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Research Article

β-Lactoglobulin Variants in Awassi and Morkaraman Sheep and their Association with the Composition and Rennet Clotting Time of the Milk

Şerafettin ÇELİK[1,*], Salih ÖZDEMİR[2]

[1]Department of Food Engineering, Faculty of Agriculture, Harran University, TR 63200, Şanlıurfa - TURKEY

[2]Department of Food Engineering, Faculty of Agriculture, Atatürk University, TR 25240, Erzurum - TURKEY

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Abstract: In this study, β-lactoglobulin (β-Lg) variants in Awassi and Morkaraman sheep were studied using polyacrylamide gel electrophoresis, and their association with composition and rennet clotting time of the milk was estimated. Two alleles (A and B) and 3 different genotypes for β-Lg (AA, AB and BB) were observed in the whey from both breeds. The frequencies of β-Lg alleles were estimated as A 0.63 and B 0.37 in the Awassi breed, and A 0.56 and B 0.44 in the Morkaraman breed. These results indicate high β-Lg A frequencies in both sheep breeds. Significant relationships were obtained between β-Lg variants and some properties of the milk from both sheep breeds ($P < 0.01$). In the Awassi breed, milks with β-Lg BB had significantly higher fat content and lower lactose content, while milks with the same variant had higher protein and solid non-fat contents in the Morkaraman breed. On the other hand, milks with the β-Lg AA variant in the Awassi breed had a short rennet clotting time, while milks with this variant in the Morkaraman breed had the lowest value for rennet clotting time but the result was not statistically significant. Therefore, it could be concluded that milks with the BB genotype are of importance in terms of cheese technology.

Key Words: Awassi, Morkaraman, β-Lg, milk composition, rennet-clotting time

İvesi ve Morkaraman Koyun Irklarında β-Laktoglobulin Varyantları ve Sütün Bileşimi ve Pıhtı Oluşum Süresi ile İlişkisi

Özet: Bu çalışmada, poliakrilamid jel elektroforezi kullanılarak, İvesi ve Morkaraman koyun ırkında β-Lg genetik varyantları tespit edilmiş ve bu varyantların sütün bileşimi ve pıhtı oluşum süresi ile ilişkisi saptanmıştır. Her iki koyun ırkında 2 farklı β-Lg allel geni (A ve B) ve 3 farklı β-Lg genotipi (AA, AB ve BB) gözlenmiştir. β-Lg A ve B gen frekansları, İvesi ırkında 0,63 ve 0,37, Morkaraman ırkında ise 0,56 ve 0,44 olarak hesaplanmıştır. Bu sonuç, her iki koyun ırkında da β-Lg A geni frekansının daha yüksek olduğu göstermektedir. Her iki koyun ırkı sütlerinde de, β-Lg varyantları ile sütün bazı özellikleri arasında istatistiksel olarak önemli ilişkiler elde edilmiştir ($P < 0,01$). İvesi ırkı sütlerinde β-Lg BB varyantı, yüksek yağ ve düşük laktoz oranı ile, Morkaraman sütlerinde ise β-Lg BB varyantı yüksek protein ve yağsız kurumadde oranı ile ilişkili olduğu tespit edilmiştir. Diğer taraftan, İvesi sütlerinde β-Lg AA varyantlı sütlerde pıhtılaşma süresinin kısa olduğu; Morkaraman ırkı sütlerinde ise β-Lg BB varyantlı sütlerde pıhtılaşma süresinin daha kısa olduğu gözlenmiş, ancak sonuç istatistiksel olarak önemli bulunmamıştır. Sonuç olarak β-Lg BB varyantlı sütler, peynir teknolojisi açısından önem taşımaktadır.

Anahtar Sözcükler: İvesi, Morkaraman, β-Lg, sütün bileşimi, pıhtılaşma süresi

Introduction

The initial studies on sheep milk protein polymorphism were reported by King (1), and considerable advances have been made in the subject in recent years. Although β-Lg A and B alleles were widely reported in almost all breeds studied (1-3), β-Lg C was rare and identified in the milk of Tajik (4), Merinoland (5) and Merino (6) sheep breeds. Several studies concerning β-Lg genotypes and allele frequencies in different sheep breeds have been carried out and the results showed that β-Lg A was more common than β-Lg B (2,3,7-9), except in Tajik (4), Lacha (6) and Rhon (5) breeds.

*E-mail: scelik69@yahoo.com

There has been only limited research on the relationships between milk protein polymorphism and milk composition and technological properties of the sheep's milk from different breeds, although such milk has been widely used in cheese processing (6,9-12). Among them, the relationships between β-Lg polymorphism and characteristics of sheep's milk have been performed in different breeds (i.e. Manchega, Merino and East Friesian). However, the influence of β-Lg on milk composition is controversial, and thus the results reported for different sheep breeds' milk are inconsistent, indicating the superiority of a genotype (9,11,13) or the absence of relationships (6). An association between β-Lg variants and milk composition was also reported. For instance, with respect to AB and BB genotypes, milks with the β-Lg AA genotype were found to have higher total solid, fat and protein contents (9). No research has been reported regarding the relationships between β-Lg polymorphism and physicochemical properties of the milk from Awassi and Morkaraman breeds.

The Awassi breed is farmed commonly in the Southeast Anatolia region of Turkey, and accounts for about 2.5% of the total Turkish sheep population (14). In addition, the breed is widely raised in Iraq, Syria and Saudi Arabia (15). It is known as a milk breed and is also used for meat production. The breed has been reported to have high milk yield (108.99 kg/head per lactation) among sheep breeds raised in Turkey (8). The Morkaraman breed, which is more resistant to cold weather, is farmed in the East Anatolia region of Turkey and accounts for about 21.5% of the total Turkish sheep population (14). The milk yield of the breed was calculated as 70.64 kg/head per lactation (8). The sheep of both breeds are fat-tailed, small, and perfectly adapted to extreme environmental conditions (8,16). Both breeds have been raised since 1974 at the Atatürk University Research and Application Farm in Erzurum, Turkey, which has one of the coldest climates in the country (8,16). In Turkey, total sheep milk production was about 657,387 t in 2002, representing 7.82% of the total milk production of the country (17). All sheep breeds raised in Turkey are used for milk production, and milking was performed manually once a day. Sheep's milk is generally used for the manufacture of yogurt and traditional cheeses due to its high total solids (18).

The genetic polymorphisms of milk proteins are of great interest in animal breeding. Therefore, the aim of the present study was to determine the genetic polymorphism of whey proteins (β-Lg, α-La and serum albumin) of Awassi and Morkaraman breeds, and to investigate the possible relationships between whey protein genotypes and the chemical composition and rennet clotting time of the milk obtained from both breeds.

Materials and Methods

Materials

Individual milk samples collected from a herd of 122 Awassi and 70 Morkaraman sheep at the Research and Application Farm of Atatürk University, Erzurum, Turkey, were subjected to β-Lg genotype analysis. Among them, the milks from 87 Awassi and 32 Morkaraman sheep were analyzed for total solids, protein, fat, ash, lactose, solids non-fat contents, titratable acidity and rennet clotting time. All the sheep selected for the study had almost the same lactation and they were totally pasture fed. Studied animals were periodically health-checked every month. The first milk samples were taken when the sheep were in week 7 of lactation. This procedure was repeated 4 times at 4-week intervals during lactation, and the milking was performed manually once a day. When each sheep was completely milked, 150-200 ml of milk was sampled and cooled, and then transported to the laboratory for analysis. The analyses were done in duplicate.

Sample preparation for electrophoresis

Approximately 10 ml of the milk samples from each sheep was first subjected to fat removal by centrifuging at 290 xg for 10 min. The casein was precipitated with 0.1 M HCl at pH 4.6 and collected by centrifuging at 2000 xg for 10 min at 6 °C. The supernatant, containing whey proteins, was transferred to another test tube and stored at –20 °C until electrophoresis (19).

Separation of whey proteins

To 50 µl of aliquot of whey were added 50 µl of bromphenol blue (1%, w/w) and 50 µl of glycerol (glycerol/electrode buffer: 1/1) solutions and 10 µl of this

mixture was loaded onto the gel. Electrode buffer composition and conditions for separation of the whey proteins by native-polyacrylamide gel electrophoresis were according to Thomas et al. (2), except that 10% polyacrylamide gels were used. Electrophoresis apparatus comprised vertical slab gels (PS 500 XT, Hoefer Scientific Inst., San Francisco, CA, USA) with the dimensions of 160 mm long x 180 mm wide x 1.5 mm thick. The predominant band of each allele was considered for phenotyping.

Analytical methods

The total solids (20), total nitrogen (21), ash content and titratable acidity (22) of sheep's milk were determined as described previously. The fat content was obtained using a Milkotester (Minor A/S, Type 18410, Foss Electric, Denmark) as described by Kurt et al. (22). Protein amount was calculated as total nitrogen x 6.38. The solids non-fat and lactose contents were determined by calculating the difference. Rennet clotting time of milk was determined according to the FIL-I IDF (23) method using calf rennet (1/15,000).

Statistical analysis

The genotype and allele frequencies of the β-Lg locus were estimated by direct counting. A chi-square test was performed on the basis of the Hardy-Weinberg law for determining the differences within the breeds (24). The effect of the phenotypic structure of β-Lg on the chemical composition and rennet clotting time of the milk was analyzed with the general linear model of ANOVA. To clarify the association between β-Lg variants and the rennet clotting time of milk, the titratable acidity and chemical composition of milk were used as covariates in the variance analysis. The study was designed by completely randomized blocks with a factorial design of 2 (breeds) x 3 (β-Lg genotypes) (25). The statistical analyses were carried out using MINITAB®, software (26).

Results

Examples of the evaluation and migration profile of whey proteins of the samples on native-polyacrylamide gel electrophoresis at pH 8.3 are shown in the Figure.

The distribution of genotypes and allele frequencies of β-Lg in Awassi and Morkaraman sheep are summarized in Table 1. The results show that polymorphism existed in only the β-Lg fraction of the whey proteins from both sheep breeds studied. Only one predominant band was obtained for α-La and serum albumin; however, 3 genotypes (AA, AB and BB) were demonstrated in both breeds. The frequency of the β-Lg BB genotype was lower with respect to the other 2 genotypes (AB > AA > BB) in both breeds. Hence, the β-Lg AB genotype was more common in the milk of both breeds. Both breeds had β-Lg A and B alleles, but the β-Lg C allele was not detected. The frequencies of β-Lg A and β-Lg B genes in Awassi sheep were 0.63 and 0.37, respectively, while the corresponding frequencies in Morkaraman sheep were 0.56 and 0.44.

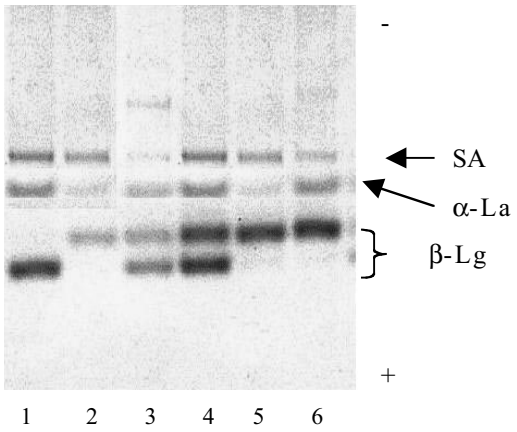

Figure. Separation of sheep whey proteins at pH 8.3 by polyacrylamide gel electrophoresis. Lane 1, whey samples including β-Lg AA genotype; lanes 2, 5 and 6, whey sample containing β-Lg BB genotype; lanes 3 and 4, whey samples containing β-Lg AB genotype. SA: serum albumin; α-La: α-lactalbumin; β-Lg: β-lactoglobulin.

The results for the populations of both breeds showed that there was a good agreement between the observed and expected frequencies on the basis of the Hardy-Weinberg law, indicating that these sheep populations are in genetic equilibrium (Table 1). Statistically significant differences ($P < 0.05$) were not observed between Awassi and Morkaraman sheep ($\chi^2 = 2.20$) with respect to β-Lg genotypes.

The estimated mean and standard deviation of studied properties of milks from Awassi and Morkaraman sheep with different β-Lg genotypes using ANOVA are given in Table 2. Highly significant differences were observed among the β-Lg variants with regard to the protein and

Table 1. The distribution of β-Lg genotypes and allele frequencies of Awassi and Morkaraman breeds.

Breed		Genotypic frequencies			Allele frequencies		
		AA	BB	AB	χ^2	A	B
Awassi	Observed	44	13	65	2.38	0.63	0.37
(n = 122)	Expected	48.42	16.7	56.88	DF = 1		
Morkaraman	Observed	20	12	38	0.71	0.56	0.44
(n = 70)	Expected	21.95	13.55	34.5	DF = 1		

Note: χ^2: chi-square value; n: total number of sheep; DF: degrees of freedom

Table 2. The chemical composition and rennet clotting time in milks with different variants of β-Lg from Awassi and Morkaraman breeds.

	Awassi breed (n = 87)			Morkaraman breed (n = 32)		
	AA (n = 30) EM ± SD	AB (n = 46) EM ± SD	BB (n = 11) EM ± SD	AA (n = 9) EM ± SD	AB (n = 16) EM ± SD	BB (n = 7) EM ± SD
TS	17.1 ± 0.13	17.1 ± 0.11	17.1 ± 0.24	16.5 ± 0.27	16.6 ± 0.19	17.0 ± 0.31
Protein	5.2 ± 0.05 ^{bc}	5.3 ± 0.04 ^{b}	5.2 ± 0.09 ^{bc}	5.0 ± 0.10 ^{c}	5.2 ± 0.07 ^{bc}	5.6 ± 0.11 ^{a}
Fat	5.7 ± 0.07 ^{b}	5.7 ± 0.06 ^{b}	6.1 ± 0.14 ^{a}	5.4 ± 0.15 ^{c}	5.4 ± 0.11 ^{c}	5.2 ± 0.17 ^{c}
Lactose	5.3 ± 0.05 ^{a}	5.2 ± 0.04 ^{a}	4.9 ± 0.10 ^{b}	5.3 ± 0.11 ^{a}	5.1 ± 0.08 ^{a}	5.3 ± 0.13 ^{a}
SNF	11.4 ± 0.09 ^{ab}	11.4 ± 0.07 ^{ab}	11.0 ± 0.16 ^{b}	11.2 ± 0.18 ^{b}	11.3 ± 0.13 ^{b}	11.8 ± 0.21 ^{a}
Ash	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.02	0.9 ± 0.02	1.0 ± 0.01	0.9± 0.02
RCT	4.7 ± 1.16	6.7 ± 0.44	5.5 ± 0.60	9.2 ± 1.12	8.2 ± 0.85	8.1 ± 1.37

Note: The differences are statistically significant between means marked with a different letter in the same row. EM: estimated mean; TS: total solids; SNF: solids non-fat; RCT: rennet clotting time (min); n: total number of milk samples/milking; SD: estimated standard deviation. Total solids, protein, fat, lactose, SNF and ash contents were measured as g/100 g milk.

solids non-fat contents of the milk of both breeds ($P < 0.01$). Significant differences were also obtained in the fat and lactose contents of milk related to different β-Lg genotypes in Awassi sheep ($P < 0.05$). However, significant differences were not found in the remaining properties of the milk with the variants of β-Lg ($P < 0.05$). In the Awassi breed, milks with β-Lg AB had higher estimated mean protein content than did other milks containing either AA or BB genotypes (AA = BB). The mean fat content of milks with the BB genotype was higher while the solids non-fat and lactose contents were lower than those of the milks of the other 2 genotypes (AB and AA). On the other hand, milks with the β-Lg BB genotype had significantly higher mean protein and solids non-fat contents than did milks with AA or AB genotypes in the Morkaraman breed.

Although the result was not significant, the findings indicated that milks with the β-Lg AA genotype in the Awassi breed showed a shorter rennet clotting time than the milks of other 2 genotypes, whereas in the Morkaraman breed the milks of the BB genotype clotted faster than did the milks with either AA or AB genotypes. In addition, it was observed that the titratable acidity and protein content of milk had a significant effect on the rennet clotting time of milk ($P < 0.01$), and a negative significant correlation was obtained between the rennet

clotting time and titratable acidity of milk ($P < 0.01$; $r = -0.514$).

Discussion

The distributions of allele frequencies in our study showed that the frequencies of β-Lg A were higher than those of β-Lg B in both sheep breeds. Similar results were also reported for Comisana (27), Hyfer, BLXM (2), Manchega (3), Awassi (8) and East Friesian (9) sheep breeds. Nevertheless, our results contradicted those reported for Lacha (6), Tajik (4) and Rhon (5) breeds of sheep. This difference could be due to the breed properties such as those of fat-tailed, meat or milk breeds.

A short rennet clotting time is preferred for cheese production since a smaller amount of rennet and shorter time are required for the clotting of cheese milk (28). Furthermore, a reduction in the rennet clotting time of milk generally results in an increase in curd firmness, eliminating problems arising from soft curd, and decreasing the manufacturing cost (28). Therefore, these conditions should be regarded as additional benefits in cheese production, and it can be suggested that genotypes related to shorter rennet clotting time are more suitable for cheese processing.

The relationship between β-Lg variants and milk composition and coagulation properties in dairy cows was investigated, and a positive relationship was obtained (6). However, in sheep's milk, the results obtained so far are still preliminary and concern mainly β-Lg polymorphism. It was reported that Massese milks with the β-Lg AA genotype had a significantly shorter rennet clotting time than milks containing the BB genotype (29), whereas no relationships were obtained between β-Lg polymorphism and rennet clotting time (6). On the other hand, the β-Lg BB genotype had a positive effect on the rennet coagulation properties of milk (30). Several studies have shown that sheep's milk with β-Lg AA was more suitable for cheese processing due to the best rennet clotting time and the best yield in cheese (11-13).

The relationships between β-Lg variants and milk composition and coagulation properties in sheep's milk are still preliminary, and further investigation is needed. It was concluded that the β-Lg AB genotype and allele A were more common in both sheep breeds. Regarding milk composition, higher fat and lower lactose contents in the milks of the Awassi breed, and higher protein and solids non-fat contents were obtained in the milks containing the β-Lg BB genotype in the Morkaraman breed, indicating that the BB genotype in both breeds could be more suitable milk for cheese production. Nevertheless, there is a need for further studies on the genetic polymorphism of casein in sheep's milk and their relationships with milk's constituents.

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