ASSOCIATION BETWEEN GROWTH HORMONE GENE POLYMORPHISM AND ECONOMIC TRAITS IN PIGS

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Summary. Growth hormone (GH) regulates growth, development and various metabolic activities. The objective of this study was to investigate the effect of single nucleotide polymorphisms in growth hormone (GH) gene on performance traits in pigs. Genotypes of growth hormone gene (*GH*) were established with PCR-RFLP technique using *FokI* endonuclease. Porcine GH gene AA genotype was found with frequency 0.121, AG genotype – 0.474 and GG genotype with frequency 0.405. Animals with GG genotype had less body fat amount and higher muscularity percent, compared to AG and AA genotype animal. Pigs with GG genotype had the lowest age while reaching 100 kg.
Keywords: Growth hormone (GH), restriction fragments length polymorphism (RFLP), pigs.

KIAULIŲ AUGIMO HORMONO GENO POLIMORFIZMO ĮTAKA EKONOMINIAMS POŽYMIAMS

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Santrauka. Augimo hormonas (GH) reguliuoja augimą, vystymąsi ir įvairius metabolinius procesus. Šio darbo tikslas buvo ištirti augimo hormono geno (GH) vieno nukleotido polimorfizmo įtaką kiaulių produktyvioms savybėms. Augimo hormono geno genotipai nustatyti PGR-RFIP metodu, naudojant *FokI* restrikcinį fermentą. Kiaulių GH geno AA genotipas buvo rastas 0,121 dažniu, AG genotipas – 0,474, o GG genotipas pasireiškė 0,405 dažniu. GG genotipo gyvulių lašiniai buvo plonesni ir raumeningesni už AG ir AA genotipo gyvulių. GG genotipo kiaulės 100 kg masę pasiekė greičiau.
Raktažodžiai: augimo hormonas (GH), restrikcinių fragmentų ilgio polimorfizmas (RFIP), kiaulės.

Introduction. Meat is one of the main sources of protein in the human diet, and pork is one of the most produced and consumed worldwide. One of the concerns during pork production is its quality (Band, 2005). Eating quality of meat depends on several important characteristics, including appearance, colour, taste, fat content, texture, and tenderness. Meat quality depends on species, genetic background, the protein complement of the muscle, and environmental factors (Ciobanu, 2004). For many years, one of the major objectives of the swine industry was to increase the lean: fat ratio of pork carcasses. As a result, dramatic improvements in the body composition of pigs have been made through genetic selection (Huff – Lonergan, 2002).

Advances in different areas of animal production (management, nutrition, environment, sanitary control and genetic breeding) have led to improvements in the pig production chain. Genetic breeding of herds have been carried out by selecting animals with high production potential based on their phenotype. One alternative for the selection of the best animals to be used as parents of the next generation is to identify genes or loci controlling economically important traits and to incorporate this information into traditional breeding methods.

In the last few years there have been many important advances in molecular genetics and biotechnology. Pigs represent a good animal model for genetic studies due to their short gestational period, large number of piglets per litter and short generation interval (Faria, 2006).

There are a number of genes affecting pork production quality and quantity. It is myostatin, leptin, growth hormone, growth hormone receptor, stress and other genes.

The GH gene pathway contains various interdependent genes, such as IGF1 (insulin – like growth factor), PIT1 (pituitary – specific transcription factor), GHRH (growth hormone releasing hormone receptor) and others. These genes are potential candidate markers because of their important physiological effects associated with economic traits (Franco, 2005).

Growth hormone (GH) is a peptide hormone with about 190 residues which regulates growth, development and various metabolic activities. In all mammals, the GH gene extends over 2~3 kb and comprises five exons split by four introns. Injections of GH into growing pigs increassed growth rate and the percentage of muscle and fat accretion was decreased. This gene controls growth and fat deposit in pigs (Song, 2003; Jing, 2006). The growth hormone gene is localized on chromosome pair 12 (in the p1.4 region) (Rejduch, 2008).

Lean weight of animals is related to the number of myofibers in their muscles. Pigs with more muscle fibers grew faster and had greater muscle mass. Mutations disrupting normal protein function of the myostatin gene seem to cause the double-muscling phenotype. However, other genes can also affect myofiber numbers. Disruption of the myogenin gene also affects myofiber numbers (Pas,

1999).

Fatness in pigs is of economic importance because of market incentives for production of lean pork and because increasinsing fatness increases the feed costs of production. Leptin, a 16 – kDa protein secreted from white adipocytes, is involved in regulation of food intake, energy expenditure, and whole – body energy balance (Jiang, 1999).

Rendement Napole (RN) gene is a swine gene found to cause low ultimate pH and water holding capacity in pork. The gene is also commonly called the „acid meat gene" or „Hampshire effect". The negative effects of the RN gene on pork quality result in economic losses in the pork industry (Houde, 2002).

The aim of the study was to investigate the association between polymorphisms in the GH gene and the fattening and meat traits in pigs of Lithuania.

Material and methods. 116 various breed pigs from State Pig Breeding Station, were used in this study. Pigs were dependent Lithuanian White (34), old - type Lithuanian White (11), Large White (18), Landrase (11), Yorkshire (22), Large White hybrid (20).

Analyses were done in Lithuanian University of Health Sciences, K. Janušauskas Laboratory of Animal Genetic. Chemicals were purchased from „Fermentas". DNA were extracted from hair roots using DTT (1M), Chelex 100, Protenase K (20mg/ml) chemicals. GH gene was analysed by PCR-RFLP method, using GH1 forward 5'-TTATCCATTAGCACATGCCTGCCAG-3' and GH2 reverse 5'-CTGGGGAGCTTACAAACTCCTT-3' oligonucleotide primers (10pmol), 0.2 mM dNTP, 25 mM $MgCl_2$, 10x Taq Buffer $(NH_4)SO_4$, 2U Taq DNA polymerase, BSA (20 mg/ml). Polymerase chain reaction (PCR) was done with the following conditions: 95 ^{O}C for 3 min, followed by 35 cycles of 94 ^{O}C for 45 s, 59 ^{O}C for 45 S and 76 ^{O}C for 1 min, with a final extension at 76 ^{O}C for 10 min. After amplification, 10 µL of the PCR amplification (604 bp) was digested with *FokI* restriction enzyme by incubation 55^{O} C for 3 h. The genotyping was done in 3 % agarose gel stained with ethidium bromide and photographed under UV light (Song, 2003).

After PCR was obtained 604 bp fragment. After digestion with restriction enzymes we got A allele – 604 bp and G allele – 345 bp and 259 bp (Figure 1).

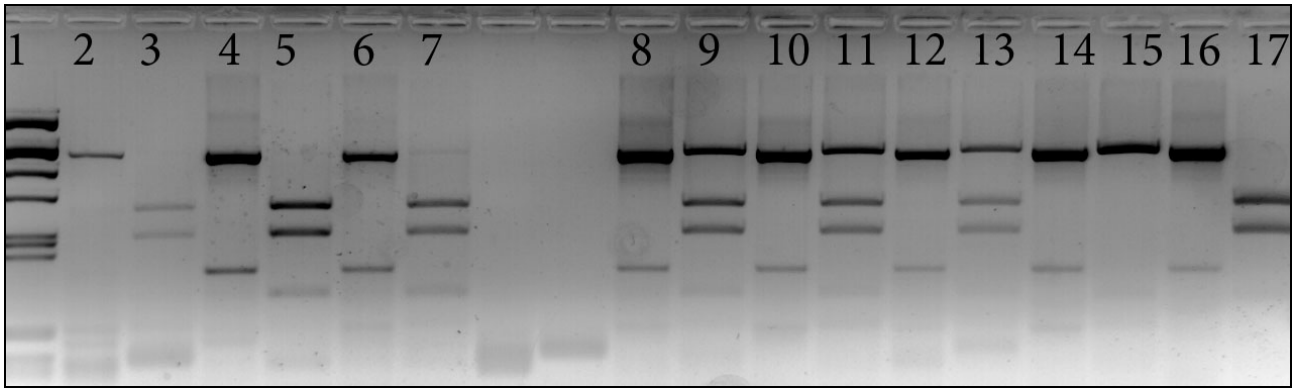

Figure 1. **Pig GH gene polymorphism studies**:1- DNA marker

(pBR322 DNA/AluI Marker, 20); 2, 4, 6, 8, 10, 12, 14, 16,– PCR; 15 - AA genotype; 9, 11, 13 – AG genotype; 3, 5, 7, 17 – GG genotype.

To assess the influence of GH gene for production characteristics and fattening parameters a data base of following phenotipic characteristics was formed – the hot carcass weight, lenght of carcass halves, lenght of bacon halves, loin muscle area, weight of ham, fat thickness at 6-7 rib, fat thickness at 10th rib, fat thickness behind the last rib, fat thickness at Fat_2 point, muscle thickness at Fat_2 point, muscularity, age at slaughter, age at 100 kg, daily gain, feed consumed during the fattening period, feed consumption of 1 kg weight gain. The data was obtained from State Pig Breeding Station, Kaunas department.

Statistical analyses. Statistical analyses were done using R statistical package (http://cran.r-project.org/bin/windows/base/). In the data analysis the following indicators were calculated: gene and genotype frequencies, actual and predicted heterozigosity, gene influence for each indicator.

Results and discussion. After examination of 116 pigs it was found, that GH gene is polymorfic in investigated group of pigs, two allelis of this gene – A (604 bp) and G (345 bp and 259 bp) were found. AA genotype was found with frequency 0.121, AG genotype – 0.474 and GG genotype with frequency 0.405. A allele was found with frequency 0.358, and G allele – 0.642 (Table 1).

Table 1. **Pig GH gene genotypes and allele frequencies**

Genotype	n	Frequency	Allele	Frequency
AA	14	0.121	A	0.358
AG	55	0.474	G	0.642
GG	47	0.405		
	116	1		1

AA genotype with the highest frequency was found in the Large White and their crossbred (0.188) and was not found in Landrace pig breed. GG genotype with highest

frequency was found in Lithuanian White pig breed (0.406) and least- in the old -type Lithuanian White (0.031). AG genotype with the highest frequency was found in the Lithuanian White (0.531) pigs, and least frequency was in the Yorkshire (0.094) pig breed.

Table 2. **Actual and theoretical heterozygosity in investigated group of pigs**

Heterozygosity	Investigated group of pigs
Actual heterozygosity	0.385
Theoretical heterozygosity	0.356
χ^2 – criterion value	1.78 (p=0.182)
(P – value)	

Evaluating actual and theoretical heterozygosity across the investigated group of animals, the actual heterozygosity was found higher than theoretical, indicating the sufficient amount of genetic diversity of studied loci, although the difference were not statistically significant (Table 2).

Pigs with GG genotype had the lowest age while reaching 100 kg (178.8 days), the lowest consumption of feed during fattening period had pigs with GG genotype (176.3 kg), and the lowest feed consumption of 1 kg weight gain had animals with GG and AG genotypes (2.7 kg), while pigs with AA genotype 1 kg overweight had 2.8 kg feed. The largest daily gain reached pigs with GG genotype (777.4 g), and animals with AA genotype had lowest daily gain (743.7 g) (Table 3).

Pig GH gene G allele increased length of bacon halves, muscle thickness and muscularity. The lowest fat thickness was characterized by GG genotype pigs (13.8 mm at Fat_2 point), and the highest – animals of AG genotype (17.9 mm at Fat_2 point). Animals with GG genotype had bigger muscularity (56.3 %), and the lowest – animals with AG genotype (53.8 %). Weight of ham was the same in all genotypes (11.8 kg), also differed slightly length of carcass halves (AA – 98.9 cm, AG – 98.6 cm and GG – 98.8 cm) and bacon halves (AA – 77.9 cm, AG – 77.6 cm, GG – 77.7 cm). The highest area of back muscule has pigs with GG genotype (40.6 cm²), and the lowest has pigs with AA genotype (39.4 cm²) (Table 4).

Table 3. **GH gene influence on the fattening properties of pig**

Genotype	n	Age at slaughter, days	Age at 100 kg, days.	Daily gain, g	Feed consumed during the fattening period, kg	Feed consumption for 1kg weight gain kg
AA	14	174.9±0.98	182.1±1.01	743.7±11.28^a	180±0.45^a	2.8±0.01^a
AG	55	174±0.25	179.6±0.27	760.8±1.89^b	178.1±0.19^b	2.7±0.01^b
GG	47	173±0.33	178.8±0.35	777.4±2.35	176.3±0.2	2.7±0.01

a,b – averages in the column of table, marked by different letters, statistically significant differs with each other ($p<0.05$)

Table 4. **Pig GH gene influence on meat traits**

Genotype	n	Warm carcass weight, kg	Lenght of carcass halves, cm	Lenght of bacon halves, cm	Area of back muscule, cm²	Weight of ham, kg	Fat thickness at 6–7 rib, mm	Fat thickness at 10 rib, mm	Fat thickness at last rib, mm	Fat thickness at Fat_2 point, mm	Muscle thickness at Fat_2 point, mm	Lean meat content, %
AA	14	75.3± 0.05	98.9± 0.12	77.9± 0.14	39.4± 0.34	11.8± 0.02	18.2± 0.19	17± 0.22	17.9± 0.19^a	15.4± 0.19	45.1± 0.651	54.7± 0.208
AG	55	75.3± 0.01	98.6± 0.04	77.6± 0.04	40.1± 0.09	11.8± 0.01	19.1± 0.08^a	17.7± 0.08^a	18.2± 0.08^a	16.4± 0.08^a	45.5± 0.136	53.8± 0.078^a
GG	47	75.2± 0.02	98.8± 0.04	77.7± 0.04	40.6± 0.06	11.8± 0.01	16.3± 0.07^b	15.1± 0.07^b	15.6± 0.07^b	13.8± 0.06^b	46.4± 0.117	56.3± 0.06^b

a,b – a,b – averages in the column of table, marked by different letters, statistically significant differs with each other ($p<0.05$)

A,B – between the averages in the column of table, marked by different letters, established a statistically significant difference trend ($0.1<p<0.05$)

GH plays a key role in regulating tissue growth and metabolism in animal. Although most amino acids of the GH protein are conserved, there are still many single nucleotide polymorphisms which are reported in traits of growth, lean rate and milk production (Jing, 2006). Growth hormone gene may be potential candidate marker for marker assisted selection programs. The sequence of porcine GH was identified by Vice and Wells (1987), and the total length of porcine GH gene is 2231 bp, containing four introns and five exons. Several growth hormone gene polymorphic sites had been reported and the effects of some sites on growth performance were investigated (Song, 2003). Song et. al. (2003) found pig breed differences in 506 bp fragment of GH. They found Landrace and Meishan pigs lacked allele G3 of MspI site, Meishan pig lacked allele G1 of ApaI site. Pierzchała et. al. (2004) studied two single nucleotide polimorphisms sites in GH gene. They found GH/MspI and GH/HaeII genotypes significantly related to the weight of ham, weight of ham meat and ham content of carcass. Nearly significant differences between GH/MspIn genotypes were found for mean fat thickness, fat thickness at lower back and over the loin, and for loin eye height. Rybarczyk et.al (2007) did not found any relationship between the GH gene polymorphism and the carcass slaughter traits determined with meatiness measuring apparatus CGM and the meat quality and meat basic chemical composition in PIC hybrid porkers. Knorr et al. (1997) demonstrated significant association of GH genotypes with eight carcass fatness traits in Meishan x Pietrain pigs, whereas did not confirm this fact in the hybrids of wild boar x Pietrain pigs. Wang et al. (2003) showed an association of GH/ApaI gene polymorphism with carcass meat content in Yorkshire pigs, however not confirming this in Nanchang White pigs. Pierzchala et al. (1999; 2004), Krenkova et al. (1999), Kuryl et al. (2003), Franco et al. (2005) point to significant association of different *GH* gene variants with carcass fatness and meatiness in different pig breeds and lines (Rybarczyk, 2007).

In conclusion, our results demonstrate the potential of the GH gene as a candidate for the investigation of quantitative traits in pigs. This GH gene polymorphism could be tested for its possible application in marker – assisted selection programs in pigs.

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Received 22 June 2011
Accepted 15 November 2011