Bulletin UASVM Animal Science and Biotechnologies, 68(1-2)/2011
Print ISSN 1843-5262; Electronic ISSN 1843-536X

Effect of α_{S1}-casein Polymorphisms on Milk Composition and Cheesemaking Efficiency in Carpathian Goat Breed

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Abstract. In goat milk, among the four caseins, α_{S1}-casein (α_{S1}-CN) locus is the most polymorphic. Eighteen alleles with four different expression levels have been identified so far in different goat breeds, having a significant influence on milk protein, casein, fat contents, manufacturing properties and cheese yield.

In Carpathian goat breed, which exhibits a high heterogeneity concerning milk quality, we already reported a high degree of α_{S1}-CN polymorphism. However, no studies were done in this breed so far to quantify the effect of this locus polymorphism on milk parameters. In this respect, four different experimental lots with different genotypes in α_{S1}-CN locus (AA, EE, FF and a control heterozygous lot-HET) were organized in 2009. In 2010, monthly controls were conducted in the goats from the experimental lots. Differences between α_{S1}-CN genotypes were observed in analyzed milk quality parameters: fat content, whole protein and whole casein (AA>HET>FF>EE), unfatty dry matter (AA>FF>HET>EE), lactose (FF>AA> EE> HET), urea (FF>HET>AA>EE) and somatic cells (FF>HET>EE>AA).

In 2010 season one hundred liters of milk from each genotype group (AA, EE, FF and HET) were processed in green cheese in the same conditions. Differences between genotypes concerning the cheese yield were observed: AA-EE=17,33%; AA-FF=16,48%; FF-EE=5,84%. EE cheese type was jugged as having the strongest goaty flavour, AA the weakest, FF being intermediate. Some results obtained in our study (PN II Project no. 52104/2008) are in contrast with those obtained in other goat breeds.

Keywords: Carpathian goat, α_{S1}-casein, polymorphism, milk, cheese, genetic markers

INTRODUCTION

In goat milk, among the six major milk proteins, α_{S1}-casein (α_{S1}-CN) exhibits the most increased polymorphism, like in no other specie, 18 alleles with four expression levels being known so far: strong alleles: A, B, C producing 3.5g α_{S1}-CN /l; intermediate alleles: E with 1.1g α_{S1}-CN /l; weak alleles: F with 0.45g αS1-CN /l and null alleles characterized by the absence of α_{S1}-CN in the milk of homozygous animals (Grosclaude *et al.*, 1987; Martin *et al.*, 2002; Ramunno *et al.*, 2005; Balteanu *et al.*, 2010). In the other three casein loci alleles with different expression levels were also identified: α_{S2}-casein 0-2g/ l milk/allele, β-casein 0-5g/l milk/allele, K-casein 4-6 g/l milk/allele.

In α_{S1}-CN A and B alleles appear more frequently in goat breeds from Southern Italy (Sacchi *et al.*, 2005), F allele has a higher frequency in Northern Italian breeds (Sacchi *et al.*, 2005). E allele is more frequent in Spanish and French breeds (Jordana *et al.*, 1996; Grosclaude *et al.*, 1997), F alleles are more frequent in French breeds (Grosclaude *et al.*, 1997) and null allele in Norwegian breeds (Devold *et al.*, 2010).

The effect of α_{S1}-CN polymorphism on goat milk quality was studied in French breeds (Mahé *et al.*, 1993; Manfredi *et al.*, 1995; Delacroix *et al.*, 1996; Ricordeau *et al.*, 2000).

These studies concluded that A allele has a significant positive effect on whole milk protein, casein, fat contents and manufacturing properties, in comparison with F allele, E allele having an intermediary effect between the previous two. The cheese obtained from AA genotypes had a less pronounced goat flavour in comparison with that obtained from FF genotypes, due to different fatty acid profiles (Delacroix *et al.*, 1996). Similar studies were performed in Spanish breeds (Sánchez *et al.*, 1998, Caravaca *et al.*, 2011), Italian breeds (Meggiolaro *et al.*, 2000), Norwegian breeds (Devold *et al.*, 2010) and USA (Clark *et al.*, 2000).

In 1987 low expression alleles were predominant in French goat breeds (Grosclaude *et al.*, 1987), having a negative influence on milk quality parameters and its manufacturing properties (Mahé *et al.*, 1993). The benefits of combining information provided by this genetic marker with traditionally breeding schemes appeared soon, the genetic progress recorded in France concerning goat milk quality, having a major impact on cheese production.

In Romania from about 700.000 existing goat heads, 75% of population is represented by the unimproved Carpathian breed. The remainder of 25% is represented by White Banat breed, an improved breed with higher milk production. Unfortunately approximately 74.11% of the flocks are reared in small farms with less than 20 heads (Pascal *et al.*, 2006). Carpathian goat breed was never submitted to an intense breeding process, therefore there is a high heterogeneity concerning milk quality (fat 3.2-6.9% and protein 2.5-5%) and quantity (200-700l/lactation). This variability represents a high breeding potential.

In Carpathian goat breed we already reported a high degree of α_{S1}-CN polymorphisms in Carpathian goat breed (Balteanu *et al.*, 2007;Balteanu *et al.*, 2010a, b; Balteanu, 2010c; Vlaic *et al.*, 2010), which could be the cause of milk quality parameters variation. However, no studies were done so far to quantify its effect on milk composition, cheese yielding capacity and specific its goaty flavour. Considering this, our goal is to investigate the effect of α_{S1}-CN polymorphisms on milk quality and cheese making efficieny in this breed (PN II Project no. 52104/2008).

MATERIALS AND METHODS

I. Milk samples collection. In order to evaluate the influence of α_{S1}-CN polymorphism on milk composition and cheese yield, four controls in 2010 lactation were performed. The experimental lots were already established in 2009 on different genotypes in α_{S1}-CN locus: Lot 1: AA-blue tags; Lot 2: EE-yellow tags; Lot 3: FF-green tags (Balteanu *et al.*, 2010a). A number of 80 females belonging to the three studied genotypes groups (AA, EE and FF) were sampled during these 4 months. Furthermore, a control lot of 15 females (white tags) having different allelic combinations in α_{S1}-CN (AE, AF, EF, EF) were sampled during the same period. All four milk controls were made on the same females during the 2010 season. After a complete milking of each individual and milk homogenisation a 50 ml samples were collected from each female for further analysis.

The milk samples were analysed in an authorised laboratory (FCCL Floresti, Cluj County) in a Milkoscan 6000 and a Fossomatic equipments. The following milk quality parameters were determined: fat content, whole protein, whole casein, unfatty dry matter, lactose, pH, and somatic cells. The freezing point was determined by cryoscopy method.

II. Cheese making experiment. In order to evaluate the influence the cheese yielding capacity of each milk type, the remaining milk form each individual after sampling was mixed according to the corresponding genotype, being obtained four mixtures of milk: AA type, EE type, FF type and HET type. A sample was taken from each milk mixture for physicochemical analysis.

From each milk mixture, a fresh cheese type was produced by classical procedure of rennet adding. Twenty five litters were processed each time on each genotype group (100 litters/lactation/genotype group). The cheeses were dried over a 48 hours period and then they were weight.

To evaluate some sensory properties of the obtained cheeses, in particular the goaty flavour, cheeses gustation were organised on each control and questionnaires were distributed to 30 subjects.

RESULTS AND DISCUSSION

I. Evaluation of goat α_{S1}-CN genotypes influence of milk quality parameters

In order to have a general picture on 2010 control season, concerning the α_{S1}-CN genotypes influence of milk quality parameters in the goats from the four experimental lots (α_{S1}-CN AA, EE, FF and HET), the average values of seven milk parameters (with a direct influence on milk composition), obtained in the four controls (April, May, July and October), were calculated (Tab1).

Tab. 1

The average of individual values of seven determined goat milk quality parameters obtained on the 4 controls/ each α_{S1}-CN genotypes groups / 2010 lactation

Milk component/ Method of analysis	α_{S1}-CN genotypes groups			
	AA	EE	FF	HET
Fat (g/100g) (FIL IDF 141C:2000)	4.57	3.72	4.19	4.22
Protein (g/100g) (FIL IDF 141C:2000)	4.05	3.55	3.58	3.80
Casein (g/100g) (Milkoscan)	2.99	2.59	2.63	2.77
Lactose (g/100g) (FIL IDF 141C:2000)	3.99	3.96	4.18	3.88
Unfatty dry matter (g/100g) (Milkoscan)	8.86	8.34	8.59	8.53
Ureea mg/dl (Milkoscan)	43.13	41.18	52.75	45.98
NCS/ml x1000 (SR EN ISO 13366-2: 2007)	2700.67	2858	3941.67	3145.33

Furthermore the differences between genotypes concerning these milk parameters were also calculated on the entire 2010 season (Tab.2).

Tab. 2

Differences obtained between α_{S1}-CN genotypes groups/ 2010 lactation concerning goat milk quality parameters

Milk component	Differences between α_{S1}-CN genotypes groups					
	AA- EE	AA-FF	EE-FF	AA- Het	EE- Het	FF- Het
Fat	0.85	0.38	-0.47	0.35	-0.50	-0.03
Protein	0.49	0.47	-0.02	0.25	-0.24	-0.22
Casein	0.40	0.36	-0.04	0.22	-0.18	-0.14
Lactose	0.02	-0.19	-0.22	0.10	0.08	0.30
Unfatty dry matter	0.51	0.27	-0.24	0.33	-0.18	0.06
Ureea	1.95	-9.63	-11.58	-2.85	-4.80	6.78
NCS	-157.33	-1241	-1083.67	-444.67	-287.33	796.33

Therefore in the 2010 control season, a net superiority of the α_{S1}-CN AA genotypes as compared with the other studied genotypes was observed, concerning fat, whole protein and whole casein (AA>HET>FF>EE). The average content of unfatty dry matter was also superior in AA genotypes as compared with the others (AA>FF>HET>EE). The lactose content was superior in FF genotypes as compared with the others (FF>AA>EE>HET). The

urea content was higher in FF genotypes as compared with the other (FF>HET>AA>EE). Finally the somatic cell score was the highest in FF genotypes (FF>HET>EE>AA) (Tab.1, 2).

II. Evaluation of goat α_{S1}-CN genotypes influence on cheese yield (year 2010)

The quantities of cheeses/genotype group on the entire 2010 season were obtained by summation of the quantities obtained in April, May, July and June (Tab 3).

Tab. 3

The summation of cheeses quantities obtained from 100 litters of milk/ each α_{S1}-CN genotype group/ 2010 season and differences obtained between genotypes

α_{S1}-CN genotypes groups	Kg cheese/ season/100l of processed milk	Differences between α_{S1}-CN genotypes (kg of cheese)					
		AA-EE	AA-FF	EE-FF	AA-Het	EE- Het	FF-Het
AA	16.782	4.957	3.491	-1.466	2.136	-2.820	-1.354
EE	11.826	Differences between α_{S1}-CN genotypes (%)					
FF	13.292	AA-EE	AA-FF	EE-FF	AA-Het	EE-Het	FF-Het
HET	14.646	17.33	16.48	-5.84	6.80	-10.65	-4.85

Concerning the cheese yield the highest quantitative difference was observed between AA and EE genotypes: 4.957 kg/100 litters of processed milk/season (17.33%). The observed difference between AA and FF genotypes was 3.491 kg/100 litters of processed milk/season (16.48%). The difference between EE and FF genotypes was 1.466 kg/100 litter of processed milk/season (5.84%) in favour of FF genotype. The HET genotypes were superior concerning cheese yield to EE and FF genotypes, but inferior to AA genotypes. The superiority of HET genotypes as compared with EE and FF is not surprising since they contain a certain proportion A allele in heterozygous condition. The high differences obtained in cheese yielding capacity of each milk type (α_{S1}-CN AA, EE, FF and HET), confirmed one more time the influence on this locus polymorphism on milk quality parameters, with significant repercussion on cheese yield. These differences are mainly due to the differences in allelic expression of α_{S1}-CN locus.

To conclude, a net superiority of α_{S1}-CN AA genotypes was observed concerning all analysed milk quality parameters and cheese yield. What is surprising is that we observed a superiority of FF milk, as compared with EE milk, concerning the main quality parameters and cheese yield. These results are in contrast with those obtained in other studies (mentioned in the introduction section), according to which EE genotypes are superior to FF genotypes.

III. Evaluation of goat α_{S1}-CN genotypes influence on cheese goaty flavour (year2010)

The centralisation of data obtained following cheeses degustation revealed surprising results. The A type cheese was frequently appreciated as cattle cheese, the majority of the subjects being unable to detect a specific goaty flavour. The F type cheese was jugged as having a medium goaty flavour, HET type cheese being intermediate between A and F. Surprisingly the EE cheese type was jugged as having the strongest specific goaty flavour: EE>FF>HET>AA. The results concerning goaty flavour evaluation by degustation are in contrast with the results obtained in French breeds, were EE had a intermediate goaty flavour between A and F: FF>EE>AA. The calculation of allele and genotypes frequency in α_{S1}-CN was already done in goat population from central part of Romania (Balteanu *et al.,* 2007; Balteanu *et al.*, 2010a,b; Balteanu, 2010c; Vlaic *et al.*, 2010), northern and eastern part (Balteanu, 2010 unpublished data). According to these studies the calculated frequency of E and F allele in α_{S1}-CN locus was 0.340 in the goat populations studied from central part of Romania, 0.447 in the eastern populations and 0.429 in the northern populations. The average frequency of E and F allele calculated in 1300 genotyped individuals so far is 0.405.

Considering that in Romania there are about 700.000 goats and the frequency of medium and low expression allele in 40.5%, we can estimate that about 238.500 Carpathian goats are carriers of E and F alleles. These data corroborated with the fact that, as was proven in the present study, E and F allele have a negative influence on milk quality and cheese yield, we can easily deduce that economic losses are pretty important.

Because on the European market there is a high deficit of goat dairy products and therefore there is no milk quota production limit, Romania has a big potential to satisfy these demands. One of the latest national norms, formulated by the Romanian National Agency for Livestock Improvement and Reproduction concerning this specie, is the improvement of milk quality and its manufacturing properties. This goal could be easily achieved by introduction in the selection criteria of goat populations of α_{S1}-CN as a genetic marker.

We can conclude that the study of major milk protein polymorphism in Carpathian goat breed (especially the polymorphism of α_{S1}-CN) and its influence on milk quality and its manufacturing properties (PN II Project no. 52104/2008), will have a major importance in the future nation breeding programs, because it provides a valuable molecular tool for rapid improvement of milk composition of this valuable breed, neglected for many years.

CONCLUSIONS

An experiment on evaluation of α_{S1}-CN polymorphism influence on milk quality, cheese yield and cheeses flavour was conducted in 2010 in four different experimental lots belonging to Carpathian Goat with different genotypes in α_{S1}-CN locus (AA, EE, FF and a control heterozygous lot-HET). In 2010, monthly controls were conducted in the goats from the experimental lots. Differences between α_{S1}-CN genotypes were observed in analysed milk quality parameters: fat content, whole protein and whole casein (AA>HET>FF>EE), unfatty dry matter (AA>FF>HET>EE), lactose (FF>AA>EE> HET), urea (FF>HET>AA>EE) and somatic cells (FF>HET>EE>AA).

In 2010 season one hundred litres of milk from each genotype group (AA, EE, FF and HET), were processed in green cheese in the same conditions. Differences between genotypes concerning the cheese yield were observed: AA-EE=17.33%; AA-FF=16.48%; FF-EE=5.84%. EE cheese type was jugged as having the strongest goaty flavour, AA the weakest, FF being intermediate. Some results obtained in our study (PN II Project no. 52104/2008) are in contrast with those obtained in other goat breeds.

Considering the major influence of α_{S1}-CN polymorphism in Carpathian goat breed, observed in study, its use in the selection schemes is could represent a valuable tool for rapid improvement of milk composition of this valuable but unimproved breed.

Acknowledgments. This work was supported by UEFISCDI, Project PNII 52104/2008, Code 1086.

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