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POLYMORPHISMS IN THE GENE OF *UCP3* IN DAIRY CATTLE

POLIMORFIZM W GENIE *UCP3* U BYDŁA MLECZNEGO

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Streszczenie. Celem prowadzonych badań było oszacowanie wpływu genotypów w regionie polimorficznym genu *UCP3* na wybrane cechy użytkowości mlecznej (wydajność mleka, białka, i tłuszczu oraz zawartość białka i tłuszczu). Badano stado krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czerwono-białej liczące 169 krów. Analizę genotypów poszczególnych osobników przeprowadzono z wykorzystaniem metody PCR-RFLP. Analizując frekwencję alleli i genotypów dla *UCP3/Maell* w badanym stadzie krów rasy polskiej holsztyńsko-fryzyjskiej odmiany czerwono-białej można stwierdzić, że genotyp CC występował z największą częstością (0,66). Frekwencja allelu C wynosiła 0,79. Analiza otrzymanych wyników wykazała statystycznie istotne zależności między genotypami *UCP3/Maell* a poszczególnymi cechami użytkowości mlecznej.

Key words: dairy cattle, gene polymorphism, milk production traits, *UCP3*.

Słowa kluczowe: bydło mleczne, cechy użytkowości mlecznej, polimorfizm genu, *UCP3*.

INTRODUCTION

Poland is one the largest milk producers in Europe. Recent years have shown a tendency of decline in the population accompanied by an increase in the productivity of milk. The increase of breeding efficiency is affected by the application of scientific achievements in the field of genetics, in combination with proper nutrition strategy. Genetic markers are becoming ever more important in the breeding programs. Researchers seek the correlation between their presence and traits affecting the milk production (Ogorevc et al. 2009). One of the marker class is an SNP (*single nucleotide polymorphism*). The analysis of polymorphism of microsatellite sequences and their coupling with quantitative traits (QTL – quantitative trait *locus*) allows to determine genes and regions responsible for economically important productivity traits. Researchers have identified several *loci* determining milk performance traits, such as: milk yield, protein and fat yield, protein and fat content in milk, by using microsatellite markers in bovine animals (Kwaśnicki and Frankowski 2007, Jędraszczyk 2010).

In recent years, researchers' attention was drawn by the family of uncoupling proteins (UCP) located in the inner mitochondrial membrane, as factors of metabolic control. The physiological role of these proteins is not yet fully understood, but the discovery of regulating mechanisms of their activity will help to understand the physiology of animals' mitochondria (Krauss et al. 2005).

So far, researchers have conducted numerous analyses, aiming at verification of the relationship between cases of single nucleotide polymorphism (SNP) of the *UCP3* gene and their influence on the fatty acids metabolism (Chen et al. 2011).

During lactation the level of *UCP3* protein expression in the muscles decreases, which is the result of metabolic adaptation to the conditions of breastfeeding and associated energy expenditure. Identifying genotypes in the polymorphic region of *UCP3* gene, responsible for the increase of traits of milk performance traits of bovine animals, would provide benefits to breeders (Pedraza et al. 2001).

The undertaken study aimed at determining the frequencies of alleles and genotypes with regard to the polymorphism in the *UCP3* gene (intron 2) and establishing association between the genotypes and some milk production traits of cows.

MATERIAL AND METHODS

A herd of 169 Polish Red-and-White Holstein-Friesian cows was tested. All tested animals were kept in similar environmental conditions in the south-western region of Poland. The entire herd of cows has finished three consecutive lactations. Animals were fed with standardized nutritional doses, with the possibility of using a pasture during spring and summer. The documentation of milk performance of each cow included milk yield, fat and protein content and their percentage share. Milking was carried out twice a day, and the test milkings were performed in accordance with A4 method.

The peripheral blood, collected from the jugular vein of each tested cow to the vacuum tubes containing K3EDTA anticoagulant, was used for the isolation of DNA. It was carried out using the kit *MasterPure* from Epicenter Technologies for DNA isolation from whole blood, according to the manufacturer's instructions.

It involved the analysis of the SNP polymorphic site in intron 2 (T/C) at position 679 (GenBank AF127030). A fragment of *UCP3* gene was amplified by PCR, with adequate specific primers sequences. The fragment of *UCP3* gene 281 bp in length was amplified using a pair of primers with the following nucleotide sequences: forward: 5' – GAG CTT GGG CAA GTC ACA GAC – 3' and reverse: 5' – GGT ACT GGG CAC TCC GAG – 3'.

The reaction of the amplification of the *UCP3* gene fragment had the following thermal profile: initial denaturation – 95°C for 5 minutes, then successively in 30 cycles: 30 seconds at 94°C (denaturation), 45 seconds at 55°C (annealing of the primers) and 45 seconds at 72°C (extension of the DNA chain), and then final extension of the chain at 72°C for 8 minutes.

The next step of the analysis was to subject PCR products to digestion with the restrictive enzyme *Maell* at 37°C for at least 3 hours. Received PCR products and restrictive fragments were analyzed and archived using the kit of documentation and analysis of the agarose gels.

The statistical analysis of the correlation between *UCP3/MaeII* polymorphism and milk performance traits was carried out in the program STATISTICA 7.1 using a linear model of GLM package (General Lineal Model):

$$Y_{ijkl} = \mu + a_i + b_j + c_k + d_l + e_{ijkl}$$

where:

Y_{ijkl} – is the value of the observed trait in an individual;

μ – average value of a trait in the tested herd;

a_i – a fixed effect of *UCP3/MaeII* genotype ($i = 1, 2, 3$), I, II and III lactation;

b_j – a fixed effect of the year of calving ($j = 1, 2, 3, \dots, 7$) I, II and III lactation;

c_k – a fixed effect of the month of calving ($k = 1, 2, 3, \dots, 12$) I, II and III lactation;

d_l – a random effect of the father ($l = 1, 2, 3, \dots, 35$) I, II and III lactation;

e_{ijkl} – a random error.

Duncan's Test was used to trace the differences between the average values of the analyzed traits.

RESULTS

Genotypes CC, TC and TT were identified after the restrictive analysis of a fragment of intron 2 of *UCP3* gene 281 bp in length, using the restrictive enzyme *MaeII*. Their presence was conditioned by two alleles, T allele (restrictive fragment 281 bp in length) and C allele (two restrictive fragments 161 bp and 120 bp in length). The frequency of individual genotypes and *UCP3/MaeII* alleles was as follows: CC – 0.66, TC – 0.25, TT – 0.09 and C – 0.78 i T – 0.22.

The table shows the average values and standard deviation for the individual milk performance traits, in relation to *UCP3/MaeII* genotypes. By considering the individual average traits, it was found that individuals with homozygous CC genotype in the course of the I and III lactation were characterized by high productivity of milk (respectively +247 kg and +102 kg), protein yield (+6.2 kg and +1.9 kg), and in three consecutive lactations these individuals attained the highest fat yield (+1.0 kg, +4.4 kg and +9.7 kg). Cows with a heterozygous TC genotype during the II lactation showed the highest milk yield (+146 kg) and protein content (+3.2 kg). In the three analyzed lactations tested in a cow herd it can be observed that heterozygous TC individuals attained the lowest fat content of milk (–21.8 kg, –8.8 kg and –21.8 kg). Bovine animals with TT genotypes were characterized by the lowest milk yield during the three lactations (–851 kg, –426 kg, –257 kg) and the lowest protein content in the II lactation (–12.4 kg). However, in the I and II lactation these individuals attained the highest percentage of fat in milk (+0.16% and 0.11%). By analyzing the protein content, it was demonstrated that homozygous individuals, as well as the heterozygous ones, attained results similar to the herd's average value. In case of the I lactation, the differences between the milk yields of cow with various genotypes were statistically confirmed ($P \leq 0.05$; ≤ 0.001).

Table 1. Means and standard deviations (SD) of milk production traits in relation to *UCP3* / *Maell* genotypesTabela 1. Wartości średnie i odchylenie standardowe dla cech użytkowości mlecznej w odniesieniu do genotypów *UCP3* / *Maell*

Lactation Laktacja	Genotype Genotyp	n	Milk yield Wydajność mleka	Protein Białko		Fat Tłuszcz	
			[kg]	[kg]	[%]	[kg]	[%]
I	CC	111	6978 ±1346 ^{AB}	227.0 ±44.2	3.34 ±0.26	281.2 ±67.9	4.12 ±0.50
	TC	42	6401 ±1107 ^A	208.9 ±35.4	3.32 ±0.21	249.4 ±49.0	3.97 ±0.54
	TT	16	5880 ±1621 ^B	208.8 ±31.8	3.38 ±0.22	259.2 ±41.9	4.26 ±0.47
	Total Ogółem	169	6731 ±1362	220.8 ±41.8	3.34 ±0.25	271.2 ±62.9	4.10 ±0.51
II	CC	111	8387 ±1619	278.7 ±44.7	3.38 ±0.23	348.9 ±76.1	4.23 ±0.56
	TC	42	8526 ±1600	281.3 ±44.4	3.43 ±0.42	335.7 ±75.0	4.07 ±0.47
	TT	16	7954 ±1249	265.7 ±42.8	3.40 ±0.20	337.0 ±58.6	4.31 ±0.37
	Total Ogółem	169	8380 ±1582	278.1 ±44.4	3.39 ±0.02	344.5 ±74.2	4.20 ±0.04
III	CC	111	8978 ±1541	290.3 ±46.3	3.33 ±0.24	393.2 ±93.0	4.47 ±0.68
	TC	42	8704 ±1512	283.2 ±46.7	3.35 ±0.26	361.7 ±89.0	4.23 ±0.61
	TT	16	8619 ±1143	289.1 ±39.1	3.42 ±0.21	374.0 ±60.3	4.42 ±0.38
	Total Ogółem	169	8876 ±1500	288.4 ±45.6	3.34 ±0.24	383.5 ±90.0	4.40 ±0.64

n – the number of individuals; ^A P ≤ 0.05 ^B ≤ 0.001.n – liczba osobników; ^A P ≤ 0,05 ^B ≤ 0,001.

DISCUSSION

The extension of information on QTL for economically important productivity traits will significantly improve the selection of bovine animals.

The following paper undertook a study which aimed at determining the influence of identified genotypes in the polymorphic region of *UCP3* gene on selected milk performance trait of the tested cow herd. The *UCP3* protein was selected for the study because it is involved in the energy metabolism, especially the metabolism of fatty acids and ATP-dependent processes (Solanes et al. 1997, Chung et al. 2011). The location of the *UCP3* protein in skeletal muscles may indicate its participation in thermogenesis at rest (Nabben and Hoeks 2008, Musa et al. 2012).

This gene was mapped in pigs with chromosome 9 (9p21–p24) adjacent to the region rich in QTL (quantitative trait *loci*) for the traits of adiposity and intramuscular fat content (Chen et al. 2011). *Locus* of the bovine *UCP3* gene is located on the fifteenth chromosome, BTA15 (Solanes et al. 1997). The presence of SNP in the *UCP3* gene, affecting the adiposity traits, was found in chickens and pigs. A significant association between mutations within this gene and traits of feed efficiency was observed, such as: average daily gain, feed conversion ratio and yield increase (Liu et al. 2005, Chen et al. 2011).

So far, numerous analyses were carried out, aiming at the verification of the correlation between cases of single nucleotide polymorphism (SNP) of the *UCP3* gene and their influence on the metabolism of fatty acids (Chen et al. 2011). mRNA *UCP3* expression is positively correlated with the body metabolism at rest (Liu et al. 2005).

Ramsay et al. (2008) demonstrated, in the in vitro examination, that the expression of the porcine *UCP3* gene can be regulated with somatotropin. This hormone is a potent inducer of lipid metabolism and inhibitor of the *UCP3* protein expansion in skeletal muscles.

In contrast, the thyroid hormones and leptin affect the growth of *UCP3* expansion in muscles. Studies have shown that supplementation of leptin during breastfeeding or rapid weaning of puppies, returns changes in mRNA expression of *UCP3* in skeletal muscles of rats to the previous state (Xiao et al. 2004). The increase in *UCP3* expression in muscles is also noticeable in physiological and nutritional conditions, when fatty acids are the primary source of energy. During lactation, the level of *UCP3* protein expansion in muscles decreases, which is the result of metabolic adaptation to the conditions of breastfeeding and associated energy expenditure. Identification of genotypes in the polymorphic region of the *UCP3* gene, responsible for the growth of bovine animals' milk performance trait, would provide breeders with tangible benefits (Pedraza et al. 2001).

The physiological regulation of food intake, growth and energy balance in bovine animals (and other animals) are under the control of many genes, which may become important components of the variability of performance traits from an economic point of view.

CONCLUSIONS

Based on obtained results it can be stated that there is a correlation between analyzed genotypes and selected milk performance traits in the tested cow herd, in case of milk yield in the I lactation the differences were statistically confirmed. In order to use the above study in the breeding of dairy cows, one should continue the study of the influence of individual *UCP3* genetic variations on milk performance traits, which would have covered a much larger group of animals of different dairy breeds.

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Abstract. The aim of the following study was to determine the influence of genotypes in the polymorphic region of *UCP3* gene on the selected traits of milk performance (milk yield, protein, fat and the content of protein and fat). A herd of 169 Polish Red-and-White Holstein-Friesian cows was studied. The analysis of genotypes of individuals was performed using the PCR-RFLP method. By analyzing the frequency of alleles and genotypes of *UCP3/Maell*, in the tested herd of Polish Red-and-White Holstein-Friesian cows, one can state that the CC genotype occurred with the greatest frequency (0.66). The frequency of the C allele was 0.79. The analysis of obtained results showed statistically significant correlations between the *UCP3/Maell* genotypes and individual traits of milk performance.