Acta Universitatis Latviensis, 2009, Vol. 753, Biology, pp. 117–124

Genetic diversity in milk proteins among goats bred in Lithuania

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Abstract

The aim of the study was to determine the main plymorphisms in AlfaS1-casein, AlfaS2-casein, Kapa-casein and Beta-lactoglobulin genes in goats bred in Lithuania and compare these among breeds. The study was based on determination of single polymorphisms among individuals tested by PCR-RFLP and A-PCR methods. Allele, genotype and haplotype frequencies were calculated and the Hardy-Weinberg equilibrium test was performed. Lithuanian native, Zaanen, Czech White and German White breeds were found to be polymorphic for AlfaS1-casein, AlfaS2-casein, Kapa-casein and Beta-lactoglobulin genes. The AlfaS1-casein gene B allele (0.981) and BB genotype (0.83) as well as Beta-lactoglobulin C allele (0.913) and CC genotype (0.87) had the highest frequency in Lithuanian native breed. Eight different casein locus haplotypes were found in the studied breeds. Haplotypes B/A/AB and B/B/AB were the most common among the Lithuanian goat population. B/B/C and E/A/C haplotypes were found solely in two breeds – Lithuanian Native and Zaanen. The haplotype E/B/C may be unique, as it was found only in one the Zaanen breed.

Key words: allele, genotype, goat, haplotype, milk protein genes, PCR-RFLP.

Introduction

It is widely known that animal productivity and other agricultural traits, such as milk quantity, composition, fat and protein quantity and chemical composition, suitability for processing and production of individual milk, milk discharge velocity during the milking process, succulence, speed of growth, etc., are inherited from generation to generation. However, their formation and functional features are determined by genes. The advances in molecule genetic technologies, especially DNA markers, over the last few decades have had an enormous influence on the compilation of gene maps, which has allowed to identify genes that control the changing part of multigenic features.

The detection of milk protein genetic variants gave rise to the analysis of milk protein polymorphisms in many animal species, such as cattle, sheep and goat. In the first stage of analysis cattle milk proteins were in the focus of attention; the aim was to indicate the relationship between milk protein variants, milk productivity and technological features. Cow Kapa-casein A and B variants were shown to improve milk productivity and milk quality. Kapa-casein BB genotype cow milk has more fat, protein and caseins (Ng-Kwai Hang 1998; Di Stacio et al 2000; Miceikienė et al 2005) and has better industrial features

– shorter coagulation time with aided by rennet performance, better consistence for curd formation and higher cheese output (Schaar 1985; Marziali et al. 1986).

The main focus of goat milk analysis was onthe AlfaS1-casein. „Strong" (A, B, C) alleles, „medium" (E), „low" (F, D) and „zero" alleles were identified in the AlfaS1-casein, associated with the high (3.6 g L^{-1}), medium (1.6 g L^{-1}), low (0.6 g L^{-1}) and zero AlfaS1-casein quantity in milk (Jordana et al. 1991; Feligini et al. 2005). Much research has been recently conducted on the AlfaS1-casein polymorphic relationship with the goat milk productivity, physico-chemical and technological features. However, the number of studies on goat AlfaS2-casein and Beta-lactoglobulin has been relatively low, perhaps due to low polymorphism (Folch et al 1994; Lagonigro et al 2001). However, goat Kapa-casein polymorphism was demonstrated using protein electrophoresis (Di Luccia et al. 1990), chromatography (Law et al. 1993) and capillary electrophoresis (Recio et al. 1997) and gene variants have been identified by molecular methods in different goat breeds (Caroli et al. 2001; Yahyaoui et al. 2001; Angiolillo et al. 2002; Feligini et al. 2002; Miceikienė et al 2007).

The aim of this study was to determine the main plymorphisms in AlfaS1-casein, AlfaS2-casein, Kapa-casein and Beta-lactoglobulin genes in goats bred in Lithuania and compare these among breeds.

Materials and methods

Samples for identification of milk protein genetic polymorphism were taken from unrelated individuals from Lithuanian Native (30), Zaanen (74), Czech White (29) and German White (18) goat breeds. DNA was extracted from hair roots.

AlfaS1-casein gene polymorphism was investigated by A-PCR method (Feligini et al. 2005). Primer sequences were following: forward BE 5'-CAA-CCT-CAA-ATT-GAA-GGC-ACT-3'; forward E 5'-CAA–CCT-CAA-ATT-GAA-GGC-ACT-3'; reverse R 5'-CAA-GCT-CTT-AGG-ACA-ATT-TCA-CTT-3'.

AlfaS2-casein gene polymorphisms were identified by PCR-RFLP (Cosenza et al. 1998). Primer sequences used: forward 5'-GCCATT-CAT-CCC-AGA-AAG-3' and reverse 5'-CTC-TTC-ATT-TGC-GTT-CCT-TA-3'. The digestion of PCR product was performed using endonuclease MseI (MBI Fermentas, Lithuania).

Kapa-casein gene polymorphisms were identified by PCR-RFLP (Yahyaoui et al. 2001). Primer sequences used were: forward 5'-TGT-GCT-GAG-TAG-GTA-TCC-TAG-TTA-TGG-3' and reverse 3'-GAT-TCC-TCT-GTA-GTT-TCT-CCT-GTT-GCG-5'. The digestion of PCR product was performed using endonucleases *Alw44*I (MBI Fermentas, Lithuania) and *BseN*I (MBI Fermentas, Lithuania).

The beta-lactoglobulin gene polymorphism was assessed by PCR-RFLP (Yahyaoui et al. 2000). Primer sequences were following: forward 5'GTCACT TTCCCGTCCTGGGG-3 and reverse 5'GGCCTTTCATGGTCTGGGTGACG-3'. The digestion of PCR product was performed using endonuclease SmaI (MBI Fermentas, Lithuania).

The PCR reactions were carried out using the GeneAmp PCR System 2700 (AppliedBiosystem). The reaction products were analyzed by electrophoresis on 3 % agarose gel. EtBr was added to gels to visualize the analysis results under UV light in the Heliorab system.

Goat AlfaS1-casein, AlfaS2-casein, Kapa-casein and Beta-lactoglobulin allele,

genotype, haplotype frequencies in each breed and in all investigated group, expected and observed heterozygosities per locus and population, deviations from Hardy-Weinberg equilibrium were calculated using the R statistical package (http://www.r-project.org/).

Results and discussion

B and E alleles were identified in Lithuanian goat breed milk protein AlfaS1-casein. B „strong" allele, which conditions a larger quantity of AlfaS1-casein, was found very frequently (0.643 - 0.891) in the goats of all analyzed Lithuanian breeds, and was most frequent (0.89) in one Lithuanian goat breed (Table 1). This allele ranges among Italian goat breeds from a frequency of 0.32 to 0.52 (Feligini et al. 2002). In Hungarian milking goat breeds, B allele was identified along with the A allele and was found in 61 % of the goat population studied (Veress et al. 2004). Goat selection prefers „strong" alleles, which provide high output of making cheese out of such milk. „Low" effect or zero alleles are also important; they are not useful for milk manufacture industry, but are demanded by goat milk consumers, since this milk is suitable for allergic people. E allele had the highest frequent range (0.36) in the Lithuanian Zaanen goat population and the lowest frequency in the Lithuanian native goat breed (0.12; Table 1). These data are consistent with the frequencies of E allele in Zaanen goat (41 %) in France (Grosclaude et al. 1987) and Zaanen goat (46 %) in Italy (Ramunno et al. 2001). In the Spanish Canaria goat breed, the E allele was found at a 0.20 frequency range (Jordana et al. 1991). The Hungarian milking goat breed possessed this allele only in 8 % of all animals, in the Polish white goat breed – 5 % (Krolikowska et al. 2002). Therefore, the „strong" A and B alleles were most frequent in goat breeds of the Mediterranian region as well as in Lithuanian goat breeds; whereas, „medium"(E) and „low"(F) alleles were more common in French, Italian, Swiss and Spanish goat breeds (Jordana et al. 1996; Enne et al. 1997; Grosclaude et al. 1997).

In the local Lithuanian breed, AlfaS1-casein BB genotype was found at a high frequency,

Table 1. Allele frequencies of AlfaS1-casein, AlfaS2-casein, Kapa-casein and Beta-lactoglobulin genes in Lithuania goat breed

Milk protein allele types	Lithuanian Native	Zaanen	Czech White	German White
AlfaS1-casein				
B	0.891	0.643	0.875	0.778
E	0.109	0.357	0.125	0.222
AlfaS2-casein				
A	0.565	0.690	0.675	0.667
B	0.435	0.310	0.325	0.333
Kapa-casein				
A+B	0.826	0.857	0.925	1.000
C	0.174	0.143	0.075	0.000
Beta-lactoglobulin				
C	0.913	0.738	0.725	0.722
T	0.087	0.282	0.275	0.278

Table 2. Genotype frequencies of AlfaS1-casein, AlfaS2-casein, Kapa-casein and Beta-lactoglobulin genes in goats bred in Lithuania

Milk protein genotypes	Lithuanian Native	Zaanen	Czech White	German White
AlfaS1-casein				
BB	0.83	0.48	0.80	0.72
BE	0.13	0.33	0.15	0.11
EE	0.04	0.19	0.05	0.17
AlfaS2-casein				
AA	0.35	0.62	0.50	0.45
AB	0.43	0.14	0.35	0.45
BB	0.22	0.24	0.15	0.10
Kapa-casein				
A+B/A+B	0.74	0.80	0.85	1.00
A+B/C	0.17	0.10	0.15	0.00
CC	0.09	0.10	0.00	0.00
Beta-lactoglobulin				
CC	0.87	0.57	0.55	0.56
CT	0.09	0.33	0.35	0.34
TT	0.04	0.10	0.10	0.10

and the EE genotype at low frequent range (Table 2). In total, 30 % of Polish white goat breed possessed the BB genotype and 25 to 38 % of the Italian goat breed (Feligini et al. 2002). No BE and EE genotypes were found in the Italian Montefalkone breed (Belivacqua et al. 1999).

The analysis of distribution of the milk protein AlfaS2-casein gene variant showed that Lithuanian goat breeds possess the same milk protein AlfaS2-casein alleles as other European goat breeds – A and B. The A allele frequency in Lithuanian goat breeds varied from 0.56 in Lithuanian Native to 0.69 in the Zaanen breed with a mean of 66.3 % (Table

Table 3. Frequencies of casein haplotypes in goats bred in Lithuania. *, Haplotype was composed from AlfaS1-casein, AlfaS2-casein and Kapa-casein alleles

Haploytpe*	Lithuanian Native	Zaanen	Czech White	German White
B/A/AB	0.32	0.37	0.42	0.58
B/A/C	0.17	0.06	0.05	-
B/B/AB	0.24	0.17	0.32	0.26
B/B/C	0.13	0.06	-	-
E/A/AB	0.06	0.14	0.05	0.11
E/B/AB	0.04	0.08	0.16	0.05
E/A/C	0.04	0.06	-	-
E/B/C	-	0.06	-	-

Table 4. Observed and expected heterozigosity for four milk protein loci in Lithuanian goat breeds

	Investigated population	Lithuanian Native	Zaanen	Czech White	German White
Observed H	0.325	0.293	0.238	0.213	0.222
Expected H	0.341	0.313	0.397	0.297	0.298
χ2- test	1.852	1.226	0.015	0.428	0.184
(P-value)	(0.6035)	(0.2682)	(0.9014)	(0.5131)	(0.6684)

1). In the Italian Alpine goat breed the A allele was found at a 0.68 frequency (Cosenza et al.1998), in the French Alpine goat breed at a 0.85 frequency (Bouniol et al. 1994) and in the South Italian locals in 31 % of the animals (Ramunno et al. 2001). The recently found E allele was found in two Italian goat breeds of five (Lagonigro et al. 2001). It is possible that the E allele could be used as a breed marker to identify from what breed milk goat milk products are made. Thus it seems important to study this allele in Lithuanian goat breeds.

The AlfaS2-casein AA genotype was prevalent in Zaanen and Czech White breeds. In the Native Lithuanian breed, the AlfaS2-casein AB genotype was found at a high frequency, whereas the BB genotype at a low frequency. The frequency of AA genotype was 49 %, AB genotype – 35 %, and BB genotype – 16 % of tested goats (Table 2).

The Kapa-casein gene has been analyzed among Italian, Spanish, German, French and Hugarian goat breeds (Caroli et al. 2001; Sacchi et al. 2001; Yahyaoui et al. 2001; Feligni et al. 2002; Veress et al. 2004). Our results correspond with these provided by other scientists. The Kapa-casein C allele frequency varied among Lithuanian goat breeds from 0 in German White breed to 0.17 in Lithuanian Native, similarly, in Hungarian, Spanish and French goat breeds with a frequency of 0 to 0.15. The Kapa-casein A+B allele frequency in Lithuanian breeds varied from 0.83 to 1, whereas in Hungarian, Spanish and French goat breeds from 0.85 to 1 (Table 1). In the French Zaanen goat breed, the frequency of C allele was similar to the Lithuanian Zaanen breed, respectively 0.11 and 0.14 (Yahyaoui et al. 2001; Veress et al. 2004). The frequency of the A allele in Italian breeds varied from 0.44 to 0.67, while in German goat breeds this allele was found at a higher frequency range. A total of 90 % of the German Ionika goat breed possessed this allele, compared to 100 % in Togenburgo breed (Caroli et al. 2001). The A variant was found in all other breeds, but was not found among Italian Teramina and Montefalcone breeds. Excepting the E allele, which has a rather high frequency in the Montefalcone breed (0.41), the frequency range

Table 5. Observed and expected heterozigosity in Kapa-casein milk protein loci in Lithuanian goat breeds

	Investigated population	Lithuanian Native	Zaanen	Czech White	German White
Observed H	0.183	0.522	0.143	0.000	0.000
Expected H	0.258	0.423	0.278	0.180	0.000
χ2- test	7.0	1.24	4.95	20.0	
(P-value)	(0.0303)	(0.5381)	(0.0840)	(0.0000)	

of other variants is comparatively low – under 15 %. The C variant was common in the Zaanen breed, whereas F alleles have been found only in Italian Teramina, Girgentana and Sarda breeds. Similarly, the D, F, G and E alleles were not found in the Spanish and French breeds (Yahyaoui et al. 2001; Jann et al. 2004).

The AB genotype of Kapa-casein gene locus was the most prevalent in all tested Lithuanian goat breeds. The CC genotype was not found in Czech White and German Czech White breeds. In total, 84 % of goats had the AB genotype and only 5 % had the CC genotype (Table 2).

Beta-lactoglobulin C and T alleles were identified in West European milky goat breeds. The C was obseved to be common among Spanish breeds such as Malagvena (0.75), Pajoja (0.73) and Canaria breed (1.00). In the French Zaanen goat breed this allele was found at a 73 % frequency, in Hungarian milky – 88 % (Veress et al. 2004). In Lithuanian goat breeds the mean C allele frequency was 0.78. Lithuanian Native goats had the highest frequency (0.91). In the Lithuanian Zaanen goat breed, the C allele was at the same frequency as in French goat breeds (Table 1). 65 % of the investigated goats had Beta-lactoglobulin CC genotype, 27 % CT genotype and only 8 % had the TT genotype (Table 2).

In the Native Lithuanian breed, AlfaS1-casein BB genotype was found at a high frequency, while EE genotype at a low frequency (Table 2). 30 % of the Polish white goat breed had the BB genotype; the Italian goat breed had this genotype in 25 - 38 % (Feligini et al. 2002). No BE and EE genotypes were found in the Italian Montefalkone breed (Belivacqua et al. 1999).

Caseins are coded by four related genes that form a cluster: AlfaS1-casein, AlfaS2-casein, Beta-casein and Kapa-casein, found in the 6th goat chromosome. Allele combinations in the casein locus (haplotypes) are closely related and inherited as one genetic unit. Therefore, the casein locus genotype is extremely important in successful selection. In total eight different haplotypes were found in goats bred in Lithuania. The haplotypes B/A/AB were the most common among the Lithuanian goat population. B/B/C and E/A/C haplotypes were found solely in two breeds – Lithuanian local and Zaanen. The haplotype E/B/C was unique, as it was found only in one breed – Zaanen (Table 3).

All Lithuanian goat breeds had a similar level of genetic diversity and comparable number of different alleles found in the milk protein locus. No unique alleles found in one breed can be used as breed genetic markers to serve as marker for goat milk and products of this breed were found. The evaluation of milk protein allele diversity and distribution proved genetic balance in all Lithuanian goat breeds, while observing a lesser heterozygosity than expected by the Hardy-Weinberg equilibrium law. The mean heterozygosity found in the goat group was 0.325 (Table 4). According to literature data, the Kapa-casein locus was found in genetic balance in German (Caroli et al. 2001), Italian and Spanish goat breeds, except Alpine, Frontalakska and Sarda breeds, where a deficiency of heterozygote genotypes was observed (Feligini et al. 2005). In our investigated goat breeds, Lithuanian Native, Zaanen breeds had the Kapa-casein locus in the genetic balance, whereas the Czech White goat breed contained a significant deviation of the Kapa-casein gene locus from the Hardy-Weinberg law as a result of heterozygosity deficiency (Table 5).

The goat breeds can be characterized with a relatively reduced level of artificial selection, in comparison with cattle and sheep breeds. This conditions a high level of genetic variability in goat casein genes. Some breeds or populations might possess unique alleles or allele combinations that no other breeds possess, which might be useful

as the source of genetic diversity for commercial domestic breeds. This emphasises the importance of preservation of a wide genetic diversity in these populations and the need of genetic sources preservation programmes.

High genetic variability, indicated by casein locus, and relation to milk features provide the opportunity to derive goat breeds that produce milk suitable for different manufacture technologies and special consumers needs (Rando et al. 2000).

The application of genetic markers in the selection of agricultural animals opens the possibility to asses animals and fully use the residing beneficial agricultural features. Genetic markers can be used for identifying both one gene or gene group influencing a trait or trait group. One more advantage of the usage of genetic markers in the selection is the fact that this method of assessment is reliable and economical; it allows identifying genes that control selection and technological value of the animal in young age, and to estimate genetic variability in agricultural animals and opportunity to avoid undesirable features. The usage of genetic markers in selection might accelerate the selection process, improve the quality of agricultural production, reduce the production prime cost and make the production more competitive in markets.

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