ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY ZEMĚDĚLSKÉ A LESNICKÉ UNIVERZITY V BRNĚ

Volume LVI 20 Number 4, 2008

EFFECT OF GROWTH HORMONE-RELEASING HORMONE GENE POLYMORPHISM (*GHRH/HAE*III) ON MILK PERFORMANCE IN POLISH HOLSTEIN-FRIESIAN COWS

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Abstract

SZEWCZUK, M., ZYCH, S., CHABERSKI, R.: *Effect of growth hormone-releasing hormone gene polymorphism (GHRH/HaeIII) on milk performance in polish holstein-friesian cows.* Acta univ. agric. et silvic. Mendel. Brun., 2008, LVI, No. 4, pp. 177–182

The objective of this study was to evaluate the relationship between the polymorphism within *GHRH* gene (*GHRH/Hae*III) and milk production traits for Polish Holstein-Friesian using PCR-RFLP technique for genotyping. The molecular background of this mutation was defined as the A→C transversion at the intron 2. A total of 220 cows were examined. The following frequencies were established: 0.0227 for genotype *AA*, 0.3227 for *AB*, and 0.6546 for *BB*; 0.1841 for the allele $GHRH^A$ and 0.8159 for $GHRH^B$. A highly significant association was found between the *GHRH/Hae*III polymorphism and milk performance. The BB-genotype cows were characterised by a higher fat yield and percentage ($P \leq 0.01$).

dairy cattle, milk production traits, PCR-RFLP, somatoliberin

Molecular genetics has created new means of animal improvement. A range of genetic markers associated with milk performance have been discovered. New genes that encode the proteins that take part in the enzymatic pathway of milk components biosynthesis (i.e. milk proteins genes expression regulation), or those involved in the development and metabolism of the mammary gland functional cells are being found and identified. The genes that encode the somatotropic axis proteins are considered to be crucial for these processes (PARMENTIER et al., 1999). Growth hormone-releasing hormone *(GHRH),* or somatoliberin, belongs to the group of hormones that take part in this complex structure. The primary role of this pituitary-gland hormone is to release the growth hormone from the anterior pituitary.

The bovine *GHRH* gene, which is composed of five exons separated by four introns, has been mapped at the chromosome 13 (BARENDSE et al., 1994). The unprocessed protein precursor of the hormone comprises 106 amino-acid residues encoded by the exons 2–5. However, the target molecule of bovine somatoliberin is composed of 44 amino acids whose sequence is determined by the exons 3 and 4 of the *GHRH* gene (MONTERO et al., 2000).

The polymorphism of the *GHRH/Hae*III, first described by MOODY et al. (1995), is one of potentially important polymorphic sites. The literature published so far does not bring much information on this issue. For example, the molecular background of the mutation remains unknown. Thus, it seams purposeful to carry out thorough and in-depth studies on the possible effects of the *GHRH/Hae*III gene polymorphism on milk performance in dairy cattle.

MATERIALS AND METHODS

The studies involved 220 Polish Holstein-Friesian cows bred on a farm located in the Greater Poland. The blood was collected from the external jugular vein into K3EDTA-containing test tubes. DNA was isolated from the peripheral blood using the *MasterPure™ Genomic DNA Purification Kit* (Epicentre Technologies).

The following primers designed by DYBUS et al. (2003) were applied:

GHRH F 5' TTCCCAAGCCTCTCAGGTAA 3'
GHRH R 5' GCGTACCGTGGAATCCTAGT 3'

A 297-bp PCR product was amplified using the *Biometra* thermal cycler. The PCR mixture contained 90 ng of genomic DNA, 10 pmol of each primer, 2 µl 10x buffer with $(NH_4)_2SO_4$ (750 mM Tris-HCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 1.2 µl 25 mM $MgCl_2$, 2 µl 2 mM dNTP mix, and 0.5 unit of Taq DNA polymerase (*MBI Fermentas*); deionized water was added to obtain a volume of 20 µl. The following thermal cycling conditions were applied: initial denaturation at 94 °C for 5 min.; 35 cycles (denaturation, 94 °C/1 min., annealing, 60 °C/50 s., extending, 72 °C/40 s.), final extension at 72 °C/5 min.

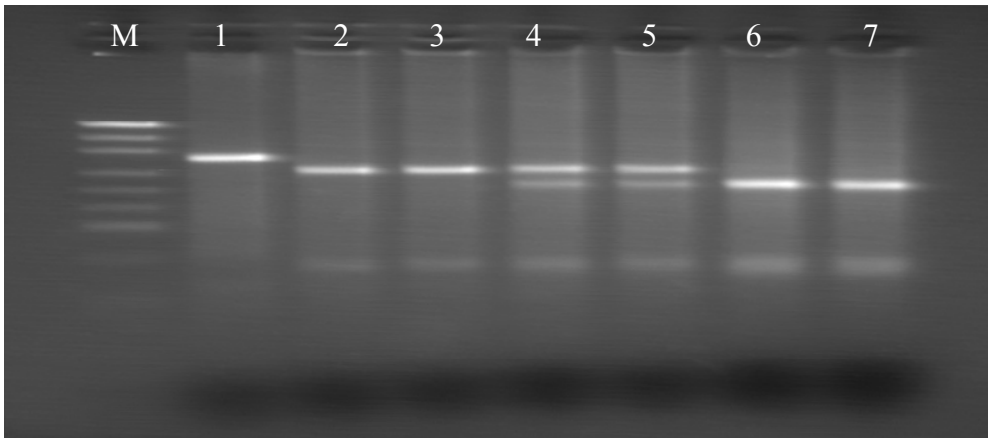
The genotypes were identified by restriction analysis. The PCR-amplified DNA was digested with 5 units of the *Hae*III restriction enzyme (15 U/µl, GG↓CC, *EURx*). The product was separated on 2% agarose gel (*Prona Basic, le GQT*) with ethidium bromide staining. The electrophoresis was carried out in 1 x TBE buffer. The gels were visualized under UV light (312 nm) using the *Vilber Lourmat* transilluminator.

An association of the *GHRH*/*Hae*III polymorphism with milk yield (in kg), milk fat and protein yield (in kg), as well as milk fat and protein content (percentage) was analysed. The analysis of milk performance was based on the data obtained from the official milk recording performed with the A_4 method. The significance of differences between particular traits was tested using a one-way ANOVA with the Tukey test.

In order to determine the molecular background of the mutation, selected PCR products were sequenced (IBB PAN, Warsaw).

RESULTS AND DISCUSSION

Two alleles (*GHRH*^{A} and *GHRH*^{B}) and three genotypes (*AA*, *AB*, *BB*) were found in the analysed herd of dairy cows. The following restriction fragments: 242 bp and 55 bp for genotype *AA*, 242 bp, 194 bp, 55 bp and 48 bp for genotype *AB*, and 194 bp, 55 bp and 48 bp for genotype *BB* were obtained (Fig. 1).



1: *Gel image of GHRH/HaeIII restriction fragments*
Lane M, DNA size marker (pUC19/*MspI*); lane 1, PCR product; lanes 2 and 3, genotype *AA*; lanes 4 and 5, genotype *AB*; lanes 6 and 7, genotype *BB*

I: Presents the frequencies of the genotypes and alleles of the somatoliberin gene in the studied herd of dairy cows in the experimental design with *Hae*III restriction endonuclease.

I: *Frequencies of GHRH/HaeIII genotypes and alleles in the studied population of cows*

Polymorphism *GHRH/Hae*III	Genotype			Total	Allele	
	AA	AB	BB		*GHRH*^{A}	*GHRH*^{B}
n	5	71	144	220	0.1841	0.8159
frequency	0.0227	0.3227	0.6546	1.0000		

The *GHRH*^{A} allele was found with the frequency of 0.1841, whilst *GHRH*^{B} with the frequency of 0.8159. Higher frequencies of rare *GHRH*^{A} allele were reported by DYBUS and GRZESIAK (2006) (0.2480) as well as KLAUZIŃSKA et al. (2004) (0.2400). Besides, frequencies for the *GHRH*^{A} allele considerably differed between breeds. MOODY et al. (1995) reported that frequencies for the *GHRH*^{A} allele were 0.07 and 0.7 for Hereford and Angus sires, respectively. For Limousine cattle DYBUS et al. (2003) estimated the frequency at 0.01.

The *AA* genotype occurred in as few as 5 cows of the studies population, which was reflected by the lowest frequency, i.e. 0.0227. The *AB* genotype oc-

curred with the frequency 0.3227, whereas the majority of the cows were characterised by the *BB* genotype, with its frequency being 0.6546. Similar frequencies of these genotypes were found by DYBUS and GRZESIAK (2006) (*AA*, 0.0545; *AB*, 0.3133; *BB*, 0.6322).

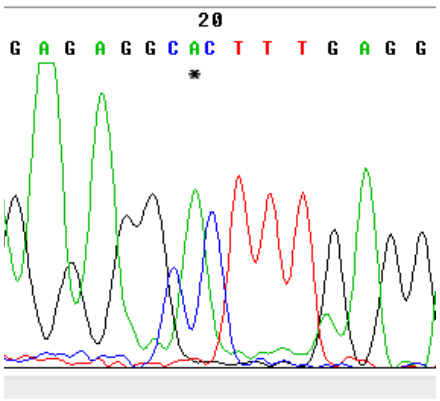
Means, standard deviations, and coefficients of variability of the 305-day milk production traits of the 220 Polish Holstein-Friesian cows sample are presented in Tab. II.

II: *Means (X), standard deviations (SD), and coefficients of variability (V) for milk performance traits in the analysed herd of cows in relation to GHRH/HaeIII genotypes*

Trait		Genotype		
		AA	AB	BB
Milk [kg]	X	7948	7911	7884
	SD	1135.4	1255.9	1219.0
	V	14.3	15.9	15.5
Fat [kg]	X	299.6^{AB}	326.8^{B}	330.0^{A}
	SD	60.8	56.3	54.3
	V	20.3	17.2	16.4
Protein [kg]	X	259.8	253.1	252.7
	SD	42.8	40.4	38.0
	V	16.5	16.0	15.1
Fat [%]	X	3.77^{AB}	4.14^{B}	4.20^{A}
	SD	0.44	0.38	0.47
	V	11.7	9.3	11.2
Protein [%]	X	3.27	3.21	3.22
	SD	0.25	0.22	0.20
	V	7.6	6.9	6.2

^{AB} Means in rows marked with capital letters differ at $P \leq 0.01$

As a result of DNA sequencing (IBB PAN, Warsaw), we have obtained fluorograms which were used to determine the molecular background of the mutation (Fig. 2). The sequence of bovine *GHRH* intron 2, with regard of the polymorphic site, has been deposited in GenBank under accession number EF210074.



2: *A part of the fluorogram of intron 2 of bovine GHRH gene for rare AA genotype (* mutation site)*

Statistically significant differences between the *GHRH/Hae*III polymorphism and milk production traits were found only in relation to fat yield and content. The highest fat yield (kg) and fat content (%) were achieved by the *BB* cows (330 kg and 4.20%), the *AB* cows were in the middle (326.8 kg and 4.14 %), whereas the *AA* genotype showed the poorest results (299.6 kg and 3.77%) ($P \leq 0.01$). Our results are therefore in contradiction to the preliminary data published by PARMENTIER et al. (1999). According to these authors, the rare *AA* genotype was significantly associated with the discussed traits ($P \leq 0.01$). DYBUS and GRZESIAK (2006) also found that cows with one or two *GHRH*^{A} alleles might produce milk of a higher fat percentage, although this was not statistically significant. However, a low number of the individuals with the *AA* genotype strongly limits the conclusions. Studies by BESWICK and KENNELLY (1998) may confirm the role of GHRH in regulation of milk fat synthesis. These authors measured the level of mRNA and protein abundance of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Both, the growth hormone and growth hormone-releasing hormone, the starting components of the so-

matotropic axis, significantly influenced the synthesis of these enzymes in the mammary gland. The GH receptor (GHR) mRNA was highly expressed in all examined stages (mammogenesis, lactogenesis, galactopoiesis and involution) (PLATH-GABLER et al., 2001). Moreover, the receptor protein was also demonstrated in the mammary gland samples by *in situ* hybridisation and immunohistochemistry in the epithelial and stromal compartments (SINOWATZ et al., 2000). Therefore, a direct effect of growth hormone (and indirect GHRH) on development and function of the bovine mammary gland seems to be indicated.

The *AA* cows, on the other hand, were characterised by slightly higher milk (7948 kg) and protein (259.8 kg) yields as compared with the *AB* (respectively, 7911 kg and 253.1 kg) or *BB* (7884 kg and 252.7 kg) cows. This has not been a significant difference, however. DYBUS and GRZESIAK (2006) also reported that *AA* cows were characterised by a higher milk yield (for the 2nd and 3rd lactation), although differences were non-significant either.

MOODY et al. (1995) and several other elaborations did not specify exactly localization of discussed mutation. DYBUS and GRZESIAK (2006) only suggested that this polymorphism is located at the intron 2. Their findings were based on the complete sequence of the bovine *GHRH* (GenBank accession number AF242855) and probably on localisation of two *Hae*III restriction sites within PCR product. A comparison of our results with the sequences deposited in GenBank (GenBank accession numbers AF242855, U29611 and AF168686) confirms that the *GHRH*/*Hae*III polymorphism is located at intron 2. Moreover, the specificity of the mutation has been determined, that is the transversion A→C located at the initial part of intron 2, at the position A44C (GenBank accession number EF210074). A DNA polymorphism located at the non-coding part (intron in this case) does not directly influence the protein structure. However, mutations in splicing consensus sequences, as well as in the neighbouring regions, may affect the process of exon splicing. *GHRH*/*Hae*III polymorphism has been located at the intron that precedes the exon 3, which – like the exon 4 – encodes the target molecule of the hormone.

CONCLUSIONS

The preliminary results presented in this study disqualify the *GHRH*/*Hae*III polymorphism as a useful marker in the selection of Polish Holstein-Friesian cows for improved milk yield or milk protein yield or percentage, but an effect of this polymorphism on milk performance of dairy cows has not been fully documented. It has been suggested instead that the polymorphism could be used in selection for higher fat yield and percentage; however, due to lack of evident results, a low frequency of the *AA* genotype, and the polygenic regulation of milk synthesis, it should be verified in further research.

SOUHRN

Efekt polymorfismu genu růstového hormonu (*GHRH*/*Hae*III) na mléčnou užitkovost holštýnsko-fríských krav v Polsku

Cílem publikace bylo vyhodnocení vztahů mezi polymorfismem *GHRH* genu (*GHRH*/*Hae*III) a znaky mléčné užitkovosti u holštýnsko-fríských krav v Polsku s využitím PCR-RFLP metod pro genotypování. Molekulární základ sledované mutace byl definován jako A→C transverze na intronu 2. Do sledování bylo zahrnuto celkem 220 dojnic. Získány byly následující frekvence: 0,0227 pro genotyp *AA*, 0,3227 pro *AB*, a 0,6546 pro *BB*; 0,1841 pro alelu $GHRH^A$ a 0,8159 pro alelu $GHRH^B$. Vysoce signifikantní vztah byl potvrzen mezi *GHRH*/*Hae*III a produkcí mléka. Pro krávy genotypu BB byla charakteristická vyšší produkce i obsah tuku ($P \leq 0,01$).

dojnice, holštýn, mléčná užitkovost, somatoliberin

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