

EFFECT OF CSN2, CSN3 AND LGB GENES ON MILK PRODUCTION TRAITS OF MONTBÉLIARDE DAIRY CATTLE

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Abstract

The aim of this study was to investigate the effects of CSN2 (coding beta-casein), CSN3 (coding kappa-casein) and LGB (coding beta-lactoglobulin) genes polymorphisms on milk production traits in a Montbéliarde herd from a Romanian dairy farm.

In order to achieve the objective, 34 cows with first lactation finished were sampled. Milk samples were analyzed for fat, lactose and total solids by infrared absorption methodology. Genetic variants of milk proteins were identified by using polyacrylamide gel electrophoresis containing dithiothreitol and urea. For testing the state of genetic equilibrium in this population, according to Hardy-Weinberg theory, we used chi-square test (χ^2). The highest observed frequencies were for beta-casein A2 allele (β -CN*A2), K-CN*B, respectively β -LG*B. Cows with β -CN A2A2, K-CN AB and β -GB AA genotypes produced a significantly higher milk yield. Beta-casein genotype A1A2 positive effect may be used in order to obtain higher fat, protein and casein content in milk.

Key words: CSN2, CSN3, LGB, effect, milk production traits, Montbéliarde, polymorphism

INTRODUCTION

In bovine milk, there are six major milk proteins [1]. The caseins alpha-casein S1, beta-casein, alpha-casein S2 and kappa-casein (α S1-, β -, α S2- and κ -CN) are encoded by autosomal genes (CSN1S1, CSN2, CSN1S2 and CSN3) [2] mapped on chromosome 6 [3]. On the other hand, the whey proteins alpha-lactalbumin and beta-lactoglobulin (α -lactalbumin and β -lactoglobulin) are encoded by LALBA, respectively LGB genes [4] located on chromosome 5 [5], respectively on chromosome 11 [6]. In these milk proteins corresponding genes, amino acids changes occur and cause mutations in the protein structure [7]. These mutations, named polymorphisms (genetic variants named by letters) were fully characterized [8]. It is known that besides seasonal variations, breed and other factors, some of these genetic

variants are affecting the milk composition and milk production traits. Thus, in the last decade, different studies have suggested possible connections between some genetic variants of milk proteins and milk production traits. For instance, some researchers [9, 10, 11, 12, 13] reported that A2 allele from CSN2 locus has a positive effect on milk yield. On the other hand, β -CN*A1 affects milk's fat [14, 15, 16], protein [14, 17, 13, 16], casein [14] and lactose content [16]. Among dairy cows, the allele A from CSN3 locus is associated with a higher milk yield [13, 18, 19, 20], while κ -CN*B allele is associated with higher milk protein and fat content [14, 21, 18, 22, 23, 20]. At this locus there are some contradictory results, as some studies have shown positive associations between κ -CN*A and milk fat [24, 23, 17, 25], respectively protein content [15, 26].

Available data concerning the BLG polymorphisms in relation to milk production traits shows that β -LG*A allele is associated with a higher milk yield and protein content [14, 27, 13, 21, 28, 29], respectively that

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allele B is responsible for higher milk fat content [30, 31, 32, 33] and casein content [14, 18].

The aim of this study was to investigate the effects of β -, κ -casein and β -lactoglobulin milk protein genotypes on the milk production traits of a Montbéliarde herd from Romania.

MATERIAL AND METHODS

Individual milk samples from 34 imported cows (from a total number of 89) with first lactation finished were collected. Milk samples were analyzed for fat, lactose and total solids by infrared absorption methodology. Somatic cells were electronically counted (SomaScope, Foss Electric, Netherlands), using cytometry flow methodology.

The selected individuals were genotyped by isoelectric focusing using 4% ultrathin (0.5 mm) polyacrylamide gel with 8M urea and a 3 ampholytes mix: pH=2.5-5, pH=4.2-4.9, pH=5.0-7.0, in an Multiphor II Electrophoresis System (GE Healthcare, Sweden). The entire protocol is described in our previous publications [34, 10]. After electrophoresis, in order to color the IEF profiles, Coomassie Brilliant Blue stain was used.

Further, in order to establish the genetic structure, the allele frequencies were calculated with Pop Gene v.1.32. The χ^2 -test [35] was carried out to determine whether the observed genotype frequencies in our herd deviated significantly from the Hardy-Weinberg-Equilibrium (HWE). According to this test, results are statistically different at a level of 5% significance. Investigated traits were milk yield and percentages of fat, protein, casein and lactose. For basic statistical analyses One Way Anova (SPSS v.19 for Windows) was used. For multifactorial analysis of variance we used the C.O.P. & R.G. software [36].

RESULTS AND DISCUSSION

Cows were genotyped for all six milk major proteins loci but our population was monomorphic for α_{S1} -CN, α_{S2} -CN and α -LA. The genetic variants of milk proteins obtained by isoelectric focusing (IEF) in polyacrylamide gel are shown in Figure 1. The distribution of β -CN, κ -CN and β -LG genotypes and alleles frequencies, respectively the state of genetic equilibrium (HWE) in the studied Montbéliarde population are presented in Table 1.

Table 1 The distribution of β -, κ -casein and β -lactoglobulin genotypes and alleles frequencies, respectively Hardy-Weinberg equilibrium in Montbéliarde population

Locus ¹	Genotype	HWE		Allele	Allele frequency
		Observed	Expected ²		
CSN2	A ₁ A ₂	7	6.03	A1	0.37
	A ₂ A ₂	11	12.36	A2	0.47
	A1B	3	2.50	B	0.15
	A ₂ B	12	10.25		
	BB	1	2.13		
		$\chi^2 = 1.041$	$p^3 = 0.307$		
CSN3	AA	3	4.24	A	0.35
	AB	18	15.23	B	0.65
	BB	13	14.23		
		$\chi^2 = 0.861$	$p = 0.353$		
LGB	AA	3	1.88	A	0.24
	AB	10	12.24	B	0.76
	BB	21	19.88		
		$\chi^2 = 1.135$	$p = 0.286$		

¹ β -casein, κ -casein and β -lactoglobulin; ²calculated according to the Hardy-Weinberg law;

³P signifies overall level of significance between observed and expected genotype frequencies determined by the chi square test (χ^2), where χ^2 = chi-square value; $p > 0.05$ = not significant: the population is in genetic equilibrium according to Hardy-Weinberg law

In all three polymorphic loci there was no significant difference ($P>0.05$) between the observed and expected frequencies, suggesting that the studied population is in genetic equilibrium according to Hardy-Weinberg-law.

CSN2 locus

At this locus three alleles were detected: A_1 , A_2 and B. Allele A_2 which is the most common in β -CN [8] had the highest frequency (Table 1).

All frequencies calculated for this locus were within the literature range [31, 37, 38] for Montbéliarde breed. This population analyzed for CSN2 locus was in genetic equilibrium according to Hardy-Weinberg theory.

The associations between milk protein genetic variants and milk production traits are shown in table 2.

We found some positive effects of β -CN genotypes ($A_2A_2>A_1A_2>A_2B>A_1B$) on milk yield, with highly significant differences between A_2A_2 and A_1B (1509 kg/lactation), our results being in agreement with other studies [13, 21, 11, 12].

In terms of milk quality traits, β -CN genotypes had a significant effect on fat, protein, casein ($A_1A_2>A_2A_2>A_2B$) and lactose

content (A_1B). These results are similar with previous findings [15, 9, 23, 16, 13, 19].

CSN3 locus

At CSN3 locus, allele B had a higher frequency compared to A, as reported in a previous paper [20], this finding being in agreement with other results [39]. This population analyzed for CSN3 locus was in genetic equilibrium according to Hardy-Weinberg theory.

We found very significant effects of CSN3 genotypes on milk yield ($AB>BB>AA$), these results being similar to those reported recently [40]. Heterozygous cows κ -CN*AB produced with 839 kg more milk/lactation compared to the homozygous cows for allele A.

Highly significant differences were also found for fat content ($BB>AB>AA$) this finding being in accordance with another recent study [41]. Protein and casein content were not affected although there are slightly differences between κ -CN genotypes for these traits. On the other side, highly significant differences were recorded between κ -CN genotypes ($AB>BB$) for the lactose content of milk.

Table 2 Associations between milk protein genetic variants and milk yield, respectively milk composition in Montbéliarde cows with a mean value of lactation period = 309.66 days (Mean \pm standard deviation)

Milk protein	Genotype	N	Trait (kg)	Milk composition (%)			
			Milk yield/TLP	Fat	Protein	Casein	Lactose
	A_1A_2	7	8396.86 ^a	4.30 \pm 0.05 ^a	3.51 \pm 0.09 ^a	2.71 \pm 0.08 ^a	4.45 \pm 0.10 ^d
	A_1B	3	7139.33 ^d	3.69 \pm 0.19 ^d	3.41 \pm 0.06 ^a	2.61 \pm 0.06 ^b	4.65 \pm 0.07 ^a
β-CN	A_2A_2	11	8648.55 ^a	4.08 \pm 0.14 ^a	3.41 \pm 0.05 ^a	2.62 \pm 0.03 ^b	4.53 \pm 0.06 ^c
	A_2B	12	8356.75 ^a	3.93 \pm 0.10 ^a	3.39 \pm 0.04 ^c	2.64 \pm 0.03 ^a	4.59 \pm 0.06 ^b
	BB	1	8732.00 ^a	4.15 \pm 0.00 ^a	3.23 \pm 0.00 ^d	2.60 \pm 0.00 ^b	4.68 \pm 0.00 ^a
	p^1		***	***	***	**	***
	AA	3	7776.67 ^c	3.43 \pm 0.15 ^d	3.41 \pm 0.06 ^a	2.64 \pm 0.04	4.57 \pm 0.16 ^a
κ-CN	AB	18	8615.28 ^a	3.90 \pm 0.09 ^c	3.42 \pm 0.03 ^a	2.64 \pm 0.02	4.58 \pm 0.05 ^a
	BB	13	7972.15 ^d	4.36 \pm 0.06 ^a	3.46 \pm 0.06 ^b	2.66 \pm 0.05	4.51 \pm 0.06 ^c
	p^1		***	***	*	n.s.	***
	AA	3	9151.00 ^a	4.01 \pm 0.24 ^a	3.45 \pm 0.06 ^a	2.61 \pm 0.07 ^b	4.50 \pm 0.07 ^d
β-LG	AB	10	8910.40 ^a	3.92 \pm 0.15 ^c	3.38 \pm 0.04 ^b	2.66 \pm 0.03 ^a	4.66 \pm 0.05 ^a
	BB	21	7968.14 ^d	4.04 \pm 0.09 ^a	3.41 \pm 0.04 ^a	2.69 \pm 0.05 ^a	4.50 \pm 0.05 ^d
	p^1		***	**	*	*	***

¹ p signifies overall level of significance between each locus and yield as well as compositional traits of milk (F-Test), n.s. - not significant; *Significance at $p<0.05$; **Significance at $p<0.01$; ***Significance at $p<0.001$. Means within columns for each milk protein locus with different superscripts differ at $p<0.01$ ($a>b>c>d$)

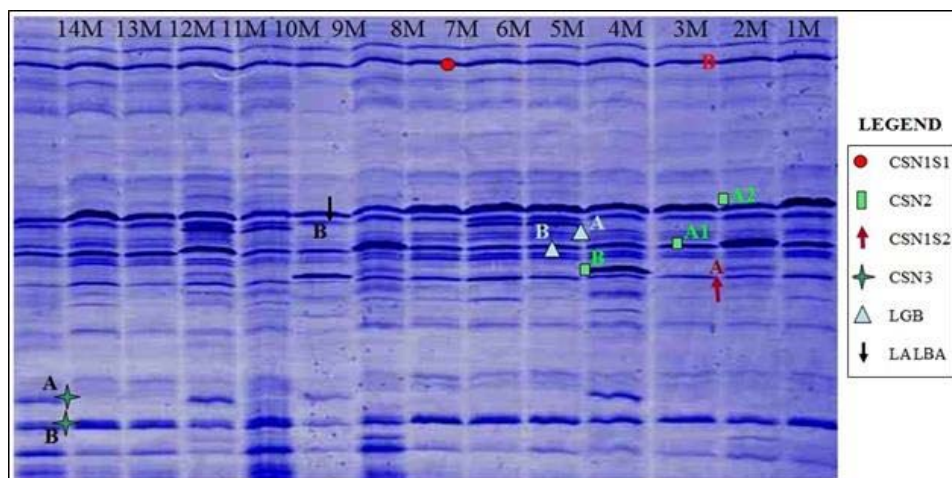


Fig. 1 Electrophoretic profile of tested Montbéliarde cows

LGB locus

At this polymorphic locus two alleles A and B were detected, respectively three genotypes presented in Table 1. We calculated the allele frequencies and compared with other studies, which showed similar results [30]. β -LG*B allele had the highest frequency (Table 1).

This population analyzed for LGB locus was in genetic equilibrium according to Hardy-Weinberg theory. Our results are showing that higher milk yields are associated with β -LG (AA>AB>BB) and comes in agreement with several studies [9, 27, 21, 28, 29, 26] and in contradiction with other authors on the other hand [9].

The homozygous cows for β -LG*A allele produced 1.183 kg more milk per total lactation when compared to BB cows ($p \leq 0.001$), which produced 942 kg less milk compared to heterozygous AB cows ($p \leq 0.001$).

The genotypic effect on fat content was distinctly significant ($p \leq 0.01$) between BB and AB genotypes for this trait (Table 2), our result being similar with previous reports [9, 30, 32, 33]. Milk sampled from homozygous AA cows had significantly higher protein content (AA>BB>AB) compared to milk produced by heterozygous AB cows ($p \leq 0.05$). Similar associations were reported by several authors [13, 18, 29].

The casein content was significantly affected ($p \leq 0.05$) by β -LG BB genotype (BB>AB>AA), this result being in accordance with other research [15], suggesting that β -LG*B allele has a greater influence on milk's casein content.

CONCLUSION

For CSN2 locus, β -CN*A2 allele is positively associated with higher milk yield, while the β -CN*A1 and A2 alleles are favorable in selection in order to obtain higher fat, protein and casein content in cow milk.

The K-CN*A allele from CSN3 locus is associated with higher milk yield, while K-CN*B allele is favorable for obtaining a higher fat, protein and casein content. We recommend selection for K-CN*A allele for increasing milk yield and selection of K-CN*B allele in order to improve the milk quality traits.

At LGB locus, in order to obtain a higher milk yield, we recommend selection for β -LG*A genetic variant, respectively for β -LG*B allele to improve milk quality traits such as fat, protein and casein content in Montbéliarde breed.

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