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Association od the *Dde*I growth hormone gene polymorphism with some performance traits in Polish Large White and Czech Large White × Polish Large White pigs

Lenka PUTNOVÁ[1], Leona KŘENKOVÁ[1], Irena VRTKOVÁ[1], Josef DVOŘÁK[1], Arkadiusz PIETRUSZKA[2], Roman CZARNECKI[2]

[1]Department of Genetics, Mendel University of Agriculture and Forestry Brno, Czech Republic
[2]Department of Pig Breeding, Agricultural University of Szczecin, Poland

Abstract. The genotypes of growth hormone gene polymorphisms (*GH-Dde*I, *GH-Msp*I, *GH-Hae*II, *GH-Apa*I, *GH-Cfo*I) were determined in 78 pigs [Czech Large White sires ($CLW_{sire\ line}$) × Polish Large White (PLW) sows, Polish Large White sires × Polish Large White sows], by the PCR-RFLP method. Preliminary studies found only *GH Dde*I polymorphism to be associated with performance traits. The associations of this polymorphism with growth and carcass traits were investigated. The linear model included the effects of candidate genes, genetic groups, sex and linear covariables of age at slaughter and body weight at weaning. The *Dde*I polymorphism of the *GH* gene showed associations with carcass length ($P \leq 0.05$), average daily gain from birth to weaning ($P \leq 0.05$) and average daily gain from weaning to slaughter ($P \leq 0.05$). The association of *GH* genotypes with feed conversion was near significance. Sex influenced average daily gain from birth to weaning. The genetic groups (Czech or Polish sires) improved fat thickness at *sacrum* point 3, average daily gain from birth to weaning, lean meat content, weight of chop, weight of shoulder, weight of neck and average daily gain from weaning to slaughter. The regression on age at slaughter and on body weight at weaning influenced the majority of production traits.

Key words: carcass traits, *GH* gene, growth traits, pigs, polymorphism.

Introduction

Growth hormone (somatotropin, somatotropic hormone) secreted from the anterior pituitary gland has a central position in the regulation of growth. The gene of

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Correspondence: L. PUTNOVÁ, Department of Genetics, Mendel University of Agriculture and Forestry Brno, Zemìdìlská 1, 613 00 Brno, Czech Republic, e-mail: putnova@mendelu.cz

growth hormone (*GH*) participates in the variability of pig meat production to a great extent. The *GH* gene interferes with proteosynthesis of structural proteins. Its effects are intensively studied with regard to their possible use in selection programmes and commercial application. Anabolic growth hormone's effects are mediated via the production of insulin-like growth factor I (IGF-I). IGF-I (somatomedin C) and IGF-II (somatomedin A) are now recognised as potent mitogens stimulating DNA synthesis and cell proliferation in a wide variety of cell types by growth hormone administration (BRAMELD et al. 1996). A polymorphism within intron 2 of the porcine *IGF*-II gene was identified by KNOLL et al. (2000). NEZER et al. (1999) mapped an imprinted QTL with a major effect on muscle weight and fat deposition to the *IGF*-II locus in pigs.

Growth hormone appears to play an important metabolic role during late pregnancy and in lactation maintenance (ESCALADA et al. 1997). Growth hormone, prolactin, placental lactogen and other polypeptide hormones form a family based on amino acid sequence homology and are reported to have arisen by duplication of an ancestral gene. The recent identification of the complementary DNAs encoding *PRL* and *GH* receptors has led to the discovery that the receptors, as well as the hormones themselves, form a gene family (KELLY et al. 1991). The prolactin and growth hormone receptors are homologous to receptors of members of the cytokine superfamily (CLEVENGER et al. 1998).

The porcine *GH* gene (*pGH*) was localized to chromosome 12 with a regional assignment to 12p1.2-p1.5 (YERLE et al. 1993). Precise localization using nonradioactive *in situ* hybridization mapped the gene to band 12p1.4 (CHOWDHARY et al. 1994). It comprises five exons with a total transcribed length of 1.7 kb (VIZE, WELLS 1987). SCHELLANDER et al. (1994) described *Msp*I and *Hae*II polymorphism in a 506 bp fragment from base 384 in the first intron to base 889 in the third exon. HANDLER et al. (1996) amplified a 605 bp region from the first intron (base 65) to the second exon (base 487) of the *pGH* gene with the use of primers described by LARSEN, NIELSEN (1993) and found *Apa*I and *Cfo*I polymorphisms of the gene. Base substitution at position 546 of the second exon can be detected by the *Dde*I restriction enzyme (DWORAK et al. 1996).

*Cfo*I polymorphism of the *GH* gene was found to be associated with daily gain and feed efficiency in Piétrain and with lean meat content in Large White (HARDGE et al. 1997). *Dde*I and *Hae*II *GH* genotypes were not associated with the expression of any trait in Yorkshire, Landrace and Hampshire (CASAS-CARILLO et al. 1997). In the two families derived from crossing Piétrain with either wild boar or Meishan, eight carcass composition traits were significantly associated with *Apa*I-*GH* variants in a family Meishan × Piétrain (KNORR et al. 1997). The *Msp*I genotypes differed significantly in lean meant content and backfat thickness determined by PIGLOG (ca. 80 kg) and in last chest vertebra backfat thickness, average backfat thickness and lean meat content determined post mortem in a hybrid population (Large White × Landrace) × hybrid boars (KŘENKOVÁ et al. 1998). KŘENKOVÁ et al. (1999) found associations of *GH-Hae*II with the weight of the right carcass half, daily gain to weaning and

body weight at weaning in hybrid pigs (Large White × Landrace) × one Large White boar from the sire line or one hybrid boar (Large White × Piétrain).

Material and methods

Material

Altogether 34 male and 44 female pigs, obtained by crossing Czech Large White sires ($CLW_{sire\ linc}$) × Polish Large White (PLW) sows, or Polish Large White sires × Polish Large White sows, were included in this study.

The following carcass traits were evaluated: longissimus muscle area [LMA] (cm^2), live weight at slaughter [LWS] (kg), carcass length [CL] (cm), fat thickness over the shoulder [FSH] (cm), backfat thickness [FB] (cm), fat thickness at *sacrum* point 1 [T_1] (cm), fat thickness at *sacrum* point 2 [T_2] (cm), fat thickness at *sacrum* point 4[T_3](cm), average daily gain from birth to weaning [ADGBW] (g), lean meat content [LMC] (%), weight of the chop [WCH] (kg), weight of ham [WHA] (kg), weight of the shoulder [WSH] (kg), weight of the neck [WNE] (kg), pH_1 measured 45 min *post mortem*, average daily gain from weaning to slaughter [ADGWS] (g), average daily gain from birth to slaughter [ADGBS] (g), feed conversion [FC] (kg feed per kg gain).

PCR-RFLP analysis

Genomic DNA was isolated from blood with the use of QIAamp® Kit (QIAGEN). Genotypes were determined by PCR-RFLP on the basis of literature and a modification used in our laboratory. After digestion with restriction endonucleases and electrophoretic analysis, amplified DNA showed the following genotypes:

605 bp PCR product length:
*GH-Dde*I *D* 148, 335, 122 bp; *d* 148, 457 bp (DWORAK et al. 1996);
*GH-Apa*I a_1 449, 101, 55 bp; a_2 316, 133, 101, 55 bp (HANDLER et al. 1996);
*GH-Cfo*I c_1 605 bp; c_2 497, 108 bp; c_3 448, 157 bp; c_4 448, 108, 49 bp (HANDLER et al. 1996);
506 bp PCR product length:
*GH-Msp*I "+" 222, 147, 137 bp; "–" 284, 222 bp (SCHELLANDER et al. 1994);
*GH-Hae*II "+" 333, 173 bp; "–" 506 bp (SCHELLANDER et al. 1994).

Statistical analysis

The statistical analysis was carried out by the GLM (SAS, 1988) procedure. The following linear model was employed:

$$y_{ijklmno} = \mu + RYR1_i + GH(DdeI)_j + MYF4_k + PIT1_l + P_m + S_n + b_1 a_{ijklmn} + b_2 w_{ijklmn} + e_{ijklmno},$$

where: $y_{ijklmno}$ is the *ijklmno*-th observation, μ is a general mean, $RYR1_i$ is the effect of the *i*-th *RYR*1 genotype (i = 1,2), $GH(DdeI)_j$ is the effect of the *j*-th *GH* genotype at the *Dde*I locus (j = 1,2,3), $MYF4_k$ is the effect of the *k*-th *MYF*4 genotype (k = 1,2,3), $PIT1_l$ = is the effect of the *l*-th *PIT*1 genotype (l = 1,2,3), P_m is the effect of the *m*-th genetic group (m = 1,2), S_n is the effect of the *n*-th sex (n = 1,2), b_1 and b_2 ore linear regression coefficients, $a_{ijklmno}$ is the slaughter age of the *ijklmno*-th individual, $w_{ijklmno}$ is the body weight at weaning of the *ijklmno*-th individual, $e_{ijklmno}$ is the residual effect.

Significant associations between the *GH* genotype identified with *Dde*I RFLPs, genetic groups, sex and carcass traits were classified according to individual groups of genotypes and calculated values of LSM ± SE (least square mean ± standard error).

Information about the degree of polymorphism of *RYR*1, *MYF*4, *PIT*1 genes presented in the linear model and the relation of these candidate genes to carcass traits will be the subject of another study.

Results

The frequencies of alleles of the growth hormone gene polymorphisms (*GH-Dde*I, *GH-Msp*I, *GH-Hae*II, *GH-Apa*I, *GH-Cfo*I) in 78 pigs are presented in Table 1. Preliminary studies of *GH* polymorphisms revealed significant differences between genotypes only in the polymorphic site of *Dde*I. So we discovered only *Dde*I polymorphism associations with production traits. The frequencies of genotypes and alleles of *GH Dde*I polymorphism divided into two parts according to sex and genetic groups are given in Table 2.

Results of GLM analysis according to the model equation mentioned above were as follows. The *Dde*I polymorphism of the *GH* gene showed associations with carcass length ($P \leq 0.05$), average daily gain from birth to weaning ($P \leq 0.05$) and average daily gain from weaning to slaughter ($P \leq 0.05$). The association of *GH* genotypes with feed conversion was near significance. Sex influenced average daily gain from birth to weaning ($P \leq 0.05$). The genetic groups were associated with fat thickness at *sacrum* point 3 ($P \leq 0.01$), average daily gain from birth to weaning ($P \leq 0.05$), lean meat content ($P \leq 0.05$), weight of the chop ($P \leq 0.05$),

Table 1. Frequencies of alleles of growth hormone gene polymorphisms in a population of 78 pigs

Polymorphisms	Frequency of alleles									
	+	–	*D*	*d*	a_1	a_2	c_1	c_2	c_3	c_4
*Dde*I			0.44	0.56						
*Msp*I	0.67	0.33								
*Hae*II	0.47	0.53								
*Apa*I					0.34	0.66				
*Cfo*I							0.20	0.13	0.43	0.24

Table 2. Frequencies of genotypes and alleles of GH DdeI polymorphism in a population of 78 pigs divided into two parts according to sex and genetic groups

Genotype	Sex		Genetic groups	
	Male (n=34)	Female (n=44)	CLW × PLW (n=54)	PLW × PLW (n=24)
			Frequencies of genotypes	
DD	0.18	0.23	0.21	0.21
Dd	0.44	0.50	0.44	0.54
dd	0.38	0.27	0.35	0.25
Allele			Frequencies of alleles	
D	0.40	0.48	0.43	0.48
d	0.60	0.52	0.57	0.52

CLW = Czech Large White, PLW = Polish Large White

weight of the shoulder ($P \leq 0.05$), weight of the neck ($P \leq 0.01$) and average daily gain from weaning to slaughter ($P \leq 0.05$). The regression with the age at slaughter was significant for carcass length ($P \leq 0.05$), pH_1 value ($P \leq 0.01$), feed conversion ($P \leq 0.001$), average daily gain from birth to slaughter ($P \leq 0.001$) and average daily gain from weaning to slaughter ($P \leq 0.001$). The regression on body weight at weaning was significant for carcass length ($P \leq 0.01$), average daily gain from birth to weaning ($P \leq 0.001$), feed conversion ($P \leq 0.001$), and average daily gain from weaning to slaughter ($P \leq 0.001$).

Significant associations between *GH* genotype identified with *Dde*I RFLPs and carcass traits are shown in Table 3. Significant associations of genetic groups and sex with growth, carcass value and meat quality traits were determined (Table 4). There were statistically significant differences between *DD* and *Dd* genotypes. Homozygotes *DD* had a higher average carcass length by 1.74 cm ($P \leq 0.05$) and average daily gain from weaning to slaughter by 19.86 g ($P \leq 0.01$), as compared to heterozygotes *Dd*. Heterozygotes *Dd* had a higher average daily gain from birth to weaning by 8.61 g ($P \leq 0.01$) and feed conversion by 0.17 kg/kg

Table 3. Least square mean (standard deviations) of genotypes for growth, carcass value and meat quality traits

Traits		Growth hormone *Dde*I genotype		
		DD	*Dd*	*dd*
CL	(cm)	$78.93^{a} \pm 0.63$	$77.19^{b} \pm 0.48$	78.06 ± 0.55
ADGBW	(g)	$341.67^{Aa} \pm 2.81$	$350.28^{B} \pm 2.15$	$349.48^{b} \pm 2.46$
ADGWS	(g)	$648.19^{A} \pm 7.01$	$628.33^{B} \pm 5.37$	634.64 ± 6.13
FC	(kg/kg)	$3.76^{a} \pm 0.07$	$3.93^{b} \pm 0.05$	3.86 ± 0.06

^{a,b} = $P \leq 0.05$, ^{A,B} = $P \leq 0.01$

CL = carcass length, ADGBW = average daily gain from birth to weaning, ADGWS = average daily gain from weaning to slaughter, FC = feed conversion.

Table 4. Least square mean (standard deviations) of genetic groups and both sexes for growth, carcass value and meat quality traits

Traits	Genetic group		Sex	
	CLW × PLW	PLW × PLW	Male	Female
T_3 (cm)	$2.03^{A} \pm 0.12$	$2.59^{B} \pm 0.15$	–	–
ADGBW (g)	$343.22^{a} \pm 2.23$	$351.07^{b} \pm 2.80$	$344.79^{a} \pm 2.17$	$349.50^{b} \pm 2.03$
LMC (%)	$52.30^{a} \pm 0.75$	$49.19^{b} \pm 0.94$	–	–
WCH (kg)	$7.13^{a} \pm 0.17$	$7.84^{b} \pm 0.22$	–	–
WSH (kg)	$5.95^{a} \pm 0.08$	$5.64^{b} \pm 0.10$	–	–
WNE (kg)	$4.58^{A} \pm 0.09$	$5.07^{B} \pm 0.11$	–	–
ADGWS (g)	$646.42^{a} \pm 5.56$	$627.69^{b} \pm 6.99$	–	–

$^{a, b}$ = P≤ 0.05, $^{A, B}$ = P≤ 0.01

T_3 = fat thickness at *sacrum* point 3, ADGBW = average daily gain from birth to weaning, LMC = lean meat content, WCH = weight of chop, WSH = weight of shoulder, WNE = weight of neck, ADGWS = average daily gain from weaning to slaughter

($P \leq 0.05$) than homozygotes *DD*. The females had the average daily gain from birth to weaning higher by 4.71 g than had the males ($P \leq 0.05$). Polish sires' offspring showed a higher average fat thickness at *sacrum* point 3 by 0.56 cm ($P \leq 0.01$), average daily gain from birth to weaning by 7.85 g ($P \leq 0.05$), weight of the chop by 0.71 kg ($P \leq 0.05$) and weight of the neck by 0.49 kg ($P \leq 0.01$) than the Czech sires' offspring. The Czech sires' offspring had a average higher lean meat content by 3.11 % ($P \leq 0.05$), weight of the shoulder by 0.31 kg ($P \leq 0.05$) and average daily gain from weaning to slaughter by 18.73 g ($P \leq 0.05$) than had the Polish sires' offspring.

Discussion

Investigations into the association of gene variants in the *GH* gene with quantitative performance traits in pigs suggest that the *GH* gene is a QTL for growth rate and is a major endocrine factor regulating muscle development (CASAS-CARILLO et al. 1992, NIELSEN et al. 1995).

It is well known that treating pigs daily with pituitary growth hormone markedly enhances growth rate, improves feed efficiency, decreases adipose tissue growth and increases muscle growth. The mechanisms by which *GH* influences adipose tissue growth differ from those affecting muscle. The effects that *GH* has on muscle growth probably are the results of *GH* directly and indirectly via *IGF*-I. The effects of *GH* on adipose tissue growth in the pig are direct, not mediated by *IGF*-I (EVOCK et al. 1988). PIERZCHALA et al. (1999) found that piglets carrying the "+ +/– –" (*Hae*II/*Msp*I) haplotype should be recommended as a most useful material for fattening. *Dde*I-*GH* genotype was not associated with the expression of any trait in Yorkshire, Landrace and Hampshire (CASAS-CARILLO et al. 1997).

Our results showed that *Dde*I polymorphism of the porcine growth hormone gene affects average daily gain from birth to weaning, average daily gain from weaning to slaughter, carcass length and feed conversion in a population of Polish Large White and Czech Large White × Polish Large White pig crosses. The significant differences between genetic groups can be explained by some degree of heterosis. The study did not reveal any significant differences between genotypes in polymorphic sites of *GH-Msp*I, *GH-Hae*II, *GH-Apa*I and *GH-Cfo*I.

Conclusion

The results of this study indicated that the *Dde*I polymorphism of the growth hormone gene is association with carcass length, average daily gain from birth to weaning, average daily gain from weaning to slaughter, and feed conversion. The genetic groups differed in fat thickness at *sacrum* point 3, average daily gain from birth to weaning, lean meat content, weight of the chop, weight of the shoulder, weight of the neck and average daily gain from weaning to slaughter.

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