Association of SNPs in Porcine Estrogen Receptor Gene with Carcass Traits

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Abstract

The aim of this study was to assess the influence of estrogen receptor gene polymorphism on carcass traits in pigs' population. In association analysis was evaluated the effect of *ESR/PvuII* genotypes on back fat thickness, proportion of valuable meat parts, area of *musculus longisimus thoracis* (MLT) and proportion of thigh. In total 180 genomic DNA samples of crossbreeds (Large White x Landrace) was genotyped by using PCR-RFLP method and restriction enzyme *Pvu*II. In population was observed prevalence of heterozygous individuals (45.56%). The lowest proportion was detected for homozygous BB genotype (13.89%). Genotype frequencies in analysed population caused the higher frequency of A allele (0.63±0.03) and relative high level of observed heterozygosity (0.46). The medium level of polymorphic information content (0.36) was found for selected locus. Association analysis was carried out by One-Way ANOVA procedure. The results showed that genotype of *ESR* gene influenced significantly only proportion of thigh and area of MLT ($P<0.01$). Average values of both traits observed for each genotype indicated positive effect of A allele present in genotype. Our results demonstrate the significant role of *ESR* gene for economically important traits that can be used for application in marker assisted selection programs of pigs.

Keywords: associations' analysis, porcine *ESR* gene, polymorphism, production trait

1. Introduction

For the pig breeders, the clear requirement is to produce quality lean pork at minimum cost and in a manner that is acceptable to the public [1]. The genetic background of economically important traits in pig breeding is under control of many genes. The candidate gene approach allows the detection of polymorphisms in genes likely to cause variation in trait based on physiological, immunological or endocrine evidences [2]. The SNPs identification can help to the better understanding of genetic background and predisposition for traits of interest among analysed individuals and also can contribute to the understanding of biological processes that are involved in forming of these traits. The marker assisted selection (MAS) has been proposed alongside traditionally pig breeding schemes as one of the approaches that can be used to enhance the rate of genetic improvement of these kinds of traits [3].

Estrogens are hormones participating in many processes that have important functions for reproduction, growth, differentiation and physiology of the reproduction [4]. Estrogen receptors, similarly as other nuclear receptors, are transcription factors, which after binding to a proper ligand (17b-estradiol, estron or estriol) are capable of regulating transcription of target genes. Due to the functions that estrogens play in the regulation of reproduction, development of the mammary gland, growth and differentiation of cells, estrogen receptors and their genes are considered as candidate markers for production and functional traits in farm animals [5].

The protein coded by the estrogen receptor 1 gene (*ESR*) promotes the expression of different transcription factors involved in the reproductive

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function of female tissues [6]. In males *ESR* has impact on beginning and maintaining of spermatogenesis on different levels of hormone regulation [7]. Several allele variants of the porcine gene encoding *ESR*, associated with single nucleotide polymorphisms, located in both of its exons and introns have been described [8]. In the *ESR* gene the *Pvu*II polymorphism was found by Rothschild et al. [9]. This single nucleotide polymorphism located in the intron region is mainly described as genetic marker for reproduction performances [10]. Subsequently, in coding region of *ESR* gene were found also other mutations SNP 1227 C > T (exon 5), 1452 C > T (exon 7), 1665 T > C (*Ava*I polymorphism), and 1755 A > G (exon 8) [8]. The study of *ESR* gene showed associations between polymorphism in *ESR*/*Ava*I, *ESR*/*Pvu*II, and *ESR*/*Msp*A11 locus and litter size in pigs. More recently, some studies revealed an effect of *ESR* gene also on semen characteristics of boars [7]. Only several studies evaluated the associations between polymorphisms in *ESR* gene and production traits [11; 12; 13].

The aim of this study was to analyse the genetic structure of crossbred population (Large White x Landrace) based on identification of *ESR*/*Pvu*II polymorphism and evaluate its effect on selected carcass traits.

2. Materials and methods

For analysis of *ESR*/*Pvu*II polymorphism effect on selected production traits in total 180 crossbreeds (Large White x Landrace) was used. The genomic DNA was extracted from blood samples according to Miller et al. [14], and concentration and purity of extracted genomic DNA were estimated by the spectrophotometer measurements. The PCR-RFLP method and restriction endonuclease *Pvu*II was used for detection of SNP in *ESR* gene. The 120 bp fragment of intron in porcine *ESR* gene was amplified using primers according to Kamiński et al. [15]. The polymerase chain reaction was performed in a 25 µl reaction mixtures, containing: 1 x 20 x PCR reaction buffer, 2.5 mM $MgCl_2$, 2 mM dNTP, 100 pmol/µl of each primer, 0,5 U/µl Taq polymerázy, 50 ng genomic DNA. Thermal cycling conditions included: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 60 sec, 72°C for 30 sec and a final extension at 72°C for 7 min. The PCR products of *ESR* gene were subsequently digested with 1 µl of restriction enzyme *Pvu*II and separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) stained with GelRed (Biotium) in order to visualization under UV light.

After successfully genotyping of individuals the genetic structure of population was determined. The frequency of alleles and genotypes was calculated by direct counting and tested for deviations from Hardy – Weinberg equilibrium using Chi-square (χ^2) test. The observed heterozygosity (H_e) and homozygosity (H_o), polymorphic information content (PIC) and effective allele number (N_e) were calculated according Liu and Muse [16]. Moreover, the Wright's fixation index (F_{IS}) was determined in analysed population.

The effect of *ESR*/*Pvu*II genotype on selected carcass traits: back fat thickness (BFT), proportion of valuable meat parts (LM), area of *musculus longisimus thoracis* (MLT) and proportion of thigh (TP) was tested firstly by parametric t-test. Secondly the analysis of *ESR*/*Pvu*II polymorphism impact on selected carcass traits was performed by One-Way ANOVA procedure of SAS Enterprise Guide 4.2 software [17].

3. Results and discussion

The SNP genotyping of analysed pigs' population was performed by PCR-RFLP method according to Kamiński et al. [15]. After digestion of PCR products with restriction enzyme *Pvu*II three genotypes were detected. The homozygous AA individuals were identified based on 120 bp fragments, the presence of AB heterozygotes characterized 120, 65, and 55 bp fragments and the BB homozygous genotype was identified based on 65 and 55 bp fragments. In selected crossbreeds population was found the prevalence of heterozyotes (46%). The lowest proportion was observed for homozygous BB genotype (14%). Higher frequency of AA and AB genotype was transferred to the distribution of allele A which was in population more frequent than allele B (Table 1). The observed value of homozygosity was the result of the heterozygotes proportion in analysed population. The SNP *ESR*/*Pvu*II reached in population median level of polymorphic

information content. The allele impact in population was balanced. The value of F_{IS} index close to zero indicated sufficient proportion of heterozygous individuals and also Hardy-Weinberg equilibrium, which was found based on Chi-square test.

Table 2 summarized the observed average values of analysed production traits. The statistical significant differences in relation to the *ESR*/*Pvu*II genotypes were found for back fat thickness, proportion of thigh and MLT area (Table 3). The average value observed in animal groups with different *ESR*/*Pvu*II genotypes indicated positive effect of B allele. The significant effect of *ESR*/*Pvu*II polymorphism on proportion of thigh and MLT area was also confirmed using One-Way ANOVA method. Based on *ESR*/*Pvu*II genotypes effect we were able to describe the variability of selected production traits only in average on 2.5%.

The estrogen receptor gene has been studied as an important marker for genetic improvement of different traits, mainly reproduction performance, in swine breeding programs, especially in Large White, Meishan and Yorkshire purebreds or derived crossbreds [4]. The B allele frequency of *ESR*/*Pvu*II loci detected in our population of crossbreeds (Large White x Landrace) was relatively higher in comparison with studies of different breeds of pig [8; 18; 19]. Matoušek et al. [20] and Trakovická et al. [21] found comparable alleles distribution in population of Czech Large White pigs and Slovak crossbreeds Large White x Landrace. The estrogen receptor gene was primarily evaluated in relation to the reproduction traits. The effect of *ESR* polymorphisms on various hormonal and histo-anatomical changes in the reproductive system has been [22]. The impact of *ESR* polymorphisms was investigated in relation to reproduction in Large White [23], Prestice Black Pied [24], and Landrace [18]. Only several studies were aimed to explanation of molecular mechanism and role of the *ESR* locus in biochemical processes of meat and fat metabolism [11; 12; 13].

Table1. Frequency of alleles and genotypes

Genotype frequency			Allele frequency		$\chi 2$ test	H_o	H_e	PIC	N_e	F_{IS}
ESR^{AA}	ESR^{AB}	ESR^{BB}	ESR^{A}	ESR^{B}	0.07	0.54	0.46	0.36	1.87	0.02
0.40	0.46	0.14	0.63± 0.02	0.37±0.02						

P > 0.05

Table 2. Basic statistical variation measurements carcass traits in pigs

Trait	Boars					Sows				
	n	mean	SD	min	max	n	mean	SD	min	max
BFT [mm]	88	17.47	3.77	11.33	27.00	90	17.16	3.16	11.33	24.33
LM [%]	90	55.15	2.08	49.74	59.68	90	55.41	1.69	50.16	58.46
TP [%]	90	22.67	1.41	19.65	26.67	90	22.61	1.10	19.59	24.84
MLT area [cm^2]	90	44.38	4.11	37.20	61.70	90	43.63	4.23	37.10	61.70

Table 3. The effect of *ESR*/*Pvu*II genotypes on carcass traits in pigs

Trait	Genotype					
	ESR^{AA}		ESR^{AB}		ESR^{BB}	
	n	mean±SD	n	mean±SD	n	mean±SD
BFT [mm]	72	16.73±3.39*	82	17.89±3.51*	24	17.09±3.38
LM [%]	73	55.51±1.77	82	55.18±1.91	25	54.91±2.17
TP [%]	73	22.86±1.24*	82	22.57±1.29	25	22.21±1.13*
MLT area [cm^2]	73	44.16±4.23*	82	44.48±4.31*	25	41.99±2.94*

*P < 0.05

4. Conclusions

In our study was evaluated the effect of *ESR*/*Pvu*II polymorphisms on selected production traits in crossbred pigs population (Large White x Landrace). The analysis of genetic population structure showed prevalence of heterozygous individuals. The A allele was in population more frequent than B allele. The higher frequency of AA and AB genotype was transferred to the observed population heterozygosity. The analysis of *ESR*/*Pvu*II polymorphisms impact on carcass traits showed significant influence on two traits – proportion of thigh and MLT area. For both traits was found positive effect of B allele. The *ESR*/*Pvu*II locus seems to be relevant to the genetic improvement of economically important traits in pis. The more extensive association analysis on greater sample with involvement of other pigs breed is needed for confirmation of our assumption.

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