



Polymorphism of lactoferrin gene in Egyptian goats and its association with milk composition traits in Zaraibi breed

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Abstract

The objectives of this study were to identify polymorphisms in the lactoferrin gene among three Egyptian goat breeds (Barki, Zaraibi, and Damascus) and to investigate the effect of *LF* genotype, parity, and lactation stage on milk composition traits of Zaraibi goats. One hundred and thirty-two blood samples were collected for DNA extraction, with 53 from Zaraibi, 40 from Damascus, and 39 from Barki breeds. Fat, protein, total solids, solids-not-fat, and lactose percentages were determined in Zaraibi goat milk using an automatic milk analyzer. Two genotypes, GG and GA, in the lactoferrin gene were identified using single-strand conformation polymorphism and were confirmed by direct sequencing technique. The Zaraibi breed recorded the highest heterozygosity (0.272) and effective number of alleles (1.369), while the Damascus breed recorded the lowest values. The G/A SNP showed a significant association with protein, solids-not-fat, and total solid content of Zaraibi goat milk. Protein, solids-not-fat, and total solid content in our study were significantly higher at early and late parities. Lactose percentage decreased significantly from early to late parity. Fat, protein, solids-not-fat, and total solid content were significantly higher at early and late stages of lactation, and our results encourage the utilization of Zaraibi goat milk in cheese and butter processing at these stages. Moreover, the G/A SNP of the *LF* gene may be a useful marker for assisted selection programs to improve goat milk composition.

Keywords Goat · Lactoferrin gene · Milk composition · Lactation stage · Parity

Introduction

Goats are widely distributed globally, and they are considered one of the most economically important livestock in Egypt. There are approximately 4.3 million goats in Egypt, and the major native breeds are Baladi and Barki, which are reared for meat production; and Zaraibi, which is reared for milk production (Galal et al. 2005). Goats play an important role in the production of meat, milk, skin, and fiber for communities living in arid and semi-arid regions. They are considered one of the

most efficient ruminant species, as they can adjust to both tropical and desert environments (Metawi 2011; Kaliber et al. 2016). Moreover, goats are less expensive and easier to keep than other livestock in developing countries, like Egypt, where space and capital are limited (Khalil et al. 2013). The Damascus goat, also known as Shami, is a native breed of Syria. This breed is considered one of the best dual-purpose breeds in the Middle East, combining high prolificacy with high milk production (Al-Atiyat and Aljumaah 2014; Abd El-Hamid et al. 2017). The Ministry of Agriculture in Egypt imported bucks and does of the Damascus breed for crossing with Barki goats to improve their milk production. Goat milk can be consumed as is, but a large proportion of it is also processed to make butter, cheese, ice cream, and other dairy products. However, the uneven composition and quality of the milk may be reflected in butter and cheese quality (Pavić et al. 2002). Previous studies have shown that the yield and composition of goat milk are influenced by genetic and non-genetic factors, including the genetic type of the animal, age, lactation stage, parity, and management (Bhosale et al. 2009; Mahmoud et al. 2014; Šlyžius et al. 2017; El-Tarabany et al. 2018).

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Lactoferrin (LF) is an 80 kDa glycoprotein belonging to the serum transferrin gene family (Guo et al. 2010). This iron-binding glycoprotein is found in many exocrine secretions, including milk, semen, saliva, and tears (Wakabayashi et al. 2006). LF is produced by epithelial cells of the mucosa in various mammalian species, including humans, mice, and livestock, such as cattle, buffalo, camels, sheep, and goat (Kang et al. 2011; Elijah and Ayoadele 2017). LF plays an important role in both innate and adaptive immune responses. The activities of LF against bacteria (González-Chávez et al. 2009), viruses (Małaczewska and Rotkiewicz 2007), inflammation (Wakabayashi et al. 2006), and tumors (Małaczewska and Rotkiewicz 2007) have been reported. The concentration of LF in goat milk is slightly higher than in cow milk (Hiss et al. 2008). Due to its high digestibility, immunological properties, and similarities between human and goat LF, goat milk is highly recommended for infants, the elderly, and convalescents (Le Parc et al. 2014; Kanwar et al. 2015).

The gene encoding LF is a multifunctional gene involved in economically important traits, like milk protein composition and skeletal structure in goats (Guo et al. 2010; García-Montoya et al. 2012; Ali and Al-Samarai 2018). Genetic polymorphisms have been used by goat breeders as a tool for the selection of the best animals. Single-strand conformation polymorphism (SSCP) is considered a powerful molecular technique for the detection of mutations in amplified DNA fragments and may be used as a tool for the selection of economically important traits (Caroli et al. 2007). However, studies of the effects of *LF* gene polymorphisms on milk composition traits in goats are scarce (Guo et al. 2010; Ali and Al-Samarai 2018). Therefore, the objectives of this study were first, to identify polymorphisms in exon II of the *LF* gene among three Egyptian goat breeds (Barki, Zaraibi, and Damascus) and second, to investigate the effect of *LF* gene polymorphisms and the effect of non-genetic factors, such as lactation stage and parity, on milk composition traits in Zaraibi goats.

Materials and methods

Animals

Blood samples were collected from 132 milking dame goats belonging to the three goat breeds reared in Egypt. Of the goats included in this study, 53 were Zaraibi (EL-Serw experimental station), 40 were Damascus (El-Gemeza experimental station), and 39 were Barki breeds (Borg El-Arab experimental station). These stations belong to the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture in Egypt.

Herd management and feeding

Goats were housed in semi-open pens and fed on Egyptian clover (*Trifolium alexandrinum*) from December to May. For the rest of the year, they were fed on conserved rice straw and green fodder, if available, and a concentrate mixture. The components of the concentrate mixture were maize, cotton seed cake, rice bran, wheat bran, sodium chloride, and calcium carbonate, with an average composition of 16% crude protein, 3% crude fat, and 15% crude fiber. Goats were fed each day for 2 weeks before the mating season and from the second to the fourth week of pregnancy; dame goats were supplemented with 0.5 kg of concentrate mixture. Furthermore, milking goats were supplemented with 1 kg of concentrate mixture daily. The Zaraibi flocks were divided into two groups for two mating seasons per year (June and October). During the October mating season, Zaraibi dames were randomly divided into mating groups and each was randomly assigned to a fertile buck. The mating period lasted for 45 days, and the pregnancy period lasted for 145–150 days. Young females were first mated at the age of 18 months. At kidding, newborns were tagged and their type of birth, gender, and pedigree were recorded. Moreover, milk production by their dames was recorded daily until drying off.

Analysis of milk composition

Zaraibi dairy goats were examined daily for clinical signs of mastitis. Any dairy goats suffering from mastitis were eliminated from the study. Milk yield was recorded on a daily basis throughout the experiment for each individual goat, but milk composition was evaluated weekly. Lactation stage was divided into 7 months, and milk composition data were collected across five parities. Animals were hand-milked daily in the morning, and the yield from each doe was determined by weighing the milk. Milk samples of approximately 100 mL were collected aseptically, preserved using the Broad Spectrum Microtabs II tablets (Advanced Instruments, Norwood, MA, USA), and stored at refrigerator temperatures until analyzed. The composition of milk samples, including fat, protein, and lactose content was assessed using a Milko-Scan instrument (MilkoScan 6000, Foss Electric A/S, Hillerød, Denmark). Milk solids-not-fat (SNF) and total solids (TS) were measured according to the previously described methods (Cipolat-Gotet et al. 2013).

DNA extraction and polymerase chain reaction

DNA was extracted from goat blood using a GeneJET Genomic DNA Purification Kit, following the manufacturer's protocol (#K0721, Fermentas, Waltham, MA, USA). A DNA fragment of 247 bp from exon II of the *LF* gene was amplified using previously published primers

(Guo et al. 2010). Each PCR mix consists of 1.0 μ M upper and lower primers, 0.2 mM dNTPs, and 1.25 U of Taq polymerase. Aliquots of the reaction mix were added to PCR tubes containing 100 ng of goat DNA. Reactions were performed using the following cycling conditions: initial denaturation for 5 min at 95 °C; 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s; and a final extension at 72 °C for 7 min. Amplification was verified by electrophoresis on 2% agarose gels in a 1 $\times$ Tris-borate EDTA (TBE; 0.09 M Tris-boric acid and 0.002 M EDTA) buffer alongside a GeneRuler™ 100 bp Ladder (Thermo Fisher Scientific, Waltham, MA, USA) as a molecular weight marker to confirm the length of the PCR products. Gels were stained with ethidium bromide (Gibco-BRL, Waltham, MA, USA) and visualized on a UV transilluminator.

Single-stranded conformation polymorphism

LF gene PCR products were genotyped by SSCP analysis according to the method of Orita et al. (1989). The different conformation patterns were detected and transformed into corresponding genotypes (GG and GA) according to sequenced control samples. PCR product aliquots of 5 μ L were mixed with 5 μ L of denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanol, and 0.025% bromophenol blue), heated for 10 min at 98 °C, and then chilled on ice. Denatured DNA was subjected to polyacrylamide gel electrophoresis (80 $\times$ 73 $\times$ 0.75 mm) in 1 $\times$ TBE buffer at a constant voltage (200 V) for 2.5–3.0 h. Gels were stained with ethidium bromide in 1 $\times$ TBE buffer, visualized under UV light, and then photographed with an FX Molecular Imager apparatus (BIO-RAD, Hercules, CA, USA).

DNA sequencing

To convert different SSCP conformation patterns into corresponding genotypes (GG and GA), DNA sequencing was performed for the four control samples, with two samples

from each of the conformation patterns. The amplified products were purified using a PCR Purification Kit (Roche, Mannheim, Germany), and the resulting products were sequenced using the same primers as those used for PCR. Samples were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), according to the standard protocol, and electrophoresed on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). BLAST software (Altschul et al. 1990) was used for sequence identification and confirmation. The FinchTV 1.4.0 (Geospiza, Inc., Seattle, WA, USA, <http://www.geospiza.com>), MEGA 6 (Kumar et al. 2008), and Bioedit 7.0.5.3 (Hall 1999) software applications were used for sequence alignment and polymorphism detection.

Statistical analysis

Estimations of genotypic and allelic frequencies, heterozygosity (H_e), number of effective alleles, and Hardy-Weinberg equilibrium were carried out using a GENALEX version 6.0 (Peakall and Smouse 2006). The association of different *LF* genotypes with milk composition traits (fat, protein, lactose, TS, and SNF) was performed using a two-way analysis of variance with genotype, parity, and lactation stage as the fixed effects in a general linear model (GLM) using the SAS v9.1.3 statistical package (Institute 2004).

The following linear model was used for the studied traits:

$$Y_{ijkl} = \mu + G_i + Pa_j + LS_k + Pa (LS)_{jk} + e_{ijkl}$$

where Y_{ijkl} is the milk composition measurement, μ is the overall mean, G_i is the fixed effect of the i th genotypes (2 genotypes; GG and GA), Pa_j is the fixed effect of the j th parity (5 parities; 1st, 2nd..., and 5th), LS_k is the fixed effect of the k th lactation stage (7 stages; 1, 2..., and 7), $Pa (LS)_{jk}$ is the effect of the interaction between parity and lactation stage, and e_{ijkl} is the random error assumed to be normally distributed with a mean = 0 and a variance = σ^2_e .

Fig. 1 PCR-SSCP conformation patterns showing the two different patterns of Zaraibi goat *LF* gene; conformation pattern I represents genotype GG and conformation pattern II represents genotype GA

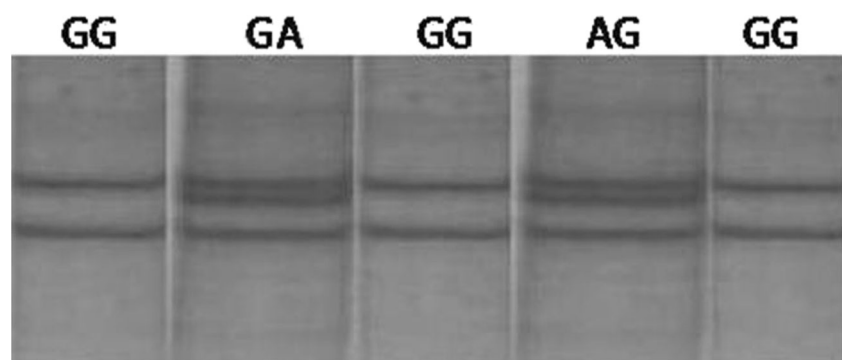
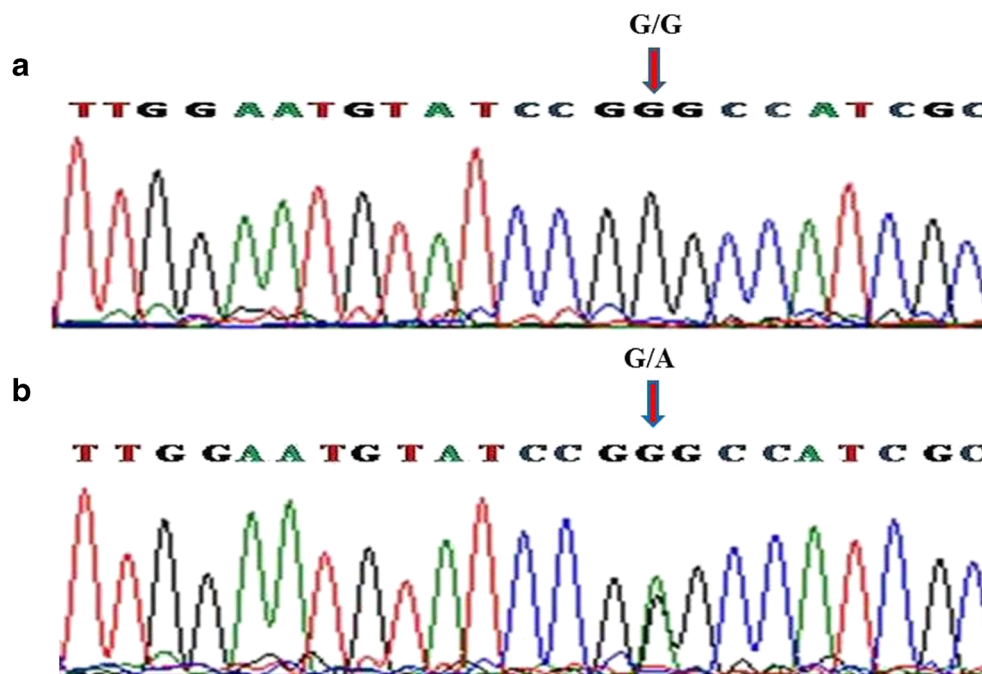


Fig. 2 Sequence chromatogram of *LF* gene exon II (247 bp) in Egyptian Zaraibi goats. **a** Genotype GG. **b** Genotype GA



Results

Polymorphism in the *LF* gene in Egyptian goat breeds

The results of the SSCP analysis showed the presence of two different conformation patterns (two genotypes) in the three investigated goat breeds (Fig. 1). Among the three tested goat breeds, the GG genotype showed the highest frequency of 72%, while the GA genotype occurred at a frequency of 28%, and the AA genotype was not present. Direct sequencing of PCR products confirmed the presence of the two genotypes (GG and GA) as shown in Fig. 2. The genotype and allele frequencies, *He*, number of effective alleles, and Hardy-Weinberg equilibrium results are presented in Table 1. The Zaraibi breed showed the highest values for A allele frequency (0.160), GA genotype frequency (0.321), *He* (0.272), and the effective number of alleles (1.369), while the Damascus breed showed the lowest values for these parameters. A chi square test showed that Egyptian goat populations were in Hardy-Weinberg equilibrium.

Effect of genotype at the *LF* gene G/A SNP on milk composition in Zaraibi goats

Results of the association between *LF* genotype and milk chemical composition (fat, protein, lactose, TS, and SNF) in the Zaraibi goat breed are presented in Table 2. The results showed that the G/A genotype had a significant effect on protein and SNF content, with higher values observed in individuals goats carrying the GA genotype. However, the *LF* gene G/A genotype had no significant effect on fat, lactose, or TS content.

Effect of parity and lactation stage on milk composition of Zaraibi goats

Results for the effect of parity on milk composition in the Zaraibi goat breed are presented in Table 3. Protein, TS, and SNF content were significantly higher at early and late parities. Lactose content decreased significantly from early to late parity. On the contrary, there was no significant effect of parity

Table 1 Genetic polymorphism of *LF* gene in three Egyptian goat breeds

Breeds	No.	Observed genotype frequencies			Expected genotype frequencies			Allele frequencies		<i>He</i>	<i>Ne</i>	Hardy-Weinberg equilibrium	
		GG	GA	AA	GG	GA	AA	G	A			χ^2 test	<i>P</i> value
Zaraibi	53	0.679	0.321	0.0	0.705	0.269	0.026	0.840	0.160	0.272	1.369	1.934	0.164 ^{ns}
Damascus	40	0.800	0.200	0.0	0.810	0.180	0.010	0.900	0.100	0.182	1.220	0.494	0.482 ^{ns}
Barki	39	0.692	0.308	0.0	0.716	0.260	0.024	0.846	0.154	0.264	1.352	1.289	0.256 ^{ns}
Total	132	0.720	0.280	0.0	0.739	0.241	0.020	0.860	0.140	0.242	1.318	3.507	0.061 ^{ns}

No. number of animals, *He* expected heterozygosity, *Ne* effective number of alleles, *ns* not significant

Table 2 Effect of *LF* genotypes on milk compositions in Zaraibi goat breed

Genotype	No.	Fat LSM ± SE	Protein LSM ± SE	Lactose LSM ± SE	Total solid LSM ± SE	Solids-not-fat LSM ± SE
GG	36	4.468 ± 0.049	2.733 ^b ± 0.016	4.431 ± 0.019	12.385 ± 0.064	7.870 ^b ± 0.030
GA	17	4.498 ± 0.073	2.919 ^a ± 0.023	4.494 ± 0.028	12.594 ± 0.094	8.102 ^a ± 0.040
<i>P</i> value	53	0.738 ^{ns}	0.001***	0.074 ^{ns}	0.069 ^{ns}	0.001***

Within column, means bearing different superscripts differ significantly

No. number of observed genotypes, *LSM* least square mean, *SE* standard error, *ns* not significant

****P* < 0.001

on fat percentage. Data regarding the variation in milk composition according to lactation stage for Zaraibi goats are presented in Table 4. Fat, protein, TS, and SNF content were significantly higher in the early and late stages of lactation. Although, lactose content was significantly affected by lactation stage, it showed a fluctuating trend. This interaction between parity and lactation stage was highly significant for all studied milk components (fat, protein, lactose, TS, and SNF; data not shown).

Discussion

In recent years, a growing demand for goat milk and processed products, like cheese, has driven the commercialization of dairy goat farming (Mestawet et al. 2012; El-Tarabany et al. 2018). The production of high-quality goat milk is an essential step towards successful goat milk and goat dairy product processing industries (Park and Guo 2006). Our study aimed to identify *LF* gene polymorphisms in Egyptian goat breeds and determine their association with the composition of Zaraibi goat milk. Two genotypes were identified in exon II of the *LF* gene using SSCP in our study. In the three investigated goat breeds, the GG genotype occurred at a frequency of 72% and the AG genotype occurred at a frequency of 28%.

Similar results were reported by Guo et al. (2010), who identified three *LF* genotypes, GG (92.7%), AG (4.6%), and

AA (2.7%), in two Chinese dairy goat breeds using SSCP and direct sequencing techniques. The frequencies of the G and A alleles were very similar between Egyptian (0.86 and 0.14) and Chinese (0.95 and 0.05) goat breeds. Our results showed that Zaraibi goats with heterozygous *LF* genotypes had significantly higher protein and SNF values than those homozygous for the G allele. The association between *LF* genotype and milk composition and body measurement traits has also been studied in the two Chinese dairy goat breeds, Guanzhong and Xinong Saanen. In contrast to our results, Guo et al. (2010) found a significant association between the GA genotype of the *LF* gene and protein and acidity traits in Guanzhong and Xinong Saanen dairy goats, with animals carrying the AA genotype having more favorable traits than those with the GA genotype.

Our results showed that Zaraibi goats produced milk with significantly higher protein, TS, and SNF content, but no significant difference in fat percentage at earlier and later parities. Similar results were obtained by Mioč et al. (2008), who reported a no significant effect of parity on fat percentage in milk from Croatian goats. Olechnowicz and Sobek (2008) and Šlyžius et al. (2017) showed similar results to those in our study, with protein and fat content being higher at earlier and later parities, and the lowest values were recorded at the third parity. By contrast, Bhosale et al. (2009) showed that protein, fat, TS, and SNF content increased significantly with the increase in parity order. The higher values for milk fat and

Table 3 Effect of parity on milk compositions traits in Zaraibi goat breed

Parity	No.	Fat LSM ± SE	Protein LSM ± SE	Lactose LSM ± SE	Total solid LSM ± SE	Solids-not-fat LSM ± SE
1	16	4.632 ± 0.079	2.844 ^{ab} ± 0.026	4.592 ^a ± 0.031	12.846 ^a ± 0.102	8.136 ^a ± 0.048
2	13	4.416 ± 0.085	2.731 ^c ± 0.027	4.454 ^b ± 0.032	12.301 ^b ± 0.109	7.881 ^b ± 0.051
3	11	4.381 ± 0.092	2.819 ^b ± 0.029	4.477 ^b ± 0.036	12.435 ^b ± 0.118	7.991 ^b ± 0.056
4	7	4.519 ± 0.108	2.910 ^a ± 0.034	4.334 ^c ± 0.042	12.451 ^b ± 0.139	7.954 ^b ± 0.066
5	6	4.465 ± 0.094	2.827 ^b ± 0.030	4.455 ^b ± 0.036	12.415 ^b ± 0.121	7.971 ^b ± 0.057
<i>P</i> value	53	0.271 ^{ns}	0.023*	0.001***	0.006*	0.011*

Within column, means bearing different superscripts differ significantly

No. number of parities, *LSM* least square mean, *SE* standard error, *ns* not significant

P* < 0.05, **P* < 0.001

Table 4 Effect of lactation stage within the parity on milk compositions in Zaraibi goat breed

Lactation stage (monthly)	No.	Fat LSM $\pm$ SE	Protein LSM $\pm$ SE	Lactose LSM $\pm$ SE	Total solid LSM $\pm$ SE	Solids-not-fat LSM $\pm$ SE
1	53	6.036 ^a $\pm$ 0.108	2.781 ^d $\pm$ 0.034	4.620 ^a $\pm$ 0.042	14.201 ^a $\pm$ 0.138	8.112 ^a $\pm$ 0.065
2	53	4.861 ^c $\pm$ 0.108	2.473 ^e $\pm$ 0.034	4.469 ^{ab} $\pm$ 0.042	12.542 ^c $\pm$ 0.138	7.648 ^{cd} $\pm$ 0.065
3	53	3.881 ^d $\pm$ 0.108	2.519 ^e $\pm$ 0.034	4.373 ^c $\pm$ 0.042	11.495 ^d $\pm$ 0.138	7.597 ^c $\pm$ 0.065
4	53	3.741 ^d $\pm$ 0.108	3.044 ^b $\pm$ 0.034	4.576 ^a $\pm$ 0.042	12.116 ^c $\pm$ 0.138	8.397 ^a $\pm$ 0.065
5	53	3.818 ^d $\pm$ 0.108	2.754 ^d $\pm$ 0.034	4.408 ^{bc} $\pm$ 0.042	11.668 ^d $\pm$ 0.138	8.305 ^{cd} $\pm$ 0.065
6	53	3.711 ^d $\pm$ 0.108	2.933 ^c $\pm$ 0.034	4.361 ^c $\pm$ 0.042	11.692 ^d $\pm$ 0.138	7.858 ^{cb} $\pm$ 0.065
7	53	5.330 ^b $\pm$ 0.108	3.281 ^a $\pm$ 0.034	4.430 ^{bc} $\pm$ 0.042	13.712 ^b $\pm$ 0.138	7.991 ^a $\pm$ 0.065
<i>P</i> value		0.001***	0.001***	0.001***	0.001***	0.001***

Within column, means bearing different superscripts differ significantly

No. number of lactation stage, *LSM* least square mean, *SE* standard error

****P* < 0001

protein content at the earlier and later parities in our study may be explained by the fact that goat milk yields at the beginning and the end of lactational parities are lower than in the middle parities, and this indicator correlates negatively with milk fat and protein content in goat breeds (Olechnowicz and Sobek 2008).

In agreement with most similar studies, lactose content was significantly reduced in goat milk at the late parities of the animal's life compared with the early and middle parities (Olechnowicz and Sobek 2008; Bhosale et al. 2009). Fat, protein, TS, and SNF content in Zaraibi goats in our study were significantly higher at the beginning and at the end of the lactation stage, probably due to decreased milk yield at these stages. This result indicated that Zaraibi milk should be used in cheese processing at either the early or the late stage of lactation. Our results were in agreement with those reported by Mestawet et al. (2012) in Ethiopian goats, by Mahmoud et al. (2014) in Damascan goats, and by Idamokoro et al. (2017) in South African goats. On the contrary, El-Tarabany et al. (2018) reported relatively stable protein and fat content with no significant differences at different stages of lactation in Egyptian Baladi goats.

Previous studies have reported conflicting results for protein and fat content at different stages of lactation. Some studies have reported that protein and fat content decrease significantly from early to late lactation stages (Olechnowicz and Sobek 2008; Strzałkowska et al. 2009; Mahmoud et al. 2014). However, others have found that protein, fat, TS, and SNF content increase as lactation stages progress (Strzałkowska et al. 2009; Ibnelbachyr et al. 2015; El-Tarabany et al. 2018). In contrast, our study found that lactate content significantly fluctuated with the stage of lactation. However, other authors have reported that lactose content significantly decreases from early to late lactation stages (Olechnowicz and Sobek 2008; Mahmoud et al. 2014; El-Tarabany et al. 2018). Moreover, others have reported that lactose content is higher

in the early and late stages of lactation than in other stages (Noutfia et al. 2014; Ibnelbachyr et al. 2015; Idamokoro et al. 2017).

In conclusion, our study showed that parity and lactation stage played significant roles in the profitability of Zaraibi goats through their effect on milk composition. Zaraibi goats produced milk with a higher percentage of fat, protein, TS, and SNF, at the beginning and at the end of lactation and at earlier and later parities of their productive life. Accordingly, these data suggest that their milk should be used for cheese and butter processing at these periods. Our results suggested that the G/A SNP of the *LF* gene significantly affected milk composition in Zaraibi goats. Such a polymorphic locus may be useful as a marker in assisted selection programs for the improvement of goat milk composition.

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Compliance with ethical standards

Statement of animal rights All procedures performed in studies 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.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abd El-Hamid, I., Ibrahim N., Farrag B., Younis F., and Wahba I., 2017. Reproductive and Productive Efficiency of Damascus and Baladi goats under Egyptian arid conditions, Research Journal of Animal and Veterinary. 9: 6-14.

- Al-Atiyat, R., and Aljumaah R., 2014. Genetic relatedness between Ardi, Black Bedouin and Damascus goat breeds, *Genetics and Molecular Research*. 13: 4654–65.
- Ali, H., and Al-Samarai W., 2018. Association of lactoferrin gene polymorphism with milk production and composition, *The Iraqi Journal of Agricultural Science*. 49: 200–204.
- Altschul, S.F., Gish W., Miller W., Myers E.W., and Lipman D.J., 1990. Basic local alignment search tool, *Journal of molecular biology*. 215: 403–410.
- Bhosale, S., Kahate P., Kamble K., Thakare V., and Gubbawar S., 2009. Effect of lactation on physico-chemical properties of local goat milk, *Veterinary world*. 2: 17–19.
- 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 α s1-casein polymorphism, *Journal of Dairy Science*. 90: 2989–2996.
- Cipolat-Gotet, C., Cecchinato A., De Marchi M., and Bittante G., 2013. Factors affecting variation of different measures of cheese yield and milk nutrient recovery from an individual model cheese-manufacturing process, *Journal of Dairy Science*. 96: 7952–7965.
- Elijah, A.F., and Ayoadele O.O., 2017. Genetic diversity of lactoferrin gene in-silico on selected Mammalian species, *Biotechnology in Animal Husbandry*. 33: 171–180.
- 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 journal of biological sciences*. 25: 1632–1638.
- Galal, S., Abdel-Rasoul F., Anous M.R., and Shaat I., 2005. On-station characterization of small ruminant breeds in Egypt, ICARDA, Aleppo, Syria.
- García-Montoya, I.A., Cendón T.S., Arévalo-Gallegos S., and Rascón-Cruz Q., 2012. Lactoferrin a multiple bioactive protein: an overview, *Biochimica et Biophysica Acta (BBA)-General Subjects*. 1820: 226–236.
- González-Chávez, S.A., Arévalo-Gallegos S., and Rascón-Cruz Q., 2009. Lactoferrin: structure, function and applications, *International journal of antimicrobial agents*. 33: 301–308.
- Guo, B. et al., 2010. A novel polymorphism of the lactoferrin gene and its association with milk composition and body traits in dairy goats, *Genetics and Molecular Research*. 9: 2199–2206.
- Hall, T.A., editor BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series*; 1999: [London]: Information Retrieval Ltd.
- Hiss, S., Meyer T., and Sauerwein H., 2008. Lactoferrin concentrations in goat milk throughout lactation, *Small Ruminant Research*. 80: 87–90.
- Ibnelbachyr, M., Boujenane I., Chikhi A., and Noutfia Y., 2015. Effect of some non-genetic factors on milk yield and composition of Draa indigenous goats under an intensive system of three kiddings in 2 years, *Tropical animal health and production*. 47: 727–733.
- 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: 13.
- Institute, S., 2004. SAS/ETS 9.1 User's Guide, SAS Institute, Kaliber, M., Koluman N., and Silanikove N., 2016. Physiological and behavioral basis for the successful adaptation of goats to severe water restriction under hot environmental conditions, *animal*. 10: 82–88.
- Kang, J.-F., Li X.-L., Zheng H.-Q., Zhou R.-Y., Li L.-H., and Zhao H.-Y., 2011. Sequence Analysis of the Lactoferrin Gene and Variation of g. 7605C → T in 10 Chinese Indigenous Goat Breeds, *Biochemical genetics*. 49: 63–72.
- Kanwar, J. et al., 2015. Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions, *Molecules*. 20: 9703–9731.
- 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, *Egyptian Journal of Sheep and Goat Sciences*. 8: 29–42.
- Kumar, S., Nei M., Dudley J., and Tamura K., 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences, *Briefings in bioinformatics*. 9: 299–306.
- 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.
- 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, *Journal of Animal Production Advances*. 4: 355–362.
- Małaczewska, J., and Rotkiewicz Z., 2007. Laktoferyna–białko multipotencjalne, *Medycyna Weterynaryjna*. 63: 136–139.
- Mestawet, T., Girma A., Ådnøy T., Devold T., Narvhus J., and Vegarud G., 2012. Milk production, composition and variation at different lactation stages of four goat breeds in Ethiopia, *Small Ruminant Research*. 105: 176–181.
- Metawi, H., 2011. Economic sustainability of goat production under different production systems in Egypt, *CIHEAM-Options Méditerranéennes*. 100: 185–190.
- Mioč, B. et al., 2008. Factors affecting goat milk yield and composition, *Mljekarstvo*. 58: 305–313.
- Noutfia, Y., Zantar S., Ibnelbachyr M., Abdelouahab S., and Ounas I., 2014. Effect of stage of lactation on the physical and chemical composition of Drâa goat milk, *African Journal of Food, Agriculture, Nutrition and Development*. 14: 1981–1991.
- Olechnowicz, J., and Sobek Z., 2008. Factors of variation influencing production level, SCC and basic milk composition in dairy goats, *Journal of Animal and Feed Sciences*. 17: 41.
- 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.
- Park, Y., and Guo M., 2006. Goat milk products: processing technology, types and consumption trends. *Handbook of Milk of Non-Bovine Mammals*. YW Park and GFW Haenlein, eds. Blackwell Publishers. Ames, Iowa and Oxford, England, 2006, Blackwell Publishers, Iowa and Oxford, England., p 59–106
- Pavić, V., Antunac N., Mioč B., Ivanković A., and Havranek J., 2002. Influence of stage of lactation on the chemical composition and physical properties of sheep milk, *Czech journal of animal science*. 47: 80–84.
- Peakall, R., and Smouse P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research, *Molecular ecology notes*. 6: 288–295.
- Šlyžius, E., Šlyžienė B., and Lindžiūtė V., 2017. Factors affecting goat milk fat yield, *Žemės ūkio mokslai*. 24: 91–100.
- Strzałkowska, N. et al., 2009. Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation, *Animal Science Papers and Reports*. 27: 311–320.
- Wakabayashi, H., Yamauchi K., and Takase M., 2006. Lactoferrin research, technology and applications, *International Dairy Journal*. 16: 1241–1251.