IDENTIFICATION OF MILK COMPOSITION AND PROTEINS POLYMORPHISM CORRELATED WITH CHEESE QUALITY, TO SELECT THE BEST CARPATINA GOATS

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Abstract

The purpose of this study was to identify the proteins polymorphism in Carpatina goat in order to select the best individuals which exhibit the most increased polymorphism for αs1-caseins. Milk composition was analysed for fat (5.08%) and protein (4.14%). Protein percentage didn't display high variability and ranged between 4.82% and 3.22%. SDS-PAGE analysis revealed, after electrophoresis, five protein fractions in milk samples: αs1, β, k-CN, and β-LG, and α-LA. Different expressions of the same type of protein fraction were discovered by densitometry analysis for each investigated goat. The casein group, composed by three fractions α s1-, β- and k-casein (17.5%, 48.8%, 10.9%), shows in fact that they are 77.2% of the total milk protein and 22.8% are albumins, similar with other studies for goats. Three levels of expression were recorded for each milk protein in every milk sample: 19.04% of the goats manifested strong expression for α s1-CN and medium and low expression for 57.15% and 23.81% of it. In case of the β-LG, strong expression level was found for 38.09% of the goats and medium and low expression level for 47.62% and 14.09%, respectively. Further investigations will be necessary to confirm the results by PCR-RFLP analysis to identify Carpatina goat caseins polymorphism with the genetic variants for each protein locus. This will allow keeping for reproduction the most suitable animals with strong genetic expression and high content in caseins very well correlated with milk quality and quantity.

Key words: Carpatina, dairy goats, milk composition, milk proteins polymorphism

INTRODUCTION

Milk production has a major importance in cheese industry and especially goat milk from which derive many traditional products like yogurt, cheese, with recommendation for human health, like children and adult people. Goat milk has non allergenic quality and is very similar with human milk. A number of studies were dedicated to discover caseins in dairy goats breeds in Spain, Italy, Portugal which prefers traditional milk and cheese products [5], [17]. The demand for this type of products has increased in the last years because of the belief that goat milk has unique properties, it is biologically active very good for therapy and health [10]. Goat milk is more digestible than cow milk, and it is more suitable for children and adults suffering from milk allergies and intestinal problems [3]. Carpatina breed has a body conformation for milk production and it is growing in mounting area having an average milk production of 500-700 kg in almost 7-9 mouths dairy production and 160% prolificacy. Romania is on the 4th place regarding goat population after England, Spain and Greece. The purpose of this study was to investigate and to identify the protein polymorphism in Carpatina goat in order to select the best individuals which propose αs1-caseins which exhibits the most increased polymorphism. There are studies who investigated caseins and found genes that have strong expressions (A, B, C) producing 3.5 g αs1-CN/l, medium expression allele (E) with 1.1 g αs1-CN/l and weak allele (F) with 0.45 g αs1-CN/l [12]. The effect of αs1-CN polymorphism was investigated in French breeds and was discovered that A allele has a significant positive effect on the total milk protein, casein, fat content and manufacturing properties increasing by 15% the cheese quality and

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quantity [13]. Similar studies were done on Spanish [14], and Italian [11] goat breeds. That way we consider an opportunity to investigate milk quality and protein polymorphism which is very well correlated with cheese quantity and quality in Carpatina breed.

MATERIAL AND METHOD

Animals Milk samples from 21 Carpatina goats from Buzau County were used to determine milk composition and protein polymorphism.

Sample preparation for total milk protein polymorphism determination: 50 ml milk from each sample were centrifuged at 5000 rpm at a temperature of 4^0C for 30 minutes in order to allow fat milk to be separated at the surface. The skimmed milk was preserved at -20^0C until analysis. *Protein determination*: milk total proteins were determined using Bradford method [9]. Skimmed milk samples were diluted 1:40 using plates with 96 wells (Corning, Sigma, Redox, Romania) and used to determine the total milk protein. The wells were filled with 5µl sample and 250 µl Bradford reagent. The samples were incubated at room temperature 45 minutes. The absorbance was measured at 595 nm using a microplate reader (TECAN SUNRISE, Austria) compared to blank.

Milk composition was determined with Ekomilk M analyser (Bulteh 2000 Ltd, EON Trading LLC, USA).

Milk protein electrophoresis protein polymorphism were determined by vertical polyacrylamide gel electrophoresis (Mini-Protean 3 BioRad system, Romania) using migration gel with 15% polyacrylamide and concentration gel with 5% polyacrylamide, migration time 1 h and 30 minutes at 200 V. Precision Plus Protein standards ladder contains ten recombinant protein bands that were used as reference for milk caseins and albumins which are migrating between 25 and 19 kDa (beta-CN 24 kDa, kappa-CN 19-20 kDa, alfa-CN 23-25 kDa). After protein migration, the gels were stained by immersion in dye solution (Coomassi Blue 250 R BioRad, Romania) for 45 minutes and were decoloured by immersion in a solution with 10% acetic acid and 2.5% methanol. *Gel visualisation* was done with transluminator Benchtop 2UV and analysed with Image J software.

RESULTS AND DISCUSSION

Table 1 shows that the fat percentage of the milk samples ranged between 8.89 % and 1.58%. Protein percentage has no high variability and ranged between 4.82% and 3.22% like in other goat breeds cited in scientific literature [7], [1], [4].

Table 1 Milk composition in Carpatina goats

	Fat (%)	SNF (%)	Protein (%)
Mean	5.08	9.21	4.14
Standard deviation	1.77	0.53	0.43
Error	0.35	0.11	0.09
Variability coefficient	34.75	5.71	10.28
Maxim	8.89	10.1	4.82
Minim	1.58	8.13	3.22

The SDS-PAGE analysis revealed after electrophoresis five protein fractions in milk samples from Carpatina goat (figure 1). These proteins are α s1-casein, β-casein, k-casein, β-lactoglobulin, and α-lactalbumin. The casein group composed by tree fractions α s1-casein, β-casein and k-casein (17.5%, 48.8%, 10.9%) show in fact that they are 77.2% of the total milk protein and 22.8% are albumins similar with other studies for goats [16], [6] and sheep [12].

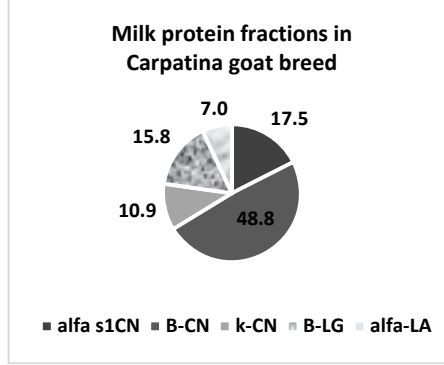

Fig. 1 Milk protein fractions in Carpatina goat

Using densitometry analysis we determined the different expression of the

same type of protein fraction for each goat investigated (Tables 2, 3; Figures 2, 3). The purpose of this study was to quantify the expression of milk protein fractions which are with strong, medium and low expression.

Table 2 Densitometry of the Carpatina goat milk proteins (samples 1-10)

Protein fractions	1	2	3	4	5	6	7	8	9	10
α s1CN	13245	14242	11786	13536	14899	14238	13268	14671	13226	15059
B-CN	42989	32047	37842	40686	30103	35867	36838	32136	36496	40734
k-CN	8178	9846	6892	7565	9579	9983	11532	8996	7098	7898
B-LG	11834	10505	15371	14143	17856	11573	12797	12976	11799	16573
alfa-LA	6131	6699	4728	7042	7231	4533	6233	6973	5917	4558

Table 3 Densitometry of the Carpatina goat milk proteins (samples 11-21)

Protein fractions	11	12	13	14	15	16	17	18	19	20	21
α s1CN	13784	15294	11784	17555	10959	15269	16281	10820	13085	11014	7264
B-CN	48778	42472	40663	40732	31950	32943	32734	32835	39991	37670	36258
k-CN	7459	7448	6556	11126	4556	10781	6934	7946	7642	9385	8334
B-LG	14702	8621	9109	15030	6122	11775	13402	7622	11615	11221	8447
α LA	6048	5069	3388	8347	3473	4941	5339	3595	4709	4356	3350

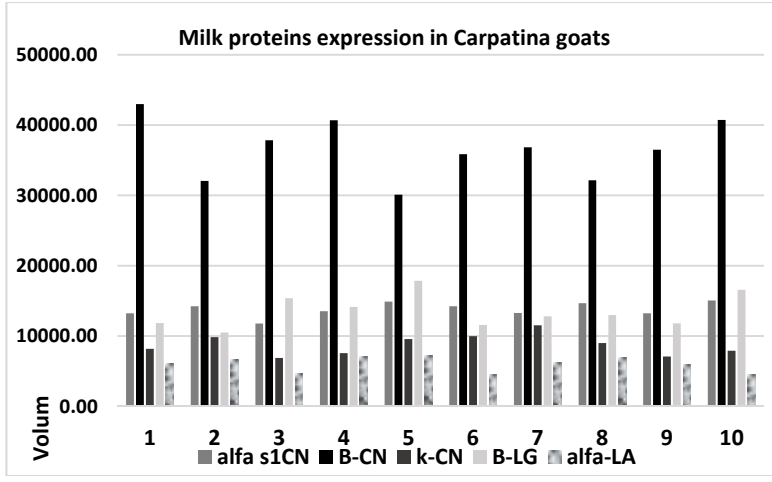

Fig. 2 Milk proteins expression in Carpatina goats sample 1-10

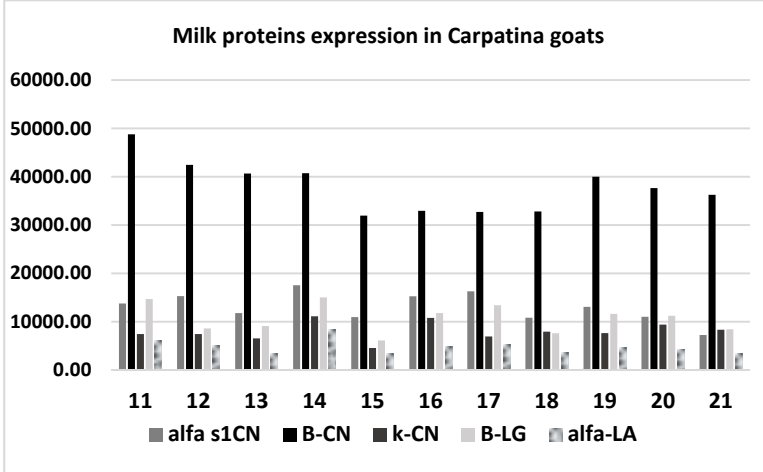

Fig. 3 Milk proteins expression in Carpatina goats sample 11-21

The strong expression level of α s1-casein recorded at Carpatina goat in this study was 19.04%, almost three times higher compared with that obtained in other similar research on genetic variability of goats (6%) [1]. Medium and low expression for α s1-casein was found in 57.15% and 23.81 % of the analysed Carpatina goats. The second protein fraction investigated in the present study was β-casein with strong expression level in 4.76% of the goats. Medium expression level was found for β-casein in the majority of the analysed Carpatina goats (80.95%) and 14.29% of it had low expression level (Table 4).

Table 4 Protein expression in Carpatina goats

Protein fractions	Level of protein expression			
	Strong expression (%)	Medium expression (%)	Simple expression (%)	No expression (%)
α s1-CN	19.04	57.15	23.81	-
β -CN	4.76	80.95	14.29	-
k-CN	33.34	47.62	19.04	-
β -LG	38.09	47.62	14.29	-
α -LA	57.14	33.34	9.52	-

For k-casein fraction, 33.34% of the goats manifested strong expression level, while medium and low expression level was found in 47.62% and 19.04% of the goats. The genetic polymorphism was investigated in a similar study in Tunisian goats to identify k-caseins and three genotypes were discovered (AA or BB, CC and AC or BC) with the frequencies 12.55%, 60.5% and 27% [8]. Tow albumin protein fractions were analysed in Carpatina goats. In this study three expression levels were manifested by milk samples for β-lactoglobulin, with strong, medium and low expression (38.09%, 47.62% 14.09%). Another albumin fraction found in the milk of Carpatina goats was α-lactalbumin. This milk protein had a strong expression for 57.14% of the individuals. One third of the investigated individuals for α-lactalbumin manifested medium level expression for 33.34% of goats and for 14.29% it had low level expression (table 4). Figures 4, 5 and 6 show milk proteins bands migrated in SDS-PAGE vertical gel. In each polyacrylamide gel, first well is for the marker with ten recombinant proteins as reference for caseins and albumins followed by the milk samples.

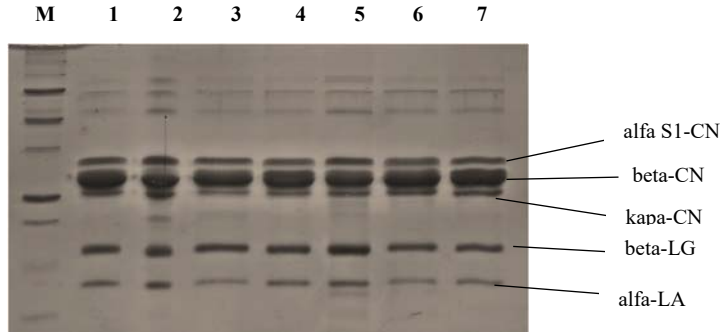

Fig. 4 Milk proteins expression in Carpatina goats sample 1-7

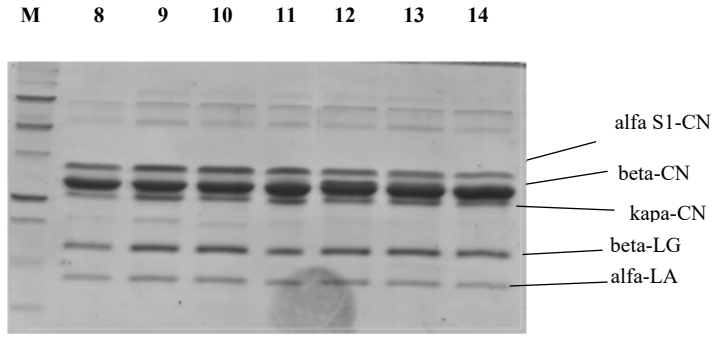

Fig. 5 Milk proteins expression in Carpatina goats sample 8-14

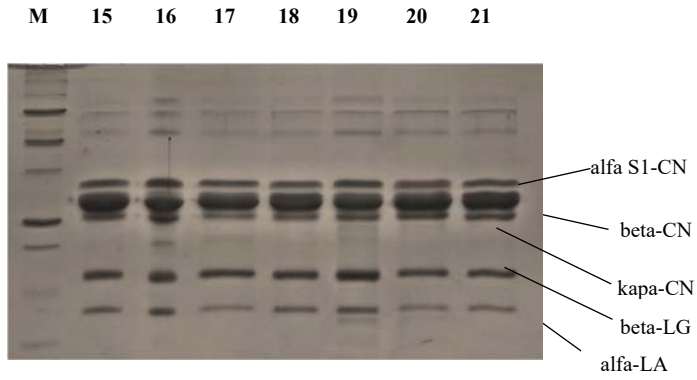

Fig. 6 Milk proteins expression in Carpatina goats sample 15-21

CONCLUSIONS

In this study a strong expression level of α s1-casein was recorded at Carpatina goat (19.04%), almost three times higher compared with that obtained in other similar research on genetic variability of goats. That why further investigations will be necessary to confirm results of the present study using PCR-RFLP analysis in order to identify Carpatina goat caseins polymorphism with the purpose to reveal the genetic variants at the locus of each protein. These will allow to

keep for reproduction the most suitable animals with strong genetic expression and high content in caseins very well correlated with milk quality and quantity.

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