Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **41(1)** (**2008**), Timişoara

GENETIC POLYMORPHISM OF SOME PROTEINS IN THE MILK OF CARPATHIAN GOAT

STUDIUL POLIMORFISMULUI GENETIC AL UNOR PROTEINE DIN LAPTELE DE CAPRĂ CARPATINĂ

ZAULET MIHAELA*, KEVORKIAN STELIANA *, ENACHE MIHAELA * , GHITA ELENA**, LAZAR CRISTINA**, REBEDEA MARIANA *

** University of Bucharest, Faculty of Biology, Romania*
***INCDBNA Balotesti, Romania*

The paper presents some aspects of the polymorphism of some Carpathian goat milk proteins. The Carpathian breed is the main breed of goats reared in Romania. The optimal working conditions were determined for the identification of the casein phenotypes. The technique of the polyacrylamide gel electrophoresis was used. The milk samples were processed to remove the fat and whey and the migration was done in the presence of a standard sample which contained proteins with different molecular weights. The interpretation of the electrophoresis migrations revealed the presence of two genotypes, the homozygous genotype β-Cn BB and the heterozygous β-Cn AB. The homozygous genotype β-Cn AA was not identified in any individual. The heterozygous genotype β-Cn AB displayed a high frequency (59%) and it was observed in 10 individuals. The homozygous genotype β-Cn BB was observed in 7 individuals and it had a frequency of 41%. The homozygous genotype β-Cn AA has not been identified in the studied population. The distribution of these genotypes showed that allele β-CnB was predominant (70%) over allele β-can (30%).
Key words: goats, milk polymorphism, casein

Introduction

The Carpathian breed is the main goat breed reared in Romania accounting for about 80% of the total stock of goats. The goat is reared in southern Romania extending up to the hill and mountain areas. The breed is characterised by a large variability of the conformation, productivity and colour having an outer look specific to the primitive animals, with robust body, lively behaviour, high mobility and agility.

The individuals of this breed are noteworthy for their special biological particularities due to their level of rustification and to their capacity to adapt to climacteric stress; they are resilient to certain contagious diseases such as tuberculosis, brucellosis and have very low mortality rates; they strive on roughages. The biological longevity of the breed is 10-12 years (maximum 18 years) and the productive longevity is of 7-8 years. The research carried out in

Romania by Taftă (1996) gives us some information of the morpho-productive traits of the breed. Thus, the live weight is 38.5-52.5 kg in females and about 56 kg in males; their height is 61.7-69.72 cm. The milk yield in about 9 months is 240-280 l, with a high of 450 l and a record of 800 l milk. Milk fat amounts to 4.5-5 %. Milk protein amounts to 3.3 %, lactose to 4.5 %, and the minerals amount to 0.7 %. The meat is produced only by suckling kids obtained in early spring (age of 1-2 months and a live weight of 8-12 kg) and by reformed goats. The prolificacy of the Carpathian breed is 140 %, the female kids having 2.9 kg at birth, while the males have 3.1 kg.

Goat rearing is, for the Romanian farmers, an opportunity in their competition with the European Union farmers because goat milk is not submitted to quotas and it is demanded on the European market due to curative treatments. Goat milk has six main proteins, four casein proteins and two non-casein proteins. The casein proteins are α_{S1}, α_{S2}, β and k – casein; the non-casein proteins are α-lactalbumin and β–lactoglobulin, but these forms differ through the genetic polymorphism and the frequency of appearance in goat populations. The presence of $\alpha\ s_1$ casein has been studied frequently in recent years, when 6 different forms of it were discovered in goat milk: A, B, C, E, F and „0" (G.F.W. Haelein, 2003).

The research of Yahyaoui et al. (2003) aimed to characterize and genotype the variants of the k-casein in the goat. K-casein is the milk protein which determines the size and specific function of the milk myceles and their separation by chimosine accounting for milk curdling. Four variants of k-casein were characterized in Spanish, French and Italian goat breeds.

This paper aims to identify by electrophoresis some casein proteins in the Carpathian goat's milk.

Materials and Methods

Milk samples were collected from 17 Carpathian goats reared at ICDCOC Palas Constanta. The milk samples were collected from the following goats (sample number / tag number): 1-689, 2-120, 3-456, 4-69119, 5-659, 6-991, 7-695, 8-695, 9-880, 10-478, 11-964, 12-986, 13-688, 14-519, 15-029, 16-498, 17-998.

Several working variants were used to determine the optimal conditions of electrophoretic migration and to separate the milk caseins.

Polyacrylamide gel electrophoresis employed casein assays in the laboratory

Method principle. Sample preparation

The fat and whey had to be removed before determining β casein gene polymorphism in goat milk.

- The fat was removed by centrifuging at 3000 × g, at 4^0 C, for 10 minute and filtration through filter paper (J.F. Medrano and L. Sharrow, 1989).

- The casein was separated from the whey through isoelectric precipitation at pH 4,6 with acetate buffer, at 40^0C for 20 minutes followed centrifuging at 3000 × g, at 4^0 C, for 10 minute (J.F. Medrano et L. Sharrow, 1989).

The Bradford method. Method principle

This technique relies on the observation of the maximum absorption of the acid solution of Coomassie Brilliant Blue G-250 dye which changes from 465 nm to 595 nm when it binds to proteins. The hydrophobic and ionic interactions stabilise the anionic structure of the dyestuff causing a change of absorption detectable in VIS. Having a constant absorption coefficient of the dyestuff-albumin in a wide domain, this method can be used mainly to determine the protein concentration from cell fractions for electrophoretic analyses.

The working protocol requires:

1. The use of TECAN UV/VIS multireader for electrophoretic determinations.
2. To dilute the samples to be analysed so that they contain between 5 and 100 µg protein.
3. To prepare BSA (bovine serum albumin) standards containing between 0 and 1.4mg/mL protein in a volume of 140 µl.
4. To add the samples to be analysed and the standards of 5µl over 250µl of Bradford reagent.
5. Sample incubation for 30 minutes at room temperature.
6. Measure the absorbance using TECAN multireader at 595 nm.

Figure 1
BSA standard curve

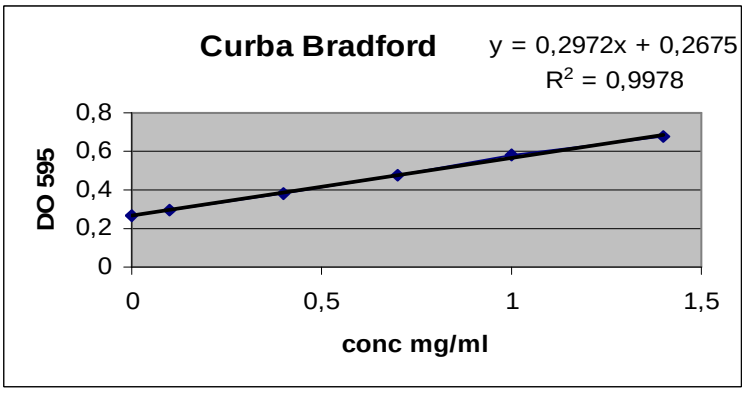


Figure 1 shows the curve for BSA standard. The efficiency coefficient of 0.9978 shows a good standard curve. Protein concentration was assayed for 2 samples (Table 1).

Table 1

Concentrations assayed for samples 1 and 2 with dilution 40x, 50x and 60x

Sample/dilution	Concentration, µg/µl
1/40x	21.02288
1/50x	22.7961
1/60x	22.71198
10/40x	29.21938
10/50x	30.21534
10/60x	31.93809

Electrophoresis. Two gels were poured for a good electrophoretic migration: a gel for actual migration with 12.5% polyacrylamide concentration and a gel for sample concentration with 3% polyacrylamide concentration. Table 2 shows the working protocol.

Table 2

Reagents and amounts required for protein electrophoresis

	Migration gel 12.5%	Concentration gel 3%
Polyacrylamide 30%	3.125 ml	0.25 ml
TRIS pH 8.8	1.875 ml	-
TRIS pH 6.8	-	0.625 ml
APS 10%	0.025 ml	0.0125 ml
TEMED	0.005 ml	0.0025 ml
Distilled water	2.47 ml	1.61 ml

- Electrophoresis buffer: SDS 1X – Tris 0.125M, glycine 0.96M, SDS 0.5%. Prepared as stock solution 5X which is diluted before using.
- Sample buffer: β-mercaptoethanol 2x: 1ml glycerol, 0.1 ml β-mercaptoethanol, 3ml SDS 10%, 1.25ml TrisHcl 1M, pH-6.7, 1.2 mg brom phenol blue.
- Buffer 4x TRIS pH 6.8: 0.5 M TRIS, 0.4% SDS. Brought to pH with HCl.
- Buffer 4x TRIS pH 8,8: 1,5 M TRIS, 0,4% SDS. Brought to pH with HCl.
- Dyestuff: 1g copper sulphate, 1g Coomasie Blue, 50 ml perchloric acid, 950ml distilled water
- Discoloration solution : acetic acid : methanol : water / 1:3:6

Preparation of the polyacrylamide gel:

- mount the plates in holders;
- pour the gel over the migration gel and allow it to polymerise; pour distilled water to prevent the gel from drying;
- remove the water and pour the concentration gel;
- mount the combs to form the wells and allow it to polymerise again;
- each sample of goat milk is mixed with the "sample buffer" and loaded into the wells with a Hamiltonian pipette;
- electrophoresis is done at a constant tension of 80V (about 3V/cm) and at the temperature of 4^0C, for 200 minutes. We used a Mini-Protean II system (BioRad);
- after migration finishes, the gels are coloured by immersion in the dyestuff for one hour;
- remove the dyestuff and pour the discolouration solution over the gels;
- the proteins are viewed on a UV BIO-PROFIL (Vilber Lourmat) transiluminator

Results and Discussions

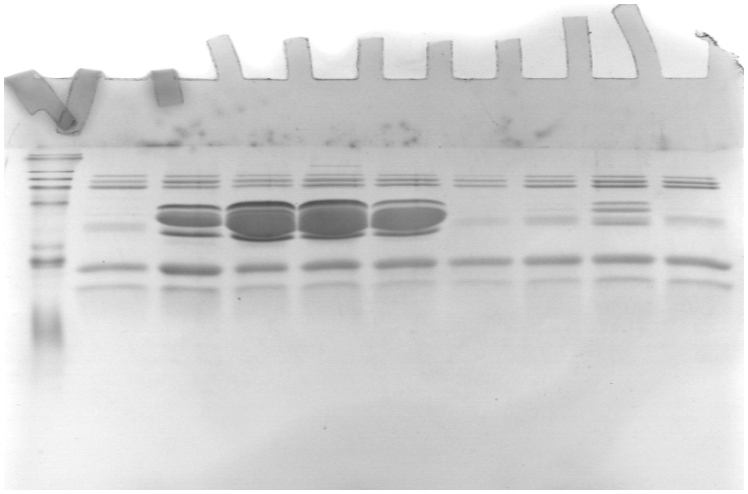
Figures 2 and 3 show the electrophoregrams of the 17 samples of goat milk processed according to the protocol described. Migration was done in the presence of a standard sample which consists of the following proteins with different molecular weights: Myosine-250 kDa, Phosphorilase-148 kDa, BSA-98 kDa, Glutamic dehydrogenase-64 kDa, Alcohol dehydrogenase-50 kDa, Carbonic anhydrase -36 kDa, Myoglobin-22 kDa, Lysosime- 16 kDa, Aprotinine-6 kDa, Insulin-4 kDa. The molecular weight of the casein ranges between 25 and 19 kDa (beta Cn 24 kDa, kappa Cn19-20 kDa and alpha Cn 23-25 kDa).

After the electrophoretic migrations have been interpreted, two genotypes were identified in the goat population studied: the homozygous genotype β-Cn BB and the heterozygous genotype β-Cn AB. The homozygous genotype β-Cn AA has not been detected in any individual.

Table 3 shows allele and genotype frequency at the locus β-Cn which has been identified in the population studied.

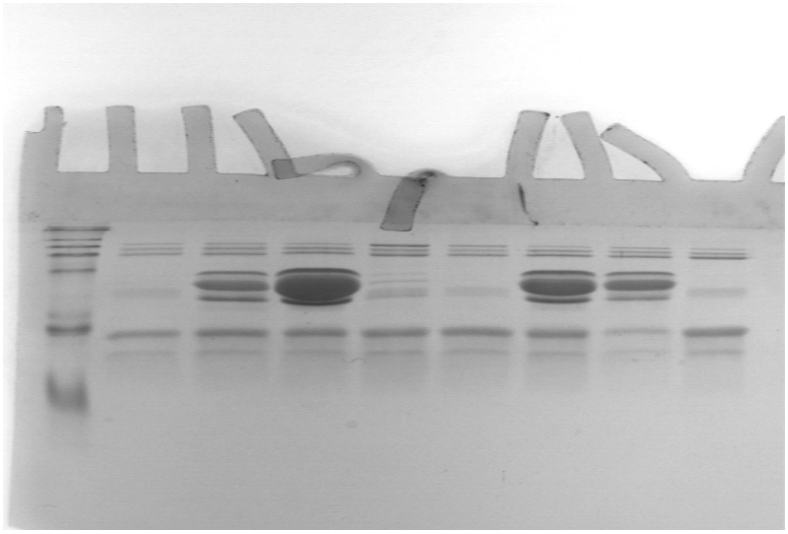
Table 3
Allele and genotype frequency at the locus β-Cn in the population studied

Alleles	Allele frequency	Genotypes	Genotype number	Genotype frequency
A	0.300	AA	0	0.000
B	0.700	AB	10	0.590
		BB	7	0.410



C 1 2 3 4 5 6 7 8 9

Figure 2 Electrophoregram with the control sample and with the milk samples **1 - 9.**



C 10 11 12 13 14 15 16 17

Figure 3 Electrophoregram with the control sample and with the milk samples 10 -17.

The highest frequency among genotypes was observed in the heterozygous genotype β-Cn AB, which has been identified in 10 individuals (59%). The homozygous genotype β-Cn BB has been identified in 7 and had a frequency of 41%. The homozygous genotype β-Cn AA has not been detected in the population studied. Consistent with the genotype distribution observed, the β-CnB allele

clearly predominated with a value of 70%, compared to 30 %, recorded for allele β-CnA

Conclusions

The polyacrylamide gel electrophoresis performed in order to determine the phenotypes at the β-Cn locus in the 17 samples of Carpathian goat milk revealed:

- the concrete conditions for a proper migration of the milk samples;
- two genotypes have been identified at the β-Cn locus of the studied population, the homozygous genotype β-CnBB (7 individuals) and the heterozygous genotype β-Cn AB (10 individuals);
- the frequency of these genotypes was 59% for the heterozygous genotype β-Cn AB and 41% for the homozygous genotype β-CnBB;
- the frequency of gene β-CnB was 70%, compared to 30%, the frequency of gene β-CnA.

Bibliography

1. **Maria Ballester**, **Armand Sanchez and Josep M., Folch** (2005) – *Polymorphisms in the goat β-lactoglobulin gene*, Jour. of Dairy Research, , 72 379-384

2. **S. Chessa, E. Budelli, K. Gutscher, A. Caroli and G. Erhardt** (2003)– *Short comunication: simultaneous identification of five k-casein (CSN3) alleles in domestic goat by polymerase chain reaction – Single strand conformation polymorphism* , J. Dairy Sci., 86:3726-3729

3. **Christine Leroux**, **Fabienne Le Provost, Elisabeth Petit, Laurence Bernard, Yves Chilliard, Patrice Martin** (2004) – *Real – Time RT-PCR and cDNA macroarray to study the impact of the genetic polymorphism at the α_{s1} - casein locus on the expression of genes in the goat mammary gland during lactation* , Reprod. Nutr. Dev. 43, 2003, 459-469, INRA, EDP Sciences

4. **Ajay Kumar, Pramod Kumar Rout, Ramadhar Roy** (2006)– *Polimorphism of β-lactoglobulin gene in Indian goats and its effect on milk yield* , J. Appl Genet 47 (1)

5. **Hiroyuki Tomotake, Ryoko Okuyama, Mitsuaki Katagiri and col (**2006).- *Comparison between Holstain cow`s milk and Japanese – Saanen goat`s milk in fatty acid composition, lipid digestibility and protein profile* , Biosci. Biotechnol. Biochem. 70 (11),(2771-2774)

6. **M. H. Yahyaoui**, **A. Angiolillo, F. Pilla, A. Sanchez, and J. M. Folch** (2003)– *Characterisation and genotyping of the caprine k – casein variants*, J. Dairy Sci., 86:2715-2720