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Genetic variants of kappa-casein gene in Busha and Brown Swiss breeds of cattle

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Abstract

The genetic variants of kappa-casein gene were found in different frequencies in various cattle breeds. These genetic variants cause differences in quality and composition of the milk. The objective of this work was to identify the genotypes and allelic frequencies of kappa-casein locus in Brown Swiss as an exotic breed and Busha cattle as indigenous cattle breed in Montenegro. The DNA of 21 blood samples of Busha breed cattle and 19 blood samples of Brown Swiss cattle were genotyped for polymorphisms at the kappa-casein gene by a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) essay. A 351 bp fragment of kappa casein was amplified and digested with HinfI restriction endonuclease. Three genotypes were identified (AA, AB and BB) with frequencies of 0.117, 0.450 and 0.433 respectively in Brown Swiss breed and 0.274, 0.499 and 0.227 in Busha breed. The frequencies of alleles were 0.316 (A) and 0.684 (B) in Brown Swiss and 0.524 and 0.476 (respectively) in Busha cattle. The genotyping of kappa-casein alleles (A and B) is of practical importance, since B allele is in positive correlations with commercially valuable parameters of cheese yielding efficiency. The results of very high frequency of B allele in Brown Swiss cattle confirmed good performance of this breed in Montenegrin population. This determination of A and B allele frequencies in population of Busha breed is the first research of this kind in Montenegro. It could be used for possible increasing the frequency of desired alleles and genotypes by including in the programs of selection and preservation of the Busha breed in Montenegro, as an important animal genetic resource.

Keywords: kappa-casein, PCR-RFLP, Busha, Brown Swiss

1. Introduction

Cattle production, in addition to meat, provides 90% of the total amount of milk used for human population. Yield and quality of milk in modern breeding programs can often be expressed through the quantity of milk, fat and proteins. However, identifying the polymorphic gene variants of milk proteins associated with productive traits opens additional possibilities for improvement of production of different breeds [1].

Genetic polymorphisms of bovine milk proteins induce a significant scientific interest, mainly associated with their evolution, population structure, breeding and hybridization. Over the last decades, studies have been concentrated on the influence of genetic variants of the major milk proteins on quantitative and qualitative milk traits and their technological properties [2, 3].

Kappa-casein as one of the four milk casein proteins is determined by the gene positioned at the 6th bovine chromosome with a length of 13 kb that is divided into 5 exons [4, 5]. It is the most important and highly studied protein genes. So far, in cattle population 11 variants of kappa-casein gene have been described: A, B, C, E, F1, F2, G1, G2, H, I and J. However, variants A and B are the most common and the most studied [6]. A and B variants differ in the amino acids 136 and 148. At position 136, threonine is replaced by isoleucine, while at position 148, aspartic acid is replaced by alanine, for A and B, respectively [4, 7, 8]. In the most of the kappa-casein studies, the association of the B allele variant with some quantitative and milk processing traits was identified. The cheese yield from cows with genotype BB is 10% higher compared to AA genotype. The B allele of kappa-casein not only promotes an increase in cheese yield and improves cheese quality, but is positively correlated with other valuable parameters of milk productivity as protein content and milk yield [9, 10, 11]. The effect of the kappa-casein B allele on the protein content of milk has also been reported in many studies involving different cattle breeds from many countries. The genetic variants of kappa-casein gene were found in different frequencies in various cattle breeds. B allele of kappa-casein gene is integrated into cattle breeding programs in many countries. The development of PCR-RFLP (Polimerase Chain Reaction - Restriction Fragment Lenght Polymorphism) technique enabled fast analysis of the polymorphisms of virtually unlimited number of genes, including those coding kappa-casein gene. The objective of this work was to identify the genotypes and allelic frequencies of kappa-casein locus in the Brown Swiss as an exotic breed and Busha as an indigenous and endangered cattle breed in Montenegro.

2. Material and Methods

Research of polymorphism of kappa-casein was carried out on Brown Swiss and Busha breed of cattle. Brown Swiss breed played very important role in Montenegrin cattle production in second half of XX century. As the alpine dual purpose breed (for milk and meat), Brown Swiss has been used for crossing and improvement of production traits of cattle population in Montenegro. First import of Brown Swiss in Montenegro was in the sixties of XX century, and after that it has occasionally been imported either as live animals or semen [12]. Current share of BS in total cattle population is up to 15%. Busha breed is brachyceros autochthonous breed in the area of Balkan peninsula. Busha population had been the most numerous populations of cattle (approx. 95%) in Montenegro till the middle of XX century. In the second half of XX century, during intensive development of cattle production, Busha breed had been crossed or just replaced by these much more productive breeds. Due to the constant negative trend in population size, this breed is endangered by extinction [13]. Current Montenegrin Busha population is less than 300 animals, so it is included in the program of in situ conservation of animal genetic resources.

The blood samples of Brown Swiss cattle breed were collected on several farms in the area of municipality Podgorica in south and in the municipality BijeloPolje in north part of Montenegro. Blood samples of Busha cattle, as the lowest numerous breed, are collected on the farms in different areas of Montenegro (Ulcinj, Niksic, Plav, Andrijevica). In total 23 blood samples were collected of Busha and 20 blood samples of Brown Swiss breed. Samples collected in 3 ml vacuum tubes, with EDTA used as an anticoagulant, from the left jugular vein. The blood samples were maintained at -20 ^{0}C until used for DNA extraction. Genomic DNA was isolated from 200 µl of blood using phenol chloroform protocol, following the method described by [14]. The quality of DNA was checked by Nano-Vue Spectrophotometer taking ratio of optical density (OD) value at 260 and 280 nm.

PCR and RFLP Procedure: The kappa casein genotype was determined using PCR-RFLP. Amplification of 351 bp fragment of kappa gene was done using the polymerase chain reaction (PCR) with primers: forward (5'-TT TAT GGC CAT TCC ACC AA-3') and reverse (5'-ATT AGC CCA TTT CGC CTT CT-3') as reported by Dogru and Ozdemir 2009. The amplification was performed in 30 µl of final volume with 100 ng of genomic DNA. The reaction mix contained 10xPCR buffer, MgCl2 (1.5mM), dNTP nucleotides (100 µM), 1 µM of each primers and AmpliTaq Gold polymerase (0.5 U). Amplification was performed in Mastercycler Pro (Eppendorf) gradient programmed for initial denaturation at 94°C for 5 min (initial denaturation), followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 60°C for 45 sec, extension at 72°C for 60 sec, and final extension at 72°C for 7 min. PCR products were checked for amplification by electrophoresis on 1% agarose gel, in parallel with 50 and 100 bp DNA marker.

Genotyping was done using PCR-RFLP analysis [15]. For genotyping PCR product was digested with Hinf I restriction enzyme which was used for the determination of kappa-casein alleles. The total reaction mixture of 15 µL comprised of 10 µL of PCR product, 1.5 µL of enzyme buffer, 5 U of restriction enzyme and 3.2 µL of dH2O. After digestion at 37°C for three hours the digested products were resolved by electrophoresis on 2,5%agarose gel in parallel with a 100 bp DNA marker. The genotype patterns were visualised under UV light by Quantum ST400, after staining with ethidium bromide. The genotypes of cattle kappa-casein gene were identified on the base of restricted fragments size.

Statistical analysis: Allele and genotype frequencies for the genetic variants of kappa-casein gene analyzed by Hardy-Weinberg equilibrium calculator including analysis for ascertainment bias [16]. The chi-square statistic was used to check whether the populations were in Hardy-Weinberg equilibrium.

3. Results and Discussion

Genotypes and allelic frequencies distribution for kappa-casein gene in 21 Busha and 19 Brown Swiss breeds were detected (Table1). PCR amplification of the 351-bp fragment of the kappa-casein gene produced a clear and distinct band on the gel, which is of major importance for successful digestion step of PCR products (Figure 1). Digestion of the PCR products with endonuclease Hinf I generated presence of three fragments: 261, 131 and 89 bp. Genotype AA identified by presence of two fragments (131 and 89 bp), BB genotype by fragments of 261 and 89 bp, while AB genotype was identified by presence of three fragments (261, 131 and 89 bp)

The genotype AB had highest frequencies in both breeds (0.499 and 0.450) of all genotypes. The frequency of BB genotype was much higher in Brown Swiss than in Busha breed (0.433 and 0.227, consequently). In both investigated breeds observed heterozygotes was higher than expected, indicating the possible excess of outbreeding. According to χ^2 test, both breeds were found to be in Hardy-Weinberg equilibrium that suggesting that the kappa-casein gene in investigated population was not influenced by selection. Higher share the of observed AB and BB genotype determined in Brown Swiss breed it is assumed could be a consequence of use of homozygous bulls (semen) to "desirable" allelic polymorphic B form. Milk produced by BB genotype cows yielded significantly more cheese than that produced by AA genotype [17]. The frequency of AA genotype was very low for Brown Swiss breed (0.117).

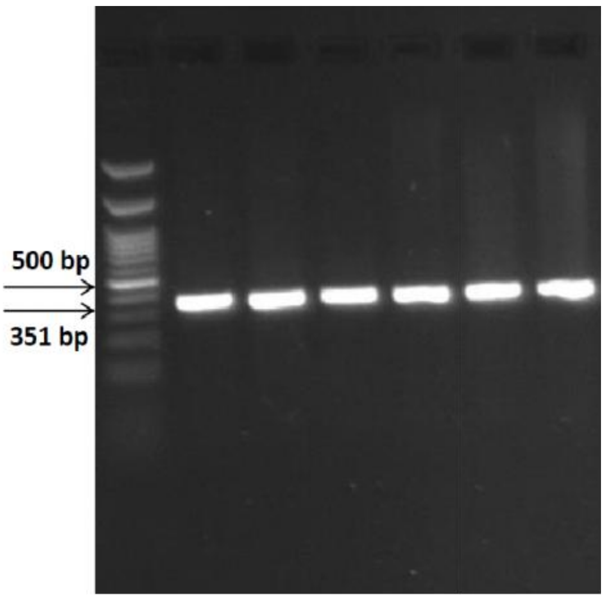

Figure 1: Electrophoresis of kappa-casein gene polymorphim PCR products on 1% agarose gel

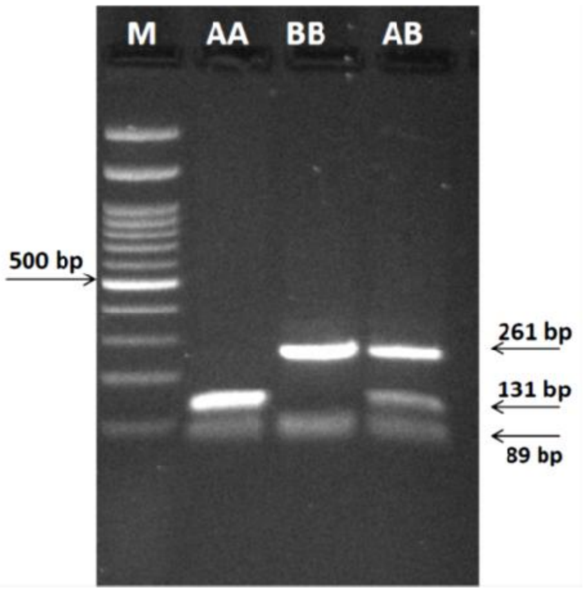

Figure 2: PCR-RFLP of kappa-casein gene using Hinf I on 2.5% agarose gel

Table 1. Frequencies of genotypes and alleles and Hardy Weinberg equilibrium test for kappa-casein locus of Busha and Brown Swiss cattle breeds

Breed	Kappa – casein genotype				Allele frequencies		¡ ^{2} **P**
	Parameters	AA	AB	BB	A	B	
Busha	Ho	5	12	4	0.524	0.476	$\chi^2 = 0.444^{ns}$
	He	5.76	10.48	4.76			
	Frequency	**0.274**	**0.499**	**0.227**			P = 0.505
Brown Swiss	Ho	2	9	8	0.342	0.658	$\chi 2 = 0.052^{ns}$
	He	2.22	8.55	8.22			
	Frequency	**0.117**	**0.450**	**0.433**			P = 0.819

Ho: observed heterozygotes, He: expected heterozygotes on the basis of Hardy-Weinberg, χ2 =chi-square value

Previous studies suggest that milk allelic variants A of kappa-casein requires a longer time of milk rennet and less chees yield [3]. By determination kappa-casein gene polymorphism slightly higher frequencies of A allele was identified in Busha breed (0.524) than of allele B (0.476), while in Brown Swiss breed frequency of allele B was much higher than A (0.658 and 0.342 consequently). The similar ratio of A and B alleles was reported for Brown Swiss breed reared in Turkey [15]. B allele of kappa-casein was a favorable in cattle population, because it significantly affected both milk and milk protein yield of Holstein and Native Iranian breed [8]. Cows of AB and BB genotypes showed higher milk fat content when compared to the AA genotype [18]. Because of the evident effects of kappa-casein genetic variants on cheese yield, selection of animals with the favorable kappa-casein B allele is considerable.

The higher frequency of A allele than allele B of Montenegrin Busha breed determined in present study is in agreement with the earlier observations for Busha breed reared in other countries of West Balkan, as it was reported for Busha in Serbia [1, 19] for Busha reared in Bosnia and Herzegovina [20]. However our results are not in accordance with the results for Busha breed in Croatia where had found lower frequency of A than B allele [21, 22].

Kucerova et al. (2006) also reported that More frequently A allele (0.62) than allele B (0.38) is presented in population of Czech Simmental cattle breed [5]. Similar findings of the allele and genotype frequencies identified in Czech Simmental (Fleckvieh) cattle population [23], as well as in Holstein Friesian cattle in Macedonia and for more commercial cattle breeds in Croatia [21, 10].

4. Conclusions

The results of PCR-RFLP analysis showed, as it was expected, that three genotypes (AA, BB and AB) were found in the population studied for kappa-casein gene. The results of very high frequency of B allele in Brown Swiss cattle implied that this breed should have a good performance in the milk traits related to the B allele of kappa-casein gene. Hence, future studies should be focused on the association between cattle genotype and milk composition characteristics.

Determination of A and B allele frequencies in population of Busha breed is the first one of this kind in Montenegro. It could be used in the programs of selection and preservation of the Busha breed, as important animal genetic resource in Montenegro, in order to increase frequency of desired alleles and genotypes.

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