THE STUDY OF α_S1 CASEIN GENETIC MARKER POLYMORPHISM IN CARPATHIAN GOAT BREED: SYNTHESIS OF 2009 RESEARCH (PROJECT PN II 52104/2008)

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Abstract

This paper presents the synthesis of 2009 research of PNII 52104/2008 project, which has as a major objective the evaluation of α_S1 casein genetic polymorphism on milk quality, cheese making efficiency and their specific goat flavour in Carpathian goat breed. In the phases 2 and 3 we had as goals the establishing of experimental lots and performing controlled mating in docks, in order to obtain the F1 progeny. Following genotyping (by IEF and PCR), of some goat populations from central part of Romania, 136 females and 6 males on the 3 homozygous genotypes categories (AA, EE, FF) in $\alpha_S1$casein. Following controlled mating in docks 2 reproduction indexes were established: fecundity index (94,85%) and abortion index (4,65%). In order to study the allele frequency in all 6 loci codifying major milk proteins, we continued the milk samples collection from the 2 planned regions in these 2 phases. Therefore 916 samples in all were collected form 5 farms from northern part of Romania (468 samples) and 5 farms from eastern part (448 samples). The obtained results so far are in accordance with the proposed objectives for these 2 phases.

Key words: Carpathian goat, α_{S1}casein, milk, polymorphism, genetic marker

INTRODUCTION

Since the discovery of four alleles categories in goat α_{S1}casein (α_{S1}-CN) locus, associated with different synthesis levels [4], goat milk protein polymorphisms received a considerable research interest. Genotyping in goat α_{S1}-CN locus became an important tool for improving milk quality and its manufacturing properties, in different breeds. The effects of α_{S1}-CN polymorphisms on goat milk composition, renneting properties, cheese yield and their specific goaty flavour, have been intensively studied in French breeds [5], Spanish breeds [8], Italian breeds [6], Norwegian breeds [9] and in USA breeds [3]. This genetic marker was significantly associated with high milk quality and superior manufacturing properties; A allele, with high expression (3,6g/kg of milk) improves milk whole protein, casein, fats content, manufacturing properties and cheese making efficiency with up to 20%, in comparison with weak expression allele F (0,6g/kg of milk), E allele with medium expression (1,6g/kg of milk), having an intermediary effect between previous two.

The major objective of the project PNII 52104/2008 is to establish the impact of α_{S1}-CN polymorphism in Carpathian goat on milk quality, cheese making efficiency and their goaty flavour, comparing three genotypes categories from this locus (AA with EE, AA with FF and EE with FF). Trough the characterization of local goats populations (including Carpathian and White Banat breeds), we wish to elaborate a pure breeding methodology based on this genetic marker (MAS), applicable on the national level and compatible with traditionally breeding systems from Romania.

In 2009 our goals in the 2 phases were: in Phase II- Genotyping of she and he goats parents in α_{S1}-CN locus, the establishing of experimental lots on the three genotypes categories (AA, EE, FF), the elaboration of equilibrated fodder ration; in Phase III- Performing the controlled mating in docks, in order to obtain the F1 progeny and the establishment of reproduction indexes.

MATERIAL AND METHOD

I. Genotyping of some goat populations in order to establish the experimental lots

Milk samples collection was done from goat individuals belonging to Carpathian goat breed from eastern part of Romania: Botosani, Iasi, Vaslui, Bacau, Neamt Counties (448 samples), northern part: Maramures, Bistrita-Nasaud, Salaj Counties (468 samples), but was continued in central part (being collected 600 samples). This was done directly from udder in 50 ml sterile tubes, with no preservatives, and stored during transportation at 4°C and then frozen at -20°C.

Ia. She goats genotyping from milk samples by IEF

Only the samples collected in central part of Romania were further analysed by isoelectric focusing technique (IEF), in order to choose the she-goats from the experimental lots on the three genotypes categories wanted in α_{S1}-CN (AA, EE, FF), the others being planned to be analysed in 2010 in accordance with project working plan. The genotyping was carried out from collected milk samples by IEF, as described before [1]. The genotypes were established in all 6 loci codifying the six major milk proteins: α_{S1}-CN, ß-CN, α_{S2}-CN, k-CN, ß-lactoglobulin and α-lactoalbumin.

Ib. Blood samples collection and DNA extraction

Blood samples were collected from jugular vein on K-EDTA anticoagulant from 300 she-goats with desired genotypes indentified by IEF and 60 he-goats in order to confirm/identify the genotypes in α_{S1}-CN locus. The DNA extraction was done as described before [4].

Ic. Genotyping of he-goats in α_{S1}-CN locus and confirmation of she-goats genotypes by PCR-RFLP, AS-PCR and sequencing

The PCR- RFLP genotyping was first done from reference samples, using primers specifically designed from intron 8 (CR-F) and intron 9 respectively (CR-R), including entire exon 9, as described before [7]. In this region are located several mutations which are differentiating the common genetic variants in α_{S1}-CN locus, being possible the identification of A, B and F alleles, following the digestion with PdmI enzyme. The differentiation of E allele from the others (especially B) was done by amplification with specific primers (CBE-F, CBE-R) of the transposable element from exon 19 (3'untranslated region), characterizing this allele. The PCR products obtained from reference samples with both protocols were sequenced, in order to confirm the mutations which are differentiating the common allele in these amplified regions.

In order to establish the genotypes in α_{S1}-CN locus in he-goats (60 individuals) and to confirm the genotypes observed by IEF in females (300 individuals), all individuals were genotyped by PCR-RFLP and AS-PCR.

II. Establishing the experimental lots

Choosing the she-goats had as first selection criteria the genotype in α_{S1}-CN locus. Because we target the evaluation of α_{S1}-CN locus polymorphism impact on milk quality and cheese making efficiency, were chosen individuals with similar genotypes in the other 5 loci codifying major milk proteins, genotypes obtained by IEF. In order to obtain results close to reality, were chosen representative individuals for Carpathian breed (pretty heterogeneous), from all point of views (conformation, constitution, milk production, etc). Therefore from the 300 candidate she-goats, 136 on the three genotypes categories on α_{S1}-CN locus were chosen for F1 generation obtaining: Lot 1: 48 she-goats AA; Lot 2: 43 she-goats EE; Lot 3: 45 she-goats FF.

Choosing the he-goats was done on the same criteria as for she goats, but with particularities specific to reproductive males. From the 60 genotyped males, 4 on each

genotype categories in α_{S1}-CN locus were chosen (12 in all). These males were tested for reproduction behaviour, being selected 2 on each genotype categories (AA, EE and FF).

III. The elaboration of fodder ration

In elaboration of fodder ration were chosen forages which are currently used in majority of goat farms from Romania. Indeed an equilibrated ration, could lead to a better expression of genetic potential, but the results would not correctly reflected the reality existing in Romanian goat farms and could not be objectively used in selection. Therefore we choose to feed the animals in similar conditions as those existing in the majority Romanian goat farms.

IV. Establishment of mating plan and making controlled mating

The controlled mating were done between 15 September-31 October 2009, experimental lots being organized as follows: Lot 1 - 48 she-goats AA: Dock 1 - he-goat 1 AA - 24 she-goats AA; Dock 2 - he-goat 2 AA - 24 she-goats AA; Lot 2 - 43 she-goats EE: Dock 3 - he-goat 3 EE - 22 she-goats EE; Dock 4 - he-goat 4 AA - 21 she-goats EE; Lot 3 - 45 she-goats FF: Dock 5 - he-goat 5 FF - 23 she-goats FF; Dock 6 - he-goat 6 FF - 22 she-goats FF. All animals were supplementary marked with colour and numbered tags. For the controlled mating she-goats were introduced in docks in accordance with tag colour and number. The mating was performed during the night, after the afternoon milking.

RESULTS AND DISCUTIONS

I. Genotyping results obtained by IEF

By IEF electrophoresis profiles analysis of milk samples collected form central part of Romania, common allele in the 6 loci were identified, described also in the literature and in our previous work [1]. The she goat females were chosen on the 3 homozygous genotypes categories in α_{S1}-CN locus (AA, EE and FF) and with similar genotypes in the other 5 loci.

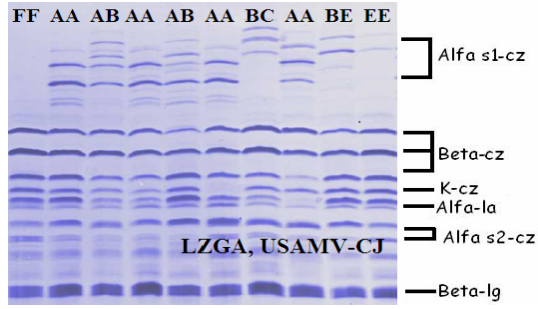

Figure 1 IEF electrophoresis profiles belonging to some individuals from Carpathian goat breed evidencing the genotypes in the 6 loci codifying goat major milk proteins

II. Genotyping results in α_{S1}-CN locus obtained in he-goats and confirmation of genotypes obtained by IEF of chosen she-goats, by PCR-RFLP and AS-PCR

The restriction map of the 213/223/224 bp PCR products, obtained following amplification from α_S1-CN gene, was done for PdmI enzyme. In the case of A, B and E allele the amplified fragment contains one restriction site for this enzyme, located in the same position. The restriction of A allele is leading to the obtaining 2 fragments: 150bp and 63bp (due to a 11bp deletion from the structure of this variant). In the case of B and E allele the fragments size were: 161bp and 63bp, therefore they cannot be differentiated because they are identical in this region. In the case of F allele the deletion of a cytosine from the amplified fragment abolish the restriction site for this enzyme, therefore the amplified fragment in not digested by this enzyme (Figure 2 left). The amplification of the transposable element characteristic to E allele leaded to the obtaining of a 547 bp product (Figure 2 right).

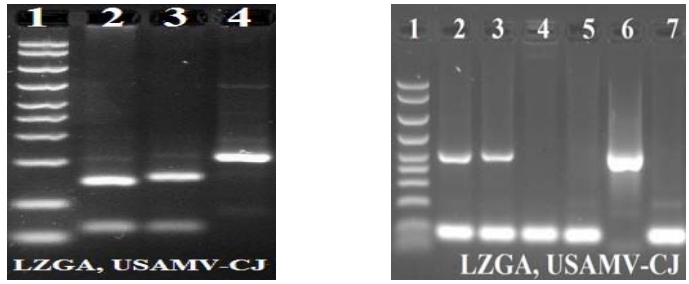

Figure 2 Left picture: Genotypes identification in α_S1-CN locus by PCR-RFLP. Lane 1: Ampli Size™ Molecular Ruler (BioRad Laboratories, Hercules, CA, USA); lane 2: AA genotype; lane 3: BB, BE and EE genotypes; lane 4: FF genotype; Right picture: Identification E allele by amplification of the transposable element from exon 19: lane 1 Ampli Size™ Molecular Ruler (BioRad Laboratories, Hercules, CA, USA); lanes 2 and 3 heterozygous genotypes carrying E allele; lanes 4,5,7 other genotypes than E; lane 6: EE homozygous genotypes

In the case of EE genotype a single 547 bp fragment (containing the 457 bp transposable element) was obtained and in BE genotypes a 547 bp fragment together with a 90bp fragment (signalling the absence of this insertion) were obtained. In homozygous non E individuals a single 90 bp fragment was obtained (Figure 2 right).

Sequencing of all PCR products confirmed the appartenance of these amplified fragments (obtained by both protocols) to the α_S1-CN gene and the mutations characterising each allele. Comparing the obtained genotypes by IEF, PCR-RFLP, AS-PCR and sequencing we concluded that IEF is a very useful technique for identification of genotypes in the 6 loci codifying milk proteins. However E allele can be correctly identified by IEF just in homozygous condition. In order to obtain early information about the genotypes in α_S1-CN locus in both males and females, these PCR tests proved to be faster and more efficient.

III. The elaboration of equilibrated fodder ration

In the winter-spring period (February-April 2009), autumn-winter (November-December 2009), the fodder ration was composed of alfalfa hay 3 kg/head/day, 0.5 kg/head/day corn and wheat grains and salt at pleasure. Between May-October, fodder ratio was composed exclusively by pasture, with vitamin-mineral premixes before and in mating season. The males were stimulated in mating season by 0.5 kg/head/day of grains.

IV. Results concerning some reproduction parameters in she goats from experimental lots

Reproduction indexes were calculated according to the formulas existing in literature, in order to evaluate the success of used mating plan. The fecundity index was calculated using formula: Fm%= number of pregnant she-goats/whole number of mated she-goats x 100 and the abortion index using the formula: A%= number of she goats with abortion/ number of pregnant she goats x 100. In Table 1 are presented the values of the two indices based on the data existing at the end of November 2009.

Table 1. Reproduction indices in she-goats from experimental lots

Breed	Number of whole mated females	Number of pregnant she-goats	Fecundity index	Number of she-goats with abortion	Abortion index
Carpathian	136	129	94,85%	6	4,65%

The she-goats mated in cycles one and two which did not passed in the 3rd cycle were considered pregnant, confirmed by the echography performed at 2 months with HK Pregnancy Diagnosis Unit. These indexes are situated between normal limits for this specie

CONCLUSIONS

We collected in this two phases of 2009 a number of 916 samples from eastern and northern part of Romania, in order to indentify in the following phase 4 the genotypes in the 6 major milk proteins loci. From the 600 milk samples collected from central part were chosen following genotyping by IEF and PCR 136 females on the 3 desired genotypes categories in α_{S1}1-CN locus (AA, EE and FF). From the 60 genotyped males and after their testing for reproduction aptitudes 2 on each genotype were chosen for controlled mating. Following mating two reproduction parameters were established, which are situated in normal limits for this breed

ACKNOWLEDGMENTS

This research was supported by Romanian Ministry of Education, Research and Innovation trough CNMP Project PNII: 52104/2008-2011.

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