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Differentiation of fatteners carcass quality traits depending on *GH*/*Msp*I polymorphism

Zróżnicowanie cech jakości tuszy tuczników w zależności od polimorfizmu *GH/Msp*I

Summary. The aim of the present study was to analyze the traits of fattening and slaughter performances of fatteners according to *GH*/*Msp*I polymorphism. A total of 369 fatteners from one herd were investgated. The fatteners belong to five different breed groups: Landrace, Landrace × Duroc, Landrace × Yorkshire, (Landrace × Yorkshire) × Duroc, (Landrace × Yorkshire) × (Duroc × Pietrain). The frequencies of *C* and *T* alleles of *GH*/*Msp*I polymorphism were, respectively: *C* – 0.864 and *T* – 0.136. Comparing the numbers of individuals in *GH*/*Msp*I genotype gropus and the number calculated theoretically according to the Hardy-Weinberg law, no statistically significant differences were found. The conducted studies demonstrated a statistically significant influence of the breed group on the examined traits of fattening and a slaughter performances and a statistically significant interaction between the breed group and *GH*/*Msp*I polymorphism for loin eye area. It was shown that *GH*/*Msp*I polymorphism itself does not differentiate in any statistically significant way any of the examined fattening and slaughter performances of studied fatteners.

Keywords: pigs, DNA polymorphism, growth hormone, carcass quality

INTRODUCTION

For many years swine breeding programmes have been focused on improvement of fattening and slaughter performances, what resulted in obtaining carcass with high meat content at fine daily gain of body weight and sufficient pasture use. The fattening and slaughter improvement contributed to obtain notably meat breeds and lines, with fast growth, however with low quality of meat [Eikelenboom *et al*. 1996, Kirchheim *et al*.

1997]. Despite the improvement of meat content is accompanied by the increase of aberrations in meat quality features, there is no other way in improvement of swine's on the economical ground. This situation is determined by practical requirements as demand for lean meat and higher profitability pig production. With the new genotypes and genes identification techniques and the crossing program based on its results, that prevents from simultaneous increase of meat defects frequency, the high-meat fatteners with satisfying quality of meat will be able to obtain [Kaczorek *et al*. 1998]. The dynamic progress of molecular genetics methods let to know location, structure and function of genes responsible for quantitative traits. By use of the new laboratory methods it is possible to analyze the influence of particular point mutations in candidate genes, which products take part in physiological processes determining specified feature [Terman *et al*. 2008]. Progress of molecular genetics techniques and realization of animal genome mapping allow to discover the gene structure and their polymorphism, and it lets to know the influence of gene heterogeneity on animal utility traits significant for breeding. Molecular genetics gives a chance to reach the fast progress in breeding work. In relation to pigs, the scientists' interest is focused on genes responsible for growth rate, meat and carcass quality, and traits related to reproduction. To the genes belong i.a. growth hormone gene. Growth factors of somatotropic axis and their receptors belong to an interesting group of height and body mass development regulators. Growth hormone secretion to blood is under hypothalamic neurohormones control – GHRH and STS. *GH* works by its specific cell membrane receptors (GHR) and it stimulates the activity of other hormones produced mainly in a liver and muscle tissue- so called somatomedines (growth substance similar to insulin – IGF). The gene encoding the porcine growth hormone (GH) has been localized to the q-arm of chromosome 12 [Yerle *et al*. 1993] and it consists of 5 exons with total transcribed length is 1.7 kb [Vize and Wells 1987]. Genetic polymorphism of porcine *GH* gene was noticed and about 20 genetic variations of the gene in different breeds and lines of swine was described [Seeburg *et al*. 1983, Vize and Wells 1987, O'Mahony *et al*. 1989, Kirkpatrick and Huff 1990, Nielsen and Larsen 1991, Kirkpatrick *et al.* 1993, Shellander *et al*. 1994, Handler *et al*. 1995, Jiang *et al*. 1996, Knorr *et al*. 1997]. Dependence between individual genetic variation of porcine *GH* gene and carcass fatness and meat quality [Gelderman *et al*. 1996, Knorr *et al*. 1997] and loin eye area and fatness [Casas-Carrilo *et al*. 1994] was described, showing that porcine *GH* gene is a candidate gene for fatness [Knorr *et al*. 1997], especially AA/BB (*Hae*II/*Msp*I) haplotype – Putnova *et a*l. [2001].

In connection with numerous communications about dependence between genetic variations of *GH* gene and porcine meat utility traits our studies are focused on: 1. detection of the mutation in *GH* gene, 2. determination of the genetic structure of research material pursuant to individual genotype and *GH* alleles frequency in examined material, 3. analysis of dependence between *GH*/*Msp*I polymorphism and traits of fattening and slaughter performances in examined group of fatteners.

MATERIAL AND METHODS

The amount of 369 fatteners that belong to 5 different breed groups: Landrace – L (75), Landrace × Duroc – L × D (107), Landrace × Yorkshire – L × Y (68), (Landrace ×

Yorkshire) × Duroc – (L × Y) × D (75), (Landrace × Yorkshire) × (Duroc × Pietrain) – (L × Y) × (D × P) (44) constituted the research material. The fatteners were similar as for the conditions of breeding, feeding, their sex, dead weight (mtc = 85 kg), slaughter conditions (automatic electric stunning; 250 V, Inarco line of Duch company – STORK), and carcass treating after slaughter. All individuals were slaughtered in a meat establishments, and then they were evaluated in as far as fattening time, meat deposition and fatness of the carcass. The carcass was evaluated in accordance with methods used in Porcine Slaughter Utility Control Station. Daily gains were calculated from body mass differences at birth and on the slaughter day. DNA in the analysis was isolated from whole blood and it was used for detection of the *GH* gene polymorphism. In the PCR reaction used the primer sequences designed by Kirkpatrick [1992]. Gene amplification product of a 506 bp was digested with *Msp*I restriction enzyme for 3 hours in 37°C. Products of the restriction analysis were electrophoretically separated on agarose gel containing Ethidium Bromide. The electrophoresis was conducted in 1 × TBE buffer. Results of the separation were visualized in UV light and they were statistically analyzed. Using the computation suite STATGEN the analysis of a genetic structure of the fatteners herd was conducted, describing: 1. *GH*/*MspI* allele and genotype frequency and their expected frequency, 2. Frequency of homo- and heterozygous genotypes and their expected frequency, 3. Significance of differences was verified with chi-square test. Analysis of the correlation was conducted by means of two-factor analysis of variance in non-orthogonal design allowing breed group and examined polymorphism influence. Group of 351 of fatteners, immune from $RYR1^T$ allele (gene with an influence on carcass quality features and quality of meat) was under analysis. The fatteners with *TT* genotype were not considered because of their low frequency (only 3 fatteners in whole group).

RESULTS AND DISCUSSION

Primer sequences used in the analysis let to amplify *GH* gene fragment of a 506 bp, which was digested with *Msp*I restriction enzyme to identify point mutation in intron 2 of *GH* gene. After electrophoresis performed on 1.5% agarose gel with pUC19/*Msp*I existence of 284 and 222 bp fragments (*CC* genotype), 284, 222, 147, 137 bp fragments (*CT* genotype), and 222, 147, 137 bp fragments (*TT* genotype) was revealed.

In the examined herd of fatteners three *GH*/*Msp*I genotypes (*CC*, *CT* and *TT*) determined by two alleles (*C* and *T*) were found. Allele *C* frequency was 0.864, whereas allele *T* frequency was 0.136. In the analyzed herd allele *C* was more frequent than in breed group of inseminating boars examined by Kmieć *et al*. [2007] and in a group of PIC hybrid porkers examined by Rybarczyk *et al*. [2007] and much more frequent than in a herd of Polish Large White sows studied by Kmieć *et al*. [2008], and less frequent than in a herd of Duroc pigs studied by Urban *et al*. [2002]. In the examined herd of porkers *CC* genotype frequency of the analyzed polymorphism *GH*/*Msp*I was 0.737, *CT* genotype – 0.255, and *TT* genotype – 0.008 Table 1. Less frequent *CC* genotype (0.03) was found in the herds of White Large Polish breed, Pietrain (0.20), and Zlotnicka Pstra breed (0.04) by Kurył *et al*. [2003]. Also low frequency of *CC* genotype was determined by Kmieć *et al*. [2007] in a group of inseminating boars (0.03) and in the herd of White Large Polish herd (0.467) [2008], and by Rybarczyk *et al*. [2007] in the group of PIC

hybrid porkers (0.119). Analysis of an individual *GH*/*Msp*I genotypes frequency in the examined group of fatteners showed statistically significant differences in a frequency of *CC* and *CT* genotypes. Whereas a *TT* genotype frequency in individual breed groups of fatteners were low and differences between them too small to be statistically confirmed.

Table 1. Frequency of genotypes and *GH/Msp*I alleles among examined breed groups of fatteners

Tabela 1. Frekwencje genotypów i alleli *GH/ Msp*I w badanych grupach rasowych tuczników

Breed group Grupa rasowa	n	*GH/Msp*I genotypes Genotypy *GH/Msp*I			Alleles *GH/Msp*I Allele *GH/Msp*I	
		CC	*CT*	*TT*	*C*	*T*
Landrace	75	0.573^{ABa}	0.427^{ABC}	0.000	0.787^{aA}	0.213
Landrace × Yorkshire	68	0.765^{A}	0.235^{A}	0.000	0.882^{a}	0.118
Landrace × Duroc	107	0.832^{B}	0.150^{B}	0.019	0.907^{A}	0.093
(L × Y) × Duroc	75	0.747^{a}	0.240^{C}	0.013	0.867	0.133
(L × Y) × (D × P)	44	0.727	0.273	0.000	0.864	0.136
Total/Ogółem	369	0.737	0.255	0.008	0.864	0.136

Frequency in columns marked with the same letter are statistically different. Significance of the differences at $P \leq 0.05$ was marked with small letters, and significance of the differences at $P \leq 0.01$ was marked with capital letters.

Częstości w kolumnach oznaczone tą samą literą różnią się między sobą istotnie. Małymi literami oznaczono istotność różnic przy $P \leq 0,05$, dużymi literami oznaczono istotność różnic przy $P \leq 0,01$.

In the examined group of porkers no disturbance of genetic equilibrium was noticed because there was no statistically significant differences when comparing the numbers of individuals observed in the *GH*/*Msp*I genotype groups and the number calculated theoretically according to the Hardy-Weinberg law for any of breed group of analyzed porkers.

When analyzing frequency of *GH*/*Msp*I homozygous genotypes it was shown that Landrace × Duroc fatteners were characterized by the highest homozygosity, whereas Landrace fatteners were characterized by the lowest one. Differences in frequency of *GH*/*Msp*I homozygous genotypes between animals from individual breed groups of porkers were confirmed statistically ($P \leq 0.01$) – Table 3.

The analysis of variation in quality traits of pork carcass was conducted depending on *GH*/*Msp*I genotypes. The analyzed traits are: rate of growth (g/day), age on slaughter day (days), the middle carcass length (cm), mean backfat thickness in 5 measurements (cm), neck meat mass (kg), shoulder mass (kg), bacon mass (kg), ham mass (kg), loin mass (kg), area of a loin eye (cm^2), meat mass in particular primary cuts (kg), meat content in carcass according to SKURTCH (%) – Table 5.

Table 2. Expected (exp.) and observed (obs.) abundance of the *GH/Hae*II genotypes among examined breed groups of fatteners
Tabela 2. Liczebność obserwowana (obs.) i oczekiwana (oczek.) genotypów *GH/Msp*I w badanych grupach rasowych tuczników

Breed group Grupa rasowa	Overall obs./exp Razem obs./oczek.	*GH/ Msp*I genotypes Genotypy *GH/Msp*I			Significance of the differences Istotność różnic
		CC	*CT*	*TT*	
Landrace	75/75.0	43/46.4	32/25.2	0/3.4	0.0951^{ns}
Landrace × Yorkshire	68/68	52/52.9	16/14.1	0/0.9	0.5736^{ns}
Landrace × Duroc	107/107.0	89/87.9	16/18.1	2/0.9	0.5829^{ns}
(L × Y) × Duroc	75/75.0	56/56.3	18/17.3	1/1.3	0.8551^{ns}
(L × Y) × (D × P)	44/44.0	32/32.8	12/10.4	0/0.8	0.5610^{ns}

n.s. – statistically non-significant differences
n.s. – różnice statystycznie nieistotne

Table 3. Frequency of homo- and heterozygous *GH/Msp*I genotypes occurrence among examined breed group of fatteners
Tabela 3. Częstość występowania homo- i heterozygotycznych genotypów *GH/Msp*I w badanych grupach rasowych tuczników

Breed group Grupa rasowa	N	Homozygous/Homozygoty		Heterozygous/Heterozygoty	
		n	frequency częstość	n	frequency częstość
Landrace	75	43	0.573^{ABC}	32	0.427
Landrace × Yorkshire	68	52	0.765^{A}	16	0.235
Landrace × Duroc	107	91	0.850^{B}	16	0.015
(L × Y) × Duroc	75	57	0.760^{C}	18	0.240
(L × Y) × (D × P)	44	32	0.727	12	0.273

Frequency in columns marked with the same letter are statistically different. Significance of the differences at $P \leq 0.05$ was marked with small letters, and significance of the differences at $P \leq 0.01$ was marked with capital letters.
Częstości w kolumnach oznaczone tą samą literą różnią się między sobą istotnie. Małymi literami oznaczono istotność różnic przy $P \leq 0,05$, dużymi literami oznaczono istotność różnic przy $P \leq 0,01$.

Table 4. Abundance of individual genotypes of analyzed *GH/Msp*I polymorphism
Tabela 4. Liczebności poszczególnych genotypów analizowanego polimorfizmu *GH/Msp*I

*GH/Msp*I genotypes Genotypy *GH/Msp*I	Breed group / Grupa rasowa					Total Razem
	L	L × D	L × Y	(L ×Y) × D	(L×Y) × (D×P)	
CC	43	89	52	56	20	260
CT	32	16	16	18	9	91
TT	0	2	0	1	0	3
Total/Razem	75	107	68	75	29	354

Table 5. Results of variance analysis allowing of an influence of breed group and *GH/Msp*I polymorphism on examined carcass quality traits
Tabela 5. Wyniki analizy wariancji uwzględniającej oddziaływanie grupy rasowej i polimorfizmu genu *GH/Msp*I na badane cechy jakości tuszy

Trait Cecha	Mean Średnia	Influence/Oddziaływanie		
		breed group grupa rasowa	genotype genotyp	interaction interakcja
Rate of growth (g/day) Tempo wzrostu (g/dzień)	680.06 ±78.73	13.40^{**}	0.41^{ns}	0.78ns
Age on slaughter day (days) Wiek w dniu uboju (dni)	159.32 ±14.11	22.42^{**}	0.76^{ns}	1.35^{ns}
Middle carcass length (cm) Długość środkowa tuszy (cm)	82.06 ±2.73	7.20^{**}	0.56^{ns}	1.04^{ns}
Mean backfat thickness in 5 (cm) Średnia grubość słoniny z 5 pomiarów (cm)	2.01 ±0.29	5.33^{**}	1.30^{ns}	1.06^{ns}
Neck meat mass (kg) Masa karkówki (kg)	5.37 ±0.52	2.33^{ns}	1.77^{ns}	1.62^{ns}
Wing bone mass (kg) Masa łopatki (kg)	6.04 ±0.39	2.00^{ns}	0.98^{ns}	1.55^{ns}
Bacon mass (kg) Masa boczku (kg)	6.55 ±0.58	12.67^{**}	2.79^{ns}	0.99^{ns}
Ham mass (kg) Masa szynki (kg)	10.34 ±0.58	10.86^{**}	0.98^{ns}	0.23^{ns}
Loin mass (kg) Masa polędwicy (kg)	8.47 ±0.74	32.00^{**}	0.36^{ns}	2.16^{ns}
Area of a loin eye (cm^2) Powierzchnia oka polędwicy (cm^2)	51.82 ±5.79	2.38^{ns}	0.66^{ns}	2.88*
Meat mass in particular primary cuts (kg) Masa mięsa wyrębów podstawowych (kg)	23.36 ±1.01	3.24*	0.83^{ns}	1.17^{ns}
Meat content in carcass (%) Zawartość mięsa w tuszy (%)	56.51 ±2.47	1.88^{ns}	2.28^{ns}	1.45^{ns}

*P ≤ 0.05; ** P ≤ 00.1; n.s. – statistically non-significant differences
*P ≤ 0,05; ** P ≤ 00,1; n.s. – różnice statystycznie nieistotne

The analysis in variation in quality traits of pork carcass depending on *GH*/*Msp*I genotypes was conducted by use of STATISTICA computation suite and by means of two-factor analysis of variance in non-orthogonal design. Whole analysis of correlation was conducted in a group of 351 fatteners immune from $RYR1^T$ allele (gene with an influence on carcass quality features and quality of meat), and the abundance of individual genotypes in genetic groups is shown in Table 4. In the analysis of the association the fatteners with *TT* genotype were not considered because of their low frequency (only 3 fatteners in whole group).

Two-factor analysis of variance showed that breed group has no influence on neck meat mass, shoulder mass, area of a loin eye, and meat content in carcass, whereas there was a statistically significant influence of breed group on other examined traits of fattening and slaughter performances ($P \leq 0.05$ and $P \leq 0.01$) – Table 5.

Table 6. Mean values and standard deviations of examined traits of fatteners carcass quality according to *GH*/*Hae*II polymorphism

Tabela 6. Wartości średnie i odchylenia standardowe badanych cech jakości tuszy tuczników w zależności od polimorfizmu *GH*/*MIPS*

Trait Cecha	Mean Średnia	*GH/Msp*I genotypes Genotyp *GH/Msp*I		$F_{emp.}$
		CC	*CT*	
n	351	260	91	
Rate of height (g/day) Tempo wzrostu (g/dzień)	680.06 ±78.83	674.94 ±75.09	699.42 ±88.41	0.41^{ns}
Age at slaughter day (days) Wiek w dniu uboju (dni)	159.97 ±13.03	160.79 ±12.37	157.41 ±14.75	0.76^{ns}
Middle carcass length (cm) Długość środkowa tuszy (cm)	82.05 ±2.74	81.92 ±2.70	82.41 ±2.84	0.56^{ns}
Mean backfat thickness in 5 measurements (cm) Średnia grubość słoniny z 5 pom. (cm)	2.02 ±0.29	2.01 ±0.28	2.03 ±0.31	1.30^{ns}
Neck meat mass (kg) Masa karkówki (kg)	5.36 ±0.52	5.39 ±0.51	5.30 ±0.55	1.77^{ns}
Wing bone mass (kg) Masa łopatki (kg)	6.04 ±0.39	6.03 ±0.39	6.06 ±0.36	0.98^{ns}
Bacon mass (kg) Masa boczku (kg)	6.55 ±0.58	6.51 ±0.58	6.66 ±0.60	2.79^{ns}
Ham mass (kg) Masa szynki (kg)	10.34 ±0.59	10.35 ±0.58	10.31 ±0.58	0.98^{ns}
Loin mass (kg) Masa polędwicy (kg)	8.47 ±0.74	8.48 ±0.75	8.41 ±0.72	0.36^{ns}
Area of a loin eye (cm^2) Powierzchnia oka polędwicy (kg)	51.85 ±5.80	51.61 ±5.79	52.54 ±5.81	0.66^{ns}
Meat mass in particular primary cuts . (kg) Masa mięsa wyrębów podstawowych (kg)	23.36 ±1.01	23.39 ±0.99	23.27 ±1.06	0.83^{ns}
Meat content in carcass (%) Zawartość mięsa w tuszy (%)	56.50 ±2.48	56.60 ±2.46	56.22 ±2.53	2.28^{ns}

*P ≤ 0,05; ** P ≤ 00,1; n.s. – statistically non-significant differences

*P ≤ 0,05; ** P ≤ 00,1; n.s. – różnice statystycznie nieistotne

It was shown that *GH*/*Msp*I polymorphism does not constitute the statistically significant source of variability in proportion to all analyzed traits of fattening and slaughter per formances in examine group of fatteners.

The results does not confirm communications of other authors, who showed statistically significant differences in traits of fattening and slaughter performances in case of [Urban *et al*. 2002], Torhyb line [Kurył *et al*. 2003] and White Large Polish × Zlotnicka Pstra pigs [Pierzchała *et al*. 1999).

Differences in average values of examined traits of fattening and slaughter performances between animals with different *GH*/*Msp*I genotypes presented in Table 6 were small and they were not confirmed statistically. However other authors studied correlation between production traits and genotypes of animals in *GH* gene, e.g. meat utility traits (fat content, meat quality and resistance for stress) of F2 generation of half-breeds originated by crossing Pietrain or Meishan breeds, and wild boar [Geldermann *et al*. 1996; Knorr *et al*. 1997]. Casas-Carrilo *et al*. [1994] showed that somatotropin gene polymorphism was the reason of variability of loin eye area and traits connected with fatness grade. It was conceded that swine *GH* gene is a candidate gene to fatness grade [Knorr *et al*. 1997].

CONCLUSIONS

Conducted studies proved prevalence of single nucleotide polymorphism in intron 2 of *GH* gene, identified by use of *Msp*I endonuclease in all examined breed group of fatteners. It was noticed that individual alleles and *CC* and *CT* genotypes frequencies were significant statistically different between individual breed groups of porkers ($P \leq 0.05$ and $P \leq 0.01$). In analyzed breed groups disturbance of genetic equilibrium was not noticed. Comparing the numbers of individuals observed in *GH*/*Msp*I genotype groups with the number calculated theoretically according to the Hardy-Weinberg law no statistically significant differences were found. Conducted studies showed statistically significant influence of breed group on studied traits of fattening and slaughter performances and statistically significant interaction between a breed group and *GH*/*Msp*I polymorphism to area of a loin eye of fatteners. However *GH*/*Msp*I polymorphism itself did not differentiate in statistically significant way any of analyzed traits of fattening and slaughter performances in examined group of fatteners.

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Streszczenie. Celem badań była analiza zmienności cech użytkowości tucznej i rzeźnej tuczników w zależności od polimorfizmu *GH*/*Msp*I. Materiał badawczy stanowiło stado 369 tuczników należących do pięciu grup rasowych: landrace, landrace × duroc, landrace × yorkshire, (landrace × yorkshire) × duroc, (landrace × yorkshire) × (duroc × pietrain). Częstość występowania alleli polimorfizmu *GH/Msp*I wynosiła odpowiednio: *C* – 0.864 i *T* – 0.136. Porównując liczebności obserwowane w grupach genotypowych *GH*/*Msp*I z liczebnościami teoretycznie skalkulowanymi zgodnie z regułą Hardy'ego-Weinberga nie stwierdzono różnic statystycznie istotnych. Przeprowadzone badania udowodniły istotny statystycznie wpływ grupy rasowej na badane cechy użytkowości tucznej i rzeźnej oraz statystycznie istotną interakcję między grupą rasową a polimorfizmem *GH*/*Msp*I dla powierzchni oka polędwicy. Wykazano, że sam polimorfizm *GH*/*Msp*I nie różnicował w sposób statystycznie istotny żadnej z analizowanych cech użytkowości tucznej i rzeźnej badanej grupy tuczników.

Słowa kluczowe: świnie, polimorfizm DNA, hormon wzrostu, jakość tuszy