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PRL and CSN1S2 Genes and Milk Traits in Damascus and Zaraiby Goats

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GOAT MILK is considered one of the most important types of food in the world, as it is easy to digest for children and adults, and expensive cheese is made from it. Prolactin (PRL) and alpha casein 2 (CS1N2) genes are considered as biomarker selection for milk production in farm animals. The present study aimed to identify different genotypes and single nucleotide polymorphisms (SNPs) in PRL and CS1N2 genes correlated with milk traits in Damascus and Zaraiby goats. Sixty milk and blood samples were collected from Damascus (n=30) and Zaraiby (n=30) goat farm in Kafr El-Sheikh, Egypt. Different milk traits were determined in collected milk samples using biochemical methods. Different genotypes of PRL, CS1N2a and CSN1N2b genes were detected by SSCP-PCR technique. The results of biochemical analysis for milk samples showed that fat, protein, lactose, and solid non-fat (SNF) levels were (3.83, 3.7, 4.3, and 7.7) in Zaraiby goat milk, higher than Damascus goat milk (3.1, 3.3, 4.2, and 7.4). While pH levels were (6.63) in Damascus goat milk, higher than Zaraiby goat milk (6.62), the statistical analysis showed that differences between Damascus goat and Zarariby goat were significant in fat content and non-significant in other components PRL, CS1N2a and CSN1N2b fragments were detected by specific primers at 208 bp, 270 bp, and 230 bp, respectively. The results of SSCP-PCR analysis showed that PRL, CS1N2a, and CS1N2b were monomorphic patterns. This study recommends examining other places in these genes to detect new SNPs to explain the reasons for increased milk composition in Zaraiby goats compared to Damascus goats.

Keywords: PRL, CSN1S2, Gene, Milk composition, Goats.

Introduction

Goats are one of the most important types of livestock which produce a variety of products, such as milk, fibre, meat and leather [1]. Zaraiby goats are double purpose animals, for production of milk and meat [2]. Recently, the role of goat's milk in human nutrition has increased, due to its chemical, physical, sensory and nutritional properties that make it an effective substitute for cow's milk. Goat's milk differs from cow's milk mainly because of its smaller size of fat globules, which results in better digestibility and a higher proportion of short and medium-chain fatty acids, which are partly responsible for its stronger flavour [3,4]. Genetic variations in candidate genes correlated with economic traits are considered as genetic selection to determine evolutionary relationships in different livestock breeds [5]. The PRL gene plays a major role in growth, the hair

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growth cycle, and lactation [6]. Several studies indicated that the PRL polymorphism is correlated with cashmere traits or wool in goats and sheep [7]. Other researchers have studied the relationship of PRL polymorphisms with milk traits such as milk yield and milk protein yield in cattle breeds [8]. Moreover, many researchers have suggested the importance of PRL gene in improving reproduction in different sheep breeds [9]. CSN1S2 is one of a cluster of genes named CSN1S1, CSN2, CSN3 on chromosome 6 [10]. It accounts for more than 40% in cow's milk [11], and ranges from (0 to 25%) in goat's milk due to a polymorphism in the gene that codes for this protein [12]. Many investigations had showed the effect of CSN1S2 gene polymorphism on composition, production [13], technological properties [14], and fatty acid content of goat milk [15]. The presence of an extensive polymorphism in CSN1S2 has led to differences in casein synthesis [12], as well as its importance for the application of milk protein research in the dairy industry [16]. There are 7 known alleles at the CSN1S2 locus, and these alleles are associated with different levels of alpha-casein in goat milk [17]. Therefore, this study was focused on the genotypes of CSN1S2 and PRL genes in Zaraiby and Damascus goats to find favourable alleles that might be correlated with high contents of milk composition.

Experimental design

Samples collection and DNA extraction

Sixty blood samples were collected from Zaraiby (n=30) and Damascus (n=30) goats in tubes 10 ml containing EDITA, also, 60 milk samples were collected in plastic bottles (250ml) from Zaraiby and Damscus goats from Kafr El-Sheikh farm in Egypt. DNA was extracted from blood samples using mini kit blood DNA extraction (Biovission, Korea).

Biochemical analysis

Total protein measurement in milk

Total protein was measured by the semi-micro kjeldahl distillation method according to Kjeldahl, [18]. In briefly, the acidic digestion step was performed using conc. H_2SO_4. The nitrogen-containing milk sample was weighed in a long-necked digestion flask to converse into NH_4+ ions. Kjeldahl flasks with a capacity for 500–800mL and gas or electric heating have been used for the digestion. The digest was included residual H_2SO_4 to retain the NH_3 as NH_4. The flask was heated after the addition of water and alkali to the digested sample, in order to distill a volume of distillate and NH_3 were collected in the acidic distillation receiver. The ammonia from the distillation was collected in an excess of standard acid and determined by a back titration with standard alkali solution.

Determination of Fat, lactose, and pH in Milk

Total fat was measured in milk samples according to Gerber Method. In briefly, 10.75 ml of milk sample was added to the butyrometer containing ten milliliters of H_2SO_4, then one milliliter of amyl alcohol was added. The butyrometer was shacked in the shaker stand until no white particles were seen and invert it a few times. The butyrometer was heated in water bath for 5 min, then centrifuged at 13000 rpm for 4 min. lactose and pH were determined by using an infrared spectrophotometer

SSCP-PCR

The primers (10 pmol/lμ) of PRL, CSN1S2a and CSN1S2b, respectively, were added in 12.5 μl of master mix and 50 ng of DNA in separate PCR tube. The PCR conditions were one cycle at 95°C for 3 min., 33 cycles at 95°C for 30 seconds, annealing temperature 52°C for 30 seconds, 72°C for 30 seconds, and finally 72°C for 5 minutes. 10 μL of PCR products were diluted in 40 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 acrylamide: bisacrylamide gels (29:1) according to Darwish et al. [19].

Statistical analysis

Data obtained from milk composition analysis in Zaraiby and Damascus goats were analyzed using independent t-test using the SPSS program version 18. The results were significant at $P \leq 0.05$.

Results and Discussion

Fat, protein, lactose, and solid non-fat (SNF) levels were (3.83, 3.7, 4.3, and 7.7) in Zaraiby goat milk higher than Damascus goat milk (3.1, 3.3, 4.2, and 7.4). While pH levels were (6.63) in Damascus goat milk higher than Zaraiby goat milk (6.62), the statistical analysis showed that differences between Damascus goat and Zarariby goat were significant in fat content and non-significant in other components (Table 2). In another study, protein, lactose, fat, total solid, and ash were 3.5± 0.07%, 3.6±0.08%, 4.3±0.12%, 12.2±0.16%, 0.77±0.02% in the Damascus goats [20]. While, content of protein, lactose, fat, total solid, and ash was recorded 3.01±0.03, 4.62±0.03, 4.06±0.06, 12.44±0.012, and 0.75±0.02, respectively in Zaraiby goats [21]

TABLE 1. Primer sequence, annealing temperature, and PCR product size

Name	Sequences	Size (bp)	Annealing Tm.
PRL	F: 5'CGACTGTCAGGTGTCCCTTC3' R: 5'TTCATGGTGGGTCTGTTGGG3'	208	52
CSN1S2a	F: 5'-GCTTATCGTCCACAGTAATCTT-3' R:5'-GGACTCTAAATATACTTAATGAATT-3'	270	52
CSN1S2b	F: 5'-TTTTTATTACAAAAGACAACT-3' R: 5'-GGTTAGGTCTAGGTGTTCTGA-3'	230	52

TABLE 2. Milk composition analysis in Zaraiby and Damascus goats

Breeds	pH	Fat	Protein	Lactose	SNF
Zaraiby	6.63±0.01	3.83±0.1	3.7±0.08	4.2±0.06	7.4±0.2
Damascus	6.62±0.01	3.1±0.2	3.3±0.05	4.3±0.03	7.7±0.1
P-Value	0.55	0.04	0.1	0.2	0.32

Polymerase chain reaction was performed using specific primers to detect PRL, CSN1S2a, and CSN1S2b genes at 208 bp, 270 bp, and 230 bp, respectively in Zaraiby and Damascus goats (Fig. 1).

PRL, CSN1S2a, and CSN1S2b genes were recorded monomorphic pattern in Zaraiby and Damascus goats using polyacrylamide gel of SSCP-PCR method (Fig. 2).

In the current study, the polymerase chain reaction amplification of PRL gene fragment (208 bp) showed a monomorphic pattern in polyacrylamide gel of SSCP-PCR method in Damascus and Zaraiby goats (Fig. 2). These results are consistent with those reported by Aravindakshan, [20] in Indian goats. The RFLP method showed three genotypes in PRL gene fragment (196 bp); AA with frequency (0.10, 0.25, and 0.00), AB with frequency (0.85, 0.75, and 0.90), and BB with frequency (0.05, 0.00, and 0.10) in Barki, Damascus, and Zaraiby goat, respectively [21]. Also, there three genotypes (CC, CA, and AA) were detected by SSCP-PCR in PRL gene of Chinese indigenous goat breed [22], they detected new mutation (g.576C > A) by using sequencing analysis. The present study showed three SNPs at positions 41G<T, 69A<G, and 80C<T, as well as an insertion with a C base at position 40 in the AB and BB genotypes. The present study showed monomorphic patterns in two types (a, b) of CSN1S2 gene in Damascus and Zaraiby goats by SSCP-PCR technique (Fig. 3 and Fig. 4). In another study was performed on Saanen goats, there three genotypes (AA, AB, and BB) were observed with frequency 26.67%, 33.33%, and (40.00%), respectively [22]. Dancil et al. [22] showed that milk yield was significantly affected by A-B alleles in Saanen goats. As they indicated positive correlation between AB genotype and high levels of protein, lactose, fat, total solid, solid not fat, and casein. In this study, milk composition in Zaraiby goats was higher than Damascus goats. Therefore, it is must to investigate other places in these genes to find SNPs may explain elevation of milk composition in Zaraiby goats compared with Damascus goats.

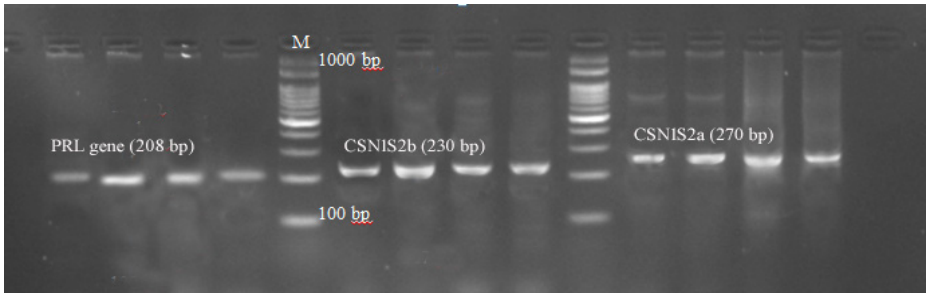

Fig. 1. Detection of PRL, CSN1S2a and CSN1S2b genes by PCR

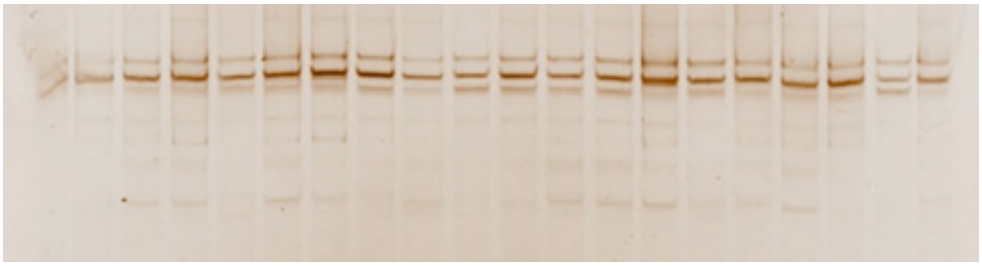

Fig. 2. Detection of monomorphic patterns of PRL gene in Zaraiby and Damascus goats by SSCP-PCR

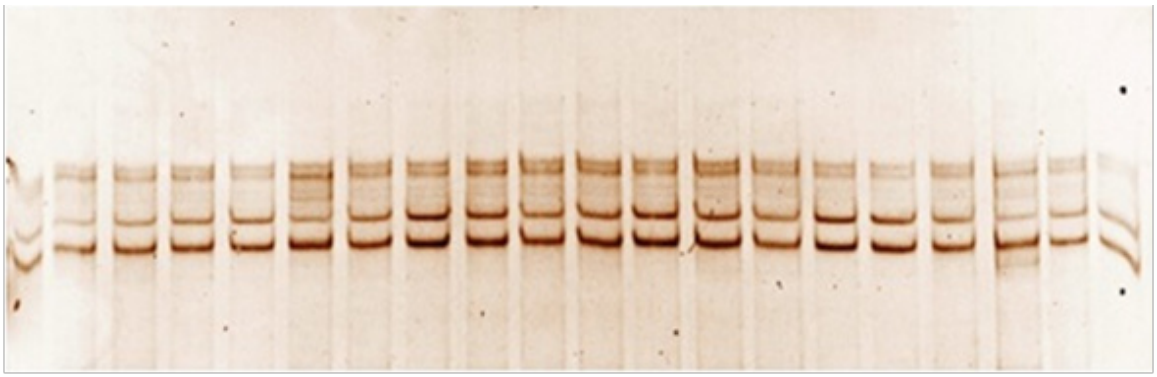

Fig. 3. Detection of monomorphic patterns of CSN1S2a gene in Zaraiby and Damascus goats by SSCP-PCR

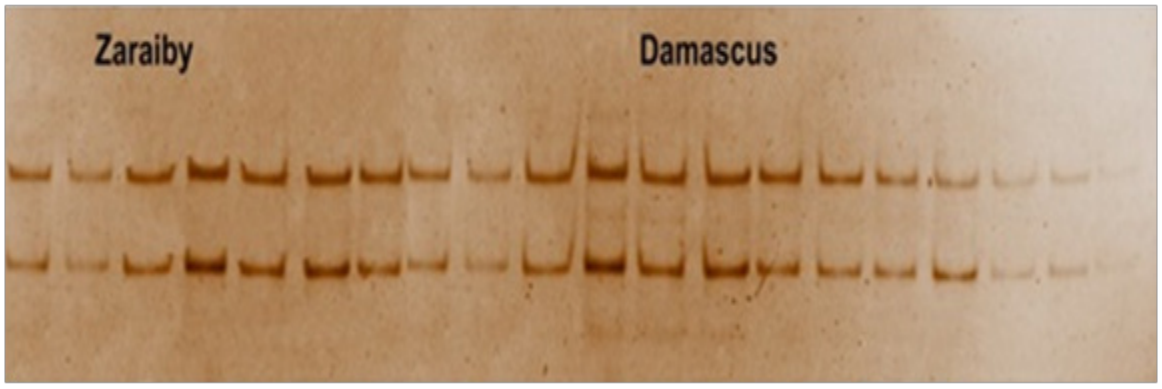

Fig. 4. Detection of monomorphic patterns of CSN1S2b gene in Zaraiby and Damascus goats by SSCP-PCR

Conclusion

The biochemical analysis showed that milk contents of protein, fat, lactose, solid non-fat were higher in Zaraiby goats than Damascus goats. The PRL, CSN1S2a, and CSN1S2b gene fragments were detected at 208 bp, 270 bp, and 230 bp, respectively in Damascus and Zaraiby goats. These fragments recorded monomorphic patterns in polyacrylamide gel by SSCP-PC method. This study recommends examining other places in these genes to detect new SNPs to explain the reasons for increased milk composition in Zaraiby goats compared to Damascus goats.

Conflicts of interest

There are no conflicts to declare.

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جينات البرولاكتين وألفا كازين ٢ وصفات اللبن في الماعز الدمشقي والزرايبي

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تعتبر جينات الفا كازين والبرولاكتين من المعلمات الانتخابية لانتاج اللبن في حيوانات المزرعة, لذا تهدف الدراسة الى تعريف التراكيب الوراثية المختلفة لتلك الجينات وتحديد النقط الطافرة لديهم وتقييم مدى ارتباطهم بفات اللبن من خلال التحليل الاحصائي المناسب. تم تجميع عدد ثلاثين عينة دم ولبن من ماعز الزرايبي وكذلك ثلاثين عينه من الماعز الدمشقي من مزرعة الماعز بكفر الشيخ في مصر. تم تحديد كمية كل من البروتين والدهن واللاكتوز ودرجة الحموضه في كل من الماعز الزرايبي والدمشقي, وتم تحديد التراكيب الوراثية من خلال تكنيك تعدد الشكل المظهري لخيط الحمض النووي الواحد بتفاعل البلمرة المتسلسل (SSCP-PCR). أظهرت نتائج التحليل الكيموحيوي أن نسبة البروتين والدهن واللاكتوز في الماعز الزرايبي أعلى منه في الماعز الدمشقي , وأظهرت نتائج تفاعل البلمرة المتسلسل تحديد الحزمة لكل من جينات البرولاكتين والفا كازين 2 أ ، ب عند 208 bp, 230 bp 270 ,bp بالتوالي. كماأظهرت نتائج تحليل ال SSCP-PCR تحديد نمط واحد لكل من جينات البرولاكتين والفا كازين 2 أ ، ب في كل من الماعز الزرايبي والدمشقي. ولذلك توصي الدراسة بالبحث في أماكن أخرى من جينات البرولاكتين وألفا كازوين لمحاولة إيجاد نقط طافره من اجل تفسير ارتفاع محتويات لبن الماعز الزرايبي من البروتين والدهن واللاكتوز عن لبن الماعز الدمشقي.

الكلمات الدالة: جين البرولاكتين ، جين ألفا كازين ، صفات اللبن ، الماعز.