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FREQUENCY OF κ-CASEIN AND β-LACTOGLOBULIN GENOTYPES IN DAUGHTERS OF FIVE SIMMENTAL BULL SIRES

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Abstract: The aim of this study was to determine the frequency of the κ-casein and β-lactoglobulin genotypes of the daughters of 5 Simmental bulls (sires) used for insemination in Serbia. Blood samples were taken from a total of 157 cows of the Simmental breed in Toplica and Rasina districts. Cows from which blood samples were taken were the daughters of the following bulls: Rorb B033, Dionis B52, Val B064, Rondi B029 and Res 1433. In all daughters of studied bulls, the presence of all three genotypes was determined, except for daughters of bull sire Res B1433, where the absence of genotype BB was determined. In addition to determining the frequency of genotypes and alleles for both milk protein fractions, based on the $\chi2$ test, a link between the sire and the presence of genotypes was determined, and in both cases a statistically significant relationship ($p \leq 0.001$) was found.

Key words: frequency, κ-casein, β-lactoglobulin, PCR-RFLP, bulls

Introduction

Milk proteins make up 3.0-3.5% of cow's milk and thus represent an important ingredient in feeding of their progeny and the necessary source of essential amino acids and biologically active proteins.

Protein synthesis is a process by which the cell builds proteins, for which the energy and the availability of nitrogen is required. *Ostojić (2007)* defines the milk protein synthesis as a process under control of hormone and genetic mechanisms, which begins with transcription and ends with translation.

The polymorphism of β-lactoglobulin (β-Lg) and κ-casein (κ-CN) proteins is incorporated into modern cattle breeding programs, through which it aims to functionally improve populations. As the main protein of milk whey β-lactoglobulin is determined by the gene positioned on the 11th chromosome of cattle. *Caroli et al. (2009),* with two dominant polymorphic forms (A and B), also indicate nine rare polymorphic variants (C, D, E, F, G, H, I, J, W). K-casein, as one of the four casein milk proteins, is determined by the gene positioned on the 6th cattle chromosome. *Caroli et al. (2009)* state fourteen polymorphic forms of κ-casein (A, A1, B, B2, C, D, E, F1, F2, G1, G2, H, I, J) of which two polymorphic forms dominate (A and B).

Genetic polymorphism of κ-casein and β-lactoglobulin in cows of different breeds in Croatia was studied by *Ivanković et al. (2011).* Using the new analytical methods, they determined the share of the dominant allelic polymorphic variants of β-lactoglobulin and κ-casein in 3 commercial and 3 indigenous breeds of cattle: Holstein, Simmental, Brown cattle, Buša, Slavonian-Sremski Podolac and Istrian cattle. The share of allele B variant of β-lactoglobulin was dominant in all studied breeds of cattle (>52.9%). Allele A variant of κ-casein was dominant in selected breeds of cattle (60.7-76.4%), while the share of B variants of κ-casein was significantly more present in autochthonous breeds of cattle (48.2-84.1%).

Genetic polymorphism of fractions of milk proteins and their effect on the production properties of Simmental cows in Poland were studied by *Felenczak et al. (2006).* These authors state that in the β-lactoglobulin a BD genotype was also identified with a frequency of 0.024. Other genotypes had the frequency: AA 0.236, AB 0.488 and BB 0.252, and alleles: A 0.486, B 0.502, D 0.012. For κ-casein three genotypes were found, AA, AB, BB, frequency 0.281, 0.498, and 0.221, respectively, while for alleles it was: A 0.530 and B 0.470.

In the study of the genetic polymorphism of κ-casein and its influence on the production performances of Simmental cattle, the autochthonous Busha breed, and the crosses of Simmental and Red Holstein cattle in Serbia, *Đedović et al. (2015)* report the following results: the genotypic incidence of κ-casein for Simmental breed were: 42.8; 47.6 and 9.6% for AA, AB and BB genotype, for crosses: 75.0; 25.0 and 0.0% and for Busha animals: 41.7; 50.0 and 8.3%, respectively. The incidence of alleles A and B, for the observed breeds estimated on the basis of the genotype frequency, had values of 0.667 and 0.333 for Simmental breed, for crosses 0.875 and 0.125 and 0.667 and 0.333 for indigenous cattle breeds, respectively, while working with PCR-RFLP analysis of β -lactoglobulin of a small herd of Simmental cows in Serbia *Caro Petrović et al. (2017)* have found the existence of only AA β-lactoglobulin genotype.

Examination of the frequency of the κ-casein and β-lactoglobulin alleles in the Holstein-Friesian cows in Serbia was carried out by *Lukač et al. (2013).* The study covered 765 Holstein-Friesian cows, daughters of 18 bull sires, for the determination of β-lactoglobulin genotypes and 420 cows for the determination of κ-casein genotypes. The following results were obtained: from 765 cows, 172 were AA, 448 were AB and 145 cows were BB β-lactoglobulin genotypes, which meant that the frequency of AA genotypes was 0.23, AB 0.58 and BB only 0, 19. The frequency of alleles A and B was 0.52 and 0.48; of 420 cows, 105 were AA, 219 AB and 96 BB κ-casein genotypes. The frequency of genotypes AA, AB and BB ranged within values of 0.25, 0.52 and 0.23, while in alleles A and B, it was 0.51 and 0.49.

Material and Method

Blood samples for genetic testing were taken from 157 cows of Simmental breed in BD Vacutainer® K2EDTA tubes in an amount of 6 ml from the tail vein (v. Caudalis), after which they were stored at a temperature of 4ºC until the DNA was isolated. The isolation of the DNA was performed using isolators according to the manufacturer's instructions. The multiplication of the portion of the κ-casein gene containing polymorphic sequence was done using the following primers (*Mitra et al., 1998*): Casein FW 5′ CAC GTC ACC CAC ACC CAC ATT TATC - 3′ and casein REV 5′ TAA TTA GCC CAT TTC GCC TTC TCT GT - 3′ (Invitrogen-Thermo Fisher Scientific Inc., USA). The multiplication of the portion of the β-lactoglobulin gene containing the polymorphic sequence was done using the following primers (*Medrano I Aguilar-Cordova, 1990)*: β-lactoglobulin FW-GTC CTT GTG CTG GAC ACC GAC TAC A- 3′ and β-lactoglobulin REV-CAG GAC ACC GGC TCC CGG TAT ATG A- 3′ (Invitrogen-Thermo Fisher Scientific Inc., USA).

The following steps were applied in the PCR reaction: denaturation at 95ºC for 2 minutes, 30 cycles of denaturation at 95ºC for 1 minute, 30 cycles of hybridization at 57ºC (61ºC for β-lactoglobulin) for 30 seconds and 30 cycles of polymerization at 72ºC for 1 minute. The completion was followed by final elongation at 72°C for 7 minutes for κ-casein and 10 minutes for β-lactoglobulin. Identification of polymorphism in the genes for κ-casein and β-lactoglobulin was performed using a method based on restriction fragment size polymorphism (RFLP). The amplification products were purified by precipitation and treated with a restriction enzyme Hinf I that specifically recognizes the 5'GANTC-3 'sequence, which includes polymorphism in the κ-casein gene and Hae III that specifically recognizes the 5'GGCC-3' sequence comprising polymorphism in the gene for β-lactoglobulin according to the manufacturer's instructions. Polymorphism of restriction fragment size was analyzed by agarose gel electrophoresis.

Statistical processing was performed in the SPSS Statistics for windows, Version 23.0 program, which included determining the frequency of genotypes for both investigated genes. By using the $\chi 2$ test, the correlation between the sires and the genotype frequency was examined.

Results and Discussion

The research was carried out on 157 cows of Simmental breed on the territory of Toplica and Rasina district. In order to detect genotypes for κ-casein and β-lactoglobulin, specific primers were used. Polymorphism of restriction fragment size was analyzed by agarose gel electrophoresis, when three genotypes for both protein fractions were discovered.

The length of the restriction fragments of κ-casein for the AA genotype was 156, 132 and 91 base pairs, for AB 288, 156, 132 and 91 base pairs, while for the genotype BB the restriction fragment was 288 and 91 base pairs.

Table 1. Frequencies of κ-casein genotypes and alleles and the influence of bulls on the frequency

Sire, HB	Genotype	No. of animals	Frequency	Alleles	Frequency
Rorb,B033	AA	18	0.529		
	AB	14	0.412	A	0,735
	BB	2	0.059	B	0.265
Dionis,B52	AA	7	0.219		
	AB	19	0.593	A	0.516
	BB	6	0.188	B	0.484
Val, B064	AA	5	0.167		
	AB	15	0.500	A	0.417
	BB	10	0.333	B	0.583
Rondi, B029	AA	10	0.323		
	AB	16	0.516	A	0.581
	BB	5	0.161	B	0.419
Res, 1433	AA	13	0.433		
	AB	17	0.567	A	0.717
	BB	0	0.000	B	0.283
Total	**AA**	**53**	**33.8**		
	AB	**81**	**51.6**	**A**	**0.596**
	BB	**23**	**14.6**	**B**	**0.404**
χ2=110.955		df=8	p value=0.000		
χ2 = hi square test; df = degrees of freedom; p = significance					

From Table 1 it can be seen that the presence of three genotypes for κ-casein with the frequency AA 0.529, AB 0.412 and BB 0.059 was found in daughters of Rorb HB V-033 bull sire, or by the number of cows AA 18, AB 14 and BB 2. The frequency of alleles for the daughters of this bull was 0.735 for allele A and 0.265 for allele B. The daughters of Dionis HB B52 had the following genotypes: AA 0.219, AB 0.593 and BB 0.188, while in 32 studied/tested duaghters of this bull, the frequency of allele A was 0.516 and the frequency of allele B 0.484.

Genotypes AA, AB and BB were also found in daughters of bull sire Val HB V-064 with a frequency of 0.167, 0.5 and 0.333, respectively. In 30 daughters of this bull, the incidence of alleles A and B was 0.417 and 0.583, respectively and it was the only bull that was examined in this study whose daughters had a higher share of allele B compared to allele A for κ-casein. The same genotypes were also found in daughters of Rondi HB V-029 with frequencies of AA 0.323, AB 0.516 and BB 0.161. Allele A had a frequency of 0.581 while for Allele B it was 0.419. Daughters of bull sire Res HB 1433 are the only ones in this study in which BB genotype was not represented. The frequency of the other two genotypes was 0.433 for AA and for AB 0.567. The frequency of alleles A in daughters of this bull was three times higher in relation to allele B and amounted to 0.717 compared to 0.283 allele B.

If we look at the frequencies of κ-casein genotypes on the total population, they are roughly the same as reported by *Đedović et al. (2015)*, *Falenczak et al. (2007)* and *Lukač et al. (2013)*, while *Ivanković et al. (2011)* obtained slightly different results of the frequency of genotypes.

Based on the χ2 test of the independence of the trait, it was found that there was a statistically significant ($p \leq 0.001$) relation between the sire and the presence of genotypes for κ-casein.

The length of restriction fragments of β-lactoglobulin for the AA genotype was 144 and 108 base pairs, for AB 144, 108, 74 and 70 base pairs, while for the genotype BB the restriction fragment was 108, 74 and 70 base pairs.

In Table 2, it can be seen that in 34 daughters of Rorb HB V-033, three genotypes for β-lactoglobulin with a frequency of AA 0.371. AB 0.486 and BB 0.143 were found. The allele frequency was 0.632 and 0.368. Bull sire Dionis HB B52 had 32 examined daughters with a genotype frequency of 0.313 AA, 0.406 AB and 0.281 BB. Allele A had a frequency of 0.515 and allele B 0.484.

As with the previous two bulls, in daughters of bull sire Val, HB V-064, the highest frequency was recorded for AB genotype of 0.5, slightly lower for BB 0.4 while AA genotype had the lowest frequency of 0.1. The allelic frequency ranged from 0.35 for allele A to 0.65 for allele B. The frequency of the genotypes was tested in 31 daughters of the bull Rondi HB V-029. The AA genotype had a frequency of 0.387, somewhat higher frequency was record for genotype AB. 0.548, while the BB genotype had the lowest frequency of 0.065. Alleles A and B. expressed the distinctive difference in the frequency with values of 0.661 and 0.339.

As with κ-casein, in case of β-lactoglobulin, the BB genotype has not been identified in 30 daughters of the bull Res HB 1433, and of the remaining two genotypes the higher frequency was recorded for AB genotype of 0.533 while the AA genotype had a frequency of 0.467. The absence of the BB genotype for κ-casein and β-lactoglobulin in daughters of the bull Res HB 1433 leads to the conclusion that this bull is an AA genotype, which is very important if we know that the genotypes of these protein fractions affect the production and the composition of the milk.

If we look at the frequency of genotypes for β-lactoglobulin on the total population, they are about the same as reported by *Lukač et al. (2013) in* their research, while *Ivanković et al. (2011), Caro Petrović et al. (2017)* and *Falenczak et al. (2007)* show somewhat different results of the frequency of genotypes.

Based on the $\chi 2$ test of the independence of the trait, it was found that there was statistically significant ($p \leq 0.001$) relation between bull sire and the presence of β-lactoglobulin genotypes.

Table 2. Frequencies of β-lactoglobulin genotypes and alleles and the influence of bulls on the frequency

Sire HB	Genotype	No. of animals	Frequency	Alleles	Frequency
Rorb. B033	AA	13	0.371		
	AB	17	0.486	A	0.632
	BB	4	0.143	B	0.368
Dionis.B52	AA	10	0.313		
	AB	13	0.406	A	0.516
	BB	9	0.281	B	0.484
Val. B064	AA	3	0.100		
	AB	15	0.500	A	0.350
	BB	12	0.400	B	0.650
Rondi. B029	AA	12	0.387		
	AB	17	0.548	A	0.661
	BB	2	0.065	B	0.339
Res. 1433	AA	14	0.467		
	AB	16	0.533	A	0.733
	BB	0	0.000	B	0.267
Total	**AA**	**52**	**32.48**		
	AB	**78**	**50.32**	**A**	**0.580**
	BB	**27**	**17.20**	**B**	**0.420**
χ2=129.074		df=8	p value=0.000		
χ2 = hi square test; df = degree of fredom; p = significancet					

Conclusion

Determination of the κ-casein and β-lactoglobulin genotypes in bulls used for insemination of cows of specific population is very important because by monitoring various forms of milk proteins and their frequencies we can increase the frequency of those genotypes that exhibit a beneficial effect on the milk properties and fulfill the goals that a breeder has for the particular populations of cows. By favoring suitable genotypes, i.e. by the planned selection of parents of desirable genotypes, first of all sires (bulls), a faster genetic improvement of the population is realized, as well as improvements in both production and milk composition and reduction in production losses.

Frekvencija genotipova κ-kazeina i β-laktoglobulina kod ćerki pet bikova simentalske rase goveda

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Rezime

Cilj ovog istraživanja bio je da se utvrdi frekvencija genotipova κ-kazeina i β-laktoglobulina ćerki 5 bikova (očeva) sementalske rase koji se koriste za osemenjavanje u Srbiji. Uzorci krvi uzeti su iz ukupno 157 krava simentalske rase u Topličkom i Rasinskom okrugu. Krave od kojih su uzeti uzorci krvi bile su ćerke sledećih bikova: Rorb B033, Dionis B52, Val B064, Rondi B029 i Res 1433. Kod svih ćerki, ispitivanih bikova, utvrđeno je prisustvo sva tri genotipa, osim kod ćerki bika Res B1433, kod kojih je utvrđeno odsustvo genotipa BB. Pored određivanja frekvencija genotipova i alela za obe frakcije proteina mleka, na osnovu $\chi 2$ testa urađeno je ispitivanje veze između očeva i zastupljenosti genotipova, i u oba slučaja je utvrđeno da postoji statistički značajna veza ($p \leq 0,001$).

Ključne reči: frekvencija, κ-kazein, β-laktoglobulin, PCR-RFLP, bikovi

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