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Associations between *Alu I* Polymorphism in the Prolactin Receptor Gene and Reproductive Traits of Slovak Large White, White Meaty and Landrace Pigs*

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ABSTRACT : We investigated the effect of the prolactin receptor gene (PRLR) 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. The frequency of A allele was 0.48, 0.49 and 0.47 in LW, WM and L, respectively. We found numerous highly significant effects of PRLR locus on TNB ($p \leq 0.01$; $p \leq 0.05$) in all tested breeds. The most marked difference of +1.31±0.45 pigs/L was found between AA and BB genotypes in WM. Within the other breeds the difference between the homozygous genotypes reached up to +0.94±0.3 and +1.21±0.19 pigs per litter in LW and L, respectively. We also identified significant differences between AA and AB genotypes related to TNB in L. Similarly NBA, as well as NW traits were significantly affected ($p \leq 0.01$; $p \leq 0.05$) by the genotype just in LW and L. The homozygous genotype AA was favourable in all breeds and traits. Our results showed the possibility of PRLR utilization in marker-assisted selection within breeding programs to increase reproductive traits of pigs in Slovakia. (**Key Words** : Prolactin Receptor Gene, PRLR, Reproductive Traits, Litter Size, Pigs)

INTRODUCTION

Prolactin is an anterior pituitary peptide hormone involved in many different endocrine activities. It is essential for reproductive performance, mammary development and lactation. The hormone exerts its physiological effects via the prolactin receptor (PRLR) which has been detected in various tissues in many mammalian species (Kelly et al., 1991). The porcine PRLR gene has been mapped to chromosome 16 (Vincent et al., 1997). An importance of the gene was confirmed in transgenic mice experiments - mice homozygous for a null mutation in the PRLR gene were sterile due to a failure of embryonic implantation, demonstrated irregular cycles, reduced fertilization rates and defective embryonic development (Ormandy et al., 1997). These characteristics make PRLR a strong candidate gene for reproductive traits. Vincent et al. (1998) reported an identification of *Alu I* polymorphism in the PRLR gene that was associated (A allele) with increased litter size in Large White, Landrace, Duroc and Large White×Meishan crossbreeds. Allelic additive effects of A allele on reproductive traits have been reported also for six PIC lines (Rothschild et al., 1998; van Rens et al., 2003), Synthetic lines of Large White, Landrace and Meishan (Southwood et al., 1999), Duroc (Drogemüller et al., 2000), Large White×Meishan F_2 crossbred gilts (van Rens and van der Lende, 2002), and Polish Large White× Landrace sows (Terman, 2005). In the Czech Republic, *Alu I* polymorphism has been shown to be associated with litter size mainly in Landrace breed (Putnova et al., 2002). Moreover the authors revealed a new *Hpa II* polymorphism in the PRLR gene which was also in association with reproductive traits with the effects exceeding two pigs per litter in Landrace and 1 pig per litter in Large White populations.

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

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Table 1. Basic statistics for all tested traits

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.

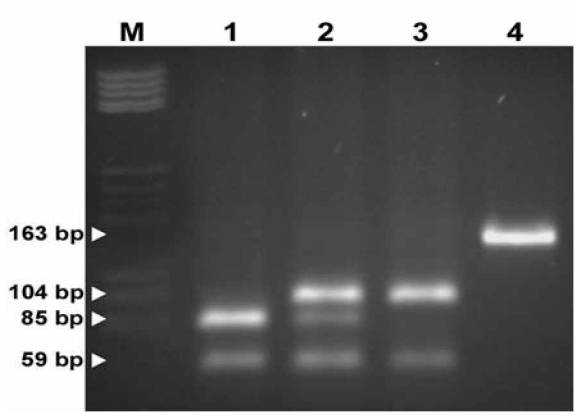

Figure 1. Representative results of PRLR/Alu I detection by RCR-RFLP technique. Lane M = Molecular weight marker pBR322/Hae III; lane 1 = Genotype AA; line 2 = Genotype AB; line 3 = Genotype BB; line 4 = PCR product.

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.

Alu I genetic polymorphism at PRLR gene was detected by PCR-RFLP method according to Drögemüller et al. (2001). 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 1,664 litters from 421 sows (1^{st}-8^{th} 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 PRLR genotypes and litter size traits were evaluated for each breed separately by linear model as follows (SAS® v. 8.2; 2002):

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

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, $b_1\times agef_{ijklm}+b_2\times agef_{ijklm}^2$ = 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 generally utilized in routine genetic evaluation of litter size traits in pigs in Slovakia. Additional effects of genotype (according to the PRLR gene) were included in the model.

RESULTS

Representative results of PRLR/Alu I polymorphism detection by PCR-RFLP method is showed in Figure 1. 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 PRLR genotypes and alleles in tested pigs are given in Table 2. The most abundant genotype was the heterozygous one. We

Table 2. Frequencies of the PRLR genotypes and alleles in Large White (LW), White Meaty (WM) and Landrace (L) sows

Breed	Herd	Number of sows	Genotypes (%)			χ^2	Alleles	
			AA	AB	BB		A	B
LW	I	76	25.00	47.37	27.63	0.21	0.487	0.513
	II	79	24.05	48.10	27.85	0.11	0.481	0.519
	Total	155	24.52	47.74	27.74	0.30	0.484	0.516
WM	III	65	23.08	52.31	24.61	0.14	0.492	0.508
	IV	69	23.19	50.72	26.09	0.02	0.486	0.514
	Total	134	23.13	51.49	25.38	0.12	0.489	0.511
L	V	47	17.02	57.45	25.53	1.16	0.457	0.543
	VI	85	18.82	56.47	24.71	1.51	0.471	0.529
	Total	132	18.18	56.82	25.00	2.65	0.466	0.534

χ^2 = chi-square test.

Table 3. Effect of the PRLR genotypes (least square means±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)

Breed	Genotype	NL	TNB (LSM±S_E)	NBA (LSM±S_E)	NW (LSM±S_E)
LW	AA	164	12.63±0.52^{B}	11.45±0.41^{Bb}	9.24±0.34^{b}
	AB	333	11.81±0.50^{A}	10.92±0.40^{a}	8.86±0.33^{a}
	BB	143	11.69±0.52^{A}	10.62±0.41^{A}	8.79±0.34^{a}
			+0.47±0.17	**+0.42±0.18**	**+0.23±0.12**
WM	AA	105	10.84±1.05^{B}	9.20±1.04	8.56±0.73
	AB	252	10.29±1.04^{b}	8.72±1.03	8.42±0.72
	BB	132	9.53±1.04^{Aa}	8.40±1.03	8.30±0.73
			+0.66±0.28	**+0.40±0.25**	**+0.13±0.18**
L	AA	104	11.60±0.58^{B}	11.06±0.58^{B}	9.83±0.54^{Bb}
	AB	308	10.81±0.57^{Ab}	10.42±0.57^{A}	9.40±0.52^{a}
	BB	123	10.39±0.57^{Aa}	10.14±0.57^{A}	9.16±0.53^{A}
			+0.61±0.17	**+0.47±0.12**	**+0.33±0.11**

NL = Number of litters; small letters (a, b) denoted a significance difference p≤0.05, capital letters (A, B) denoted a significance difference p≤0.01, additive genetic effects of A allele are bold-marked.

found an equal distribution of the genotypes in all breeds. The loci were in Hardy-Weinberg equilibrium in all tested herds. The frequency of PRLR-A allele, which is supposed to be positively associated with litter size in some populations (Vincent et al., 1998; Drogemüller et al., 2000; Terman, 2005) ranged from 0.466 (L) to 0.484 (LW).

Effects of the PRLR genotypes on reproductive traits are presented in Table 3. We observed numerous highly significant effects of PRLR locus on TNB (p≤0.01; p≤0.05) in all tested breeds. The most marked difference of +1.31±0.45 pigs/L was found between AA and BB genotypes in WM, resulting in the additive genetic effect of +0.66±0.28 pigs per copy of A allele. Within the other breeds the difference between the homozygous genotypes reached up to +0.94±0.3 and +1.21±0.19 pigs per litter in LW and L, respectively. In the last mentioned breeds we also identified significant differences between AA and AB genotypes in TNB. In these breeds the additive effects were +0.47±0.17 and +0.61±0.17 pigs per A allele copy in LW and L, respectively. Similarly NBA, as well as NW traits were significantly affected (p≤0.01; p≤0.05) by the genotype just in LW and L. The homozygous genotype AA was favourable in all breeds and traits. The additive effects of +0.42±0.18 (LW) and +0.47±0.12 (L) were calculated for NBA. Within the NW we also found higher additive effect in L (+0.33±0.11 pigs per A allele copy); the effect of +0.23±0.12 pigs/copy of A allele was calculated in LW.

DISCUSSION

The identification of genes or genetic markers associated with reproductive traits in pigs could have a great economic impact on pork production. Many polymorphic genes have been analyzed up to date including those for key hormones and their receptors (Rothschild et al., 1998; Linville et al., 2001; Xiong et al., 2004) or other important proteins (Niu et al., 2006). Such revealed genetic polymorphisms could be employed in marker-assisted selection programs to improve reproductive efficiency.

In our study the AA genotype of PRLR showed the highest values in all tested traits and, vice versa, the BB genotype was associated with the lowest ones. The differences between the genotypes were significant in many traits in all breeds. Vincent et al. (1998) reported the differences between homozygous genotypes from 0.66 to 1 pig per litter in a dependence on studied breed and lines (Large White, Landrace, Duroc and Large White×Meishan crossbreeds). In the study of Southwood et al. (1999) an increase from 0.1 to 0.8 pigs per litter in TNB was identified in A-allele carriers of five Landrace-based lines. Putnova et al. (2002) found associations between PRLR/Alu I polymorphism and litter size mainly in Landrace pigs. An increase of 2.23 (TNB), 1.42 (NBA) and 0.11 (NW) pigs per litter per copy of allele A was estimated. Allele A was also associated with NBA across the first parity in LW (+1.05 pigs/L). The authors analyzed populations of pigs from the Czech Republic, which are closely related to the ones from Slovakia according to the same origin. This fact could be supported by similar frequencies of alleles found in both studies in LW. However, our data achieved in L and LW breeds showed smaller effect of A allele in TNB and NBA in comparison with the results by Putnova et al. (2002). The authors also revealed a new *Hpa II* polymorphism in the PRLR gene which was associated with reproductive traits with the effects exceeding two pigs per litter in Landrace and 1 pig per litter in Large White populations. Positive effect of A allele on TNB and NBA was also observed in studies of van Rens and van der Lende (2002). Homozygous genotypes differed in TNB (2.6 piglets/L) and NBA (2.4 piglets/L), respectively. Moreover, the effect of PRLR locus on average weight of placenta was identified. In Poland, Korwin-Kossakowska et al. (2003) found a significant

effect of the PRLR gene on NBA in sows during first parity. More recently, Terman (2005) identified differences in litter size between sows carrying different PRLR genotypes in favour of genotype AA. In first parity the effect was statistically significant.

On the other hand, some other studies did not show significant associations between PRLR genotypes and litter size. Drögemüller et al. (2001) found a positive effect of B allele on NBA in Landrace and Duroc lines. Similarly Linville et al. (2001) did not observe an association between PRLR and ovulating rate, as well as NBA. No significant effect of the locus on reproductive traits was also found in Jinhua pigs, however. TNB and NBA were significantly affected by gene-gene interaction between PRLR and the estrogen receptor gene (Xu et al., 2003). These findings could be supported by results of Isler et al. (2000) who found B allele to be significantly associated with different traits related to litter size (the number of fetuses per uterine horn, average fetal weight and total fetal weight) in Yorkshire×Large White crossbreed sows.

Some studies have been focused on some associations of PRLR gene with reproductive traits of boars. However, while Kmiec and Terman (2006) and Huang et al. (2006) found positive associations of the locus with semen quality, e.g. ejaculate volume, sperm concentration, percentage of live sperm and number of live sperm in the ejaculate, Lin et al. (2006) observed no significant effects on sperm quality and fertility in boars.

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, similarly to other studies, the magnitude of the PRLR effects on reproductive traits varied in individual populations. The data from our study, as well as the other ones (e.g. Putnova et al., 2002) indicate that *Alu I* polymorphism is not a causative mutation and possibly the polymorphism is in linkage disequilibrium with different alleles of the 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 PRLR utilization in marker-assisted selection (MAS) to increase litter size. The effects of the gene on litter size were more various and significant than in a case of the estrogen receptor gene in the same populations (Omelka et al., 2005; Omelka et al., 2006; Chvojková and Hraška, 2008). However, the number of analysed pigs was limited in our study. Moreover, no association analysis of PRLR gene with production traits was realized. As it has been reported (Alonso et al., 2003) the PRLR locus could affect the daily average weight in Landrace pigs. In case of negative PRLR effect on production traits the application of the marker in MAS could be limited. An examination of a larger sample population and associations to production traits could bring a more conclusive evaluation of the PRLR effect on litter size, as well as a possibility of PRLR utilization in breeding programs. 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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