

Genotipization of κ -Casein in Holstein-Friesian Cattle in Macedonia and its Association With Some Milk Properties

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Abstract – Kappa (κ)-casein is frequently used as an additional trait in the selection of dairy cows. The gene coding this protein is polymorphic and different κ -casein genotypes correlate with the milk yield and its processing properties. The aim of this study was to determine the presence of κ -casein genotypes in Holstein-Friesian cattle in Macedonia. In order to use the data from this study to improve the productivity of the dairy farms, the particular genotypes were associated with the lactation time, the milk yield and its clotting time that are important parameters in the cheese production. Determination of the κ -casein polymorphisms was done on 172 black-white Holstein-Friesian dairy cows by PCR-RFLP method using three different restriction enzymes (*Hinf* I, *Bsu* RI and *Tai* I). The κ -casein genotype frequencies were 54.7, 36.0 and 9.3 % for AA, AB and BB genotype, respectively. The allele frequency was 0.727 : 0.273 of the alleles A and B indicating the majority of the allele A. Statistical analyses showed that there is a significant influence of κ -casein genotype on milk yield, extracted curd after the coagulation and the fat content in extracted curd. Also there is a significant high influence of κ -casein genotype on the coagulation time during the cheese making process. Dairy cows with BB and AB genotype of κ -casein have 3 % higher milk yield than those with AA genotype. Milk produced by cows with BB genotype has shortest time for the initial and final coagulation in contrast with AA genotype. Milk yielded by cows with AB genotype showed an intermediate effect. Fat contents and proteins in the extracted whey in AA genotype were present more than 35 % compared to the milk produced by cows with AB and BB genotypes.

Keywords – κ -casein, Polymorphism, Holstein-Friesian, Milk, Traits.

I. INTRODUCTION

Studies on genetic polymorphisms in cattle are closely related to production traits and for this purpose, techniques capable for detecting small variations in the DNA molecule are used. These molecular techniques enabled to obtain more data about the individual genome structure [1]. Therefore, the recombinant DNA technology became

an efficient tool in the process of selection within the dairy cattle breeding programmes [2].

Kappa (κ)-casein as one of the four milk casein proteins is determined by the gene positioned at the 6th bovine chromosome [3]. The κ -casein plays an essential role in stabilizing the casein micelles and therefore has a significant influence on milk manufacturing properties [4]. Genetic variants of κ -casein have been extensively studied in cattle at the protein and DNA levels, and numerous alleles have been revealed [5]. Caroli [6] reported fourteen polymorphic types of κ -CN (A, A1, B, B2, C, D, E, F1, F2, G1, G2, H, I, J). Two of them, A and B, are particularly frequent in the cattle population. According to their allele frequency, the A allele is considered to be the initial type of allele [7].

The polymorphisms that appear in the κ -casein gene are associated with the milk yield and its technological properties [8]. Identification of these polymorphisms opens possibilities of further improvements in the dairy industry.

κ -casein polymorphism was one clear reason for the variation in milk coagulation process [9]. In most of the κ -casein studies, the association of the B allele variant with some quantitative and milk processing traits was identified. The milk with κ -CN BB genotype demands shorter curdling time [10] and it has also been assessed that using the BB type casein milk results with about 10% more cheese than AA type [11]. However, several other studies have shown that the B allele compared with the A allele, has a more favourable effect on coagulation or cheese-making properties, but also in the total the amount of the casein and κ -casein protein contents [12], [13], [14], [15], [16].

Different variants of κ -casein have an effect on the milk yield as well. Thus, the individuals of AB genotype produce more than 150 kg milk per lactation in comparison with AA genotype individuals [17].

The use of κ -casein as a molecular marker in the selection of dairy cows was the cause for this study in order to presume the presence of κ -casein genotypes in

Holstein-Friesian breed in Macedonia. The other aspect of this study was to estimate the influence of κ -casein variants in the milk production and its processing traits.

II. MATERIALS AND METHODS

In this study, 172 samples from first lactating dairy cows belonging to Holstein-Friesian breed were genotyped for κ -casein polymorphism. After genotyping the κ -casein region, twelve cows were selected for milk coagulation (clotting time) experiment. The milk samples were clustered in three groups and each group has different genotype of κ -casein (AA, AB and BB, respectively) with samples taken from four representative animals.

Genotyping the κ -casein

DNA from 172 blood samples of dairy cows was extracted using Promega kit for genomic DNA extraction from leucocytes

Amplification of the polymorphic site of the κ -casein gene was performed by PCR. The reaction mixture contained: 1x PCR buffer (10 mM TRIS-HCl pH 8.3, 50 mM KCl), 20 pM of each primers: Forward 5'-TGT GCT GAG TAG GTA TCC TAG TTA TGG-3' and Reverse 5'-GCG TTG TCT TCT TTG ATG TCT CCT TAG-3', 200 nM dNTP mix, 2 mM MgCl₂, 200 ng of DNA and 2 U of Taq Polymerase. The PCR was performed using the following program: hot start 95°C – 3 min, denaturation 95°C – 1 min + annealing 65°C – 1 min + polymerization 72°C – 1,5 min in 30 cycles and terminal extension 72°C – 5 min. The size of the resulting product (amplicon) was 453 bp and it was electrophoretically analysed on 1.5% agarose gel in TBE buffer. In order to identification the A, B, C and E alleles of κ -casein, the amplicons were digested using the Polymerase Chain Reaction - Restriction Fragment Length Polymorphism technique (PCR - RLFP) with 5U/l of *Hinf* I, *Bsu* RI and *Tai* I restriction enzymes [5]. The size of the resulting fragments were analysed by electrophoresis on 1.5% agarose gels, in TBE buffer.

Milk Coagulation Experiment

Twelve milk samples of selected cows with different κ -casein genotype were used for the analyses of the milk coagulation properties. All samples were included in the study in triplicates. The initial amount of 500 ml milk from each sample was placed in an appropriate container. After the milk was warmed at 32 °C, 0.5 ml rennet was added. The initial coagulation time was measured starting from this moment until the appearance of first milk flakes. The time measurement continued until the milk coagulated completely and it was assigned as final coagulation time. After that, 1 cm³ of the coagulated milk was taken out and dried for 15 min. Remaining curd was analysed for its dry contents, fats and proteins using standard methods: as follow: dry matter - EN ISO, 2004, fat - EN ISO, 2008b, protein - FIL-IDF, 1993.

Quantitative Daily Records

Data regarding the milk yield and the duration of lactation season were provided by the farmers and were part of the records used for the statistical analysis.

Statistical Analysis

Genotype distribution and allele frequencies were determined by individual and allele counting. The effect of κ -casein genotype on the milk yield in first lactation was evaluated by generalized linear model. In the model the genotype was treated as fixed effect, while the duration of lactation was coverable (in days). All statistical analyses were done with SPSS 6.1 for Windows Student Version. Chicago, USA, 1994.

III. RESULTS AND DISCUSSION

Genotyping the κ -casein was performed using PCR - RLFP method. Digestion of the PCR products was done separately with three different restriction enzymes, *Hinf* I, *Bsu* RI and *Tai* I. Identification of the alleles A and B, was done using the enzyme *Hinf* I with restriction site G^AANTC, that do not exist in the allele B, because of the change of the aspartic acid with alanine on position 148 in the polypeptide chain. For this reason, the digestion results with three fragments of 326, 100 and 27 bp of the allele A and two fragments of 426 and 100 bp of the allele B (Figure 1).

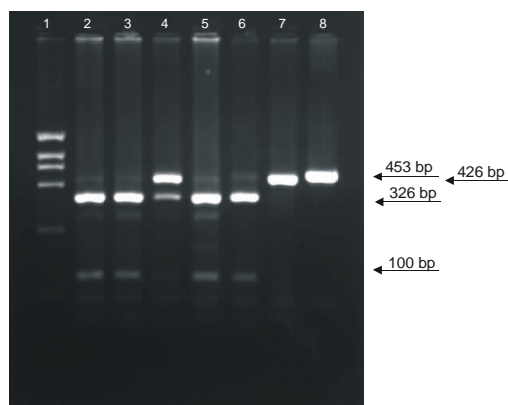


Fig. 1. 1.5% Agarose gel electrophoresis of digested PCR products using *Hinf*I enzyme. #1 DNA ladder, #2, #3, #5 and #6 AA genotype, #4 AB genotype; #7 BB genotype, #8 undigested amplification.

Distinguishing the alleles A and E was performed by digestion with *Bsu* RI restriction enzyme. In this way the presence of the polymorphic site, when the amino acid serine in κ -casein A with glycine in κ -casein E in the 155 position, has been detected. After the complete digestion, the allele E resulted with three fragments of 230, 145 and 78 bp, while the A allele showed two fragments of 230 and 223 bp that were overlapping and visualized as one band in the 1.5% agarose gel (Figure 2).

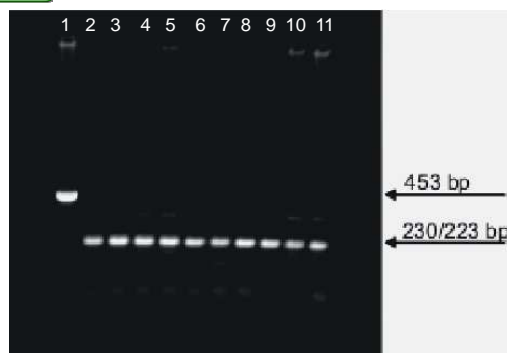


Fig. 2. 1.5% Agarose gel electrophoresis of digested PCR products using *Bsu* RI enzyme. #1 undigested amplification, #2-#11 non AE, non BE and non EE κ -casein samples.

The presence of the allele C was checked by digestion with the restriction enzyme *Tai* I. The restriction site for this enzyme ACGT^A, doesn't exist in allele C due to the substitution of the amino acid arginine with histidine in the 97th position in the polypeptide chain. The A and B allele result with two fragments of 254 and 199 bp (Figure 3)

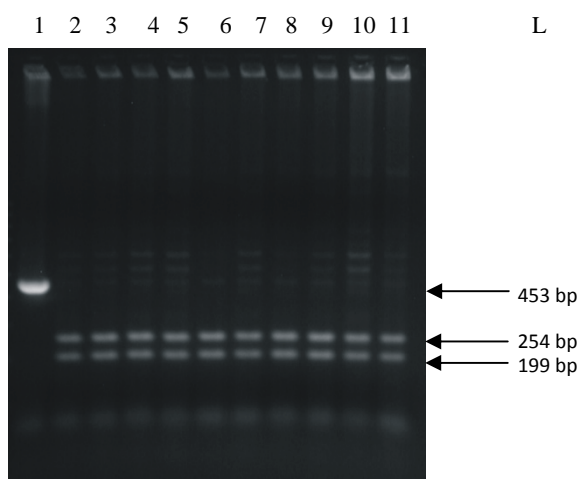


Fig. 3. 1.5% Agarose gel electrophoresis of digested PCR products using *Tai* I enzyme. #1 undigested amplification, #2-#11 non AC, non BC and non CC κ -casein samples. L – ladder 50bp-1000 bp

Distribution of the κ -casein genotypes and the allele frequency are shown in Table 1.

Table 1. A) Genotypes distribution and B) Allele frequencies of κ -casein in the investigated cattle (n=172)

Genotype	Number of animals / %
AA	94 (54.66)
AB	62 (36.04)
BB	16 (9.30)

Allele	Allele frequency
A	0.727
B	0.273

The results suggested that κ -casein genotypic frequencies for analysed cows belonging to Holstein-Friesian breed in Macedonia were: 54,66; 36,04 and 9,30% for AA, AB and BB genotype, while frequencies of A and B alleles, in examined breeds, assessed on the basis of genotype frequencies, were 0.727 and 0.273. It showed that the domination of κ -casein -A allelic variant was confirmed in this breed, while the frequency of desirable κ -casein -B allelic variant was lower. In estimated DNA samples was not found the case of C and E allele of κ -casein gene. A significant domination of κ -casein A allelic variant in the Holstein populations in other countries and regions was observed as well [18], [19].

In the studies on bovine breeds genetic characterization, it was found out that the κ -casein-B allele occurs at higher frequencies in breeds originating from *Bos taurus* than in those of *Bos indicus* origin [20].

In this study genotypes for κ -casein were matched with some lactation traits, milk yield and coagulation properties. Possible associations of milk yield in first lactation and the duration of lactation with the κ -casein genotype in all 172 analysed cows are shown in Table 2.

Table 2 Mean values of total serum proteins, milk yield and duration of lactation in estimated cows associated with different genotype for κ -casein (n = 172)

Genotype	AA	AB	BB
Milk yield in first lactation (l)	6332±672	6550±543	6536±601
Duration of lactation (days)	269±26	268±32	285±28
Effect	p-value		
Milk yield in first lactation	0.042*		
Duration of lactation	0.330 ^{NS}		
NS p>0.05; * p<0.05, ** p<0.01			

The κ -casein genotype influenced significantly ($p \leq 0.05$) the milk yield in a first lactation, but it didn't influenced significantly ($p > 0.05$) the duration of lactation and total serum protein. Statistical analyses show that dairy cows with BB and AB genotype of κ -casein has about 3 % higher yield of milk than those with AA genotype. It is in contrast with the finding of Sitkowska [21] where the κ -casein AA genotype cows from Simmental breed obtained the highest yield of milk. Alipanah [22], also indicated that in Red-pied cows, statistically significant differences for milk yield where AA genotype cows had higher milk yield. Our study showed that the differences in duration of the lactation compared with the κ -casein genotype weren't statistically valid.

The effects of κ -casein genetic variants on the renneting properties of individual milks were studied. Milk coagulation experiment was realised with selected 12 milk samples in triplicate (four from each κ -casein genotype AA, AB and BB). Possible associations of κ -casein genotype with data obtained during the milk coagulation experiment and biochemical analysis of remained curd are shown in Table 3.

Table 3. Mean values of the parameters analysed during the milk coagulation experiment associated with different genotype for κ -casein (n = 12)

Genotype	AA	AB	BB	p-value
Initial coagulation (min : sec)	11:35	10:30	9:30	0.008**
Final coagulation (min : sec)	46:15	44:06	33:30	0.009**
Extracted curd (ml)	264.50	301.25	307.50	0.027*
Curd fats (g/l)	0,465	0,285	0,260	0.034*
Curd proteins (g/l)	1,2850	1,2525	1,0450	0.23 ^{NS}
Dry content in curd (%)	7,2825	7,3250	7,2375	0.19 ^{NS}

NS p > 0.05; * p < 0.05, ** p < 0.01

Milk yielded by cows with BB genotype need shortest time for the initial and final coagulation in the cheese making process compared with milk obtained from cows with AA genotype. Milk yielded by cows with AB genotype showed intermediate effect. It showed significantly high influence of genotype for κ -casein on renneting time. The results show that the renneting properties of individual milks are to a considerable degree genetically controlled.

During the cheese making process was found that the amount of extracted curd is significantly influenced by κ -casein genotype, whereas the amounts of extracted curd obtained from κ -casein milk samples with AB and BB genotypes are significantly higher compared with those from milk samples with AA κ -casein genotype. Also, the amount of fats in the extracted curd from milk samples with AA genotype were presented more than 35 % comparing with milk samples with AB and BB genotypes. The only one analysed parameter related to extracted curd which didn't showed significant influence of κ -casein genotype were amount of proteins and dry content. However the milk of BB genotype cows produced 10% higher yield of cheese in relation to the milk of AA genotype cows [4].

IV. CONCLUSIONS

Genotyping of the κ -casein in Holstein-Friesian dairy cattle in Republic of Macedonia showed that the allele A is dominant and it is an unfavorable factor in the cheese production. In all analysed samples, C and E alleles were not found.

Dairy cows with BB and AB genotype of κ -casein has 3 % higher yield of milk than those with AA genotype. Milk yielded by cows with BB genotype need shortest time for the initial and final coagulation in the process of cheese making in contrast with AA genotype. Milk yielded by cows with AB genotype showed intermediate effect. Amount of fats in the extracted whey in AA genotype were present more than 35 % comparing with the milk obtained from cows with AB and BB genotypes.

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