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GH/HaeII and GH/MspI Restriction Polymorphism in a Herd of Polish Large White Sows

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Abstract: The study covered a herd of 300 polish large white sows kept in one pigsty in the West-Pomeranian Region in Poland. The polymorphism of a selected 506-bp GH gene fragment was detected by the PCR-RFLP method using specific primer sequences and endonuclease HaeII and MspI. In the studied herd of sows, there were found to be three GH/HaeII genotypes (h^-h^--4.7%, h^+h^--54% and h^+h^+-41.3%) and three GH/MspI genotypes (m^-m^- 30%, m^+m^--23.3% and m^+m^+-46.7%), each of them determined by two alleles. The GH/HaeII allele frequencies were 31.7% for allele h^- and 68.3% for allele h^+, whereas the GH/MspI allele frequencies were 41.7% for allele m^- and 58.3% for allele m^+. The genetic equilibrium in the herd under study was found to be disrupted as the number of individuals observed in the GH/HaeII and GH/MspI genotype groups was significantly different from their expected number calculated according to the Hardy-Weinberg law. The study proved the existence of GH/HaeII and GH/MspI polymorphisms in a selected GH gene sequence in Polish Large White sows and revealed an association between the GH/HaeII and GH/MspI genotypes and reproductive traits of the sows.

Key words: Growth hormone gene, GH, polymorphism, reproductive traits, polish large white sows

INTRODUCTION

The major role of growth hormone is to stimulate the development of cells and tissues as well as the growth of the body as a whole. Growth hormone secretion varies throughout the individual's life. The highest growth hormone concentration is observed at puberty and afterwards it decreases, being always higher in males and depending on the physiological condition of the individual (Izadyar *et al*., 1999). Growth hormone stimulates particular target cells directly or indirectly. Its indirect function consists in promoting the release of other growth hormone-dependent hormones, which are produced mainly in the liver (Enright, 1988). Growth hormone was found to have an effect on milk production in females, better feed conversion, testis growth and development (Spiteri-grech and Nieschlag, 1992), spermatogenesis (Laron and Klinger, 1998) as well as the growth, maturation and number of ovarian follicles (Yoshimura *et al*., 1994; Gong *et al*., 1993; Izadyar *et al*., 1999). The Growth Hormone (GH) gene in pigs was localized on chromosome 12 within region 12p1.-pl.5 (Yerle *et al*., 1993) and it comprises 5 exons of a total transcript sequence length of 1.7 kbp (Vize and Wells, 1987). Single nucleotide changes were detected within introns and exons as well as within the regulatory sequences of the GH gene in pigs (Kamiñski and Wachek, 2002). NIELSEN and Larsen (1991) detected GH/DraI and GH/TaqI polymorphism in local breeds of pigs kept in Denmark and Sweden, whereas Kirkpatrick (1992) found GH/MspI and GH/HaeII polymorphism in herds of pigs that he studied. Nielsen *et al*. (1995) found two variants of the TATA box promoter sequence in Meishan × Pietrain crossbred pigs and reported changes in growth hormone concentration in blood plasma, which might indicate that there are differences in the GH gene expression.

The aim of this study was to identify mutations in a selected growth hormone gene fragment by using endonuclease HaeII and MspI, to determine the genetic structure of the studied herd of Polish Large White sows and to establish possible associations between GH/MspI and GH/HaeII genotypes and the analyzed reproductive traits of the sows.

MATERIALS AND METHODS

The study covered 300 Polish Large White sows kept in identical environmental conditions in one pigsty in the West-Pomeranian Region in Poland and fed standard rations. The DNA used in the analysis was isolated from approx. Three milliliter samples of whole blood collected into vacuum test tubes containing K_3EDTA as anticoagulant. DNA isolation was performed using the specialist MasterPure™ kit (Epicentre®). The PCR-RFLP method was applied to determine the polymorphism consisting in C→A substitution at position 566 within

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exon 2 and the polymorphism consisting in C→T substitution at position 742 within intron 2 of the pig GH gene. A 506 bp fragment of the GH gene under analysis was amplified using a pair of primers designed by Kirkpatrick (1992) and containing the following nucleotide sequences:

pGH1 - Forward: 5' - GCC AAG TTT TAA ATG TCC CTG-3'
pGH1 - Reverse: 5' - CTG TCC CTC CGG GAT GTA G- 3'

The total volume of the PCR reaction mix was 20 μL and the reaction was carried out under the following temperature profile: initial denaturation of DNA templates at 95°C for 3 min followed by 35 cycles of proper denaturation at 95°C for 45 sec, primer annealing at 59°C for 45 sec, synthesis of PCR products at 72°C for 2 min and final synthesis at 72°C for 4 min. Afterwards, the amplified DNA fragment of 506 bp was digested at 37°C for 24 h with 2 units of endonuclease HaeII, which recognizes the sequence 5'...PuGCGC/Py...3' where Py = T/C, or restrictase MspI, which recognizes the sequence 5'...C/CGG...3'. The digested DNA fragments were analyzed electrophoretically on 1.5% agarose gels containing ethidium bromide with pUC19/MspI as a DNA fragment size marker.

After the electrophoresis process, the gels were visualized under UV light using a set of instruments for electrophoresis gel documentation and analysis by Vilber Lourmat.

The results obtained in the PCR-RFLP analysis of the GH gene fragment were analyzed statistically. Using the STATGEN statistical software package, the genetic structure of the studied herd of sows was analyzed to determine: The frequencies of the GH/MspI and GH/HaeII alleles and genotypes and their expected frequencies, the frequencies of homo- and heterozygous genotypes and their expected frequencies and the significance of differences verified with a Chi-square test. The statistical analysis concerning the associations between GH/MspI and GH/HaeII polymorphism and sow reproductive traits (sow's age at farrowing, number of piglets born, number of piglets weaned, piglet mortality rate in the rearing period and duration of the rearing period) was carried out using the STATISTICA 96 data analysis software according to the following model:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + d_l + (ab)_{ij} + (Ac)_{ik} + (bc)_{jk} + e_{ijkl}$$

Where, Y_{ijkl} - trait; μ - overall mean; a_i - fixed effect of GH/HaeII genotype (i = 1, 2, 3) and GH/MspI genotype (i = 1, 2, 3); b_j - fixed effect of year (j = 1997, 1998, 1999, ... 2002); c_k - fixed effect of season (k = 1, 2, ..., 12); d_l - fixed effect of RYR1 genotype (l = 1, 2) ; $(ab)_{ij}$ - effect of GH x year interaction; $(ac)_{ik}$ - effect of GH genotype x season interaction; (bc)jk - effect of year x season interaction; e_{ijkl}- random error.

The data obtained in the statistical calculations were compiled in tables. Allele and genotype frequencies, arithmetic means and their standard deviations were also included.

RESULTS AND DISCUSSION

The primer sequences used in the study allowed to amplify a 506-bp fragment of the GH gene, which was then digested with restriction enzyme HaeII. After electrophoresis performed on a 2% agarose gel with pUC19/MspI as a DNA fragment size marker, the following DNA fragments were identified: a 506-bp fragment - h^-h^- genotype; 506, 333 and 173-bp fragments-h^+h^- genotype and 333 and 173-bp fragments-h^+h^+ genotype. Thus, three GH/HaeII genotypes (h^-h^-, h^+h^- and h^+h^+) were found in the studied herd of Polish Large White sows, each of the genotypes being controlled by two alleles (h^- and h^+). Allele h^- frequency was 31.7% whereas allele h^+ frequency was 68.3%. Genotype frequencies in the studied herd were found to be as follows: h^-h^- genotype-4.7%, h^+h^- genotype-54% and h^+h^+ genotype-41.3% (Table 1). A significantly higher h^-h^- genotype frequency had been previously reported by Kuryl *et al.* (2003) for Zlotniki Spotted pigs (36%), whereas no such genotype had been found by these authors in Pietrain pigs. A significantly higher h^-h^- genotype frequency (21%) had also been reported by Urban *et al.* (2002) for Duroc pigs. A higher h^+h^- genotype frequency (58%) had been observed by Kuryl *et al.* (2003) in synthetic Torhyb line pigs, similar (57%)- in Zlotniki Spotted pigs and significantly lower (37%)- in Pietrain pigs. Furthermore, a h^+h^- genotype frequency comparable with the one calculated in this study had been reported by Urban *et al.* (2002) for Duroc pigs (51%). As far as h^+h^+ genotype is concerned, it was significantly more frequent in the herd of polish large white pigs under this study than in Zlotniki Spotted pigs (7%) and significantly less frequent than in Pietrain pigs (63%) studied by Kuryl *et al.* (2003). A significantly lower frequency of h^+h^+ genotype (28%) had been reported by Urban *et al.* (2002) for Duroc pigs. The genetic equilibrium in the herd under study was found to be disrupted as the number of individuals observed in each GH/HaeII genotype group was statistically significantly different compared with the expected n umber calculated according to the Hardy-inberg law ($\chi^2 = 18.42$).

Table 1: Frequencies of GH/HaeII and GH/MspI genotypes and alleles in the studied herd of sows

	Genotype			Allele	
Polymorphism	Genotype	n	Frequency (%)	Allele	Frequency (%)
GH/HaeII	h^-h^-	14	4.7		
	h^+h^-	162	54.0	h^-	31.7
	h^+h^+	124	41.3	h^+	68.3
	Total	300	100	Total	100
GH/MspI	m^-m^-	90	30.0		
	m^+m^-	70	23.3	m^-	41.7
	m^+m^+	140	46.7	m^+	58.3
	Total	300	100	Total	100

Table 2: Values of the reproductive traits analyzed in the studied herd of sows in relation to GH/HaeII polymorphism

			Sow's age [days]		No. of piglets						Duration of the piglet rearing period[days]	
					Born		Weaned		Dead			
Genotype GH/HaeII	Litter	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
h^-h^-		14	394	44	9.00^{a}	1.86	6.92	2.74	2.08^{ab}	2.35	35	2.0
h^+h^-	I	162	397	47	8.67	2.41	7.39	2.45	1.29^{a}	1.53	35	3.0
h^+h^+		124	403	52	8.30^{a}	2.45	7.21	2.35	1.08^{b}	1.30	35	3.0
h^-h^-		12	575	63	9.67	2.96	8.58	2.74	1.08	1.24	35	2.0
h^+h^-	II	109	565	68	8.85	2.85	7.87	2.58	0.98	1.59	35	3.0
h^+h^+		86	576	62	9.24	2.41	8.10	2.07	1.14	1.12	35	2.0
h^-h^-		8	739	72	9.87	4.12	8.50	3.42	1.37	1.41	35	2.0
h^+h^-	III	83	722	72	9.96	2.49	8.47	2.21	1.49	1.45	35	3.0
h^+h^+		58	737	67	10.34	2.34	8.93	2.02	1.41	1.40	35	2.0
h^-h^-		20		-	9.35	2.06	7.80	1.91	1.55	1.23	34	3.0
h^+h^-	Subsequent	306		-	9.63	2.65	8.11	2.33	1.52	1.29	35	2.0
h^+h^+	Litters	145		-	10.04	2.34	8.37	1.94	1.67	1.34	35	2.0

The means marked with the same suberscript letter differ significantly. Small letters denote significance of difference with $P \leq 0.05$, whereas capital letters denote significance of difference with $p \leq 0.01$

The amplified 506-bp fragment of the GH gene was subjected to digestion with restriction enzyme MspI and afterwards to electrophoresis performed on a 1.5% agarose gel with pUC19/MspI as a DNA fragment size marker, which allowed to identify the following DNA fragments: 284 and 222 bp fragments for m^-m^- genotype; 284, 222, 147 and 137 bp fragments for m^+m^- genotype; 222, 147 and 137 bp fragments for m^+m^+ genotype. The genotype frequencies calculated for the herd under study were as follows: m^-m^- genotype-30%, m^+m^- genotype-23.3%, m^+m^+ genotype-46.7%. Allele m^- frequency was 41.7%, whereas allele m^+ frequency was 58.3% (Table 1). For comparison, Kuryl *et al.* (2003) had reported a significantly higher frequency of m^+m^+ genotype in a herd of Polish Large White pigs (85%), similar in a herd of Zlotniki Spotted pigs (48%) and significantly lower in Pietrain pigs (23%). On the other hand, the frequency of m^+m^+ genotype observed by Urban *et al.* (2002) in a herd of Duroc pigs was significantly lower (72%) compared with the frequency of this genotype in polish large white sows under this study. The genetic equilibrium in the studied herd was found to be disrupted as the differences between the number of individuals observed in each GH/MspI genotype group and the expected number calculated according to the Hardy-Weinberg law were statistically significant at $p \leq 0.01$ ($\chi^2 = 75.65$).

Table 2 shows the values of the analyzed reproductive traits in the sows under study in relation to the GH/HaeII genotypes. As can be seen from the data given in this table, no statistically significant differences were found in the age at farrowing between sows with different GH/HaeII genotypes. The piglet rearing period in sows with different GH/HaeII genotypes was also similar in all the studied litters. However, the analysis of the number of piglets born in the first litters of sows with different GH/HaeII genotypes showed that h^-h^- genotype sows gave birth to 0.70 piglets more ($p \leq 0.05$) than h^+h^+ genotype sows and 0.33 piglets more than h^+h^- genotype sows. Similarly, h^-h^- genotype sows had the largest number of piglets in the second litter: they gave birth to 0.82 piglets more ($p \leq 0.05$) than sows with h^+h^- genotype and 0.43 piglets more ($p \leq 0.05$) than sows with h^+h^+ genotype. The largest third and subsequent litters were observed in h^+h^+ genotype sows whereas the smallest ones-in h^-h^- genotype sows. The analysis of the number of piglets weaned from sows with different GH/HaeII genotypes showed that in the first litter the largest number of piglets was weaned from sows with the heterozygous h^+h^- genotype whereas in the second litter- from sows with h^-h^- genotype. In the third and subsequent litters, the largest number of piglets was weaned from sows with h^+h^+ genotype. Furthermore, the

Table 3: Values of the reproductive traits analyzed in the studied herd of sows in relation to GH/MspI polymorphism

Genotype			Sow's age [days]		No. of piglets						Duration of the piglet rearing period[days]	
					Born		Weaned		Dead			
GH/HaeII	Litter	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
m^-m^-		90	402	54	8.64^a	2.32	7.53	1.91	1.11	2.35	35	2.0
m^+m^-	I	70	398	53	8.20^a	2.33	7.43	2.40	0.77^a	1.04	35	3.0
m^+m^+		140	396	41	8.55	2.48	7.28	2.20	1.27^a	1.26	35	2.0
m^-m^-		68	567	67	9.00	2.72	7.75	2.52	1.25^A	1.73	35	2.0
m^+m^-	II	62	572	65	8.87	2.74	8.19	2.42	0.68^A	1.22	34	2.0
m^+m^+		104	572	63	9.18	2.61	8.12	2.21	1.06	1.13	35	3.0
m^-m^-		54	736	74	10.02	2.46	8.57	2.12	1.45	1.53	35	2.0
m^+m^-	III	48	730	74	9.95	2.19	8.64	1.98	1.31	1.22	35	2.0
m^+m^+		73	730	65	10.31	2.43	8.69	2.08	1.63	1.52	34	2.0
m^-m^-		92		-	9.83	2.60	8.40	2.19	1.42^a	1.30	35	2.0
m^+m^-	Subsequent	132		-	9.46	2.85	8.04	2.64	1.42^b	1.14	35	2.0
m^+m^+	Litters	205		-	9.92	2.32	8.17	1.91	1.75^{ab}	1.42	35	2.0

The means marked with the same suberscript letter differ significantly. Small letters denote significance of difference with $P \leq 0.05$, whereas capital letters denote significance of difference with $p \leq 0.01$

analysis of piglet mortality rate during the rearing period in sows with different GH/HaeII genotypes showed that the highest mortality rate among piglets from the first litter was in the group of h^-h^- genotype sows and this rate was found to be statistically significantly higher ($p \leq 0.05$) than in the group of sows with other GH/HaeII genotypes. On the other hand, the analysis of piglet mortality rate during the rearing period in the second and subsequent litters showed only minor differences between sows of different GH/HaeII genotypes.

Table 3 shows the values of the analyzed reproductive traits in the sows under study in relation to the GH/MspI genotypes. As can be seen from the data given in this table, no statistically significant differences were found in the age at farrowing between sows with different GH/MspI genotypes. The piglet rearing period in sows with different GH/HaeII genotypes was also similar in all the studied litters. The analysis of the number of piglets born by sows with different GH/HaeII genotypes showed that in the first litter the largest number of piglets was born by m^-m^- genotype sows, whereas the smallest one-by sows with the heterozygous m^+m^- genotype. The difference amounted to 0.44 piglets and was confirmed statistically at the 0.05 significance level. In the second, third and subsequent litters, the largest number of piglets per litter was born by m^+m^+ genotype sows but the differences in the number of piglets born in these litters by sows of different GH/MspI genotypes were small and could not be confirmed statistically. The analysis of the number of piglets after weaning showed very small, statistically non-significant differences in the values of this trait in sows with different GH/MspI genotypes. Finally, the analysis of piglet mortality rate during the rearing period in sows with different GH/MspI genot ypes showed that the highest mortality rate among piglets from the first as well as third and subsequent litters was in the group of m^+m^+ genotype sows and this rate was found to be statistically significantly higher ($p \leq 0.05$) than in the group of sows with other genotypes. As far as the second litter is concerned, the highest piglet mortality rate was observed in the group of m^-m^- genotype sows and this rate was found to be statistically significantly higher ($p \leq 0.01$) than in the group of sows with the heterozygous m^+m^- GH/MspI genotype.

CONCLUSION

In this study, polymorphism of the analyzed 506-bp GH gene fragment was detected by using endonuclease HaeII and MspI. The studied herd of 300 Polish Large White sows was found to have three GH/HaeII genotypes (h^-h^-, h^+h^- and h^+h^+) and three GH/MspI genotypes (m^-m^-, $m+m^-$ and m+m+), each of them determined by two alleles (alleles h^- and h^+ and alleles m^- and m^+, respectively). Genetic equilibrium in the herd under study was found to be disrupted as the number of individuals observed in the GH/HaeII and GH/MspI genotype groups was significantly different from the expected number calculated according to the Hardy-Weinberg law. The analysis of associations between GH/HaeII and GH/MspI polymorphism and the reproductive traits under study showed that the values of reproductive traits differ depending on the genotype.

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