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*GHRH/Hae*III gene polymorphism and its associations with milk production traits in Polish Black-and-White cattle
(short communication)

Abstract
The objective of this paper was to evaluate the relationship between the polymorphism of the *GHRH* and milk production traits of Polish Black-and-White. A total of 881 cows were included in the study. A PCR-RFLP method was used to genotyping. The frequencies of the genotypes and alleles were as follows: 0.0545 for *AA*, 0.3133 for *AB* and 0.6322 for *BB*, and 0.2111 for GHRH^{A} and 0.7889 – *GHRH*^{B}. There were no significant associations between *GHRH/Hae*III polymorphism and milk production traits of the analysed cows.

Key Words: *GHRH* gene, PCR-RFLP, milk production traits, dairy cattle

Zusammenfassung
Titel der Arbeit: **Polymorphismus des *GHRH/Hae*III-Gens und sein Zusammenhang zwischen den Milchleistungsmerkmalen bei schwarzbunten Kühen** (Kurzmitteilung)
Zusammenhänge zwischen dem Polymorphismus des *GHRH*-Gens wurden bei schwarzbunten Kühen analysiert. Die Untersuchungen wurden an 881 Kühen durchgeführt. Angewandt wurde die PCR-RFLP-Methode. Die Genotyp- und Allelfrequenz war wie folgt: für *AA* 0.0545, für *AB* 0.3133 und für *BB* 0.6322 sowie für *GHRH*^{A} 0.2111 und 0.7889 für GHRH^{B}. Zwischen dem Polymorphismus von *GHRH/Hae*III-Gen und den Milchleistungsmerkmalen bei untersuchten Kühen wurden keine Zusammenhänge festgestellt.

Schlüsselwörter: *GHRH-G*en, PCR-RFLP, Milchrind, Milchleistungsmerkmale

Introduction
Identification of the genes underlying livestock production traits (quantitative trait loci – QTL) is likely to lead to more efficient the traditional breeding programs and is a promising way to improve production traits of farm animals. Among the putative candidate genes, milk protein loci have been studied most extensively (PARMENTIER et al., 1999). Another group are genes related to the somatotropic axis (RENAVILLE et al., 1997; SØRENSEN et al., 2002; DYBUS et al., 2003, 2005).
Growth hormone releasing hormone (GHRH) stimulates both synthesis and secretion of pituitary growth hormone (GH) binds to specific receptors on somatotrophs (FROHMAN et al., 1992). Bovine GHRH increased the serum concentration of endogenous GH (LØVENDAHL et al., 1991) and increased milk production (LAPIERRE et al., 1988). VANDERKOOI et al. (1995) reported that bGHRH stimulates milk synthesis through the same mechanisms as bGH. BESWICK and KENNELLY (1998), who measured mRNA and protein abundance of the acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) in the mammary gland and adipose tissue, presented a potential role of GHRH in the lipid metabolism of the mammary gland.

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Bovine *GHRH* gene was linked to *CSSM30* on chromosome 13 and represents U11 synteny group (BARENDSE et al., 1994). The bovine *GHRH* gene consists of five exons separated by four introns (ZHOU et al., 2000).
In a preliminary study on 89 artificial insemination (AI) Holstein-Friesian bulls, MOODY et al. (1995) observed that the rare (7.7%) *AA* genotype, identified with PCR-RFLP *Hae*III restriction enzyme improved both fat percentage and fat yield.
The aim of this study was to estimate the allelic frequencies at the *GHRH-Hae*III *locus* and to investigate the relationship of this polymorphism and milk production traits of Polish Black-and-White cows.

Materials and methods

A total of 881 Black-and-White (B&W) cows were genotyped. The *GHRH-Hae*III genotypes were analysed using the PCR-RFLP method. Crude DNA was isolated from blood samples using *MasterPure*^{TM} kit (Epicentre Technologies). A 297-bp fragment of the *GHRH* gene was amplified using methodology of DYBUS et al. (2003). The following cycles were applied: initial denaturation, 94ºC/5 min, followed by 30 cycles: denaturation, 94ºC/40 sec, primer annealing, 60ºC/40 sec, PCR products synthesis, 72ºC/40 sec, and final synthesis, 72ºC/5 min. The amplified DNA was digested with 5 units of *Hae*III enzyme (MBI Fermentas). The digestion products were separated by horizontal electrophoresis (90 volts, 50 minutes) through 3% agarose gels (Prona) in 1 x TBE and 0.5 µM ethidium bromide.
Data for 305-day milk production in the first, second and third lactation were obtained from farm records. Statistical calculations were performed using procedures of STATISTICA (2005). The effect of the *GHRH/Hae*III genotypes on the milk production traits were analysed using a General Linear Model (GLM). The following statistical model was used:

$$y_{ijklmno} = \mu + G_i + s_j + D_k + H_l + YS_m + b_1(x_n - A_n) + b_2(x_o - HF_o) + e_{ijklmno}$$

where:

$y_{ijklmno}$ – analyzed trait; μ – overall mean; G_i – fixed effect of GHRH genotype (i=1, …3); s_j – random effect of sire (j=1, …175/147/103); D_k– fixed effect days of milk (k=1, 2); H_l – fixed effect of barns (l=1, …5); YS_m – fixed effect year-season (m=1, …17/15/13*); b_1 – linear regression coefficient of calving age; x_n – calving age in month of a cow (n=1,2); A_n – mean calving age; b_2 – linear regression coefficient of percentage of HF genes; x_o – percentage of HF genes in a cow (0=1, 2); HF_o – mean percentage of HF genes; $e_{ijklmno}$ – random error*
** - in the first/second/third lactation*

Results

The following restriction fragments were obtained: 242 and 55 bp for the *AA* genotype, 242, 194, 55, 48 for the *AB* genotype, and 194, 55, 48 bp for the *BB*.
The table shows the influence of the *GHRH-Hae*III polymorphism on milk production traits in the Black-and-White cows.
In the analysed population of B&W cattle, the *BB* genotype was the most frequent (0.6322), followed by the *AB* (0.3133), whereas the *AA* was the least frequent (0.0545), respectively. The frequency of $GHRH^A$ estimated for analysed cattle was 0.2111. A higher frequency of the $GHRH^A$ (0.70) in Angus cattle, and lower (0.07 and 0.1) in Hereford and Limousine cattle (MOODY et al., 1995; DYBUS et al., 2003).

Table
Means and standard deviations of milk production traits in Black-and-White cows carrying different *GHRH-Hae*III genotypes (Mittelwerte und Standardabweichungen der Milchleistungsmerkmale bei Kühen mit verschiedenen Genotypen *GHRH-Hae*III)

Lactation	*GHRH* Genotype	n	Milk yield (kg)	Fat (kg)	Fat (%)	Protein (kg)	Protein (%)	F&P (%)
1st	*AA*	48	5247 (1648)	224.3 (77.25)	4.24 (0.45)	166.3 (54.80)	3.16 (0.19)	7.40 (0.57)
	AB	276	5240 (1252)	217.9 (55.05)	4.16 (0.41)	165.3 (42.85)	3.14 (0.21)	7.30 (0.53)
	BB	557	5281 (1382)	217.4 (62.74)	4.11 (0.44)	167.2 (46.49)	3.16 (0.19)	7.28 (0.55)
Total		881	5267 (1358)	218.0 (61.28)	4.14 (0.43)	166.5 (45.83)	3.16 (0.19)	7.29 (0.54)
F value (genotype effect)			0.21	0.09	0.19	0.43	1.04	0.19
2nd	*AA*	35	5784 (1542)	262.9 (78.52)	4.53 (0.61)	185.8 (53.56)	3.20 (0.23)	7.74 (0.71)
	AB	176	5758 (1195)	244.9 (57.07)	4.26 (0.45)	185.2 (41.92)	3.20 (0.23)	7.46 (0.55)
	BB	368	5732 (1310)	235.0 (63.66)	4.09 (0.52)	186.1 (46.02)	3.23 (0.21)	7.32 (0.61)
Total		579	5743 (1289)	239.7 (63.06)	4.16 (0.52)	185.8 (45.23)	3.22 (0.22)	7.39 (0.61)
F value (genotype effect)			0.39	0.25	1.87	0.15	0.98	1.66
3rd	*AA*	20	6322 (1167)	286.7 (59.55)	4.54 (0.47)	196.0 (38.86)	3.10 (0.20)	7.64 (0.55)
	AB	115	6238 (1421)	263.3 (69.88)	4.21 (0.56)	199.4 (47.46)	3.17 (0.21)	7.38 (0.66)
	BB	213	6029 (1353)	244.0 (68.58)	4.02 (0.52)	194.3 (46.23)	3.21 (0.18)	7.23 (0.60)
Total		348	6115 (1367)	252.9 (69.44)	4.11 (0.54)	196.1 (46.20)	3.19 (0.19)	7.30 (0.63)
F value (genotype effect)			0.21	0.22	1.02	0.32	2.19	1.03

n – number of cows; F&P – sum of fat and protein

Discussion

A number of strategies can be envisaged to identify candidate gene markers. One of them uses a candidate gene approach. This strategy consists of the study of mutations/polymorphisms of different genes potentially involved in a physiological process. Various authors have found QTLs for different traits of cattle and linked them to numerous chromosome regions (HEYEN et al., 1999; KUHN et al., 2003). SCHROOTEN et al. (2000) reported a QTL influencing fore udder attachment near the *TGLA23* marker on BTA13. The authors indicate that quantitative trait loci for udder traits on chromosomes 13 may also affect somatic cell score and mastitis resistance. On BTA13, ASHWELL et al. (2001) detected a QTL for canonical conformation traits. In the other study, HIENDLEDER et al. (2003) performed a whole genome scan in a granddaughter design and detect QTL on chromosome 13 affecting teat length.

It is interesting where the region of *GHRH*/*Hae*III mutation is located, especially since the rare *AA* genotype of *GHRH* was significantly favourable for fat percentage (MOODY et al., 1995). These authors located the polymorphism in the exon 3 of *GHRH* comparing the sequence of the amplified fragment (GenBank, U29611) with an analogical sequence in the human (homology 91%) and murine (77%) *GHRH* gene. If we compare the sequence published by MOODY et al. (1995) with the complete *GHRH* sequence (AF242855 – GenBank 2000), it turns out that the fragment amplified by the authors does not correspond to exon 3, but it covers a part of exon 2, the entire intron 2, and a part of exon 3; the analysed polymorphic site is located in intron 2.

Our experiment din not confirms the associations found by MOODY et al. (1995). No associations between *RFLP* in *GHRH* gene and milk production traits were found. The lack of significant differences between the particular GHRH genotypes was a result of both the effect of the selected factors on the studied traits and the covariates used in the model. However, the results obtained in the study indicate that cows with one or two $GHRH^A$ alleles could yield more fat in their milk. It should be verified in further studies with the more balanced populations of dairy cattle.

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