



Changes in Reproductive Traits of Large White Pigs after Estrogen Receptor Gene-based Selection in Slovakia: Preliminary Results*

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ABSTRACT : We investigated the effect of ESR gene-based selection on an improvement of litter size in the herds in real (non-experimental) conditions. The pigs were selected for three years. In the tested population the pigs were mated according to a breeding scheme where the individuals with at least one ESR-B allele were preferred in the selection. In the control group (CP; n = 140) the pigs were mated just according to a breeding scheme without knowledge of the ESR genotype. We observed a significant increase in litter size (total number of born, number of born alive and number of weaned piglets per litter) in the final tested ESR-selected population (LP; n = 184) and an insignificant increase in CP as compared with the original population (OP; n = 155). After the selection we could observe a significant increase in the frequency of allele B in LP. Frequency of the genotypes AB and BB increased in both LP and CP; the distribution of the genotypes changed significantly only in LP. An association analysis of the ESR gene effects on reproductive traits in LP showed no significant differences between the genotypes. The results of our study suggest that ESR gene-based selection can be successful also in small herds, under real (non-experimental) conditions with a respect for general breeding principles and limitations and during a short period. An examination of a larger sample population as well as an analysis of selection consequences on other traits (meat and carcass quality) could bring a more conclusive evaluation of ESR-based selection. Nevertheless, the results are encouraging especially for small breeding farms taking a perspective of better litter size improvement. (**Key Words :** ESR Gene, Litter Size, Pigs, Selection)

INTRODUCTION

The key porcine reproductive traits, including litter size, play important role in the successful production of farm animals. However, the traits display low heritability (Lukovic et al., 2007) which makes a rather difficult improvement of them by selective breeding. Therefore, the identification of genes controlling reproduction or quantitative trait loci (QTL) and genetic markers associated with reproductive traits could bring a possibility of utilization of the genes (QTL) in marker-assisted selection programs in pigs to improve reproductive efficiency. A number of polymorphic genes with significant effects on litter size have been identified in pigs up to date (Buske et al., 2006). The analyzed genes usually code key hormones and their receptors (Omelka et al., 2001; Rothschild et al., 2007) or other important proteins (Niu et al., 2006). The

QTL mapping is also still in progress (Rothschild et al., 2007).

The estrogen receptor (ESR) gene has been identified as a major gene for litter size in Meishan and Large White breeds. Rothschild et al. (1996) described a Pvu II polymorphism in the third intron of the gene which produces two common alleles, A and B. The favourable B allele was associated with a first parity increase of 1.15 pigs per litter for each copy of the allele in Meishan crosses. In later parities, the effect of the B allele was about 0.5 pigs per litter. The B allele also segregates in Large White breed and the effect varies from 0.31 to 0.42 pigs per litter in first and later parities (Short et al., 1997). Many ESR-based studies have been done up to date involving different breeds in relatively large (hundreds to thousands of individuals), as well as small (100-200 individuals) herds. Effects of the ESR gene (Pvu II polymorphism) have also been analyzed in Slovak (Central European) populations of Large White (LW), White Meaty (WM) and Landrace (L) pigs (Omelka et al., 2005a; Omelka et al., 2006). In LW the authors revealed a highly significant effect of ESR locus just on number of weaned piglets.

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Table 1. Basic statistics for tested traits in analyzed populations of Large White pigs

	n	nL	TNB	t-test	NBA	t-test	NW	t-test
OP	155	640	11.93±1.55		10.95±0.99		9.19±0.76	
OP (L 1-2)	155	279	11.02±1.06	OP-LP**	10.29±0.78	OP-LP**	8.93±0.58	OP-LP***
LP (L 1-2)	184	311	12.14±2.51	LP-CP*	11.42±2.13	LP-CP*	11.23±1.65	LP-CP**
CP (L 1-2)	140	247	11.62±2.39		10.88±2.07		9.61±1.45	

OP = Originated population; LP = The last analyzed population; CP = Control group; L 1-2 = Litters 1-2.

nL = Number of litters; TNB = Total number of born; NBA = Number of born alive; NW = Number of weaned piglets.

The values of TNB, NBA, NW are expressed as least square means±standard deviation; * p≤0.05, ** p≤0.01, *** p≤0.001.

After these studies, three years long, the ESR gene-based selection was followed in the Large White herds with a respect for general breeding principles. The aim of this paper was to evaluate the effect of this selection on an improvement of litter size in the small herds in real (non-experimental) conditions.

MATERIAL AND METHODS

The originated population (OP) used for a production of next filial generations involved pigs previously analyzed in the studies by Omelka et al. (2005a) and Omelka et al. (2006). A total number of studied animals included 155 sows and 38 boars of Large White (LW) pigs from two Slovak nucleus herds. Within the herds 68 pigs were randomly selected to create a control group (CP). Data of total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets per litter were calculated across the nucleus herds and used for further evaluation of a selection impact.

The pigs were selected for three years. During the selection, only healthy and positively evaluated pigs were used for mating. In tested population the pigs were mated according to a breeding scheme developed by the breeders, in which information about the ESR genotype was incorporated. The pigs with at least one ESR-B allele were preferred in the selection (AB and BB genotypes). However, the selection was performed with a respect for general breeding principles and limitations and therefore the ESR genotype preference was not kept in some cases (e.g. due to relationship of the animals; insufficient breeding value of B-allele carrier; limited number of boars with genotype BB). Each of the generation was used to produce the next one, therefore all individuals were tested for ESR genotype by the method of Omelka et al. (2004), or the genotype was determined according to the known genotypes of parents. In the control group the pigs were mated just according to a breeding scheme without knowledge of the ESR genotype. During the selection we observed two to three new generations in both groups. The last analyzed population (LP) of pigs consisted of 168 sows and 16 boars; the control one involved 128 sows and 12 boars.

After the selection, the data of TNB, NBA and NW were collected across the nucleus herds within LP and CP.

Basic statistics for tested traits is given in Table 1. Because of the fact that only two litters of LP and CP parents are available, just first two litters from OP were involved in a statistical analysis of reproductive traits improvement by t-test for independent samples. Allele and genotype frequencies in LP, as well as in CP were calculated and Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies as well as the frequencies before and after the selection using a chi-square goodness of fit test. Besides, effects of the ESR gene on reproductive traits were analyzed. A total of 311 litters from 184 sows (LP; 1st-2nd litters) of LW were included in the litter size analyses in which TNB, NBA and NW were recorded.

The associations between ESR genotypes and analyzed traits were evaluated by linear model as follows (SAS[®] v. 8.2; 2002):

$$y_{ijklm} = GS_i + GB_j + PAR_k + hys_l + anim_m + b_1 * agef_{ijklm} + b_2 * agef_{ijklm}^2 + e_{ijklm}$$

where y: trait value, GS: genotype of sow, GB: genotype of mated boar, PAR: parity effect, hys: random herd-year-season effect, anim: random effect of animal, $b_1 * agef_{ijklm} + b_2 * agef_{ijklm}^2$: linear and quadratic regression on age at farrowing and e: random error.

The variance components for random effects were estimated by MIXED procedure and REML method. Differences of Least Squares Means (LSM) were tested by Scheffe multiple range test. Linear model used in our study was close to the statistical model used in routine genetic evaluation of litter size traits in pigs in Slovakia. It contained the effects which are known to significantly affect litter size in Slovak populations (Peškovičová et al., 2002). Additional effects of genotype (according to the ESR gene) were included in the model.

RESULTS

According to the basic statistics (Table 1) we observed an increase in TNB, NBA and NW in LP and CP as compared with OP. However, the differences were significant only between OP-LP, as well as LP-CP.

Table 2. Frequencies of the ESR genotypes and alleles in Large White (LW) pigs in analyzed populations

	Herd	Number of pigs	ESR genotypes (%)			χ^2	χ^2 (BG)	ESR alleles		
			AA	AB	BB			A	B	
OP	I	76	46.1	47.4	6.5	1.49	OP-LP	0.70	0.30	
	II	79	39.2	49.4	11.4	0.49		8.93*	0.64	0.36
	Total	155	42.6	48.4	9.0	0.82		0.67	0.33	
LP	I	85	37.5	37.5	25.0	5.31	OP-CP	0.56	0.44	
	II	99	28.6	58.3	13.1	4.40		1.10	0.58	0.42
	Total	184	29.4	56.5	14.1	2.97		0.58	0.42	
CP	I	62	35.4	52.3	12.3	1.10	LP-CP	0.62	0.38	
	II	78	42.0	46.4	11.6	0.05		3.74	0.61	0.39
	Total	140	38.8	49.3	11.9	0.38		0.63	0.37	

OP = Originated population; LP = The last analyzed population.

χ^2 = Chi-square test (observed vs. expected frequencies according to HW equilibrium); χ^2 (BG) = Chi-square test between groups OP, LP, CP. * $p \leq 0.05$.

Table 3. Effect of the ESR genotypes (least square means \pm standard error) of sows on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets in Large White, LP

ESR genotypes	Number of litters	TNB		NBA		NW	
		LSM \pm SE	p value	LSM \pm SE	p value	LSM \pm SE	p value
AA	99	11.71 \pm 0.53		10.95 \pm 0.41		8.95 \pm 0.32	
AB	136	11.85 \pm 0.52		10.86 \pm 0.39		8.81 \pm 0.32	
BB	76	12.01 \pm 0.66		10.96 \pm 0.53		8.95 \pm 0.42	
AB-AA		+0.14 \pm 0.21	0.52	-0.09 \pm 0.18	0.61	-0.15 \pm 0.14	0.30
BB-AA		+0.30 \pm 0.44	0.49	+0.02 \pm 0.38	0.97	0.00 \pm 0.29	0.99
BB-AB		+0.17 \pm 0.42	0.69	+0.11 \pm 0.36	0.77	+0.15 \pm 0.28	0.60

Table 4. Effect of the ESR genotypes (least square means \pm standard error) of sows on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets in Large White, OP (litters 1-2)

ESR genotypes	Number of litters	TNB		NBA		NW	
		LSM \pm SE	p value	LSM \pm SE	p value	LSM \pm SE	p value
AA	86	10.98 \pm 0.32		10.27 \pm 0.57		9.08 \pm 0.40	
AB	180	11.01 \pm 0.49		10.28 \pm 0.31		8.84 \pm 0.37	
BB	13	11.23 \pm 0.86		10.46 \pm 0.71		9.07 \pm 0.56	
AB-AA		+0.03 \pm 0.15	0.60	+0.02 \pm 0.14	0.55	-0.24 \pm 0.42	0.61
BB-AA		+0.25 \pm 0.35	0.51	+0.19 \pm 0.44	0.83	-0.02 \pm 0.19	0.76
BB-AB		+0.22 \pm 0.46	0.73	+0.18 \pm 0.29	0.87	+0.23 \pm 0.28	0.20

The frequencies of the ESR genotypes and alleles in tested populations are given in Table 2. The loci were in Hardy-Weinberg equilibrium in all tested herds. In OP the frequency of ESR-B allele, which is supposed to be positively associated with litter size according to many authors (e.g. Rothschild et al., 1996; Short et al., 1997), was 0.33. Heterozygous genotype was the most abundant genotype in the population, genotype BB was found in a frequency of 9%. After the selection with a preference of B-carriers we could observe a significant increase in a frequency of allele B (0.42). Frequency of the genotypes AB and BB also increased. Despite of an increase in BB genotypes in both LP and CP, the distribution of the genotypes changed significantly only in LP.

Effects of the ESR genotypes on reproduction traits (least square means with standard errors) are presented in Table 3, 4 and 5. Unlike the studies previously mentioned we did not observe a significant effect of ESR locus on any reproductive trait in any analyzed population. However, the

ESR-B allele showed a tendency to insignificantly improve TNB. Gene substitution effect for B allele in LP was +0.15 \pm 0.48, +0.01 \pm 0.24 and +0.007 \pm 0.33 for TNB, NBA and NW, respectively.

DISCUSSION

In the present study, an increase in litter size parameters in LP with statistical significant differences was found out as compared with OP and CP. In general, it is not easy to explain the differences in quantitative traits since genetic and environmental factors should be taken into account. The test used in the analysis just compares the means of the populations. Therefore a control group of pigs was established where the same selection scheme was applied as in LP without incorporation of ESR information. Both LP and CP populations were kept in the same conditions and care. The CP-LP differences suggest an effect of ESR gene on improvement of the traits. After the selection the

Table 5. Effect of the ESR genotypes (least square means±standard error) of sows on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets in Large White, CP

ESR genotypes	Number of litters	TNB		NBA		NW	
		LSM±SE	p value	LSM±SE	p value	LSM±SE	p value
AA	84	11.21±0.62		10.89±0.42		9.58±0.33	
AB	104	11.64±0.54		10.78±0.41		9.19±0.33	
BB	59	12.04±0.75		11.25±0.47		10.17±0.77	
AB-AA		+0.43±0.51	0.24	-0.11±0.17	0.59	-0.39±0.31	0.77
BB-AA		+0.83±0.79	0.09	+0.36±0.29	0.21	+0.59±0.33	0.11
BB-AB		+0.40±0.39	0.47	+0.47±0.48	0.62	+0.98±0.72	0.08

frequency of B allele was 0.09 higher in comparison with the OP status and, despite of CP, the results were significant. The distribution of the genotypes also changed in favour of B allele carriers. According to the increase in B allele frequency we could calculate a positive selection coefficient of 0.43 for B allele. Although the B allele frequency was higher after the selection, it did not reach the value found by Short et al. (1997) who noted a frequency of ESR-B allele ranging from 0.64 to 0.74. In the Czech Republic, where populations of pigs are closely related to Slovak ones, the ESR-B allele frequency varies from 0.27 to 0.40 in LW (Vrtková and Dvořák, 2001; Matoušek et al., 2003).

An improvement of economically important traits including reproductive ones by selection is in the centre of interest within breeding programs all over the world (Bidanel et al., 1994; Li et al., 2004). Many of them, especially aimed at meat quality and carcass, have been more successful because of higher heritability of the traits (Cameron and Curran, 1995; Nguyen and McPhee, 2005). First experiments focused on litter size used "hyperprolific selection scheme" (Bidanel et al., 1994). The selection process took 20 years resulting in 2.6 piglets per litter increase. Due to a low heritability the use of genetic markers associated with reproductive traits is often applied to increase rates of genetic response and bring more economic profit to pig industry (Omelka et al., 2001; Buske et al., 2006). The ESR gene is the most utilized genetic marker with effects on litter size (Alfonzo, 2005). It has been analyzed as a candidate gene for reproductive traits in many studies in which populations of pigs in experimental herds have usually been used. However, the magnitude of the ESR effects on reproductive traits varied in individual experimental as well as real populations. In this study we did not find significant differences between the genotypes; however, the number of analysed pigs was limited. Other analyses of related populations from Central European region revealed both a significant (Matoušek et al., 2003; Goliášová and Wolf, 2004) and insignificant (Kmieć et al., 2002) effects. Despite of non-significant effect of ESR genotype on the traits, an improvement of litter size was sometimes observed in selected herds (Noguera et al., 2003). In addition to Pvu II polymorphism, an Ava I polymorphic site in the ESR gene is also analyzed in association with

reproductive traits (Kaminski et al., 2003; Omelka et al., 2005b).

The results of our study suggest that an increase in B allele in a population can be followed by an improvement of litter size. The ESR gene-based selection can be more or less successful also in small herds, real (non-experimental) conditions and during short period. However, the number of tested pigs was limited in our study. Besides, no effect of the selection on other traits (meat and carcass quality) was studied. An examination of a larger sample population could bring a more conclusive evaluation of the ESR-based selection. Nevertheless, the results could be encouraging especially for small breeding farms to make a perspective of better litter size improvement.

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