EFFECT OF THE POLYMORPHIC COMPOSITE FORMS OF BETA-LACTOGLOBULIN ON THE MILK YIELD AND CHEMICAL COMPOSITION IN MAXIMUM LACTATION

WPŁYW GENOTYPU BETA-LAKTOGLOBULINY NA WYDAJNOŚĆ I SKŁAD CHEMICZNY MLEKA W LAKTACJI MAKSYMALNEJ

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ABSTRACT

On the basis of polymorphism in beta-lactoglobulin gene, a genetic structure of 155 cows bred in the Kujavian-Pomeranian voivodeship was detemined. Genotypes marking was conducted using the PCR-RFLP method. The results of the analysis, as regards polymorphism in beta-lactoglobulin gene, indicate that in the population under analysis BLG AB heterozygotes (50.32%) are twice as frequent as BLG AA (22.58%) and BLG BB (27.10%) homozygotes. Within beta-lactoglobulin small majority of frequency of gene B over A was shown, which is highly desired in dairy industry. The highest milk yield and its basic components was noted in maximum lactation for cows with BLG AA genotype, whereas milk of BLG BB cows exceeded milk obtained from cows with BLG AA and BLG BB genotypes as regards fat and protein content.

Key words: cows, milk proteins, beta-lactoglobulin gene

STRESZCZENIE

Na podstawie polimorfizmu w genie beta-laktoglobuliny określono strukturę genetyczną populacji 155 krów utrzymywanych na terenie województwa kujawsko-pomorskiego. Oznaczenie genotypów przeprowadzono przy użyciu metody PCR-RFLP (Medrano, Aguilar-Cordova). Wyniki przeprowadzonych badań pod względem polimorfizmu beta-laktoglobuliny wskazują na ponad dwukrotnie większy udział w badanej populacji heterozygot BLG AB (50,32%) niż homozygot BLG AA (22,58%) i BLG BB (27,10%). W obrębie beta-laktoglobuliny wykazano niewielką przewagę częstości genu B nad A, co jest pożądane przez przemysł mleczarski. Najwyższą wydajność mleka i jego podstawowych składników stwierdzono w laktacji maksymalnej u krów o genotypie BLG AA. Natomiast mleko krów BLG BB pod względem zawartości tłuszczu i białka przewyższało mleko pozyskiwane od krów o genotypach BLG AA i BLG AB.

Słowa kluczowe: krowy, białka mleka, gen beta-laktoglobuliny

STRESZCZENIE SZCZEGÓŁOWE

Analizy przeprowadzono na podstawie wydajności maksymalnej 155 krów rasy holsztyńsko-fryzyjskiej odmiany czarno-białej, użytkowanych w dwóch stadach położonych w województwie kujawsko-pomorskim. Metodą PCR-RFLP wg Medrano and Aguilar-Cordova określono genotypy krów pod względem genu beta-laktoglobuliny. Obliczono frekwencję genów i genotypów BLG w badanej populacji. Dane o użytkowości mlecznej krów pochodziły z bazy danych systemu SYMLEK. Obliczenia statystyczne wykonano przy zastosowaniu jednoczynnikowej analizy wariancji (SAS). Przeanalizowano wpływ genotypu beta-laktoglobuliny na wydajność i skład chemiczny mleka w laktacji maksymalnej. Wyodrębniono trzy grupy krów pod względem polimorfizmu beta-laktoglobuliny: AA, AB i BB. Udział heterozygot AB w badanej populacji był ponad dwukrotnie większy niż homozygot AA i BB. W układzie BLG stwierdzono następującą frekwencję genów: genu B (0,52), genu A (0,48). Najwyższą wydajnością mleka i jego podstawowych składników charakteryzowały się krowy o genotypie BLG AA. W badanej populacji krowy homozygotyczne BB produkowały w laktacji maksymalnej w porównaniu z homozygotami AA o 453 kg mniej mleka oraz o 9 kg mniej tłuszczu i białka. Wydajność mleka, tłuszczu i białka u krów heterozygotycznych wyniosła odpowiednio: 7618 kg, 347 kg i 251 kg. Między uzyskanymi wynikami nie stwierdzono różnic istotnych statystycznie, jednak na ich podstawie można wnioskować o korzystnym efekcie oddziaływania allelu BLG A na wydajność mleka i jego podstawowych składników w badanej populacji krów. Zaobserwowano również, że w zależności od wariantu genetycznego beta-laktoglobuliny, krowy o genotypie BLG BB charakteryzowały się wyższą o 0,12–0,16 % zawartością tłuszczu i o 0,04–0,08 % zawartością białka w porównaniu z homozygotami AA i heterozygotami AB.

INTRODUCTION

Protein yield and content are most highly rewarded in cattle breeding programs carried out at present. It results from, among others, inclination towards advanced milk processing. Therefore, its chemical composition is of primary importance. Furthermore, price of milk, profitability and economy of its production depend on it. One of the methods of improving the content and milk technological properties is the use of information on polymorphism of single genes engaged in shaping the importance of these properties [4]. Recent years have shown more frequent attempts to demonstrate the relation between milk protein polymorphism and yield, chemical composition and technological parameters of cow's milk [3, 5, 6, 7, 12, 13, 14].

Beta-lactoglobulin is a basic component of whey protein, the value of which is estimated to be 9 % of milk nitrogen compounds [1, 10, 13]. Polymorphism of beta-lactoglobulin is determined by a series of codominant alleles. Most often, three variants are identified: AA, AB i BB [7, 8, 13]. The information on bulls beta-lactoglobulin and kappa-casein genotypes is considered a potential marker of milk performance and placed in annual catalogues of bulls published by, among others, the Animal Breeding and Insemination Station in Bydgoszcz [4, 13].

The research aimed at estimating genes and beta-lactoglobulin genotypes and their effect on milk yield and chemical composition of milk in maximum lactation for cows bred in the Kujavian-Pomeranian voivodeship.

MATERIALS AND METHODS

The research included 155 Holstein-Friesian Black-and-White cows bred in two herds in the Kujavian-Pomeranian voivodeship. Blood from jugular vein was collected in ethylenediaminetetraacetic acid (EDTA)-treated plastic tube. High molecular weight DNA was extracted from the whole blood using MasterPure^{TM} DNA Purification Kit from Blood (Epicentre Technologies) following manufacturer's instructions. To identify BLG genotypes based on polimorhisms at exon IV nucleotide position (T→C), RFLP analysis using restriction enzyme HaeIII (Fermentas) [9]. PCR amplification of a 262 bp fragment was performed in 25µl reaction volume containing approximately 200ng of genomic DNA, 2.5pmol of each primer, 200µM of each dNTP, 1.5 mM $MgCl_2$ and 0.625 U Taq polymerase (Fermentas) in 1-fold reaction buffer. Temperature profile of the reaction was: denaturation at 94^{o}C for 3 min followed by 34 amplification cycles of 30s at 94^{o}C, 30s at 60^{o}C, 30s at 72^{o}C and final extension of 5 min at 72^{o}C. For digestion of the 262 bp fragment with restriction enzyme HaeIII samples were incubated for 4 h at 37^{o}C. Restriction fragments obtained were then split in 3.5-percent agarized gels with ethidine bromium (0.5 µg·ml^{-1}), with the presence of DNA pUC19/MspI pattern.

On the basis of obtained results, the characteristics of genetic structure of examined cows was conducted. For these purposes, the frequency of genes and BLG genotypes was calculated. The data on cow's milk performance – maximum performances – were taken from the SYMLEK system database. Statistical calculations were made using one-factor analysis of variance (SAS)

[11]. The significance of differences was verified using Scheffe statistical test.

RESULTS AND DISCUSSION

In own research three groups of cows regarding polymorphism of beta-lactoglobuline were distinguished: AA, AB and BB. The data in Table 1 reveals that the participation of AB heterozygotes in examined population was two times higher compared with AA and BB homozygotes, while the greater number of BLG BB homozygotes was noted in comparison with AA homozygotes. The results obtained correspond to those reported by other authors [2, 4 8, 13]. Litwińczuk et al. [8] demonstrated similar percentage participation of BLG genotypes in four different types of farms. Authors estimated the participation of BLG AA, AB and BB for Central-Eastern Poland: 13.3, 53.4 and 33.3 respectively, while for Vilnius region in Lithuania: 20.6, 58.6 and 21.1. In the studies of Czeniawska-Piątkowska and Kamieniecki [2], BLG AB phenotypes were most frequent in a group of cows with the higher (75.1–100 %) participation of hf genes, while in a group of cows with 50.1–75.0 % genotype authors did not state the occurence of BLG AA variant.

In the poulation under analysis in BLG configuration, the higher participation of gene B (0.52) rather than A (0.48) was noted (Table 1). Similar tendency of genes determining polymorphism BLG was observed also by Czerniawska-Piątkowska [2], Litwińczuk et al. [8], Kamiński [4]. Litwińczuk et al. [7] point to, quoting other authors, diversity of frequency of genes A and B occurence depending on cattle breed. Dobicki et al. [3] estimated for the population of Red-and-White cows the frequency of A and B allele to be 0.295 and 0.705 respectively. For Black-and-White bulls admitted for reproduction in Poland between 1994 and 2000, the frequency of gene A was 0.41, while for gene B it was B 0.59 [4].

Table 2 shows the results of productivity of cows in maximum lactation depending on genetic variant of beta-lactoglobulin. It was noted that the highest milk yield and its basic component was characteristic of cows with BLG AA genotype. In the examined population homozygotic cows BB produced 453 kg less milk and 9 kg less fat and protein than BB homozygotes in maximum lactation. Milk, fat and protein yield for heterozygotic cows were 7618 kg, 347 kg and 251 kg respectively. Own studies correspond with the results obtained by Kamiński and Zabolewicz [5] and Dobicki et al. [3]. Litwińczuk et al. [7] quote after Henderson and Marshall, as well as Król, that in case of beta-lactoglobulin AB genotype is connected with higher yield. This was not confirmed statistically in own studies. Nonetheless, it can be concluded that the effect of BLG A allele on milk yield and its basic components is positive in the population of cows under analysis.

Depending on genetic variant of beta-lactoglobulin, cows with BLG BB genotype were characterized by fat content higher by 0.12–0.16 % and protein content higher by 0.04–0.08 % when compared with AA homozygotes and AB heterozygotes. Own results correspond with the results obtained by Dobicki et al. [3], and with those of other authors quoted by Litwińczuk et al. [7]. Kamiński and Zabolewicz [5] noted higher fat and protein content (4.32 and 3.51 %) in milk produced by cows with BLG AA genotype, whereas the values of these traits in the case of AB and BB genotypes were identical: 4.27 and 3.33 % respectively. According to Litwińczuk et al. [7], milk protein polymorphism was recognized to such an extent that in breeding industry genetic variants of milk protein can successfully be introduced as additional selection parameters. The autors point out that it concerns mainly genetic variants of beta-lactoglobulin and kappa-casein and their effect on protein content and milk technological properties. Selection of dairy cattle using genetic markers would largely respond to increasingly higher requirements of dairy industry that prefers milk with higher technological parameters for cheese production. In practice, according to Kamiński [4], in order to ensure the use of desired effects of some milk protein genotypes, selective semen distribution should be introduced, consisting in using semen of bulls with favourable genotypes, e.g. beta-lactoglobulin in the regions with large dairy processing factories. The semen of remaining bulls would be used in regions where milk is purchased mainly for direct consumption.

CONCLUSIONS

The results of research conducted as regards polymorphism in beta-lactoglobulin gene indicate that in examined population BLG AB heterozygotes (50.32%) are twice as frequent as BLG AA (22.58%) and BLG BB (27.10%) homozygotes. Within beta-lactoglobulin the advantage of gene B over gene A frequency was proved, which is highly desired in dairy industry. The highest milk yield and its basic components was displayed in maximum lactation for cows with BLG AA genotypes. Milk of BLG BB cows exceeded milk obtained from BLG AA and BLG AB cows as regards fat and protein content.

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Table 1. The frequency of beta-globulin genes and genotypes in the examined population
Frekwencja genów i genotypów beta-laktoglobuliny w badanej populacji.

Genotype Genotyp	Number of genotypes Liczba genotypów	Frequency of genotypes Frekwencja genotypów	Frequence of allele Frekwencja allelu	
			A	B
AA	35	22.58		
AB	78	50.32	0.48	0.52
BB	42	27.10		

Table 2. Analysis of milk characteristic of examined population estimated for maximum lactation depending on genetic variant of beta-lactoglobulin
Analiza cech mleka badanej populacji oszacowanych dla laktacji maksymalnej w zależności od wariantu genetycznego beta-laktoglobuliny.

Genotype Genotyp	Milk yield (kg) Wydajność mleka (kg)	Fat (kg) tłuszcz (kg)	Fat (%) Tłuszcz (%)	Protein (kg) Białko (kg)	Protein (%) Białko (%)
AA	7932.40	349.83	4.45	258.97	3.27
AB	7618.50	346.79	4.50	251.15	3.31
BB	7479.80	341.03	4.61	249.74	3.35
Mean Średnia	7651.78	344.57	4.51	252.53	3.32

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