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Original scientific article

THE INFLUENCE OF GENETIC β -LACTOGLOBULIN POLYMORPHISM ON THE QUANTITY AND QUALITY OF MILK OF THE SIMMENTAL BREED IN SERBIA

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The study of the link between genes controlling protein polymorphism and milk performance traits of domestic animals has great economic importance from a selection point of view, as it reduces the generation interval, leading to increased productivity in livestock. The objective of this paper was to establish the influence of genetic β -lactoglobulin polymorphism on the quantity and quality of milk of the simmental breed in Serbia. For the research blood samples were taken from a total of 157 Simmental cows. The genotypes of Simmental cows for β-lactoblobulin and their effect on quantitative milk performance traits were determined using the PCR-RFLP analysis. The variability of traits influenced by the genetic polymorphism of β-lactoblobulin was statistically very highly significant (p <0.0001) for milk yield in standard lactation and milk fat and protein yields, while it showed no statistically significant variability (p> 0.05) for content of milk fat and protein. The AB genotype cows achieved a 121 kg and 338 kg increase in milk production compared to the BB genotype and AA genotype cows, respectively.

Keywords: polymorphism, ***β-l***actoblobulin***,*** *PCR-RFLP, Simmental breed*

INTRODUCTION

After ASCHAFFENBURG and DREWRY (1955) discovered the existence of a polymorphism for cow milk β-lactoglobulin in the middle of the last century, CAROLI *et al*. (2009) have found that β-lactoglobulin is determined by a gene positioned on the 11th bovine chromosome with two

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dominant polymorphic forms (A and B) and nine rare polymorphic variants (C, D, E, F, G, H, I, J, W).

After the discovery of polymorphism of the most important constituents of milk protein, its effect on the quantitative and qualitative properties of cow's milk, lactation properties and processing properties of milk has become the subject of numerous studies (ANTUNAC *et al.*, 1991). A large number of studies have found a positive effect of the AA genotype of β-lactoglobulin on milk yield (MAYER *et al.*,1990; JAKOB and PUHAN, 1992; VAN DER BERG *et al.*,1992; HILL, 1993; IKONEN *et al.*,1999; KAMINSKI *et al.*, 2002; CAROLI *et al.*, 2004; KUČEROVA *et al.*, 2006), although some authors (TSIARAS *et al.*, 2005; KARIMI *et al.*, 2009; DOKSO *et al.*, 2014), attribute in their research advantage to the AB genotype of β-lactoglobulin, especially for protein production (MOLINA *et al.*, 2006a). Positive influence of AA genotype on the qualitative milk properties, i.e. on the concentration of milk components is established in following studies NG KWAI-HANG *et al.*, 2002b; ROBITAILLE *et al.*, 2002; MOLINA *et al*., 2006b; DOKSO *et al.*, 2014, while BOTARO *et al.* (2008) has found that cows of the AA genotype realize higher casein production (2,067g/100g of milk) compared to cows of the BB genotype (1,949g/100g of milk). A more recent study confirmed the beneficial effect of the BB β-lactoglobulin genotype on milk fat content (TSIARAS *et al.*, 2005; BALCAN *et al.*, 2007; KARIMI *et al.*, 2009), milk casein content (BRAUNSCHWEIG *et al.*, 2000; ROBITAILLE *et al.*, 2002), total protein content (BOBE *et al.*, 1999; KUČEROVA *et al.*, 2006; BALCAN *et al.*, 2007), total solids (CELIK, 2003) and higher cheese yield (LUNDEN *et al.*, 1997; STRZALKOWSKA *et al.*, 2002) which is very important for local dairies and the processing industry. NEUBAUEROVÁ (2001) and NG KWAI-HANG *et al.* (2002a), CURI *et al.* (2005) have found no link between qualitative and quantitative traits of milk and β-lactoglobulin genotypes in their studies, while on the other hand KAMINSKI *et al.* (2002) have obtained the results of the dependence of β-lactoglobulin polymorphism and milk protein yield. The knowledge of the link between polymorphism of protein fractions and quantitative milk properties, through adequate selection of bulls for artificial insemination, can lead to the most favourable and fastest genetic variant that will lead to realization of the breeding goal. Therefore, presently, in many countries, β-lactoglobulin protein polymorphism (β-Lg) and κ-casein (κ-CN) embedded in modern bovine breeding programs through which functional efforts are made to improve cattle populations.

MATERIAL AND METHOD

DNA isolation

Blood samples from 157 Simmental cows were taken for genetic analysis. Blood samples from the tail vein (v. Caudalis) were collected into BD Vacutainer® K2EDTA tubes in an amount of 6 ml, after which they were stored at 4°C until DNA isolation. DNA isolation was done using an UltraClean® BloodSpin® DNA Isolation Kit (MO BIO Laboratories Inc., USA), according to the manufacturer's instructions. Polymerase chain reaction (PCR) in 20 µl reaction required: sterile deionized water 13.4 µl, PCR pufer (1X) 2 µl, $MgCl_2$, dNTP (200 µM) 0.5 µl, 1 µl each (0.4 µM) from each primer, Taq polymerase (0.02 U/µl; Kapa B 0.1 µl; Kapa Biosystems, USA) and 2 µl of isolated DNA each. Replication of a portion of the β-lactoglobulin gene containing the polymorphic sequence was done using the following primers (MEDRANO and 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).

Table 1. Expected fragment sizes

Protein	Genotype	Fragment length
	AA	144 and 108
β-lactoglobulin	AB	144, 108, 74 and 70
	BB	108, 74 and 70

PCR-RFLP

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. Completion was followed by final elongation at 72ºC for 10 minutes for β-lactoglobulin. Identification of polymorphisms in genes for β-lactoglobulin was done using a restriction fragment size polymorphism (RFLP) method. This method consists of identifying a polymorphism by treating PCR products with an appropriate restriction enzyme and comparing the size of the bands on an agarose gel. The amplification products were purified by precipitation and treated with the Hae III restriction enzyme (New England Biolabs Inc., USA) that specifically recognizes the sequence 5′GGCC-3′ that encapsulates polymorphism in the β-lactoglobulin gene according to the manufacturer's instructions. The size restriction fragment polymorphism was analysed by agarose gel electrophoresis.

Statistical analysis

Statistical processing of genotype and allele frequency determination and subsequent influence of fixed factors on the analysed milk performance traits were performed using the GLM procedure within the SAS software package (version 9.3- SAS Inst. Inc., Cary, NC, USA) using the following fixed model:

$$Yijkl = \mu + Bi + Gj + Sk + Al + eijkl$$

Where:

Yijkl – phenotypic expression of the trait examined
μ- general population average
Bi- fixed effect of the i genotype of β-lactoglobulin
Gj- fixed effect of k calving year
Sk–fixed effect of l calving season
Al- fixed effect of m age at first calving
eijkl-random error.

For traits that showed statistically significant dependence on the β-lactoglobulin genotype, the least significant difference test (LSD test) was performed.

RESULTS AND DISCUSSION

This study showed the frequency of β-lactoglobulin genotypes and alleles for the total cow population studied. The frequency obtained for the AA, AB, and BB genotypes for β-lactoglobulin was 33.10%, 49.70%, and 17.20%, respectively, meaning that of 157 cows, 52 had genotype AA, 78 genotype AB and 27 genotype BB. The frequency of alleles A and B resulting from genotype frequencies was 58.00% for allele A and 42.00% for allele B.

The AB genotype cows had the highest milk production in standard lactation relative to the other two genotypes (Table 2). They produced 6624.29 kg of milk in 305 days, or 338.29 and 120.94 kg more than cows of AA and BB genotypes, respectively. The positive influence of the AB genotype on milk production in standard lactation was observed by TSIARAS *et al.* (2005), KARIMI *et al.* (2009) and DOKSO *et al.* (2014).

Table 2. Mean values of milk yield per β-lactoglobulin genotypes and p-value of studied effects

Performance traits	Genotypes of β-lactoglobulin	$\bar{X}$	SD	SE	Effect(p) Genotype β-lg Lactation Season
Milk yield in standard lactation (kg)	AA	6286.00	498.41	32.31	0.0001***
	AB	6624.29	597.18	31.22	0.0004***
	BB	6503.35	577.97	48.50	0.2005^{nz}
Milk fat content (%)	AA	4.02	0.11	0.01	0.1181^{nz}
	AB	3.98	0.12	0.01	0.0001***
	BB	4.04	0.13	0.01	0.889^{nz}
Milk fat yield (kg)	AA	252.17	17.61	1.14	0.0001***
	AB	262.99	19.83	1.04	0.1095 ^{nz}
	BB	262.31	21.36	1.79	0.1237 ^{nz}
Protein content (%)	AA	3.25	0.09	0.01	0.1859 ^{nz}
	AB	3.24	0.09	0.00	0.0399*
	BB	3.23	0.11	0.01	0.8257 ^{nz}
Protein yield (kg)	AA	204.46	18.12	1.18	0.0001***
	AB	214.66	20.52	1.07	0.0115*
	BB	210.50	20.18	1.69	0.2195^{nz}

***- p ≤0.001; ** - p ≤0.01; * - p ≤ 0.05; nz – p >0.05

From the presented table it can be concluded that the investigated influence of the β-lactoglobulin genotype on milk performance properties had a statistically significant (p≤0.001) effect on milk production in standard lactations. This result differs significantly from the results obtained by authors who found no significant changes in milk yield depending on the genotype for β-lactoglobulin, namely LIN *et al.* (1989), AHMADI *et al.* (2005), ÇARDAK (2005) and DOKSO *et al.* (2014), in cows of the Simmental breed. At the same time, these authors conclude that there is a statistically significant dependence of the β-lactoglobulin genotype and milk yield in the Holstein Friesian breed.

The milk fat content was the highest in the BB genotype cows (4.04%), that is by 0.02% higher than the AA cows and by 0.06% higher than the AB genotype cows (Table 2). The AB and BB genotype cows achieved a milk fat yield of 262 kg, which was about 10 kg more than the AA genotype cows. The results obtained are similar to those obtained by TSIARAS *et al.* (2005) for milk fat yield (BB and AB>AA) and milk fat content (BB>AA and AB), but differ from the results reported by NG KWAI-HANG *et al.* (2002b), ROBITAILLE *et al.* (2002), MOLINA *et al.* (2006) and DOKSO *et al.* (2014).

Protein content was slightly higher for the AA genotype compared to the other two, but protein yield was the highest for the AB genotype cows. They produced 214.66 kg of protein in 305 days, 4 kg more than BB cows and 10 kg more than the AA genotype cows. These results coincide with those of TSIARAS et al. (2005), MOLINA et al. (2006), DOKSO et al. (2014) and HILL (1993), who favours the AA genotype in terms of protein content and yield.

By examining the effect of β-lactoglobulin genotype polymorphism on milk fat and protein content and yield, statistically very highly significant effect on yield of milk components ($p \leq 0.001$) was found, which is identical to the results of TSIARAS et al. (2005), but not their content ($p > 0.05$), which agrees with the results of DOKSO et al. (2014), as can be seen in Table 2. Based on the results of the Least Significant Difference Test (LSD), the following was determined:

- the difference in milk yield in standard lactation is significant between the AB and BB genotypes at $p \leq 0.05$, the AA and AB genotypes at $p \leq 0.001$, and the AA and BB genotypes at $p \leq 0.001$;
- the difference in milk fat yield is significant between the AA and BB genotypes and the AA and AB genotypes at $p \leq 0.001$;
- the difference in protein yield is significant between the AA and BB genotypes at $p \leq 0.01$, the AA and AB genotypes at $p \leq 0.001$, and between AB and BB genotypes at $p \leq 0.05$.

Table 3. Differences in the average values of tested traits by β-lactoglobulin genotype (LSD test)

Milk yield in standard lactation (kg)	Genotype AB	Genotype BB
Genotype AA	-338,288***	-217,349***
Genotype AB		120,939*
Milk fat yield (kg)	Genotype AB	Genotype BB
Genotype AA	-10,820***	-10,139***
Genotype AB		0,680 ^{nz}
Milk protein yield (kg)	Genotype AB	Genotype BB
Genotype AA	-10,199***	-6,034**
Genotype AB		4,165*

CONCLUSION

The results obtained showed that cows of the AB genotype achieved the highest milk yield as well as the highest milk fat and protein yield. The realization that a particular genotype, in this case the AB genotype, has a statistically significant effect on the increase in milk yield, milk fat and protein yield compared to other two genotypes (AB and BB) has exceptional economic importance from the aspect of selection, because by favouring individual animals of this genotype the increase of productivity and economic profitability of production could be achieved. Therefore, it is extremely important to continue to study the links between polymorphisms of genes controlling certain traits of domestic animals and selecting animals with superior traits at an early age, thereby reducing the generation interval and accelerating population genetic progression.

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UTICAJ GENETIČKOG POLIMORFIZMA β-LAKTOGLOBULINA NA KOLIČINU I KVALITET MLEKA

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Izvod

Ispitivanjem veze između gena koji kontrolišu polimorfizam proteina i osobina mlečnosti domaćih životinja, ima veliki ekonomski značaj sa aspekta selekcije, jer se na taj način smanjuje generacijski interval, što dovodi do povećanja produktivnosti u stočarstvu. U ovom istraživanju uzorci krvi uzeti su iz ukupno 157 krava simentalske rase. Genotipovi krava simentalske rase za β-laktoblobulin i njihov uticaj na kvantitativne osobine mlečnosti određeni su na ukupno 157 grla pomoću PCR-RFLP analize. Varijabilnost osobina pod uticajem genetskog polimorfizma β-laktoblobulina bila je statistički vrlo visoko značajna ($p<0.0001$) za prinos mleka u standardnoj laktaciji i prinose mlečne masti i proteina, dok na sadržaje mlečne masti i proteina nije ispoljila statistički značajnu varijabilnost ($p>0.05$). Krave genotipa AB ostvarile su za 121 kg veću proizvodnju mleka od krava genotipa BB i 338 kg od krava AA genotipa.

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