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POLYMORPHISM IN THE *GHRL* GENE AND MILK PRODUCTION TRAITS IN JERSEY CATTLE

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Abstract. The aim of this study was to assess potential relationships between *GHRL 4491A>G* genotypes and selected milk production traits, including milk yield, protein and fat content, and protein and fat yield. The study involved 180 Jersey cows. Genotypes of individual animals were determined using the ACRS and PCR-RFLP methods. The allele frequencies were as follows: *A* – 0.98 and *G* – 0.02. The conducted research revealed statistically significant relationships ($P \leq 0.05$) between *GHRL* genotypes and milk protein content, and tendencies were observed regarding the association of genotypes with analyzed milk production traits.

Key words: *GHRL*, ghrelin, dairy cattle, single nucleotide polymorphism.

INTRODUCTION

Ghrelin is a peptide hormone characterized by serine modification at the third position by n-octanoyl acid residues (Anderson et al. 2005). This hormone primarily participates in the regulation of the body's energy balance (Abizaid et al. 2008). Ghrelin is predominantly produced and secreted in the stomach, but it has been shown to be produced in the kidneys, intestines, placenta, pituitary gland, pancreas, and brain (Anderson et al. 2005).

As demonstrated by Casanueva and Dieguez (2002), ghrelin is the most potent stimulator of growth hormone secretion known to date. On the other hand, the growth hormone itself is the only pituitary hormone that is tightly regulated by the metabolic environment. Therefore, ghrelin serves as a bridge connecting growth and body composition with overall metabolism. This hormone exhibits strong systemic effects, and its blood concentration increases before major meals, signaling the need for food intake (Jin et al. 2010).

The gene encoding ghrelin in cattle is located on chromosome 22 (BTA22) and consists of 5 exons and 4 introns (Colinet et al. 2009). In the case of the *GHRL* gene, alternative splicing has been demonstrated, resulting in the potential generation of multiple end products, including obestatin, which exhibits anorexigenic effects (Grala et al. 2010). The precursor of ghrelin in cattle is proghrelin, which consists of 116 amino acids (Anderson et al. 2005).

In the study by Nakahara et al. (2003), it was shown that among females subjected to ghrelin stimulation, there was an increase in milk yield. Ghrelin stimulates milk synthesis by

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increasing blood flow in the mammary gland, which is associated with the growth and development of blood vessels necessary for increased blood supply to the gland (Nakahara et al. 2003). Ghrelin present in circulation indirectly affects milk production by increasing growth hormone secretion, which is known to play a significant role during lactation (Roche et al. 2008).

The conducted research aimed to identify the *4491A>G* single nucleotide polymorphism (SNP) in the gene encoding ghrelin and assess potential relationships between specific genotypes and selected milk production traits.

MATERIAL AND METHODS

The study included a herd of 180 Jersey cows located in the Greater Poland region. All animals were kept under similar environmental conditions. The cows were fed a standard diet, and during the spring-summer period, they were maintained on pastures. Milk yield in the herd was assessed using the A4 method. Among the analyzed cows in the herd, all individuals had completed their first lactation, 151 had completed their second lactation, and 96 had completed their third lactation. Milk production records were maintained for all cows, including milk, protein, and fat yield (kg), and protein and fat content in milk (%).

Peripheral blood served as the DNA source, collected into vacuum tubes containing K3EDTA as an anticoagulant. DNA isolation was performed using the MasterPure™ DNA Isolation Kit (Epicentre®) following the isolation protocol provided with the kit.

Genotyping of individuals was performed using the PCR-ACRS method. In the studied *GHRL* gene, the *4491A>G* SNP in the ghrelin coding gene (GenBank AM691749) was analyzed. Amplifications were conducted using appropriately designed primers, resulting in a specific product of 187 base pairs. The sequences of the used primers (the modification of ACRS at position 4489 C→T is included in the forward primer sequence) were as follows: forward: GTG GGG ATC TTA AGT TCC C**T**A, reverse: AGG GTG GGA GAA CGG ACA GGT.

The *GHRL* gene fragment was amplified in a reaction following a properly selected thermal profile: initial denaturation at 94°C for 5 minutes, followed by 29 cycles of denaturation at 94°C for 30 seconds, primer annealing at 53°C for 1 minute, DNA chain extension at 72°C for 30 seconds, and final extension at 72°C for 5 minutes. After 1.5% agarose gel electrophoresis with ethidium bromide, the amplification products were viewed in UV light. The next step involved the digestion of the obtained amplification products using the restriction enzyme *Fsp*BI. The restriction enzyme digestion was carried out in a volume of 20 µl at the time and temperature recommended by the manufacturer. The obtained fragments were then separated using horizontal electrophoresis on 3% agarose gels stained with ethidium bromide and visualized under a UV transilluminator.

The obtained genotyping results were subjected to statistical analysis. Calculations were performed to determine the frequency of *GHRL*/*Fsp*BI genotypes and the frequency of individual alleles. The subsequent step was to assess potential relationships between individual genotypes and milk production traits during three subsequent lactations. The following traits were analyzed: milk, protein, and fat yield (kg), and protein and fat content in milk (%). The statistical analysis of the relationships between the SNP polymorphism in the ghrelin coding gene and milk production traits was conducted using the STATISTICA program and General Linear Model (GLM) software packages. Differences between mean trait values were analyzed using Duncan's multiple range test.

The statistical analysis was performed using the following linear model:

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

where:

Y_{ijklmn} – the value of the observed trait in the individual,
μ – average value of the trait in the tested herd,
a_i – the effect of the *4491A>G* genotype (i = 1, 2) for I, II, and III lactation,
b_j – the effect of the year calving (j = 1, 2, 3, …, 7) for I, II, and III lactation,
c_k – the effect of the month calving (k = 1, 2, 3, …, 12) for I, II, and III lactation,
d_l – the random effect of the father (l = 1, 2, 3, …, 35) for I, II, and III lactation,
e_{ijkl} – random error.

RESULTS

Restriction analysis of the investigated fragment of *the GHRL* gene, which had a length of 187 base pairs, using the *Fsp*BI enzyme allowed for the identification of two out of the three possible genotypes: *AA* (187 bp, no recognition site for the restriction enzyme) and *AG* (187 bp, 167 bp, and 19 bp). These genotypes were determined by two alleles: *A* and *G*. The frequency of the individual allele occurrence was as follows: *A* – 0.98 and *G* – 0.02, while the genotype frequencies were as follows: *AA* – 0.97 and *AG* – 0.03. No presence of the *GG* genotype was observed in the studied herd of cows.

The influence of the *4491A>G GHRL* polymorphism on milk production traits such as milk, protein, and fat yield (kg), and protein and fat content (%) in milk was analyzed. The average values and standard deviations for these milk production traits in relation to *GHRL*/*Fsp*BI genotypes are presented in Table 1.

Table 1. Mean values and standard deviation for milk production traits in references to *GHRL* genotypes

L	Genotype	n	Milk	Protein		Fat	
			kg	kg	%	kg	%
I	*AA*	174	4004 ±651	154,4 ±22,0	3,89 ±0,30	224,6 ±33,1	5,65 ±0,53
	AG	6	4046 ±570	158,0 ±14,7	3,93 ±0,27	210,0 ±18,2	5,24 ±0,59
	total	180	4006 ±647	154,5 ±21,7	3,89 ±0,30	224,1 ±32,8	5,64 ±0,54
II	*AA*	145	4322 ±695	173,3 ±23,2	3,98 ±0,25	256,4 ±38,7	5,71 ±0,57
	AG	6	4511 ±657	179,7 ±25,3	4,02 ±0,12	241,8 ±20,3	5,67 ±0,62
	total	151	4507 ±658	179,5 ±25,2	3,98 ±0,25	255,8 ±38,3	5,71 ±0,57
III	*AA*	91	4794 ±711	194,4 ±25,9	4,00 ±0,23*	277,7 ±38,5	5,73 ±0,60
	AG	5	4877 ±725	201,4 ±31,2	4,20 ±0,07*	275,0 ±39,5	5,74 ±0,24
	total	96	4872 ±721	194,8 ±26,1	4,00 ±0,23	277,6 ±38,4	5,73 ±0,58

L – lactation; n – number of cows; * mean values in the columns differ statistically ($P \leq 0.05$).

Regarding the average milk yield, it was found that cows with a heterozygous genotype had slightly higher values in the first lactation compared to the mean value (+40 kg). However, in the second and third lactations, individuals with the *AG* genotype showed lower milk yield values compared to the mean value (respectively: −185 kg and −78 kg). Analysis of the average protein yield indicated that in the first and third lactations, cows with a heterozygous genotype had higher protein yields than the mean value (+3.5 kg and +6.6 kg, respectively), while in the second lactation, individuals with the homozygous *AA* genotype had lower protein yield than the mean value (−6.2 kg). In terms of average fat yield, cows with the *AG* genotype had lower fat yield values in all three lactations (−14.1 kg, −14.0 kg, and −2.6 kg, respectively). Analyzing the protein content in milk, it was found that cows with the *AG* genotype achieved the highest values for this trait in all three subsequent lactations (+0.04%,

+0.04%, and 0.20%, respectively); in the case of the third lactation, the difference was statistically significant (P ≤ 0.05). Analysis of fat content in milk showed that in the first and second lactations, individuals with a heterozygous genotype had lower values for this trait (−0.4% and −0.04%, respectively), while in the third lactation, individuals with *AG* and *AA* genotypes achieved similar fat content values in milk.

DISCUSSION

Ghrelin plays a role in milk production by causing changes in the secretion of metabolic hormones and the distribution of nutrients necessary for milk production. Furthermore, ghrelin, through its presence in maternal milk, affects developmental changes in offspring, contributing to their maturation (Itoh et al. 2005).

Lactation is a period in a female's life that requires significant energy investment. During this time, mothers increase their food intake and utilize fat reserves to provide nourishment for themselves and their offspring. These transformations correlate with a decrease in leptin concentration and changes in the expression of peptides that regulate food intake and energy balance (Abizaid et al. 2008). During the first and final stages of pregnancy, it has been observed that there is a decrease in maternal plasma ghrelin concentrations. Exogenous delivery of ghrelin leads to increased growth hormone production, increased dry matter intake, and consequently, increased milk production in cattle (Roche et al. 2006).

The *4491A>G GHRL* polymorphism has also been studied in other cattle breeds, and a similar frequency of allele *A* has been observed: Holstein cattle – 0.97; Belgian Blue cattle – 0.81; and Simmental cattle – 0.76 (Colinet et al. 2009), Chinese Holstein cattle – 0.80 (Sun et al. 2011). Similar frequencies of allele *A* were observed in the case of the *375 A>G GHRL* polymorphism in Polish Holstein-Friesian cattle of the Red-and-White variety – 0.93 (Kowalewska-Łuczak et al. 2011).

Polymorphism in the ghrelin gene, concerning various traits in livestock, has been analyzed in cattle (Sherman et al. 2008; Sun et al. 2011; Liu et al. 2020), goats (Jin et al. 2010), pigs (Wojtysiak and Kaczor 2011; Tyra et al. 2019), sheep (Bahrami et al. 2013; Al-Shuhaib et al. 2019), and chickens (Jin et al. 2014; Sanda et al. 2021; Yang et al. 2022). In the studied animal species, associations have been found between the analyzed SNPs and traits such as feed conversion rate, growth rate, and meat quality.

CONCLUSIONS

The studies presented in the above manuscript demonstrated that individuals with the heterozygous genotype exhibited higher protein content in milk (statistically significant difference at P ≤ 0.05 in the third lactation) and lower fat yield.

Considering the fact that only two out of three possible genotypes were identified in this study, further analysis of this polymorphic site in the *GHRL* gene is necessary to assess potential relationships between specific genetic variants and selected milk performance traits. Analyses conducted on numerous cattle herds, including different breeds, would allow for the verification of the obtained research results.

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POLIMORFIZM W GENIE *GHRL* A CECHY UŻYTKOWOŚCI MLECZNEJ BYDŁA RASY JERSEY

Streszczenie. Celem prowadzonych badań było oszacowanie ewentualnych zależności pomiędzy genotypami *GHRL 4491A>G* a wybranymi cechami użytkowości mlecznej (wydajność mleka, białka i tłuszczu oraz zawartość białka i tłuszczu). Badaniami objęto 180 krów rasy jersey. Genotypy poszczególnych osobników oznaczano przy użyciu metody ACRS oraz PCR-RFLP. Frekwencja alleli była następująca *A* – 0,98 i *G* – 0,02. W prowadzonych badaniach wykazano statystycznie istotne zależności ($P \leq 0,05$) pomiędzy genotypami *GHRL* a zawartością białka w mleku oraz zaobserwowano tendencje do utrzymywania się powiązania genotypów z analizowanymi cechami użytkowości mlecznej.

Słowa kluczowe: *GHRL*, grelina, bydło mleczne, polimorfizm pojedynczego nukleotydu.