COMPARISON OF DAILY MILK YIELD AND ITS CHEMICAL COMPOSITION BETWEEN COWS FROM SELECTED GENETIC GROUPS OF BETA-LACTOGLOBULIN AND KAPPA-CASEIN GENES

PORÓWNANIE DZIENNEJ WYDAJNOŚCI MLEKA ORAZ JEGO SKŁADU CHEMICZNEGO MIĘDZY KROWAMI WYBRANYCH GRUP GENETYCZNYCH W ZAKRESIE GENÓW BETA-LAKTOGLOBULINY I KAPPA-KAZEINY

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ABSTRACT

The objective of the research was to analyse the influence of selected genetic groups on the daily milk yield and its chemical composition in black and white Holstein-Friesian cows. The genotyping of beta-lactoglobulin and kappa-casein genes was conducted by means of the PCR-RFLP technique. The collected numeric data were analysed statistically with the covariance analysis. The joint effects of beta-lactoglobulin and kappa-casein genotypes were estimated. The greatest amount of milk was obtained from the genotype combination AAAB, whereas the milk of the genotype combination AABB was characterized with the greatest fat content. The smallest number of samples was collected from cows with genes that are preferred in terms of technological processing, combination BBBB. During test milking, the BBBB genotype combination was characterised by high milk yield and protein content, but also the highest content of somatic cells in milk samples under analysis.

KEYWORDS: milk cows, trial milking, LGB, CSN3

STRESZCZENIE

Celem badań była analiza wpływu wybranych grup genetycznych genów beta-laktoglobuliny (LGB) i kappa-kazeiny (CSN3) na dzienną wydajność i skład chemiczny mleka krów rasy holsztyńsko-fryzyjskiej odmiany czarno-białej utrzymywanych w jednym ze stad województwa kujawsko-pomorskiego. Genotypowanie genów LGB i CSN3 przeprowadzono za pomocą techniki PCR-RFLP z użyciem enzymów, odpowiednio: HaeIII i HinfI. Zebrane dane liczbowe opracowano statystycznie za pomocą analizy kowariancji, do porównań między grupami wykorzystano analizę kontrastów (ANOVA/MANOVA STATISTICA 8.0). Został zbadany łączny efekt genów beta-laktoglobuliny i kappa-kazeiny. Najwięcej mleka otrzymano od krów o kombinacji genotypów AAAB, natomiast mleko zwierząt o kombinacji genotypów AABB charakteryzowało się najwyższą procentową zawartością tłuszczu. Najmniejszą ilość prób zgromadzono od krów o preferowanym pod względem technologicznego przetwórstwa układzie genów BBBB. Podczas próbnych udojów, grupa krów o kombinacji genotypów BBBB charakteryzowała się wysoką wydajnością mleka i zawartością białka, ale jednocześnie najwyższą zawartością komórek somatycznych w ocenianych próbach mleka.

SŁOWA KLUCZOWE: dzienna wydajność mleka, skład chemiczny mleka, LGB, CSN3

DETAILED ABSTRACT

Do genów białek mleka o szczególnym znaczeniu dla wyników ekonomicznych hodowli bydła należą geny β-laktoglobulina (LGB) i κ-kazeina (CSN3). Polimorfizm beta-laktoglobuliny warunkuje seria kodominujących alleli. Najczęściej identyfikowane są jej trzy warianty AA, AB i BB. Locus LGB oddziałuje głównie na skład mleka i jego jakość, szczególnie allel B został wskazany jako wpływający na jakość mleka w europejskiej hodowli bydła. Natomiast allel A genu LGB jest wiązany z parametrami wydajności mlecznej.

Analiza wpływu poszczególnych wariantów genotypów na użytkowość mleczną oraz skład mleka była przedmiotem badań wielu autorów. Kombinacja BBBB opisywana jest jako najbardziej korzystna a jednocześnie najrzadziej spotykana - stanowi ona zaledwie kilka procent badanych populacji. Udział genotypów kombinacji BBBB zmniejsza się w populacji aktywnej wraz ze wzrostem procentowego udziału genów rasy holsztyńsko-fryzyjskiej. W związku z czym w wielu pracach pomija się ją w szczegółowych analizach statystycznych.

Celem badań była analiza wpływu wybranych grup genetycznych genów beta-laktoglobuliny (LGB) i kappa-kazeiny (CSN3) na dzienną wydajność i skład chemiczny mleka krów rasy holsztyńsko-fryzyjskiej odmiany czarno-białej utrzymywanych w jednym ze stad województwa kujawsko-pomorskiego. Genotypowanie genów LGB i CSN3 przeprowadzono za pomocą techniki PCR-RFLP z użyciem enzymów, odpowiednio: HaeIII i HinfI. Zebrane dane liczbowe opracowano statystycznie za pomocą analizy kowariancji, do porównań między grupami wykorzystano analizę kontrastów. (ANOVA/ MANOVA STATISTICA 8.0) [15]. Najwięcej mleka otrzymano od krów o kombinacji genotypów AAAB, natomiast mleko zwierząt o kombinacji genotypów AABB charakteryzowało się najwyższą procentową zawartością tłuszczu (tab. 1). Najmniejszą ilość prób zgromadzono od krów o preferowanym pod względem technologicznego przetwórstwa układzie genów BBBB. Podczas próbnych udojów, grupa krów o kombinacji genotypów BBBB charakteryzowała się wysoką wydajnością mleka i zawartością białka, ale jednocześnie najwyższą zawartością komórek somatycznych w ocenianych próbach mleka (tab. 1).

W badaniach własnych stwierdzono, że wiek krowy w dniu pierwszego wycielenia oraz kategoria genotypu pod względem genów LGB/CSN3 wpłynęły istotnie statystycznie na wydajność mleka, procentową zawartość białka i laktozy w mleku oraz LnLKS (tabela 2). Analiza kontrastów wykazała istotne różnice w wydajności mleka oraz zawartości komórek somatycznych (LnLKS) w mleku krów między osobnikami należącymi do kategorii BBBB a ABAB oraz BBBB a AAAA (tab. 3).

INTRODUCTION

The β-lactoglobuline (LGB) and κ-casein (CSN3) are milk genes of key importance for economic results of cattle breeding. The polymorphism of beta-lactoglobuline is conditioned by a series of codominant alleles. The AA, AB and BB variants are among the most widely recognized ones [10, 11, 19]. The LGB locus primarily influences milk content and its quality, and it was the B allele that was identified to affect milk quality in European cattle breeding [17]. The A allele of the LGB gene is in turn considered to be related to milk yield parameters.

The analyses of CSN3 genotype's influence revealed that milk from cows with BB genotypes of κ-casein is characterized with the shortest coagulability time, highest thermal stability, and higher cheese yield by as much as 8%, whereas milk containing the AA κ-casein displays much longer coagulability time [3, 5, 6, 8]. In addition, in cows with the CSN3 BB genotype, higher milk, fat and protein content was found, while in the cows with the AB kappa-casein genotype, higher fat and protein content in 305-day lactation was observed [3]. Numerous research in the field have also shown that in milk obtained from the BB and AB CSN3 genotypic cows, when compared with the AA CSN3 cows, clot firmness is 20-100% higher. These parameters characterize milk with a measurable economic value. The results of more than 30 years of research conducted in Europe, USA, Canada, Australia, and Poland proved that the CSN3 gene can be considered favourable and demonstrating strong pleiotropic activity. According to Strzałkowska et al. [16, 17], there is a significant interaction between CSN3 and LGB genes, which justifies studying their effects collectively. There is a likelihood of synergic or antagonistic relationship between respective polymorphic forms of the LGB and CSN3 and milk yield and its chemical composition [16]. The participation of BBBB genotypes decreases in an active population as the percentage share of Holstein-Friesian breed genes increases.

The analysis of the influence of respective genotype variants on milk performance and milk content was researched by numerous authors [2, 3, 4, 7, 9, 10, 14, 16, 17]. The BBBB combination is described as the most favorable one, but, at the same time, the least frequent, comprising just a few percent of the population under study. The participation of the BBBB genotypes decreases in an active population as the percentage share of Holstein-Friesian breed genes increases.

Thus, many papers omit them in detailed statistical analyses. The aim of the present paper is to present the results of the milk yield in the BBBB group in comparison with the AAAA and ABAB genotypic cattle using a contrast analysis.

MATERIALS AND METHODS

In the research the data from 2729 records of the milk yield of 155 black-and-white Holstein-Friesian cows kept in a barn in the kujawsko-pomorskie province were utilized. The cows were kept in a two-row building on shallow-litter stalls. Threefold milking was performed using wired milking machines, and the cows were fed using the TMR system, and a forage trailer was incorporated.

The research included the cow milk yield during trial milking and first three lactations were taken into consideration. The information on milk performance traits of cows was collected from the SYMLEK database provided by the Polish Federation of Cattle Breeders and Dairy Farmers.

The material for a molecular analysis, i.e. blood, was taken from a jugular vein to EDTA test tubes. High-quality DNA was obtained with the MasterPure™ DNA purification kit. The LGB gene was identified based on polymorphism in exon 4 in (T→C) position, the RFLP analysis was conducted using the HaeIII restrictive enzyme (Fermentas) [12]. A fragment of 262 base pairs was amplified using the PCR method with the HaeIII enzyme.

The molecular research on the CSN3 gene was conducted by analysing polymorphism in exon 4 of the gene in position (A→C); the RFLP analysis was performed using the restriction enzyme HinfI (Fermentas) [12]. A 350 bp fragment was etched using the HinfI enzyme. The restriction fragments obtained for both LGB and CSN3 genes were then separated in 3.5-percent agarized gels containing ethidium bromide (0.5 µg·ml-1), in the presence of the pUC19/MspI DNA marker.

The genotypes of cows depending on the arrangement of the LGB and CSN3 genes were split into nine categories. Since empiric distribution of somatic cells count did not fulfill the requirements of standard distribution, the data were transformed using logarithmic function (natural logarithm was used, i.e. LnLKS). The numerical data collected was processed statistically using the covariance principle, and the importance in differences between selected groups (BBBB a AAAA and BBBB a ABAB) was highlighted with contrast analysis [15]. Its assumptions regarding standard distribution of residues (excluding somatic cells count), homogeneity of variance in groups and the assumption that experimental activity does not

Table 1. Mean adjusted values of cow milk parameters depending on CASK and BLG genes (including the age factor).
Tabela 1 Średnie skorygowane wartości parametrów mleka krów w zależności od układu genów CASK i BLG (zastosowana poprawka na wiek).

Genotype LGB/CSN3 Genotyp LGB/CSN3	n	Milk yield Wydajność mleka (kg)		Fat content Zawartość tłuszczu (%)		Protein content Zawartość białka (%)		Lactose content Zawartość laktozy (%)		Ln SCC LnLKS	
		$\bar{x}$	±95% Przedział ufności Confidence interval	$\bar{x}$	±95% Przedział ufności Confidence interval	$\bar{x}$	±95% Przedział ufności Confidence interval	$\bar{x}$	±95% Przedział ufności Confidence interval	$\bar{x}$	±95% Przedział ufności Confidence interval
BBBB	48	19.68	17.55-21.79	4.47	4.21-4.73	3.46	3.33-3.59	4.79	4.76-4.81	12.65	12.19-13.10
ABBB	70	14.78	13.03-16.53	4.16	3.95-4.38	3.29	3.18-3.39	4.79	4.77-4.80	12.64	12.32-12.97
AABB	47	12.68	10.47-14.87	4.75	4.49-5.01	3.43	3.30-3.57	4.84	4.82-4.85	11.92	11.56-12.27
BBAB	152	15.41	14.20-16.61	4.50	4.36-4.65	3.43	3.36-3.50	4.82	4.78-4.85	12.51	12.28-12.74
ABAB	322	13.06	12.22-13.89	4.65	4.55-4.75	3.34	3.29-3.39	4.78	4.75-4.80	11.60	11.47-11.72
AAAB	163	19.71	18.56-20.84	4.56	4.42-4.7	3.43	3.36-3.50	4.75	4.71-4.78	11.70	11.46-11.94
BBAA	547	15.83	15.20-16.45	4.60	4.53-4.68	3.44	3.40-3.48	4.78	4.72-4.82	12.08	11.98-12.18
ABAA	874	15.31	14.81-15.79	4.59	4.53-4.65	3.41	3.38-3.44	4.76	4.70-4.81	12.00	11.91-12.09
AAAA	506	16.24	15.59-16.88	4.41	4.33-4.49	3.41	3.37-3.45	4.73	4.68-4.78	12.07	11.95-12.19

Table 2. F_{emp} value and the level of the effect of the factors studied on dairy milk yield
Tabela 2. Wartość F_{emp} oraz istotność wpływu badanych czynników na wydajność mleka

Factors Czynniki	Fat content Zawartość tłuszczu (%)	Lactose content Zawartość laktozy (%)	Factors Czynniki	Milk yield Wydajność mleka(kg)	Protein content Zawartość białka/ (%)	LnLKS Ln SCC
Age at first calving Wiek pierwszego wycielenia	35.3	250.62***	Genotype LGB/CSN3 * Age at first calving Genotyp LGB/CSN3 * Wiek pierwszego wycielenia	2.85**	22.00***	12.24***
Genotype LGB/CSN3 Genotyp LGB/CSN3	4.4	4.35***	Genotype LGB/CSN3 Genotyp LGB/CSN3	2.05*	3.00**	2.85**

*** - p≤ 0,001
** - p≤ 0,01
* - p≤ 0,05

influence concomitant variable were all confirmed. Depending on the test results of parallelism of regression line within the groups, two models were applied:

1. Model of identical slopes (for percentage content of fat and lactose in milk)

$$Y_{ij} = \mu + \alpha_i + \beta x_{ij} + e_{ij}$$

where:

α_i – effect of genetic group

βx_{ij} – effect of concomitant variable related to ith factor and jth observation

e_{ij}– sampling error

2. Model of different slopes (for milk field, percentage content of protein in milk and LnLKS)

$$Y_{ij} = \mu + \alpha_i + \beta_i x_{ij} + e_{ij}$$

where:

α_i – effect of genetic group

$\beta_i x_{ij}$ – effect of concomitant variable related to ith factor and jth observation

e_{ij}– sampling error

The tables show concomitant variable adjusted mean values, the results of an F-test for covariance analysis, t-test values for contrast analysis and corresponding p levels [15].

RESULTS AND DISCUSSION

Table 1 presents the cow's daily milk yield as well as milk content, and characteristics depending on the combination of the LGB and CSN3 genotypes. The smallest groups were the cows from the genotypic groups AABB and BBB respectively. The total number of samples obtained from the cows which had the CSN3 gene in the form of the BB homozygote was 165. The samples from cows with homozygotic distribution of protein genes BB LGB equaled 747 (See Table 1).

The greatest number of trial milkings was obtained from cows with the CSN3 gene as homozygote AA, totaling 1927 (Table 1), whereas a comparable group of trial milking for the LGB gene was 716 trials (Table 1). Low participation of LGB BB and CSN3 BB genotypic cows in the population under study was also confirmed in the research of other authors [2, 3, 4, 7, 9, 10, 16]. Pytlewski et al. [14] found very low participation of cows with BBBB combination of genes, as this group was only 1.3% of the whole analysed population. In the study of Strzałkowska et al. [17], the cattle with the BBBB variant was excluded from detailed analyses due to insufficient number of such cows. Czerniawska-Piątkowska et al. [3] studied the frequencies of genes in two herds depending on the participation of the Holstein-Friesian breed genes in cows' genotype, and concluded that the CSN3 allele was less frequent than the CSN3 A allele. The low count of homozygotic CSN3 BB cows was also proved in the research of Juszczak et al.[4]. The studies on black and white cattle in a number of countries, including Poland, showed that the frequency of the cases of cattle with desirable genotypes of κ-casein is equally low and decreases systematically [2, 5, 6, 16]. Thus, there is a strong need to take action in order to grow the B allele of the CSN3 gene in a population of milk cows in Poland. Also the studies conducted by Lien et al. [9] on determining the frequency of milk protein genes in populations of different breeds of Nordic cattle indicated the advantage of the A allele of the CSN3 gene in young cattle breeds over older ones.

Table 3 Contrast analysis - t and p values.
Tabela 3 Analiza kontrastów – wartości t i p.

Variable Zmienna	Genotype LGB/CSN3 Genotyp LGB/CSN3	t	p
Milk yield	BBBB – ABAB	-5.68	0.0000
Wydajność mleka(kg)	BBBB – AAAA	-3.12	0.0018
Fat content	BBBB – ABAB	1.28	0.2008
Zawartość tłuszczu (%)	BBBB – AAAA	-0.44	0.6590
Protein content	BBBB – ABAB	-1.53	0.1252
Zawartość białka (%)	BBBB – AAAA	-0.53	0.5940
Lactose content	BBBB – ABAB	0.31	0.7597
Zawartość laktozy (%)	BBBB – AAAA	1.13	0.2569
Ln SCC	BBBB – ABAB	-4.06	0.0000
LnLKS	BBBB – AAAA	-2.17	0.0301

A similar, when compared with present research, tendency of frequency of genes determining LGB polymorphism was observed by Czerniawska-Piątkowska and Kamieniecki [2], Litwińczuk et al. [10] and Kamiński [7]. In black and white bulls approved for reproduction in Poland between 1994 and 2000, the frequency of the A allele of the LGB gene was 0.41, whereas for the B gene it was 0.59 [7].

The highest milk yield was obtained from cows belonging to the category AAAB and BBBB (Table 1). The results obtained should not be compared directly with those of other authors, as some of the said papers concentrated on analysing the influence of a single milk protein gene, and not a combination thereof. Strzałkowska et al. [17] stated that in their research the greatest count of FCM milk was obtained from cows with genotypes combination of AA AA. Bovenhuis et al. [1] noted that there is a dependency between the AA LGB genotype and milk yield, whereas the B variant of the CSN3 gene was associated with higher milk yield by Van Eenennaam and Medrano [20].

Cows with genotype having gene distribution of AAAB gave in the present research the largest amount of milk with high fat and protein content, and with lower (when compared with other genetic groups) content of somatic cells. The research conducted by Strzałkowska et al. [16] allowed authors to conclude that higher milk yield is dependent on the form of AA of each examined protein, i.e. LGB and CSN3. The research of Ziemiński et al. [21] proved in turn that the highest milk yield was obtained in case of cows with the BBAB genotypes combination. Pytlewski et al. [14] concluded that the most favourable variant of genotype as regards milk yield, lactose and high somatic cell content occurred in BBAA. Tsiaras et al. [18] in turn observed statistically significant difference between milk yield (AB>AA), fat yield (BB and AB>AA), fat content (BB>AA and AB) and lactose yield (AB>AA) in case of the LGB genotype. In present research the percentage content of lactose in milk in all tested genetic groups was on a comparable level, and the content in excess of 4.8% was found only in groups BBAB and AABB (Table 1). In the research of Strzałkowska et al. [16] it was also concluded that the milk of cows with genotype of BBAB was characterized with the highest percentage of lactose. At the same time these authors [16] concluded that the BBAB cows produced milk that had higher protein thermo stability and the best usability for cheese production.

Percentage fat content in cow milk in individual categories fluctuated between 4.16-4.75%. The error was 0.03 to 0.13. The highest percentage fat content was found in trial milking of the group cows with genotype AABB, whereas the lowest in the cows with genotype ABBB. The content of protein in most of the studied groups was 3.4%, and the highest content was observed in milk of the genotype BBBB (3.46%), whereas the smallest was noted for group ABBB (3.29%). Ng-Kwai-Hang et al. [13], upon examining the population of 8000 cows of the Holstein-Friesian breed, failed to note any differences in milk content among cattle with different CSN3 genotype. Tsiaris et al. [18] indicated statistically significant influence of CSN3 genotype on milk yield and protein content in cow milk. In the studies of Pytlewski et al. [14] and Bovenhuis et al.[1] the greatest percentage of protein content was found in the AAAB combination group.

The natural logarithm of somatic cells (LnLSK) fluctuated, depending on the group, from 11.60 to 12.65. The quality of milk obtained from cows belonging to the category ABAB was higher than that from the ABBB category cows. Pytlewski et al. [14] deemed the AABB genotype the worst genetic distribution of the LGB and CSN3 variants for the majority of trial milking traits under study. This was confirmed in present research in case of the milk yield.

In this research it was concluded that the factors under study, i.e. cow's age on the day of the first calving and category of genotype regarding genes LGB/CSN3 had a statistically significant influence on the milk yield, percentage protein and lactose content in milk and LnLKS (Table 2). The analysis of contrasts revealed that there are significant differences in the milk yield and content of somatic cells (LnLKS) in cow's milk between the cows belonging to category BBBB and ABAB, and between BBBB and AAAA; the differences were statistically significant (tab. 3). In case of remaining properties, the differences of statistic significance between the genotypic groups were not observed (Table 3).

CONCLUSIONS

To conclude, the examinations confirmed high milk yield in cows with the BBBB gene combination, at the same time indicating that the milk of cows from this group was characterized with the highest number of somatic cells. The samples obtained from the group with the BBBB gene arrangement comprised nearly 2% of the analyzed results of milk recording.

The highest milk yield during trial milking was obtained from cows with the genotype variant AAAB, whereas the milk of cows from the AABB was characterized with the highest percentage fat content.

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