THE EFFECT OF LGB GENES ON QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF MILK HOLSTEIN BREED IN CROATIA

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Scientific paper

Summary

Among whey proteins β-lactoglobulin is of particular importance. The gene (LGB) responsible for synthesis of β-lactoglobulin in Bos taurus is located on the eleventh chromosome genome. LGB is in two common variants, A and B polymorphic allelic variants, and several rare variants. The aim of this study was to determine the impact of LGB genotypes on milk production characteristics of the Croatian population Holstein breed. For the 112 cow in third lactation underwent genotyping for the LGB using RFLP-PCR method. Data on the qualitative and quantitative characteristics of milk are obtained from the HPA. There was a positive effect of AA genotype on milk yield and BB genotypes on fat content, while effect of genotype was not a significant on protein content. The results presented in this paper can be used in programs of selection and crossing of cattle in order to achieve efficient production of milk in terms of quantity and the useful substances in milk.

Key words: *cows, beta-lactoglobulin, milk production, milk characteristics*

INTRODUCTION

In milk there are two major proteins groups, casein and whey proteins. Caseins account for ca. 80 % of bovine milk protein, whereas both major whey proteins constitute about 14 % (Roginski, 2003).
Study of the genetic polymorphism of milk proteins has received considerable research interest in recent years because of possible association between milk protein genotypes and economically important traits in dairy cattle. The polymorphism of LGB gene has been associated to differences in milk composition, processing and quality (McLien at al., 1984) and also with to yield characteristics (Sitkowska at al., 2009).
This study has the objective to observing the occurrence of polymorphism in the beta-lactoglobulin gene of Holstein cows in Croatia, looking for associations with economical characteristics of milk, such as: milk yield and content fat and protein.

MATERIALS AND METHODS

Beta-lactoglobulin (LGB) genotypes were identified in 112 cows of the Croatian population Holstein breed.
DNA was isolated from the root hairs with the use of Sigma-Aldrich™ GenEluete Mammalian Genomic DNA Miniprep kit, according to a modified manufacturer's instructions. Genotyping of LGB was performed using PCR-PFLP. The 247 bp LGB promoter fragment was PCR amplified. Identification allele A and B of LGB was done according Medrano and Aguilar-Cordova (1990).
The sequences of primers used for amplification of LGB gene were as follows (GenBank Acc.No. X14712):

Forward: 5`GTCCTTGTGCTGGACACCGACTACA3` and
Reverse: 5`CAGGACACCGGCTCCCGGTATATGA 3`.

Amplification reaction were done in a final volume of 20 µL containing DNA, 10 mol of each primer, 10X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTP and 0,5 U/ µL Taq DNA polymerase. The PCR conditions for LGB were 4 min. at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 30 s at 72°C. In the last cycle extension (72°C) was extended to 10 minutes.
The PCR products were digested with *HaeIII* restriction endonuclease in 20 µL of reaction separately. The reaction mixture comprised of 4.89 µL H_2O MiliQ, 2.25 µL enzyme buffer R and 0.4 µL restriction enzyme (4 units). The digestion reactions were incubated at 37°C for 2.5 hours. After digestion, the digested products were analyzed on 3% agarose gel with ethidium bromide at 100V for 50 min. The gels were visualized under UV light.
Data on the qualitative and quantitative characteristics of milk were obtained from the HPA (Croatian Agricultural Agency).
Statistical data processing was performed using software package SAS (Statistical Alalysis Softwere SAS/STAT).

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RESULTS AND DISCUSSION

The genotyping of the LGB was successfully performed by PCR-RLFP technique for all samples. After the action of enzymes obtained fragments of the size of 153, 109, 79 and 74 bp.
Genotype and allele frequencies are shown in Table 1.

Tab. 1: Genotype and allelic frequencies of LGB gene

N	Genotype frequencies (%)			Allelic frequencies (%)	
	AA	AB	BB	A	B
112	13.22	59.50	27.27	42.97	57.02

The results show that the allele A is represented in higher frequency than allele B in Holstein breed in Croatia. Frequency of these alleles in accordance with numerous studies carried out with Holstein breed (Aleandri at al. 1990; Ng-Kwai-Hang at al. 1990; Sabour at al. 1993)
The influence of the effects of LGB genotypes on milk production parameters (milk yield, protein and fat contents) in the third lactation is presented in Table 2.

Table 2: Effect of genotypes of LGB gene on milk production traits

Genotype	Milk yield (kg)	Fat (%)	Protein (%)
AA	10577.06 ± 456.86	4.25 ± 0.17	3.29 ± 0.06
AB	10022.52 ± 217.92	4.25 ± 0.08	3.32 ± 0.02
BB	9652.14 ± 605.54	4.44 ± 0.11	3.32 ± 0.04

The highest values for milk yield were associated with genotype AA, the average milk yield higher for 554.54 kg than genotype AB, and 924.92 kg than cows with genotype BB. Positive effect of genotype AA on milk yield was recorded by Sitkowska at al. (2009).
This study found a positive effect of BB genotype on fat content (+ 0.19 %), while the effect of LGB genotype on the proteins contents was not recorded. The positive effect of BB genotype on fat content was recorded by Ng-Kwai-Hang at al. (1984), Karimi at al. (2009), Sitkowska at al. (2009).
A positive effect of LGB alleles B on protein contents was reported by Miceikiene et al. (2005), while Ng-kwai-Hang at al. (2002) claimed that milk B variant of LGB was associated with better coagulation properties, shorter coagulation time, higher curd firmness, higher yield of cheese and better cheese quality compared to A variant.

CONCLUSIONS

The results of this study indicate that certain genotypes of the LGB have an impact on the certain characteristics of cow's milk.
The genotype AA of LGB gene was associated with higher milk yield then AB and BB genotypes.
The positive association between BB genotype and fat content were confirmed.
The results of the study could be used as source of information for producers and breeders, as well as for selection of desirable genotypes suitable for a certain type the dairy industry.

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