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ASSOCIATION BETWEEN BETA-LACTOGLOBULIN (LGB) POLYMORPHISM AND YIELD AND COMPOSITION OF MILK OF HOLSTEIN-FRIESIAN COWS IMPORTED FROM SWEDEN

Ewa Czerniawska-Piątkowska, Małgorzata Szewczuk, Aleksandra Olszewska, Ewa Chociłowicz

West Pomeranian University of Technology in Szczecin, Poland

Abstract. The study involved 102 Holstein-Friesian cows imported from Sweden, kept on a farm in the West Pomerania Province. Beta-lactoglobulin (LGB) genotypes were determined using PCR (Polymerase Chain Reaction) method according to Medrano and Aquilar-Cordova [1990]. It was found that cows with the LGB AA genotype had the highest milk yield in all analysed 305-day lactations. The differences were significant in the third lactation ($P \leq 0.01$, $P \leq 0.05$). For the same genotype, the highest milk protein yield and content for the three analysed lactations were recorded. The highest milk fat content and yield were found in the BB homozygotes for all the analysed lactations.

Keywords: beta-lactoglobulin, HF, milk proteins, milk yield, polymorphism

INTRODUCTION

Beta-lactoglobulin (LGB) is a whey protein found in the milk of ruminants. Due to the effect it has on the formation of physicochemical properties of milk, it is associated with its utility by many researchers. Confirmation of these relationships enabled utilization of LGB as a marker in the selection of animals characterized by the best production traits [Oprządek et al. 2006, Czerniawska-Piątkowska et al. 2007].

Polymorphism of milk proteins and their genetic variants are determined by the multiple co-dominant alleles, that is, the presence of two or more alleles in one locus [Litwińczuk et al. 2003]. In this way, the genetic variants are formed, which differ in the sequences of amino acids, that is, the primary structure. These changes have significant effect on the chemical composition and physicochemical properties of milk as a material.

Corresponding author – Adres do korespondencji: dr hab. inż. Ewa Czerniawska-Piątkowska, Department of Ruminant Science, West Pomeranian University of Technology in Szczecin, Doktora Judyma 10, 71-460 Szczecin, Poland, e-mail: Ewa.Czerniawska-Piatkowska@zut.edu.pl

Krzyżewski et al. [1997] presented relationships between genetic variants of milk proteins and milk traits as well as milk quality. According to them, variant B of beta-lactoglobulin is characterized by the lower content of whey proteins and higher content of casein proteins compared to variant A, without significant effect on the total protein content or the formation of a more compact casein curd in the process of cheese production and is characterized by a higher content in cheeses, in the range from 1 to 4%. However, Walawski [1993] found a significant effect of beta-lactoglobulin on the yield, acidity, coagulability and thermal stability of milk.

In the detection of polymorphism, a significant part is played by genetic markers which do not influence the level of quantitative trait by themselves but are associated with it through the localization, in the same chromosome, of the genes determining the marker and the selected quantitative trait [Miciński et al. 2008]. Due to them it is possible to find relationships between polymorphic forms of beta-lactoglobulin and milk yield, its chemical composition as well as determine their effect on the technological value of milk. Many stations performing insemination of dairy cattle in Europe, among others, in Poland as well as in North America already place a given genotype (*CSN2, CASK, BLG*) of each bull as a molecular genetic marker in the commercial catalogues.

Research on the polymorphism enables the selection of the best individuals, characterized by the high parameters in respect of the content of individual constituents in milk, as well as recording of genetic changes. According to Litwińczuk et al. [2006], beta-lactoglobulin is the most diverse polymorphic form and the following three its variants are identified most frequently: AA, AB and BB. This fraction, besides kappa-casein, arouses the greatest interest among researchers, although the results of experiments are not always concurrent and sometimes they are even contradictory. This justifies the necessity of continuing the research on the utilization of genetic markers in the improvement of milk performance of cows in herds with high milk production.

The aims of this study were to establish the frequencies of genes and genotypes determining beta-lactoglobulin variants in Holstein-Friesian cows imported from Sweden as well as to establish the relationships between production traits (milk, protein and fat yield, fat and protein content) and the polymorphism in beta-lactoglobulin loci (LGB).

MATERIAL AND METHODS

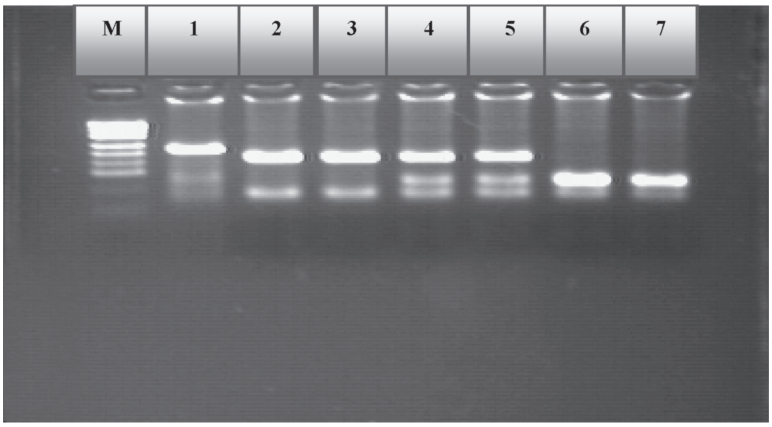
The research was conducted in the West Pomerania Province on 102 Holstein-Friesian cows imported from Sweden. The blood for the tests was collected from the external jugular vein into the vacuum test tubes with K3EDTA anticoagulant using *MasterPure*™ *Genomic DNA Purification Kit* provided by *Epicentra Technologies*. Beta-lactoglobulin (LGB) genotypes were determined using PCR (Polymerase Chain Reaction) according to the method by Medrano and Aquilar-Cordova [1990]. The obtained DNA fragments were digested using the *Bsu*RI (*Hae*III) restriction enzyme (gg ↓ cc) provided by Fermentas. Restriction fragments were separated electrophoretically in a 2% agarose gel with ethidium bromide in the presence of 1*TBE buffer and visualized in the transilluminator provided by Vilber Lourmat.

The analysis of the milk yield was performed based on the data from the official milk recording using A4 method, taking into consideration the yield of milk, fat and protein (kg) as well as their percentage content in milk (%).

The data were analyzed statistically calculating the mean values ($\overline{x}$), standard deviation (S) and coefficient of variation (V%). The significance of differences between the group means was determined using Duncan's test by means of Statistica® 9 PL software.

RESULTS AND DISCUSSION

In Fig.1 the electrophoretic separation of the LGB/*Hae*III restriction fragments of the bovine beta-lactoglobulin gene is presented. Specific PCR products of 191 bp were obtained. In the examined herd of dairy cows, two alleles of the bovine beta-lactoglobulin gene (LGB A and LGB B) and three genotypes (AA, AB and BB) were identified (Fig. 1).



M – DNA size marker (pUC19/*Msp*I); lane 1 – PCR product; lanes 2, 3 – genotype AA; lanes 3, 4 – genotype AB; lanes 6, 7 – genotype BB.
M – marker wielkości DNA (pUC19/*Msp*I); tor 1 − produkt PCR; tory 2, 3 − genotyp AA; tory 4, 5 − genotyp AB; tory 6, 7 − genotyp BB.

Fig. 1. Electrophoretic restriction fragments LGB/*Hae*III of bovine beta-lactoglobulin gene
Rys. 1. Elektroforetyczny rozdział fragmentów restrykcyjnych LGB/*Hae*III genu beta--laktoglobuliny bydła

The conducted research showed (Table 1) that 52% of the population had the BB genotype, 40% had the AB genotype and 8% had the AA genotype. The observed numbers and frequencies of alleles in the examined loci were in accordance with Hardy-Weinberg distribution. Results of the previous studies by Barłowska [2001], Ziemiński et al. [2005] and Miciński et al. [2008] show the lower proportion of the LGB AA homozygotes in the herds of Holstein-Friesian cattle. The cited authors emphasize the advantage of heterozygous (AB) animals over homozygous (AA, BB) ones.

Table 1. Beta-lactoglobulin gene and genotype frequencies in HF cows imported from Sweden
Tabela 1. Rozkład genów i genotypów polimorficznych beta-laktoglobuliny (LGB) u krów rasy hf importowanych ze Szwecji

Genotypes Genotypy	Number of genotypes Liczba genotypów	Frequency Frekwencja		Chi²	P
		genotype genotypu	gene genu		
AA	8	AA = 0.08	A = 0.28		
AB	40	AB = 0.40	B = 0.72	0.006	0.973
BB	52	BB = 0.52			

In the present study, the frequency of allele B (0.72) was considerably higher compared to allele A (0.28), which has been confirmed by other authors: Barłowska [2001], Ziemiński et al. [2005], Litwińczuk et al. [2006] and Miciński et al. [2008]. Different results were obtained by Kulig [2005].

The highest milk yield (Table 2) in the first lactation was characteristic of cows with the AA genotype (6458 kg), then those with the BB genotype (6434.8 kg) and the AB genotype (6416.5 kg). Similarly, in the second and third lactations, the highest milk yield was found in cows with the AA genotype (6688.6 kg and 6781.4 kg, respectively) and the lowest one (5072.6 kg) in the AB heterozygotes in the third lactation.

Table 2. Milk yield and composition depending on LGB genotype in 305-d lactations
Tabela 2. Wydajność mleka i jego skład w zależności od genotypu LGB w laktacjach 305-dniowych

Lactations Laktacje		I			III			III		
Genotypes LGB Genotypy LGB		AA	AB	BB	AA	AB	BB	AA	AB	BB
Detailed list – Wyszczególnienie										
Milk, kg Mleko, kg	$\bar{x}$	6458.0	6416.5	6434.8	6688.6	6662.2	6639.2	6781.4^{Aa}	5072.6^{Ab}	6239.9^{ab}
	s	793.7	524.5	615.6	463.6	1373.4	1015.0	546.0	719.2	725.8
Fat, kg Tłuszcz, kg	$\bar{x}$	242.9	260.4	262.7	277.1	288.4	293.2	278.3^{AB}	216.1^{A}	259.4^{B}
	s	15.2	37.3	40.1	35.7	65.0	42.4	26.0	31.8	33.0
Protein, kg Białko, kg	$\bar{x}$	225.8	220.6	221.2	249.8	228.6	232.8	234.6^{AB}	171.3^{AC}	204.7^{BC}
	s	24.8	18.4	22.2	16.1	49.9	32.3	20.0	25.1	26.5
Fat, % Tłuszcz, %	$\bar{x}$	3.79^{ab}	4.04^{a}	4.07^{b}	4.13^{c}	4.34	4.44^{c}	4.10	4.27	4.16
	s	0.35	0.35	0.35	0.36	0.41	0.30	0.19	0.29	0.24
Protein, % Białko, %	$\bar{x}$	3.50	3.44	3.44	3.74^{Aa}	3.44^{A}	3.52^{a}	3.46^{b}	3.38	3.29^{b}
	s	0.13	0.14	0.15	0.12	0.32	0.22	0.20	0.24	0.24

A, B – statistically significant difference at P≤0.01– A, B – różnica istotna na poziomie P≤0,01.
a, b – statistically significant difference at P≤0.05 – a, b – różnica istotna na poziomie P≤0,05.

Statistically significant differences (P≤0.01 and P≤0.05) between the analysed groups of cows in the third lactation were recorded. Similarly, in the study by Oprządek et al. [2006] and Czerniawska-Piątkowska et al. [2007] conducted on Holstein-Friesian cows, animals with the AA genotype were characterized by the highest lactation yield in the first lactation. In subsequent lactations, the results were divergent. Czerniawska-Piątkowska et al. [2007] found the highest lactation yield in the second lactation in cows with the AA genotype, whereas Oprządek et al. [2006] found it in the BB homozygotes. Oprządek et al. [2006] recorded the highest lactation yield in the AB heterozygotes in the third lactation. The differences could have been caused by the application of different statistical methods.

In the present study, the highest milk fat yield was found in cows with the BB genotype in the first and second lactation (262.7 kg and 293.2 kg, respectively). However, in the third lactation, cows with the AA genotype were characterized by the highest milk fat yield (278.3 kg). Significant differences were found between the analysed genotypes of cows (P≤0.01). In the case of milk protein, the highest yield for the three analysed lactations was found in cows with the AA genotypes (225.8 kg, 249.8 kg and 234.6 kg, respectively). Statistically significant differences were observed in the third lactation (P≤0.01).

In the study by Oprządek et al. [2006], the highest milk fat and protein yield was recorded in the milk of cows with the AB genotype (343.8 kg and 266.5 kg, respectively) in the third lactation. In another study by Czerniawska-Piątkowska et al. [2007], it was found that the AA homozygotes were characterized by the highest milk fat and protein yield in the first lactation (331.1 kg and 250 kg, respectively).

Analysing the milk fat content (Table 2), the highest values were recorded in cows with the BB genotype in the first and second lactation (4.04% and 4.44%, respectively). The differences were significant (P≤0.05). In the third lactation, the highest content of fat was found in the AB heterozygotes (4.27%). On the other hand, the percentage content of protein was the highest in cows with the AA genotype in all the analysed lactations. Statistically significant differences (P≤0.01 and P≤0.05) between the analysed genotypes of cows were found in the second and third lactation.

From the study by Czerniawska-Piątkowska et al. [2007], it appears that the highest milk fat and protein content was characteristic of the first lactation. The highest milk fat content was recorded by these authors for the milk of cows with the AA genotype (4.23%), whereas the highest milk protein content was found in the BB homozygotes (3.24%). Oprządek et al. [2006] showed that the highest content of milk fat and protein is characteristic of milk from cows with the BB genotype (4.26% in the third lactation and 3.36% in the second lactation, respectively).

The present research showed (Table 3) that cows with the AA genotype were characterized by the highest milk yield (6642.7 kg), whereas the AB heterozygotes had the lowest milk yield (6050.4 kg). This is in accordance with the studies by other authors, among others, Juszczak et al. [2000], Ziemiński et al. [2005], Litwińczuk et al. [2006] and Miciński et al. [2008]. Different results were obtained by, among others, Henderson and Marshall [1996], Czerniawska-Piątkowska et al. [2002] and Król [2003].

Table 3. Milk yield and composition depending on LGB genotype for the first three lactations in HF cows
Tabela 3. Wydajność i skład mleka dla trzech laktacji w zależności od genotypu beta-laktoglobuliny u krów rasy hf

Detailed list Wyszczególnienie		Genotypes – Genotypy		
		AA	AB	BB
Milk, kg Mleko, kg	$\overline{x}$	6642.7	6050.4	6438.0
	s	605.9	1170.5	814.7
Fat, kg Tłuszcz, kg	$\overline{x}$	266.1	254.9	271.8
	s	30.7	55.4	41.4
Protein, kg Białko, kg	$\overline{x}$	236.7	206.8	219.56
	s	22.15	42.2	29.51
Fat, % Tłuszcz, %	$\overline{x}$	4.01^{ab}	4.21^{a}	4.22^{b}
	s	0.34	0.37	0.34
Protein, % Białko, %	$\overline{x}$	3.57	3.42	3.42
	s	0.19	0.24	0.23

a, b – statistically significant difference at P≤0.05 – a, b – różnica istotna na poziomie P≤0,05.

In the case of milk fat yield, in the present study it was found the LGB BB homozygotes (271.8 kg) were characterized by the highest yield, whereas the lowest one was found in the AB heterozygotes (254.9 kg). This has been confirmed by the results obtained by Litwińczuk et al. [2006].

The milk of cows with the AA genotype was characterized by the highest milk protein content (236.7 kg), whereas that of cows with the AB genotype by the lowest one (206.8 kg). This has been confirmed by the results obtained by other authors, among others, Ziemiński et al. [2005], Litwińczuk et al. [2006] and Sitkowska et al. [2009].

From the present study, it appears that the highest milk protein content was recorded in the milk of cows with the LGB AA genotype (3.57%), which has been reflected in the studies by Michalak [1997] and Ziemiński et al. [2005].

In the present study, it was also found that the homozygous BB cows had slightly higher milk fat content (4.22%) in comparison with the heterozygous LGB AB (4.21%) and homozygous LGB AA (4.01%) animals. The differences were significant (P≤0.05). Similar results were obtained by, among others, Strzałkowska et al. [1999] and different ones by Miciński et al. [2008].

In the study concerning beta-lactoglobulin polymorphism, the highest milk yield was found in cows with the LGB AA genotype, whereas the highest milk fat content was recorded in the LGB BB homozygotes. These results have been confirmed by other authors, among others, Strzałkowska [2000], Litwińczuk et al. [2006], Oprządek et al. [2006] or Sitkowska et al. [2009].

CONCLUSIONS

Milk protein is its most important constituent, determining the usefulness of milk in processing. It is also the main factor affecting its physicochemical properties. Moreover, the high nutritive value of the milk protein substances is the reason for the continuous search for the effective methods of increasing their content in cow milk. It was found that cows with the LGB AA genotype were characterized by the highest milk yield in all lactations. In the third lactation, these differences were significant ($P \leq 0.01$ and $P \leq 0.05$). For the same genotype, the highest milk protein yield and content in the analysed three lactations were found. In the second and third lactation, the differences were significant ($P \leq 0.01$ and $P \leq 0.05$). On the other hand, in cows with the LGB BB genotype, the highest milk fat yield and content for the three lactations were found. For the milk fat content, the differences were significant ($P \leq 0.05$).

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ZWIĄZEK POLIMORFIZMU BETA-LAKTOGLOBULINY (LGB) Z WYDAJNOŚCIĄ I SKŁADEM MLEKA KRÓW RASY HOLSZTYŃSKO-FRYZYJSKIEJ IMPORTOWANYCH ZE SZWECJI

Streszczenie. Badania przeprowadzono na 102 krowach rasy holsztyńsko-fryzyjskiej importowanych ze Szwecji, w gospodarstwie rolnym w województwie zachodniopomorskim. Genotypy beta-laktoglobuliny (LGB) oznaczono metodą PCR (ang. Polymerase Chain Reaction) wg metodyki Medrano i Aquilar-Cordova [1990]. Stwierdzono, że krowy o genotypie LGB AA uzyskały najwyższą wydajność mleka we wszystkich analizowanych 305-dniowych laktacjach. W trzeciej laktacji różnice były istotne (P≤0,01; P≤0,05). Dla tego samego genotypu zanotowano najwyższą wydajność i zawartość białka w mleku dla trzech analizowanych laktacji. Najwyższą zawartość i wydajność tłuszczu stwierdzono u homozygot BB dla wszystkich badanych laktacji.

Słowa kluczowe: beta-laktoglobulina, białka mleka, hf, polimorfizm, wydajność mleka

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