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Association study of *PIT1* and *GHRH* SNPs with economically important traits in pigs of three breeds reared in Poland

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Using the GLM procedure an association was analysed between *PIT1* and *GHRH* SNP_S and economically important traits in pigs of three breeds reared in Poland. Significant effect of *GHRH/ Alu*I SNP was observed on several quality traits such as water-holding capacity and meat colour (A, B and L*) in Polish Large White pigs (P<0.05), with the differences between alternative homozygotes being 8.1%, and 5% (meat colour), and 16% and 3% (WHC), respectively. With respect to the *PIT1* gene polymorphism, it was found that pigs carrying *AA* genotype presented lower values of growth traits such as feed:gain ratio, daily feed intake and number of days on test compared to *BB* animals (P<0.05) as well as lower pH_{24} in loin and ham. In turn, heterozygous pigs (*AB*) had the highest level of fat and the lowest values of meat traits when compared to both homozygotes. It was concluded that polymorphisms in *GHRH* and *PIT1* genes were not directly associated with quality and carcass traits, and likely they are linked to genetic markers localized on chromosomes 17 and 13. Therefore, further investigations should aim at thorough testing of *GHRH* and *PIT1 loci*.

KEY WORDS: gene / *GHRH* / pigs / PIT1/ polymorphism

The PIT1 (or POU1F1) protein belongs to a family of pituitary-specific transcription factors. This transcription factor is required for expression of growth hormone (GH), prolactin and thyroid stimulating hormone genes [Yu *et al.* 1995, Cogan and Phillips,

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1998a]. The mutations in *PIT1* gene were first observed in mice with growth disorder and later in humans in pituitary hormone-deficient patients [Radovick *et al.* 1992]. Parks *et al.* [1993] suggested that PIT1-protein was synthesized in somatotroph, lactotroph and tireotroph nuclei and influenced *GH* expression. Therefore, mutation in *PIT1* gene may also change their expression.

The porcine *PIT1* gene is located in pig chromosome 13 [Archibald *et al.* 1995]. Yu *et al.* [1995] studying an effect of *PIT1* on performance traits in pigs suspected, that it *PIT1* could be a candidate gene for one of the major genes influencing quality traits. The group of Stancekova and colleagues [1999] drew the similar conclusion after analysing the association of polymorphism in *PIT1* with pig traits. They tested two different polymorphisms: *PIT1/Rsa*I and *PIT1/Msp*I in Large White breed and Large White x Landrace hybrid and reported that pigs with *DD* genotype (*PIT1/MspI*) were fattier when compared to *CD* and *CC* animals. In another study the progeny of Polish Landrace × Polish Large White crossbred sows and of Polish Landrace, Polish Large White, Duroc and Pietrain boars were investigated regarding the influence of *PIT1/Rsa*I polymorphism on economically important traits [Pierzchała *et al.* 2003]. They observed a significant effect of the polymorphism 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 (%). Different type of study, in which the impact of *PIT1* polymorphism on *GH* expression has been analysed [Franco *et al.* 2005a] showed that pigs of AB genotype had higher level of *GH* mRNA compared to *AA* genotype animals (P=0.034).

GHRH, also known as growth hormone-releasing factor (GRF, GHRF), somatoliberin or somatocrinin, is a releasing hormone for growth hormone. It plays an important role in growth metabolism according to interaction with various independent genes such as *GH* (growth hormone), *IGF1* (insulin-like growth factor 1), *PIT1, GHRHR* (growth hormone releasing hormone receptor) and *GHR* (growth hormone receptor) [Cogan and Phillips 1998b]. In pigs, *GHRH* gene is located in chromosome 17 (SSC17) - Baskin and Pomp [1997].

An association between genotypes of *GHRH* gene and average daily body gain, backfat thickness, feed conversion, body length and carcass meat percent in 352 pigs (112 Duroc, 132 Landrace and 108 Yorkshire) was analysed using PCR-RFLP method [Cho *et al.* 2009]. They observed, that *GHRH* polymorphism was dependent on breed ($P<0.01$) and associated with meat percentage of carcass. Pierzchała and others [2003] on the other hand, showed significant effect of this polymorphism on fat thickness over shoulder (cm) and meat content of carcass (%). Franco *et al.* [2005b] demonstrated an effect of *GHRH/Alu*I polymorphism on average daily gain (P=0.0001).

GHRH and *PIT1* are potential candidate genes, influencing quality traits as they encode proteins, which cause an important effects on physiological functions. Facing the above the aim of the present study was to confirm or deny the associations between polymorphisms in these two genes and economically important traits of three pig breeds reared in Poland.

Materials and methods

The PCR-RFLP analyses were performed on 458 gilts of breeds: Pietrain, Polish Large White (PLW) and Polish Landrace (PL) – (83, 176 and 198 animals respectively). The pigs were free of *RYR1* gene mutation and were maintained at the Pig Testing Stations (Pawłowice, Rossocha, Mełno and Chorzelów, Poland) of the National Research Institute of Animal Production under uniform housing and feeding conditions. The gilts were fed *ad libitum* from 30 up to 100 kg body weight then slaughtered and dissected. During the test average daily gain (ADG g/day), feed gain ratio (FGR kg/kg), daily feed intake (DFI kg/day) and number of days on test (NDT, day). ADG was calculated as off-test weight (approx. 100 kg) minus on-test weight (30 kg) divided by number of days on test.

During the dissection, several carcass composition and meat quality traits were evaluated – lean meat percent (LMP), lean meat content (LMC, kg), weight of loin (WL) and ham without backfat and skin (WH, kg), average backfat thickness (ABT, cm) measured in five points of backfat and loin eye area (LE, cm^2). The pH was measured 45 min and 24 h post-slaughter in the *Longissimus dorsi* by the last rib and *Semimembranosus* muscles according to standard applied in Pig Test Stations, colour of meat (A, B and L*) was estimated by MINOLTA, intramuscular fat (IMF) was assessed in thawed *Longissimus* homogenate by the Soxhlet method using Soxtherm SOX 406-Gerhardt [Soxhlet, 1879] and water-holding capacity (WHC) using Grau-Hamm method [Sałyga *et al.* 2007]. Immediately after the slaughter, blood samples were collected in EDTA-coated vacuum tubes, then frozen and stored at -20°C. DNA was extracted from whole blood samples using the Genomic Wizard Purification Kit (PROMEGA, Madison, WI, USA) following instructions provided by the manufacturer's protocol.

The PCR-RFLP method was used to determine genotype frequencies of polymorphisms in *PIT1* and *GHRH* genes in three pig breeds. For *PIT1* gene region *Rsa*I restriction enzyme was used and *Alu*I for the determination of *GHRH* polymorphism [Pierzchała *et al*. 2003]. Restriction digestions were carried out at uniform conditions at 37^0C overnight. The PCR products were analysed after restriction digestion in 3% agarose gel. The SNP in *PIT1* gene was localized in intron 4 (dbSNP rs80904061), in turn polymorphism in *GHRH* gene in intron 2 [Baskin and Pomp 1997].

The GHRH and PIT1 polymorphism data was analysed using SAS MIXED procedure (SAS Institute Inc.). The joined analysis was conducted according to the mixed model:

$$Y_{ijkl} = \mu + a_i + b_j + d_k + s_l + e_{ijkl}$$

where:

Y_{ijkl} – corresponds to the observed trait (ADG, DFI, FGR NDT, LMC, LMP, ABT, LE, WH, WL, meat colour, pH24, pH45 and WHC) in the fixed effect of k-th genotype group;

μ – overall mean;

a_i – the *GHRH* (i = *AA, AB, BB*) and *PIT1* (i = *AA, AB, BB*) genotypes;

b_j – fixed effect of *j*-th breed (Polish Landrace, Polish Large White and Pietrain);

d_k – random effect of slaughtering day ($k = 1, \ldots, 175$);

s_l – random sire effect ($l = 1, \ldots, 125$);

e_{ijkl} – random residual effect.

The effect of these genes was parameterized: for additive effect as -1, 0 and 1 for genotypes *AA, AB* and *BB,* respectively and for dominance effect as 1, -1 and 1 for *AA, AB* and *BB* genotypes, respectively [Liu, 1998]. Additive and dominance genetic effects for *GHRH* and *PIT1* genes were estimated using formula respectively and tested by t-test for significant deviation from zero. The Bonferroni correction in LSMEANS statement was used to counteract the problem of multiple comparison adjustment of the P-values for pair-wise comparisons of means. All analyses were performed for the complete data set, where all animals were analysed jointly and for each breed separately using the same model of excluded fixed effect: b_j.

Results and discussion

A total of 457 pigs were genotyped for polymorphisms in *PIT1* and *GHRH* genes. The genotypes and allelic frequencies are shown in Table 1. The alleles of *GHRH/AluI*

Table 1. Genotype and allele frequencies for *PIT1* and *GHRH* genes

Gene	Breed	Genotype	Genotype frequency	Allele	Allele frequency	Hardy-Weinberg equilibrium
PIT1	PLW (n=176)	AA (n=79)	0.449	A	0.662	P=0.53
		AB (n=75)	0.426	B	0.338	
		BB (n=22)	0.125			
	PL (n=197)	AA (n=132)	0.670	A	0.835	P<0.05
		AB (n=65)	0.330	B	0.165	
	Pietrain (n=83)	AA (n=30)	0.361	A	0.247	P=0.21
		AB(n=35)	0.422	B	0.753	
		BB (n=18)	0.217			
GHRH	PLW (n=176)	AA (n=28)	0.159	A	0.398	P=0.96
		AB (n=84)	0.477	B	0.602	
		BB (n=64)	0.364			
	PL (n=197)	AA (n=5)	0.025	A	0.129	P=0.78
		AB (n=41)	0.208	B	0.871	
		BB (n=151)	0.776			
	Pietrain (n=83)	AA (n=20)	0.241	A	0.452	P=0.18
		AB(n=35)	0.422	B	0.548	
		BB (n=28)	0.337			

PLW – Polish Large White; PL – Polish Landrace.

locus were in Hardy-Weinberg equilibrium in all breeds except for Polish Landrace. The least squares means of traits and standard errors for each genotype are shown in Tables 2 and 4. Additive and dominance effects are presented in Table 3 and 5.

At the *GHRH*/*Alu*I *locus* the genotype frequencies in all breeds analysed jointly were 11.6% for *AA*, 35% for *AB* and 53.4% for *BB* (53,160 and 244 pigs, respectively) – Table 1. Several significant associations of *GHRH* polymorphism with some of the growth traits were observed. The pigs with *BB* genotype showed the highest daily gain, which was associated with the lowest number of days in test, but results were significant only in the joined analysis, not in the analysis of individual breeds. Heterozygous pigs (AB) did not present intermediate values, but the lowest daily gain and the highest number of days in test (-27 g; +3 days compared to *BB* pigs), which was consistent with the observed dominance effect ($P<0.05$, Tab. 3). Moreover, for quality traits several significant differences were observed, namely meat colour (A, B and L*) and WHC in Polish Large White pigs ($P<0.05$), with the differences between alternative homozygotes being 8.1 and 5% (meat colour), and 16 and 3% (WHC), respectively. Animals with *BB* genotype were characterized by the darkest and the lowest colour saturation of red and yellow parameters of meat. Also these pigs had the lowest WHC. It was confirmed by the significant additive effect ($P<0.01$). The similar association was obtained in Polish Landrace and in Pietrain gilts, but the results were not significant (Tab. 2). In relation to carcass traits, *AA* pigs had the thickest backfat, which was observed in Polish Landrace (*AA* – 1.73, *AB* – 1.42 and *BB* – 1.50, $P<0.05$). Meanwhile, heterozygous gilts seemed to have the heaviest loin and ham. However, significant results were obtained only for loin in Polish Landrace gilts and for ham, when all pigs were analysed jointly ($P<0.05$). Moreover, other measured parameters of meat varied between the pigs with different genotypes: heterozygous gilts were characterized with the highest loin eye area (+1.5 cm^2 in Polish Large White when compared to the lowest values, $P<0.05$) and share of meat in lean (1.1% and 500 g in Polish Landrace when compared to the lowest values, $P<0.05$), but only in several cases the significant values were noted (Tab. 2). In general, pigs with *AB* genotype seemed to have higher level of meat content, though these effects were not observed in all breeds.

At the *locus PIT1*/*Rsa*I, the genotype frequencies in the combined analysis were 52.7 for *AA*, 38.3 for *AB* and 9 for *BB*. The associative analysis of *PIT1*/*Rsa*I mutation and pig traits presented significant effect on two growth traits such as daily feed intake and feed:gain ratio. The pigs with *AA* genotype were found more valuable as regards feed efficiency (in Pietrain - *AA* – FGR – 2.77; DFI – 2.27 and Pietrain *BB* pigs FGR – 2.95; DFI – 2.45, $P<0.05$, in Polish Large White – similar trends were observed in Polish Landrace genotype *BB* was absent, Tab. 4). Moreover, the additive effect for feed to gain ratio was significant ($P<0.05$, Tab. 5). The analysis of the quality traits, demonstrated an effect of *PIT1*/*Rsa*I polymorphism on pH_{24} hours after slaughter. Pigs carrying *AA* genotype had overall lower pH than *AB* and *BB* animals. Consequently, analysis of the effects showed that substitution $A \rightarrow B$ increases pH_{24} hours after

Table 2. Least squares means±SE for chosen carcass traits by *GHRH* genotypes

Traits	Genotype GHRH	Polish Large White	Polish Landrace	Pietrain	Total
NDT (days)	AA	83±2.8	81±4.9	89±2.3	86±2.0
	AB	86±2.2^{a}	83±4.0^{a}	87±1.9	86±1.6^{A}
	BB	81±2.2^{a}	81±1.6^{a}	85±2.1	83±1.6^{A}
TDG (kg/day)	AA	913±27.8	937±49.3	818±27.9	872.5±18.5
	AB	889±19.8	931±38.0	846±25.1	873±13.8^{A}
	BB	937±20.5	958±36.8	840±26.3	900±13.4^{A}
IMF	AA	1.64±0.094	1.36±0.15	1.49±0.18	1.53±0.071^{a}
	AB	1.59±0.072	1.52±0.067	1.74±0.16	1.59±0.055
	BB	1.69±0.074	1.54±0.051	1.67±0.17	1.64±0.054^{a}
Meat colour (A*)	AA	16.0±0.38^{ab}	16.3±1.13	16.9±0.79	16.1±0.38
	AB	15.18±0.26^{a}	16.0±0.81	16.0±0.61	15.6±0.28
	BB	15.19±0.28^{b}	15.7±0.80	15.6±0.67	15.7±0.27
Meat colour (B*)	AA	3.47±0.33^{a}	2.40±0.74	3.44±0.53	3.34±0.27
	AB	3.30±0.46	2.43±0.56	3.10±0.47	3.28±0.22
	BB	2.91±0.28^{a}	2.59±0.55	3.15±0.50	3.27±0.21
Meat colour (L*)	AA	55.6±0.71^{a}	52.5±3.46	53.1±2.40	54.8±1.21
	AB	54.5±0.46	52.6±2.35	51.5±1.84	53.6±0.78
	BB	54.1±0.48^{a}	51.8±2.32	53.03±2.00	54.0±0.75
WHC (mg)	AA	39.3±1.33^{ab}	38.6±2.19	36.5±1.55	38.2±0.97
	AB	36.6±0.95^{a}	36.8±1.12	34.4±1.34	35.9±0.79
	BB	36.1±1.00^{b}	35.9±0.90	35.3±1.50	35.5±0.73
WL (kg)	AA	6.28±0.12	6.38±0.28	6.47±0.14	6.40±0.09
	AB	6.39±0.08	6.58±0.21^{a}	6.60±0.11	6.50±0.07
	BB	6.26±0.09	6.40±0.20^{a}	6.50±0.13	6.37±0.06
WH (kg)	AA	8.87±0.14	8.82±0.33	10.23±0.15	9.34±0.11
	AB	8.97±0.10	8.97±0.25	10.08±0.12	9.39±0.08^{a}
	BB	8.82±0.10	8.85±0.24	10.02±0.14	9.27±0.08^{a}
ABT (cm)	AA	1.53±0.07	1.73±0.16^{ab}	1.17±0.09	1.43±0.05
	AB	1.53±0.05^{a}	1.42±0.12^{a}	1.18±0.07	1.39±0.04
	BB	1.44±0.05^{a}	1.50±0.11^{b}	1.16±0.08	1.38±0.04
LE (cm2)	AA	52.7±1.22	50.8±3.16	63.6±1.50	56.4±0.97
	AB	53.2±0.90^{a}	53.7±2.34	64.2±1.15	56.9±0.72
	BB	51.7±0.93^{a}	53.6±2.27	63.2±1.37	56.1±0.70
LMP (%)	AA	58.7±0.62	58.2±1.50	67.4±0.86	61.7±0.47
	AB	59.0±0.44	59.1±1.09^{a}	66.7±0.73	61.8±0.34^{a}
	BB	58.5±0.46	58.0±1.06^{a}	66.8±0.81	61.2±0.33^{a}
LMC (kg)	AA	23.4±0.34	23.4±0.83	26.5±0.34	24.5±0.26
	AB	23.8±0.25^{a}	23.9±0.62	26.3±0.28	24.7±0.20
	BB	23.3±0.26^{a}	23.5±0.60	26.1±0.31	24.3±0.19

NDT –number days in test, IMF – level of intramuscular fat, Meat colour – A- redness; B – yellowness, L – lightness, TDG – test daily gain, WHC – water holding capacity, WL– weight of loin without backfat and skin (kg), WH – weight of ham without backfat and skin (kg), ABT – average backfat thickness (cm), LE – loin eye area, LMP – lean meat percentage (%), LMC – lean meat content (kg).

$^{aA...}$Values with the same superscripts show significant differences between genotypes: small letters – $P<0.05$; capitals – $P<0.01$.

Table 3. Additive and dominance effects (with SE) obtained for the *GHRH*

Trait	Additive effect			Dominance effect		
	value	SE	P	value	SE	P
NDT	-1.23	1.58	0.70	1.84	1.08	**0.005**
TDG	14.0	16.7	0.96	-13.1	11.3	**0.017**
IMF	0.06	0.06	0.38	-0.003	0.040	0.15
Meat colour (A*)	-0.23	0.36	0.19	-0.25	0.24	0.93
Meat colour (B*)	-0.04	0.22	0.79	-0.03	0.15	0.95
Meat colour (L*)	-0.41	1.14	0.28	-0.82	0.74	0.59
WHC	-1.33	0.86	**0.008**	-0.96	0.58	0.53
WL	-0.01	0.08	0.22	0.12	0.06	**0.02**
WH	-0.04	0.10	0.62	0.085	0.07	0.057
ABT	-0.03	0.05	0.34	-0.02	0.03	0.84
LE	-0.14	0.89	0.53	0.70	0.60	0.16
LMP	-0.26	0.45	0.83	0.36	0.30	**0.04**
LMC	-0.10	0.24	0.41	-0.04	0.03	0.24

Trait symbols are explained at the bottom of Table 2.
The significant results of effect are bolded.

Table 4. Least squares means±SE for chosen carcass traits by *PIT1* genotypes

Trait	Genotype PIT1/RsaI	Polish Large White	Polish Landrace	Pietrain	Total
FGR (kg/kg)	AA	2.83±0.05	2.85±0.06	2.77±0.13^{ab}	2.83±0.04^{a}
	AB	2.88±0.05	2.91±0.07	2.95±0.12^{a}	2.89±0.04^{a}
	BB	2.91±0.08	-	2.95±0.13^{b}	2.90±0.06
DFI (kg/day)	AA	2.59±0.06	2.55±0.07	2.27±0.09^{a}	2.48±0.04
	AB	2.61±0.06	2.60±0.07	2.40±0.08	2.53±0.04
	BB	2.62±0.09	-	2.45±0.09^{a}	2.54±0.07
PH24 loin	AA	5.48±0.05^{a}	5.52±0.03	5.62±0.03	5.52±0.02^{a}
	AB	5.55±0.05^{a}	5.55±0.04	5.56±0.03	5.56±0.02^{a}
	BB	5.50±0.09	-	5.58±0.04	5.55 ± 0.04
PH24 ham	AA	5.48±0.06^{a}	5.60±0.03^{a}	5.62±0.04	5.55±0.02^{a}
	AB	5.65±0.09^{a}	5.64±0.03^{a}	5.58±0.03	5.62±0.02^{a}
	BB	5.72±0.22	-	5.63±0.04	5.65±0.04
ABT (cm)	AA	1.50±0.05	1.54±0.06^{a}	1.13±0.07	1.38±0.04^{a}
	AB	1.53±0.05	1.63±0.06^{a}	1.14±0.07	1.44±0.04^{a}
	BB	1.47±0.08	-	1.12±0.08	1.38±0.06
LE (cm^2)	AA	53.5±0.89	53.2±1.18	64.5±1.50	57.3±0.7^{a}
	AB	52.6±0.90	52.2±1.31	62.5±1.06	56.3±0.7^{a}
	BB	51.5±1.32	-	64.1±1.67	55.9±1.1
LMP (%)	AA	59.3±0.43^{a}	59.4±0.54	66.8±0.85	61.9±0.32
	AB	58.5±0.44^{a}	58.9±0.60	66.7±0.71	61.4±0.32
	BB	58.5±0.68	-	67.3±0.91	61.4±0.54

FGR – feed:gain ratio; DFI – Daily feed intake; pH24 (loin and ham) – pH measured 24 hours after slaughter; ABT – average backfat thickness (cm); LE – loin eye area (cm^2); LMP – lean meat percentage.
$^{aA,\ldots}$Values with the same superscripts show significant differences between genotypes: small letters – $P<0.05$; capitals – $P<0.01$.

Table 5. Additive and dominance effects (with SE) obtained for *PIT1*

Trait	Additive effect			Dominance effect		
	value	SE	P	value	SE	P
FGR	0.04	0.03	**0.05**	0.03	0.06	0.22
DFI	0.03	0.03	0.16	0.02	0.06	0.33
pH24 loin	0.02	0.02	**0.035**	0.02	0.04	0.40
pH24 ham	0.05	0.02	**0.003**	0.02	0.05	0.052
ABT	-0.003	0.03	0.056	0.06	0.06	0.93
LE	-0.72	0.58	0.08	-0.30	1.07	0.18
LMP	-0.25	0.29	0.07	-0.29	0.54	0.37

Trait symbols are explained at the bottom of Table 4.
The significant results of effects are bolded.

slaughter in loin by 0.02 and in ham by 0.05 (P<0.05). In addition, meat carcass traits: LE and LMP seemed to be lower in pigs with *BB* genotype compared to *AA* animals. While *AA* pigs presented the lower level of backfat thickness when compared to *AB* animals (Polish Landrace 1.54 and 1.63, respectively, P<0.05). Polish Large White gilts tended in the same direction (Tab. 4).

A progress of work in the last twenty years in the field of molecular genetics led to the discovery of a number of interesting groups of genes and genetic markers linked to genes responsible for economically important traits in farm animals. These include single genes that cause strong phenotypic effects, which are called the major genes and Quantitative Trait *Loci* (QTL). Through a functional genomics approach to understand the molecular basis of meat quality, we can gain further insight the complex interplay of gene expression events involved in the development of meat quality [Pierzchała *et al*. 2011]. Discovering and gathering new genetic markers bring us closer to understanding the physiological basis of miogenesis process. Nevertheless, novel genetic markers should be thoroughly tested before they can be included into the estimation of a breeding value of animals. In our study we have chosen the *PIT1* and *GHRH* genes, which belong to the genes associated with expression of the growth hormone. The GHRH is an endogenous stimulator of somatothropin secretion. It stimulates the proliferation of pituitary somatothrophic cells during the development, regulating the production and secretion of GH, while PIT1 has been shown to be a positive regulatory factor of growth hormone [Frajman *at al.* 2008]. Because of this the genes encoding these proteins were suggested as candidate genes, which affect the farm animal traits. In the present investigation the GLM procedure was used to show the influence of mutations in *PIT1* and *GHRH* genes on the formation of animal traits. Animals carrying different genotypes were compared for carcass composition, growth performance and meat quality traits.

Associations between *GHRH* and *PIT1* polymorphisms and economically important pig traits were earlier described by Pierzchała *et al.* [2003] and Cho *et al.*

[2009]. We have attempted to estimate the effect of *PIT1* and *GHRH* polymorphisms once more in breeds maintained in Poland.

Our investigation concerned three pig breeds bred in Poland. Polish Landrace (PL) and Polish Large White (PLW) are the most popular pigs in Poland used as a dam-lines. They demonstrate a high prolificacy, which means that they have high litter sizes and good lactation parameters. They are of similar leanness, even though their genetic origin is different. They were selected mainly based on the litter size and in 40% on carcass and growth traits. Both were/are unbiased for the mutation in *RYR1* gene [Milewska 2006]. The third line used in this study, the Pietrain pigs are used as a sire-line in Polish breeding. Therefore, they have undergone selection for meat content of carcass. The Pietrain pigs are characterized by an exceptional muscularity and leanness, what particularly it relates to the meat content in ham. However they reveal poor growth characteristics such as daily gain and feed to gain ratio [Różycki *et al*. 2009].

In the present study we observed significant effects of *GHRH* and *PIT1* genes polymorphisms on certain carcass traits (Tab. 2).

The distribution of genotypes of *GHRH* gene was in Hardy-Weinberg equilibrium in all investigated breeds, even though they were undergone of strongly selection. This could suggest that *GHRH*/*Alu*I polymorphism is not linked to the carcass pigs' traits, taken into account as selection criteria in Polish breeding. However, the associations of *GHRH/Alu*I *locus* genotype with the examined characteristics of pigs, were comparable to the results obtained in earlier studies when growth, quality and carcass traits were considered [Franco *et al.* 2005, Pierzchała *et al.* 2003]. Our results demonstrated that the *AB* heterozygotes reached the weight of 100 kg three days later than animals of all other genotypes, indicating the phenomenon of over-dominance. The similar results were obtained by Franco *et al.* [2005b], where the heterozygotes revealed the lowest daily gain (AA – 900g, AB – 858; $P<0.01$). Cho *et al*. [2009] analysed one more growth trait: feed conversion ratio, but they did not observe any association. In the present study we did not detect any influence of the polymorphism in *GHRH* gene on feeding traits. But it was identified the influence of this polymorphism on several quality traits concerning meat color. The pigs with *AA* genotype presented the brightest color of meat (L*), which may be due to a high level of WHC by the meat of these pigs. Previous reports did not deliver this kind of information. On the other hand, when carcass traits are considered, pigs with AA genotype had the highest level of fatness. The highest meatness was detected for heterozygous *AB* pigs, which was consistent with the observed dominance effect, in *AB* pigs that had +0.4% LMP than others pigs (Tab. 2). Pierzchała *et al.* [2003] showed similar associations: *AA* pigs had thicker fat over shoulder ($P<0.05$), while pigs carrying *AB* genotype at *GHRH/Alu*I *locus* presented a higher share of meat in ham ($P\leq0.05$) than these of *AA* genotype. However, the Chinese research, which analysed Landrace, Duroc and Yorkshire pigs, reported that the *AA* genotype had the highest meatness (*AA*- 58.46, *AB*-57.83 and *BB*-57.24, $P<0.01$) and the lowest backfat thickness (*AA*- 1.39, *AB*-1.40 and *BB*- 1.44) – [Cho *et al.* 2009].

In turn, the chi-square test showed one deviation from the Hardy-Weinberg equilibrium at *PIT1 locus* in Polish Landrace breed, even though these pigs were selected with the same direction as Polish Large White. It should be confirmed that *PIT1* polymorphism is not linked with traits, which are selection criteria in dam-line [Tyra and Żak 2012]. Moreover, we observed that pigs with *AA* genotype at *PIT1/Rsa*I *locus* in Pietrain gilts obtained the lowest results of daily feed intake and feed to gain ratio, indicating that they have better feed conversion and their maintenance costs less than of pigs of the other genotypes. Similar but not significant results were obtained for Polish Large White and Polish Landrace gilts. The teams of Pierzchała *et al.* [2003] and Franco *et al*. [2005b] reported that pigs with *AA* genotype had higher daily gain, what confirmed the better results for growth traits. In addition, the analysis of association of PIT1/*Rsa*I SNP and porcine meat quality provided information suggesting, that investigated polymorphism could have an influence on pH_{24} after dissection both in ham and loin. The lowest pH_{24} was detected in heterozygous *AB*, which was consistent with the additive effect. The lower pH_{24} significantly influences proteolytic processes [Łyczyński *et al.* 2009], which in turn determine important quality traits such as firmness or other parameters associated with meat tenderness, and also influenced on meat storage [Boler *et al* 2010]. Previous reports did not deliver any information on the impact of polymorphism in *PIT1* on meat quality traits. Pierzchała *et al.* [2003] and Franco *et al*. [2005b] suggested that *PIT1/Rsa*I *locus* may be a carcass indicator. The former authors observed that pigs with *BB* genotype had higher share of meat in the carcass and in ham ($P<0.05$, 0.01, respectively), while thicker fat over loin and higher weight of fat covering ham were associated with *AA* genotype. In the second report, they detected the lowest fat thickness in heterozygous pigs. Our results are not consistent with previous studies, because they found the highest backfat thickness to be associated with *AB* pigs, and *AA* pigs obtained the highest results according to meat (carcass) traits (LE and LMP, $P<0.05$). Stancekova *et al.* [1999], reported a significant association, but only for *PIT1/Msp*I with mean backfat thickness and lean content of carcass. They observed no such association for *PIT1/Rsa*I genotypes. Nowadays authors, who want to solve the problem: what is genetic basis of meat formation, focus on quality and taste of meat, because it is required by consumers. Therefore, the detection of new molecular markers associated with meat quality is necessary in order to improve programs in traditional animal breeding.

The presented associations of *PIT1* and *GHRH* polymorphisms and economically important pigs traits are not so obvious. We did not observe correlations in all of the examined breeds, and even if the differences between genotypes had been present, they often were not statistically significant. The reason for this situation could be that these breeds were undergone strong selection.

Our results suggest that the effects of the *PIT1* and *GHRH* mutations on growth and quality traits vary depending on the breed analysed. However, the effect of these mutations on growth traits confirmed results obtained previously in other studies. Moreover, our study revealed that these mutations affect meat quality traits. Results

presented above provide new information on the usefulness of these markers for gene-assisted selection in Polish pig breeding. The desirable genotypes will vary depending on customer needs. Positive selection of the *A* allele in *GHRH* polymorphism may lead to an improvement of growth traits, what could subsequently be used as selection criteria in sires lines. Increasing an *A* allele frequency could possibly improve lightness of meat. While a *B* allele at *PIT1/Rsa*I *locus* might influence the pH_{24}, what is important during the storage of meat. These polymorphisms might may also be used in estimating the breeding value by microarray SNP chips.

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