Preliminary data on beta-lactoglobulin genetic polymorphisms in Hungarian Awassi and Racka sheep

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Abstract

In Hungary, just as in other parts of Europe, most of the dairy sheep are dual purpose with incomes originating partly from meat and in about 65-75% from milk. Since there is an increase of demand for goat and sheep dairy products one of the main breeding goals is to increase milk yield.

Milk is one of the main protein sources in human nutrition. The two main milk protein types are the caseins (α_{S1}, α_{S2}, β, κ) and the whey proteins (α-lactalbumin, β-lactoglobulin). These milk proteins show genetic polymorphisms, and the different genetic variants not only influence the physical and chemical properties of milk but also milk yield and protein and fat content.

Within the framework of our research we studied the β-lactoglobulin genotypes of Hungarian Awassi and Gyimesi Racka sheep using individual milk samples and isoelectric focusing in the presence of carrier ampholytes. In total 147 Awassi and 203 Racka ewes were typed, and genotype and gene frequencies were calculated for both populations. In Awassi the genotype frequencies are 0.2313 (AA), 0.4966 (AB), and 0.2721 (BB), in the Racka breed 0.3251 (AA), 0.5025 (AB) és 0.1724 (BB). The frequency of allel A was 0.4796 in Awassi and 0.5764 in Racka, and allele B was 0.5204 and 0.4236, respectively. The β-lactoglobulin BB genotype frequencies (P=0,0343) and the allele frequencies (P=0,0140) showed significant differences between the two examined sheep populations. The observed and expected genotype frequencies showed no significant differences, according to the Hardy-Weinberg law both populations approximate genetic equilibrium.

Key words: milk protein genetic polymorphism, sheep, β-lactoglobulin, genotype and gene frequencies, CA-IEF

1. Introduction

Hungary is the fifth largest sheep milk producer country in Central and Eastern Europe (30000 tonnes/year, FAO, 2005). The Awassi flock at Bakonszeg is a significant population of this breed within the EU boundaries. Since Assaf and Awassi breeds can be regarded as the most productive dairy breeds, which are able to produce 600-700kg milk per lactation, therefore characterisation and identification of the most valuable individuals by the help of genetic markers have outstanding economic importance. The Awassi flock was established by partly using imported Awassi ewes and partly mating Hungarian Merino ewes with Awassi rams and systematic backcrossing with Awassi rams. Based on this structure, big differences in the individual production are present in the flock providing a good base to find association between the phenotype and the genotype. On the other hand, the ancient Hungarian Racka population might also carry so far unrecognized genetic variants which does not occur in the current commercial

breeds, but could have a special value in future breeding programs.

2. Materials and methods

We have collected 252 Awassi and 218 Racka milk samples from the breeds of the Awassi Zrt (Bakonszeg, Hungary). The dairy animals were selected randomly, the milk samples were kept on -20C. The milk samples were defatted with centrifugation and lyophilized. The lyophilized milk samples were solved in aquous solution and isoelectric precipitated at pH 4.6 to separate the casein and whey fractions. The isolectric focusing in the presence of ampholytes and urea was performed as previously described [1]... The separation was performed on CA-IEF gels with the help of a Multiphore II electrophoresis apparatus (Pharmacia). Statistical analysis was performed by *Fisher-test* (QuickCalcs, http://www.graphpad/quickcalcs).

3. Results and discussion

The main selection criterion for dairy sheep is milk yield standardised for lactation length. However given that dairy industries are paying according to milk composition, traits related to composition should be considered as selection criteria. Furthermore the negative correlations between milk yield and contents increased the need to pay attention to these traits in the selection schemes. Notably recent data suggest a QTL in the telomeric region of ovine chromosome 6 in the neighbourhood of the casein region [2]. For dairy sheep research on genetic polymorphisms of caseins and whey proteins is mainly limited to examine the relation between αs1-casein and β-lactoglobulin loci and dairy traits or technological properties. Both of them gave less conclusive results than in goats [2]. Information on the polymorphisms of other main sheep milk components is limited (Table 1). Moreover milk protein polymorphisms has not been published either in the Awassi or in the Racka breeds, therefore either of them might carry so far unidentified variants with influence on protein yield or technological properties.

Table 1. Ovine milk protein variants (Moioli et al. 1998)

Locus	Protein variants milk analysis.	DNA polymorphisms
a-s1 casein	A, B, C, D _Welsh , E .	*Eco*RI and *Taq*I polymorphic patterns by using DNA *a*-s1 bovine probe
a-s2 casein	A, B	*Eco*RV polymorphic pattern by using a cDNA *a*-s2 bovine probe
β-casein	Nothing existing	*Eco*RV, *Hin*dIII and *Taq*I polymorphic patterns by using a cDNA *b*-casein bovine probe
κ-casein	Nothing existing	*Hin*d III, *PÍu*II and *Pst* I polymorphic patterns by using a cDNA *k*-casein bovine probe
α-lactalbumin	A, B	Nothing existing
β-lactoglobulin	A, B, C	A, B, C

Contradictory data were published on the influence of β-lactoglobulin genotypes on milk production and composition, however the majority of authors underline the positive role of AA and AB genotypes on milk production parameters e.g. protein and fat content (Table 2).

As a first step the β-lactoglobulin genotypes were determined in 147 Awassi and 203 Racka ewes. The genotype and gene frequencies were determined (Table 3). The AB genotype was the most frequent in both breeds.

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***Table 2.*β-Lactoglobulin genetic variants in sheep**

Gene	*Milk protein variant*	*DNA polymorphisms*
β-laktoglobulin (BLG)	A [Tyr-38, Arg-166] B [His-38, Arg-166] C [Tyr-38, Gln-166] (Ali et al., 1990; Erhardt, 1989; Kolde & Braunitzer, 1983)	A, B allél, C/T (Schlee et al., 1993; Feligini et al., 1998) C allél, G/A (Prinzenberg & Erhardt, 1998)

[ ] = type and position of amino acid change in the precursor molecule

The genotype frequencies in the Awassi breed were AB>BB>AA, and AB>AA>BB in Racka sheeps. The Racka like other examined sheep breeds revealed higher allele frequency for β-lactoglobulin A [3]. On the contrary in the Awassi population the B allele was more frequent. Interestingly similar tendency was described in the dairy Italian Sarda and Czeh Romanov sheep breeds. Among the Racka milk samples three samples resulted a so far unknown protein band on the CA-IEF gel. Mass spectrometry analysis underlined that the new band is β-lactoglobulin. Detailed characterization of the newly found variant at DNA level is currently on the way in our laboratory.

4. Conclusions

Within the framework of our research we studied the β-lactoglobulin genotypes of Hungarian Awassi and Racka sheep using individual milk samples and isoelectric focusing in the presence of carrier ampholytes. The β-lactoglobulin BB genotype frequencies (*P*=0,0343) and the allele frequencies (*P*=0,0140) showed significant differences between the two examined sheep populations.

***Table 3.*Genotype and gene frequencies in the examined sheep populations**

BLG	**Awassi**		**Racka**		***P***
Genotype	*Freq.*	*Number of ewes*	*Freq.*	*Number of ewes*	
AA	0,2313	34	0,3251	66	*P*=0,0565
AB	0,4966	73	0,5025	102	NS
BB	0,2721	40	0,1724	35	*P*=0,0343
Allél					
A	0,4796		0,5764		*P*=0,0140
B	0,5204		0,4236		*P*=0,0140
Összes		*147*		*203*	

Freq = frequency

NS = not significant

We performed analysis of milk proteins from Awassi and Racka milk samples by CA-IEF method, because this method gave us the highest probability to identify so far unrecognised allelic variant of β-lactoglobulin. Indeed in some Racka ewes we found a novel, so far unidentified variant. Our ultimate aim is to perform a complete sequencing analysis with DNA samples of the same animals. In future term, we aim to develop a simple and economical method e.g. PCR-RFLP or AS-PCR for large scale screening of the flock. Our data contribute to developing a new breeding strategy for improved milk production in the examined breeds.

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