Journal of Central European Agriculture, 2011, 12(4), p.737-744 DOI: 10.5513/JCEA01/12.4.984

Ghrelin gene polymorphism in dairy cattle

Polimorfizm w genie greliny u bydła mlecznego

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Abstract

The aim of this experiment was to estimate possible associations between *GHRL G375A* genotypes and some milk performance traits (yields of milk, protein and fat, and protein and fat content). The study included Polish Holstein–Friesian strain Red-and-White cows. The ACRS and PCR-RFLP method was used to identification genotypes. *GHRL G375A* frequencies were as follow: *AA* – 0.86, *AG*- 0.14, and GG – was not found, while allele frequencies were: *A* – 0.93 and *G* – 0.07. In this study, no statistically significant correlation between *GHRL* genotypes and analyzed traits was found, however, a tendency to maintain a relationship of genotypes with milk production traits is shown.

Keywords: dairy cattle, ghrelin, milk production traits, ACRS

Abstract

Celem prowadzonych badań było oszacowanie ewentualnych zależności pomiędzy genotypami *GHRL G375A* a wybranymi cechami użytkowości mlecznej (wydajność mleka, białka, i tłuszczu oraz zawartość białka i tłuszczu). Badaniami objęto 169 krów holsztyńsko-fryzyjskiej odmiany czerwono-białej. Genotypy poszczególnych osobników oznaczano przy użyciu metody ACRS oraz PCR-RFLP. Frekwencja genotypów i alleli była następująca: *AA* – 0,86, *AG* – 0,14, *GG* – nie stwierdzono oraz *A* – 0,93 i *G* – 0,07. W prowadzonych badaniach nie wykazano statystycznie istotnych zależności pomiędzy genotypami *GHRL* a analizowanymi cechami, jednakże zaobserwowano tendencje do utrzymywania się powiązania genotypów z analizowanymi cechami użytkowości mlecznej.

Słowa kluczowe: bydło mleczne, gen greliny, cechy użytkowości mlecznej, ACRS

Detailed abstract

Badaniami objęto 169 krów rasy holsztyńsko–fryzyjskiej odmiany czerwono–białej. Do izolacji wykorzystano krew pobraną od każdego osobnika z żyły szyjnej do próżniowych probówek zawierających czynnik antykoagulacyjny K_3EDTA. Oznaczenia genotypów poszczególnych osobników dokonano przy użyciu metody PCR – ACRS. W badanym genie greliny analizie poddano miejsce polimorficzne

(tranzycja A/G; *A375G* – licząc od początku sekwencji intronu) znajdujące się na 3 intronie genu *GHRL* (GenBank AY455980). Przy użyciu zaprojektowanych starterów przeprowadzono amplifikacje, której efektem był specyficzny produkt o długości 187 par zasad. Podczas projektowania sekwencji starterowych w sekwencji startera forward uwzględniono modyfikację ACRS w pozycji 373 C→T; licząc od początku intronu 3. Po reakcji PCR otrzymany amplikon trawiono enzymem restrykcyjnym *Fsp*BI. Wyniki genotypowania, jakie uzyskano poddano analizie statystycznej. Wykonano obliczenia częstości występowania genotypów *GHRL A375G* oraz częstości występowania poszczególnych alleli. Kolejnym krokiem było oszacowanie ewentualnych zależności między poszczególnymi genotypami a cechami użytkowości mlecznej w trzech kolejnych etapach laktacji. W analizie restrykcyjnej fragmentu intronu 3 genu greliny zidentyfikowano dwa z trzech możliwych genotypów: *AA* oraz *GA*. Obecność tych genotypów warunkują dwa allele: G oraz A. Frekwencja poszczególnych genotypów i alleli *GHRL* została przedstawiona w tabeli 1.Tabela 2 przedstawia wartości średnie oraz odchylenia standardowe dla poszczególnych cech użytkowości mlecznej w odniesieniu do dwóch genotypów *GHRL A375G* (*AA* i *GA*). Analizując średnią wydajność mleka stwierdzono, że zarówno podczas I jak i II i III laktacji krowy o genotypie *GA* produkowały najwięcej mleka W odniesieniu do kolejnej analizowanej cechy – wydajności białka w mleku stwierdzono, że najwyższą średnią wydajność osiągały zwierzęta o genotypie heterozygotycznym we wszystkich trzech kolejnych laktacjach. W przypadku średniej wydajności białka w mleku w laktacji I krowy o genotypie heterozygotycznym i homozygotycznym *AA* osiągały podobną średnią wartość analizowanej cechy, natomiast w laktacji II i III nieznacznie wyższą zawartością białka w mleku charakteryzowały się krowy o genotypie *GA*. Analiza statystyczna wydajności tłuszczu pozwoliła na stwierdzenie, że w kolejnych trzech laktacjach zwierzęta o genotypie heterozygotycznym osiągały najwyższe wartości badanej cechy. Rozpatrując zawartość tłuszczu w mleku wykazano, że w I laktacji krowy o genotypie heterozygotycznym osiągały najwyższą wartość tej cechy, natomiast w laktacji II i III osobniki o genotypie homozygotycznym *AA* charakteryzowały się najwyższą zawartością tłuszczu w mleku. Zaobserwowano pewne tendencje między poszczególnymi badanymi genotypami a analizowanymi cechami. We wszystkich 3 kolejnych laktacjach osobniki heterozygotyczne charakteryzowały się najwyższymi średnimi wartościami większości analizowanych cech. Ze względu na brak obecności genotypu homozygotycznego *GG* w analizowanym stadzie krów trudno jest oszacować czy miałby on wpływ na analizowane cechy.

Introduction

Ghrelin (*GHRL*) is a peptide hormone characterized by the presence of the n-octanoyl group at position 3 (usually serine). Ghrelin precursor in cattle is proghrelin consisting of 116 amino acids [2].

Ghrelin is a hormone involved in the regulation of the energetic balance of an organism [1]. It is secreted mainly by stomach but it is also produced in kidneys, intestines, placenta, pituitary gland, pancreas and brain [2]. This hormone has strong

systemic effect and its concentration in blood increases before main meals signalling the necessity of food intake [7].

Ghrelin is the strongest of the growth hormone secretion stimulators discovered so far. Growth hormone is the only pituitary hormone that is strictly controlled by the metabolic environment. Ghrelin is a bridge connecting growth and composition of the body with general metabolism [3].

An increase in the milk yield in females subjected to stimulation with ghrelin has been observed. Ghrelin stimulates milk synthesis through an increase in blood flow in the mammary gland, which is associated with a growth and development of blood vessels necessary for an increased blood supply in the gland [8]. Growth hormone plays an important role during lactation, whereas ghrelin present in the blood circulation indirectly affects milk production by increasing GH secretion. Growth hormone regulates the lipid metabolism in the adipose tissue whereby contributing to the reduction in body weight during lactation [10].

Bovine *GHRL* gene has been localized on chromosome 22 (BTA22) and it is completely sequenced – it consists of 5 exons and 4 introns – 532 bp in total [4]. There are several ways of alternative splicing of the primary transcript of ghrelin gene, which manifests itself in the possibility of producing a larger number of final products, among others, obestatin that shows anorexigenic effect [5].

Material and methods

The study included 169 Polish Holstein–Friesian strain Red-and-White cows. All testes animals were kept in identical environmental conditions in the south-western region of Poland. All cows completed three consecutive lactations. Cows were standard fed, and seasonally (in spring and summer) put out to pasture. DNA was isolated from blood samples collected into test tubes containing K_3EDTA. The genotypes of individuals were determined using PCR-ACRS method. In the studied *GHRL* gene polymorphic site (transition A/G; *G375A* - counting from the beginning of the intron) located in intron 3 (GenBank AY455980) was analyzed. Using the designed primers amplifications were carried out and received a specific product with a length of 187 base pairs. Sequences of used primers (forward primer in the sequence included ACRS mismatched nucleotide of position 373 C→T; counting from the beginning of the intron 3) were as follow:

forward: GTG GGG ATC TTA AGT TCC CTA ,

reverse: AGG GTG GGA GAA CGG ACA GGT.

The reaction was performed as follows: initial denaturation at 94°C for 5 min, 29 cycles of denaturation (94°C, 30 s), annealing (53°C, 60 s), elongation (72°C, 30 s), final extending (72°C, 5 min). The next step was to digest obtained amplification products with the restriction enzyme *Fsp*BI. The restriction fragments obtained were analyzed on a 2.5% agarose gel stained with ethidium bromide. The gels were

visualized under UV light and documented with the use of imaging system. Next, the obtained genotyping results were statistically analyzed. The initial stage was estimation frequencies of alleles and genotypes of *GHRL A375G*. The next stage involved an analysis of associations between the ghrelin genotypes and milk production traits in three consecutive lactations. Analyzed the following traits: milk yield (kg), protein yield (kg), fat yield (kg), milk protein content (%), and milk fat content (%).The analysis of associations between the *GHRL* genotypes and milk production traits was performed according to the GLM procedure using Statistica software, version 7.1. [12].

Results

In the restriction analysis of the 187 bp fragment of intron 3 of ghrelin gene, the *Fsp*BI restriction enzyme was used, which allowed identification of the two of three possible genotypes: *AA* (187 bp, no restriction site) and *GA* (187 bp, 167 bp and 19 bp). The occurrence of these genotypes is determined by two alleles: G and A. The frequencies of individual *GHRL* genotypes and alleles are presented in Table 1. The conducted analysis of the herd of Holstein-Friesian cows of Red-and-White strain revealed that *AA* is the most frequently occurring genotype (0.86), following by *AG* genotype (0.14), whereas the occurrence of *GG* was not found in the analyzed herd.

Table 1 Frequency of genotypes and alleles of *GHRL A375G*
Tabela 1 Frekwencja genotypów i alleli *GHRL A375G*

	Genotype / Genotyp		Allele / Allel	
	AA	GA	A	G
frequency	0.86	0.14		
frekwencja			0.93	0.07
number	145	24		
liczebność				

Table 2 presents mean values and standard deviations for individual milk performance traits with regard to two *GHRL G375A* genotypes (*AA* and *GA*).

When analyzing the mean milk yield, it was found that cows with the *GA* genotype, in the first as well as the second and third lactation produced most milk (+77, +127, +137, respectively). In terms of the next analyzed trait – milk protein yield, it was found that the highest mean yield was obtained by animals with the heterozygous genotype in all the three consecutive lactations (+2.6, +3.9, +1.3 for the first, second and third lactation, respectively). In the case of the mean milk protein yield in the first

Table 2 Mean values and standard deviation (SD) of studied traits in references to *GHRL A375G* genotype; L – lactation
Tabela 2 Wartości średnie i odchylenia standardowe (SD) badanych cech użytkowości w odniesieniu do genotypów *GHRL A375G*; L – laktacja

L	Genotype genotyp	n	Milk yield wydajność mleka	Protein Białko		Fat Tłuszcz	
			[kg]	[kg]	[%]	[kg]	[%]
I	*AA*	145	6720±1378	220.4±42.4	3.34±0.25	268.7±55.4	4.04±0.44
	GA	24	6797±1291	223.0±38.9	3.34±0.23	271.7±64.2	4.10±0.53
	Total Ogółem	169	6731±1362	220.8±41.8	3.34±0.25	271.2±62.9	4.10±0.51
II	*AA*	145	8362±1600	277.5±44.7	3.39±0.30	343.5±72.9	4.20±0.52
	GA	24	8489±1496	281.4±43.2	3.37±0.24	350.7±83.1	4.17±0.62
	Total Ogółem	169	8380±1582	278.1±44.4	3.39±0.02	344.5±74.2	4.20±004
III	*AA*	145	8856±1534	287.3±41.8	3.27±0.20	382.5±89.2	4.41±0.63
	GA	24	8993±1299	288.6±46.3	3.36±0.25	390.0±96.8	4.39±0.74
	Total Ogółem	169	8876±1500	288.4±45.6	3.34±0.24	383.5±90.0	4.40±0.64

lactation, cows with the heterozygous and homozygous *AA* genotype reached a similar mean value of the analyzed trait, whereas in the second and third lactation cows with the *GA* genotype were characterized by somewhat higher milk protein content. Statistical analysis of milk fat yield allowed us to conclude that, in the three successive lactations, animals with the heterozygous genotype reached the highest values of the analyzed trait (+2.4, +7.2, +7.5, respectively). When analyzing milk fat content, it was shown that, in the first lactation, cows with the heterozygous genotype reached the highest value of this trait, whereas in the second and third lactation individuals with the homozygous *AA* genotype were characterized by the highest milk fat content. Some trends between the examined genotype and individual analyzed traits were observed. In all the three successive lactations, heterozygous individuals were characterized by the highest mean values of most analyzed traits. Due to the lack of the homozygous *GG* genotype in the analyzed herd of cows, it is difficult to estimate its effect on the analyzed traits.

Discussion

Ghrelin plays a certain part in the milk production through the induction of changes in the secretion of metabolic hormones and distribution of nutrients essential for the food production. Moreover, due to its occurrence in the dam's milk, it affects developmental changes of the offspring contributing to reaching maturity by them [6].

Lactation is a period during the life of a female that requires considerable energetic expenditure. At that time, dams increase food intake and use fat reserves in order to provide nourishment for themselves and their offspring. These changes correlate with a decrease in the leptin level and with the changes in the expression of peptides regulating food intake and energetic balance [1]. In the initial and final stage of lactation, a decrease in the ghrelin concentration in the dam's plasma occurs. Exogenous administration of ghrelin results with increased production of growth hormone, increased intake of dry matter and increased milk production in cattle associated with it [9]. Initiation of lactation dramatically changes the metabolism of many organs so that the mammary gland can be equipped with the nutrients essential for milk synthesis. Females during lactation must generate enough energy to maintain their own homeostasis and that of their offspring. During lactation, an increase in the food intake and, at the same time, decrease in energetic expenditure occurs, which is possible due to the utilization of the energy stored in fat for milk synthesis. Therefore, it is possible to maintain the ghrelin concentration at the basic level [10].

SNP in intron 3 of *GHRL* has also been analyzed on other cattle breeds and the similar frequency of the *G* allele was found: 0.15, 0.12, 0.03, 0.19 and 0.24 for Angus, Charolais, Holstein, Belgian Blue and Simmental cattle, respectively [4, 11]. In the bovine ghrelin gene itself, eight SNPs have been detected so far. All of them are located in the non-coding parts of the gene [4]. Also in goats, SNP in the *GHRL* gene was found and it is also located in the non-coding sequence [7].

Polymorphism in the gene coding for ghrelin with regard to various traits of an organism has only been analyzed in cattle [11] and goats [7] so far. In the case of both studied species, an association between the analyzed SNPs and traits such as feed conversion index or growth rate was found.

Conclusion

The results of the present work did not show any statistically significant differences between individual *GHRL G375A* genotypes in Holstein-Friesian cattle of Red-and-White strain with regard to the aforementioned traits. However, this research allowed indication of the individuals characterized by the highest and the lowest values for the given traits.

Due to the fact that only two out of the three possible genotypes were identified in the present study, a further analysis of this polymorphic site within the *GHRL* gene will be

necessary in order to estimate potential associations between individual genetic variants and selected milk performance traits. Analyses that would be carried out on the basis of numerous cow herds and on herds of cattle of various breeds would allow verification of the obtained results.

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