DOI: 10.21005/AAPZ2024.70.2.6

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2024, 371(70)2, 60–67

Received 23 Feb 2024
Revised 19 Mar 2024
Accepted 21 Mar 2024

Inga KOWALEWSKA[1], Volodymir KOSTIUK[2], Wasyl KOSTENKO[2], Ewa CZERNIAWSKA-PIĄTKOWSKA[3], Marcjanna WRZECIŃSKA[3], Alicja KOWALCZYK[4], Sonia HILLER[3]

UCP3 GENE POLYMORPHISM AND MILK PRODUCTION TRAITS IN CATTLE

[1] Department of Genetics, West Pomeranian University of Technology, Szczecin, Poland
[2] National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
[3] Department of Ruminant Science, West Pomeranian University of Technology, Szczecin, Poland
[4] Department of Environmental Hygiene and Animal Welfare, Wrocław University of Environmental and Life Sciences, Wroclaw, Poland

Abstract. The protein UCP3 can perform several different functions such as regulating lipid metabolism in mitochondria, protecting against oxidative stress, and regulating energy metabolism, and proper metabolism is crucial in cows during lactation. The aim of the study was to determine the frequency of genes and alleles and their influence on milk production traits for four SNP (*rs467440799, rs446854207, rs444645335,* and *rs440638775*) polymorphisms in the *UCP3* gene. The experiment was conducted in a herd of Polish Holstein-Friesian black and white cows, and genotypes were determined using the PCR-RFLP method. The conducted research showed that the analyzed SNPs significantly ($p \leq 0.05$) influence milk production traits such as milk yield, fat yield, and protein yield.

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

INTRODUCTION

Dairy cattle breeding plays a significant role worldwide for economic, social, and food-related reasons. Currently, dairy farming and milk production constitute a highly complex area of agricultural production due to the influence of both environmental and genetic factors. Additionally, various molecular methods contribute to improving the genetic component. The use of marker-assisted selection can accelerate genetic progress by shortening generations, as it enables more precision selection based on genetic data instead of relying solely on phenotypic observations. This approach can benefit both breeders, by increasing breeding efficiency, and consumers, by improving product traits. Utilizing information from genotyping, for example, allows to obtain animals that are increasingly efficient or produce milk with consumer or cheese industry-desired parameters.

Understanding the interrelationships between energy metabolism, thermogenesis, and milk production is crucial for optimizing feeding strategies, management practices, and breeding

Correspondence author: Inga Kowalewska, Department of Genetics, West Pomeranian University of Technology, Piastów 45, 70-311 Szczecin, Poland, e-mail: inga.kowalewska@zut.edu.pl.

programs aimed at maximizing milk yield efficiency while maintaining cow health and welfare. Research is ongoing to elucidate the specific mechanisms underlying these processes in dairy cattle to establish new goals for improving milk production and metabolic health. In dairy cattle, particularly in colder conditions or during periods of stress (e.g. lactations, high milk production), thermogenesis becomes a key for maintaining body temperature within a narrow range conducive to proper physiological functions (Liu et al. 2019). Brown adipose tissue (BAT) is a specialized tissue involved in thermogenesis, primarily through the action of uncoupling proteins (UCPs), such as UCP1, and UCP3 (Sharma et al. 2014).

Uncoupling protein 3 (UCP3) belongs to a family of proteins that act as membrane carriers of anions in mitochondria. The main function of uncoupling proteins is to uncouple oxidative phosphorylation from ATP synthesis by creating a proton leak across the inner mitochondrial membrane. Through uncoupling oxidative phosphorylation, uncoupling proteins regulate metabolic rate and energy expenditure. They can be regulated in response to various stimuli, such as cold exposure, certain hormones (e.g. thyroid hormone), and dietary factors. Although the functions of UCP3 are not fully understood, research suggests several potential roles for this protein, such as regulating lipid metabolism by increasing fatty acid oxidation in mitochondria, protecting against oxidative stress by reducing the production of reactive oxygen species, and regulating energy metabolism by influencing energy expenditure (Ledesma et al. 2002; Pohl et al. 2019). As is widely known, proper metabolism is particularly necessary during lactation when there is a high energy demand in the cow's body (Tufarelli et al. 2023).

Therefore, the gene encoding *UCP3* appears to be an interesting candidate for research on the impact of polymorphism in this gene on the traits of cattle utility. Hence, studies were conducted to analyze the influence of four polymorphisms in this gene on the fundamental parameters of milk utility.

MATERIAL AND METHODS

The research was conducted in a herd of Polish Holstein-Friesian cattle maintained in the area of Greater Poland. The herd comprised 446 animals, all kept under similar environmental conditions. The cows were fed using the TMR system (Total Mixed Ration) and had free access to fresh water, and during the spring and summer months, they were maintained on pastures.

DNA was isolated from whole peripheral blood collected into tubes containing anticoagulant factor (K_3EDTA) using a commercially available isolation kit, according to the methodology provided by the manufacturer. The next step involved developing appropriate primer sequences to conduct genotyping for four selected single nucleotide polymorphisms (SNPs) in the gene encoding uncoupling protein 3. The analyzed mutations are missense mutations: M89I (*rs467440799*), T132A (*rs446854207*), L232M (*rs444645335*), and F271L (*rs440638775*). Based on the *UCP3* gene sequence from the Ensembl database, individual primer sequences were designed using the Primer 3 program (http://bioinfo.ut.ee/primer3-0.4.0/) (Table 1). Genotyping was performed using the restriction fragment length polymorphism (RFLP) method, employing appropriately selected restriction enzymes – *Sau3*AI (*rs467440799*), *Pci*I (*rs446854207*), *Fat*I (*rs444645335*), and *Rsa*I (*rs440638775*). Amplification of individual *UCP3* gene fragments was carried out in commercially available ready-to-use PCR Mixes (2xPCR Mix) according to the manufacturer's instructions, using the appropriate primers for each SNP. Reactions were conducted under standard PCR thermal conditions, with annealing temperatures as specified in Table 1. The obtained PCR products

were digested with the appropriate restriction enzymes, and genotypes were identified by separating digested products in agarose gel electrophoresis.

Table 1. PCR-RFLP conditions for the analyzed SNPs in the UCP3 gene and information on the loci and substitution of amino acids

SNP	Location/ AA change	Primer sequence (5′–3′)	AT	AS
rs467440799 c.267G>C	exon 3 Met89Ile	F: CTCATACTCTGCGGGGTGTC R: AGCTCTGCCTCTGAGTCTGC	55°C	318
rs446854207 c.394A>G	exon 4 Thr132Ala	F: CTACACCCCCAAGGGATCGGA R: TCTGTGGGCTGGGCACATG	59°C	351
rs444645335 c.694C>A	exon 6 Leu232Met	F: GGGAAATCCCATGGGCAGAGGG R: TCCACCGGGGAGGCCACCA	61°C	283
rs440638775 c.813C>G	exon 6 Phe271Leu	F: GGGAAATCCCATGGGCAGAGGG R: GGAGGGGAGCTCACCCCTTGTA	61°C	405

AT – annealing temperature, AS – amplicon size.

The next step in the research was the analysis of the obtained genotyping results. Allele and genotype frequencies for all studied polymorphisms were calculated using POPGENE software (Yeh and Boyle 1997). Subsequently, using Statistica 13.0. software, a statistical analysis was conducted to relate the genotyping results to utility parameters obtained from breeding documentation: milk yield, fat, and protein content, and fat and protein yield in milk.

RESULTS

Restriction analysis of individual DNA fragments allowed for the identification of all three possible genotypes for all four polymorphisms, with the presence of these genotypes being determined by specific alleles. For the *rs467440799* polymorphism, following *Sau3A*I enzyme digestion, allele *G* (203, 37, 32, 25, 21 bp) and allele *C* (138, 65, 37, 32, 25, 21 bp) were identified. For *rs446854207,* the *Pci*I enzyme was used, resulting in allele *A* (332, 19 bp) and allele *G* (351 bp). For *rs4 44645335*, the *Fat*I enzyme was employed, revealing allele *C* (186, 88, 9 bp) and allele *A* (165, 88, 21, 9 bp). Lastly, for *rs440638775*, the *Rsa*I restriction enzyme was utilized, demonstrating the presence of allele *C* (324, 81 bp) and allele *G* (324, 60, 21 bp). Allele and genotype frequencies are presented in Table 2.

The obtained genotyping results underwent statistical analysis, and the outcomes are summarized in Table 3. Analysis of the results for the polymorphism mapped in exon 3 (*rs467440799*) showed that this polymorphism significantly ($p < 0.05$) affects milk, fat, and protein yield. Animals with the homozygous *GG* genotype exhibited the highest milk and fat yield, while cows with the homozygous *CC* genotype showed the lowest average protein yield. No differences were observed in the mean values for individual genotypes for the other two analyzed utility traits, fat, and protein content.

The next SNP examined was *rs446854207*. In the case of this substitution, the statistical analysis revealed that cows with the homozygous *AA* genotype produced more milk than animals with other genotypes, and individuals with the homozygous *GG* genotype showed the lowest fat yield. These observed differences were statistically confirmed ($p < 0.05$). Regarding the other analyzed traits, it was noted that for protein yield, cows with the homozygous

GG genotype exhibited the lowest average value of this trait, although the difference was not statistically significant. However, for fat and protein content, all genotyped individuals achieved similar mean values of these traits.

Table 2. Genotype and allele frequencies of *UCP3* gene

SNP	Genotype	n	Frequency	Allele	Frequency
rs467440799	*GG*	102	0.23	*G*	0.41
	GC	161	0.36	*C*	0.59
	CC	183	0.41		
rs446854207	*AA*	67	0.15	*A*	0.41
	AG	236	0.53	*G*	0.59
	GG	143	0.32		
rs444645335	*CC*	151	0.34	*C*	0.42
	CA	73	0.16	*A*	0.58
	AA	222	0.50		
rs440638775	*CC*	92	0.21	*C*	0.51
	CG	268	0.60	*G*	0.49
	GG	86	0.19		

n – number of cows.

Table 3. Mean values and standard deviation for milk production traits in references to *UCP3* genotypes

SNP	Genotype	Milk yield [kg]	Fat yield [kg]	Protein yield [kg]	Fat content [%]	Protein content [%]
rs467440799	*GG*	8331.56 ± 1343.26^{a}	324.75 ± 51.01^{a}	268.05 ± 40.52^{a}	3.87 ± 0.09	3.20 ± 0.10
	GC	8096.01 ± 1280.44^{b}	317.38 ± 52.06^{b}	263.18 ± 42.41^{a}	3.86 ± 0.12	3.20 ± 0.07
	CC	8010.56 ± 1379.19^{b}	312.09 ± 5139^{b}	257.31 ± 41.81^{b}	3.86 ± 0.12	3.18 ± 0.10
rs446854207	*AA*	8207.51 ± 1678.54^{a}	319.09 ± 62.95^{a}	262.84 ± 51.34	3.86 ± 0.09	3.19 ± 0.05
	AG	8172.12 ± 1265.55^{b}	318.55 ± 48.86^{a}	264.15 ± 40.18	3.87 ± 0.12	3.19 ± 0.10
	GG	7977.36 ± 1275.93	313.13 ± 48.86^{b}	257.70 ± 39.58	3.86 ± 0.11	3.20 ± 0.09
rs444645335	*CC*	8026.57 ± 1486.45	311.15 ± 55.03^{a}	258.70 ± 43.39	3.85 ± 0.10	3.19 ± 0.05
	CA	7933.26 ± 1327.68^{a}	310.38 ± 51.58^{a}	258.68 ± 42.82	3.85 ± 0.12	3.22 ± 0.09
	AA	8235.17 ± 1226.27^{b}	322.96 ± 48.75b	265.11 ± 40.39	3.88 ± 0.12	3.19 ± 0.11
rs440638775	*CC*	8134.21 ± 1388.25^{a}	316.72 ± 50.63	263.60 ± 40.31	3.86 ± 0.10	3.21 ± 0.03
	CG	8079.08 ± 1327.58^{b}	315.99 ± 52.99	260.93 ± 43.22	3.86 ± 0.12	3.19 ± 0.11
	GG	8205.26 ± 1331.42^{c}	319.90 ± 48.96	263.00 ± 39.50	3.87 ± 0.11	3.19 ± 0.05

a, b, c – the least squares means represented by different letters vary significantly ($p < 0.05$).

For the *rs444645335* polymorphism, statistically significant differences ($p < 0.05$) were observed for two out of five analyzed milk utility traits, namely milk and fat yield. Cows with the homozygous *AA* genotype exhibited the highest milk and fat yield, while cows with the heterozygous genotype showed the lowest milk yield. For protein yield, it was observed that animals with the homozygous *AA* genotype produced the most protein, but this observation was not statistically confirmed. Regarding fat and protein content in milk, no differences were noted in the mean values of these traits for animals with different genotypes.

For the last of the examined polymorphisms, *rs440638775*, it was also demonstrated that it significantly ($p < 0.05$) affects milk yield – cows with the homozygous *GG* genotype produced the most milk, while cows with the heterozygous genotype produced the least milk. For the other analyzed utility traits, no differences were found in the mean values of individual traits.

DISCUSSION

Uncoupling proteins, such as UCP3, which are present in various tissues including skeletal muscles and likely in mammary glands, can uncouple the process of oxidative phosphorylation from ATP synthesis, leading to the dissipation of energy as heat instead of ATP production. This process is particularly important in brown adipose tissue (BAT) due to heat generation but may also occur in other tissues, potentially influencing overall energy metabolism (Demine et al. 2019). Proper energy metabolism and thermogenesis are essential to meet the high energy demands associated with milk synthesis and lactation in dairy cattle. Any disturbances or inefficiencies in these processes may lead to reduced milk production or other metabolic issues. Polymorphisms in genes related to energy metabolism and thermogenesis, such as *UCP3*, may affect the efficiency of energy utilization and heat production, potentially influencing milk yield and composition in this way (Favorit et al. 2021).

One of the earliest studies focusing on the association of individual polymorphic sites in the *UCP3* gene with utility traits in cattle, such as growth and performance indicators, feed utilization, and carcass parameters, was conducted by Sherman et al. (2008). In this study, the authors focused on three single nucleotide substitutions mapped in introns 2, 3, and 6 and demonstrated the influence of this gene on traits such as average daily gain, feed conversion ratio, and partial efficiency of growth.

Another study on *UCP3* gene polymorphism is by Zhang and Li (2011), conducted on three native Chinese cattle breeds, where the analyzed polymorphism was shown to affect body weight, withers height, and body length at 6 months ($p < 0.05$ or < 0.01), body length at 18 months ($p < 0.05$), and body length and heart girth at 24 months ($p < 0.01$) in Nanyang cattle.

Meanwhile, Chung et al. (2011) conducted their research on native Korean Hanwoo cattle and identified ten SNPs in introns 3, 4, and 5, but growth-related analyses were performed only for one polymorphism in intron 5 (g.3076A>G), showing that this SNP significantly influenced the degree of meat marbling.

Gui and Jia (2018) mapped another polymorphism in intron 1 (g.6821C>T) of the *UCP3* gene and associated the genotyping results for this SNP with carcass quality traits in Chinese Qinchuan cattle. The authors demonstrated that the studied polymorphism significantly affected ($p < 0.05$) intramuscular fat content.

Finally, Jiang et al. (2020) conducted research on Simmental×Native Yellow Cattle crossbreeds and assessed the impact of five SNPs (exons 3 and 6) in the *UCP3* gene on growth

parameters. They showed that two out of the five analyzed polymorphisms significantly influenced ($p < 0.05$ or $p < 0.01$) parameters such as body weight, body length, body height, and chest circumference at birth, weaning, six, twelve, and eighteen months.

There are fewer publications concerning the relationship between the *UCP3* gene and dairy utility traits. In one study (Głosińska et al. 2015), polymorphism in intron 2 was analyzed about basic dairy utility parameters in Holstein-Friesian cattle, demonstrating a significant ($p \leq 0.05$) impact of this polymorphism on milk yield.

In another study (Kowalewska-Łuczak et al. 2018), the influence of polymorphism in intron 2 of the *UCP3* gene on dairy utility traits was also estimated in herds of Jersey and Holstein-Friesian cows. This study found a significant ($p \leq 0.05$; $p \leq 0.01$) impact of the studied SNP on milk yield in both herds and the case of Holstein-Friesian cows, a significant ($p \leq 0.05$) impact on milk fat content was observed.

The gene encoding uncoupling protein 3 has been studied in other livestock species as well, including pigs and poultry. In the case of pigs, the polymorphism g.946C>T in the *UCP3* gene was analyzed, showing a significant impact on abdominal fat weight and backfat thickness (Cieślak et al. 2009). Additionally, Chen et al. (2011) investigated two polymorphisms across eight pig breeds, demonstrating their significant influence on intramuscular fat content in Pietrain×Jinhua crossbreeds. In poultry, Jin et al. (2018) examined six polymorphisms in the *UCP3* gene regarding growth parameters and feed efficiency. They found that individual SNPs studied had an impact on traits such as body weight gain from 49 to 70 days of age, body weight at 70 days of age, feed conversion ratio from 49 to 70 days of age, and feed intake from 49 to 70 days of age.

The conducted study demonstrated that all four analyzed polymorphisms in the gene encoding uncoupling protein 3 significantly ($p \leq 0.05$) affect milk yield in the examined herd of Polish Holstein-Friesian cows. Additionally, three SNPs (*rs467440799*, *rs446854207*, *rs444645335)* also significantly ($p \leq 0.05$) influence fat yield, while only one polymorphism (*rs467440799*) showed a significant ($p \leq 0.05$) effect on protein yield. Based on the obtained genotyping results, it appears beneficial to prefer individuals with the *AA* genotype for the *rs467440799*, *rs446854207,* and *rs444645335* polymorphisms when aiming to improve dairy utility traits in cattle. Although the obtained results seem favorable and promising, they are preliminary due to the limited number of studies analyzing the influence of the *UCP3* gene on dairy utility traits.

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POLIMORFIZM W GENIE *UCP3* A CECHY UŻYTKOWOŚCI MLECZNEJ BYDŁA

Streszczenie. Białko UCP3 może pełnić kilka różnych funkcji, takich jak regulacja metabolizmu lipidów w mitochondriach, ochrona przed stresem oksydacyjnym oraz regulacja metabolizmu energetycznego, a prawidłowy metabolizm jest kluczowy dla krów w okresie laktacji. Celem pracy było oszacowanie częstości występowania alleli i genotypów oraz określenie wpływu poszczególnych genotypów na cechy produkcyjne mleka dla czterech polimorfizmów (*rs467440799*, *rs446854207*, *rs444645335* i *rs440638775*) w genie *UCP3*. Doświadczenie przeprowadzono w stadzie krów rasy polskiej holsztyńsko-fryzyjskiej czarno-białej, a genotypy określono metodą PCR-RFLP. Przeprowadzone badania wykazały, że analizowane pojedyncze podstawienia nukleotydowe (SNP) w istotny ($p \leq 0,05$) sposób wpływają na cechy użytkowości mleka, takie jak wydajność mleka, wydajność tłuszczu i wydajność białka.

Słowa kluczowe cechy użytkowości mlecznej, bydło, UCP3, polimorfizm genu.