGAGACCCCTGTAGCCG-3'	290 bp

Table 2: Milk analysis.

Species	Genotypes	pH	Fat	Protein	Lactose	SNF
Damascus	Pattern I (CC)	6.62±0.01	1.4±0.24	2.73±0.15	4.2±0.05	7.5±0.13
	Pattern II (CT)	6.61±0.02	1.84±0.22	2.6±0.1	4.3±0.03	7.68±0.1
	P-Value	0.624	0.185	0.463	0.061	0.438
Zaraiby		6.6	1.33	2.68	4.1	7.3

Milk composition analysis

Mean of pH, fat, protein, lactose, and SNF in Zaraiby goats were 6.6, 1.33, 2.68, 4.1, and 7.3, respectively. Mean of pH, lactose and SNF percentage in Pattern II (CT genotype) and Pattern I (CC genotype) of Damascus goat are the same value approximately. Mean of fat percentage insignificant increase in Pattern II, CT genotype, (1.84) compared with Pattern I, CC genotype (1.4) while mean of protein percentage insignificant reduce in Pattern II, CT genotype, (2.6) compared with pattern I, CC genotype, (2.7) (Table 2).

In respect of *β*-lactoglobulin gene, SSCP analysis showed only one genotypic pattern in both Damascus and Zaraibi goats. In other words, *β-LG* gene was monomorphic in the two studied breeds. Sequence analysis of a 177-base-pair fragment of the exon 7*β*-LG gene indicated the absence of SNPs in sequenced analysis. The present investigation failed to find out a significant linkage between the obtained *β-LG* genotypes and measured milk traits; milk pH, fat, protein, lactose and solids-not-fat (SNF) percentages.

Similarly, Gharedaghi et al. (2016) confirmed that exon 7 *β-LG* gene variation exhibited no significant effect on milk yield or fat percentage in Indian Mahabadi does. As well, Pakistani goats (Beetal and DDP) were disclosed polymorphic for *β-LG* locus by (Mahmood et al., 2016), with richness of A allele and heterozygous AB genotype. All three found *β-LG* milk protein isoforms lacked statistically the association with milk yield and components. Likewise, (Palayukan et al., 2020) viewed no connection between the obtained genotypes of a SNP (G4179A) in *BLG* exon 2 with milk yield or constituents in Senduro does. Earlier, El-Shazly et al. (2012) who discovered that the *β-LG* genotype had no significant effect on milk yield or composition but milk protein content, which was high in *BLG* genotype A in some Saudi sheep breeds. Additionally, Ambarwati et al. (2019) affirmed that the association of Saanen *BLG-Sac*II/RFLP genotypes (AA, AG, GG) with milk protein and milk production did not differ significantly. Furthermore, Kahilo et al. (2014) and El-Shazly et al. (2017) suggested that the connection between caprine *β-LG* polymorphism and milk yield is conflicting, and might be breed-dependent (i.e. relaying on the studied breed itself), and this could be helpful in using the high milk yield genotype, inside each breed, in breeding programs.

On the other hand, there are numerous contradictory reports, showing the significant association between *BLG* variants and dairy traits. Gharedaghi et al. (2016) assessed, in Indian Mahabadi goats, the fellowship between exon 7 *β-LG* gene variations and high milk protein fraction. Also, Kumar et al. (2006) described that *BLG* gene in AA genotype produced higher milk yield than those of AB genotypes in Indian local goats. More, El-Hanafy et al. (2010) uncovered the relationship between the polymorphism of *BLG* exon 7 gene and dairy performance in three Egyptian local goat breeds. Rendering to them, *BLG* genotype AA had a higher milk yield than genotype AB. In addition, El-Hanafy et al. (2015) found, in Saudi goats, that *BLG* gene AA genotype correlated with higher milk yield than AB and BB. Moreover, Isik et al. (2017) reported that *BLG*/S1S1 genotype of 427 bp of *β-LG* exon 7 disclosed higher lactation milk yield in Saanen doe.

With respect to (*LEP*) gene, SSCP analysis showed only one genotypic pattern (monomorphic) in Zaraibi does. However, it recorded two patterns in Damascus (polymorphic). Sequence analysis of a fragment of 275 bp length from exon 3 of *Leptin* gene displayed only one SNP in Pattern II (CT genotype) of Damascus goat at position 194 C>T. Means of milk pH, lactose, protein, fat and solids-not-fat (SNF) percentages in Pattern II (CT genotype) and Pattern I (CC genotype) of Damascus breed did not differ significantly. Consequently, the present inquiry failed to manifest a significant correlation between the obtained *Leptin* genotypes and measured dairy traits; milk pH, protein, lactose, fat and solids-not-fat (SNF) percentages. Similar results appeared by Avondo et al. (2019), who stated that the *LEP* intron 1 genetic polymorphism had

no significant effects on milk yield or gross composition in Girgentana lactating does. In like manner, Marchitelli et al. (2013), who did not expose any significant relationship between a SNP in the *LEP* gene (g. 1180C > T) and the analyzed fatty acid traits in three different bovine breeds. Likewise, Grădinaru et al. (2020) did not note any significant relations of *LEP* A1620G genotypes with milk, fat, and protein yields in Romanian Cows. More, there was no relationship between *LEP-Sau*3AI polymorphism with daily and 305-days milk yields in Simmental Swiss cows (ÜÇME and AKYÜZ, 2021).

On the contrary, Maletic et al. (2019) disclosed *LEP* gene polymorphism on exon 3 (A59V locus) and intron 2 (SAU3AI locus) in Busha cattle, and experimented their associations with milk traits; the first locus significantly impacted only solids-not-fat (SNF) fraction. In parallel, Abousoliman et al. (2020) observed a significant association between rs420693815 in exon 3 of ovine *LEP* gene and milk yield ($p \leq 0.1$). As ewes of GG genotypes had a higher milk yield than ewes bearing AG and AA genotypes. Ibrahim et al. (2020) exposed the alliance between the caprine *LEP* gene polymorphism within a 356 bp region in exon 1 and various Barki dairy traits. They recognized that the noticed *Leptin* variants exhibited significant ($P < 0.05$) additive effects on milk pH and high significant ($P < 0.01$) dominance, influencing the milk yield (MY) and protein content (PRO). As well, caprine *LEP* intron 1 genotype of the SNP (c.483T>A) established a significant effect on the fatty acid constituents (the levels of mono- and polyunsaturated fatty acids, desaturation index and favorable atherogenic index) in Girgentana lactating goats (Avondo et al., 2019).

CONCLUSIONS AND RECOMMENDATION

Analysis of SSCP-PCR and sequencing showed that Zaraiby goats had one genotype in β-globuline and *Leptin* genes while Damascus goats had one genotype in β-globuline, and two genotypes in *Leptin* gene with one SNP at position 194 C>T. This SNP causes an insignificant increase in milk fat percent in Damscus goat. Therefore, the T allele may help improve milk fat content through breeding programs.

NOVELTY STATEMENT

Damascus goat has mutant genotype in leptin gene may be associated with increase the fat percentage in milk. So, it is useful to enter the Damascus goat into the breeding program.

AUTHOR'S CONTRIBUTION

This study was done in collaboration with all authors. A.D. designed this study. M.E.I. collected the blood and milk samples. A.D. and D.M.M. extracted DNA from blood samples. A.M.A. analyzed the milk composition. H.R.D. performed the SSCP-PCR. A.D. and H.R.D. analyzed the data. M.A.A. and A.D. drafted the manuscript. A.D. and I.M. F. critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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