Milk protein polymorphisms and effect of herds on cows' milk composition

Polimorfizm genów białek mleka oraz efekt stada a skład mleka krów

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ABSTRACT

Kappa-casein (CSN3) and beta-lactoglobulin (LGB) loci affect milk yield traits and the quality of milk protein. Their polymorphisms explain the role of genetic variance and facilitate estimation of breeding value. Our research was aimed at estimating the effect of beta-lactoglobulin and kappa-casein genotypes on milk yield and the chemical composition of milk, for cows bred in three herds in the kujawsko-pomorskie province. We divided the cows into three groups depending on their betalactoglobulin and kappa-casein polymorphisms: AA, AB and BB. The percentage of AB LGB heterozygotes in the examined population was the highest, at 44%; whereas the frequency of both gene alleles was at a similar level. In the kappa-casein gene, we found that there was a predominance of AA CSN3 genes (0.69), and a small percentage of BB CSN3 genotypes (0.07). We also observed a higher frequency of the A allele (0.81) as compared to the B allele (0.19), which is desired in breeding. Analysing the influence of the examined factors, we established a statistically significant influence of the barn as well as interactions between the barn and the LGB and CSN3 genotype on the percentage of fat and protein in milk from all examined lactations. Milk yield is influenced by the interaction between examined genotypes and the LGB genotype. The highest fat and protein content in the first lactation was found in animals with the AB LGB genotype: 4.53% of fat and 3.41% of protein. In lactations 2 and 3, the highest values for these parameters were noted for cows with the AA LGB genotype. As to the kappa-casein gene, we observed that cows with the AA CSN3 genotype constituted the largest group. Heterozygous animals produced the highest amount of milk with higher fat and protein yield as compared to the other groups.

KEYWORDS: beta-lactoglobulin, housing system, kappa-casein, milk composition, milk yield

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STRESZCZENIE

Loci kappa-kazeiny (CSN3) i beta-laktoglobuliny (LGB) wpływaja na wydajność mleczna oraz jakość białka w mleku. Ich polimorfizm wyjaśnia role warjancji genetycznej oraz ułatwia ocene wartości hodowlanej zwierzat. Celem badań było oszacowanie wpływu genotypów beta-laktoglobuliny i kappa-kazeiny na wydajność mleczna i skład chemiczny mleka krów hodowanych w trzech stadach na terenie województwa kujawsko-pomorskiego. Wyodrebniono trzy grupy krów pod względem polimorfizmu beta-laktoglobuliny oraz kappa-kazeiny: AA, AB i BB. Udział heterozygot AB LGB w badanej populacji był największy, sięgał 44%, natomiast frekwencja obu alleli genu była na podobnym poziomie. W przypadku genu kappakazeiny, stwierdzono przewagę genotypów AA CSN3 (0,69) i niewielki udział genotypów BB CSN3 (0.07). Zaobserwowano wieksza frekwencją allela A (0.81) nad pożądanym w hodowli allelem B (0,19). Analizując wpływ badanych czynników stwierdzono wysoko istotny statystycznie wpływ obory oraz interakcji miedzy obora i genotypem LGB oraz CSN3 na procentowa zawartość tłuszczu i białka w mleku we wszystkich badanych laktacjach. Natomiast na wydajność mleka wpływ miała interakcja między badanymi genotypami oraz genotyp LGB. Najwyższą zawartość tłuszczu i białka w pierwszej laktacji stwierdzono w grupie osobników o genotypie AB LGB odpowiednio: 4,53% tłuszczu i 3,41% białka. W laktacji 2 i 3 najwyższe wartości tych parametrów osiągnęły krowy o genotypie AA LGB W przypadku genu kappakazejny stwierdzono, że krowy o genotypie AA CSN3 były najliczniejszą grupą, osobniki heterozygotyczne produkowały najwięcej mleka z wyższą wydajnością białka i tłuszczu w stosunku do pozostałych porównywanych grup.

SŁOWA KLUCZOWE: beta-laktoglobulina, system utrzymania, kappa-kazeina, skład mleka, wydajność mleczna

DETAILED ABSTRACT

We conducted numerous analyses of the effects that milk protein genes have on traits related to milk yield. However, it is difficult to determine the best genotype for breeding, despite the fact that we know which gene combinations are particularly preferred in connection with technological processing of milk. The aim of the study was to estimate the frequency of beta-lactoglobulin and kappa-casein genes as well as genotypes, and their impact on milk yield and chemical content in cows maintained in three herds in the kujawsko-pomorskie province. Our research was conducted on 398 Polish Holstein-Friesian black and white cows, maintained in three selected herds in the kuiawsko-pomorskie province. Statistical calculations were made with the use of multiple analysis of variance. Significance of differences was verified with the Scheffe's test. In our own examinations, we divided the cows into three groups based on their beta-lactoglobulin and kappa-casein polymorphism: AA, AB and BB. The percentage of AB LGB heterozygotes in the examined population was the highest, at 44%; whereas the frequency of both gene alleles was at a similar level. In the kappa-casein gene, we found that there was a predominance of AA CSN3 genes (0.69), and a small percentage of BB CSN3 genotypes (0.07). We also observed a higher frequency of the A allele (0.81) as compared to the B allele (0.19), which is desired in breeding. Analysing the influence of the examined factors, we established a statistically significant influence of the barn as well as interactions

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between the barn and the LGB and CSN3 genotype on the percentage of fat and protein in milk from all examined lactations. The highest fat and protein content in the first lactation was found in animals with the AB LGB genotype: 4.53% of fat and 3.41% of protein. In lactations 2 and 3, the highest values for these parameters were noted for cows with the AA LGB genotype. As to the kappa-casein gene, we observed that cows with the AA CSN3 genotype constituted the largest group. Heterozygous animals produced the highest amount of milk with higher fat and protein yield as compared to the other groups.

INTRODUCTION

About 95% of proteins contained in ruminant milk is coded by 6 highly polymorphic genes which are characterized by several nonsynonymous and synonymous mutations, with up to 47 protein variants identified (Caroli et al., 2009; Martin et al., 2002). The frequency of preferred genetic variants differs according to breeds; but, generally, the most expected forms are rarer in research populations.

Almost 40 years ago it was discovered that CSN3 in milk is related to milk yield, milk usability for processing, and, in particular, to cheese production. The CSN3 B allele is related to the technological parameters, i.e. coagulation time is shorter by 10-30%, coagulum firmness greater by 20-100%, and both fresh and mature cheese efficiency are higher by 5-8% (Buchberger, Dovč, 2000; Kamiński, Zabolewski, 2000). According to many authors (reviewed by: Buchberger, Dovč, 2000; Caroli et al., 2009) the BB CSN3 genotype is associated with a higher content of casein, whey proteins and total protein in milk.

Research on milk protein genes conducted in recent years (Czerwińska-Piotrowska, Kamieniecki, 2004; Czerwińska-Piątkowska et al., 2004; Feleńczak et al., 2000; Juszczak et al., 2001; Miciński et al., 2008; Pytlewski et al., 2004; Tsiaras et al., 2005; Ziemiński et al., 2005; Litwińczuk et al., 2006) yielded ambiguous results, which were mainly affected by the living conditions and nutrition of animals, as well as used statistical models.

Many authors are pointing to environment, as limiting possibilities of the production capacity of cows. Dymnicki (1987) reported that milk yield in cows is in 70% dependent on environmental factors, and the genetic potential of cattle is not fully exploited, mainly due to unfavorable conditions of maintaining and feeding. As a result, it is difficult, or even impossible, to unambiguously determine the "perfect" genotype for breeding purposes, even though we know which gene combinations are particularly preferred in connection with technological processing of milk. While working on genetic improvement of dairy cattle breeding, we examined how significantly an analysis of the breeding process affects the presence of milk protein genes and their interaction with environmental conditions in which animals are kept.

The research aimed at estimating the effect of beta-lactoglobulin and kappa-casein genotypes on milk yield and chemical composition of milk in cows bred in three herds in the *kujawsko-pomorskie* province.

MATERIAL AND METHODS

The research covered 398 Polish Holstein-Friesian black and white cows, kept in three selected herds from the *kujawsko-pomorskie* province. In all of the three herds,

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the animals were kept in buildings which were especially designated for cows. The housing, nutrition, and milking conditions were different across the herds, and are compared in Table 1.

Table 1: Main information about herds

Tabela 1: Podstawowe informacje o stadach

Characteristics	Herd A	Herd B	Herd C
Charakterystyka	Stado A	Stado B	Stado C
Housing system System utrzymania	Tie-stall	Free-stall	Tie-stall
	<i>Uwięziowy</i>	Wolnostanowiskowe	<i>Uwięziowy</i>
Stall type	Shallow bed	Deep litter	Shallow bed
Stanowiska	Ściołowe płytkie	Głęboka ściółka	Ściołowe płytkie
Milking system System doju	Direct to can milking system Dojarka bańkowa	Milking parlour Hala udojowa	Milking pipeline system Dojarka przewodowa
Nutrition basis Podstawa żywienia	Maize silage during winter, and green forage during summer Zimą kiszonki z kukurydzy, latem zielonki łąkowe	TMR	Maize and alfalfa silage during winter, and green forage during summer Zimą kiszonki z kukurydzy i lucerny, latem zielonki łąkowe
Number of cows Liczba krów	207	69	122

TMR – Total Mixed Ration (pełnoporcjowa, mieszana dawka żywieniowa)

The material for molecular analysis – the blood was taken from the jugular vein into test-tubes containing EDTA. The LGB gene was identified based on polymorphism in exon 4 position (T \rightarrow C) and the RFLP analysis was performed with the use of the HaelII restriction enzyme (Fermentas) (Medrano, J.F., Aguilar-Cordova E., 1990b). In order to digest a 262bp-long segment with the use of a HaelII enzyme, the test tubes were incubated for 4 h at 37°C. Molecular examinations of the CSN3 gene were carried out by analysing the polymorphism in the gene's exon 4 position (A \rightarrow C); and the RFLP analysis was performed with the use of the Hinfl restriction enzyme (Fermentas) (Medrano, J.F., Aguilar-Cordova E., 1990a). A 350bp-long segment was digested with the use of Hinfl enzyme for 6 h at 37°C. The obtained restriction fragments, both for the LGB as well as CSN3 gene, were subsequently separated in 3.5-percent agarose gels with the addition of ethidium bromide (0.5 μ g·ml $^{-1}$) and in the presence of the pUC19/Mspl DNA pattern.

The genetic structure of the examined population in terms of milk proteins polymorphism was determined by calculating allele and gene frequency of LGB and CSN3. The details of milk composition were taken from the SYMLEK database; the first lactation of all examined cows took place at the turn of 2006 and 2007.

To perform the statistical calculations, we adopted the multiple analysis of variance (SAS, 2008) and used the following statistical model:

$$Y_{ijk} = \mu + b_i + c_j + f_k + b_i * c_j + b_i * f_k + c_j * f_k + e_{ijk}$$

Where:

Y_{ijk} – observed trait μ – total mean value *b_i* – constant herds effect

c_i – constant LGB genotype effect

 f_k – constant CSN3 genotype effect

 $b_i * c_j$ – interaction between herd and LGB genotypes

 $b_i * f_k - interaction between herd and CSN3 genotypes$

 $c_i * f_k$ – interaction between LGB and CSN3 genotypes

e_{iik} – random error.

The significance of differences was verified by means of the Scheffe's test (SAS, 2008).

RESULTS

In the authors' own research, three groups of cows were created according to the polymorphism of the beta-lactoglobulin and kappa-casein genes: AA, AB, and BB (Table 2). The data in Table 2 suggests that the AB LGB heterozygotes were the most common in the examined population, and their participation was at 0,44; the frequency of both gene alleles was at a similar level, with the B allele being slightly more frequent (Table 2). In the case of the kappa-casein gene, the AA CSN3 (0.69) genotypes were found to be more frequent, and there was a small participation of the BB CSN3 (0.07) genotypes. It was also observed that that the frequency of the A allele was higher (0.81) as compared to the desired B allele (0.19).

The highest frequency of A allele of LGB was determined in herd B (0.61), while the frequencies of B allele were highest in herds A (0.57) and C (0.55) (Table 2). In the analysed population, in the CSN3 system, we found the highest participation of the AA genotypes in herd B, and at the same time, this herd had no individuals with the BB CSN3 genotype (Table 2).

Table 2: The frequency of beta-globulin and kappa-casein genes and genotypes in the examined population

Tabela 2: Frekwencja genów i genotypów beta-laktoglobuliny i kappa-kazeiny w badanej populacji.

Genotype	Number of genotypes	Frequency of genotypes		y of allele cja allelu
Genotyp	Liczba genotypów	Frekwencja genotypów	Α	В
	HERD	A – STADO A		
LGB				
AA	42	0.20		
AB	96	0.46	0.43	0.57
BB	69	0.33		
CSN3				
AA	147	0.71		
AB	42	0.20	0.81	0.19
BB	18	0.09		
	HERD	B – STADO B		_
LGB				
AA	33	0.48		
AB	18	0.26	0.61	0.39
BB	18	0.26		
CSN3				
AA	51	0.74	0.87	0.13

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Genotype	Number of genotypes	Frequency of genotypes		y of allele cja allelu
Genotyp	Liczba genotypów	Frekwencja genotypów	Α	В
AB	18	0.26		
BB	0	0		
	HERD	C – STADO C		_
LGB				
AA	24	0.20		
AB	62	0.51	0.45	0.55
BB	36	0.30		
CSN3				
AA	78	0.64		
AB	36	0.30	0.79	0.21
BB	8	0.07		
	TOTA	L – ŁĄCZNIE		
LGB				
AA	99	0.25		
AB	176	0.44	0.47	0.53
BB	123	0.31		
CSN3				
AA	276	0.69		
AB	96	0.24	0.81	0.19
BB	26	0.07		

Analysing the effect of the cow-house the animals came from, and the genotypes in terms of the LGB and CSN3 genes, we found a statistically significant effect of the cow-house on milk yield in the first lactation. Also, the influence of the interaction between the cow-house and the examined genotypes on the percentage of fat in the milk in all of the examined lactations proved to be highly significant (Table 3). Furthermore, milk yield was influenced by the interaction between the examined genotypes, which was confirmed statistically (Table 3).

Table 3: Value of $F_{\text{empirical}}$ and the effect of the factors studied on dairy milk yield and its composition

Tabela 3: Wartość F_{empiryczne} oraz istotność wpływu badanych czynników na wydajność mleka i jego skład

Factors – Czynniki	MY (kg)	FY (kg)	FC (%)	PY (kg)	PC (%)
		Lactat	ion – <i>Lak</i>	tacja 1	
Herd – <i>Stado</i>	23.71***	32.12***	3.25*	39.71***	37.86***
Genotype – Genotyp LGB	3.59*	3.10*	0.45	3.70*	0.72
Genotype - Genotyp CSN3	0.47	1.94	5.54**	0.73	3.15*
Herd*Genotype LGB Stado * genotype LGB	1.45	1.44	4.31**	1.10	9.88***
Herd*Genotype CSN3 Stado* Genotyp CSN3	2.53	4.77**	9.38***	1.70	3.12*
LGB*CSN3	4.97***	1.73	3.45**	3.94**	4.03**
		Lactat	ion – <i>Lak</i>	tacja 2	
Herd – Stado	1.61	1.95	2.10	1.07	0.42
Genotype - Genotyp LGB	0.90	1.26	1.47	0.76	0.04
Genotype - Genotyp CSN3	0.98	0.80	0.07	1.40	1.02

Factors – Czynniki	MY (kg)	FY (kg)	FC (%)	PY (kg)	PC (%)
Herd*Genotype LGB Stado*Genotyp LGB	1.29	0.96	13.87***	1.02	0.45
Herd*Genotype CSN3 Stado* Genotyp CSN3	1.24	0.98	3.12*	2.22	1.17
LGB*CSN3	2.69*	1.61	2.67*	2.41*	0.28
		Lacta	tion – <i>Lakt</i>	acja 3	
Herd – <i>Stado</i>	3.39*	2.33	1.07	2.46	0.72
Genotype – Genotyp LGB	0.82	0.58	1.35	0.80	0.53
Genotype – Genotyp CSN3	1.20	3.22*	1.35	0.86	0.99
Herd*Genotype LGB Stado*Genotyp LGB	2.27*	3.15*	6.71***	2.42*	2.53*
Herd*Genotype CSN3 Stado*Genotyp CSN3	0.69	1.12	1.73	0.74	0.84
LGB*CSN3	2.51*	1.46	1.88	2.14	0.68

^{***-} p ≤ 0.001

Description for tables 3-7:

MY (kg) – Milk yield – Wydajność mleka

FY (kg) – Fat yield – Wydajność tłuszczu

FC (%) – Fat content – Zawartość tłuszczu

PY (kg) – Protein vield – Wydainość białka

PC (%) – Protein content – Zawartość białka

The analysis of milk yield and milk components in each herd (Table 4) proved that the animals of herd A produced, in the first lactation, the highest amount of milk and of all its examined components, in comparison to the other groups as well as to the mean value found for the whole examined population. In the subsequent two lactations, the situation was different. More milk was obtained from cows in cowhouse C. The lowest yield of milk and its components was established in herd B-it was the only free-stall herd, and the smallest one in the research.

Table 4: The analysis of milk characteristics in the examined population estimated according to the housing-system

Tabela 4: Analiza cech mleka badanej populacji oszacowanych w zależności od obory

Lactation	Traits	Entire	population	Herd	4	Herd I	3	Herd	С
Laktacja	Cechy	Cała p	oopulacja	Stado	A	Stado	В	Stado	C
		n	X	n	х	n	х	n	х
	MY (kg)	398	6009.69	207	6487.32	69	6126.56	122	5133.20
	FY (kg)	398	260.75	207	284.39	69	254.26	122	224.31
1	FC (%)	398	4.36	207	4.39	69	4.20	122	4.39
	PY (kg)	398	197.08	207	219.09	69	199.65	122	158.27
	PC (%)	398	3.27	207	3.38	69	3.26	122	3.08
	MY (kg)	344	5963.89	174	5892.09	57	5459.10	113	6329.07
	FY (kg)	344	263.07	174	265.88	57	226.84	113	277.02
2	FC (%)	344	4.42	174	4.48	57	4.18	113	4.45
	PY (kg)	344	197.28	174	199.34	57	181.68	113	201.98
	PC (%)	344	3.26	174	3.30	57	3.29	113	3.18
	MY (kg)	251	6600.15	111	6544.27	36	5995.50	104	6869.09
	FY (kg)	251	300.02	111	303.08	36	264.08	104	309.19
3	FC (%)	251	4.40	111	4.35	36	4.58	104	4.40
	PY (kg)	251	217.30	111	221.22	36	199.25	104	219.38
	PC (%)	251	3.18	111	3.21	36	3.32	104	3.11

^{** -} p ≤ 0.01

^{*-} $p \le 0.05$

Analysing the yield according to the housing system, the LGB gene genotype, and lactation, we discovered that results were significantly different in certain herds and lactations. Generally, it may be assumed that in housing system B and C, the animals with AA LGB genotype (except in Farm C in second lactation, Table 7) obtained the highest milk, protein, and fat yields; and in housing system A, AB individuals had the best production characteristics only in the first lactation, while in the second and third, the best production was recorded in BB LGB homozygotes (Table 5,6,7). We found the highest content of milk components in the first and second lactations in housing system A, in the individuals with the AB LGB genotype: 4.53% of fat and 3.41% of protein; and for fat in the second lactation: 4.70. As for the third lactation, the cows from housing system B, also with the AB LGB genotype, reached the highest values of these parameters (Table 6).

In the case of kappa-casein gene, it was established that the cows with the AA CSN3 genotype constituted the most numerous group. However, in a number of examined cases, the heterozygous animals produced the greatest amount of milk with higher protein and fat yield (Tables 5, 6, 7). In herd B, only two genetic groups were found in terms of the CSN3 gene – AA and AB. The highest percentage of fat was generally found in heterozygous animals – only in herd C, the group with the highest fat percentage was the AA CSN3 homozygous group (Table 7). In the analysed population, the percentage of protein in all CSN3 form groups was at a similar level; only in herd C the level was slightly different (Table 7). In herd A, among BB CSN3 homozygotes, in the third lactation, the upward trend is visible for percentage increase of protein content in milk, from 3.34% in the second lactation to 3.56% in the third lactation (Table 5). A similar trend was observed in herd C.

Table 5: Milk yield in herd A depending on the LGB and CSN3 genotype
Tabela 5: Wydajność mleczna krów w stadzie A zależności od genotypu LGB i CSN3

	T:4-						Herd A -	- Stade	o A				
Lactation	Traits		Gend	- Genotyp	}	Genotype - Genotyp CSN3							
Laktacja	Cechy		AA		AB	AB BB			AA		AB	BB	
		n	X	n	X	n	X	n	X	n	X	n	x
	MY (kg)	42	6231.07	96	6553.84	69	6550.74	147	6433.81	42	6650.35	18	6543.83
	FY (kg)	42	277.71	96	297.37	69	270.39	147	275.73	42	319.07	18	274.16
1	FC (%)	42	4.46	96	4.53	69	4.14	147	4.29	42	4.78	18	4.22
	PY (kg)	42	211.28	96	224.03	69	216.96	147	218.65	42	221.28	18	217.50
	PC (%)	42	3.39	96	3.41	69	3.31	147	3.40	42	3.32	18	3.32
_	MY (kg)	36	6131.83	75	5539.56	63	6174.76	132	5893.36	27	5500.88	15	6585.00
	FY (kg)	36	270.25	75	257.44	63	273.43	132	264.50	27	257.22	15	293.60
2	FC (%)	36	4.42	75	4.70	63	4.23	132	4.43	27	4.69	15	4.44
	PY (kg)	36	205.16	75	188.24	63	209.24	132	200.56	27	181.66	15	220.40
	PC (%)	36	3.34	75	3.33	63	3.22	132	3.30	27	3.23	15	3.34
	MY (kg)	24	6357.75	33	6102.36	54	6897.22	90	6550.70	12	6665.00	9	6319.00
3	FY (kg)	24	274.37	33	287.45	54	325.39	90	304.33	12	315.00	9	274.66
	FC (%)	24	3.84	33	4.24	54	4.63	90	4.29	12	4.77	9	4.42
	PY (kg)	24	213.62	33	210.27	54	231.28	90	220.90	12	220.75	9	225.00
	PC (%)	24	2.94	33	3.13	54	3.37	90	3.15	12	3.32	9	3.56

Table 6: Milk yield in herd B depending on the LGB and CSN3 genotype
Tabela 6: Wydajność mleczna krów w stadzie B zależności od genotypu LGB i CSN3

	Tueite		•		•	He	erd B - Sta	ndo B	}				
Lactation Laktacia	Traits	Genotype - Genotyp LGB								Genotype - Genotyp CSN3			
Laklatja	Cechy		AA		AB		BB		AA		AB	В	ВВ
		n	x	n	x	n	x	n	x	n	x	n	X
	MY (kg)	33	6251.54	18	5860.83	18	6163.16	51	6282.41	18	5685.00	-	-
	FY (kg)	33	261.63	18	247.83	18	247.16	51	254.41	18	253.83	-	-
1	FC (%)	33	4.25	18	4.23	18	4.09	51	4.08	18	4.54	-	-
	PY (kg)	33	197.18	18	198.33	18	205.50	51	203.58	18	188.50	-	-
	PC (%)	33	3.14	18	3.39	18	3.34	51	3.24	18	3.32	-	-
	MY (kg)	27	5797.77	15	4899.00	15	5409.60	42	5276.28	15	5971.00	-	-
	FY (kg)	27	265.33	15	193.40	15	191.00	42	214.85	15	260.40	-	-
2	FC (%)	27	4.77	15	3.81	15	3.50	42	4.11	15	4.39	-	-
	PY (kg)	27	195.44	15	161.40	15	177.20	42	173.35	15	205.00	-	-
	PC (%)	27	3.30	15	3.31	15	3.25	42	3.25	15	3.39	-	-
	MY (kg)	18	6783.33	9	3962.33	9	6453.00	27	5539.66	9	7363.00	-	-
	FY (kg)	18	329.33	9	165.66	9	232.00	27	228.55	9	370.66	-	-
3	FC (%)	18	4.86	9	5.12	9	3.49	27	4.43	9	5.05	-	-
	PY (kg)	18	233.83	9	134.00	9	195.33	27	183.22	9	247.33	-	-
	PC (%)	18	3.44	9	3.39	9	3.00	27	3.31	9	3.35	-	-

Table 7: Milk yield in herd C depending on the LGB and CSN3 genotype
Tabela 7: Wydajność mleczna krów w stadzie C zależności od genotypu LGB i CSN3

Lactation Laktacia	Traits	Genotype – Genotyp LGB							Genotype - Genotyp CSN3						
Lakiacja	Cechy		AA	٠.	AB		ВВ		AA		AB		BB		
		n	х	n	х	n	х	n	х	n	х	n	Х		
	MY (kg)	24	5121.62	62	5132.80	36	5141.58	78	5084.30	36	5234.50	8	5154.00		
	FY (kg)	24	209.12	62	229.59	36	225.33	78	224.23	36	226.83	8	213.75		
1	FC (%)	24	4.08	62	4.49	36	4.39	78	4.42	36	4.35	8	4.18		
	PY (kg)	24	156.50	62	157.03	36	161.58	78	157.65	36	160.53	8	153.87		
	PC (%)	24	3.05	62	3.06	36	3.13	78	3.09	36	3.06	8	2.98		
	MY (kg)	24	6676.75	56	6580.87	33	5648.90	75	6077.20	33	6878.18	5	6483.00		
	FY (kg)	24	277.62	56	287.26	33	259.18	75	270.80	33	296.00	5	245.00		
2	FC (%)	24	4.19	56	4.41	33	4.69	75	4.53	33	4.34	5	3.95		
	PY (kg)	24	211.25	56	209.19	33	183.00	75	192.32	33	223.81	5	202.80		
	PC (%)	24	3.16	56	3.18	33	3.18	75	3.14	33	3.27	5	3.13		
	MY (kg)	24	8055.00	56	6147.76	24	7366.25	66	7112.22	33	6504.72	5	6064.40		
	FY (kg)	24	324.12	56	288.78	24	341.87	66	316.90	33	302.00	5	254.80		
3	FC (%)	24	4.04	56	4.44	24	4.62	66	4.50	33	4.21	5	4.20		
	PY (kg)	24	257.37	56	196.92	24	233.75	66	226.36	33	207.45	5	205.80		
	PC (%)	24	3.20	56	3.04	24	3.15	66	3.19	33	2.89	5	3.41		

DISCUSSION

The results obtained in this research for the LGB gene frequency correspond with the results of other authors (Czerniawska-Piątkowska and Kamieniecki, 2000; Juszczak et al., 2001; Kamiński, 2001; Litwińczuk et al., 1999). Czerniawska-Piątkowska et al. (2004b) examined gene frequency in two herds based on the content of the Holstein-Friesian genes in the cow genotype, and found out, similarly to the results of this research in herds A and C, a lower content of the LGB A allele over the LGB B allele. Ng-Kwai-Hang et al. (1990), having examined 8000 cows from 546 milk cattle herds, reported that the highest frequency in terms of the LGB gene was present in the AB heterozygotes, at the level of approximately 50%; it was 40% in the case of the BB homozygotes. As for the CSN3 gene frequency, the highest number of animals had the A type gene: there were approximately 55% of the AA homozygotes, 40% of the AB heterozygotes, and nearly 4% of the BB individuals. Low presence of the BB

CSN3 homozygous animals was also observed in our examinations, as well as those conducted by Czerniawska-Piątkowska et al. (2004b), and Juszczak et al. (2001). Litwińczuk et al. (1999) proved a similar percentage of the LGB genotypes in the four farm types. The authors estimated for the Central and Eastern Poland that the participation of AA, AB and BB LGB was respectively 13.3, 53.4, and 33.3. Whereas for the region of Vilnius, Lithuania, these numbers equalled 20.6, 58.6, and 21.1. In black and white bulls, approved for reproduction in Poland in the years 1994 to 2000. the frequency of the A allele of the LGB gene was 0.41; whereas for the B gene it was 0.59 (Czerniawska-Piatkowska and Kamieniecki, 2004a). As highlighted by Czerniawska-Piątkowska and Kamieniecki (2004a), there is a great need for inducing growth of the B allele of the CSN3 gene in the Polish population of dairy cows. Sawa et al. (2008), analysing the lifetime performance of cows in two housing systems (tie-stall and free-stall), came to the conclusion that in the cow-houses where there were 50-200 cows, the free-stall animals obtained higher lifetime performance in respect of milk. Zdziarski et al. (2002) obtained very similar results. However, these authors point that in herds of more than 200 cows, the animals in tiestall cow-houses were better reproduction cows. Szarek (1998) emphasises the fact that milking frequency also influences cow performance. Weglaszy et al. (2009), who examined the influence of milking frequency on milk yield and milk composition over the first 100 days of lactation, also confirms this fact. Our research has proved that the yield for milk from half of the udder milked three times was significantly higher in statistical terms than when produced by the other half milked twice. Bovenhuis et al. (1992) found, similarly to our research, a positive relation between the AA LGB genotype and milk yield. Czerniawska-Piatkowska et al. (2011) stated that cows with the AA LGB genotype had the highest milk yield in all analyzed 305day lactations. However, Ziemiński et al. (2002) reported that cows with the BB LGB genotype were characterised by the highest lifetime performance. Juszczak et al. (2001) found statistically significant influence of the genotype in relation to the percentage of fat in milk (BB LGB > AB LGB > AA LGB and BB CSN3 > AA CSN3). The presented results are different from those obtained in the discussed research. However, Tsiaras et al. (2005) observed a statistically significant difference between milk yield (AB>AA), fat (BB and AB>AA), fat content (BB>AA and AB), and lactose yield (AB>AA) in the case of the LGB genotype. These authors also point to the statistically significant influence of the CSN3 genotype on protein content in milk and the performance of these traits. According to Tsiaras et al. (2005) animals with the AB genotype of the CSN3 gene are more productive than AA homozygotes. Kamiński and Zabolewicz (2000) proved higher fat and protein content (4.32% and 3.51%) in milk from cows with the AA LGB genotype. Whereas for the AB and BB genotype, the values for these traits were at the same level and amounted to 4.27 and 3.33% respectively. Results obtained by these other researchers correspond with the results presented in this study.

The research carried out by Lin et al. (1989) on the influence of kappa-casein on the traits of milk yield in subsequent lactations, proved that cows with the BB genotype gave more milk than cows with the other genotypes. Van Eenennaam and Medrano (1991) also associated the B variant of the CSN3 gene with higher milk yield. Feleńczak et al. (2000) found that cows with the AB and BB genotype of kappa-casein were characterised by higher content of total protein, casein, and fat in milk. Bovenhuis and Weller (1994) also proved that milk from cows with the BB genotype, as for CSN3, contained more protein than that from the other compared groups. At the same time, the research suggests significantly higher technological usefulness of

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milk from cows with the BB genotype in terms of the CSN3 gene for cheese production.

Strzałkowska et al. (2000) report that the highest amount of fat corrected milk was obtained from cows with AA CSN3 and AA LGB combinations. Pytlewski et al. (2004) stated that the most favourable genotype variant of milk yield, lactose, and high content of somatic cells was found with the BB LGB/AA CSN3 system.

CONCLUSION

The presented research results prove that the cow-house as well as lactose and genotype affect the level of the examined traits. Milk yield grew in subsequent lactations. As for LGB genotype, heterozygous animals were the most frequent. Whereas in the case of the CSN3 genotype – the most frequent were AA homozygotes. Milk yield and content differed depending on the herd and lactation. Therefore, it is impossible to clearly determine which genotypes guarantee the highest level of milk traits in a given population.

REFERENCES

- Bovenhius H., Van Arendonk J., Korver S., (1992) Associations between milk protein polymorphism and milk production traits. Journal of Dairy Science 75, 2549-2559.
- Bovenhuis H., Weller J. I., (1994) Mapping and Analysis of Dairy Cattle Quantitative Trait Loci by Maximum Likelihood Methodology Using Milk Protein Genes as Genetic Markers. Genetics 137 (1), 267-280.
- Buchberger J., Dovč P., (2000) Lactoprotein Genetic Variants in Cattle. Food Technology and Biotechnology 38, 2, 91-98.
- Caroli, A.M., Chessa, S., Erhardt, G.J., (2009) Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science, 92, 11, 5335-5352.
- Czerniawska-Piątkowska E., Kamieniecki H., (2000) Frekwencja genów i genotypów białek mleka krów w wielkostadnym gospodarstwie z terenu Pomorza Zachodniego. Folia Universitatis Agriculturae Stetinensis, Zootechnica 227, 44, 35-40.
- Czerniawska-Piątkowska E., Kamieniecki H., (2004a) Polimorfizm białek mleka u bydła. Medycyna Weterynaryjna 60(7), 692-694.
- Czerniawska-Piątkowska E., Kamieniecki H., Pilarczyk R., Rzewucka E., (2004b) A comparison of protein polymorphisms in milk produced by two dairy farms in West Pomerania. Archive Tierzucht 47, 155-163.
- Czerniawska-Piątkowska E., Szewczuk M., Olszewska A. Chociłowicz E., (2011) Association between beta-lactoglobulin (LGB) polymorphism and yield and composition of milk of Holstein-Friesian cows imported from Sweden. Acta Scientiarum Polonorum, Zootechnica 10, 1, 9-18.
- Dymnicki E., (1987) Wpływ czynników genetycznych i środowiskowych na użytkowość mleczną krów rasy czarno-białej w kolejnych laktacjach w zależności od poziomu produkcyjnego stada. Rozprawa 8, IGiHZ PAN, Jastrzębiec.

- Feleńczak A., Gil Z., Ormian M., (2000) Kappa-kazeina jako wskaźnik przydatności technologicznej mleka. Roczniki Naukowe Zootechniki Suplement 8, 9-13.
- Juszczak J., Erhardt G., Kuczaj M., Ziemiński R., Panicke L., (2001) Zusammenhang zwischen K-Casein und ß-Lactoglobulin-Varianten mit der Milchleistung und der Nutzungsdauer von Rindern der Rassen Schwarzbuntes Rind und Polnisches Rotvieh. Archive Tierzucht 44, 239-249.
- Kamiński S., Zabolewicz T., (2000) Associations between bovine beta-lactoglobulin polymorphism within coding and regulary sequences and milk performance traits. Journal of Applied Genetics 41, 2, 91-99.
- Kamiński S., (2001) Polimorfizm genów białek mleka u bydła. Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego. Rozprawa UWM Olsztyn.
- Lin, C. Y., McAllister A. J., Ng-Kwai-Hang K. F., Hayes J. F., Batra T. R., Lee A. J., Roy G. L., Vesely J. A., Wauthy, J M: Winter, K A, (1989) Relationships of milk protein types to lifetime performance. Journal of Dairy Science 72 (11), 3085-3090.
- Litwińczuk A., Litwińczuk Z., Tumienie M., Barłowska J., (1999) Polimorfizm białek mleka krów cb z wybranych rejonów Polski i Litwy. Prace i Materiały Zootechniczne 54, 101-106.
- Litwińczuk A., Barłowska J., Król J., Litwińczuk Z., (2006) Białka polimorficzne mleka jako markery cech użytkowych bydła mlecznego i mięsnego. Medycyna Weterynaryjna 62, 1, 6-10.
- Martin, P., Szymanowska M., Zwierzchowski L., Leroux C., 2002. The impact of genetic polymorphisms on the protein composition of ruminants milks. Reproduction Nutrition Development 42, 433-459.
- Medrano, J.F., Aguilar-Cordova E., (1990a) Genotyping of bovine kappa-casein loci following DNA sequence amplification. Bio/Technology, 8, 144-146.
- Medrano J.F., Aquilar-Cordova E., (1990b). Polymerase chain reaction amplification of bovine beta-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. Animal Biotechnology 1, 73-77.
- Miciński J., Mariusz Jastrzębski M., Klupczyński J. (2008) Yield and composition of milk from Polish Holstein-Friesian and Jersey cows in particular months of the first lactations as dependent on milk protein polymorphism. Archive Tierzucht 51, 3, 216-223.
- Ng-Kwai-Hang K. F., Monardes H. G., Hayes J. F., (1990) Association between genetic polymorphism of milk proteins and production traits during three lactations. Journal of Dairy Science 73, 3414.
- Pytlewski J., Antkowiak I., Dorynek Z., Stanisławski D., (2004) Polimorfizm LGB i CSN3 a dzienna wydajność, skład i wybrane wskaźniki fizyczne mleka krów czarno-białych. Zeszyty Naukowe Przeglądu Hodowlanego 72 (1), 195-204.
- SAS Institute Inc. (2008) SAS/STAT User's guide, Version 9.2, Cary, NC, SAS Institute Inc.
- Sawa A., (1998) Genetyczne i środowiskowe uwarunkowania użytkowości krów w poszczególnych okresach życia. Rozprawa ATR Bydgoszczy.
- Sawa A., Bogucki M., Jankowska M., (2008) Wpływ systemu utrzymania krów na

- Sitkowska et al.: Milk Protein Polymorphisms And Effect Of Herds On Cows` Milk Composition wyniki ich życiowej użytkowości. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego 4 (4), 77-85.
 - Strzałkowska N., Krzyżewski J., Ryniewicz Z., (2000) Wpływ genotypu betalaktoglobuliny i kappa-kazeiny na wydajność, skład chemiczny i podstawowe parametry technologiczne mleka krów cb. Prace i Materiały Zootechniczne 56, 107-119.
 - Szarek J., (1998) Osiągnięcia nauki zaprezentowane na 49 kongresie Europejskiej Federacji Zootechnicznej. Zeszyty Naukowe Przeglądu Hodowlanego 38, 45-55.
 - Tsiaras A. M., Bargouli G. G., Banos G., Boscos C. M., (2005) Effect of Kappa-casein and Beta-Lactoglobulin Loci on Milk Production Traits and Reproductive Performance of Holstein Cows. Journal of Dairy Science 2005; 88(1), 327-334.
 - Węglarzy K., Bereza M., Szarek J., (2009) Wpływ częstości doju na wydajność i skład mleka krów za pierwsze 100 dni laktacji. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego, 2 (5), 91-97.
 - Van Eenennaam A., Medrano J.F., (1991) Milk Protein Polymorphisms in California Dairy Cattle. Journal of Dairy Science 74, 1730-1742.
 - Zdziarski K., Grodzki H., Nałęcz-Tarwacka T., Brzozowski P., Przysucha T., (2002) Wpływ systemu utrzymania i genotypu krów na długość ich użytkowania i życiową użytkowość mleczną. Zeszyty Naukowe Przeglądu Hodowlanego 62, 29-35.
 - Ziemiński R., Juszczak J., Walawski K., (2002) Association between milk protein polymorphisms and life production traits in herds of Black-and-White and Red-and-White cattle improved by Holstein-Friesian sires. Annals of Animal Science2 (1), 29-4.
 - Ziemiński R., Juszczak J., Czarnik U., Ćwikła A., Zabolewicz T., Walawski K., (2005) Związek między polimorfizmem białek mleka i zróżnicowaniem wydajności oraz składu mleka krów utrzymywanych w stadzie bydła rasy czarno-białej kombinatu rolnego Kietrz. Acta Scientiarum Polonorum, Zootechnica 4(1), 163-170.