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SUMMARY OF THE PhD THESIS

ASSESSING THE $\alpha_{S1}$-CASEIN POLYMORPHISM EFFECT ON MILK QUALITY AND CHEESEMAKING EFFICIENCY IN CARPATHIAN GOAT BREED

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The assessment of the effect of the $\alpha_{S1}$-caseinei polymorphism in Carpatină goats on milk quality and cheese yield

UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE CLUJ-NAPOCA
DOCTORAL SCHOOL

FACULTY OF ANIMAL SCIENCE AND

To......................................................................................................................

With distinguished consideration we invite you in 28.09.2012, 8:00 o’clock in the Council Hall of Animal Science and Biotechnology Faculty, UASMV Cluj-Napoca, Mănăştur 3-5 st., to take part to the public meeting gathered for the presentation of the doctoral thesis entitled "ASSESSING THE $\alpha_{S1}$-CASEIN POLYMORPHISM EFFECT ON MILK QUALITY AND CHEESEMAKING EFFICIENCY IN CARPATHIAN GOAT BREED", elaborated by eng. POP Felician Dorin, with the aim of awarding the title of DOCTOR IN ANIMAL SCIENCE.

The Componence of the Doctoral Comission is the following:
1. Prof. Vioara MIREŞAN Ph.D., UASMV Cluj-Napoca - President
2. Prof. Augustin VLAIC Ph.D., UASMV Cluj-Napoca – Scientific coordinator
3. Prof. Ioan PĂDEANU Ph.D., UASMVB Timișoara - Referee
4. ProfIoan BENCSIK Ph.D., UASMVB Timișoara - Referee
5. Associate professor Viorica COŞIER Ph.D., UASMV Cluj-Napoca - Referee
The assessment of the effect of the αS1-casein polymorphism in Carpatină goats on milk quality and cheese yield

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**Keywords:** Carpatin breed, polymorphism, caseine, milk quality, cheese producing yield.

**INTRODUCTION**

The goat rearing and exploitation recorded an ascendant development worldwide, due to its exceptional biological and economical particularities. The research and development efforts developed in last 20 years, for a more efficient use of present genetic resources, radically changed the judgement concerning the role of the goat specie in this context.

The goat milk quality represented and still represents the main objective in many countries, due to its biological active particularities. This led to the development and fundamentation of research in the field of selection based on genetic markers, with the aim of improving and selecting the individuals with best productive performances.

In goat, the qualitative milk parameters, coagulation time, coagulum stiffness, cheese obtaining yield and flavour are directly influenced by the accentuated polymorphism noted at $\alpha_{S1}$-casein locus (CSN1S1).

Even though, in our country, the goat species was never the objective of an sustained and continuous action of selection. This is prooved by their heterogeneity concerning milk production, the quantity of fat and protein in milk, body conformation and development, mane presence or absence, variety of the pilose coat colour, etc. (TAFTĂ, 1996, 2008; ZAMFIRESCU, 2009). We have to notice that as time passes by, more serious attention was paid to enhanced interest for goat rearing, and to problems concerning the increase of productivity in this specie.

The knowledge of the polymorphism of the proteins from the goat milk and their impact on milk quality, will open new perspectives in our country, to a better valuation of goat milk, increasing the yield of cheese obtaining and possibility of autenticity certification of the goat milk products.
PART I
THE BIBLIOGRAPHICAL STUDY

CHAPTER I
THE IMPORTANCE, EVOLUTION AND DYNAMICS OF GOAT BREADING

In this chapter, the economical importance of the goat rearing is presented, in context of the globe population increasing, and supplying easily assimilable food, with superior nutritional value. There is also presented, the dynamics of goat sector development in last 20 years, worldwide, and also in our country, including the improvement programmes put into practice in countries with tradition in goat rearing. In few pages are presented the morphoproduc tive traits of both genuine goat breeds (Carpathian and Albă de Banat), emphasizing their heterogeneity and importance of enhancing their productive performances.

CHAPTER II
THE CROMOZOMIAL LOCATION OF THE GENES ENCODING THE MAJOR MILK PROTEINS IN GOAT MILK AND ITS BIOLOGICAL PROPERTIES

In this chapter, the localization on cromozomes of the genes codifying the main proteins in the milk from the goat specie: αs1-caseine (αS1-CN), β-caseine (β-CN), αs2-caseine (αS2-CN), κ-caseine (κ-CN), α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and their organizing in cluster. In the second part of the chapter are detailed the main biological particularities of the goat milk and effects of the milk protein polymorphisms on its composition.
CHAPTER III
STUDY OF GOAT MILK PROTEINS POLYMORPHISMS

The chapter presents the molecular techniques used within present research for the study of the polymorphisms of the proteins from milk at DNA level (PCR-RFLP, AS-PCR techniques, DNA sequencing) and protein (IEF technique). The present stage of the research concerning the study of the genetic polymorphisms of the caseins and proteins from lactoserum, also, are chronologically presented.

CHAPTER IV
THE IMPORTANCE OF GOAT MILK PROTEINS POLYMORPHISMS STUDY

In this chapter are presented the results of the polymorphisms of the milk proteins in goat, on CSN1S1 locus, especially, on milk quality, coagulation properties, yield of cheese obtaining, sensorial traits of cheeses, and functionality of the secretory epithelial cells from the mammary gland. In the last part, it is emphasized the importance of the study of these polymorphisms, as genetic markers of identifying the authenticity of the dairy products from goat milk, sources of bioactive peptides, and also in selection of the individuals with nule genotypes at caseines loci, for obtaining a hypoalergenic milk.
II\textsuperscript{nd} PART
PERSONAL RESEARCH

CHAPTER V
THE AIM AND OBJECTIVES OF THE RESEARCH

The major objectives of the research are:

1. **Testing and improvement of some DNA tests with the aim of genotypingization of the goat sires at $\alpha_{S1}$-casein locus**
   
   Within this first objective, there were tested, optimized and validated, at DNA level, three PCR protocols (one PCR-RFLP and two AS-PCR), with the aim of genotyping all Carpathian breed sires at CSN1S1 locus.

2. **The assessing of the polymorphism of $\alpha_{S1}$-casein on goat milk quality, of the cheeses yield and their specific flavour, with the aim of recommending some genetic improvement methodologies, in pure breed, based on this marker.**

   Within this objective, we aimed to assess the effect of the polymorphism of the genetic marker of $\alpha_{S1}$-casein(CSN1S1) on the milk qualitative parameters (content of protein, casein, fat, SUN, lactose), in a representative Carpathian population, divided in three experimental groups, function of the genotype at $\alpha_{S1}$-casein locus. The study was performed by comparisons between three categories of genotypes at this locus (AA + BB + CC, EE and FF), associed in other breeds with different levels of the expression of $\alpha_{S1}$-CN protein in milk (strong, moderate, and weak).

3. **The genetic fingerprinting of the genuine goat populations concerning the polymorphisms at the six studied loci codifying the major milk proteins.**

   Within this objective, there was performed a zonal characterization of several genuine goat populations from different areas of our country, concerning the polymorphism of the major milk proteins, and of $\alpha_{S1}$-casein, especially.
5.1. MATERIAL AND METHOD

Testing and improving of the DNA tests was performed on 60 Carpathian individuals from three private farms located in the counties of Cluj and Sălaj. The DNA extraction from blood samples was performed using a protocol from INRA, Jouy en Josas, and improved within the Zonal Laboratory of Animal Genotypization. Taking into consideration that the preliminary analysis at protein level using IEF technique, indicated the majority presence of the CSN1S1 common alleles (A, B, E, F), the DNA tests were developed only for these alleles that are majority encountered in other breeds, too. Three PCR tests were selected in order to perform genotypization at DNA level: one of PCR-RFLP type, firstly described by RAMMUNO et al., 2000 and two tests AS-PCR type, firstly described by FELIGINI et al., 2005 and DETTORI et al., 2009, respectively, and improved during present research development (POP et al., 2008a). In order to perform genotypization at genic expression level, in lactating females, the isoelectric focization technique (IEF) was used.

Our study aims the assessment of the polymorphism of the genetic marker CSN1S1 of milk qualitative parameters (total protein, casein, fat, dry matter and lactose) and of cheese AA, EE and FF genotypes, on a representative Carpathian goat population. For this reason, five quantifications of these parameters were performed, during July - August 2009, May - July 2010, respectively, with a single quantification by month.

The data were statistically processed using OriginPro 8.5 programme. The significance of the differences between the averages obtained by each category of genotype was tested.

The assessment of the specific goat flavour in prepared cheese, was performed by organizing tastings within the Unit of Genetics and animal breeding and within the Traditional Products Fair organized by USAMV Cluj. The test was applied on 30 subjects. The characterization of the polymorphisms of the major proteins from milk was performed using IEF technique, on 830 Carpathian individuals, from 14 farms distributed in 11 counties from our country.
The assessment of the effect of the αS1-caseine polymorphism in Carpatină goats on milk quality and cheese yield

THE PRACTICAL IMPORTANCE OF THE RESEARCH

the study of the polymorphism of the major proteins from goat milk, will contribute to the development of the knowledge in the area, and the transferr of the results in practice, by:

1) elaboration of the future national programmes of genetic markers assisted selection (MAS) and improvement.

2) reducing of production costs of cheese, by processing milk with higher protein and fat content, and understood superior processing yields.

3) increasing of producttion and diversification of traditional goat assortments, function of the preferences of the consumers concerning their flavour and consistency.

4) posibility of identifying the authenticity and protection of the integrity of the traditional products obtained from goat milk, according to: Ord. nr. 555/1020/2002, Ord. MAPDR no. 285 and 233/2004.

5) producing a natural hypoalergenic milk, by selection of individuals that are carriers of the genotypes with null expression at the main milk allergens loci.

6) valuation of some products with high content in bioactive peptides derived from the proteins from milk, in context of promoting the consumption of functional food.

CHAPTER VI

ASSAYING AND IMPROVING OF SOME DNA PROTOCOLS IN ORDER TO GENOTYPING THE BREEDING GOAT AT CSN1S1 LOCUS

RESULTS AND DISCUSSIONS

6.1. Results concerning PCR-RFLP testing and improvement

As consequence of the improvement of the PCR protocole, the amplification of the regions of the introns 8, 9 and entire exon 9 from the CSN1S1, followd by restriction for the AA genotype, 161 pb + 63 pb for the BB/EE/BE genotypes (they cannot be
differentiated by PCR-RFLP test in this region), 223 pb for the FF genotype and different combinations for heterozygous (Fig. 24).

The sequencing of the amplification products correspondent to AA, BB, EE and FF genotypes, confirmed the affiliation of *Capra* specie, *hircus* subspecie, to the CSN1S1 gene and emphasized the mutations correspondent to each allellein genuine goat breed, meaning: the deletion of the fragment of 11pb (5’-CGTAATGTTTC-3’), locted in position 75-85 from the intron 9 (for the allelle A), deletion of the citozine from the position 23 of the exon 9 (for the allelle F) and two specific substitutions for the allelle A (G→A și T→C), located in positions 19, 30 respectively, from the intron 9 (Fig. 20).
The assessment of the effect of the $\alpha_S1$-casein polymorphism in Carpatină goats on milk quality and cheese yield

Fig. 20. Part of the chromatograms obtained from PCR products sequencing belonging to CSN1S1 gene, showing the cytosine deletion specific to F allele (4A) and its presence in case of A allele (1A), B allele (2A) and E allele (3A) respectively the 11bp deletion specific to A allele (1B) and its presence in case of B allele (2B), E allele (3B) and F allele (4B) (original).

6.2. Results concerning the AS-PCR testing and improvement

In order to differentiate the individuals that are carriers of alleles B and E of $\alpha_S1$-casein (previously identified by PCR-RFLP technique) 2 AS-PCR protocols were tested.

The amplification with the CBE primers set, fanking the transposable element characteristic for allele E, led to getting of the expected products. Thus, for the EE genotypes, a 549 bp fragment was obtained, that includes the transposable element, in AE heterozygous individuals two fragments of 549 bp and 90 bp, respectively, were emphasized, and in individuals not carrier of the allele E a fragment of 90 bp was obtained (Fig. 25). Concerning the amplification of the second set of primers (Ex19), the expected products were also obtained, meaning: 445 bp for the EE genotype, 590 bp for the homozygous genotypes not carriers of the E allele, and both fragments (445 bp + 590 bp) for the heterozygous individuals of AE/BE type (Fig. 26).
The assessment of the effect of the $\alpha_{S1}$-casein polymorphism in Carpatină goats on milk quality and cheese yield

**Fig. 25.** Electrophoretic profile belonging to some Carpathian goat individuals, after amplification with CBE primers, in order to identify the LINE element specific to CSN1S1 E allele. Lane 1 - GeneRuler 100bp DNA Ladder (Thermo Scientific, Wilmington, USA), lanes 2, 3 - non E genotypes, lanes 4, 5 - EE genotypes, lane 6 - AE genotypes, lanes 7, 8, 9, 10, 11 - non E genotypes, lane 12 - EE genotypes (original).

**Fig. 26.** Electrophoretic profile belonging to some Carpathian goat individuals, after amplification with Ex primers, in order to identify the LINE element specific to CSN1S1 E allele. Lane 1 - EE genotypes, lanes 2, 3, 4 - AE genotypes, lanes 5, 6 - EE genotypes, lane 7 - AE genotypes, lane 8 - AA genotypes, lanes 9, 10 - EE genotypes, lane 11 - GeneRuler 100bp DNA Ladder (Thermo Scientific, Wilmington, USA) (original).
The assessment of the effect of the αS1-cazeinei polymorphism in Carpatină goats on milk quality and cheese yield

The sequencing of the amplicons corresponding to the CBE-F and CBE-R primers from the control samples with genotype EE, belonging to Carpatina breed, confirmed the length of the transposable element of 457 bp. It was also emphasized a substitution C → T in position 359 of the transposable element, not yet signaled in literature nor in GenBank database. We have to mention that this mutation was emphasized in all sequenced individuals (Fig. 27).

![Fig. 27. Part of the chromatograms obtained by sequencing the 549 bp PCR products, resulting from amplification with CBE primers, of some CSN1S1 EE reference samples, belonging to Carpathian goat breed. It highlights the C → T substitution at position 359 in the LINE element (original).](image)

CHAPTER VII
COMPARISON BETWEEN PCR AND IEF TECHNIQUES FOR STUDYING THE GENETIC POLYMORPHISMS AT CAPRINE CSN1S1 LOCUS
RESULTS AND DISCUSSIONS

The IEF technique allowed the identification of the variability at the protein expression level between the alleles αS1-CN. The alleles A and B with strong expression were emphasized as dense and intense cloured bands (Fig. 32, fields 3’ and 6), allelle E with moderate expression was identified as weak cloured bands (Fig. 32, field 4’), while
The assessment of the effect of the αS1-caseinei polymorphism in Carpathian goats on milk quality and cheese yield

allele F with weak expression, which migrates between first two major bands of β-CN, was difficult to be identified, as a fine band, very weak coloured (Fig. 32, field 5’).

The comparisons between the genotypes from the αS1-casein (CSN1S1) locus, obtained using the DNA analyse (presented in chapter VI), using PCR techniques, with those obtained at protein level (by IEF technique), were confirmed by the correspondence between the genotypes obtained by each technique. The PCR-RFLP protocol cannot differentiate the genotypes of the individuals that are carriers of the alleles B and E, compared to IEF technique, and for this reason, in order to confirm the carrier genotypes for allele E, testing by using AS-PCR technique is imposed (Fig. 33).

Fig. 33. Electrophoretic profiles obtained after comparative analysis by PCR-RFLP (at DNA level) and IEF (at protein level) techniques, of some reference samples with known genotypes. With red are marked individuals genotype at CSN1S1 locus. Lane 1 - Ampli Size Molecular Ruler 50-2000pb (BioRad Laboratories, CA, USA), lane 2 - unrestricted product (224 pb), lanes 3, 3’ - individuals with AA genotype, lane 4 - individual with BB/EE/BE genotype, lane 4’ - individuals with EE genotype, lane 5, 5’ - individuals with FF genotype, lane 6 - individual with AB genotype, lane 7’ - individual with BB genotype (original).

However, some cases were recorded where the PCR techniques allowed the corrections of the individuals that are carriers of the BE genotypes obtaine through IEF,
taking into consideration that alleles B and E from this locus exhibit close isolectric points, but their identification is made based in the intensity of the focalization bands.

CHAPTER VIII
ASSESSING THE CSN1S1 GENETIC MARKER POLYMORPHISM EFFECT ON MILK QUALITY PARAMETERS AND CHEESEMAKING EFFICIENCY
RESULTS AND DISCUSSIONS

8.1. Results concerning selection the individuals candidates to form the experimental groups on the three genotype categories from CSN1S1 locus
8.1.1. Genotyping results by IEF technique of some Carpathian goat populations

The genotypization of 390 Carpathian females, candidates to experimental groups formation, by IEF, led to emphasizing of the most common alleles reported for the casein loci: alleles A, B, E, F of αS1-CN, alleles A+C of β-CN, alleles A and C αS1-CN, alleles A and B of κ-CN, besides some rare recorded alleles as allele 0 of αS1-CN, allele E of β-CN and allele E of αS2-CN (Fig. 39).

![Image](image.png)

**Fig. 39.** IEF electrophoretic profile belonging to some Carpathian goat individuals, candidates to form the experimental groups.

After summarizing the obtained genotypes only individuals carriers of the AA, AB, BB, EE and FF genotypes at CSN1S1 were selected, who also had the other loci of the major proteins from milk, associated to a normal level of protein synthesis (127
individuals), as well as genotypes AA/AC/CC and EE for CSN2 (the synthesis level of 10g/L); and AA genotype for CSN3 (the synthesis level of 6g/L, because allele B is associate to a bigger casein percent, + 0.5g/L, and total protein in milk, compared to allele A) (CHIATTI et al., 2005; CARAVACA et al., 2009).

Concerning the loci of the proteins from lactoserum (α-LA and β-LB) the individuals selection easier, because these loci are monomorphs, the AA genotype being the only one present for both loci.

The individuals selected after IEF tests (127 females) were also analyzed by PCR technique, with the aim of confirmation of the genotypes obtained using IEF.

8.1.2. Results concerning genotyping by PCR techniques of preselected individuals to form experimental groups

When genotypization was performed using PCR-RFLP and AS-PCR techniques, the genotypes of all individuals previously analyzed using IEF technique, were identified and confirmed at DNA level. There were also corrected some of the genotypes of the individuals carriers of the allele B and E, determined by IEF.

The summarization of the final obtained genotypes, led to selection of 51 females for building the experimental groups, by the three categories of genotypes at the CSN1S1 locus. The groups were divided as follows:

- group 1 - 20 females with genotypes AA, AB, BB;
- group 2 - 10 females with genotype EE and
- group 3 - 21 females with genotype FF.
8.2. **Results concerning assessing the CSN1S1 genetic marker polymorphism effect on milk quality parameters, cheesemaking efficiency and cheese flavor on the three genotypes categories**

8.2.1. **Results concerning quantifying the milk quality parameters**

The results obtained for each quantitauve parameter were expressed through the average of all tests performed individually, function of the genotype category (table 15).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Analyzed parameters and method used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatness (g/100g)</td>
</tr>
<tr>
<td>AA</td>
<td>3.73±0.26</td>
</tr>
<tr>
<td>EE</td>
<td>3.59±0.12</td>
</tr>
<tr>
<td>FF</td>
<td>3.94±0.20</td>
</tr>
</tbody>
</table>

¹ solids non fat
The assessment of the effect of the αS1-caseinei polymorphism in Carpatină goats on milk quality and cheese yield

Analyze of the main milk qualitative parameters, performed at the CSN1S1 locus, by 3 categories of genotypes, of Carpathian breed emphasized that:

- concerning the average fat content, a superiority of FF genotype, compared to other genotypes (FF > and 16);

Table 16

The differences between the average values of the qualitative parameters of milk function of the genotype of the CSN1S1 locus

<table>
<thead>
<tr>
<th>CSN1S1 genotypes</th>
<th>Analyzed parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatness (g/100g)</td>
</tr>
<tr>
<td>AA - EE</td>
<td>+0.14(^{ns})</td>
</tr>
<tr>
<td>AA - FF</td>
<td>-0.21(^{ns})</td>
</tr>
<tr>
<td>EE - FF</td>
<td>-0.35(^{ns})</td>
</tr>
</tbody>
</table>

\(^{1}\) solid non fat; \(^{ns}\) - not significant differences P>0.05; \(^{*}\) -significant differences P≤0.05; \(^{**}\) -distinct significant differences P≤0.01; \(^{***}\) - very significant differences P≤0.001

- concerning the average lactose content and pH, we recorded the superiority of the FF genotype, compared to other genotypes (FF > EE > AA). The differences are distinctly significant between the EE - FF genotypes for lactose, and significant between the A - EE genotypes, very significant, respectively between AA - FF genotypes for the pH index (Tables 11 and 16);

- concerning the average protein, casein, and SUN content, a clear superiority of the AA genotype compared to others genotypes, FF genotype, especially (AA > FF > EE) was found; thus, the differences between the AA - FF genotypes
are distinctly significant for protein and casein, and significant for SUN (Tables 15 and 16).

8.2.2. Results concerning cheesemaking efficiency and cheese flavor testing

The analyse of the cheese yields from Carpathian breed, by all 3 categories of genotypes at CSN1S1 locus, emphasized that:

- between AA and EE cheese types, we record a significant difference (P≤0.05): AA genotype is superiour to EE by +3.47 kg/100 of processed milk, representing an increase by 22.10% in favour of AA genotype (Table 17, Fig. 63);
- between the AA and FF cheese types, a distinct significant difference was recorded (P≤0.01): AA is superior to FF by +3.16 kg/100 liters processed milk, representing an increase by 20.12% in favour of AA genotype (Table 17, Fig. 63);
- between EE and FF cheese a not significant difference (P≥0.05) was recorded: EE genotype is inferior to FF by -0.31 kg/100 liters processed milk, representing a decrease by 2.53% for EE genotype (Table 17, Fig. 63).

Table 17

The quantitative differences concerning quantification of the cheese yield function of genotype category

<table>
<thead>
<tr>
<th>CSN1S1 genotypes</th>
<th>Kg cheese / 100 liters of processed milk</th>
<th>Differences between genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA - EE</td>
</tr>
<tr>
<td>AA</td>
<td>15.70kg±0.11</td>
<td>+3.47kg*</td>
</tr>
<tr>
<td>EE</td>
<td>12.23kg±0.17</td>
<td>+3.16kg**</td>
</tr>
<tr>
<td>FF</td>
<td>12.54kg±0.12</td>
<td>-0.31kg&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ns</sup>-not significant differences P≥0.05; *-significant differences P≤0.05; **-distinct significant differences P≤0.01
The assessment of the effect of the αS1-cazeine polymorphism in Carpatină goats on milk quality and cheese yield

Fig. 63. Mean values graphical representation of cheesemaking efficiency, according to genotype category

The study concerning testing of the specific goat flavour of cheese, obtained by 3 categories of genotypes, was performed on a sample made up of 30 subjects, and results emphasized the followings:

- 64% of the subjects classified the AA type cheese, as having a low flavour, 28% moderate flavour, and only 8% of the subjects attributed this type of cheese a strong flavour (Fig. 65);
- 58% of the subjects classified the EE type, as having a moderate flavour, 34% strong flavour, and 8% no flavour (Fig. 65);
- 46% of the subjects classified the FF type, as having a moderate flavour, 30% no flavour, 16% low flavour, and only 8% strong flavour (Fig. 65);
- the most appreciated cheese was the A type with 65%, followed by FF type with 27% and those of EE type with 8%.
CHAPTER IX

MAJOR MILK PROTEIN POLYMORPHISM CHARACTERISATION OF THE NATIVE GOAT POPULATIONS FROM DIFFERENT COUNTRY AREAS

RESULTS AND DISCUSSIONS

9.1. Results concerning genotyping at the six loci coding for major milk protein in some goat population from different country areas

At CSN1S1 locus codifying \( \alpha_{S1}\) casein, the results indicate an average to high frequency of the allelles with strong expression (0.583) and a relative high frequency (0.390) of the allelles with moderate and low frequency (E and F), with major implication on milk quality and cheese yield.

At CSN2 locus, codifying \( \beta\) casein, allelles A and C were identified with high frequency (0.981). They cannot be differentiated by IEF technique, due to the common isoelectric points. The allele E was also identified, but with lower frequency (0.019).

At CSN1S2 locus codifying \( \alpha_{S2}\) casein, the results indicate a high frequency of the allelles C (0.40) and A (0.540), compared to allele E (0.020).
The assessment of the effect of the $\alpha_{S1}$-caseinei polymorphism in Carpatină goats on milk quality and cheese yield

At CSN3 locus codifying $\kappa$-casein results indicate a high frequency of the allelle A (0.831) compared to allelle B (0.169).

At $\alpha$-lactalbumin and $\beta$-lactoglobulin loci, monomorph profiles were identified in all analyzed individuals.

9.2. Results concerning sequencing the $\alpha_{S1}$-casein cDNA from Carpathian goat

After sequencing of the cDNA of the $\alpha_{S1}$-caseinei, prelevated from 3 Carpathian individuals, a new allele was reported. It has a $G\text{CT} - G\text{TT}$ substitution in position 32, and deletion of the CAG codon from positions 273-275 (due to its wrong removing during the process of maturation of ARNm precursor - skipping event -).

At protein level, the mutation $G\text{CT} - G\text{TT}$ from position 32 is responsible of the substitution Ala-Val from the signal peptide of the protein, while the removal of the CAG codone led to deletion of Gln from position 78 of mature protein.

The confirmation of the mutation from position 32 was performed by restriction of cDNA of $\alpha_{S1}$-casein with $Baul$ enzyme.

**Fig. 75.** Electrophoretic profile obtained after digestion with $Baul$ enzyme (lane 3) of $\alpha_{S1}$-casein cDNA amplified with $BCZS1$-F $\&$ $CCZS1$-R primers. Lane 1 - unrestricted product (692pb), lane 2 - GeneRuler 100bp DNA Ladder (Thermo Scientific, Wilmington, USA), lane 3 - restricted product belonging to individuals with AA genotype (692bp+670bp+22bp) (original).
The assessment of the effect of the αS1-cazeine polymorphism in Carpatină goats on milk quality and cheese yield

The sequence of this new allele named A1, was discharged in GenBank database, receiving the identification number JX047868.

CHAPTER X
CONCLUSIONS AND RECOMMENDATIONS

➢ As consequence of improvement and validation of PCR-RFLP and AS-PCR tests for identification of polymorphisms of milk proteins at CSN1S1 locus, they may be successfully used for precocious genotypization of genuine goat sires.

➢ The comparison of the results obtained from DNA genotypization (by PCR-RFLP and AS-PCR techniques) as well as at the level of genic expression, direct from milk (by IEF technique), of 60 individuals, led to the confirmation of the correspondence of the genotypes obtained by using each technique. Furthermore, IEF technique allows the identification of the genotypes from the other loci of milk major proteins within the same gel.

➢ The results of the statistical analyses of the main qualitative parameters of the milk of Carpathian breeds by 3 categories of genotypes at CSN1S1 locus, emphasized a clear superiority of the AA genotype on the average protein, casein and SUN content, compared to other genotypes. Concerning fat and lactose content, a superiority of the FF genotype compared to other genotypes, was reported.

➢ The results of the statistical analysis of the cheese yields in Carpathian breed, by all 3 categories of genotypes at CSN1S1 locus, emphasized a 22.10% superiority of AA genotype compared to EE, and 20.12% of AA genotype compared to FF.

➢ As result of organized tastings, the cheese from FF genotype was associated with a specific goat flavour, intermediary to other two genotyped. The most appreciated cheese was of AA type.

➢ The results of characterization of te polymorphisms of the milk major proteins from 830 Carpathian females, using IEF technique, emphasized a relatively high
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frequency (0.420) of alleles with moderate, low, and null expression (E, F and 0), with major involvements on milk quality and cheese yield.

- As a consequence of the results of the molecular, physico-chemical analyses, cheese processing yield, and tastings, we recommend the use of the CSN1S1 genetic marker codifying αS1-casein, for the improvement of the milk quality and cheese yield in Carpathian breed.

- We recommend the elaboration and implementation of a methodology destined to improve the Carpatina breed and identification of the authenticity of the traditional goat milk products based on the use of the polymorphism of the CSN1S1 genetic marker.

- In order to obtain natural hypoallergenic milk, we recommend the obtaining a goat population based on selection, with null genotypes at the loci of the main milk allergens.

BIBLIOGRAPHY


