GENETIC VARIANTS FROM THE SIX LOCI CODING THE MAJOR MILK PROTEINS IDENTIFIED ON A NUCLEUS OF MONTBELIARD BREED CATTLE EXPLOITED IN EAST OF ROMANIA

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Abstract

The aim of the current paper is to identify the genotypes, genotypes frequencies and alleles frequencies for the studied individuals. Based on our research, we identified common genetic variants for the six loci coding the major milk proteins: alpha-casein S_1 (αS_1-CN); beta-casein (β-CN); alpha-casein S_2 (αS_2-CN); kappa-casein (K-CN); beta-lactoglobulin (β-LG) and alpha-lactalbumin (α-LA). At αS1-CN locus, we identified only one genotype αS1-CN BB. For β-CN locus the A_2B genotype recorded the highest frequences (0.35), while in system αS_2-CN only one genotype was identified - AA, αS_2-CN A allele having a frequency of 1.00. For the K-CN locus, the AB genotype recorded the highest frequency (0.53). As for the whey proteins, at β-LG locus we identified three genotypes, the highest frequency being recorded by β-LG BB (0.62). In system α-LA there is monomorphism for allele α-LA B. The milk samples analysis was made by the isoelectric focusing technique (IEF). The study was carried on a total number of 34 Montbeliard cows exploited in East of Romania.

Key words: Montbéliard breed, polymorphism, milk proteins, IEF, Romania

INTRODUCTION

Since the discovering of the first genetic polymorphism at beta-lactoglobulin locus (β-LG) by Aschaffenburg et.al. [1], the researchers worldwide became interested in genetic polymorphisms of major milk proteins. As a fact, until now, numerous studies were carried in order to show the influence of the genetic variants of milk proteins on milk quality and quantity.

Nowadays at least 39 genetics variants (alleles) are known for the six major milk proteins . These alleles occur because of the substitutions or deletions of the peptidic chain aminoacids [8].

Because of some certain genetic variants impact on the quantity, quality and processing properties of milk, we can discuss about an economic importance.

Knowing the genetics polymorphisms helps in practice, because we can select through genetic tests, those individuals who might have important alleles. This way we can accelerate the improvement process of milk production [2, 7].

Major proteins polymorphisms are related to human nutrition in many ways: hypoallergenic properties of some types of milk; releasing the peptides with biological functions from milk proteins and other. [5].

The information gathered in this study may be used for milk improvement, specialy of the casein content. For example, the cheese production capacity is associated with a higher content of protein, casein and fats [4]. More than that these information can be used in order to produce a healthier milk with no negative effects on human health [3].

MATERIAL AND METHOD

Out study was carried on a total number of 34 individuals from Montbéliard breed, located in a farm from East of Romania, in Vaslui county. Milk samples were collected individually, directly from udder in 15 ml Falcon tubes, avoiding the contamination or mixing of the samples.

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In order to denaturate the proteins, we diluted 10µl of milk from every sample in 1:5 proportion using urea and dithiotreitol solution.

For analysing the milk samples, in order to identify the polymorphic profile of the milk proteins using the IEF technique, we prepared a polyacrylamide/bisacrylamide gel 4%. For obtaining the needed concentration and a ultrathin (0.5mm) gel, we prepared 18 ml of liquid gel containing acrylamide/ bisacrylamide monomers, urea and a three carrying ampholytes mixture: pH=2,5-5; pH=4,2-4.9; pH=5-7.

After the homogenization, we add two polymerization catalysts (TEMED and ammoniac persulphate) over the monomers mix. After that, the gel needs two hours to polymerize (room temperature).

After the polymerization, the gel was applied and fixed in the middle of the ceramic plate of the electrophoresis. On the anode side (A+) we applied a filter paper impregnated with H_3PO4 and on the cathode side (C-) – filter paper imbued with NaOH.

The proteins were separated due to their migration at 2000 V, for 90 minutes.

After the migration, proteins were fixed on the gel with tri chlorine acetic acid (TCA) 10% for 60 minutes. For visualising the polymorphic profile of the proteins, we used Coomassie Brilliant Blue R-250. After this stage, we have scanned the gel with Gel Doc XR (Bio-Rad) system in order to get the images.

RESULTS AND DISCUSSIONS

After genotyping the individuals from Montbéliard breed, we identified the genetic variants and genotypes for the following loci: alphaS1 casein (αS1-CN), beta-casein (β-CN), alfaS2-casein (αS2-CN), kappa-casein (K-CN), alpha-lactalbumin (α-LA) and beta-lactoglobulin (β-LG).

The analysed electrophoretic profiles revealed the presence of some ordinary genetic variants for all the six loci of the major milk proteins. The identified genotypes are shown in table 1, and the electrophoretic profile it is shown in figure 1.

At αS1-CN locus we identified one genotype was identified, formed by αS1-CN B allele.

At β-CN locus five genotypes were identified, these resulting from the combination of three alleles: β-CN A_1, A_2 and B. Based on the experimental data we calculated the frequency of alleles and genotypes for this locus (table 2). The most frequent genotypes were: A_2B, A_2A_2 and A_1A_2. The other two genotypes (A_1B, BB) have similar, reduced frequency. The ancestral allele A_2, it is associated in various studies with better milk quality and has a higher frequency compared with A_1 allele. The A_1 allele it is associated in some studies with a higher milk production and the A^1A^1 genotype it is associated with a higher quantity of fat, compared with A^2A^2 [12].

At αS2-CN locus a single allele was identified (αS2-CN A) and also a single genotype (AA). The AA genotype and the αS2-CN A allele have a frequency equal to 1.00 (table 2).

At K-CN locus 3 genotypes were observed, these were formed as a result of the combination of 2 genetic variants: K-CN A and K-CN B. Based on the experimental data, alleles and genotypes frequency (table 2) were calculated. K-CN AB genotype recorded the highest frequency and the AA genotype – the lowest. The K-CN B allele - associated in many studies with a superior milk quality has a higher frequency compared to K-CN A allele. Molina presentated in his study that κ-CN B alelle has a positive effect on milk protein quantity [11], while Lunden discovered a positive correlation between the κ-CN B allele and a higher quality of milk [10].

As for the whey proteins, for the α-LA a single allele was observed (B) and implicit a single genotype (BB), their frequencies being equal to 1.00.

At the β-LG locus 3 genotypes were identified, these were formed as a result of 2 alleles combination (β-LG A and β-LG B). The calculated frequencies for these alleles and genotypes are shown in table 2.

β-LG BB genotype was found on 21 individuals and has the highest frequency. The β-LG AA genotype has a low frequency. β-LG B allele – associated in many studied with a higher milk quality has a higher frequency then β-LG A. Also, some researchers found that the individuals having β-LG BB genotype have a higher content of protein and casein in milk [6, 9].

Table 1 The identified genotypes using IEF technique

IEF sample nr.	Animal number	Identified genotypes					
		αS1-CN	β-CN	αS2-CN	K-CN	β-LG	α-LA
1M	54	BB	A2A2	AA	BB	BB	BB
2M	58	BB	A1A2	AA	BB	BB	BB
3M	80	BB	A2A2	AA	BB	BB	BB
4M	36	BB	A2B	AA	AB	BB	BB
5M	68	BB	A2A2	AA	BB	AB	BB
6M	99	BB	A2A2	AA	BB	AB	BB
7M	41	BB	A2A2	AA	BB	BB	BB
8M	2	BB	A1A2	AA	BB	BB	BB
9M	64	BB	A2B	AA	AB	BB	BB
10M	10	BB	A2A2	AA	BB	AB	BB
11M	1	BB	A1A2	AA	AB	AA	BB
12M	16	BB	A2A2	AA	BB	BB	BB
13M	6	BB	A2A2	AA	BB	BB	BB
14M	59	BB	A2A2	AA	AB	BB	BB
15M	23	BB	A1B	AA	AA	AB	BB
16M	47	BB	A1A2	AA	AB	BB	BB
17M	67	BB	A2B	AA	AB	AB	BB
18M	65	BB	A1B	AA	AB	BB	BB
19M	90	BB	A1A2	AA	BB	BB	BB
20M	73	BB	A1B	AA	AB	AA	BB
21M	25	BB	A2B	AA	AB	BB	BB
22M	44	BB	A2B	AA	AB	BB	BB
23M	22	BB	A2B	AA	AB	AB	BB
24M	55	BB	A2B	AA	AB	AB	BB
25M	66	BB	A2A2	AA	AB	BB	BB
26M	5	BB	A2A2	AA	BB	BB	BB
27M	91	BB	A2B	AA	AB	AB	BB
28M	50	BB	A2B	AA	AB	BB	BB
29M	97	BB	BB	AA	AA	AB	BB
30M	42	BB	A1A2	AA	BB	BB	BB
31M	53	BB	A2B	AA	AB	AA	BB
32M	34	BB	A2B	AA	AA	BB	BB
33M	24	BB	A2B	AA	AB	BB	BB
34M	62	BB	A1A2	AA	AB	AB	BB

αS1-CN = alfaS1-casein; β-CN = beta-casein; αS2-CN = alfaS2-casein; k-CN = kappa casein; β-LG = beta-lactoglobulin; α-LA = alpha-lactalbumin.

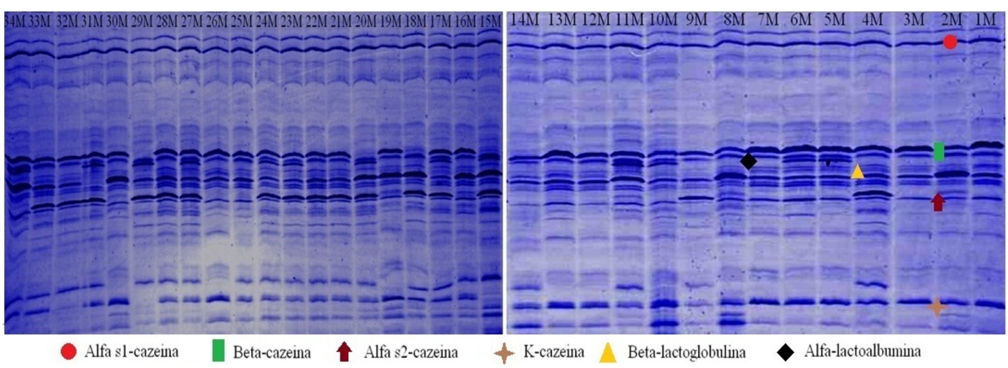

Fig. 1 – Electrophoretic profile belonging to Montbeliard breed individuals

Table 2 Frequencies of the identified genotypes and alleles

<table>
<tr><th rowspan="2">Milk proteins</th><th rowspan="2">Genotypes</th><th rowspan="2">Nr. of indiduals</th><th colspan="2">Frequency</th></tr>
<tr><th>Genotypes</th><th>Alleles</th></tr>
<tr><td>αS1-CN</td><td>BB</td><td>34</td><td>1,00</td><td>p_B=1,00</td></tr>
<tr><td rowspan="5">β-CN</td><td>A_1A_2</td><td>7</td><td>0,21</td><td rowspan="5">p_{A1}= 0,15
q_{A2} = 0,60
m_B = 0,25</td></tr>
<tr><td>A_2A_2</td><td>11</td><td>0,32</td></tr>
<tr><td>A_1B</td><td>3</td><td>0,09</td></tr>
<tr><td>A_2B</td><td>12</td><td>0,35</td></tr>
<tr><td>BB</td><td>1</td><td>0,03</td></tr>
<tr><td>αS2-CN</td><td>AA</td><td>34</td><td>1,00</td><td>p_{A1}=1,00</td></tr>
<tr><td rowspan="3">K-CN</td><td>AA</td><td>3</td><td>0,09</td><td rowspan="3">p_A=0,35
q_B=0,65</td></tr>
<tr><td>AB</td><td>18</td><td>0,53</td></tr>
<tr><td>BB</td><td>13</td><td>0,38</td></tr>
<tr><td>α-LA</td><td>BB</td><td>34</td><td>1,00</td><td>p_B=1,00</td></tr>
<tr><td rowspan="3">B-LG</td><td>AA</td><td>3</td><td>0,09</td><td rowspan="3">p_A=0,24
q_B=0,76</td></tr>
<tr><td>AB</td><td>10</td><td>0,29</td></tr>
<tr><td>BB</td><td>21</td><td>0,62</td></tr>
</table>

αS1-CN = alfaS1-casein; β-CN = beta-casein; αS2-CN = alfaS2-casein; k-CN = kappa casein; β-LG = beta-lactoglobulin; α-LA = alpha-lactalbumin

CONCLUSIONS

Our study was carried on a number of 34 Montbéliard breed individuals, from which we collected milk samples.

After genotyping the studied individuals we identified some ordinary genetic variants for major milk proteins loci.

At β-CN locus we identified 5 genotypes, but β-CN A_2B had the highest frequency. The β-CN allele which is associated in many studies with a better milk quality, had the highest frequency.

At K-CN locus we identified 3 genotypes, resulted from the combination of K- CN A K-CN B alleles. K-CN AB genotype had the highest frequency. The K-CN allele, also associated in specialty studies with a better milk quality, had the highest frequecy.

At β-LG locus 3 genotypes were identified. The highest frequency was calculated for β-LG BB genotype, which is associated in most studies with a higher content of protein and casein.

The studied individuals have some important alleles for milk production improvement, but especially for the milk quality: β-CN A_2B; K-CN B şi β-LG B.

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