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Growth rate and carcass quality in pigs as related to genotype at *loci POU1F1/Rsa*I (*Pit1/Rsa*I) and *GHRH/Alu*I*

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The aim of the study was to determine the effect of pituitary transcription factor 1 (POU1F1, Pit1) and the polymorphism of growth hormone releasing hormone (GHRH) genes on selected pig performance traits. The animals used were the progeny of Polish Landrace × Polish Large White crossbred sows and Polish Landrace, Polish Large White, Duroc or Pietrain boars. Eighteen perfor-mance traits were recorded. The *POU1F1* genotype was found to have a significant effect on mean daily live weight gain (g), ham-covering fat (kg), fat thickness over loin (cm), meat content of carcass (%) and meat content of ham (%), while the *GHRH/Alu*I genotype was significantly associated with fat thickness over shoulder (cm) and meat content of carcass (%). The results presented show that the region of chromosome 13, covering the POU1F1-encoding gene, may contain quantitative traits *loci* (QTLs) for growth rate and carcass traits. The study supplied new information on the relation between the polymorphism of GHRH-encoding gene and pig performance.

KEY WORDS: carcass / gene polymorphism / POU1F1 / GHRH / pig

Selection for increased growth rate or decreased backfat thickness is one of the most important tools used in pig breeding. The choice of animals for mating determines both growth rate during fattening and final carcass quality in their progeny. Currently, molecular genetics aims at helping breeders in making correct decisions.

Genes directly involved in regulating the expression of growth factors may be interesting as candidate markers of performance traits of farm animals. One of such

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genes is *POU1F1*, which encodes the pituitary-specific transcription factor involved in the transcription of growth hormone, prolactin and thyrotropin subunit β [Ingraham *et al.* 1990, Steinfelder *et al.* 1991, Radovick *at al.* 1992]. The POU1F1-encoding gene is mapped to pig chromosome 13 [Archibald *et al.* 1995], on which several authors have localized QTLs for growth rate and carcass fatness [Andersson *et al*. 1994, Wilkie *et al*. 1996, Moser *et al*. 1998].

Sun *et al.* [2002] showed a significant effect of porcine *POU1F1* genotype on the circulating level of growth hormone (GH) as well as a significant positive correlation between *POU1F1*-alpha mRNA and the GH plasma concentration. Yu *et al.* [1994, 1995] and later Kurył and Pierzchała [2001] indicated the presence of significant associations between the POU1F1-encoding gene polymorphism and the carcass fatness and meat deposition in pigs. Therefore, the biological importance of POU1F-encoding gene renders it possible to assume that it may have an effect on pig growth rate and carcass composition.

The growth hormone releasing hormone (GHRH) is the principal endogenous stimulator of somatotropin secretion. GHRH, the hypothalamic peptide, stimulates the proliferation of pituitary somatotroph cells during their development and regulates their ability to produce and secrete GH. These activities are mediated by a specific GHRH receptor (GHRHR). According to Salvatori *et al*. [2002], the *GHRHR* promoter is regulated by the pituitary-specific transcription factor POU1F1. They also showed that promoter mutations impairing POU1F1 binding can reduce the expression of *GHRHR* gene.

The presented study aimed at determining the relation between the polymorphism of POU1F1- and GHRH-encoding genes and performance traits in pigs.

Material and methods

The animal material, consisting of 322 animals, was obtained by mating boars of four breeds (Polish Landrace, Polish Large White, Duroc or Pietrain,) with crossbred sows (Polish Landrace × Polish Large White). Eighteen traits were recorded, including growth rate and carcass indicators.

The *POU1F1* genotyping was performed using PCR/RFLP with *Rsa*I endonucleases, according to Yu *et al*. [1994]. An amplified 1746 bp fragment encompassing *POU1F1* exons 4, 5 and 6, was digested with *Rsa*I endonuclease generating three monomorphic (774, 153 and 108 bp) and three polymorphic (710 bp – allele E, and 388 and 322 bp – allele F) fragments.

The polymorphism of the GHRH-encoding gene was identified with the PCR/RFLP method using *Alu*I endonuclease, according to Baskin and Pomp [1997]. For allele A 250 and 100 bp, while for allele B 230 and 100 bp fragments were found. Additional, smaller fragments were generated, but proved undetectable in the gels.

The association between genotypes and the 18 traits recorded (Tab. 1) was analysed using the GLM procedure (SAS Institute Inc. Cary NC., USA), according to the following model:

$$Y_{ijklm} = \mu + G_i + B_j + S(B)_{jk} + R_l + (GB)_{ij} + (GR)_{il} + \beta(\overline{wc}_{ijklm} - wc) + e_{ijklm}$$

where:
Y_{ijklm} – trait measured on *ijklm*-th animal;
μ – overall mean;
G_i – effect of *i*-th genotype (*POU1F1/Rsa*I – 1, 2, 3; *GHRH/Alu*I – 1, 2, 3);
B_j – effect of *j*-th sire breed (*j* = 1, 2, 3, 4);
$S(B)_{jk}$ – nested effect of *j*-th sire within *k*-th breed;
R_l – effect of *l*-th RYR1 genotype (*l* = 1, 2, 3);
$(GB)_{ij}$ – effect of interaction *i*-th genotype × *j*-th breed;
$(GR)_{il}$ – effect of interaction *i*-th genotype × *l*-th RYR1genotype;
β – regression coefficient on carcass weight (cold);
e_{ijklm} – random error.

Results and discussion

Least squares means for all the traits considered across the *POU1F1/Rsa*I and *GHRH/Alu*I genotypes are presented in Table 1 and 2, respectively.

At the *POU1F1/Rsa*I *locus* the genotype frequency was 42.5 for EE, 48.8 for EF and 8.7% for FF (137, 157 and 28 pigs, respectively). The higher share of meat in the carcass, higher share of meat in ham and higher mean daily live weight gain were significantly associated with genotype FF (P≤0.05, 0.01 and 0.05, respectively), whereas thicker fat over loin and higher weight of fat covering ham – with genotype EE (P≤0.01) (Tab. 1).

At *locus GHRH/Alu*I the genotype frequency was 8.4 for AA, 29.8 for AB and 61.8% for BB (27, 96 and 199 pigs, respectively). Thicker fat over shoulder was associated with genotype AA (P≤0.05). Pigs of AB *GHRH/Alu*I genotype showed a lower weight of ham fat and higher share of meat in ham (P≤0.05) than those of genotype AA (Tab. 2). The *GHRH/Alu*I genotype proved to have no significant effect on the remaining traits.

Significant relations between the *POU1F1* genotype and some carcass traits in pigs have been described by Yu *et al.* [1995]. Four years later Yu *et al*. [1999], in interval mapping, described a significant association between fat thickness and mean daily live gain and the markers localized close the gene *POU1F1*. Stancekova *et al.* [1999], reported a significant association of genotype *POU1F1/Msp*I with mean backfat thickness and lean content of carcass, but observed no such association for *POU1F1/Rsa*I genotype.

Brunsh *et al*. [2002] reported that 14 traits of growth rate and carcass composition were associated with *POU1F1* genotypes in a reference family of wild boar × Pietrain

Table 1 Least square means (LSM) and their standard errors (SE) for performance and carcass traits (ADG/FCR/feed consumption/LMD)

Table 1. Least squares means (LSM) and their standard errors (SE) for performance traits across three GHRH/AluI genotypes in pigs

Trait	GHRH/AluI						Differences between genotypes (P)		
	AA		AB		BB		AA-AB	AA-BB	AB-BB
	LSM	SE	LSM	SE	LSM	SE			
Daily live weight gain (g)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Backfat thickness from 5 measurements (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Mean backfat thickness (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Backfat thickness at loin [illegible] (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Backfat thickness at loin [illegible] (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Backfat thickness at loin [illegible] (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Backfat thickness over loin (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Backfat thickness over shoulder (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
Height of loin eye (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Width of loin eye (cm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Meat content of carcass (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Meat content of ham (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		
Share of ham in carcass (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Weight of ham without bones (g)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Weight of [illegible] (g)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Weight of ham (g)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Weight of ham bones (g)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Weight of ham meat (g)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			

crosses. The present study indicates that pigs with *POU1F1* genotype EE have a significantly (P≤0.05) thicker backfat over loin than FF pigs, a suggestive association (P≤0.1) of higher fat thickness at lower back (point K1) with EE genotype, as well as significant associations (P≤0.05) of higher meat share in both ham and carcass with FF genotype. *POU1F1/Rsa*I genotypes were also significantly associated with mean daily live weight gain (Tab. 1). The earlier investigation by Kurył and Pierzchała [2001], performed on the second generation of crossbreds, derived from crossing Polish Large White with Zlotnicka Spotted pigs, showed a similar but not significant effect concerning the loin eye's area as that presented by Yu *et al.* [1995]. The significant association between the gene discussed and fat thickness over the loin [Kurył and Pierzchała 2001] was contrary to the results presented here. Also contrary, but not significant, was the effect of genotype *POU1F1/Rsa*I on meat share in carcass [Kurył and Pierzchała 2001]. In the present study a similar inverse relationship occurred for the suggestive association between genotype *POU1F1/Rsa*I and fat thickness at lower back. The higher mean daily live weight gain, associated with the FF *POU1F1/Rsa*I genotype (Tab. 1), does not corroborate the respective suggestive association presented by Yu *et al.* [1995].

Despite the results obtained earlier by Kurył and Pierzchała [2001] and Yu *et al.* [1995], as well as the difficulties in determining which of the *POU1F1/Rsa*I genotypes is desirable, it may be suggested that the SSC13 region near the POU1F1-encoding gene contributes to the variation in pig performance traits. At the moment one can only suggest that the mutation recognized here with *Rsa*I endonuclease is not a causal mutation, but is closely linked to it.

It has been known for a long time that the growth hormone (GH) significantly improves the carcass quality in pigs. It does not directly affect muscle cells, but is instead an intermediate in a series of hormonal signalling events. This includes the pituitary specific transcription factor 1 (POU1F1), GH, growth hormone releasing hormone (GHRH), the insulin-like growth factor 1 (IGF1), as well as the feedback inhibition of GH by somatostatin. Altering any one of their genes or their respective receptor genes could modify growth.

The myogenic overexpression of porcine GHRH-encoding gene, injected intramuscularly as a DNA construct containing this gene under the control of a muscle-specific synthetic promoter into piglets at the age of three weeks, increased their growth at an age exceeding 65 days [Draghia-Akli *et al.* 1999]. Although the GHRH-encoding gene has not yet been mapped, Shi and Tuggle [2001] demonstrated that it was linked to a phospholipid transfer protein (*PTLP*), localized on pig chromosome 17 with a recombination distance of 12 cM. Until now, no results have been reported that would univocally show the QTLs for carcass composition to be present on pig chromosome 17, on which only QTLs for meat quality have been described [Bidanel and Rothschild 2002]. The present results demonstrate that genotype *GHRH/Alu*I is associated with backfat thickness over the shoulder and meat share (%) in carcass. In an earlier study Kurył *et al*. [2000] reported the genotype *GHRH/Alu*I to be associated with dressing percentage. In both cases the AA *GHRH/Alu*I genotype was related to a lower dress-

ing percentage and lower meat per cent in carcass. The results presented here suggest, however, a possible linkage of gene variants with any other mutations responsible for the phenotypic level of performance traits rather than with causal mutations. Therefore, extended studies are recommended aiming at the identification of causal mutations in the GHRH-encoding gene and its relationship with growth and carcass traits in pigs. This could result in marker-assisted selection programmes becoming a reality in pig breeding.

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Wzrost świń i jakość tuszy w odniesieniu do polimorfizmu restrykcyjnego PCR-RFLP genów *POU1F1/Rsa*I i *GHRH/Alu*I

S t r e s z c z e n i e

Przedmiotem badań było określenie zależności między polimorfizmem genów *POU1F1* (przysadkowego czynnika transkrypcyjnego) i *GHRH* (somatoliberyny) a cechami użytkowymi świń. Materiałem były zwierzęta pochodzące z krzyżowania knurów czystych ras (pbz, wbp, Duroc, Pietrain) z lochami mieszańcowymi (wbp × pbz). Analizą objęto 18 cech tempa wzrostu i jakości tuszy 322 zwierząt. Istotne różnice między analizowanymi genotypami *POUF1/RsaI* w odniesieniu do badanych cech uzyskano dla procentu mięsa w tuszy, procentu mięsa w szynce, masy słoniny okrywającej szynkę ze skórą, grubości słoniny nad okiem polędwicy oraz średniego dziennego przyrostu. Istotne różnice między analizowanymi