Preliminary investigations on milk proteins polymorphism in Carpathian goats and in F1 Carpathian × Saanen hybrids

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SUMMARY

The purpose of the paper was to determine the protein concentration and the protein polymorphism in the milk from Carpathian goats and from F1 Carpathian × Saanen hybrids. Milk samples were collected from 15 Carpathian goats and from 15 F1 Carpathian × Saanen hybrids reared at ICDCOC Palas-Constanța. The average mean value for milk protein concentration in the samples of Carpathian goat milk was 1.3%, which is below the normal limits reported by the literature (2.9 – 6% total protein). The average milk protein concentration in the samples of F1 (Carpathian × Saanen) hybrids was 5%, with values which are a little bit higher than normal limits reported by the literature (average 2.7% for Saanen and 3.3 for Carpathian). This shows that the cross of the two breeds produced hybrids which yielded a higher concentration of milk protein. These results revealed that it was obtained a higher percentage of protein (3.3 – 6%) in goat than in cow milk (3.3 to 3.5%), and the other components are relatively equal, and slightly lower than in the sheep milk (5.2-6.5%). Our results also show a higher polymorphism of these proteins. The following casein proteins were determined by electrophoresis (SDS PAGE) in the milk samples from Carpathian goats and from the Carpathian × Saanen hybrids: αs2 and αs1-casein, β-casein, κ-casein, and β-lactoglobulin. To verify the results, real time PCR analysis of the polymorphic genes of the milk proteins will be performed at a subsequent stage, with the purpose to determine the genetic variants from the locus of each protein and to notice the differences between the Carpathian breed and F1 hybrids (Carpathian × Saanen).

Keywords: Carpathian breed goats, milk proteins, electrophoresis, SDS-PAGE, polymorphism

INTRODUCTION

Milk proteins consist of four casein proteins [αS1, αS2, β and k – casein (CN)] and two non-casein proteins [lactalbumin (LA) and β – lactoglobulin (β-

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LG)], which differ in the genetic polymorphism and frequency of identification in the animal populations. The presence of α S1 – CN in goat milk was frequently studied during the recent years and it was shown to have six different forms: A, B, C, E, F and „0” (Haelein, 2003).

The investigation of milk protein genetic polymorphism started more than 40 years ago and it continues to be of interest due to the relation between the milk proteins and milk quality, composition and technological characteristics. In Romania, Băltenu et al. (2007) conducted research on milk protein polymorphism, specifically regarding the frequency of αS1-CN in the Carpathian goat. A total of seven alleles (A, B, C, D, E, F and 0) were identified at αS1-CN locus, which proves to have the highest polymorphism. These alleles have different levels of expression and they fall into four categories (Boulanger et al., 1984; Grosclaude et al., 1987) as a function of their contribution to the total milk protein: alleles with a strong expression, 3.6 g/L/allele in αS1-CN with variants A, B, C; alleles with a medium expression, 1.6 g/L/allele in αS1-CN with variant E; alleles with a poor expression, 0.6 g/L/allele in αS1-CN with variants F and D; null (0) alleles, characterized by their absence from the milk of heterozygous individuals (Trujilo et al. 1999). Milk quality is influenced by casein polymorphism as seen in the differences noticed in animal genotypes. A high protein (3.71%), fat (3.99%) and dry matter (11.97%) content was noticed in the individuals with genotype AA, compared to the individuals of genotype EE, which had 3.26% protein, 3.52% fat and 11.04% dry matter (Pop et al. 2009).

Goat milk proteins, like the milk proteins from other mammalians, fall into two groups: CN (αS1, αS2, β and k –CN) and whey proteins (α-LA, β-LG and albumin, immunoglobulins).The genetic polymorphism of goat milk proteins has a strong impact on milk casein, which influences milk production characteristics, the technological properties for cheese production, its flavour and proteolysis. Generally, α-LA and β-LG have been considered to be monomorphic in goats. However, goat caseins, αS1-CN particularly, display a large qualitative and quantitative variability. The most recent genome data revealed the existence of at least 14 alleles at αS1-CN, distributed in 7 different classes of the protein variants, from A to G, associated to 4 levels of the limits of gene expression ranging between 0 (αS1-CN 0) and 3.6 g/L/allele (αS1-CN A, B and C), with variant E as intermediary quantitative gene expression (3.6 g/L/allele) and variant F associated to a low αS1-CN content (0.6 g/L/allele) (Grosclaude et al., 1987). The genetic variant AA of αS1-CN in the goat milk and the difference between it and the other variants were described (A, B, C, D, E, F and G). Variants A, B, C and E are different from variant AA or D, F and G, which show deeper structural modifications such as internal deletions. αS1-CN polymorphism, variants αS2-CN (A, B and C) and β-CN (A, B and 0) have been identified by Buniol et al., 1993, Buniol et al., 1994, Mahe et al., 1993. The results of Di Luccio et al., 1990, suggested that k –CN too has genetic polymorphism. Goat milk proteins and their polymorphism were analysed by

electrophoresis (Addeo et al., 1988, Grosclaude et al., 1987) and chromatography (Jaubert et al., 1992 and Kumagai et al., 1987). The main protein fractions and casein polymorphism have been studied and identified in goat milk by capillary electrophoresis by Recio et al. (1997). The knowledge on the primary structure of the protein molecule facilitates the understanding of its functions at the molecular level. Two general methods are presented in this study, though they have limitations.

The purpose of the paper is to determine the protein concentration and protein polymorphism in the goat milk from Carpathian goats and from F1 Carpathian × Saanen hybrids, scarcely investigated in Romania. To confirm the results, real time PCR analysis of the polymorphic genes of milk proteins will be performed, with the purpose to show the genetic variants at the locus of each protein, and to notice the differences between the Carpathian goats and F1 hybrids (Carpathian × Saanen). This will allow selecting the most appropriate variants of hybrid individuals, with the genetic expression showing a high casein content correlated quantitatively with the milk yield.

MATERIAL AND METHODS

Animals and biological samples: This study used milk samples from 12 Carpathian goats and from 15 F1 Carpathian × Saanen hybrids produced by ICDCOC Palas-Constanța, to determine milk protein polymorphism.

Sample preparation for total milk protein determination: 50 mL milk from each sample were centrifuged at 5000 rpm and at a temperature of 4°C for 20 minutes, and then kept in the refrigerator for 30 minutes, in order to allow the fat to separate at the surface. The skimmed milk was preserved at -20o C until analysis.

Protein determination: the Bradford Method (Krauspe, 1986) was used to determine the total milk proteins. The samples of skimmed milk were diluted 1:20, and 1:10 using plates with 96 wells (Corning, Sigma, Redox, Romania), and used to determine the total milk protein. The wells were filled with 255 µL fluids, of which 5 µL sample and 250 µL Bradford reagent. The samples were incubated at room temperature between 5 minutes and 1 hour. The absorbance was measured at 595nm using a microplate reader (TECAN SUNRISE, Austria) compared to the blank

Milk protein electrophoresis. The types of goat milk caseins were determined by vertical polyacrylamide gel electrophoresis (Mini-Protean 3 BioRad system, BioRad, Romania), using migration gel with 15% polyacrylamide and concentration gel with 5% polyacrylamide, migration time 1 h 30 minutes. Migration was done in the presence of a standard sample Precision Plus Protein Standars Kaleidoscope from Bio Rad which contained proteins with different molecular weight: Myosin 250 kDa, Phosphorilase 150kDa, BSA 100 kDa, Glutamic dehydrogenase 75 kDa, Alcohol

dehydrogenase 50 kDa, Carbonic anhydrase 37 kDa, Myoglobin 25 kDa, Lysosime 20 kDa, Aprotinine 15 kDa, Insulin 10 kDa. In four gels were inserted 10µl sample from the wells, with 5µg/10µl protein concentration and 10µl marker. 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 migration, the gels have been stained by immersion in the staining dye (Coomassie Blue 250 R BioRad, BioRad, Romania), for 15 minutes, and were decoloured by immersion in a solution with 10% acetic acid and 2.5% methanol.

Gel Visualisaton. The gels were visualized on a transluminator, UV BIO PROFIL (Vilber Lourmat), the density was analyzed and the results were interpreted with BIO 1D software.

RESULTS

We analyzed during this study 15 milk samples from F1 Saanen × Carpathian hybrids and 12 samples from Carpathian goats raised at the Palas-Constanta institute. Initially, we determined milk protein using the Bradford method. Tables 1 and 2 show the outcomes of the determinations.

Table 1. Protein concentration in the milk from F1 Saanen × Carpathian hybrids

Animal number	Total milk protein mg%ml milk
1	5.1
2	4.05
3	3.66
4	5.58
5	3.45
6	5.76
7	4.35
8	4.68
9	4.80
10	5.04
11	4.74
12	5.34
13	5.52
14	5.28
15	5.28
Mean	4.811
SD	0.184

Table 2. Protein concentration in the milk from the Carpathian breed

Animal number	Total milk protein mg%ml milk
1	1.123
2	1.174
3	1.020
4	1.130
5	1.060
6	1.930
7	1.500
8	1.670
9	1.980
10	1.870
11	0.850
12	1.280
Mean	1.382
SD	0.113

Protein concentration in the milk from F1 Saanen × Carpathian hybrids had a value around 5% total protein, which was in agreement with the literature data for goats (Boudier et al., 1981 Georgescu at all., 2000). The hybridization of the

Carpathian goats with Saanen goats improved the total milk protein percentage (Saanen goats have 2.7% proteins, Carpathian goats have 3.3% proteins), probably due to the heterosis effect.

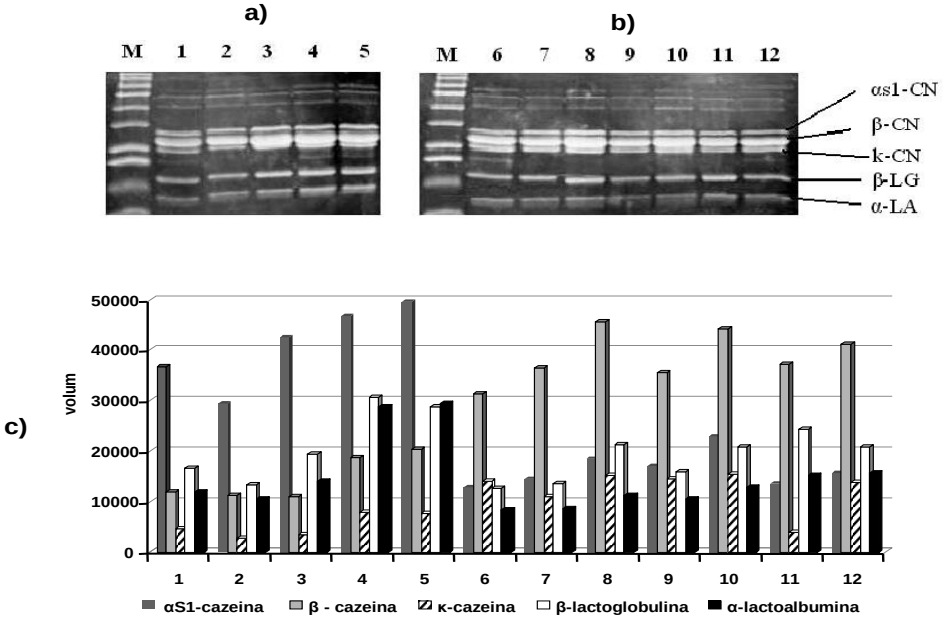

Figure 1a, b, c. Expression of the milk proteins in the Romanian Carpathian goats

Protein concentration in the milk from Carpathian goats, 2% total protein, was in agreement with the literature data (Boudier at all., 1981, Georgescu at all., 2000). There have been samples below the normal milk protein level.

The SDS-PAGE analysis revealed the presence of several protein bands in the milk from Carpathian goats (Fig. 1a and 1b). The identified proteins were αs1-casein, β-casein, k-casein, β-lactoglobulin and α-lactalbumin. The densitometry analysis revealed different expressions of the same type of protein function of the analysed individual animal. Thus, the expression of αs1-casein peaked at goat number 5, and had the lowest level at animal number 6. The intensity of the protein bands obtained for all 12 goats were grouped on three levels of expression: strong (goats 5, 3, 4 and 1), medium (goats 2, 8 and 10) and poor (goats 6, 7, 9, 11 and 12). For β – casein the peak value was determined in sample number 8, and the lowest value in sample number 3. Grouping the goats according to the expression of the β – casein, we noticed that goats 10, 9, 8, 7, 6, and 12 had maximal values; goats 4 and 5 had medium values, while goats 1, 2, 3 and 11 had poor values. For the κ-casein the peak value of the expression was determined in sample number 5, and the lowest expression was determined in sample number 6. Grouping the goats according to the level of expression, animals 5, 1, 3 and 4 displayed a strong expression; animals 2, 8 and 10 displayed a medium expression, while animals 6, 7, 9, 11

and 12 displayed a poor expression. For β-lactoglobulin the peak expression was determined in goat number 4, and the minimal expression in goat number 6. The levels of expression for this protein were just medium (goats 1, 2, 3, 6, 7, 9, 10 and 12) and strong (goats 5, 8 and 11). For α-lactalbumin the peak expression was determined in goat number 5 and the minimal expression in goat number 6. The levels of expression for this protein were strong (goats 5 and 4), medium (goats 1, 3, 8, 10, 11 and 12) and poor (goats 2, 6, 7 and 9). Table 3 and Fig. 1c show these values.

The SDS-PAGE analysis revealed the presence of several protein bands in the milk from 15 F1 Saanen × Carpathian hybrids (Fig. 2a and 2b).

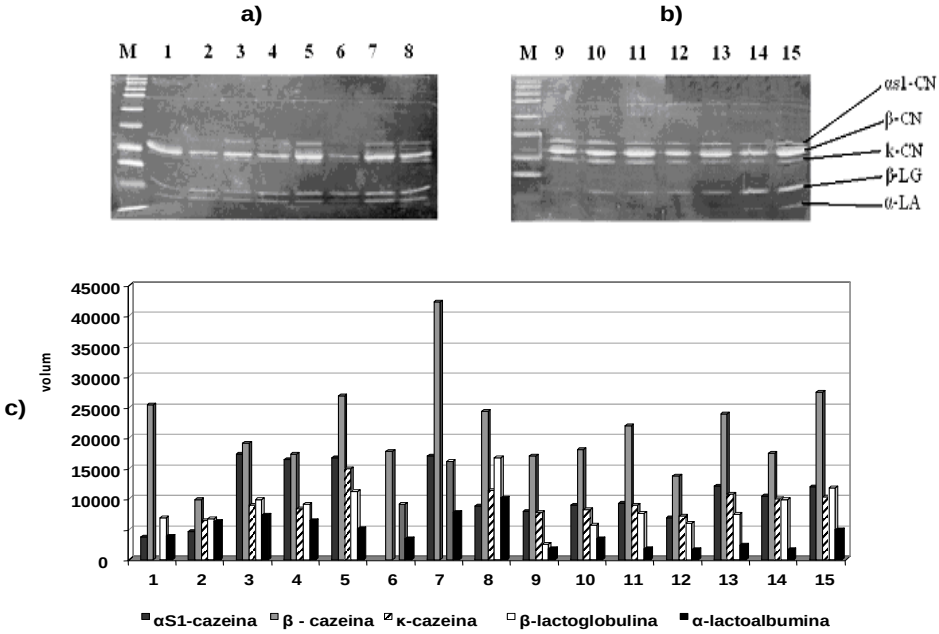

Figure 2a, b, c. Expression of the milk proteins in the F1 Saanen × Carpathian hybrids

The identified proteins were αs1-casein, β-casein, k-casein, β-lactoglobulin and α-lactalbumin. The densitometry analysis revealed different expressions of the same type of protein, function of the analysed individual animal. Thus, the expression of αs1-casein peaked at goat number 3, and had the lowest level at animal number 1. The intensity of the protein bands obtained for all 15 goats were grouped on three levels of expression: strong, medium and poor, and varied as follows.

In the milk sample from goat number 6 there was no αs1-casein. The expression of αs1-casein was strong (goats 3, 4, 5 and 7), medium (goats 8, 9, 10, 11, 12, 13, 14 and 15) and poor (goats 1 and 2). For β – casein the peak value was determined in sample number 7, and the lowest value in sample number 2.

Table 3. Densitometry of the Carpathian goats milk proteins

Protein types /individuals (Volume)	Carpathian goats											
	1	2	3	4	5	6	7	8	9	10	11	12
αS1- casein	36724	29522	42624	46688	49632	12846	14576	18432	17021	22969	13510	15633
β - casein	11992	11170	10861	18790	20263	31233	36395	45545	35444	44231	37154	41255
κ-casein	4538	2849	3388	7839	7711	14021	10873	15218	14383	15442	3880	13745
β-lactoglobulin	16669	13386	19298	30508	28677	12484	13532	21207	15849	20701	24272	20708
α-lactalbumin	11929	10428	13970	28826	29395	8319	8636	11143	10548	12791	15229	15611

Table 4. Densitometry of the F1 Carpathian × Saanen hybrids

Protein types / individuals, (Volume)	F1 Carpathian × Saanen hybrids														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
αS1- casein	3595	4582	17262	16302	16711	-	16898	8771	7907	8920	9234	6747	1204	10390	11838
β - casein	25281	9729	19112	17235	26840	17675	42299	24352	17042	18080	21849	13641	23854	17365	27504
κ-casein	-	6370	8958	8264	14899	-	-	11260	7768	8139	8952	7145	10731	9995	10216
β-lactoglobulin	6815	6583	9761	8994	11212	9034	16076	16663	2425	5637	7606	5933	7456	9809	11715
α-lactalbumin	3760	6170	7253	6319	4954	3392	7765	10089	1652	3333	1751	1537	2292	1564	4795

Grouping the goats according to the expression of the β – casein, we notice that only goat 7 had maximal value, goats 1, 3, 4, 5, 6, 8, 9, 10, 11, 13, 14 and 15 had medium values, while goats 2 and 12 had poor values. Grouping the goats according to the level of expression for κ-casein, animals 8, 5 and 13 displayed a strong expression, animals 2, 3, 4, 9, 10, 11, 12, 14 and 15 displayed a medium expression, while animals 1, 6, and 7 displayed a poor expression. For β-lactoglobulin the peak expression was determined in goat number 8, and the minimal expression in goat number 9. The levels of expression for this protein were mainly medium (goats 1, 2, 3, 4, 5, 6, 11, 13 and 14) followed by the strong expression (goats 8, 7, and 15) and poor expression (goats 9, 10 and 12). For α-lactalbumin the peak expression was determined in goat number 8, and the minimal expression in goat number 9. The levels of expression for this protein were strong (goats 8, 3 and 7), medium (goats 1, 2, 4, 5 and 15) and poor (goats 6, 9, 10, 11, 12, 13 and 14). Table 4 and Fig. 2c show these values.

In both the 12 Carpathian specimens and the 16 hybrid goats were noticed differences in the expression of the protein fraction for αS1- CN, β-CN, κ-CN, β-LG, and α-LA, which shows that this breed has the necessary potential to produce quality milk to be processed into cheese.

The strong expression of αS1-CN occurred in 33.3 % of the Carpathian goats and in only 26.66% of the hybrids; for β-CN the frequencies were 50% in Carpathians and 13.33% in the hybrids; for κ-CN the frequencies were 50% in the Carpathian goats and 20% in the hybrids, while for β-LG the frequencies were 33.33% in the Carpathian goats and 20% in the hybrids. For α-LA, the hybrids had a higher frequency of the strong expression, 20% compared to 16.66% in the Carpathian goats.

In terms of the medium protein expression, the hybrids displayed higher frequency than the Carpathian goats, as follows: for αS1- CN 53.33% compared to 25%, for β-CN, 80% in the hybrids and 25% in the Carpathian goats, while for κ-CN, 60 % in the hybrids and just 33.33 % in the Carpathian goats. The strong expression of β-LG and α-LA had a higher frequency in the Carpathian goats, 66.66% and 50%, respectively, compared to 60% and 33.33%, respectively, in the hybrids.

Table 5. Protein expression in the Carpathian breed

Protein fractions	Level of protein expression			
	Strong expression (%)	Medium expression, (%)	Simple expression, (%)	No expression (%)
α-S1- CN	33.33	25.0	41.66	-
β-CN	50.0	25.0	25.0	-
κ-CN	50.0	33.33	16.66	-
β-LG	33.33	66.66	-	-
α-LA	16.66	50.0	33.33	-

The simple quantitative expression of the protein was higher in the Carpathian goats than in the hybrids for αS1- CN (41.66 % compared to 13.33%), β-CN (25 % compared to 6.66%) and κ-CN (16.66% compared to the lack of expression). The simple quantitative expression of the protein was higher in the hybrids, 20% in β-LG and 46.66% in α-LA. Tables 5 and 6 give the proportions of protein fractions expression.

Table 6. Protein expression in the Carpathian × Sannen hybrids

Protein fractions	Level of protein expression			
	Strong expression (%)	Medium expression, (%)	Simple expression, (%)	No expression (%)
αS1- CN	26.66	53.33	13.33	6.66
β-CN	13.33	80.0	6.66	-
κ-CN	20.0	60.0	-	20.0
β-LG	20.0	60.0	20.0	-
α-LA	20.0	33.33	46.66	-

DISCUSSION

In this study we have made a comparison between the protein composition of the milk from the local Carpathian goats and from the hybrids of this breed with Saanen goats. Protein concentration in the analyzed milk samples was on average 1.3% total protein in the milk from the Carpathian goats, which is below the 3.3% protein milk mentioned in the literature, and about 5% in the hybrid goats. Thus, it resulted that hybridization improved the total milk protein, probably due to the heterosis effect. Penasa et al. (2010) conducted a similar study in which they determined the effect of heterosis on milk yield and on the qualitative traits (fat, protein) after crossing North American Holstein Friesian, Friesian, Jersey and Montbeliarde dairy cows. The highest effect of the heterosis was obtained by crossing Holstein and Jersey dairy cows, in the first generation after crossing: 477 kg more milk/lactation (305 days) and 25.3 kg more fat/lactation and 17.4 kg protein/lactation than the average productions of the parental breeds. Similar studies and determinations were performed by Prendiville et al. (2009), who estimated the hybrid vigor in the milk production, in the milk fat and milk protein, showing the effect of heterosis on the milk lactose, and on the index of milk solids (fat and protein). For instance, the milk solids in the parental breeds were 1.33 in Holstein-Friesian and 1.28 in Jersey, while generation F1 of hybrids had 1.41. Dechow et al. (2007) investigated the effect of crossing between Holstein and Swiss Brown dairy cows on the milk yield, milk fat and protein, and somatic cells count. The estimated heterosis increased the fat production by 10.38% and the protein production by 7.07%, while the heterosis for the somatic cells had a significant value, 0.22, when the

sire was Swiss Brown and non-significant, 0.43 when the sire was Holstein-Friesian.

The chemical composition of the goat milk is different from that of the cow milk by the higher protein content. Goat milk protein has about 75% casein, which is particularly useful for dairy processing. The fat content is also higher in goat milk than in cow milk, the other components being rather similar (Georgescu, 2000).

In this study, in both the milk from Carpathian goats and in the milk from Carpathian × Saanen hybrids, were identified the casein proteins αs1-casein, β-casein, κ-casein, β-lactoglobulin and α-lactalbumin, proteins which are very important in producing high quality cheese. This study showed that each type of casein has different intensities of expression identified by electrophoresis both in the milk from Carpathian and from Carpathian × Saanen hybrids.

Each protein fraction manifested in a different way at the 4 levels of the quantitative expression. The Carpathian goats had a higher proportion of the individuals with strong expression for 4 of the 5 analysed protein fractions as follows: for αS1- CN, 6.7% more in the Carpathian goats than in the hybrids, for β-CN, 36.67% more in the Carpathian goats than in the hybrids, for κ-CN, 30% more in the Carpathian goats than in the hybrids, and for β-LG, 13.33% more individuals had a strong expression of this fraction in the Carpathian goats compared to the hybrids.

In the case of α-LA, the hybrids had a higher proportion of the individuals with strong expression, by 3.34% compared to Carpathian goats.

The medium expression was more frequent in the hybrids than in the Carpathian goats, as follows: for αS1- CN, by 28.33%, for β-CN, by 55 %, and for κ-CN, by 27 %. The medium expression of β-LG and α-LA was more frequent in the Carpathian goats, by 6.66 % and 17%, respectively, than in the hybrids.

The Carpathian goats had a higher proportion of the individuals with simple expression for αS1- CN, by 28.33%, for β-CN, by 18.34% and for la κ-CN – 16.66% compared to no expression. The hybrids had a higher proportion of the individuals with simple expression, 20 % for β-LG and 46.66 % for α-LA.

The lack of expression for some protein fractions was observed in the hybrid goats: 6.66% of the hybrid goats didn’t have αS1-CN and 20% of the hybrid goats didn’t have κ-CN.

Except for a strong expression for α-LA, heterosis didn’t seem to have effect on the protein composition form the hybrid goats. However, the higher proportion of individuals with a medium expression of the protein fractions shows that the cross of Carpathian × Saanen goats was beneficial, not just in terms of the total milk protein, but also in terms of the chemical composition: over 50% of the individuals had medium expression in 4 of the 5 investigated protein fractions.

Similar investigations were performed by Marzio et al. (2009) to determine the milk protein profile in Garganica goats. By 2D electrophoresis the variability of milk casein was determined in order to find the global genotype of the casein αS1- CN, αS2-CN β-CN and κ-CN. He expressed casein profile associated with casein genotype and thus showed differences in the protein expression due to the interaction of loci. He recommended the differentiation of the individuals according to the variability at the casein locus and their exploitation function of this variability. Chiantti (2007) conducted a similar study on the effect of the protein polymorphism of κ-CN on milk composition in Orobica goats, in which he identified 3 different types of this casein which differentiates the individual goats that may be exploited in the breeding strategies for the improvement of the milk protein and milk casein, when cheese production is the main purpose of selection. Gloria de la Torre et al. (2009), investigated the effect of αS1-CN genotype on milk yield and its interaction with milk protein in Malaguena goats, and observed in the analysed population individuals with a higher or lower capacity for protein synthesis for αS1-CN; these goats were fed higher or lower dietary protein formulations. Irrespective of the diet, the milk from the goats with high capacity of protein synthesis always had higher levels of αS1-CN.

In this study we have also monitored the serum proteins β-LG and α-LA which we should consider taking into account the new aspects regarding milk investigation for hypoallergenic agents or bioactive peptides.

Real time PCR is needed for a certainty of the results on the polymorphic genes of the milk proteins from goats milk, in order to identify the genetic variants from the locus of each protein.

CONCLUSIONS

The results of this study show that following the hybridization of the local Carpathian goats with the Swiss breed Saanen, the total milk protein increased due to the heterosis effect. The cross improved also milk quality, being produced individuals with a strong expression of α-LA, individuals which can be used in reproduction to improve the qualitative and quantitative efficiency of milk production.

Except for this strong expression for α-LA, heterosis didn’t seem to have effect on the protein composition in the milk from hybrid goats. However, the high proportion of individuals with a medium expression of the protein fractions shows that the cross of Carpathian and Saanen goats had a beneficial effect on the total milk protein and on protein composition, more than 50% of the individuals displaying medium expressions for 4 of the 5 investigated protein fractions.

Real time PCR is needed for a certainty of the results on the polymorphic genes of the milk proteins from goat milk, in order to identify the genetic variants from the locus of each protein.

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