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Effect of the estrogen receptor (*ESR*) and ryanodine receptor (*RYR1*) genes on reproductive traits of Slovak Large White, White Meaty and Landrace pigs (short communication)

Abstract

We investigated the effect of the estrogen receptor (*ESR*) and ryanodine receptor (*RYR1*) genes on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets in Large White (LW), White Meaty (WM) and Landrace (L) sows from six Slovak breeding farms. We found a significant effect of *ESR* locus on NW ($P \leq 0.01$) in LW, however, we observed a negative effect of *BB* genotype on the trait. In WM, positive associations of *B* allele with TNB, NBA, NW were found but the differences were not confirmed statistically. A highly significant effect ($P \leq 0.01$) of *ESR* locus on TNB ($+0.62 \pm 0.18$ pigs per copy of *B* allele), NBA ($+0.65 \pm 0.18$) and NW ($+0.51 \pm 0.16$) was identified in L breed. With *RYR1* gene we found significantly higher TNB and NBA in heterozygous WM sows ($+1.01 \pm 0.36$ in TNB; $+0.87 \pm 0.32$ in NBA; $P \leq 0.01$). NBA was also significant in LW ($+0.41 \pm 0.19$; $P \leq 0.05$).

Key Words: estrogen receptor gene, ryanodine receptor gene, reproductive traits, litter size, pig

Zusammenfassung

Titel der Arbeit: Einfluss des Östrogen- und des Ryanodinerezeptorgens (*ESR* bzw. *RYR1*) auf Fruchtbarkeitsmerkmale beim Slowakischen Edelschwein, Weißen Fleischschwein und bei Landrasse Schweinen (Kurzmitteilung)

In der vorliegenden Studie wurde der Einfluss des Östrogenrezeptor- (*ESR*) und des Ryanodinerezeptorgens (*RYR1*) auf die Fruchtbarkeitsmerkmale insgesamt geborene (TNB), lebend geborene (NBA) und aufgezogene Ferkel (NW) bei Sauen der Rassen Edelschwein (LW), Weißes Fleischschwein (WM) und Landrasse (L), die aus sechs slowakischen Zuchtbetrieben stammten, untersucht. Es wurde ein signifikanter Einfluss des *ESR*-Lokus auf NW ($P < 0.01$) in LW ermittelt, wobei jedoch ein negativer Effekt des Genotyps *BB* beobachtet wurde. In WM wurden positive Effekte des Allels *B* auf TNB, NBA und NW ermittelt, die jedoch statistisch nicht signifikant waren. Ein hochsignifikanter Einfluss ($P < 0.01$) des *ESR*-Lokus auf TNB ($+0.62 \pm 0.18$ Ferkel pro Kopie des Allels *B*), NBA ($+0.65 \pm 0.18$) und NW ($+0.51 \pm 0.16$) wurde in der Landrasse ermittelt. Beim *RYR1*-Gen wurden signifikant höhere Werte für TNB und NBA in heterozygoten WM Sauen ($+1.01 \pm 0.36$ bei TNB; $+0.87 \pm 0.32$ bei NBA; $P < 0.01$) gefunden. NBA war ebenfalls signifikant in LW ($+0.41 \pm 0.19$; $P < 0.05$).

Schlüsselwörter: Östrogenrezeptorgen, Ryanodinerezeptorgen, Fruchtbarkeitsmerkmale, Wurfgröße, Schwein

Introduction

Reproduction plays a key role in the successful production of farm animals. Selection for increased litter size is rather difficult because of low heritability of this trait. So, the identification of genes or genetic markers associated with reproductive traits in pigs could have a great economic impact on pork production. Such genetic polymorphisms could be employed in marker-assisted selection programs to improve reproductive efficiency.

The estrogen receptor (*ESR*) gene has been identified as a major gene for litter size in Meishan and Large White breeds. ROTHSCILD et al. (1996) described a polymorphism in intron 3 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). More recently, some studies revealed an effect of *ESR* gene also on semen characteristics of boars (TERMAN et al., 2006).

The ryanodine receptor (*RYR1*) gene is one of the most frequently studied markers in relationship to malignant hyperthermia syndrome (*MH*) in pigs. FUJII et al. (1991) detected C→T point mutation at position 1843 in the *RYR1* gene associated with *MH* in six swine breeds. However, pigs with mutated *RYR1-n* allele were shown to produce fewer piglets in a litter (KURL and WRÓBLEWSKI, 1992). Better reproduction traits in sows resistant to stress were also reported in Large White and Landrace pigs (REINER et al., 1993).

The aim of our work was to determine frequencies of the *ESR* and *RYR1* genotypes and alleles and to estimate their associations with some reproductive traits in Large White, White Meaty and Landrace pigs from Slovakia.

Material and Methods

We analysed 155 sows of Large White (LW), 134 sows of White Meaty (WM) and 132 sows of Landrace (L) breeds from six Slovak nucleus herds. The groups of pigs consisted of nucleus herds and involved 37, 23 and 14 lines of LW, WM, and L, respectively. The samples were collected at random from the herds.

Genetic polymorphisms at *ESR* (*Pvu II*) and *RYR1* (*Hha I*) were genotyped simultaneously by multiplex PCR-RFLP method described by OMELKA et al. (2004). Allele and genotype frequencies were calculated and Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a chi-square goodness of fit test. A total of 1664 litters from 421 sows (1st – 8th litters) were included in the litter size analyses in which following traits were recorded: total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets. General statistics for all tested traits are given in Table 1.

The associations between *ESR*, *RYR1* genotypes and TNB, NBA, NW were evaluated for each breed and gene separately by linear model as follows (SAS[®] v. 8.2; 2002):

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

where y – trait value, GS – genotype of sow (*ESR* or *RYR1*), GB – genotype of mated boar (*ESR* or *RYR1*), PAR – parity effect, hys – random herd-year-season effect, $anim$ – random effect of animal, $b_1 * age_{ijklm} + b_2 * age_{ijklm}^2$ – 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. Additional effects of genotype (according to *ESR* and *RYR1* genes) were included in the model.

Results

According to the basic statistics (Table 1) WM breed showed the highest mean values of NBA and NW. TNB was found to be the best in LW.

The frequencies of the *ESR* and *RYR1* genotypes and alleles in tested pigs are given in Table 2. The loci were in Hardy-Weinberg equilibrium in all tested herds excepting herd IV (absence of *ESR-BB* genotype) which was reflected in significant difference in whole WM population. The frequency of *ESR-B* allele, which is supposed to be positively associated with litter size (ROTHSCHILD et al., 1996; SHORT et al., 1997), was 0.33, 0.25 and 0.08 in LW, WM and L, respectively. With *RYR1* gene we found out very low frequencies of recessive *RYR1-n* allele. In LW and WM the *RYR1-nn* genotype was absent. The frequencies were 0.09, 0.13 and 0.16 in LW, WM and L, respectively.

Table 1

Basic statistics for all tested traits (Elementare Statistik für alle geprüften Merkmale)

Trait	Large White (n=155; nL=640)				White Meaty (n=134; nL=489)				Landrace (n=132; nL=535)			
	Mean	S _D	Min	Max	Mean	S _D	Min	Max	Mean	S _D	Min	Max
TNB	11.93	1.55	6.00	18.00	11.68	1.34	4.00	18.00	10.46	0.48	5.00	15.00
NBA	10.95	0.99	4.00	17.00	11.12	1.34	4.00	16.00	10.10	0.48	5.00	14.00
NW	9.19	0.76	4.00	15.00	10.02	0.86	4.00	15.00	9.58	0.33	5.00	14.00

TNB - total number of born; NBA - number of born alive; NW - number of weaned piglets; S_D - standard deviation; Min - minimum value; Max - maximum value; nL - number of litters

Table 2

Frequencies of the *ESR* and *RYR1* genotypes and alleles in Large White (LW), White Meaty (WM) and Landrace (L) sows (Die Frequenzen des Genotyps *ESR* und *RYR1* und des Allels bei Sauen der Rassen Edelschwein (LW), Weißes Fleischschwein (WM) und Landrasse (L))

Breed	Herd	Number of sows	Genotypes (%)			χ^2	Alleles	
			ESR-AA	ESR-AB	ESR-BB		ESR-A	ESR-B
			RYR1-NN	RYR1-Nn	RYR1-nn		RYR1-N	RYR1-n
LW	I	76	46.05	47.37	6.58		0.697	0.303
			81.58	18.42	0		0.908	0.092
	II	79	39.24	49.37	11.39		0.639	0.361
			82.28	17.72	0		0.911	0.089
	Total	155	42.58	48.39	9.03		0.668	0.332
WM	III	65	81.94	18.06	0		0.910	0.090
			46.15	49.23	4.62		0.708	0.292
	IV	69	76.92	23.08	0		0.885	0.115
			59.42	40.58	0	*	0.797	0.203
	Total	134	72.46	27.54	0		0.862	0.138
L	V	47	52.99	44.78	2.23	*	0.754	0.246
			74.63	25.37	0		0.873	0.127
	VI	85	89.36	10.64	0		0.947	0.053
			70.21	27.66	2.13		0.840	0.160
	Total	132	81.18	18.82	0		0.906	0.094
			70.59	27.06	2.35		0.841	0.159
			84.09	15.91	0		0.920	0.080
			70.45	27.27	2.28		0.841	0.159

χ^2 - chi-square test; * P≤0.05

Effects of the *ESR* genotypes on reproduction traits (least square means with standard errors) are presented in Table 3. There was a highly significant effect of *ESR* locus on NW (P≤0.01) in LW - we observed a negative effect of *ESR-BB* genotype on the trait. The *ESR-AB* genotype showed a tendency to improve other tested traits but the results were not statistically significant.

A highly significant effect of *ESR* locus on TNB, NBA and NW ($P \leq 0.01$) was identified in L breed. An increase of $+0.62 \pm 0.18$ (TNB), $+0.65 \pm 0.18$ (NBA) and $+0.51 \pm 0.16$ (NW) pigs per copy of *ESR-B* allele was found. The *ESR-BB* genotype was not evaluated because of its absence.

Table 3

Effect of the *ESR* and *RYR1* genotypes (least square means \pm standard error) on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets in Large White (LW), White Meaty (WM) and Landrace (L) (Einfluss des Östrogen- und des Ryanodinegenotyps (Mittelwerte) auf die Fruchtbarkeitsmerkmale insgesamt geborener (TNB), lebend geborener (NBA) und aufgezogener Ferkel (NW) bei Sauen der Rassen Edelschwein (LW), Weißes Fleischschwein (WM) und Landrasse (L))

Breed	Genotype	NL	TNB (LSM \pm S _E)	NBA (LSM \pm S _E)	NW (LSM \pm S _E)
LW	ESR-AA	264	11.64 \pm 0.54	10.85 \pm 0.42	9.17 \pm 0.33 ^A
	ESR-AB	308	12.04 \pm 0.54	11.03 \pm 0.41	9.19 \pm 0.33 ^B
	ESR-BB	68	11.88 \pm 0.62	10.89 \pm 0.47	8.35 \pm 0.37 ^{AB}
	RYR1-NN	494	11.91 \pm 0.51	10.97 \pm 0.40 ^a	8.91 \pm 0.33
	RYR1-Nn	146	12.35 \pm 0.54	11.38 \pm 0.41 ^b	9.17 \pm 0.34
WM	ESR-AA	259	10.48 \pm 1.07	9.29 \pm 1.05	8.40 \pm 0.74
	ESR-AB	219	10.61 \pm 1.03	9.29 \pm 1.01	8.58 \pm 0.72
	ESR-BB	11	10.73 \pm 1.18	9.42 \pm 1.03	8.63 \pm 0.83
	RYR1-NN	337	9.82 \pm 1.03 ^A	8.42 \pm 1.00 ^A	8.16 \pm 0.72
	RYR1-Nn	152	10.83 \pm 1.06 ^B	9.30 \pm 1.03 ^B	8.58 \pm 0.73
L	ESR-AA	447	10.82 \pm 0.58 ^A	10.37 \pm 0.56 ^A	9.45 \pm 0.51 ^A
	ESR-AB	88	11.43 \pm 0.59 ^B	11.02 \pm 0.57 ^B	9.96 \pm 0.52 ^B
	RYR1-NN	371	10.66 \pm 0.59	10.20 \pm 0.57	9.30 \pm 0.52
	RYR1-Nn	152	10.59 \pm 0.62	10.04 \pm 0.60	9.05 \pm 0.54
	RYR1-nn	12	10.46 \pm 0.75	9.84 \pm 0.73	8.70 \pm 0.65

NL – number of litters; small letters (a, b) denoted a significance difference $P \leq 0.05$, capital letters (A, B) denoted a significance difference $P \leq 0.01$

RYR1-heterozygous LW and WM sows and *NN*-homozygous L sows showed the highest values of tested reproductive traits (Table 3). However, the differences between *RYR1-NN* and *RYR1-Nn* genotypes were significant only in LW ($+0.41 \pm 0.19$ in NBA; $P \leq 0.05$) and WM ($+1.01 \pm 0.36$ in TNB; $+0.87 \pm 0.32$ in NBA; $P \leq 0.01$) in favour of sows with heterozygous genotype.

Discussion

In our study the frequency of favourable *ESR-B* allele decreased from LW to L pigs. The results in LW could be compared with data published by SHORT et al. (1997), who found much higher frequency of *ESR-B* allele ranging from 0.64 to 0.74. Data achieved in Landrace based breeds showed very low frequency of *ESR-B* allele (0.06; KMIEĆ et al., 2002; WANG et al., 2006) or no *Pvu II* polymorphism (DRÖGEMÜLLER et al., 1999, 2001). In the Czech Republic, the *ESR-B* allele frequency varies from 0.27 to 0.40 in LW (VRTKOVÁ and DVOŘÁK, 2001; MATOUŠEK et al., 2003) and from 0.02 to 0.13 in L (VRTKOVÁ and DVOŘÁK, 2001).

With *RYR1* gene we found out very low frequencies of *RYR1-n* allele, probably as a consequence of systematic selection. Because of the negative effect on meat quality the *RYR1-n* allele has been eliminated from herds and the frequency of the allele has been decreased (O' BRIEN et al., 1993). Our results can be therefore compared with

recent publications which show the *RYR1-n* allele frequency of 0.025 - 0.045 in LW (MATOUŠEK et al., 2003) and 0.216 in Polish Landrace (KMIEĆ et al., 2000).

Statistical evaluation of the data revealed some significant effects of *ESR* and *RYR1* genes on reproductive traits. There was a significant effect of *ESR* locus on NW ($P \leq 0.01$) in LW, however, in contrast with other studies (SHORT et al., 1997) we observed a negative effect of *ESR-BB* genotype on the trait. SHORT et al. (1997) reported a significant increase ($P \leq 0.01$) of TNB and NBA of 0.42 (0.31) and 0.39 (0.31) pigs per litter in first parity (later parities), respectively. In the Czech Republic, an increase of 0.14-1.75 (TNB) and 0.13-1.96 (NBA) was found in LW (MATOUŠEK et al., 2003). The results obtained with Landrace breed can be compared with the ones published by WANG et al. (2006). The authors found a significant difference between AA and AB genotypes with advantage of over 1 pig per litter on TNB and NBA. On the other hand, in study of KMIEĆ et al. (2002) the effect was statistically insignificant. In WM we could observe a positive association of *ESR-B* allele with TNB, NBA and NW but the differences were not confirmed statistically.

With *RYR1* gene we identified significant differences between *RYR1-NN* and *RYR1-Nn* genotypes in LW and WM in favour of sows with heterozygous genotype. NYSTRÖM and ANDERSSON (1993) found out the *RYR1-NN* sows significantly produced higher litter size (mainly NW) than stress-susceptible (*RYR1-nn*) sows. Similar results were found in studies of REINER et al. (1993) and KURLY and WRÓBLEWSKI (1992). On the other hand, MATOUŠEK et al. (2003) identified significant differences ($P \leq 0.01$) between *RYR1* genotypes in TNB in favour of sows (LW) with heterozygous genotype.

In general, the use of genetic markers associated with reproductive traits can lead to increased rates of genetic response and bring more economic profit to pig industry. However, the magnitude of the *ESR* and *RYR1* effects on reproductive traits varied in individual populations. Possibly the polymorphisms are in linkage disequilibrium with different alleles of the causative mutation(s). Furthermore, because different populations differ in genetic background, allelic effects may differ as a result of epistatic effects. Nevertheless, the results of our study showed the possibility of *ESR* and *RYR1* utilization in marker-assisted selection to increase litter size. *ESR-B* allele seems to be favourable, however, Large White homozygotes *BB* produce low NW. With *RYR1* we can note a favourable effect of *N* allele in Landrace, as well as allele *n* in Large White and White Meaty pigs. However, the number of analysed pigs was limited in our study. An examination of a larger sample population could bring a more conclusive evaluation of the *ESR* and *RYR1* effects on litter size in tested breeds. Anyway, the connection of the marker testing results with conventional information and with modern statistical methods can make the selection significantly more precise.

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