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CRH GENE POLYMORPHISM IN RELATION TO MILK PRODUCTION TRAITS IN CATTLE

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Abstract. The aim of this study was to estimate the relations between the *CRH-A145G* polymorphism and milk production traits (yields of milk, protein, and fat, as well as protein and fat content) in 176 Jersey cows. The genotype and allele frequencies were estimated and they were as follows: *AG* – 0.31; *GG* – 0.69; *A* – 0.16; *G* – 0.84. Statistical analysis revealed that studied polymorphism significantly affected the fat yield, fat content (P≤0.05) and protein content in milk (P≤0.05). The results indicate that selection for the *CRH-A145G AG* animals might contribute to increase the value of these traits in Jersey cattle. However, further studies are necessary to verify the results of our study.

Keywords: *CRH* gene, dairy cattle, milk production traits

INTRODUCTION

The objective of the breeding programs is to achieve genetic progress for traits of economic importance. The traits being constantly improved in dairy cattle, due to their breeding purpose, are milk yield and quality, and the particular emphasis is placed on the milk protein yield and, then, on milk protein and fat yield. In the case of dairy cattle, a lot of attention is dedicated to the detection of QTLs (Quantitative Traits *Loci*) affecting milk production and physiological processes influencing milk performance traits. It is noteworthy that QTLs for milk performance traits have been mapped to all the bovine autosomes, mostly to autosome 6, 14 and 20 [Khatkar et al. 2004]. Moreover, mutations in candidate genes for the above-mentioned traits are identified and analyzed [Pariset et al. 2008, Ordovas et al. 2008, Javanmard et al. 2010].

Corticotropin-releasing hormone (CRH), also called corticoliberin or corticotropin-releasing factor (CRF), is a 41-amino acid peptide deriving from a 191-amino acid precursor. It is synthesized mainly in the hypothalamus, but also in other brain areas. The highest level of the *CRH* gene expression was found in the hypothalamus, but this gene is also expressed in many other places, such as placenta, uterus, ovaries, testes, liver, stomach, skin,

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immune system. CRH functions as a neuropeptide hormone participating, among others, in the stress response in vertebrates. Corticotropin-releasing hormone is also involved in controlling the energy balance of an organism, and thus can affect body weight. In addition, it participates in modulating immune and reproductive systems [Slominski et al. 2000].

The gene encoding corticoliberin has been mapped to the bovine chromosome 14 [Barendse et al. 1997], where QTLs for the postnatal growth have also been identified. Therefore, it has been considered a candidate gene for growth traits in cattle [Buchanan et al. 2005]. Taking into account the position of this gene, in the proximity of the QTL for milk performance traits, it can be considered a positional candidate gene for the above-mentioned traits. Within the bovine *CRH* gene, several single nucleotide polymorphic (SNP) sites have been identified: *C22G* causing substitution of amino acids in the signal sequence, *A145G* and *C240G*, leading to changes of amino acids in a propeptide [Buchanan et al. 2005] as well as two SNPs in exon 2 [Sherman et al. 2008].

The undertaken study aimed at determining the frequencies of alleles and genotypes with regard to the polymorphism in the gene encoding corticotropin-releasing hormone (*CRH-A145G*) and establishing association between the genotypes and some milk production traits of Jersey cows.

MATERIAL AND METHODS

The study involved a total of 176 Jersey cows from the herd kept in the Greater Poland region. The animals were fed standard feed rations and had access to pasture during the spring-summer season. Cows were of different age and in different lactation stage. All the examined individuals completed the first lactation. Among them, 164 cows completed the first and second lactation and 96 cows completed the first, second and third lactation. Cows were milked twice daily, and test day milkings were performed according to the A4 method.

The material for DNA isolation was approx. 3 ml of the peripheral blood collected from each cow from the external jugular vein into vacuum test tubes with the K_3EDTA anticoagulating factor. DNA was isolated using the MasterPure™ kit supplied by Epicentre®Biotechnologies based on the method described by the manufacturer.

The investigated SNP consists in changing adenine to guanine at position 145 in exon 1 – *CRH-A145G* (GenBank AF340152). As a result of this transition, a change of serine to asparagine occurs at the polypeptide level. The PCR-RFLP method was used for genotyping. The gene fragment of 156 bp was amplified. To conduct this reaction, a pair of primers proposed by Buchanan et al. [2005] was used. The amplification reaction thermal program was as follows: initial denaturation at 95°C for 4 min, followed by 30 cycles: 94°C for 50 s (denaturation), 58°C for 45 s (primer hybridization), 72°C for 1 min (product synthesis), and final extension at 72°C for 6 min. The PCR products were digested with 4 U of the *Dde*I restriction enzyme.

As a part of the characterization of the genetic structure of examined herd, the frequencies of the *CRH-A145G* genotypes and alleles were estimated and 2 out of the 3 possible genotypes were identified. The next stage was the analysis of the association between

genotypes and the values of the following milk production traits in the first, second and third lactation: milk yield (kg), fat yield (kg), fat content (%), protein yield (kg), protein content (%). The production records of the animals were collected from the breeding documentation carried out for herd as a part of milk recording.

When estimating the effects of the determined genotypes, the year/season of calving and sire effect were included as an additional source of variation. The following mixed-effect model of multifactor analysis of variance (ANOVA), using the GLM (General Linear Model) procedure [Statistica® 2006] was applied:

$$y_{ijk} = \mu + a_i + b_j + c_k + d(DM) + e_{ijk},$$

where:

y_{ijk} – observed trait value in *ijk*-th animal,

μ – mean trait value for herd;

a_i – effect of genotype (i = 1, 2) – I, II and III lactation,

b_j – effect of year/season of calving (j = 1, 2, 3, ..., 16 for the first lactation; j = 1, 2, 3, ..., 14 for the second and third lactation),

c_k – effect of sire (k = 1, 2, 3, …., 19 for the first lactation; k = 1, 2, 3, …., 17 for the second lactation; k = 1, 2, 3, …., 13 for the third lactation),

d(DM) – regression coefficient of the day of milking on the trait value,

e_{ijk} – random error.

RESULTS AND DISCUSSION

Restriction analysis of the amplified fragment of the *CRH* gene enabled distinguishing two genotypes: *AG* and *GG*, determined by the presence of two alleles: *A* (156 base pair – no site recognized by the enzyme) and *G* (120 and 36 bp). The lengths of the fragments obtained as a result of digestion for the individual genotypes were as follows: *AG* – 156, 120 and 36 bp, *GG* – 120 and 36 bp. The presence of the *AA* genotype was not found, which can be explained by breed specificity or too small size of the examined herd. The frequencies of the *CRH-A145G* alleles and genotypes amounted to: *AG* – 0.31; *GG* – 0.69; *A* – 0.16; *G* – 0.84. The frequencies of *CRH-A145G* alleles were also analyzed by Buchanan et al. [2005] in Charolais cattle and amounted to: *A* – 0.28 i *G* – 0.72.

In the Table 1 are presented the mean values of the examined milk production traits in the first, second and third lactation in cattle with different *CRH-A145G* genotypes. The results of the study showed statistically significant differences in the fat yield, fat content and protein content between the groups of individuals with different *CRH-A145G* genotypes in the second lactation. Cows with the *AG* genotype were characterized by significantly higher values of the above-mentioned traits compared with the *GG* cows. The differences in the fat yield, fat content and protein content between both groups of animals were 22 kg, 0.25% and 0.08%, respectively. For the remaining analyzed traits, namely, milk yield and protein yield, no statistically sig-

nificant differences were found between cows with different *CRH-A145G* genotypes, although heterozygous cows yielded slightly more milk and protein in the first and second lactation.

Table 1. Means and standard deviations (SD) of milk production traits in calves with different *CRH-A145G* genotypes

Tabela 1. Średnie i odchylenia standardowe (SD) cech użytkowości mlecznej u krów rasy jersey z różnymi genotypami *CRH-A145G*

L	Genotype Genotyp	N	Milk yield, kg Wydajność mleka, kg	Fat yield, kg Wydajność tłuszczu, kg	Fat content, % Zawartość tłuszczu, %	Protein yield, kg Wydajność białka, kg	Protein content, % Zawartość białka, %
			mean ± SD średnia ± SD	mean ± SD średnia ± SD	mean ± SD średnia ± SD	mean ± SD średnia ± SD	mean ± SD średnia ± SD
I	*AG*	55	4006 ± 678	224.6 ± 32.9	5.66 ± 0.53	154.8 ± 21.9	3.89 ± 0.22
	GG	121	3991 ± 604	223.1 ± 30.5	5.63 ± 0.55	153.9 ± 20.3	3.89 ± 0.34
	Total Ogółem	176	3995 ± 626	223.5 ± 31.1	5.64 ± 0.54	154.2 ± 20.8	3.89 ± 0.31
II	*AG*	50	4602 ± 730	272.2 ± 41.5^{A}	5.87 ± 0.54^{A}	189.0 ± 28.5	4.04 ± 0.26^{a}
	GG	114	4477 ± 620	250.2 ± 35.2^{A}	5.62 ± 0.55^{A}	176.5 ± 23.0	3.96 ± 0.23^{a}
	Total Ogółem	164	4516 ± 655	256.9 ± 38.4	5.70 ± 0.56	180.3 ± 25.4	3.98 ± 0.24
III	*AG*	26	4708 ± 681	285.7 ± 37.8	6.09 ± 0.51	193.0 ± 26.6	4.10 ± 0.17
	GG	70	4933 ± 730	274.5 ± 38.4	5.59 ± 0.55	195.4 ± 26.1	3.97 ± 0.23
	Total Ogółem	96	4872 ± 721	277.6 ± 38.4	5.73 ± 0.58	194.7 ± 26.1	4.00 ± 0.23

The means in columns marked with the same superscript letter differ significantly: ^{a} at P≤0.05; ^{A} – P≤0.01.
Średnie wartości w kolumnach oznaczone tą samą literą różnią się między sobą istotnie: ^{a} przy P≤0,05; ^{A} przy P≤0,01.
L – the number of the lactation – L – numer laktacji.
N – number of individuals with the genotype – N – liczba osobników z danym genotypem.

The approach aimed at identifying mutations in candidate genes facilitates discovering and locating major-effect genes (in particular functional mutations within these genes) for quantitative traits. The candidate genes strategy is used for various genes whose products may affect production traits of cattle. In dairy cattle, genes encoding, among others, milk proteins (e.g. αS1-casein), enzymes involved in the metabolism of fatty acids (e.g. SCD, DGAT1), some hormones (e.g. leptin, growth hormone) or transcription factors (e.g. PIT-1) are intensively analyzed. Significant associations between polymorphisms in these genes and milk performance traits have been found [Kamiński et al. 2002, Liefers et al. 2002, Zwierzchowski et al. 2002, Citek et al. 2007].

The results of the conducted study indicate that the polymorphism within the corticotropin-releasing hormone gene (*CRH-A145G*) is associated with an increased fat yield, fat and protein content in the examined herd of Jersey cows. However, due to the lack of accessible literature data that would concern the analysis of the association between the above-mentioned polymorphism and milk production traits of cattle, the verification of

the obtained results is impossible. The research on the association between the *CRH* gene polymorphism and production traits in cattle has only been conducted in beef cattle herds. Three polymorphisms within the *CRH* gene have been analyzed in association with meat related traits [Buchanan et al. 2005], but no association between the *CRH-A145G* polymorphism and the aforementioned traits in the Charolais cattle has been found. It has been shown, however, that *CRH-C22G* polymorphism interacting with the *MC4R* gene polymorphism affected significantly carcass weight, whereas the same polymorphism interacting with the *POMC* gene polymorphism had significant effect on the loin eye area [Buchanan et al. 2005]. In another study by Buchanan et al. [2005] the association between the *CRH-C240G* polymorphism and postnatal growth of cattle has been shown.

The *CRH* gene polymorphism has also been analyzed in regard to the traits associated with meat roduction of pigs, namely, the growth, carcass and meat quality traits. Significant associations between some polymorphic sites and carcass length or carcass meat content have been found [Muráni et al. 2006].

CONCLUSION

In order to use the obtained results for the improvement of milk fat yield and fat and protein content in Jersey dairy cattle, the inclusion of individuals with the *AG* genotype in the breeding work could be considered. However, due to the absence of the individuals with the *AA* genotype in the examined herd, further research involving much larger herd as well as herds of other dairy cattle breeds is necessary. It would allow to verify the obtained results prior to their potential application in selection programs of dairy cattle.

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POLIMORFIZM W GENIE *CRH* W POWIĄZANIU Z CECHAMI UŻYTKOWOŚCI MLECZNEJ BYDŁA

Streszczenie. Celem badań było oszacowanie zależności między polimorfizmem *CRH-A145G* a cechami użytkowości mlecznej (wydajnością mleka, tłuszczu i białka oraz zawartością tłuszczu i białka w mleku) bydła. Badania przeprowadzono na stadzie 176 krów rasy jersey. Oszacowano częstość występowania genotypów i alleli, które wynosiły: *AA* – 0,00; *AG* – 0,31; *GG* – 0,69; *A* – 0,16; *G* – 0,84. Analiza statystyczna wykazała, że badany polimorfizm wpływał istotnie na wydajność tłuszczu i zawartość tłuszczu (P≤0,01) oraz na zawartość białka w mleku (P≤0,05). Wyniki wskazują, że uwzględnienie w selekcji osobników z genotypem *CRH-A145G AG* mogłoby przyczynić się do zwiększenia wartości powyższych cech u bydła rasy jersey. Wymagane jest jednak kontynuowanie badań aby móc zweryfikować uzyskane wyniki.

Słowa kluczowe: bydło mleczne, cechy użytkowości mlecznej, gen *CRH*

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