ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Zootechnica 12 (4) 2013, 71–78*

AN ANALYSIS OF *PPARGC1A* GENE POLYMORPHISM IN RELATION TO CARCASS QUALITY IN PIC HYBRID FATTENERS

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Abstract. The aim of this study was to determine the association between polymorphism located in exon 8 of *PPARGC1A* gene (Cys430Ser) and carcass quality in pigs. Experiment was carried out on 350 PIC hybrid fatteners. Polymorphism was analyzed using PCR-RFLP method. The frequency of genotypes was as follows: *AA* – 0.33, *AT* – 0.57, *TT* – 0.1, however alleles: A – 0.62, T – 0.38. In the analyzed population loss of Hardy-Weinberg equilibrium was observed ($P \leq 0.01$). Statistical analysis showed that only one of the evaluated traits was associated with individual *PPARGC1A* genotypes. Cooling loss value for pig carcasses with *TT* genotype was statistically significant ($P \leq 0.05$) higher than observed in those with *AA* and *AT* genotypes.

Key words: carcass quality, hybrid fatteners, polymorphism, *PPARGC1A* gene

INTRODUCTION

In the last few years the progress in development of genetic markers associated with economically important traits in livestock is observed. In pigs production most important traits are meat quality and fatness. Hundreds of QTL (quantitative trait loci) for meat and carcass traits were mapped in swine genome [pigQTLdb]. One of them is region located on chromosome 8 (SSC8), where *PPARGC1* gene has been mapped [Jacobs et al. 2006].

The peroxisome proliferator-activated receptor-gamma coactivator-1 (PPARGC1, PGC-1α) is a transcriptional coactivator which regulates genes associated with energy metabolism. It influences thermogenesis, mitochondrial biogenesis, adipogenesis and muscle fiber-type conversion [Lin et al. 2002, Puigserver,

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Spiegelman 2003]. *PPARGC1* gene location, wide functions and genetic variations make its good candidate gene for meat and carcass traits in pigs.

A single nucleotide polymorphism (SNP) in exon 8 of *PGC-1α* gene causing amino acid substitution at position 430 (Cys to Ser) was for a first time analyzed by Kunej et al. [2005]. They reported difference in allelic distribution between Chinese and Western pig breeds. Further investigations showed that Cys430Ser polymorphism is associated with fat deposition, meat and muscle fibers traits.

The aim of this study was to analyze Cys430Ser polymorphism in relation to ten carcass quality traits in PIC hybrid fatteners.

MATERIAL AND METHODS

Investigations were carried out on 350 PIC hybrid fatteners derived from cross between a PIC377 paternal line and Camborough22 maternal line. Animals were kept in three different farms. Feeding and rearing conditions for all mentioned farms were equalized – dry, granulated fodder derived from the same feed manufacturer given *ad libitum* and shallow bedding.

DNA was isolated from longissimus lumborum collected after slaughtering by use High Pure PCR Template Preparation Kit (Roche). PCR-RFLP method was applied to estimate individual genotypes of *PPARGC1A* gene. DNA amplification were carried out by use following primer sequences [Kunej et al. 2005]:

- *forward* 5' TAA AGA TGC CGC CTC TGA CT 3'
- *reverse* 5' CTG CTT CGT CGT CAA AAA CA 3'

PCR was performed in total volume 15 µl containing: 40-50 ng of DNA template, 0.2 mmol of dNTPs mix, 1×PCR buffer with z NH4, 10 mmol of each primer, 2.5 mmol Mg^{2+}, 0.75 U Taq polymerase (*Fermentas*), loading dye and molecular grade water. Following thermal profile was applied: initial denaturation 95°C/5 min, 30 cycles of 95°C/30 s, 62°C/30 s, 72°C/30 s and final elongation 72°C/7 min. Obtained amplicons were digested by use *Alu*I enzyme in 37°C overnight. Restriction fragments were separated in 1.5% agarose gels stained with ethidium bromide. After electrophoresis gels were visualized in UV light and recorded by use *Vilber Lourmat* system.

For population genetics following parameters were calculated using PowerMarker ver. 3.25 [Liu, Muse 2005]: genotypes and alleles frequency, mean heterozygosity, gene diversity, Hardy-Weinberg equilibrium (χ^2) and PIC (polymorphic information content).

For association study following parameters were measured:

- live weight and hot carcass weight [kg];
- meatiness [%], backfat thickness and thickness muscle [mm] – was measured with a Sydel CGM optic-needle apparatus on the left half-carcasses;

- cooling loss [%] – was calculated based on the difference between the hot and cold carcass weights, expressed as per cent of the hot carcass weight;
- total protein [%] – estimated by use Kjeldahl method [AOAC 2003];
- intramuscular fat content [%] – estimated by extraction with ethyl ether (Soxhlet method) [AOAC 2003];
- ash [%] – estimated through combustion of meat sample at 550°C [AOAC 2003];
- dry matter [%] – estimated through drying of sample at 105°C to solid mass after denaturation of protein by 96% ethyl alcohol [AOAC 2003];
- marbling [score] – estimated by five trained persons on fresh meat slices. 5 point scale was applied where: 1 point – lowest fat content, 5 points – highest fat content in muscle;

Association between *PPARGC1A* gene polymorphism and performance traits were calculated using the Statistica software, ver. 8.0 (StatSoft Inc.) with GLM multiple factor mixed model. The following model was applied:

$$Y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl}$$

where: Y_{ijkl} – analyzed trait, μ – overall mean, a_i – effect of *PPARGC1A* genotype (1..3), b_j – effect of sex(1..2) , c_k – effect of farm (1..3), e_{ijkl} – random error.

RESULTS

In the studied population of pigs three genotypes and two alleles of *PPARGC1A* gene were present with following frequency: *AA* – 0.33, *AT* – 0.57, *TT* – 0.1, *A* – 0.62, *T* – 0.38. Values for mean heterozygosity and expected heterozygosity (gene diversity) were as follows: 0.57, 0.47. The comparison between the number of observed and the theoretical number of genotypes revealed significant ($P \leq 0.01$) difference what means that analyzed population was not in Hardy-Weinberg equilibrium. PIC value for *PPARGC1A* gene amounted 0.36.

Means for estimated performance traits in relation to *PPARGC1A* genotypes are given in the Table 1. Statistical analysis showed that only cooling loss was associated with different gene variants. Animals with genotype *TT* characterized by statistically ($P \leq 0.05$) higher value of this trait in comparison to those with *AA* and *AT* genotypes. Other analyzed traits had similar values or not differed statistically significant between *PPARGC1A* genotypes.

DISCUSSION

Genetic markers are valuable tools used by animal breeding. They allow to estimate the production potential in short time as distinct to conventional methods

Table 1. Mean values with standard deviations (SD) for carcass quality traits and meat composition in PIC pigs in relation to *PPARGC1A* genotypes

Tabela 1. Wartości średnie wraz z odchyleniami standardowymi (SD) dla cech jakości tuszy i składu mięsa u świń PIC w odniesieniu do genotypów *PPARGC1A*

Trait – Cecha	Genotype – Genotyp		
	AA	*AT*	*TT*
Live weight, kg – Waga żywa, kg	114.24 ± 9.36	112.51 ± 8.53	113.75 ± 8.59
Hot carcass weight, kg – Masa tuszy ciepłej, kg	87.09 ± 7.31	85.74 ± 6.68	86.48 ± 6.39
Cooling loss, % – Straty chłodzenia, %	0.98^{b} ± 0.21	0.99^{b} ± 0.17	1.13^{a} ± 0.19
Meatiness, % – Mięsność, %	56.43 ± 2.63	56.11 ± 2.84	56.03 ± 2.44
Backfat thickness, mm – Grubość słoniny, mm	14.61 ± 3.57	15.10 ± 3.58	15.56 ± 3.58
Thickness muscle, mm – Grubość mięśnia, mm	57.21 ± 5.65	57.16 ± 5.82	57.92 ± 5.52
Total protein, % – Białko ogólne, %	22.50 ± 0.66	22.46 ± 0.64	22.47 ± 0.72
Intramuscular fat, % – Tłuszcz śródmięśniowy, %	2.07 ± 0.57	2.05 ± 0.69	2.05 ± 0.72
Ash, % – Popiół, %	1.10 ± 0.06	1.11 ± 0.05	1.12 ± 0.05
Dry matter, % – Sucha masa, %	25.68 ± 0.73	25.64 ± 0.69	25.67 ± 0.84
Marbling, score – Marmurkowatość, pkt	1.08 ± 0.25	1.13 ± 0.37	1.14 ± 0.28

[a, b] Mean values marked by different superscript differ significantly at $P \leq 0.05$.

[a, b] Wartości średnie oznaczone różnymi literami różnią się istotnie na poziomie $P \leq 0,05$.

based on phenotypic traits. One of these markers in case of pig production is the *PPARGC1A* gene. The aim of the first investigations concerning this gene was to determine its chromosomal position, find polymorphic sites and compare genotype frequency in Western and Chinese pig breeds [Jacobs et al. 2005, Kunej et al. 2006] . Analysis of Cys430Ser polymorphism showed that in six Chinese breeds A allele was absent; it appeared only in Taoyuan breed in heterozygous form with frequency 0.25. In Western pig it occurred more often and ranged from 0.33 to 0.71. Similar frequency to PIC hybrid pigs was found in Yorkshire, Krskopolje and Duroc breeds (0.54–0.67). Margeta et al. [2006] on the other hand analyzed distribution of *PPARGC1A* Cys430Ser polymorphism in Croatian autochthonous pig breeds. Interestingly in Turopolje pigs T allele was absent, however in Black Slavonian its frequency was higher than observed in our research – 0.56. Another studies focused on searching for association between *PPARGC1A* gene variants and performance traits in pigs. Flisar et al. [2006] found a significant effect of Cys430Ser polymorphism on phenotypic and breeding values for backfat. In population of Slovenian Landrace breed the thickest backfat had homozygotes *AA* and the thinnest homozygotes *TT*, however in Large White breed this relation was reversed. In our results thickest backfat was observed in animals with *TT* genotypes, but difference was not confirmed statistically. Slovenian Large White pigs however characterized by similar frequency to that observed in PIC fatteners (0.64–0.68). Association between Cys430Ser variants was also investigated by

Stachowiak et al. [2007] in relation to nine fatness traits in Polish Large White, Polish Landrace breeds and line 990. Obtained results showed that T/A substitution was related to feed conversion in Polish Large White (P = 0.02). Authors also estimated allele distribution in additional three breeds. Allele A appeared with similar frequency to PIC fatteners in Duroc breed (0.67); in others it was lower except Line 990 (0.82). Another report showed lack of association between PPARGC1 polymorphism and intramuscular fat content (IMF), backfat and leaf fat in Landrace-Duroc-Yorkshire population [Erkens et al. 2009]. These results are consistent with ours where backfat thickness and IMF was not related with PGC-1α genotypes. It may be partially explained by low variability of these traits which amounted 14.61–5.56 and 2.05–2.07 respectively. By contrast, subsequent investigations proved that Cys430Ser variants were associated with IMF ($P \leq 0.01$), area and perimeter of muscle fibre in Tibetan pigs. Animals with *TT* genotypes characterized by higher IMF (4.305) in comparison to those with genotypes *AA* (2.070) and *AT* (2.816) [Liu et al. 2011]. It was not only one investigation which described analysis of muscle fibre characteristics. Kim et al. [2010] studied these traits more detailed including meat quality in Yorkshire pigs and found that Cys430Ser genotypes significantly affected number ($P \leq 0.05$) and area ($P \leq 0.01$) of type I muscle fibre and as well as muscle pH ($P \leq 0.001$) and lightness ($P \leq 0.01$). Last experiment indicated that *PPARGC1A* polymorphism was associated with pH and cooking loss in a F2 Duroc × Pietrain cross and with pH values in Italian Large White and Italian Landrace populations ($P \leq 0.05$) [Gandolfi et al. 2011].

CONCLUSIONS

Obtained results showed that polymorphism in *PPARGC1A* gene is associated with cooling loss in PIC hybrid pigs. Allele *A* seems to be favorable for this trait in homo- as well in heterozygous form.

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ANALIZA POLIMORFIZMU GENU *PPARGC1A* W ODNIESIENIU DO CECH TUSZY TUCZNIKÓW HYBRYDOWYCH PIC

Streszczenie. Celem niniejszych badań było wykazanie zależności pomiędzy polimorfizmem zlokalizowanym w 8 eksonie genu *PPARGC1A* (Cys430Ser) a cechami tuszy świń. Eksperyment został przeprowadzony na 350 tucznikach hybrydowych PIC. Polimorfizm analizowano z użyciem metody PCR-RFLP. Frekwencja genotypów była następująca: *AA* – 0.33, *AT* – 0.57, *TT* – 0.1, natomiast alleli: *A* – 0.62, *T* – 0.38. W analizowanej populacji zaobserwowano zachwianie równowagi genetycznej Hardy'ego-Weinberga ($P \leq 0{,}01$). Analiza statystyczna wykazała, że tylko jedna z ocenianych cech była powiązana z poszczególnymi genotypami *PPARGC1A*. Wartość strat chłodzenia (%) dla świń z genotypem *TT* była statystycznie istotnie ($P \leq 0{,}05$) wyższa niż obserwowana u osobników z genotypami *AA* i *AT*.

Słowa kluczowe: jakość tuszy, gen *PPARGC1A*, polimorfizm, tuczniki hybrydowe

Accepted for print – Zaakceptowano do druku: 9.11.2013