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LIPE GENE POLYMORPHISM c.442 G>A INFLUENCE ON CARCASS TRAITS IN PIGS

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Abstract: Hormone sensitive lipase is one of three enzymes involved in lipolysis process and encoded by LIPE gene. In this study we investigated LIPE gene polymorphism c.442 G>A influence on carcass traits in hybrid pigs. Genomic DNA extracted using Chelex resin, genotypes determined using RFLP-PCR. Allele A observed with frequency 0,738, allele G – 0,262. The most common genotype was AA, genotype GG was observed with lower frequency, genotype AG was rarest. While evaluating population heterozygosity, it was noticed that observed heterozygosity was only 0,075, while expected heterozygosity was 0,387. In observed pig population allele A is associated with better animal muscularity, allele G – with greater fat content.

Keywords: LIPE gene, SNP, polymorphism, c.442 G>A, carcass traits

Introduction

According to the United Nations Food and Agriculture Organization pork is the most consumed meat *per capita* in the world. Therefore, it is very important to know about the quality of consumed meat, which can be determined by animal's age, breed, genetic or environmental factors. It is known that large intakes of saturated fatty acids can cause heart and coronary diseases, type 2 diabetes or cancer (*Schwab et al. 2014*), hence consumers are choosing meat by its juiciness, tenderness, texture and properties when cooking.

Ongoing pig selection in the world is based on lowering fat content in the meat and increasing lean carcass yield (*Zimmermann et al. 2004; Tyra et al. 2011*). To maintain valuable meat traits in all generations pig selection should be based on genetic, not phenotypical properties. Accordingly, a lot of research work was done to evaluate genes candidates and their influence on carcass traits. Selected genes candidates are carefully evaluated in various animal populations, accurate enzyme

function is determined, polymorphism influence on phenotypic traits is evaluated. Selected genes are called markers; they are included in quantitative trait locus (QTL) maps.

LIPE gene encodes hormone-sensitive lipase (HSL), this enzyme plays a key role in fatty acid metabolism, so called lipolysis *(Harbitz et al. 1999; Holm et al. 2003; Chahinian et al. 2005; Thiriet et al. 2013*). HSL along with adipose triglyceride lipase and monoacyglycerol lipase synergistically affects fats in adipocyte lipid droplet and breaks down triacylglycerol to non-esterified fatty acids and glycerol. HSL is activated by glucagon or glucagon-alike enzymes (*Lampidonis et al. 2011; Siu et al. 2013*), thus lipolysis is activated when there is a lack of energy. Intronic site human LIPE gene polymorphism causes altered lipolysis in adipocytes, obesity and type 2 diabetes (*Dahlman et al. 2007*). Zidi with a team found out that LIPE genotype can determine goat milk yield and its components, as dairy animals receive most of their energy from breaking down accumulated fats (*Zidi et al., 2010*).

After *in situ* hybridization pig LIPE gene was assigned to chromosome 6 (6q12), alongside with glucose phosphate isomerase (GPI) and calcium ion channel (CIC) genes (*Chowdhary et al. 1995*). LIPE gene structure is very conservative compared to human, mouse or rat: splicing sites are fully conservative, compatible exon and intron sites are highly conservative (*Harbitz et al. 1999; Kaminski et al 2008*). There are only few pig LIPE gene polymorphisms found: polymorphic *Alu1* sequence, c.3436G<T and c.442 G>A. This research goal was to evaluate LIPE gene c.442G>A polymorphism on carcass traits in pigs in studied pig population.

Material and methods

Genetic material for study was collected at the Lithuanian Pig Breeding station. We used 40 hybrid pigs: Yorkshire x Landrace hybrids (N=16), Yorkshire x Landrace x Landrace hybrids (N=24) and Large White x Landrace x Landrace hybrids (N=10). Research was performed at the Institute of Biology Systems and Genetics in Lithuanian University of Health Sciences. Genetic material was extracted from hair follicles using Chelex resin. For one sample we used 6-10 bristle follicles, placed them in centrifuge tubes and mixed with 200 µl Chelex resin, 7.5 µl DTT and 10.7 µl proteinase K. Tubes vortexed for 30s., centrifuged at 13500 RPM for 10s and placed in thermostat for 45min at 56^{o}C (*Miceikiene et al. 2002*). Then PCR is performed, for one reaction used 15µl of mastermix (2.95µl high quality deionized water, 3µl buffer solution without $MgCl_2$, 2µl $MgCl_2$, 2.5µl dNTP, 2µl forward primer (LIPE P1 5´-CGCACRATGACACAGTCGCTGGT-3´),

2µl reverse primer (LIPE P2 5´-CAGGCAGCGRCCRTAGAAGCA -3´) (Thermo Fisher Scientific Baltics, Vilnius, Lithuania), 0.25µl BSA, 0.3µl Taq polymerase). PCR had 30 cycles. 498bp product was generated.

For restriction fragment length polymorphism reaction, we used 10µl PCR product and 10µl mastermix (7.5µl high quality deionized water, 2µl FastDigest Green buffer solution, 0.5µl *Hinf1* restriction enzyme (Thermo Scientific FastDigest *HinfI*) (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). Tubes with the mix were shortly vortexed and centrifuged. RFLP reaction was performed in thermocycler for 5min at 37^{o}C degrees. After reaction four fragments were obtained: 308bp and 190 bp for allele A; 67bp, 190bp and 241bp for allele G.

Polymorphism influence was evaluated on carcass traits: hot carcass weight and yield; carcass without head weight and yield; age at 100kg; daily gain; 1kg gain feed intake; half carcass length; half bacon length; loin area; weight of ham; fat thickness at 6-7th rib, at 10th rib, behind last rib, at last waist vertebra; "Piglog" data: fat thickness at point Fat_1 and point Fat_2, muscle thickness, muscularity. Before slaughtering pig's muscularity and fat thickness was evaluated using ultrasound device "Piglog 105". Data, related to carcass traits was obtained from National Pig Breeding Station.

Statistical data analysis was performed using *Excel* and *IBM SPSS Statistics for Windows* software. Evaluation of allele and genotype distribution, expected and observed heterozygosity and polymorphism influence on traits mentioned above was completed.

Results

After statistical data analysis and observed polymorphism evaluation all possible allele combinations were found. Most common genotype AA, observed with frequency 0.700 in 32 animals out of 50. AA genotype most commonly found in Yorkshire x Landrace x Landrace hybrids with frequency 0.714, most rarely genotype AA observed in Large White x Landrace x Landrace hybrids with frequency 0.667. Genotype GG was observed in lower frequency (0.225): Yorkshire x Landrace hybrids carried genotype GG with 0.308 frequency, Yorkshire x Landrace x Landrace with 0.238 frequency. In Large White x Landrace x Landrace population genotype GG was not detected. Rarest observed genotype was AG, frequency 0.075. Heterozygous genotype was observed in Large White x Landrace x Landrace population with frequency 0.333, Yorkshire x Landrace x Landrace – 0.048, no heterozygotes were found in Yorkshire x Landrace population.

Allele distribution in studied population is uneven: allele A was observed almost 3 times more (0.738) than allele G (0.262) (Table 1).

Table 1. Genotype and allele distribution

Genotype	n	Frequency	Allele	Frequency
AA	32	0.700	A	0.738
AG	6	0.075	G	0.262
GG	12	0.225		
	50	1		1

When evaluating population heterozygosity, it was determined that observed heterozygosity was significantly lower than expected, which shows that genetic diversity in studied population is decreased, results statistically significant. (Table 2)

Table 2. Pig population heterozygosity

Expected heterozygosity	0.387
Observed heterozygosity	0.075
X2	26.00
P value	0.0000003

Table 3 shows statistically significant results related to LIPE gene polymorphism influence on carcass traits in pigs.

Table 3. Carcass traits of pigs

Trait	Lion area cm^2	Weight of ham, kg	Overall muscularity, %	Fat thickness at 6-7^{th} rib, mm	Fat thickness at 10^{th} rib, mm	Fat thickness behind last rib, mm	Fat thickness at last waist vertebra, mm	Fat thickness at Fat_1, mm	Fat thickness at Fat_2, mm
AA	45.4±1.04^a	12.4±0.12^a	59.5±0.25^a	14.9±0.93^a	13.2±0.85^a	13.9±0.69^a	10.3±0.50^a	12.4±0.31^a	11.2±0.27^a
AG	40.3±1.14	11.9±0.10	57.0±0.94^b	24.4±5.40^b	19.1±2.57^b	20.7±3.49^b	16.4±2.43^b	14.3±1.20^b	14.0±1.00^b
GG	36.6±1.79^b	11.6±0.30^b	56.1±0.90 ^{c}	18.8±1.76	16.4±1.30	15.0±1.48^a	13.2±1.60^b	15.1±0.82	13.4±1.04^b
P	**0.0001**	**0.014**	**0.0001**	**0.008**	**0.033**	**0.026**	**0.005**	**0.002**	**0.004**

a, b, c - different letters in the column marked averages differ with each other significantly at P <0.05.

The highest muscularity was observed in pigs with genotype AA: loin area (45.4cm2±1.04) bigger than genotype GG (36.6cm2±1.79); weight of ham (12.4kg±0.12) greater than genotype GG (11.6kg±0.30); largest overall muscularity (59.5%±0.25) compared with AG (57.0%±0.94) or GG (56.1%±0.90) genotypes. Greatest fat mass is usually determined by genotype AG. Fat thickness at 6-7^{th} rib (24.4mm±5.40) greater than genotype AA (14.9mm±0.93) at 10^{th} rib (19.1mm±2.57) greater than genotype AA (13.2mm±0.85; greatest behind last rib (20.7mm±3.49) compared with AA (13.9mm±0.69) and GG (15.0mm±1.48) genotypes; at last waist vertebra greatest fat thickness was influenced by genotype

AG (16.4mm±2.43). as well as genotype GG (13.2mm±1.60). genotype AA influenced lower fat thickness at this point (10.3mm±0.50). Greater fat thickness at point Fat_1 (Piglog data) was determined by genotype AG (14.3mm±1.20). lower – genotype AA (12.4mm±0.31. However, at point Fat_2 greater fat thickness was influenced by genotype AG (14.0mm±1.00) as well as genotype GG (13.4mm±1.04), compared with genotype AA (11.2mm±0.27).

Discussion

LIPE gene encodes hormone sensitive lipase, which is one of the most important enzymes involved in accumulated fats breakdown and energy mobilization (*Ding et al., 2000*). Modifications in gene sequence can lead to altered HSL (hormone-sensitive lipase) function, which can induce incomplete lipolysis and fat accumulation in body. Pig LIPE gene polymorphism c.442 G>A was identified by American scientist Andrew Knoll and his team. In the first exon *missens* mutation occurs, when in gene sequence guanine is substituted by adenine and in enzyme sequence isoleucine is substituted to valine. After inheritance analysis both alleles (A, G) were observed in Meishan (pig breed from China known for abundance of fat) pigs, in Pietrain pigs (lean and muscular breed) G allele was fixated, Landrace (high produce of meat, low intramuscular fat content) and Large White (higher content of muscle fiber, less meat marbling) breeds were monomorphic to allele G, however Duroc breed (high content of intramuscular fat) was polymorphic (*Knoll et al., 1998*).

Scientist Lei and his colleagues conducted study data is slightly similar with our obtained data , though their investigated pig population consisted of the Great White and Meishan crossbreds. The largest back-fat thickness and intramuscular fat content determined the AG genotype, and total muscularity of animal was similar in both AA (58.4%) and GG genotypes (58.8%) (*Lei et al,. 2005*).

Wang and other scientists were investigated two local Chinese pig breeds (Nuogu bei Luobo) and Large White and Landrace crossbreeds.This scientist maintained that the animals which have AA genotype their meat properties were significantly superior nor of GG genotype animals. The results showed that the A allele was associated with the largest muscle thin and the least fat thickness of back, compared with the G allele (*Wang et al., 2012*).

When evaluating our observed population, both alleles and all genotypes were found, hence uneven allele distribution was observed. Allele A was observed almost 3 times more than allele G. Similar tendency can be observed in genotype distribution: genotype AA was most common – 32 animals out of 50, genotype GG was less common – 12 animals out of 50, homozygous genotype AG was rarest – only 6 animals out of 50. Statistical analysis was performed to evaluate genotype influence on carcass traits in pigs in studied population. We found 9 statistically

significant results: biggest loin area, weight of ham and overall muscularity was determined by genotype AA; highest fat content at 6-7th rib, at 10th rib, behind last rib and point Fat_1 was determined by genotype AG; highest fat content at last waist vertebra and point Fat_2 was determined by genotype AG, as well as genotype GG.

Conclusion

In studied pig population biggest overall muscularity, loin area and weight of ham was determined by genotype AA; higher fat content at 6-7th rib, 10th rib, at last waist vertebra, behind last rib and at both points measured with "Piglog" device was determined by both genotype AG and GG. In studied pig population, allele A is related to better animal muscularity, allele G – higher fat content.

Uticaj polimorfizma LIPE gena c.442 g> A na osobine trupa kod svinja

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Rezime

Hormon osetljiva lipaza je jedan od tri enzima koji su uključeni u proces lipolize i kodirana LIPE genomom. U ovom istraživanju smo ispitali uticaj polimorfizma LIPE gena c.442 G>A na osobine trupa kod hibridnih svinja. Genomska DNK je ekstrahovana korišćenjem Chelex-a, genotipovi određeni korišćenjem RFLP-PCR. Alele A je posmatran sa frekvencijom 0,738, alel G - 0,262. Najčešći genotip bio je AA, genotip GG je primećen sa nižom frekvencijom, genotip AG je bio najređi. Prilikom procenjivanja heterozigotnosti populacije, primećeno je da je zabeležena heterozigotnost bila samo 0,075, dok je očekivana heterozigotnost iznosila 0,387. U posmatranoj populaciji svinja, alel A je povezan sa boljom mišićavošću životinja, alelom G - sa većim sadržajem masti.

Ključne reči: LIPE gen, SNP, polimorfizam, c.442 G> A, osobine trupa

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