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THE LEPTIN GENE POLYMORPHISM AND THE PRODUCTION TRAITS IN THE YOUNG BOARS

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Abstract. The aim of the present study was to evaluate the effect of leptin gene polymorphism on the fattening and slaughter performance traits in the Polish Synthetic Line 990 young boars and to estimate the frequencies of *LEP*/*Hin*fI alleles and genotypes. Altogether, 205 young boars of the 990 line were included in the study. Based upon the carried out live evaluation, information was obtained in respect of following traits: body weight on day 21, 28, 70 and 180 of life; average daily gain; feed efficiency; mean backfat thickness; height of m. longissimus dorsi; percent meat content; and selection index. Genotype determination was performed using the PCR-RFLP technique. The lengths of the restriction fragments for respective alleles following digestion with *Hin*fI were: 152 bp (allele *T*) and 84 + 68 bp (allele *C*). Two genotypes were found, *TT* (frequency 0.8) and *TC* (frequency 0.2). The analysis of relationship between different *LEP* genotypes and all analysed traits showed small and statistically non-significant differences.

Key words: fattening and slaughter performance, leptin gene polymorphism, young boars

INTRODUCTION

Improvement of the growth rate, feed efficiency and meatiness is a chief goal of molecular diagnostics in farm animals. These are the quantitative traits determined by many genes. Molecular studies enable the identification of main genes the phenotypic effect of which is larger than that of other genes. The leptin (*LEP*) gene is a candidate for a gene with a large effect on quantitative traits, such as: backfat thickness, feed intake and growth rate [Lagonigro et al. 2003]. The expression of leptin gene depends on the weight of fat tissue and the concentration of hormones, such as estrogens, insulin, glucocorticoids, prolactin, testosterone and somatotropin. The carried out studies demonstrated that the level of leptin in the colostrum of fat pigs is approximately 306% higher than that in the pigs which are not characterised by fatness [Ramsay et al. 1998].

Jiang and Gibson [1999] carried out research on the association between the leptin gene polymorphism and the fatness in four pig breeds (Duroc, Hampshire, Landrace,

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Large White). Analysis of the sequence of four different PCR fragments showed four substitute-type mutations, namely the mutation *C/T* at position 867, *A/G* at position 1112 recognised by the *Taq*I enzyme, *C/T* at position 3469 recognised by the *Hin*fI enzyme, and the mutation *G/T* at position 3714 recognised by the *Pst*I enzyme. The two first mutations are located in introns, while the other two mutations (silent) are in the coding region. The study of polymorphism at position 3469 confirmed a significant difference in respect of fatness level only in relation to Large White breed. The obtained results are evidence of a probable existence of the association of polymorphism at position 3469 with pig fatness. Despite no effect of *C/T* substitution on the amino-acid sequence, researchers do not exclude its influence on the transcript stability or translation efficiency.

Hardge et al. [2000] demonstrated the effect of leptin gene and leptin receptor on carcass fat content in examined pig hybrids. The study of Kennes et al. [2001] suggests an association of the polymorphism 2845 ($A \rightarrow T$ substitution) and 3469 ($T \rightarrow C$ substitution) with the feed intake and growth rate in Landrace pigs. Kulig et al. [2001] showed that animals with *TC* genotype were characterized by significantly higher carcass meat content than those with *TT* genotype. The study was performed aimed at evaluation of leptin gene polymorphism on the fattening and slaughter performance traits in the Polish Synthetic Line 990 young boars and to estimate the frequencies of respective *LEP*/*Hin*fI alleles and genotypes.

MATERIAL AND METHODS

Altogether, 205 young boars of the Polish Synthetic Line 990 were included in the study. Line 990 was derived at National Research Institute of Animal Production – Experimental Station in Pawlowice, Poland, by crossing six breeds (Polish Large White, Belgian Landrace, German Landrace, Welsh, Hampshire, Duroc) and was generated in the early 80s of the last century. To this time its performance (fattening and slaughter traits) has been consolidated. In Poland, boars of Polish Synthetic Line 990 are used in breeding programms as a paternal line.

Until the weaning day, the young boars were weighed twice, i.e. on day 21 and 28 of life. Their next weighing was done when they reached the age of 70 days. On that day, all animals were put to test evaluation lasting to day 180 of life according to current methods of the National Research Institute of Animal Production [2003]. During the evaluation, the young boars were kept individually under uniform environmental conditions, and the rationed feeding with complete feed was applied. Based upon the carried out live evaluation, information was obtained in respect of following traits: body weight on day 21, 28, 70 and 180 of life; average daily gain from birth till the end of the test and average daily gain during the test (from 70 to 180 days of life). On day 180 of life, their fattening and meat values were live evaluated: feed efficiency; mean backfat thickness; height of m. longissimus dorsi; percent meat content; and selection index. The selection index considering daily gain till 180 days of age was estimated for the boars, and the percentage carcass meatiness. The meatiness was determined by means of a PIGLOG 105 ultrasound apparatus. The peripheral blood was sampled from each individual to vacuum test-tubes

with EDTA anticoagulant. From the blood samples, DNA was isolated using MasterPure™ Genomic DNA Purification Kit (Epicentre Technologies®, Madison, USA).

Genotype determination was performed using the PCR-RFLP technique. Primer sequences [Neuenschwander et al. 1996] used for the amplification of the 152-bp fragment from exon 3 of the leptin gene were:
F: 5' – TGCAGTCTGTCTCCTCCAAA – 3',
R: 5' – CGATAATTGGATCACATTTCTG – 3'.

The reaction mixture consisted of: 70 ng genomic DNA, 10 pmol of each primer, 0.2 μM of each dNTP, 1.5 μM $MgCl_2$ and 0.5 I.U of *Taq* DNA polymerase (MBI Fermentas Burlington, Ontario, Canada) in a standard PCR buffer, the entire reaction mixture was filled up with demineralized water up to the final volume of 20 μl. The thermal conditions for PCR were denaturation at 95°C for 3 min, followed by 33 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 1 min, and amplification at 72°C for 1 min; final extension at 72°C for 5 min.

The fragment was digested with the *Hin*fI restriction enzyme in order to identify respective genotypes of the 3469T>*C* polymorphism (*TT, CT* and *CC*). After digestion of PCR products, the fragments were separated and the products were analysed by electrophoresis on a 3% agarose gel. Moreover, polymorphism of *RYR1* gene was analysed [Kurył and Korwin-Kossakowska 1993] and included in statistical analysis.

Based upon the obtained trait values, the analysis of variance was conducted using the following linear model:

$$Y = \mu + a_i + b_j + c_k + S_m + e_{ijk}$$

μ – mean,
a_i – leptin gene effect (*LEP*/*Hin*fI),
b_j – *RYR1* genotype effect,
c_k – litter size effect,
S_m – effect of m-th sire,
e_{ijk} – effect of unidentified random factors (random error).

The statistical significance of differences between groups was confirmed by the Duncan's Multiple Range test. The STATISTICA®6.0 PL computer package was used for the statistical analysis.

RESULTS AND DISCUSSION

The length of the restriction fragments for respective alleles, after digestion with *Hin*fI, were 152 bp (allele *T*) and 84 + 68 bp (allele *C*) [Stratil et al. 1997]. The frequencies of respective alleles and *LEP*/*Hin*fI genotypes are presented in Table 1. Allele *T* occurred higher frequency than allele *C*. Low frequency of allele *C* in the examined group of the 990 line young boars is consistent with the results published earlier by other authors [Stratil et al. 1997, Kulig et al. 2001; Korwin-Kossakowska et al. 2002; Kmieć et al. 2003]. The analysis of *LEP* gene polymorphism showed, that a similar frequency of allele *T* was observed by

Křenková et al. [1999] who studied the Landrace, Large White and Pietrain breeds, Terman [2005] in hybrids Polish Large White x Landrace sows and Szydłowski et al. [2004] in Polish Synthetic Line 990 sows. Lower frequency (0.65) of allele *T* was observed by Urban et al. [2002] who analysed Duroc breed.

Table 1. Frequencies of genotypes and alleles in the 990 line young boars
Tabela 1. Częstość występowania genotypów i alleli u badanych knurków linii 990

Occurrence Występowanie	Genotypes *LEP/Hin*fI Genotypy *LEP/Hin*fI		Alleles *LEP/Hin*fI Allele *LEP/Hin*fI	
	TT	*TC*	*T*	*C*
Number – Liczebność	164	41	0.9	0.1
Frequency – Częstotliwość	0.8	0.2		

In the present study only two genotypes were observed *TT* and *TC*. Similar to other studies [Křenková et al. 1999; Mikolášová and Křenková 1999; Kulig et al. 2001; Korwin-Kossakowska et al. 2002; Kmieć et al. 2003], genotype *TT* in the examined young boars occurred higher frequency than genotype *TC*. Higher frequency of *TC* genotype in compare to *TT* was observed by Urban et al. [2002]. Three genotypes of *LEP* gene were identified in herd of wild boar (*Sus scrofa scrofa*) by Ernst et al. [2003].

The analysis of relationship between different *LEP* genotypes and all analysed traits showed small and statistically non-significant differences (Table 2). The body weight of young boars of the genotype groups was very similar, like in the study carried out by Kulig et al. [2001]. However Křenková et al. [1999] reported that piglets with *TT* genotype characterized by higher body weight at weaning than piglets with *TC* genotype. Higher body weight at 21, 42, 63 and 77 day of life in pigs with *TT* genotype than the animals with *TC* genotype was observed by Peixoto et al. [2006].

It was demonstrated that the *TT* homozygous animals were a slightly higher (by 1%) in average daily gain. Significant higher daily body weight gain in Polish Landrace and Duroc pigs with *TC* genotype compared to *TT* genotype was found out respectively by Kulig et al. [2001] and Urban et al. [2002]. However, Kennes et al. [2001] showed higher (by 60 g) daily body weight gain in Landrace pigs with *TT* genotype compared to animals with *TC* genotype.

Table 2. The values of fattening and slaughter performance traits in the 990 line young boars for *LEP*/*Hin*fI genotypes
Tabela 2. Wartość cech użytkowości tucznej i rzeźnej knurków linii 990 dla genotypów *LEP*/*Hin*fI

Traits Badane cechy	Genotypes Genotypy			
	TC		*TT*	
	$\bar{x}$	s	$\bar{x}$	s
Body weight on day 21 of life, kg Masa ciała w 21. dniu, kg	5.7	0.72	6.0	0.85
Body weight on day 28 of life, kg Masa ciała w 28. dniu, kg	7.7	1.1	8.0	1.24
Body weight on day 70 of life, kg Masa ciała w 70. dniu, kg	19.6	2.55	20.9	2.84
Body weight on day 180 of life, kg Masa ciała w 180. dniu, kg	109	7.82	110	8.70
Average daily gain, g Życiowy przyrost dzienny, g	609	43.07	616	45.78
Daily gain in test, g Przyrost masy ciała w teście, g	770	64.81	766	72.11
Feed efficiency in test, kg Wykorzystanie paszy w teście, kg	2.92	0.26	2.95	0.25
Mean backfat thickness, mm Średnia grubość słoniny, mm	10.1	1.58	10.2	1.73
Height of m. longissimus dorsi, mm Wysokość oka polędwicy, mm	50.5	6.43	51.5	6.09
Meatiness, % Zawartość mięsa w tuszy, %	58.6	1.88	58.7	1.90
Selection index, pkt Indeks oceny przyżyciowej, pkt.	120	10.6	122	10.4

The analysed mean backfat thickness in the genotypic groups also assumed very similar values. Similar observations were made by Jiang and Gibson [1999]. From among the four pig breeds examined by these authors (Duroc, Hampshire, Landrace, Large White), a significant association between allele *C* and backfat thickness was found in Large White pigs only. On the other hand, results with regard to the effect of polymorphism in the leptin gene on backfat thickness were observed in the study of Kennes et al. [2001]. They demonstrated that allele *C* occurred with lower frequency in Landrace pigs which had thinner backfat than in the pigs with thicker backfat. These results were not confirmed in the studies performed on a larger number of animals by those authors. Associations between polymorphism in *LEP* gene with carcass traits and meatiness pigs was analysed also by Szydłowski et al. [2004]. The authors did not observed differences between *LEP* genotype in this traits in Polish Synthetic Line 990 pigs. Kurył et al. [2003] demonstrated that Pietrain pigs appeared to be monomorphic as regards LEP^T allele. Accordingly with

authors, *TT* genotype may be more advantageous for decreasing fat deposition in the carcass than genotype *TC*, what was not confirmed by the results of present study.

CONCLUSION

In the present study, no statistically significant effect of the polymorphism 3469T>*C* in the leptin gene was found on the variation of fattening and slaughter performance traits examined in the 990 line young boars and the analysed traits in the genotypic groups also assumed very similar values.

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POLIMORFIZM W GENIE LEPTYNY A CECHY UŻYTKOWOŚCI TUCZNEJ I RZEŹNEJ KNURKÓW LINII 990

Streszczenie. Celem badań była ocena wpływu polimorfizmu w genie leptyny na cechy użytkowości tucznej i rzeźnej knurków linii 990 oraz obliczenie częstości występowania poszczególnych alleli i genotypów LEP/*Hinf*I. Łącznie badaniami objęto 205 młodych knurów linii 990. Na podstawie przeprowadzonej oceny przyżyciowej uzyskano wyniki w zakresie następujących cech: masy ciała w 21., 28., 70. i 180. dniu życia; życiowego przyrostu dziennego; wykorzystania paszy; średniej grubości słoniny; wysokości oka polędwicy; procentowej zawartości mięsa; indeksu oceny przyżyciowej. Oznaczenie genotypów przeprowadzono z wykorzystaniem metody PCR-RFLP. Długości uzyskanych w wyniku trawienia fragmentów restrykcyjnych dla poszczególnych alleli były następujące: 152 pary zasad (brak miejsca rozpoznawanego przez enzym *Hinf*I) dla allelu oznaczanego jako *T* oraz 84 i 68 par zasad (obecność miejsca rozpoznawanego przez enzym *Hinf*I) dla allelu oznaczanego jako C. Analiza restrykcyjna umożliwiła rozróżnienie jedynie dwóch genotypów: *TT* i *TC*. U badanych knurków genotyp *TT* występował z większą częstością niż genotyp *TC*. W niniejszych badaniach nie stwierdzono statystycznie istotnego wpływu polimorfizmu T3469C w genie leptyny na zmienność badanych cech użytkowości tucznej i rzeźnej u knurków linii 990, a wartości tych cech u grup genotypowych *TT* i *TC* przyjmowały bardzo zbliżone wartości.

Słowa kluczowe: młode knury, polimorfizm genu leptyny, użytkowość tuczna i rzeźna

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