GHRH/HaeIII 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 GHRHA and 0.7889 – GHRHB. There were no significant associations between GHRH/HaeIII polymorphism and milk production traits of the analysed cows.

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

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.
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 HaeIII restriction enzyme improved both fat percentage and fat yield.

The aim of this study was to estimate the allelic frequencies at the GHRH-HaeIII 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-HaeIII genotypes were analysed using the PCR-RFLP method. Crude DNA was isolated from blood samples using MasterPure™ 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, 94°C/5 min, followed by 30 cycles: denaturation, 94°C/40 sec, primer annealing, 60°C/40 sec, PCR products synthesis, 72°C/40 sec, and final synthesis, 72°C/5 min. The amplified DNA was digested with 5 units of HaeIII 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/HaeIII 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;
- \( \mu \) – 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 of 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-HaeIII 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\(^4\) estimated for analysed cattle was 0.2111. A higher frequency of the GHRH\(^4\) (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-III genotypes (Mittelwerte und Standardabweichungen der Milchleistungsmerkmale bei Kühen mit verschiedenen Genotypen GHRH-HaeIII)

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

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/HaeIII 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 analogous 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 did not confirm 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 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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