The correlation of production characteristics with the genetic variants of the encoding locus of β-lactoglobulin in three sheep breeds from Romania

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SUMMARY

The aim of this study is the genetic characterization of the β-lactoglobulin locus in the indigenous sheep breeds Karakul of Botosani, Merino Palace and Palace milk line. For animal breeders, the genetic polymorphism of milk proteins is of great interest because of milk composition and quality and yield parameters. The polymorphism of the β-lactoglobulin locus was analyzed in three breeds using the RFLP technique. In order to perform this analysis, we designated primers to amplify the polymorphic region at the level of the encoding gene for the β-lactoglobulin protein and the fragments amplified were subjected to endonuclease Rsa I digestion. The fragments resulting from restriction were analyzed by agarose gel electrophoresis, identifying thus the allele variants present. The results of the study suggest the possibility of a selection process induced in order to improve the production performance for milk. The using of genetic markers in animal selection is a successful strategy for production systems, the result of applying such methods leads to increasing productivity, product quality and of the benefits obtained in the field of livestock farming.

Keywords: sheep, β-lactoglobulin, genotyping, production parameters.

INTRODUCTION

Livestock genome study was the subject of numerous investigations aimed at clarifying some aspects of molecular structure, regulation and expression of genes that are considered to be involved in increased animal productivity and reproductive performance. Establishing a correlation between the presence of certain genes and certain characters or certain production traits means that the observed genes have a direct functional link with those characters, which allows a selection in the desired direction.

The development of molecular biology has allowed the evolution of techniques capable of identifying different variants of genes encoding for milk proteins. In the case of the DNA, polymorphisms are usually due to the

existence of point mutations. In this case the difference between individuals is detected by restriction enzymes that cut DNA only at specific sequences.

The proteins with genetic determination from sheep's milk are of two types: casein (β, α, k-casein) and non-casein (β-lactoglobulin) which differ through genetic polymorphism and frequency of their occurrence in animal populations. When it comes to the specific genes that may affect different economic characteristics in the case of sheep, β-lactoglobulin locus was the most studied. This protein has an increased polymorphism in most breeds of sheep. Until now there were three genetic variants A, B (Bell and McKenzie, 1967) and C (Erhardt, 1989). Genetic variant A differs from the genetic variant B in position 20, where variant A is Tyr and variant B is His (Gaye et al, 1987, Ali et al., 1990). In 1989, Erhardt identified variant C, which in position 148 shows an Arg-Glu substitution in opposition to variant A.

The β-lactoglobulin gene polymorphism has been extensively studied in many breeds, and the effects of the alleles identified varied widely. The AA and BB genotypes are correlated with the increased quality of milk either in terms of composition (fat and protein content) or in terms of technological properties (coagulation time and clot firmness). In the case of cheese formation process, the percentage of dry weight loss is the lowest for genotype AB and the percentage of fat loss is higher for genotype AA in comparison with the BB genotype (Rampilli et al., 1997).

Various studies conducted so far have revealed that allele A is more frequent in these populations than allele B. Thus, it appears in different breeds with the following frequencies: 0.76 in milk sheep from the Bavaria region (Schlee, 1993), 0.54 in the Pag Island sheep breed (Ivankovic and Dovc, 2004), 0.61 in the Gentile di Puglia sheep breed (Chessa et al., 2003), 0.70 in the Delle Langhe sheep breed (Leone et al., 1998) and 0.75 in the Sarda sheep breed (Pietrola et al., 2000).

This paper presents a genetic characterization of the β-lactoglobulin locus in three breeds of sheep, namely Karakul of Botosani, Merino Palas and Palas milk line and its correlation with milk production.

MATERIAL AND METHODS

Blood samples collected from three breeds (Karakul of Botosani - 43 samples from SCDCOC-Popauti/Botosani County, Merinos Palas - 25 samples and Palas milk line - 25 samples from ICDCOC Palas/Constanta County) were analyzed. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

The PCR conditions were optimized for the primer by varying the annealing temperature between 51-60˚C on a gradient thermocycler IQCycler (BioRad).

After determining the optimum temperature of 58˚C, the amplification reactions were carried out in 25 μL final volume and consisted of 1X PCR

Buffer, $MgCl_2$, 200µM dNTPs, DNA template, 0.5 units of AmpliTaq Gold DNA Polymerase, 0.5µL of each primer (F-CAACTCAAGGTCCCTCTCCA and R-CTTCAGCTCCTCCACGTACA) and nuclease free water. PCR amplifications were performed in 0.2 ml tubes using a program with 45 cycles. Denaturation was performed at 95˚C for 30 seconds, annealing at 58˚C for 30 seconds and extension at 72˚C for 1 minute. The first denaturation step was of 10 minutes at 95˚C and the final extension was of 10 minutes at 72˚C.

The PCR products obtained were digested with 1U of *Rsa I* restriction endonuclease (*Promega*) for 3 hours at 37˚C. The restriction fragments were directly analyzed by electrophoresis in agarose gel 3% stained with ethidium bromide, and visualized under UV light. The genotypes of the analyzed individuals at the β-lactoglobulin locus were established using the restriction fragments observed in the gel.

RESULTS AND DISCUSSION

In order to evaluate the genotype distribution of β-lactoglobulin in sheep populations we used the RFLP (Restriction Fragment Length Polymorphism) method. A 120 bp fragment of the ovine β-lactoglobulin gene from exon II was amplified (Figure 1). After PCR amplification, enzymatic digestion with *Rsa I* and agarose gel electrophoresis, the β-lactoglobulin A allele yields three bands of 66, 37, and 17 bp. The β-lactoglobulin B allele gives only two fragments of 103 and 17 bp, and the heterozygote has all four fragments. The 17 bp fragment results from an *Rsa I* site present in both alleles and are useful as a control procedure.

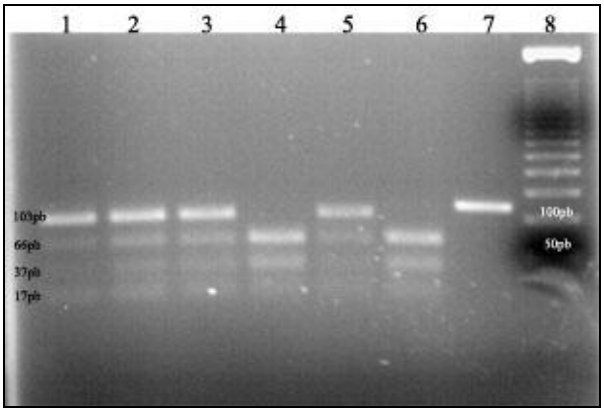

Figure 1: Results after digestion with *Rsa I*. Lanes 1, 2, 3, 5: genotype AB, lanes 4, 6: genotype AA, lane 7: uncut PCR product, lane 8: molecular weight marker (50 bp - Promega).

After determining the genotypes / phenotypes of β-lactoglobulin locus in the three sheep populations studied, we calculated the frequencies of genes, of genotypes and the state of genetic equilibrium.

In Table 1 we included the results obtained from the Karakul sheep population, where we have identified two genotypes / phenotypes: β-LG AA and β-LG AB. The heterozygous genotype β-LG AB predominated with 58% (25 individuals) and the homozygous β-LG AA genotype reached a value of 42% (18 individuals). Following the distribution of genotypes, gene frequency was 71% for β-LG A gene and 29% for β-LG B.

Table 1: Distributions of genotype and gene categories for β-lactoglobulin locus in the Karakul of Botosani breed.

Phenotypic categories	N	Weight of phenotypic categories	Categories distribution	
			A	B
AA	18	42	71	29
AB	25	58		

For the Merinos Palas individuals we identified, after interpreting the electrophoregrams, two genotypes / phenotypes: the homozygous genotype for β-LG AA, which recorded 44% (11 individuals) and heterozygous genotype for β-LG AB with a 56% value (Table 2). The frequency of the two alleles was of 72% for the β-LG A gene and 28% for the β-LG B gene.

Table 2. Distributions of genotype and gene categories for β-lactoglobulin locus in the Merinos Palas breed.

Phenotypic categories	N	Weight of phenotypic categories	Categories distribution	
			A	B
AA	11	44	72	28
AB	14	56		

In the specimens belonging to the Palas milk line, the electrophoregrams analysis for this population led to the revealing of three phenotypic / genotypic categories for the β-LG locus: homozygous gene A, heterozygous gene AB and homozygous gene B (Table 3). The frequency of these genotypes was 72% heterozygous β-LG AB genotype (18 individuals), 24% β-Lg AA homozygous genotype (6 individuals) and 4% (1 individual) homozygous β-LG BB. Regarding the gene frequency, we can clearly see that the predominant gene β-LG A recorded 60% and that the β-LG B gene has registered 40%.

Table 3: Distributions of genotype and gene categories for β-lactoglobulin locus in the Palas milk line breed.

Phenotypic categories	N	Weight of phenotypic categories	Categories distribution	
			A	B
AA	6	24		
AB	18	72	60	40
BB	1	4		

Table 4 shows several results regarding the frequency of genes in the β-LG locus in certain sheep breeds reared in Europe (Amigo et al., 2000). We may notice that the β-LG A allele is dominant, just as it is in the case of breeds from our country.

Tabel 4 Frequency of genes in β-lG locus in sheep breeds from abroad (Amigo et al., 2000).

Breed	**Country**	**N**	Allele A	Allele B
Barbaresca	Italy	62	0.62	0.38
Black Razka	Hungary	25	0.76	0.24
Wallachian	Czech Republic	61	0.84	0.16
Lacaune	France	157	0.63	0.37
Merino	Spain	340	0.58	0.41
Merino	Hungary	15	0.77	0.23
Tsigaja	Balkan	23	0.65	0.35
Lacaune	France	157	0.63	0.37

For the aim of characterizing genetically the populations studied, apart from establishing the distribution of genotype and gene categories, we were also interested in estimating the state of equilibrium, in order to establish if, in the case of this locus in the samples we have investigated, we are dealing with a transgenic or a balanced polymorphism. The results of this estimation of the state of equilibrium are presented in Table 4.

Karakul and Palas milk line populations do not have a balanced genetic structure. This is demonstrated by the fact that the comparison of the calculated value of χ^2 (7.25, respectively 6.25) with the χ^2 tabular value (5.99) we have found that the calculated value is higher, thus rejecting the premises from which we had started.

For the Merinos population, the results of the estimation show a balanced genetic structure, the calculate value χ^2 (3.77) is lower than the tabular value (5.99).

The description of the β-lactoglobulin variants was done in order to use it as genetic markers to be able to appreciate the degree of homogeneity or heterogeneity of the sheep populations in the production units and to compare the genetic fund of various breeds.

Table 5: Estimation of the state of equilibrium for the β-lactoglobulin locus in the analyzed populations.

Genotypes	No. of observed genotypes	No. of expected genotypes	d^2/A
Karakul of Botosani			
AA	18	21.68	0.62
AB	25	17.70	3.01
BB	-	3.62	3.62
Total	43	43	$\chi^2 = 7.25$
Merinos Palas			
AA	11	12.96	0.29
AB	14	10.08	1.52
BB	-	1.96	1.96
Total	25	25	$\chi^2 = 3.77$
Palas milk line			
AA	6	9	1.00
AB	18	12	3.00
BB	1	4	2.25
Total	25	25	$\chi^2 = 6.25$

Besides the fundamental importance of knowledge and of establishing the genetic structure of the samples analyzed for the β-lactoblobulin locus, a major concern was the correlation of the types of β-lactoglobulin with the production data with economic relevance, namely milk production. Thus, in Table 6 we have included information regarding the fact that the best performance concerning milk production was obtained from individuals in the population of Palas milk line specialized for milk. In this population, the β-LG locus has three identified genotypes, of which the β-LG AB heterozygous genotype recorded the highest average of milk production, followed by the β-LG homozygous genotypes β-LG AA and BB. Analyzing the other two breeds, namely the Merinos Palas and Karakul of Botosani, which are not specialized dairy breeds, we were able to discern that the highest average milk production was recorded by individuals which had the heterozygous genotypes β-LG AB. A comparative analysis of the average performance for each of the three populations (Karakul, Merino and Palas milk line) has concluded that the highest average milk production was established for the Palas milk line (0.809 liter).

Table 6: The comparative analysis of samples for the performance recorded for milk production based on the genotype for the β-lactoglobulin locus.

Genotype	Karakul of Botosani		Merinos Palas		Palas milk line	
	N	$\overline{X}\pm S_{\overline{X}}$	N	$\overline{X}\pm S_{\overline{X}}$	N	$\overline{X}\pm S_{\overline{X}}$
AA	18	0.492±0.054	11	0.499±0.054	6	0.679±0.057
AB	25	0.527±0.055	14	0.701±0.057	18	0.864±0.059
BB		-		-	1	0.607±0.000
Total	43	0.513±0.054	25	0.611±0.054	25	0.809±0.058

The results of the testing of the significance of differences between the averages of the production performances for the three populations are presented in Table 7.

Table 7: Testing the significance between lots for the analyzed population

Student test			
Feature	Mean difference	Standard deviation	Student
Karakul of Botosani			
Milk production	0.035	0.025	1.400
Merinos Palas			
Milk production	0.202	0.034	5.941
Fisher test			
Palas milk line			
	Intergroup Mean Square	Intragroup mean Square	Fisher
Milk production	0.110	0.015	7.333

For the Karakul breed sample, the tabular value t (2.021) is higher than the calculated value. Therefore we may conclude that the differences observed between the two groups are insignificant.

In the case of the Merinos breed sample, we have shown that there are significant differences between the two groups of individuals (homozygous and heterozygous) regarding the average of production performance for the characteristics analysed (t_{tab}=2.069).

For the Palas milk line population analysed, the Fisher calculated value is comparatively higher than the tabular value (F_{22}^{2} = 3.44), thus we may conclude that the differences observed between the average production performances described for the three groups of individuals are indeed significant. Therefore we have continued the Turkey test in order to determine between which groups of individuals the differences are significant. We discovered that there are significant differences between the performances recorded for the group of individuals with the AB β-lactoglobulin and those which have been identified as carrying β-lactoglobulin B (w = 0.225). The difference (0.186) between the performance recorded by homozygous and heterozygous individuals for gene A were lower than value w (0.225); thus we may conclude that the differences are not significant. Since the difference (0.072) between the average of performances recorded for the two homozygous categories does not exceed value w (0.225), the difference observed is not significant.

CONCLUSIONS

Using the RFLP technique we have analyzed the genotypes at the β-lactoglobulin locus for the Karakul of Botosani, Merinos Palas and Palas milk line sheep breeds. We have identified two genotypes (β-lG AA and β-lG AB)

for the Karakul of Botosani and Merinos Palas breeds and three genotypes (β-lG AA, β-lG BB and β-lG AB) for the Palas milk line breed. In all the breeds, the heterozygous genotype β-lG AB recorded the highest values of milk production. Regarding the allele frequency, the genetic variant A dominated in all the populations studied.

As a result of the comparative analysis of the samples regarding the performance recorded for milk production based on the genotype present at the β-lactoglobulin locus, we have noticed that the best performance in terms of milk production were obtained by the individuals from the Palas milk line population.

The results of this study suggest that the existence of a selection process induced in order to improve milk production performances is a distinct possibility.

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