Early Determination of Animals with Favorable Genes in Milk Production for Profitable Private Farms

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Abstract
The primary goal of dairy industry has been to identify an efficient and economical way of increasing milk production and its constituents without increasing the size of the dairy herd. The use of milk protein polymorphisms as detectable molecular markers has been studied intensively because of their effect on the yield and processing properties of milk and its products. Thus, molecular markers are promising alternative to the current methods of trait selection once these genes are proven to be associated with traits of interest in animals. Kappa-casein (CSN3) and beta-lactoglobulin (BLG) are two of the most important proteins in the milk of mammals that play a crucial role in the milk quality and coagulation, an essential process for cheese and butter. The A and B variant of k-casein and β-lactoglobulin were distinguished by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) analysis in 108 Romanian Simmental and 60 Holstein Friesian cattle.

Keywords: beta-lactoglobulin, kappa-casein, milk production traits, polymorphism.

1. Introduction

The initial discovery of genetic polymorphism at the BLG locus by Aschaffenburg and Drewry (1955) occurred almost 50 years ago, and the complete amino acid sequences of the genetic variants A and B of CSN3 were determined by Grosclaude et al. (1972) and Mercier et al. (1973). Since then, many studies have been carried out to determine the effect of CSN3 and BLG genotypes on milk production traits [1].
The effects of the different kappa-casein and beta-lactoglobulin alleles on the quality and the quantity of cow's milk have been widely reported and the availability of accurate and reliable protocols for the identification of the most common alleles is of great interest in breeding projects. Typing alleles A and B of those two genes is of practical importance.

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The B variant of kappa-casein is associated with an increase in milk protein as well as cheese production because plays an important role in preserving the other caseins from precipitation, with superior coagulation particularities that give the milk higher proprieties for cheese production [2]. Several studies indicate that the milk from BB genotype results in short rennet coagulation time, formation of a firmer crud and a greater cheese yield that milk from AA genotypes.
At the beta-lactoglobulin locus, variants A and B influence milk composition and its processing properties. The BB genotype of BLG is associated with higher fat contents and is, therefore, more desirable for cheese making. Many studies demonstrate that these specific genetic variants affect the manufacturing properties of milk, especially those of importance in cheese technology.
A direct selection of specific alleles involved in milk manufacturing properties has a grate importance in cattle breeding programs to improve milk production traits. To start a program of

selection using data regarding milk proteins it is necessary to determine the frequencies of the relevant alleles on the breeding which most contribute to Romanian dairy production.
The frequencies of the kappa-casein and beta-lactoglobulin alleles and genotypes have been determined in two Romanian cattle breeds, Romanian Simmental and Holstein Friesian, by means of PCR– RFLP analysis followed by digestion trough restriction enzyme. The aim of present study was to provide genetic data regarding kappa-casein and beta-lactoglobulin loci for Romanian Simmental and Holstein Friesian cattle breeds. Partial results have been presented by our group.

2. Materials and methods

The study was carried out in 2009 in 15 commercial farms located in Arad, Timis and Bihor county. The studied herds consisted of 108 Romanian Simmental and 60 Holstein Friesian cattle.
Blood sample for DNA genotyping was collected from caudal vena using 6 mL vacuum tube containing K_3EDTA. The tubes were maintained at -20^{0}C until were used for DNA extraction. Genomic DNA were isolated from blood sample (300 µl) using Wizard Genomic DNA Purification Kit (Promega, Madison, USA) and stored at 4^{0}C.
The kappa-casein specific primers (ATC ATT TAT GGC CAT TCC ACC AAA G and GCC CAT TTC GCC TTC TCT GTA ACA GA) were used to amplify a 350 bp fragment in cattle breeds [3]. Amplification reaction was done in a final volume of 25 µl containing 100 ng DNA, 25 pM of each primer and 2X DreamTaq Green Master Mix (Fermentas). The reaction was subjected to 95°C for 5 min. (initial denaturation), 35 cycles of 95°C for 30 sec., 60°C for 30 sec. and 72°C for 30 sec., followed by 72°C for 7 min. After amplification, PCR products were digested with *HindIII* restriction endonuclease in a 30 µl of restriction mixture (10U *HindIII* / reaction) and the digestion reaction were incubated at 37°C for 2 hours.
Beta-lactoglobulin gene variants were identified according to Medrano and Aquilar-Cordova [4]. DNA was amplified using the following primers: 5'-TGT GCT GGA CAC CGA CTA CAA AAA G-3' and 5'-GCT CCC GGT ATA TGA CCA CCC TCT-3'. Amplification reaction were done in a final volume of 25 µl containing 100 ng DNA, 25 pM of each primer and 2X DreamTaq Green Master Mix (Fermentas). The reaction was subjected to 95°C for 5 min. (initial denaturation), 35 cycles of 95°C for 30 sec., 63°C for 30 sec. and 72°C for 30 sec., followed by 72°C for 7 min. The amplified 247 bp long DNA fragments were digested with *HaeIII* restriction endonuclease (37°C for 2 hours, 10U *HaeIII* / 30 µl reaction).
After digestion, the digested products for CSN3 and BLG were separated electrophoretically in 3.5 agarose gel. In both CSN3 and BLG loci the variant A and B were identified. All gels were photographed and analyzed.

3. Results and discussion

Identification of A and B alleles of CSN3 and BLG was performed by PCR-RFLP technique. For CSN3 the enzyme *HindIII* cut the 350 bp PCR products in two fragments of 219/131 bp for allele B while allele A shows no restriction site. In case of BLG the genotypes were characterized as followed: genotype AA present two fragments of 148/99 bp, genotype BB two bands of 99/74 bp and three restriction fragments of 99/74/74 bp, while genotype AB showed three bands of 148/99/74 bp and four restriction fragments of 148/99/74/74 bp.
Frequencies of A and B alleles of CSN3 and BLG in the studied population was calculated and are shown in Tables 1 and 2.
At the CSN3 locus in Romanian Simmental breed the frequencies of A and B alleles were 0.761 and 0.239 and those of AA, AB and BB genotypes were 0.582, 0.359 and 0.059, respectively (Table 1), that represent a relative low frequency of B allele comparing with A allele. The same situation was observed in Holstein Friesian breed where A and B alleles frequencies were 0.842 and 0.158 (Table 2).

Table 1. Polymorphism at k-casein (CSN3) and beta-lactoglobulin (BLG) loci in the Romanian Simmental cattle

Locus	Genotype	Number of animals (frequency)	Allele frequency
CSN3	AA	60 (0.582)	p_A-0.761
	AB	37 (0.359)	q_B-0.239
	BB	6 (0.059)	
BLG	AA	12 (0.171)	p_A-0.471
	AB	42 (0.600)	q_B-0.529
	BB	16 (0.229)	

Table 2. Polymorphism at k-casein (CSN3) and beta-lactoglobulin (BLG) loci in the Holstein Friesian cattle

Locus	Genotype	Number of animals (frequency)	Allele frequency
CSN3	AA	40 (0.702)	p_A-0.842
	AB	16 (0.280)	q_B-0.158
	BB	1 (0.018)	
BLG	AA	13 (0.232)	p_A-0.473
	AB	27 (0.482)	q_B-0.527
	BB	16 (0.286)	

The frequency of CSN3 B allele in different breed ranges from 0.06 – 0.62 [1-2, 5-9]. The highest frequency was observed in Romanian Brown, Brown Swiss and Jersey with 0.62, 0.67 and 0.86, respectively [8, 10].

The frequency of BLG allele A in Romanian Simmental were 0.471, result almost similar with those obtained by other Romanian group (0.514) in the same breed [5]. For Holstein Friesian cattle breed we find similar alleles frequencies as in Romanian Simmental cattle, respectively 0.473 for A allele and 0.527 for B allele.

In different herds of dairy cattle the lower frequency of A allele was reported in Iranian Najdi cattle (0.0875) [11]. For Holstein Friesian the frequencies of BLG A allele in different herds was reported to vary from 0.37 to 0.57 [9, 12-14].

4. Conclusions

Tables 1 and 2 shows the alleles and genotypes frequencies for kappa-casein and beta-lactoglobulin in the breeds of cattle studied (Romanian Simmental and Holstein Friesian). The highest frequencies for the k-casein A allele were observed in Holstein Friesian cattle (0.842). A similar study conducted in dairy breeds showed that Holstein cattle have a higher frequency of the A allele than other breeds [2]. As shown in Tables 1 and 2, at kappa-casein locus in both breeds we observed a relatively low frequency of the B allele. Concerning to the beta-lactoglobulin locus, we find similar results for B allele frequency in Romanian Simmental and Holstein Friesian cattle breeds, respectively 0.529 and 0.527.

Allelic polymorphism of milk proteins can be studied using DNA polymorphism analysis based on PCR-RFLP technique. This procedure can be use into the dairy farms in order to increase the frequency of alleles involved in quality and quantity of cattle production. In general, B variant of both proteins was recognized as superior for milk quality in European cattle breeds. Thus, it may be concluded that kappa-casein and beta-lactoglobulin genotypes, when used as genetic markers in selection programs, contribute to the improvement of milk production traits in cattle.

Acknowledgements

This work was supported by the project no.299392 / MADR and World Bank, A.G. 141557/22.04.2008

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