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Agricultural University of Szczecin, Department of Genetics and Animal Breeding, Laboratory of Molecular Genetics, Poland

HANNA KULIG, WILHELM GRZESIAK and IWONA SZATKOWSKA

# Effect of leptin gene polymorphism on growth and carcass traits in pigs (short communication)

## Summary

The polymorphism of the porcine leptin gene and its relation with some performance traits (body weight, average daily gains, lean meat content) were analysed in 131 sows of Polish Landrace. The frequencies of detected genotypes TT, TC and CC were 0.763, 0.222 and 0.015 respectively. The estimated frequencies of alleles were 0.874 for allele T and 0,126 for allele C. Lean meat content and average daily gains in TC pigs were statistically significantly higher then those of TT individuals. No statistically significant differences in body weight were found among sows of the above genotypes.

Key Words: pigs, leptin, polymorphism, performance traits

## Zusammenfassung

Titel der Arbeit: **Einfluss des Leptingenpolymorphismus auf Wachstum und Schlachtmerkmale beim Schwein** (Kurzmitteilung)

Es wurden Zusammenhänge zwischen dem Leptingenpolymorphismus und ausgewählten Merkmalen wie Körpergewicht, tägliche Zunahme und Fleischanteil der Schlachthälften bei 131 Sauen der Polnischen Landrasse untersucht. Die drei Genotypen TT (homozygot), TC (heterozygot) und CC (homozygot) traten mit der Frequenz 0,763, 0,222 und 0,015 auf. Die Allelfrequenzen betrugen für T 0,874 und für C 0,126. Hinsichtlich der täglichen Zunahme und des Fleischanteils erreichten die Genotypen TC signifikant höhere Werte, während sich diese im Merkmal Körpergewicht nicht unterschieden.

Schlüsselwörter: Schwein, Leptingenpolymorphismus, tägliche Zunahme, Fleischanteil

## Introduction

High level of pork consumption in Poland results from culinary traditions. Several features, such as rapid growth rate or good feed conversion, determine the usefulness of pigs for meat production. Due to the market demand for low-fat products, obesity in pigs becomes an important problem. Additionally, in economical terms, increased fatness is undesirable as it increases feed costs of production. Therefore breeders aim at an increase of lean pork production. It is also important to investigate genetic markers that could be useful for testing obesity and meat performance related features. The leptin gene can probably be such a marker.

Leptin, the protein encoded by the obese gene (*ob*), belongs to the cytokine family, which includes interleukin 2 (IL-2) and growth hormone. Like all the members of the family, leptin has its characteristic, three-dimensional 4-α-helix bundle structure (HOUSEKNECHT et al., 1998). Leptin features make it a secretory protein with hormonal properties (ZHANG et al., 1994). As a plasma protein, it is responsible for fat reserves in humans and mice (HOUSEKNECHT et al., 1998). Leptin is secreted

into the blood by adipocytes (RAMSAY et al., 1998) and in pigs it consists of 167 amino acids with a predicted molecular weight of 18.661-kDa (MENDIOLA et al., 1997).

The *ob* gene has been mapped to porcine chromosome 18 (NEUENSCHWANDER et al., 1996; SASAKI et al., 1996) and it consists of three exons: the first one is a short untranslated sequence and the amino acid coding sequence is located in the second and third exons (BIDWELL et al., 1997). The gene structure and the amino acid sequence of the protein are highly conserved in several mammalian species. The amino acid sequence of the porcine leptin is in 95%, 92% and 89% homologous to respectively bovine, human and murine sequences (BIDWELL et al., 1997). Additionally, cDNA sequence data collected by Ramsay et al. [1998] demonstrate 92%, 88% and 85% homology with the sequences of the above species. Very similar results were reported by MENDIOLA et al. (1997). Data obtained on mice studies suggest some association between the mutations in leptin gene and the profound obese phenotype of the *ob/ob* mouse (ZHANG et al., 1994). At least four single base polymorphisms were found in the porcine leptin gene caused by C/T, A/G, C/T and G/T substitutions at the positions 867, 1112, 3469 and 3714 respectively. The first two mutations appeared in introns and the second two – in exons, however they were silent. The last three substitutions change the recognition sites for enzymes *TaqI*, *HinfI*, *PstI* respectively (STRATIL et al., 1997; JIANG and GIBSON, 1999).

The obesity gene expression, which is regulated by adipose tissue mass and hormones such as insulin and glucocorticoids, is restricted to adipose tissue and placenta in mammals (HOUSEKNECHT and PORTOCARRERO, 1998). Some data show that it could also be related to preadipocytes recruitment as well as fat cell size (CHEN et al., 1997).

Physiological functions of leptin, which were observed mainly in humans and rodents, concern the regulation of food intake, stimulation or inhibition of energy expenditure and reproductive functions (BARB et al., 1998; CUNNINGHAM et al., 1999; HOUSEKNECHT et al., 1998; HOUSEKNECHT and PORTOCARRERO, 1998). Leptin receptors are localised, among others, in the regions of the hypothalamus (DYER et al., 1997; TARTAGLIA, 1997), where two primary regulators of growth hormone secretion (GH-releasing factor and somatostatin) are also produced (LESHIN et al., 1994). This could suggest the role of leptin in growth processes. The same regions of brain are also involved in food intake regulation. Because of high level of leptin molecule homology among several species, it is possible that this hormone may influence similar features in pigs.

All the above data and suggestions have brought the authors to conduct the studies on the C/T *HinfI* polymorphism of the leptin gene in Polish Landrace and to determine its possible relations with growth and carcass traits.

## Material and methods

The study covered 131 sows of Polish Landrace which had been bred under similar environmental conditions. The blood from an external jugular vein was collected into tubes with EDTA. Genomic DNA was then isolated with MasterPure kit, according to the producer's instructions. Genotypes analyses were performed using the PCR-RFLP

method. The PCR primers that were used to amplify the desired fragment of the porcine leptin gene (152 bp) had the following nucleotide sequences (NEUENSCHWANDER et al., 1996):
5' – TGC AGT CTG TCT CCT CCA AA – 3'
5' – CGA TAA TTG GAT CAC ATT TCT G – 3'
The PCR was performed in a total volume of 20 µl containing 50-100 ng DNA, PCR buffer, 0.75 mmol of $MgCl_2$, 10 pmol of each dNTP, 25 pmol of each primer and 0.5 U *Taq* polymerase. The PCR conditions were as follows: 95°C for 2 min., 35 cycles of 95°C for 1 min., 55°C for 1 min. and 72°C for 1 min.; the final step was at 72°C for 5 min. (STRATIL et al., 1997). The PCR products were digested with *HinfI* endonuclease and then separated on 3% agarose gels (45 min, 90 V) with 1 µg/ml of ethidium bromide.
The following performance traits parameters of 170–202 day-old animals, with minimal body weight not less than 70 kg, were analysed in the experiment: body weight (kg), average daily gains (g) and lean meat content (%). All the data came from farm documentation. Lean meat content and average daily gains data were recorded on live animals using "Piglog 106", ultrasonic instrument, according to the method worked out in the Institute of Animal Breeding in Balice (ECKERT and ŻAK, 1999).
Statistical analysis of lean meat content and average daily gains was done using the t-test, whereas the non-parametrical Mann-Withney's U test was used for body weight analysis, due to the negative result of data distribution normality test (Shapiro-Wilks' W test).

## Results and discussion

The *HinfI* endonuclease digests the wild type allele C into two fragments: 84 and 68 bp, while the mutated allele T stays undigested (152 bp). In consequence, the *HinfI*–RFLP allows recognition of three different genotypes: TT (152 bp fragment), TC (152, 84 and 68 bp) and CC (84 and 68 bp) (Figure).


Fig.: *HinfI* polymorphism in porcine leptin gene (Polymorphie *HinfI* im Leptingen bei Schweinen) M – DNA marker (pUC19/*MspI*); lane 1 – homozygote TT (152 bp); lane 2 – heterozygote TC (152, 84, 68); homozygote CC (84, 68)

Among the studied animals, the frequencies of each genotype were as follows: TT – 0.763, CT – 0.222 and CC – 0.015. Alleles T and C appeared with frequencies (respectively) 0.874 and 0.126, which was in accordance with reference data (KŘENKOVÁ, 1999; MIKOLÁŠOVÁ and KŘENKOVÁ, 1999).

STRATIL et al. (1997) noted a considerably higher occurrence of allele T in Landrace, Hampshire, Large White, Black Pied Pöeštice, Pietrain and Czech Meat Pig. The allele T did not appear in Meishan pigs. Examining the populations of crossbred pigs, Landrace x BU and BU x Pietrain, KŘENKOVÁ (1999) determined the frequencies of alleles and genotypes as follows: allele T 0.925, allele C 0.075 and genotype TT 0.850, TC 0.150. None of the tested animals carried the CC genotype. MIKOLÁŠOVÁ et al. (1999) obtained similar results for crossbred pigs BU x L: T frequency was 0.930, C – 0.070, genotype TT – 0.865 and genotype CT – 0.135. The experiment presented in this paper also confirmed higher frequency of allele T and genotype TT in Polish Landrace.

According to the cited above data on leptin (leptin gene expression, physiological functions and high homology of leptin), the influence of this protein on the regulation of food intake, energy expenditure and another similar features in pigs could be suggested. The localisation of leptin receptors also indicates its role in the modulation of growth hormone secretion (BARB et al., 1998). The experiments data indicate that leptin could be an important link between the growth process, metabolic status and neuroendocrine system (BARB et al., 1998).

BIDWELL et al. (1997) reported higher levels of leptin mRNA in subcutaneous adipose tissue in finished pigs (with body weight at 136 kg) than in growing ones (60 kg). Higher level of leptin mRNA and approximately 306% higher levels of protein in sera was noted by RAMSAY et al. (1998) in obese pigs comparing to contemporary nonobese individuals.

In this experiment, the differences in lean meat content and average daily gains between the pigs carrying TT and TC genotypes were observed (Table). The lean meat content in the TT pigs was statistically significantly lower ($P \leq 0.05$) than in the TC pigs (in 1.01%). The average daily gains of the previous group were also statistically significantly lower ($P \leq 0.05$) than of the TC pigs (in 26.34 g). There were no statistically significant differences in body weight between both genotypes.

The CC homozygous pigs had considerably higher mean body weight and average daily gains than the TT and TC animals, however, because of a small number of observations (only two CC pigs), that group was not used for statistical comparison.

Table

Relations between leptin genotype and some performance traits in pigs (Zusammenhang zwischen dem Leptin-Genotyp und ausgewählten Nutzeigenschaften bei Schweinen) (SD –standard deviation, $\bar{x}$ - mean value of the feature, n – number of observations among the group)

| Feature | Genotype TT | | | TC | | | CC | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | $\bar{x}$ | SD | n | $\bar{x}$ | SD | n | $\bar{x}$ | SD |
| Body weight [kg] | 94 | 99.86 | 11.53 | 29 | 101.21 | 9.54 | 2 | 114.00 | 5.66 |
| Average daily gain [g] | 100 | 558.56* | 51.73 | 29 | 584.90* | 47.79 | 2 | 596.50 | 33.23 |
| Lean meat content [%] | 100 | 56.35* | 1.99 | 29 | 57.36* | 2.26 | 2 | 55.30 | 1.98 |

* - significance of differences at $P \leq 0.05$

MIKOLÁŠOVÁ et al. (1999) did not establish any relation between leptin allele and lean meat content or average daily gain but the latter feature was nearly at the significance level (P=0.06). HARDGE et al. (1998) described considerable associations between leptin gene and meat to fat ratio, and the back fat thickness; TT pigs had higher values of both features than TC animals. However, the experiments mentioned above were conducted for different breeds.

One result of presented studies is worth mentioning: lean meat content and average daily gains increased according to the presence of the C allele in the genotype. This fact can lead to the conclusion that the C allele has a positive influence on the above features although this hypothesis needs to be statistically confirmed, considering higher number of pigs with CC genotype. According to data noted in the Communicators of Animal Breeding Institute in Balice (ECKERT and ŻAK, 1999), growth abilities and slaughter values of Polish pigs do not match up to the expectations of breeders. In such situation, the selection improving pigs' fleshiness should be applied and the leptin genotype could become one of the criteria in pig breeding.

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Author's address
MSc. HANNA KULIG, PhD WILHELM GRZESIAK, PhD DSc IWONA SZATKOWSKA
Agricultural University of Szczecin, Department of Genetics and Animal Breeding,
Laboratory of Molecular Genetics
ul. Doktora Judyma 6
71-460 Szczecin
Poland