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Associations between polymorphisms of the *UCP2* gene with milk production traits in dairy cattle

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SUMMARY

This study aimed to estimate the associations between four polymorphisms (*rs133711548*, *rs461080303*, *rs434063643*, and *rs464399127*) in the *UCP2* gene and selected milk production traits (milk, protein, and fat yield and protein and fat content). The study was conducted using 446 Polish Holstein-Friesian Black-and-White cows. The genotypes of individual animals were determined using the ACRS and PCR-RFLP methods. The frequency of the most common alleles was as follows: *rs133711548*: *C* – 0.72, *rs461080303*: *C* – 0.59, *rs434063643*: *T* – 0.69 and *rs464399127*: *G* – 0.71. Statistically significant differences ($P \leq 0.05$; $P \leq 0.01$) were found between individual genotypes of the SNPs and milk yield, protein yield, and fat yield.

KEY WORDS: gene polymorphism, dairy cattle, *UCP2* gene, milk traits

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INTRODUCTION

Uncoupling proteins (UCP) belong to the family of mitochondrial anion carriers. They are involved in dissipating energy within mitochondria by transporting protons from the intermembrane space to the mitochondrial matrix. This transport is induced by free fatty acids or lipid peroxidation products and inhibited by purine nucleotides (Cannon et al., 2006). These proteins are primarily located in the inner mitochondrial membrane and are involved in separating oxidative phosphorylation from ATP synthesis. This uncoupling leads to the dispersion of the proton gradient across the inner mitochondrial membrane, resulting in the generation of heat instead of ATP (Clemspon et al., 2011). The best known members of the UCP family are UCP1, UCP2, and UCP3.

UCP2 is the most widespread member of the UCP family among the cells of the body, including white adipose tissue, muscles, liver, pancreatic islet cells, and the nervous system (Jia et al., 2009). However, its expression levels vary depending on the tissue and physiological conditions. Since its discovery in 1997, UCP2 has been shown to be involved in various cellular processes, suggesting that it likely serves more than one function, depending on the physiological state of the cell (Ledesma et al., 2002).

One of its functions is regulation of the production of reactive oxygen species (ROS) and modulation of mitochondrial membrane potential. It also plays a role in controlling the energy balance and metabolic regulation, including lipid metabolism. This is indicated by the induction of *UCP2* gene expression under fasting conditions when circulation levels of fatty acids increase. Another indication is that when adipocytes are stimulated by the hormone leptin, UCP2 levels and enzymes involved in fatty acid oxidation increase. Therefore, this protein may play a dual role in this process: it can promote fatty acid oxidation unrelated to energy-demanding processes and/or prevent oxidative damage typically occurring under high lipid conditions (Donadelli et al., 2014; Ledesma et al., 2002).

Research on the role of the *UCP2* gene is an important element of expansion of knowledge in the field of livestock breeding. The objective of the present study was to identify four single nucleotide polymorphisms (SNPs) within the *UCP2* gene in a Polish Holstein-Friesian Black-and-White cattle herd, perform genotype analysis, determine allele and genotype frequencies, and relate the results to milk production traits in the herd.

MATERIAL AND METHODS

The study was carried out using a herd consisting of 446 Polish Holstein-Friesian Black-and-White cows kept in the Greater Poland Voivodeship. All animals were kept under similar environmental conditions. The cows were fed using the TMR (total mixed ration) system and had free access to fresh water. During the spring and summer, they grazed on pastures. Production records were kept for all cows, including milk, protein and fat yield [kg] and the protein and fat content in the milk [%].

The biological material from which DNA was isolated was whole peripheral blood collected in vacuum tubes (5 ml) containing K_3EDTA as an anticoagulant. The DNA isolation was performed using a commercial DNA isolation kit DNA (MasterPure).

Genotyping of individual cows was performed using the PCR-RFLP technique. Four single nucleotide substitutions in the gene encoding UCP2 were analysed. These SNPs are missense mutations and were mapped to exons 3, 5, and 8. Specific primer pairs for amplifying individual

UCP2 gene fragments were designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) based on DNA sequences from the Ensembl database. Three of these primer sequences required the use of the ACRS method, which enables the creation of a cutting site for the enzyme by introducing a mismatched nucleotide into the primer sequence. Detailed information regarding the SNPs analysed, including their locations, amino acid changes, primer sequences, annealing temperatures, restriction enzymes, and the size of PCR products and fragments after enzyme digestion, is provided in Table 1. To facilitate the description of the polymorphisms, they were designated SNP1, SNP2, SNP3, and SNP4, as indicated in Table 1.

Table 1.
PCR-RFLP conditions for the analysed SNPs in the *UCP2* gene and information on the loci and substitution of amino acids

SNP	*Loci*	location/ AA change	primer sequence (5′–3′)	AT	AS	RE	PCR–RFLP pattern (bp)
SNP1	*rs133711548* c.34*A*>*C*	exon 3 Thr12Pro	F: *AGGCCACAGATGTGCCCCGT* R: *GGCCCCTGCCTTTCTCCTTGG*	62°C	293	*Rsa*I	272,21/ 293
SNP2	*rs461080303* c.383*C*>*G*	exon 5 Ala128Gly	F: *ATGGCTTTCCGACAGATGCT* R: *TTCAGGAGAGTGTCCTTGACG*	54°C	376	*Nla*IV	232, 59, 49, 36/ 232, 108, 36
SNP3	*rs434063643* c.596*T*>*G*	exon 5 Leu199Arg				*Bst*UI	376/ 355, 21
SNP4	*rs464399127* c.826*T*>*G*	exon 8 Ser276Ala	F: *AATGGAGACTCACAGGGGAC* R: *CTACAGGGAGCTAAGGGGTG*	54°C	407	*Aci*I	191, 147, 69/ 191, 137, 69, 10

AT – annealing temperature, AS – amplicon size, RE – restriction enzyme

PCR reactions were conducted in mixtures containing the following components: appropriate forward and reverse primers, a standard ready-to-use 2xPCR Mix, DNA, and nuclease-free water. Amplification was carried out under standard thermal conditions for PCR, with elongation temperatures as specified in Table 1. The PCR products were subsequently digested with restriction enzymes specific to each of the analysed polymorphisms. Fragments obtained after enzymatic

digestion were separated using horizontal electrophoresis on 3% agarose gels stained with ethidium bromide and visualized under a UV transilluminator.

The next step was statistical analysis of the results. The genotype and allele frequencies of the SNPs were calculated using POPGENE software version 1.32 (Yeh and Boyle, 1997). This was followed by analysis of the relationships between individual genotypes and selected milk production traits, i.e. milk yield, fat yield, protein yield, and fat and protein content in the milk. The statistical analysis of relationships between genetic variants of the *UCP2* gene and these milk production traits was carried out using STATISTICA®13.0 software (Statsoft INC., 2013). Mean values ($\bar{x}$) and standard deviation (SD) for the relationships were calculated, and Duncan's multiple range test was used for a one-way analysis of variance.

RESULTS

The analysis of genotyping results revealed that for all examined polymorphic loci, three possible genotypes were identified. Information regarding genotype and allele frequencies for each polymorphism is presented in Table 2.

Table 2.
Genotype and allele frequencies of the *UCP2* gene

SNP	Genotype	n	frequency	Allele	Frequency
SNP1	*AA*	44	0.10	*A*	0.28
	AC	159	0.36		
	CC	243	0.54	*C*	0.72
SNP2	*CC*	171	0.38	*C*	0.59
	CG	186	0.42		
	GG	89	0.20	*G*	0.41
SNP3	*TT*	234	0.53	*T*	0.69
	TG	144	0.32		
	GG	68	0.15	*G*	0.31
SNP4	*TT*	58	0.13	*T*	0.29
	TG	144	0.32		
	GG	244	0.55	*G*	0.71

n – number of cows

Table 3 presents the results of the statistical analysis of individual genotypes for the polymorphisms in relation to milk production traits, including milk, fat, and protein yield and the protein and fat content in the milk.

Table 3.
Mean values and standard deviation for milk production traits in relation to *UCP2* genotypes

SNP	Genotype	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	Fat content (%)	Protein content (%)
SNP1	*AA*	8048.64±1305.22	316.16±50.25	257.86±35.28	3.87±0.10	3.18±0.14
	AC	8017.25±1237.00	312.17±46.59	258.68±39.04	3.85±0.11	3.20±0.05
	CC	8190.32±1407.45	320.09±54.91	264.69±44.57	3.87±0.12	3.19±0.10
SNP2	*CC*	8283.23^{a}±1260.89	321.89^{a}±49.76	264.88^{A}±40.12	3.88±0.11	3.20±0.07
	CG	7965.64^{b}±1444.25	313.85^{b}±54.46	259.81^{B}±44.65	3.85±0.13	3.19±0.12
	GG	8101.54^{c}±1228.92	313.60^{b}±48.99	260.44^{C}±39.17	3.87±0.10	3.20±0.04
SNP3	*TT*	9127.81^{A}±756.82	350.25^{A}±34.43	289.1^{a}±27.83	3.86±0.12	3.20±0.09
	TG	7495.91^{B}±364.74	298.88^{B}±30.58	246.2^{a}±23.55	3.87±0.12	3.20±0.05
	GG	5954.75^{C}±696.65	240.75^{C}±33.64	201.81^{b}±31.27	3.87±0.10	3.18±0.15
SNP4	*TT*	8121.31±1459.64	316.03^{a}±54.17	263.40^{a}±43.58	3.86±0.09	3.22±0.03
	TG	8216.98±1356.73	320.22^{b}±53.16	265.98^{a}±43.02	3.85±0.11	3.20±0.05
	GG	8053.48±1300.08	315.14^{a}±50.27	259.12^{b}±40.70	3.87±0.12	3.18±0.12

A, B, C – values in rows with a capital letter differ significantly at $P \leq 0.01$.
a, b, c – values in rows with lowercase letters differ significantly at $P \leq 0.05$.

In the case of polymorphism SNP1, no statistically significant differences were found in the mean values of the traits for individual genotypes. However, cows with the homozygous *CC* genotype exhibited the highest parameters for milk, fat, and protein yield. Analysis of the results for polymorphism SNP2 revealed that individuals with homozygous *CC* genotypes had the highest milk, fat, and protein production, and these results were statistically confirmed ($P \leq 0.05$; $P \leq 0.01$). Additionally, cows with heterozygous genotypes exhibited the lowest milk and protein yield, and these differences were also statistically significant ($P \leq 0.05$; $P \leq 0.01$). For SNP3, the statistical analysis showed that cows with the homozygous *TT* genotype produced significantly more milk and fat ($P \leq 0.05$), whereas cows with the opposite homozygous genotype (*GG*) had significantly lower milk, fat, and protein yield ($P \leq 0.05$; $P \leq 0.01$). In the case of SNP4, cows with heterozygous genotypes were shown to have the highest fat and protein yield, while cows with the homozygous *GG* genotype had the lowest protein and fat yield; these differences were statistically confirmed ($P \leq 0.05$).

There were no statistically significant results for fat and protein content for any of the polymorphisms (SNP1, SNP2, SNP3 or SNP4).

DISCUSSION

Genetic markers, especially those based on single nucleotide polymorphisms (SNPs), are valuable tools in dairy cattle breeding that assist in genotype selection, enable the identification of genetic traits related to milk production, and allow breeding efforts to be directed towards obtaining animals with desirable genetic characteristics. By utilizing the selection of these markers, breeders can expedite the process of obtaining animals with desirable traits and monitor the inheritance of specific characteristics. Moreover, importantly, knowledge about specific markers enables precise determination of genotypes within a dairy cattle population. This, in turn, facilitates better adaptation of breeding programmes to changing market needs and consumer preferences. Examples of such markers that are already well known include the *DGAT1* gene, which influences milk fat composition, milk yield, and milk fat percentage, the *LGB* gene associated with beta-lactoglobulin content in milk, which affects milk protein composition, and genes encoding casein proteins such as *CSN1S1*, *CSN2*, and *CSN3*, which affect milk protein composition (Ma et al., 2021; Ogorevc et al., 2009; Weigel, 2017).

The literature on the relationship between SNPs in various genes and the utility traits of cattle contains limited information on polymorphisms mapped in the gene encoding uncoupling protein 2 and focuses mainly on meat performance traits. Shermann et al. (2008) investigated four polymorphisms in exons 2 and 4, as well as introns 2 and 5, in Angus and Charolais cattle, as well as crossbreeds of these two breeds. For three of the polymorphisms, they demonstrated significant impacts ($P < 0.05$) on lean meat yield, yield grade, dry matter intake, and body weight. Subsequent studies related to the *UCP2* gene have investigated its influence on utility traits and longevity in Holstein-Friesian cattle (Clempson et al., 2011). The authors found that this gene significantly affects the longevity of cows. In our own previous study (Kowalewska-Łuczak et al., 2018), research was conducted on the *UCP2* gene in two cattle breeds – Polish Holstein-Friesian and Jersey. The study focused on milk performance in relation to selected reproductive parameters, including age at first calving and calving interval. The polymorphism analysed in the *UCP2* gene was shown to significantly affect the calving interval. Research related to this gene has also been conducted in small ruminants – in goats. The polymorphisms studied were shown to influence the fat and protein content, dry extract yield, and lactose content in the milk of Alpine and Saanen goats (Ferreira et al., 2020).

There is a scarcity of research focusing on polymorphisms in the gene encoding uncoupling protein 2 in the context of livestock performance. UCP2 belongs to the mitochondrial carrier family. These proteins are responsible for transporting a variety of compounds into the mitochondrial matrix. The UCP2 protein plays an important role in the regulation of metabolic processes (Vozza et al., 2014). It should also be noted that the amino acid substitutions resulting from the polymorphisms studied in this research are located in a region within the mitochondrial carrier protein. Therefore, it is possible that the changes may affect the function of the protein, resulting in interference with the transport of compounds into the mitochondrial matrix.

Issues concerning genes involved in mitochondrial function, which play a crucial role in cellular energy balance and are the primary site of intracellular oxygen consumption, appear to be an

interesting topic. Issues related to negative energy balance during the periparturient period, when lactation begins, are significant challenges for high-yielding dairy cows. A negative energy balance can not only affect milk production but also lead to deterioration of the functional traits of cattle. The additional energy required for milk production is sourced from the body's reserves, leading to a decline in the cow's body condition. Postpartum energy deficiencies often exacerbate fertility outcomes; for example, oocyte development is notably impaired during negative energy balance (Mekuriaw et al., 2023; Xu et al., 2020). Therefore, it is crucial to search for genes which, by influencing the maintenance of negative energy balance during the periparturient and lactation periods, may contribute to the improvement of animals' body condition and consequently enhance functional and production traits.

CONCLUSION

The research showed statistically significant relationships ($P \leq 0.05$; $P \leq 0.01$) between the selected polymorphisms in the gene encoding UCP2 and the yield of milk, protein, and fat in the Polish Holstein-Friesian Black-and-White cattle herd.

The results of this study may be applied in the selection of Polish Holstein-Friesian Black-and-White cattle or, potentially, other dairy cattle breeds, leading to economic benefits for breeders. Of course, further research on the *UCP2* gene is necessary, not only concerning milk production traits but also in relation to the functional traits of cows. Additional research conducted in different cow herds and among various cattle breeds could verify the findings of this study and expand the pool of genes that could be recognized as genetic markers.

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