Pakistan J. Zool., vol. 54(4), pp 1781-1789, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20200706090719

Polymorphism Detection in Alpha-Lactalbumin Gene and its Association with Some Productive Traits in Egyptian Goats

Amira M. Nowier[1], Hassan R. Darwish[2], Sherif I. Ramadan[3], Nadia A. Abo El-Maaty[2] and Othman E. Othman[2]*

[1]Biotechnology Research Department, Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.
[2]Cell Biology Department, National Research Centre, Dokki, Egypt.
[3]Animal Wealth Development Department, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt.

ABSTRACT

The objectives of this study were to identify polymorphisms in exon III of the α-lactalbumin gene among three Egyptian goat breeds (Zaraibi, Damascus and Barki) and to investigate the effect of α-lactalbumin gene polymorphism, goat breed, type of birth, season of kidding, litter size and lactation stage on body weight and milk yield traits in Egyptian goats. One hundred and sixty blood samples were collected for DNA extraction; 74 from Zaraibi, 41 from Damascus, and 45 from Barki breeds. Two genotypes, TT and TC, in the α-lactalbumin gene were identified using single strand conformation polymorphism. DNA sequencing resulted in one non-synonymous MT163744: g.128T>C SNP (TCG ^{Ser}> CCG ^{Pro}) at the sixth nucleotide of α-lactalbumin gene exon III. Barki breed showed the highest heterozygosity (0.260) and effective number of alleles (1.345). The T>C SNP showed non-significant association with body weight traits. Body weights at birth, 3 months and 12 months in Damascus goat were significant and higher than those in Zaraibi. Kids born single were heavier than twins and triplets at birth age. The highest milk yield was recorded for Zaraibi does with three kids and at the first month of lactation stage. Moreover, the T>C SNP in the Zaraibi α-lactalbumin gene may be a useful marker for assisted selection programs to improve goat milk yield trait.

Article Information
Received 06 July 2020
Revised 29 August 2020
Accepted 26 September 2020
Available online 12 August 2021 (early access)
Published 22 April 2022

Authors' Contribution
AMN planned and designed the study. SIR and AMN collected the blood samples and provided the help for evaluating the milk yield and body weights measurements. OEO, HRD and NAA performed the α-lactalbumin gene genotyping. AMN, SIR and OEO analysed the data and interpreted the results. All authors wrote and revised the manuscript.

Key words
Alpha-lactalbumin gene, Body weight, Goat, Milk yield, Polymorphism

INTRODUCTION

Goats are considered one of the most important livestock especially in desert and semi-desert regions. Moreover, goats are easier to keep and less expensive than other livestock especially in developing countries, like Egypt (Khalil *et al*., 2013; Nowier *et al*., 2019). In Egypt, the main goat breeds include Baladi and Barki for meat production and Zaraibi for milk production (Galal *et al*., 2005). In addition to Damascus goats or Shami which are considered dual-purpose breed, imported from Syria to Egypt by Ministry of Agriculture (Jnied *et al*., 2013). Goat milk is highly recommended for infants, elderly, and convalescents, because of its higher nutritional value, digestibility, immunological properties, and the hypo-allergenicity compared to others (Le Parc *et al*., 2014). Goat milk can be consumed as it is, but a large proportion is processed to cheese and other dairy products producing a large amount of whey (Hejtmánková *et al*., 2012; El-Tarabany *et al*., 2018). Whey proteins have high nutritive value and considered one of the good sources of readily available proteins. α-lactalbumin (*α-LA*) is the most important and abundant whey protein in goat milk (Hejtmánková *et al*., 2012).

α-LA gene codes for α-lactalbumin and located on the fifth chromosome of goat (Ma *et al*., 2010). It has been hypothesized that the volume of milk yield is regulated by *α-LA* protein through synthesis of milk lactose which acts as a principle osmole of milk by drawing the water into the vesicles (Ma *et al*., 2010). *α-LA* is an important protein for nutrition, growth and development of the body (Layman *et al*., 2018). Therefore, *α-LA* gene is considered a candidate gene for the identification of molecular markers associated with milk yield and growth traits in farm animals. *α-LA* gene was associated with milk yield and composition traits in many farm animals such as cattle (Voelker *et al*., 1997; Kazmer *et al*., 2001), buffalo (Dayal *et al*., 2006)

* Corresponding author: othmanmah@yahoo.com
0030-9923/2022/0004-1781 $ 9.00/0

and goat (Dettori *et al*., 2015). Moreover, *α-LA* gene was associated with body size and performance traits such as chest circumference and cashmere productivity in goats (Lan *et al*., 2008; Ma *et al*., 2010). Previous studies have shown that genetic and non-genetic factors such as genetic type of the animal, type of birth and season of kidding effect on body weight of goats (Mioč *et al*., 2011; Mahal *et al*., 2013; Atoui *et al*., 2017; Tesema *et al*., 2017) and lactation stage, parity and litter size effect on milk yield of goats (Mahmoud *et al*., 2014; Pleguezuelos *et al*., 2015; El-Tarabany *et al*., 2018; Zamuner *et al*., 2020).

Single-strand conformation polymorphism (SSCP) is considered a powerful molecular technique for detection of DNA polymorphisms and may be used as a tool for the selection of economically important goat traits (Caroli *et al*., 2007). However, studies of the association of *α-LA* gene polymorphisms with milk yield and growth traits in goats are very scarce (Dayal *et al*., 2006; Zhou and Dong, 2013; Mir *et al*., 2014). Therefore, the objectives of this study were first, to identify polymorphisms in exon III of the *α-LA* gene in three Egyptian goat breeds (Zaraibi, Damascus and Barki), second, to investigate the effect of *α-LA* gene polymorphisms, breed, type of birth and season of kidding on body weight traits in Zaraibi and Damascus breeds and third, to investigate the effect of *α-LA* gene polymorphism, litter size and lactation stage on milk yield trait of Zaraibi goats.

MATERIALS AND METHODS

Statement of animal rights

All procedures performed in this study involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Committee of Animal Care and Welfare, Benha University, Egypt) with an approval number: BUFVTM 2019.

Animals and management

One hundred and sixty goats belonging to three breeds; Zaraibi (*n*= 74), Damascus (*n*= 41) and Barki (*n*= 45) were used in this study. These animals belong to Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. Semi-open pens were used for housing goats. They were fed on Egyptian clover (*Trifolum alexandrinum*) from December to May and on a concentrate mixture and rice straw during the rest of the year. The concentrate mixture was composed of cotton seed cake, maize, rice bran, wheat bran, calcium carbonate and sodium chloride, with an average composition of 16% crude protein, 15% crude fiber and 3% crude fat. During the mating season (June and October), dames were randomly divided into mating groups and each was randomly assigned to a fertile buck. First mating of young females was occurred at the age of 18 months. At kidding, newborns were tagged and their gender, type of birth and pedigree were recorded.

Measurement of live body weight of Zaraibi and Damascus kids

Data on Zaraibi and Damascus kids at different ages including live body weights at birth (BW), 3 months (3MW), and 12 months (12MW) were determined by individual weighing on an electronic scale with accuracy of± 0.05 kg.

Measurement of milk yield of Zaraibi goats

The elected Zaraibi dairy goats were primiparous at first parity of lactation with healthy and symmetrical udders. Zaraibi goats were examined daily for clinical signs of mastitis. Any dairy goats suffering from mastitis were eliminated from the study. Estimation of milk yield (MY) during suckling period was calculated biweekly for each doe by weigh-suckle-weigh method in addition to the residual milk obtained by hand milking (Benson *et al*., 1999). Kids were separated from their dams and allowed only to access milk from their dams at 08:00 and 17:00 o'clock daily. This protocol started at 3^{rd} day after birth until weaning at day 90^{th} day. The MY of the doe was calculated by adding the suckled milk at the two periods by the kid to the residual milk (Benson *et al*., 1999). After suckling period, MY was recorded on a biweekly basis for each individual goat throughout their first parity. Animals were hand milked twice weekly in the morning with two workers having comparable efficiency. The yield of each doe was determined by weighing the milk then lactation stage was divided into seven months across the first studied parity.

DNA extraction and polymerase chain reaction

Genomic DNA was extracted from blood samples of the 160 goats using a GeneJET Genomic DNA Purification Kit, following the manufacturer's protocol (#K0721, Fermentas, Waltham, MA, USA). DNA fragment of 268 bp from exon III of the *α-LA* gene was amplified using previously published primers (Cosenza *et al*., 2003). PCR mixture was consisted of 1.0 µM forward and reverse primers, 0.2 mM dNTPs, and 1.25 U of Taq polymerase. The reaction mixture was added to PCR tubes containing 50 ng of goat genomic DNA. Reactions were conducted using the following cycling conditions: initial denaturation for 5 min at 95°C followed by 30 cycles of denaturation at 95°C (60 sec), annealing at 60°C (60 sec) and extension at 72°C (120 sec) and the final extension for 10 min at 72°C. PCR amplicons were tested by electrophoresis on 2% agarose gel in 1x TBE buffer alongside a Gene Ruler TM

100 bp Ladder (Thermo Fisher Scientific, Waltham, MA, USA) as a molecular weight marker. Agarose gels were stained with ethidium bromide (Gibco-BRL, Waltham, MA, USA) and visualized on UV trans-illuminator.

Single-stranded conformation polymorphism (SSCP)

PCR products of *α-LA* gene were genotyped by SSCP analysis method (Orita *et al*., 1989). The obtained conformation patterns were identified and transformed into corresponding genotypes (TT and TC) according to sequenced samples. Equal volumes (5 µl) of PCR products and denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanol, and 0.025% bromophenol blue) were mixed and heated at 98°C for 10 min, and then chilled on ice. Denatured DNA was subjected to polyacrylamide gel electrophoresis (80×73×0.75 mm) in 1x TBE buffer at 200 voltages for 2.5-3.0 hours. Gels were visualized under UV light after staining with ethidium bromide then photographed with an FX Molecular Imager apparatus (BIO-RAD, Hercules, CA, USA).

DNA sequence

DNA sequencing was conducted for four samples; two samples from each conformation pattern for converting these patterns into the corresponding genotypes (TT and TC). The amplicons were purified by a PCR Purification Kit (Roche, Mannheim, Germany) and sequenced using the same primers. A Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) was used for sequencing the samples according to the standard protocol, and then electrophoresed on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). BLAST software (Altschul *et al*., 1990) was used for sequence identification and confirmation. Finch TV 1.4.0 (Geospiza, Inc., Seattle, WA, USA) and MEGA 7 (Kumar *et al*., 2016) softwares were used for sequence alignment and mutations detection.

Statistical analysis

Allele and genotype frequencies, heterozygosity (H_E), number of effective alleles (N_E), and Hardy-Weinberg equilibrium were estimated by using a GENALEX version 6.5 (Peakall and Smouse, 2012). The associations of different *α-LA* genotypes with body weight and milk yield traits were conducted using a two-way analysis of variance with fixed effects in a general linear model (GLM) using the SAS software ver 9.1.3 (SAS Institute Inc, Cary, NC, USA). The following linear models were used for the studied traits:

$Y_{ijkl}=\mu + G_i + B_j + T_k + S_l + B(T)_{jk} + e_{ijkl}$ Model 1

Where Y_{ijkl} is the body weight measurement, G_i is the fixed effect of the i^th^ genotype (2 genotypes; TT and TC), B_j is the fixed effect of the j^th^ breed (2 breeds; Zaraibi and Damascus), T_k is the fixed effect of the k^th^ type of birth (three types; single, twins and triplets), S_l is the fixed effect of the l^th^ season of kidding (two seasons; Autumn and Spring), $B(T)_{jk}$ is the interaction between the breed and type of birth, and e_{ijkl} is the random error assumed to be normally distributed with a mean = 0 and a variance = $\sigma^2 e$

$Y_{ijk}=\mu + G_i + L_j + M_k + M(L)_{jk} + e_{ijk}$ Model 2

Where Y_{ijk} is the milk yield measurement, G_i is the fixed effect of the i^{th} genotype (2 genotypes; TTand TC), L_j is the fixed effect of the j^{th} litter size (three; single, twins and triplets), M_k is the fixed effect of the k^{th} stage of lactation (7 stages; 1, 2, …, and 7), $M(L)_{jk}$ is the interaction between stage of lactation and litter size, and e_{ijk} is the random error assumed to be normally distributed with a mean = 0 and a variance = $\sigma^2 e$

RESULTS

Sequence alignment and phylogenetic tree

The nucleotide sequences of *α-LA* gene exon III that were conducted in this study were deposited in GenBank (NCBI accession No. MT163744.1 and MT163745.1). Alignment of the nucleotide sequences of this study via BLAST (Altschul *et al*., 1990) and Mega 7 (Kumar *et al*., 2016) software with the already published reference sequences of the caprine *α-LA* exon III gene including Spanish breeds (NCBI accession No. KF781121.1), Saudi breeds (NCBI accession No. KP940442.1), Chinese breeds (NCBI accession No. DQ673921.1 and DQ629104.1), French breeds (NCBI accession No. M63868) and Indian breeds (NCBI accession No. EU573193.1) revealed complete similarity except for nine SNPs at the start of exon III which resulted in changing of four amino acids at the beginning of exon III as shown in Figure 1.

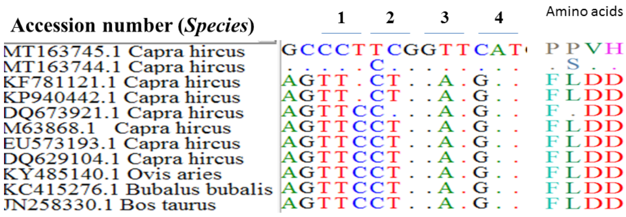

Fig. 1. Multiple sequence alignment of the first 14 nucleotides of α-LA gene exon III and their corresponding amino acids (NCBI accession No.MT163744.1 and MT163745.1) of this study with six *α-LA* reference sequences of goats retrieved from GenBank (NCBI accession No. KF781121.1 and KP940442.1 and DQ673921.1 and DQ629104.1 and M63868 and EU573193.1), *Ovis aries* (NCBI accession No. KY485140.1), *Bubalus bubalis* (NCBI accession No. KC415276.1) and *Bos taurus* (NCBI accession No. JN258330.1) species. P, Proline; V, Valine; H, Histidine; S, Serine; F, Phenylalanine; L, Leucine; D, Aspartic acid.

Neighbor-joining phylogenetic tree based on nucleotide sequences of *α-LA* gene exon III of Egyptian goats with other goats' reference sequences revealed that Egyptian sequences clustered together in one clade while others clustered in another clade with *Ovis aries* species as shown in Figure 2. The clustering pattern of *Bubalus bubalis* and *Bos taurus* might be explained by their different species type.

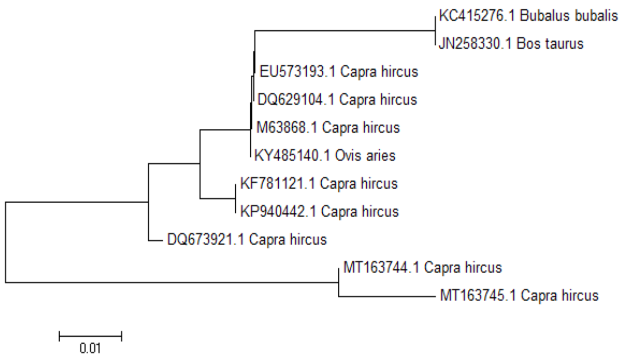

Fig. 2. Neighbor-joining phylogenetic tree based on nucleotide sequences of *α-LA* gene exon III fragments of Egyptian goats (NCBI accession No.MT163744.1 and MT163745.1), with six *α-LA* reference sequences of goats retrieved from GenBank (NCBI accession No. KF781121.1 and KP940442.1 and DQ673921.1 and DQ629104.1 and M63868 and EU573193.1), *Ovis aries* (NCBI accession No. KY485140.1). *Bubalus bubalis* (NCBI accession No. KC415276.1) and *Bos taurus* (NCBI accession No. JN258330.1) species act as out-group.

Polymorphism in the α-LA *gene in Egyptian goat breeds*

The SSCP analysis showed two different conformation patterns (two genotypes) in the investigated three Egyptian goat breeds (Fig. 3).

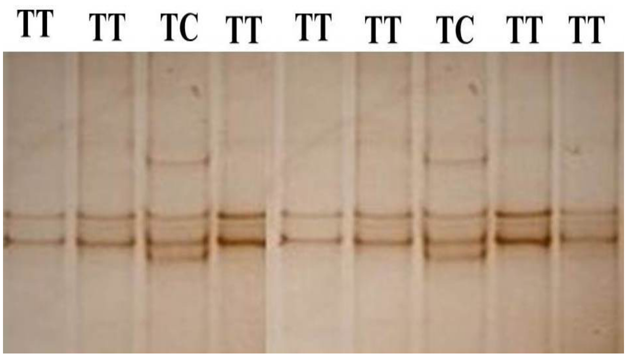

Fig. 3. PCR-SSCP conformation patterns of *α-LA* gene showing the two different patterns (genotypes) of Egyptian goat breeds; conformation pattern I represents genotype TT and conformation pattern II represents genotype TC.

DNA sequencing resulted in one non-synonymous MT163744: g.128T>C SNP (TCG ^{Ser}> CCG ^{Pro} at position 81 of the mature protein) and within the sixth nucleotide in exon 3 of *α-LA* gene (Fig. 4).

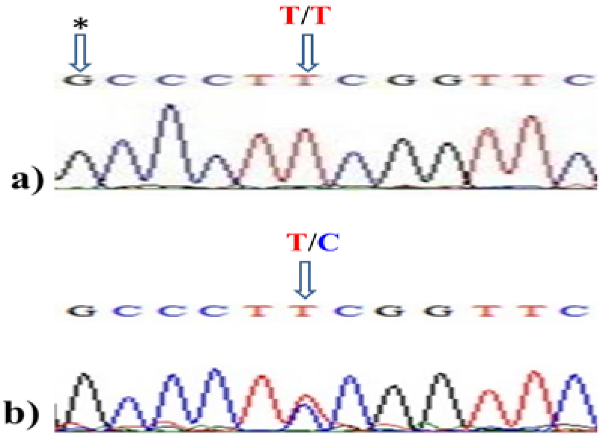

Fig. 4. Sequence chromatogram showing the *α-LA* gene exon III (268 bp) in Egyptian goat breeds. (a) Genotype TT; (b) Genotype TC, * means the start of exon III.

Among the three Egyptian goat breeds, the TT genotype showed the highest frequency of 78.1%, while the TC genotype occurred at a frequency of 21.9 %, and the CC genotype was absent. Genotype and allele frequencies, expected heterozygosity (H_E), number of effective alleles (N_E), and Hardy-Weinberg equilibrium are presented in Table I. The Damascus breed showed the highest values for T allele (0.929) and TT genotype (0.857) frequencies, while the Barki breed showed the highest H_E (0.260), and N_E (1.345). A chi square test showed that Egyptian goat populations were in Hardy-Weinberg equilibrium.

Effect of α-LA *genotype on body weight traits of Zaraibi and Damascus goat breeds*

Results of the association between *α-LA* genotype, breed, type of birth and season of kidding on body weight of Zaraibi and Damascus goat breeds are presented in Table II. The results showed that the MT163744: g.128T>C SNP of *α-LA* gene had non-significant effect on body weight traits. However, the T/C genotype recorded higher body weight at 12 months, the significance value was critical (P = 0.051). Body weights at birth, 3 months and 12 months of Damascus goat were higher and significant than those of Zaraibi. Kids born single of our study were heavier than twins and triplets at age of birth. On the contrary; there was no significant effect of kidding season on body weight traits. The interaction between the breed and type of birth was highly significant for studied body weight traits (data not shown).

Table I. Genetic polymorphism of *α-LA* gene in three Egyptian goat breeds.

Breeds	No.	Observed genotype frequencies			Expected genotype frequencies			Allele frequencies		H_E	N_E	Hardy-Weinberg equilibrium	
		TT	TC	CC	TT	TC	CC	T	C			χ^2-test	P value
Zaraibi	74	0.787	0.213	0.000	0.798	0.191	0.000	0.893	0.107	0.192	1.235	1.069	0.301^{ns}
Damascus	41	0.857	0.143	0.000	0.862	0.133	0.000	0.929	0.071	0.134	1.153	0.249	0.618^{ns}
Barki	45	0.698	0.302	0.000	0.721	0.257	0.000	0.849	0.151	0.260	1.345	1.364	0.248^{ns}
Mean ±SE	160	0.781 ±0.046	0.219 ±0.046	0.000 ±0.000	0.794 ±0.041	0.194 ±0.036	0.000 ±0.000	0.890 ±0.023	0.110 ±0.023	0.195 ±0.036	1.245 ±0.056		0.061^{ns}

No, number of animals; H_E, expected heterozygosity; N_E, effective number of alleles; ns, not significant.

Table II. Effect of α-LA genotype, breed, type of birth and season of kidding on live body weight (Kg) (LSM±SE) of Zaraibi and Damascusgoat breeds.

Independent factors	N	Weight at birth	P value	Weight at 3M	P value	Weight at 12M	P value
Genotype			0.910^{ns}		0.420^{ns}		0.051
TT	93	2.37±0.08		11.42±0.32		24.78±0.87^{b}	
TC	22	2.38±0.12		11.01±0.46		27.44±1.24^{a}	
Breed			0.001***		0.001***		0.012*
Zaraibi	74	1.63±0.09^{b}		10.03±0.35^{b}		24.07±0.96^{b}	
Damascus	41	3.13±0.12^{a}		12.39±0.48^{a}		28.14±1.30^{a}	
Type of birth			0.010**		0.132 ^{ns}		0.378 ^{ns}
Single	33	2.59±0.09^{a}		11.13 ±0.37		25.69±1.01	
Twins	74	2.54±0.08^{a}		10.63 ±0.32		25.01±0.87	
Triplets	8	2.003±0.17^{b}		11.89 ±0.66		27.63±1.79	
Season of kidding			0.269^{ns}		0.681^{ns}		0.421^{ns}
Autumn	25	2.44±0.11		11.31±0.44		26.59±1.19	
Spring	90	2.31±0.08		11.12±0.29		25.62±0.80	

Within column, means bearing different superscripts differ significantly. No, number of animals; LSM, least square mean; SE, standard error; ***P < 0001 ns, not significant; *p<0.05; **p<0.01; ***p<0.001

Effect of α-LA *genotype on milk yield of Zaraibi goat breed*

The associations between the *α-LA* genotype, litter size and lactation stage on the first parity milk yield of Zaraibi goats are presented in Table III. The results of this study showed that the MT163744: g.128T>C SNP of *α-LA* gene had a significant effect on milk yield of Zaraibi goats, with higher values observed in individual goats carrying the T/T than T/C genotypes. The triplets litter size recorded a higher and significant value for milk yield trait. Milk yield decreased significantly from early to late lactation stage. The interaction between lactation stage and litter size was highly significant for milk yield trait (data not shown).

DISCUSSION

In recent years, a growing demand for goat milk consumption has driven the commercialization of dairy goat farming and has urged the researchers for studying the factors affecting the productive traits of goats (El-Tarabany *et al.*, 2018). Our study aimed to identify *α-LA* gene polymorphism in Egyptian goat breeds and determine its association with the growth and milk yield traits. The differences between the nucleotide sequences alignment in addition to the clustering pattern in the phylogenetic tree of Egyptian goat sequences after comparing them with the reference sequences of the caprine *α-LA* gene might be attributed to the differences in the breed origin and to the different selection programs applied on Egyptian goat breeds. Two genotypes were detected in exon III of the Egyptian *α-LA* gene in this study. In the three investigated goat breeds, the T/T genotype predominated with a frequency of 78.1%. Similar results were reported by Lan *et al*. (2007), who identified only two *α-LA* genotypes; T/T and T/C with frequencies ranged from 95.3% to 100.0% and from 0.00 % to 0.047% in nine Chines goat breeds respectively. Moreover, Cosenza *et al*. (2003) reported the highest frequency of 0.83% for the T/T genotype in three Italian goat breeds. By contrast, Jain *et al*. (2008) reported only one conformational pattern of *α-LA* exon III gene in Indian Jakhrana goats.

Table III. Effect of α-LA genotype, litter size, lactation stage on milk yield (litre) (LSM±SE) of Zaraibi goat breed.

Independent factors	N	Milk yield of 1st parity	P value
Genotype			0.025*
TT	58	26.69±0.76[a]	
TC	16	24.72±1.03[b]	
Litter size			0.004**
Single	32	23.24±0.67[b]	
Twins	39	23.20±0.65[b]	
Triplets	3	30.67±1.89[a]	
Lactation stage (monthly)			0.007**
1	74	53.66±1.91[a]	
2	74	42.07±1.91[b]	
3	74	33.19±1.91[c]	
4	74	14.42±1.91[d]	
5	74	14.21±1.91[de]	
6	74	11.84±1.91[f e]	
7	74	10.54±1.91[f]	

Within column, means bearing different superscripts differ significantly. No. number of animals, LSM least square mean, SE standard error; * $p<0.05$; ** $p<0.01$.

Our results showed that T>C SNP of *α-LA* gene had non-significant effect on body weight traits. Similar results were reported by Ma *et al*. (2010) who recorded a non-significant effect of *α-LA* exon 1 gene on body size traits such as body height and height at the withers of Chinese dairy goats. Damascus goats of this study showed heavier and significant body weights at birth, weaning and yearling ages than Zaraibi ones. Youssef *et al*. (2014) reported similar results, where Damascus breed showed heavier body weights than Zaraibi at birth and weaning ages.

Birth weight of kids is considered as one of the most important contributory factors for growth and survival of goats (Tesema *et al*., 2017). Type of birth significantly effect on kids' birth weight of the current study, Kids born single were heavier than twins and triplets at birth age. Our results were in agreement with Mahal *et al*. (2013) in Bangladesh Black Bengal, Atoui *et al*. (2017) in Tunisian local goat and Tesema *et al*. (2017) in Ethiopian goats who reported heavier birth weight for kids born single. In contrast, Singh *et al*. (1990) reported a non-significant differences between birth weights of single, twins and triplets in the local and crossbred Indian kids. The differences in weights between different types of birth might be attributed to the intrauterine environment where more nutrients are available to the single kid, more space as well as lack of competition may improve growth and increase the birth weight of single born kid. The reduced birth weights of twins and triplets born kids should be corrected and compensated by extra managerial care such as longer periods of suckling before milking the extra milk. Moreover, the farmers should maintain the body weight of dams during mating and pregnancy by improving the feeding and managerial condition in order to improve kids' birth weight (Tesema *et al*., 2017). In agreement with previous studies that recorded a non-significant effect of kidding season on birth weight of Emirati goats (Al-Shorepy *et al*., 2002) and on body weights at 6 months, 12 months and 18 months of Ethiopian goats (Dadi *et al*., 2008), our study recorded non-significant effects for season of kidding on the studied traits.

Regarding the factors affecting milk yield of Zaraibi goat breed, the results of this study showed that T>C SNP of *α-LA* gene had a significant effect on milk yield, with the individual goats carrying T/T genotype yielded more milk than T/C ones. In goats, Dettori *et al*. (2015) recorded similar results to those in our study, with significant associations between two SNPs in *α-LA* promoter region gene with daily milk yield in Italian Sarda goats. By contrast, Ma *et al*. (2010) and El-Hanafy *et al*. (2016) reported non-significant associations between the *α-LA* gene exon I and exon III with milk yield in Chinese and Saudi goats respectively. Previous studies have reported significant associations between *α-LA* gene with milk yield traits in cattle (Voelker *et al*., 1997; Kazmer *et al*., 2001) and in buffalo (Kazmer *et al*., 2001).

The litter size of this study significantly affects the milk yield of Zaraibi goats with the highest volume of milk was yielded for does with three kids. Consistent with our finding, does with three or more kids recorded the highest milk yield in Australian dairy goats (Zamuner *et al*., 2020) and in Murciano-Granadina Spanish goats (Pleguezuelos *et al*., 2015). By contrast, Alkass and Merkhan (2011) reported non-significant association between litter size and milk yield of Iraqi goats. The physiological mechanisms during pregnancy in addition to the suckling reflex might explain why the does with three kids yields more milk than single and twins (Mech *et al*., 2008). Milk yield of Zaraibi goats in the current study was significant and higher at the first month of lactation stage, probably due to the increase in the number and efficiency of secretory cells at this lactation stage. Our results were in agreement with those reported by Mahmoud *et al*. (2014) in Sudanese Damascan goats, and El-Tarabany *et al*. (2018) in Egyptian Baladi goats who reported that the highest and significant milk yield were recorded during the first stage of lactation. On the contrary, Idamokoro *et al*. (2017) reported non-significant differences at different lactation stages in South African goats

In conclusion, Damascus goats of this study showed a significant and heavier body weight than Zaraibi, indicating that this imported breed still expresses its genetic potential under the Egyptian environmental conditions. The current study showed that litter size and lactation stage played significant roles in the profitability of Zaraibi goats through their effect on milk yield. Zaraibi goats with the highest milk yield were recorded for does with three kids and at the first month of lactation stage. Kids born single were heavier than twins and triplets at age of birth, so that twins and triplets born kids should be compensated by extra managerial care. Our results suggested that the T/C SNP of the *α-LA* gene significantly affected milk yield in Zaraibi goats. Such a polymorphic locus may be useful as a marker in assisted selection programs for the improvement of milk yield in the Zaraibi goats. Further cataloging of the Egyptian goats'*α-LA* gene and further studies on the possible genetic association of this gene with functional properties of whey, whey products and milk renneting in goats are eagerly anticipated.

ACKNOWLEDGMENTS

We would like to thank the goat breeders in different farms for helping the authors collect blood and milk samples. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Alkass, J. and Merkhan, K., 2011. Milk production traits of indigenous black and Meriz goats raised under farm production system. *Res. Opin. Anim. Vet. Sci*., **1**: 708-713.

Al-Shorepy, S., Alhadrami, G. and Abdulwahab, K., 2002. Genetic and phenotypic parameters for early growth traits in Emirati goat. *Small Rumin. Res*., **45**: 217-223. https://doi.org/10.1016/S0921-4488(02)00110-4

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J., 1990. Basic local alignment search tool. *J. mol. Biol*., **215**: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

Atoui, A., Hajejji, Z., Abdennebi, M., Gaddour, A. and Najari, S., 2017. Environmental factors affecting birth weight of Tunisian local goat population kids. *J. New Sci*., **38**: 2069-2076.

Benson, M., Henry, M. and Cardellino, R., 1999. Comparison of weigh-suckle-weigh and machine milking for measuring ewe milk production. *J. Anim. Sci*., **77**: 2330-2335. https://doi.org/10.2527/1999.7792330x

Caroli, A., Chiatti, F., Chessa, S., Rignanese, D., Ibeagha-Awemu, E. and Erhardt, G., 2007. Characterization of the casein gene complex in West African goats and description of a new αs1-casein polymorphism. *J. Dairy Sci*., **90**: 2989-2996. https://doi.org/10.3168/jds.2006-674

Cosenza, G., Gallo, D., Illario, R., Di Gregorio, P., Senese, C., Ferrara, L. and Ramunno, L., 2003. A MvaI PCR-RFLP detecting a silent allele at the goat α-lactalbumin locus. *J. Dairy Res*., **70**: 355-357. https://doi.org/10.1017/S0022029903006265

Dadi, H., Duguma, G., Shelima, B., Fayera, T., Tadesse, M., Woldu, T. and Tucho, T., 2008. Non-genetic factors influencing post-weaning growth and reproductive performances of Arsi-Bale goats. *Livest. Res. Rural*., **20**: 1-5.

Dayal, S., Vohra V., Kumar, P. and Sharma, A., 2006. Effect of alpha-lactalbumin gene polymorphism on milk production traits in water buffalo. *Asian Austral. J. Anim. Sci*., **19**: 305-308. https://doi.org/10.5713/ajas.2006.305

Dettori, M.L., Pazzola, M., Paschino, P., Pira, M.G. and Vacca, G.M., 2015. Variability of the caprine whey protein genes and their association with milk yield, composition and renneting properties in the Sarda breed. 1. The LALBA gene. *J. Dairy Res*., **82**: 434-441. https://doi.org/10.1017/S0022029915000461

El-Hanafy, A.A.-E.M., Qureshi, M.I., Sabir, J.S.M., Mutwakil, M., Ramadan, H.A.-M.I., El-Ashmaoui, H., Abou-Alsoud, M. and Ahmed, M.M.M., 2016. Allele mining in the caprine alpha-lactalbumin (LALBA) gene of native Saudi origin. *Biotechnol. Biotechnol. Equip*., **30**: 1115-1121. https://doi.org/

10.1080/13102818.2016.1224683

El-Tarabany, M.S., El-Tarabany, A.A. and Roushdy, E.M., 2018. Impact of lactation stage on milk composition and blood biochemical and hematological parameters of dairy Baladi goats. *Saudi J. biol. Sci*., **25**: 1632-1638. https://doi.org/10.1016/j.sjbs.2016.08.003

Galal, S., Abdel-Rasoul, F., Anous, M.R. and Shaat, I., 2005. On-station characterization of small ruminant breeds in Egypt. In: *Characterization of small ruminant breeds in West Asia and North Africa* (ed. L. Iniguez). ICARDA, Aleppo, Syria, pp. 141-193.

Hejtmánková, A., Pivec, V., Trnková, E. and Dragounová, H., 2012. Differences in the composition of total and whey proteins in goat and ewe milk and their changes throughout the lactation period. *Czech J. Anim. Sci*., **57**: 323-331. https://doi.org/10.17221/6007-CJAS

Idamokoro, E.M., Muchenje, V. and Masika, P.J., 2017. Yield and milk quality parameters at different stages of lactation from a small herd of nguni, boer and non-descript goats raised in an extensive production system. *Sustainability*, **9**: 1-13. https://doi.org/10.20944/preprints201705.0095.v1

Jain, A., Gour, D., Bisen, P., Dubey, P., Prashant, Sharma, D., Bhargava, A., Joshi, B. and Kumar, D., 2008. Single strand conformation polymorphism detection in alpha-lactalbumin gene of Indian Jakhrana milk goats. *Res. J. appl. Sci*., **58**: 205-208. https://doi.org/10.1080/09064700802609494

Jnied, A., Anous, M., Eshak, M., Rashed, M. and Kayali, I.. 2013. Molecular genetic studies for Damascus goat breed raised under Egypatian condtions. *J. appl. Sci. Res*., **9**: 360-367.

Kazmer, G., Zhou, P., Troyer, J. and Strausbaugh, L., 2001. Haplotype analysis involving a novel polymorphism in bovine alpha-lactalbumin 5'flanking region. *J. Dairy Sci*., **84**: 1542-1544. https://doi.org/10.3168/jds.S0022-0302(01)70188-9

Khalil, M., Sammour, H. and El-Wardani, M., 2013. Socio-economic and technical evaluation of sheep and goat farms in North West Coast of Egypt. *Egypt. J. Sheep Goats Sci*., **8**: 29-42. https://doi.org/10.12816/0005018

Kumar, S., Stecher, G. and Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*., **33**: 1870-1874. https://doi.org/10.1093/molbev/msw054

Lan, X., Pan, C., Chen, H., Zhang, C., Zhang, A., Zhang, L., Li, J. and Lei, C., 2007. An MspI PCR-RFLP detecting a single nucleotide polymorphism at alpha-lactalbumin gene in goat. *Czech J. Anim. Sci*., **52**: 138. https://doi.org/10.17221/2231-CJAS

Lan, X., Chen, H., Tian, Z., Liu, S., Zhang, Y., Wang, X. and Fang, X.. 2008. Correlations between SNP of LALBA gene and economic traits in Inner Mongolian white cashmere goat. *Hereditas*, **30**: 169-174. https://doi.org/10.3724/SP.J.1005.2008.00169

Layman, D.K., Lönnerdal, B. and Fernstrom, J.D., 2018. Applications for α-lactalbumin in human nutrition. *Nutr. Rev*., **76**: 444-460. https://doi.org/10.1093/nutrit/nuy004

Le Parc, A., Dallas, D.C., Duaut, S., Leonil, J., Martin, P. and Barile, D., 2014. Characterization of goat milk lactoferrin n-glycans and comparison with the N-glycomes of human and bovine milk. *Electrophoresis*, **35**: 1560-1570. https://doi.org/10.1002/elps.201300619

Ma, R., Deng, C., Zhang, X., Yue, X., Lan, X., Chen, H. and Lei, C., 2010. A novel SNP of α-lactalbumin gene in Chinese dairy goats. *Mol. Biol*., **44**: 536-540. https://doi.org/10.1134/S0026893310040059

Mahal, Z., Khandoker, M. and Haque, M., 2013. Effect of non genetic factors on productive traits of Black Bengal goats. *J. Bangladesh Agric. Univ*., **11**: 79-86. https://doi.org/10.3329/jbau.v11i1.18217

Mahmoud, N., El-Zubeir, I. and Fadlelmoula, A., 2014. Effect of stage of lactation on milk yield and composition of first kidder Damascus does in the Sudan. *J. Anim. Prod. Adv*., **4**: 355-362.

Mech, A., Dhali, A., Prakash, B. and Rajkhowa, C., 2008. Variation in milk yield and milk composition during the entire lactation period in Mithun cows (*Bos frontalis*). *Livest. Res. Rural*, **20**: 1-9.

Mioč, B., Sušić, V., Antunović, Z., Prpić, Z., Vnučec, I. and Kasap, A., 2011. Study on birth weight and pre-weaning growth of Croatian multicolored goat kids. *Vet. Arh*., **81**: 339-347.

Mir, S.N., Ullah, O. and Sheikh, R., 2014. Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. *Afr. J. Biotechnol*., **13**: 555-565. https://doi.org/10.5897/AJB2013.13216

Nowier, A.M., Darwish, H.R., Ramadan, S.I. and Othman, O.E., 2019. Polymorphism of lactoferrin gene in Egyptian goats and its association with milk composition traits in Zaraibi breed. *Trop. Anim. Hlth. Prod*., pp. 1-7. https://doi.org/10.1007/s11250-019-02099-3

Orita, M., Suzuki, Y., Sekiya, T. and Hayashi, K., 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*, **5**: 874-879. https://doi.org/10.1016/0888-7543(89)90129-8

Peakall, R. and Smouse, P.E., 2012. GENALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* **28**: 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Pleguezuelos, F.J., Fern, L. and Gonzalo, C., 2015. Variation in milk yield, contents and incomes according to somatic cell count in a large dairy goat population. *Adv. Dairy Res*., **3:** Article number 145. https://doi.org/10.4172/2329-888X.1000145

Singh, D., Singh, C. and Mishra, H., 1990. Birth weight of pure and crossbred kids under extensive system of management in villages. *Indian J. Anim. Sci*., **60**: 375-377.

Tesema, Z., Tilahun, M., Deribe, B., Lakew, M., Belayneh, N., Zegeye, A. and Aychew, D., 2017. Effect of non-genetic factors on pre-weaning growth, survivability and prolificacy of Central Highland x Boer crossbred goats in North Eastern Ethiopia. *Livest. Res. Rural*, **29**: 11-17.

Voelker, G.R., Bleck, G.T. and Wheeler, M.B., 1997. Single-base polymorphisms within the 5’flanking region of the bovine α-lactalbumin gene. *J. Dairy Sci*., **80**: 194-197. https://doi.org/10.3168/jds.S0022-0302(97)75927-7

Youssef, H.F., El-Gendy, M., Saifelnasr, E., El-Sanafawy, H.A. and Saba, F.E., 2014. Relationship between body conformation and milk yield and composition in Zaraibi and Damascus Goats. *Egypt. J. Sheep Goats Sci*., **9**: 1-12.

Zamuner, F., DiGiacomo, K., Cameron, A. and Leury, B., 2020. Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia. *J. Dairy Sci*., **103**: 954-964. https://doi.org/10.3168/jds.2019-17051

Zhou, J. and Dong, C., 2013. Association between a polymorphism of the α-lactalbumin gene and milk production traits in Chinese Holstein cows. *Genet. mol. Res*., **12**: 3375-3382. https://doi.org/10.4238/2013.September.4.3