FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin. 2013, Agric., Aliment., Pisc., Zootech. 307 (28), 11–16

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RELATIONS BETWEEN KAPPA-CASEIN POLYMORPHISM (CASK) AND MILK PERFORMANCE TRAITS IN HEIFER COWS

ZWIĄZEK POLIMORFIZMU KAPPA-KAZEINY (CASK) Z UŻYTKOWOŚCIĄ MLECZNĄ KRÓW PIERWIASTEK

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Streszczenie. Przeprowadzone badania dotyczyły ustalenia zależności pomiędzy polimorfizmem kappa-kazeiny a cechami użytkowości mlecznej 339 krów pierwiastek rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej. Genotypy kappa-kazeiny (CASK) oznaczono metodą PCR (ang. Polymerase Chain Reaction). Stwierdzono, że allel $CASK^A$ występował częściej niż allel $CASK^B$ w pierwszej 305-dniowej laktacji krów (0,831 A, 0,169 B). W badanej populacji przeważały homozygoty CASK AA (70,21%), w odniesieniu do heterozygot CASK AB (25,84%) i homozygot CASK BB (3,95%). Najwyższą wydajność mleka, tłuszczu i białka stwierdzono w mleku krów o genotypie CASK AA, a największą zawartość tłuszczu i białka odnotowano u homozygot CASK BB.

Key words: composition, cows, kappa-casein gene, milk yield.
Słowa kluczowe: gen kappa-kazeiny, krowy, skład mleka, wydajność.

INTRODUCTION

More than 95% of the proteins contained in ruminant milk are coded by 6 structural genes (Martin et al. 2002, Caroli et al. 2009). Four of them are genes encoding casein, including being the subject of this study, kappa-casein. Milk protein genes have been thoroughly investigated in cattle, known and characterized their polymorphism (Bovenhius et al. 1992, Mercier and Vilotte 1993, Zwierzchowski 1996) and identified as genetic variants of these proteins affect the field and composition of milk. Although there is still a need for such research.

Kappa-casein is a milk protein fraction, which is 10–12% total protein milk. Milk coagulation process and curd quality depend on the construction and structure of the kappa-casein gene (Feleńczak et al. 2000). It is also fraction of proteins which made the most interest because of its genetic variants are linked to traits of cows milk and suitability of milk for processing (Litwińczuk et al. 2006). Most identified three variants of CASK: AA, AB and BB.

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The aims of this study was to establish a relationship between genetic variants of kappa-casein and milk field and composition of Polish Holstein-Fresian var. Black-and-White cows in the first 305-day lactation.

MATERIAL AND METHODS

Analysis of the frequency of alleles and genotypes of kappa-casein and performance characteristics milk was conducted on 339 Polish Holstein-Fresian var Black-and-White (PHF) cows in the first 305-day lactation. The blond for tests was collected from external jugular vein into the vacuum test tubes with EDTA. DNA isolation was performer using a kit for the isolation *MasterPure™ Genomic DNA Purification Kit* provided by *Epicentre Technologies.* Genotypes of kappa-casein were determined using PCR-RFLP (Polymerase Chain Reaction-Restricted Fragments Length Polymorphism) according to the method by Kamiński (1993). The obtained DNA fragments were digested using the *HindIII* restriction enzyme (a ↓ agctt) provided by MBI Fermentas and then restriction fragments were separated electrophoretically in 2% agarose gel with ethidium bromide in the presence of 1×TBE buffer (Tris/Borate/EDTA) and visualized in the transilluminator provided by Viller Lourmat.

The analysis of milk yield and composition of cows in the first lactation was performer 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 (%).

Effect of kappa-casein for milk traits were analyzed statistically using Statistica®9 PL (StatSoft Inc. 2010) by calculating the mean values $(\bar{x})$ and standard deviation (SD). The significance of differences between genotypes was determined using multiple range Duncan's test.

RESULTS AND DISCUSSION

In the examined population of heifer cows was found that the most frequent genotype of CASK was AA genotype (70.21%), followed AB (25.84%) and the east frequent genotype was BB (3.95%). The allele $CASK^A$ appeared al most five times more likely than allele $CASK^B$ (respectively 0.831 and 0.169). The results are showed in Table 1. Similar results were also obtained by other authors. In the study by Sitkowska et al. (2008), the frequency of $CASK^A$ in PHF cattle was estimated at al most five times higher than $CASK^B$ allele (0.83 and 0.17 respectively). However, numerical genotype distribution was as follows: genotype AA (0.71), AB (0.23) and BB (0.06). In the next studies by Sitkowska et al. (2013) a similar distribution of genotypes and allele for the first lactation of cows was observed. Frequency of genotypes were: AA (0.69), AB (0.24) and BB (0.07) and alleles A (0.81) and B (0.19) in study by Sitkowska et al. (2013). However, Miciński et al. (2008) at heifer PHF found, that the most frequent genotype was heterozygote AB (0.562), followed by homozygotes AA (0.417) and the least frequent genotype was BB (0.021). The share of allele $CASK^B$ was higher than in own study and amounted 0.302, and the allele $CASK^A$ 0.698. A similar distribution of allele and genotypes was obtained by Ziemiński et al. (2005) who studied a herd of 856 Black-and-White cows. Distribution of CASK genotypes of cows in the first lactation were as follows

AA (0.536), AB (0.419) and BB (0.045) and the distribution of alleles was $CASK^A$ (0.746) and $CASK^B$ (0.254). The authors suggest that a very low proportion of homozygotes BB can probably be conditioned by the influence of HF bulls used in the improvement of the basic traits of milk.

Table 1. The frequency of kappa-casein alleles and genotypes in the investigated population
Tabela 1. Frekwencja alleli i genotypów kappa-kazeiny w badanym stadzie

Genotype Genotyp	Number of genotypes Liczba genotypów	Frequency of genotypes Frekwencja genotypów (%)	Frequence of allele Frekwencja alleli	
			A	B
AA	231	70.21	0.831	0.169
AB	95	25.84		
BB	13	3.95		
Total – Razem	339			

In Table 2 are showed results of productivity heifer cows depending on the variant of the kappa-casein gene. In the analysis of the performance traits of heifer cows not found significant differences. The highest milk (6572.0 kg), fat (270.1 kg) and protein (222.8 kg) yield was observed in milk of cows with AA CASK genotype. Milk yield was lower in heterozygotes AB milk by 242.7 kg, and homozygotes BB by 272.2 kg. Fat and protein yield for genotypes CASK AB and BB were almost identical. The present study has been confirmed by Sitkowska et al. (2008). The authors found the highest milk (6414 kg), fat (271 kg) and protein (209 kg) yield in milk of cows with AA CASK genotype. Slightly lower milk yield was recorded in milk of cows with BB CASK genotype (6398 kg). Also Litwińczuk et al. (2006) received the highest milk yield (5483.2 kg), fat (220.3 kg) and protein (173.6 kg) in milk of Black-and-White cows with a different content of HF blood with AA CASK genotype. Miciński et al. (2008) analyzed the individual components of milk showed that milk, fat and protein yield was statistically higher in milk of cows with AA genotype than in milk of cows with AB genotype. On the other hand, the result by Ziemiński et al. (2005) do not confirm the above results. The authors reported that heterozygotes AB CASK characterized the highest milk yield (6784 kg), fat (297 kg) and protein yield (224.2 kg) compared to homozygotes AA and BB CASK. Also Sitkowska et al. (2013) for the heteroygotes genotype AB CASK received the highest milk (6650.35 kg), fat (319.07 kg) and protein yield (221.28 kg) and the highest fat content in milk (4.78 %). Typically AA CASK genotype is combined with a higher milk yield in contrast to the BB genotype, and AB genotype is combined with a production of milk at an intermediate level (Bovenhius et al. 1992, Sowiński 1993, Litwińczuk et al. 1996, Walawski et al. 2003).

From the present study it appears that the highest fat (4.13%) and protein (3.44%) content was recorded in milk of cows with BB CASK genotype, which has been reflected in the studies by Ziemiński et al. (2005) (4.39% and 3.36% respectively) and Litwińczuk et al. (2006) (4.15% and 3.33% respectively). Miciński et al. (2008) showed that fat and protein content was slightly higher in milk of cows with AB genotype than AA genotype. Allele $CASK^B$ is linked with the higher content in milk proteins, including casein, fat and dry matter (Mao et al. 1992, Barłowska et al. 2000, Strzałkowska et al. 2002). On the other hand, Sitkowska et al. (2013) observed the highest protein content in milk of cows with AA CASK genotype (3.40%) compared to BB and AB genotypes (in both groups protein content was 3.32%).

Table 2. Analysis of milk characteristics estimated for the first lactation depending on genetic variant of kappa-casein (CASK)

Tabela 2. Analiza cech wydajności mlecznej oszacowanych dla pierwszej laktacji w zależności od genotypu kappa-kazeiny (CASK)

Genotype Genotyp	Statistics Statystyka	Milk yield Wydajność mleka (kg)	Fat Tłuszcz (kg)	Fat Tłuszcz (%)	Protein Białko (kg)	Protein Białko (%)
AA	$\bar{x}$	6572.0	270.1	4.11	222.8	3.39
	SD	812.0	45.1	0.38	32.4	0.19
AB	$\bar{x}$	6329.3	259.0	4.09	216.5	3.42
	SD	101.4	6.1	0.06	3.2	0.02
BB	$\bar{x}$	6299.8	260.2	4.13	216.7	3.44
	SD	1524.5	47.8	0.32	39.6	0.16

No significant differences between the examined groups tested by Duncan's test.
Brak różnic istotnych pomiędzy badanymi grupami określono za pomocą testu Duncana.

CONCLUSIONS

Results of this study indicated higher participation of homozygotes AA CASK (70.21%) compared to heterozygotes AB (25.84%) and homozygotes BB (3.95%) in tested population. The highest milk, fat and protein yield was found in milk of heifer cows with genotype CASK AA. However, the highest fat and protein content was observed in milk of cows with CASK BB genotype.

REFERENCES

Barłowska J., Litwińczuk Z., Król J., Pietras U. 2000. Związek wariantów genetycznych białek mleka z jego cechami fizykochemicznymi i zdrowotnością wymienia krów. Zesz. Nauk. Prz. Hod. 51, 333–340. [in Polish]

Bovenhius H., Van Arendonk J.A.M., Korver S. 1992. Association between milk protein polymorphism and milk production traits. J. Dairy. Sci. 75, 2549–2559.

Caroli A.M., Chessa S., Erhardt G.J. 2009. Invited review. Milk protein polymorphisms in cattle. Effect on animal breeding and human nutrition. J. Dairy Sci. 92 (11), 5335–5352.

Feleńczak A., Gil Z., Ormian M. 2000. Kappa-kazeina jako wskaźnik przydatności technologicznej mleka. Rocz. Nauk. Zootech. 8 (Suppl.), 9–13. [in Polish]

Kamiński S. 1993. Identyfikacja genotypu kappa-kazeiny u buhajów przy pomocy metody PCR-RFLP. Praca doktorska. Olsztyn, ART. [in Polish]

Litwińczuk A., Barłowska J., Florek M., Asarabowska A. 1996. Związek między polimorfizmem białek mleka a produkcyjnością krów. Ann. UMCS 14, 59–63. [in Polish]

Litwińczuk A., Barłowska J., Król J., Litwińczuk Z. 2006. Białka polimorficzne mleka jako markery cech użytkowych bydła mlecznego i mięsnego. Med. Weter. 62 (1), 6–10. [in Polish]

Mao I.L., Buttazoni P., Aleandri R. 1992. Effects of polymorphic milk protein genes on milk yield and composition traits in Holstein cattle. Acta Agric. Scandinavica 42, 1–7.

Martin P., Szymanowska M., Zwierzchowski L., Leroux C. 2002. The impact of genetic polymorphisms on the protein composition of ruminants milks. Reprod. Nutrition Develop. 42, 433–459.

Mercier J.C., Vilotte J.L. 1993. Structure and function of milk protein genes. J. Dairy Sci. 76 (10), 3079–3098.

Miciński J., Pogorzelska J., Barański W. 2008. Parametry użytkowe pierwiastek rasy hf w zależności od genetycznych wariantów wybranych białek mleka. Med. Weter. 64 (9), 1136–1140. [in Polish]

Sitkowska B., Neja W., Wiśniewska E. 2008. Relations between kappa-casein polymorphism (CSN3) and milk performance traits in heifer cows. J. Centr. Europ. Agric. 9 (4), 641–644.

Sitkowska B., Neja W., Milczewska A., Mroczkowski S., Markowska A. 2013. Milk protein polymorphisms and effect of herds on cows' milk composition. J. Centr. Europ. Agric. 14 (1), 78–90.

Sowiński G. 1993. Związek genetycznych wariantów β-laktoglobuliny, αS_1-, β- oraz κ-kazein z wydajnością, składem chemicznym i wskaźnikami technologicznej przydatności mleka krów rasy nizinnej czarno-białej. Acta Acad. Agric. AC Tech. Olst., Ser. Zootechnica 38 (Suppl. B), 3–38. [in Polish]

StatSoft, Inc 2010. Statistica (data analysis software system), version 9. www. statsoft.com.

Strzałkowska N., Krzyżewski J., Ryniewicz Z., Zwierzchowski L. 2002. Effects of κ-casein and β-lactoglobulin loci polymorphism, cow's age, stage of lactation and somatic cell Mount on daily milk field and milk composition in Polish Black-and-White cattle. Anim. Sci. Pap. Rep. 20 (1), 21–35.

Walawski K., Ziemiński R., Novokszonov A., Czarnik U., Zabolewicz T., Pareek C.S. 2003. Association between beta-lactoglobulin and kappa-casein polymorphism and variability of milk performance traits in Polish Black-and-White cow population maintained in different milk productivity herds. Ann. Anim. Sci. 3 (2), 243–253.

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 Sci. Pol., Ser. Zootechnica 4 (1), 163–170. [in Polish]

Zwierzchowski L. 1996. Structure, expression and engineering of milk protein genes. Biotech. 14, 81–94.

Abstract. The undertaken study aimed at determining relations between kappa-casein (CASK) polymorphism and milk performance traits in population of 339 Polish Holstein-Fresian var Black-and-White heifer cows. Kappa-casein genotypes were determined using PCR method. It was found that allele $CASK^A$ was more frequent than allele $CASK^B$ in the first 305-day lactation (0.831 and 0.169 respectively). In the investigated population homozygotes AA (70.21%) were dominated compared to heterozygotes AB (25.84%) and homozygotes BB (3.95%). The highest milk, fat and protein yield was found in milk of cows with AA CASK genotype, and the highest fat and protein content was observed in homozygotes BB.