

Prolactin Receptor (PRLR) Gen Polymorphism and Associations with Reproductive Traits in Pigs

^{1,3}A. Barreras Serrano, ³J.G. Herrera Haro, ¹S. Hori-Oshima, ³A. Gutiérrez Espinosa, ³M.E. Ortega Cerrilla, ³J. Pérez Pérez, ²C. Lemus Flores, ¹A.L. Kinejara Espinosa, ¹A. González Aranguré and ¹J.G. Soto Avila

¹Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California, 21100, Mexicali, Baja California, México, USA

²Posgrado en Ciencias Biológico Agropecuarias, Universidad Autónoma de Nayarit, Cd. de la Cultura Amado Nervo, 63190, Tepic, Nayarit, México, USA

³Programa Recursos Genéticos y Productividad-Ganadería, Colegio de Postgraduados, 56100, Montecillo, Edo. de México, México, USA

Abstract: The Prolactin Receptor (PRLR) gene was investigated as candidate gene for swine reproductive traits. 335 sows of 4 genetic groups: Yorkshire (Y), Landrace (L) Duroc (D) and YL were included. The traits studied were: Total Number of Born (TNB), Number Born Alive (NBA), Number of Weaned Piglets (NWP), Litter Weight at Birth (LWB) and Litter Weight at Weaning (LWW). The polymorphism was identified by PCR-RFLP. Allelic frequencies between each genetic group and Hardy-Weinberg equilibrium were tested by chi-square test. The association between PRLR genotypes with reproductive traits was evaluated by a linear model. Additive and dominance effects were estimated. The frequency of A allele was in general 0.46, with variation between genetic groups. D had the highest values for TNB. YL showed the best performance for NBA. AA genotype in D showed the best performance for NWP but no differences were found among genotypes L, YL and L. Differences in first parity were observed between genotypes for TNB, with highest value in BB (10.40 piglets). In general, additive effect per allele A resulted in a negative increase of 2.26 pigs (TNB) and positive of 0.42 kg (LWB) per litter. For TNB and LWB, dominance effect was -2.67 pigs and -0.56 kg, respectively. For LWW, additive in L resulted in -8.37 kg while dominance effect was 8.37 kg.

Key words: Prolactin receptor gene, litter size, reproduction, pigs

INTRODUCTION

Reproductive performance determines the economic efficiency in pig production systems because of its effects on productivity. Litter size is the most important economically and the most easily measured reproductive trait. Much effort has been made for its improvement (Johnson *et al.*, 1999). However, as the heritability is low (10-15%, Johnson *et al.*, 1999), the trait is limited to sex and expressed in late stages of development in the animal (Goliasova and Wolf, 2004), thus traditional genetic improvement for increased litter size has resulted only in slow genetic gain (Rothschild, 1996). The identification of individual genes controlling litter size or genetic markers associated with such trait and its use in direct selection programs could contribute to an increased rate of genetic gain in pig populations.

The candidate gene approach proposed by Rothschild and Soller (1997) is a procedure used to identify genes with significant influence on the expression of a quantitative character for possible use in genetic improvement programs. A gene is selected to be a potential candidate gene because of the physiological role it regulates in a given process or pathway (Korwin-Kossakowska *et al.*, 2003).

Prolactin Receptor (PRLR) is the specific receptor for prolactin, which is an anterior pituitary peptide hormone involved in many different endocrine activities and is essential for reproductive success (Vincent *et al.*, 1998). All actions of prolactin are mediated by its receptor (Van Rens *et al.*, 2003). The prolactin receptor, encoded by PRLR gene, is a member of the growth hormone/prolactin receptor gene family containing regions of identical sequences (Kelly *et al.*, 1991). The prolactin and

Corresponding Author: Alberto Barreras Serrano, Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California, Km 3.5 Carr. a San Felipe, Fracc. Campestre, Mexicali, B.C. México, USA

growth hormone receptors are homologous to receptors for members of the cytokine superfamily (Clevenger *et al.*, 1998). Swine ovaries and endometrium contain PRLRs, which are distributed in a pregnancy-dependent way (Young *et al.*, 1989). Endometrial prolactin receptor numbers increase on day 12 of pregnancy. The increase is stimulated by conceptus estrogen production, which allows for redirection of prostaglandin F₂ α secretion to support corpus luteum function (Pope, 1994). This implies a potential role of PRLR in preparing and maintaining a proper environment for pregnancy in pigs. Thus, based on the physiological effects, PRLR gene is a strong candidate gene for reproductive traits in pigs.

The PRLR gene was mapped in porcine chromosome 16, with an AluI PCR-RFLP polymorphism identified in porcine 157 bp-long fragment of the gene (Vincent *et al.*, 1997). An positive association was reported between AA genotype and litter size. In first litters, the AA genotype was correlated with higher numbers of piglets born alive (Rothschild *et al.*, 1998; Vincent *et al.*, 1998). Allelic additive effects (a) ranged from zero to 0.59 and 0.71 pigs per litter for total number of piglets born and number born alive, respectively (Vincent *et al.*, 1998). Associations have been reported for Landrace (Vincent *et al.*, 1998) and Duroc (Drogemuller *et al.*, 2001).

The aim of this study was to determine the allelic and genotypic frequency of PRLR gene and to study its associations with reproductive traits in a sample of sows belonging to 4 different genetic groups. Reproductive traits investigated were: Total number of born, number born alive, number of weaned piglets, litter weight at birth and litter weight at weaning.

MATERIALS AND METHODS

Animals: This study included 335 sows (6 of Duroc (D), 14 of Landrace (L), 15 of Yorkshire (Y) and 300 of Yorkshire x Landrace (YL) genetic groups) from NW region of México. A total of 300 sows belong from South of Sonora and 35 from Baja California State. All animals belonged to a population of Canadian origin. The NW region of México is characterized for an extreme climate, desert type, being the average annual rainfall <80 mm, with two seasons: summer and winter. During summer, the average temperature is 42°C. In winter, the average temperature is 14°C.

DNA samples: A total of 3 mL of blood was collected from each animal in tubes containing a buffer solution of sodium citrate as anticoagulant and used to prepare the package of white blood cells. Whole blood samples were centrifuged at 1000 rpm for 5 min and the supernatant was

eliminated. A volume of 5-10 mL of a solution of NaCl at 0.2% was added to the sediment. Then, it was mixed and centrifuged to 2000-2500 rpm for 5 min. White cells were recovered as a package and were washed using NaCl at 0.2%. The package was stored at -20°C. The extraction of DNA was done manually from whole blood using a kit (Ultra Cleanz™ DNA Blood Spin Kit, MO BIO Laboratories, Inc.). For the extraction of DNA a volume of 10 μ L of a lysis buffer (100 mM Tris-HCl, pH 7.6, EDTA 40 mM, pH 8.0, 0.5% SDS) was added, followed by a volume of 1/200 of proteinase K 20 mg mL⁻¹ being incubated at 37°C from 2 h to overnight. After 1 or 2 steps extraction of phenol (diluted solution with a buffer TE) and 1 step extraction CHCl₃, a volume of 2 of EtOH was added to obtain a precipitate containing DNA, then DNA was washed with EtOH 75% and re-suspended in sterile distilled water or solution TE buffered for storage at -20°C.

Genotyping: The genotypes of PRLR gene were identified by means of the PCR-RFLP method. The Polymerase Chain Reaction (PCR) was carried out in 0.2 mL tubes utilizing thermocycler iCycler (Bio-Rad) with primers whose sequences were proposed by Linville *et al.* (2001). Primers were as follows: the forward primer: 5' CGG CCG CAG AAT CCT GCT GC 3' and the reverse primer: 5' ACC CCA CCT TGT AAC CCA TCA TCC 3'. The PCR amplification (25 μ L final volume) was performed using 30 ng of genomic porcine DNA, 10 \times PCR buffer, 2.5 μ L each dNTP, 2 μ L each primer and 0.4 μ L Taq DNA polymerase (Nova Taq™ DNA).

Conditions were 1 cycle at 94°C for 10 min, 40 cycles (94°C, 30 seg; 60°C, 60 seg and 72°C, 30 seg), followed by 1 cycle at 72°C for 10 seg, stopped to 4°C. After PCR, 5 μ L of product was digested by 0.8 μ L of restriction enzyme *Alu* I (Fermentas Inc. USA) and the product was resolved in a agarosa gel at 2%. The AB and BB genotypes were distinguishable by the intensity of the 127-bp band, which was much darker in the AB genotype. A monomorphic band of size 35 bp comigrated with the 35-bp digestion product in the B allele.

Statistical analysis: Allele frequencies were calculated using the counting direct method consisting in the count of an allele in a genetic group divided by twice the number of observations in that genetic group. Standard error of allelic frequencies was calculated as $[p(1-p)/2n]^{1/2}$, where n is the sample size and p is allele A frequency (Spiess, 1989). The hypothesis of homogeneity of genotypic frequencies across genetic groups and Hardy-Weinberg equilibrium were tested using chi-square test.

A total of 420 litter records from 335 sows were included in the analyses. The effect of genotype of the

PRLR gene on Total Number of Born (TNB), Number Born Alive (NBA), Number of Weaned Piglets (NWP), Litter Weight at Birth (LWB) and Litter Weight at Weaning (LWW) was analyzed by least squares. The weaning in piglets was reached at 21 days of birth.

The association between PRLR genotypes with TNB, NBA, NWP, LWB and LWW was evaluated using following linear model:

$$Y_{ijklm} = \mu + G_i + P_j + YS_k + PRLR_l + e_{ijklm}$$

where:

- Y_{ijklm} = The phenotypic record of TNB, NBA, NWP, LWB and LWW
- μ = The general mean
- G_i = The effect of genetic group of sow ($i = D, L, YL, Y$)
- P_j = The effect of parity number ($j = 1, = 2$)
- YS_k = the effect of the subclass year-season of birth ($k = 1, 2, \dots, 8$)
- $PRLR_l$ = khe effect of the PRLR genotype ($l = AA, AB, BB$)
- e_{ijklm} = The random error NID ($0, \sigma_e^2$)

Moreover, all interaction effects were included. Those non-significant interactions ($p > 0.10$) were not included in the model. The analysis was performed using the GLM procedure in SAS 9.1.3 (Herrera and Barreras, 2005). Differences of least square means for PRLR genotypes were tested by Bonferroni's t-test (Kuehl, 1999). Additive (a) and dominance (d) effects of PRLR genotypes were estimated adding regression coefficients into the linear model. For additive effect a covariate of the number of favorable alleles in the genotype (0, 1, or 2) while for dominance effect a covariate with values 0, 1 and 0 in substitution of AA, AB and BB genotypes were included in the model and estimated utilizing GLM procedure of SAS.

RESULTS AND DISCUSSION

In general, AB genotype of PRLR gene accounted for 54% of the total number of sows sampled. The AA genotype represented only 19% in this study. Allele and genotypic frequencies calculated in general and for each genetic group are presented in Table 1. B allele was more abundant in contrast with A allele (0.54 vs 0.46), with standard error of 0.017. The genotypic and allelic distributions across the 4 genetic group did differ significantly ($p < 0.05$) from that expected according to Hardy-Weinberg rule. AA genotype showed higher frequency in Landrace group and was less frequently in YL group. Moreover, AB genotype frequency was similar

($p > 0.05$) in Duroc and YL. The BB genotype was not found in Landrace and Yorkshire genetic groups. Gene frequencies were found to differ ($p < 0.05$) among breeds for PRLR; frequencies for the A allele were: Landrace = 0.79, Yorkshire = 0.76, YL = 0.36 and Duroc = 0.42. The number of observed genotypes was not in Hardy-Weinberg equilibrium because the expected and observed genotype frequencies are significantly different. Independently of the genetic group, in our study the frequency of PRLR-A allele was higher than the results reported by Hernandez *et al.* (2006) in México, Kmiec and Terman (2004, 2006) and Korwin-Kossakowska *et al.* (2003) in Poland, but lower than Terman (2005) and similar to Putnová *et al.* (2002) in Large White pigs.

The allelic frequencies observed by genetic group indicate similarity to those reported by Vincent *et al.* (1997) for the Landrace group. However, frequency for the A allele in Duroc was lower than that reported by Vincent *et al.* (1997) with 0.79 in the United States, but higher in Yorkshire (0.76-0.37) of Vincent *et al.* (1997). Genotype at the PRLR locus has been shown to explain a significant portion of variation in litter size in Large White, Meishan and Landrace based lines (Vincent *et al.*, 1998). Prolactin affects production of progesterone and relaxin from the corpora lutea (Li *et al.*, 1989).

Table 2 contains least square means and standard errors for TNB, NBA, NWP, LWB and LWW in sows, by genotype, where BB genotype showed greater TNB, NBA and NWP values in comparison to AB and AA genotypes, however no significant differences between genotypes ($p > 0.05$) were observed. Similar results were published by Hernandez *et al.* (2006). In other studies, the PRLR locus was found to be associated with TNB and NBA (Rothschild *et al.*, 1998; Vincent *et al.*, 1998; Van Rens and Van der Lende, 2002). However when an interaction between genetic group x PRLR genotype was observed (Table 3), the result showed that the difference between AA and BB genotype was important ($p < 0.01$) for the number of weaned piglets and litter weight at weaning in Duroc, where AA genotype showed better performance. A similar trend was observed for NBA but with no differences ($p > 0.05$) between homozygote genotypes. For TNB trait, BB genotype showed a better reproductive performance compared to homozygous AA, however not significant differences between genotypes ($p > 0.05$) were observed. Van Rens and Van der Lende (2002) working with Large White x Meishan F2 gilts, conducted a study to determine the effects of PRLR polymorphism on reproductive traits. The polymorphism at PRLR tended to affect litter size with AA gilts having larger litters. This did not agreed with the result of Drogemuller *et al.* (2001) where the B allele indicated an

Table 1: Frequency of the Prolactin Receptor (PRLR) genotypes and alleles among sows by genetic group at Baja California and Sonora, México

State	Genetic group	n	AA	AB	BB	A	B	SE
BC	Duroc	6	0.14	0.57	0.29	0.42	0.58	0.093
BC	Landrace	14	0.59	0.41	---	0.79	0.21	0.042
BC	Yorkshire	15	0.52	0.48	---	0.76	0.24	0.038
SON	YL ^{a/}	300	0.08	0.56	0.36	0.36	0.64	0.019
	Total	335	0.19	0.54	0.27	0.46	0.54	0.017

SE = Standard Error for alleles; ^{a/}YL = Yorkshire x Landrace, BC = Baja California, SON = Sonora

additive effect on NBA trait in Duroc. Isler *et al.* (2000) also found the B allele to be favorable. They found that it influences significantly the number of fetuses per uterine horn, average fetal weight and total fetal weight in Yorkshire x Large White crossbred pigs. The BB genotype was not found in Landrace and Yorkshire genetic groups in this study. For LWB trait in YL group, BB genotype showed a better ($p < 0.01$) reproductive performance compared with homozygous AA (15.2 vs 14.5 kg, respectively). The results of this study agree partially with Vincent *et al.* (1998) whose showed that the A allele was significantly associated with increased litter size measured by TNB and NBA in three of 5 commercial lines involving Meishan, Large White, Landrace and Duroc. For TNB and LWB traits, in Landrace group, differences between genotypes were observed ($p < 0.01$), with better performance in AA genotype. Similar results were shown between genotypes for litter size traits as reported by Putnová *et al.* (2002) using Landrace in Czech Republic which affected the first parities and mainly the numbers of weaned piglets. The magnitude of the effect in Putnová *et al.* (2002) was of 2 piglets per litter in Landrace. In this study for NWP was 0.40 pigs for AA genotype but not different ($p > 0.05$) of BB genotype.

Another a study of Van Rens and Van der Lende (2002) showed that PRLR gene polymorphism affects age at first estrus, litter size and average of functional teats in LW x Meishan F2 crossbred gilts. In other study, Van Rens *et al.* (2003) found that PRLR polymorphism affects pig ovaries, uterus and placenta, which might lead to litter size differences. Thus, litter size could be affected independently from age at first estrus in a way that does not exclude the possibility of PRLR gene being the major gene rather than a marker for a closely linked major gene for litter size. A significant effect of the PRLR genotype on TNB was found for the first parity litter data. Least squares means for PRLR genotype effects in first and latter parities are shown in Table 4. Terman (2005) reported that sows with the AA genotype had the largest litters (TNB, NBA and NWP), BB the smallest and AB were in between being the differences statistically significant ($p < 0.01$) in first parity only. Korwin-Kossakowska *et al.* (2003) found the effect of PRLR genotypes to be significant on NBA for first parities. Litter sizes of AA sows were significantly lower than that

Table 2: Least square means (mean±SE) for reproductive traits in sows, by genotype and general for the Prolactin Receptor (PRLR)

Trait ^{a/}	Genotype ^{b/}			
	AA	AB	BB	General
n	83	225	112	420
TNB	10.66±0.32	10.35±0.25	10.77±0.34	10.64±0.12
NBA	9.58±0.32	9.32±0.25	9.62±0.33	10.25±0.12
NWP	8.08±0.19	8.18±0.15	8.29±0.20	8.54±0.08
LWB	14.97±0.41	14.38±0.32	14.78±0.43	14.27±0.16
LWW	52.43±1.56	52.63±1.23	52.50±1.64	56.27±0.61

^{a/}TNB = Total Number of Born, NBA = Number Born Alive, NWP = Number of Weaned Piglets, LWB = Litter Weight at Birth, LWW = Litter Weight at Weaning. ^{b/}NS ($p > 0.05$)

of BB sows. Furthermore, in sows with = 2 parities, the values for the traits TNB, NBA and NWP were significantly different depending on the genotype at the PRL locus. No significant effects of PRL genotypes on LWW were found in the analysis of the second and later sow parities data. Southwood *et al.* (1995) reported significant effect of the PRLR genotypes on litter size in Landrace. The gene effect was not significant for first parity sows but became significant for later parity sows.

The practice of genetic improvement has as goal to change the average performance of a group of animals. This is possible if we can change gene frequencies looking for an increase in favorable alleles. Considering that the parents transmit genes and not genotypes to the next generation, is necessary to know the value associated to the gene instead of the genotype, i.e., the average effect of the gene-substitution or additive effect (Falconer and Mackay, 1996). Besides, in populations with the presence of heterozygotes, is important to estimate the interaction value of alleles or dominance effect. Estimates of the additive and dominance effects of alleles and their standard errors are in Table 5. A negative increase of 2.26 pigs (TNB) and positive of 0.42 kg (LWB) per litter per copy of allele *AluI* A and different from zero ($p < 0.05$) was estimated. In the same variables, the dominance effect of PRLR was -2.67 pigs and -0.56 kg, respectively and different ($p < 0.05$) from zero. In general, the additive and dominance effects of alleles for PRLR gene in NBA, NWP and LWW resulted not different from zero ($p > 0.05$). In the analysis by genetic group, Landrace, Yorkshire and YL genetic groups showed additive and dominance effects for TNB, NBA, NWP and LWB values not different from zero ($p > 0.05$). For LWW variable,

Table 3: Least squares means and standard errors for the interaction genetic group x PRLR genotype on reproductive traits^{sd} in sows

Genetic group	Genotype	n	TNB	NBA	NWP	LWB	LWW
Duroc	AA	2	12.00±1.79a	10.50±1.75a	10.50±1.06a	15.00±1.60a	69.70±8.52a
	AB	8	6.87±0.89b	5.83±1.01b	4.62±0.53c	15.08±0.61ab	33.01±4.26d
	BB	4	12.75±1.27a	9.25±1.23a	7.75±0.75b	15.02±0.73ab	44.32±6.02cd
Landrace	AA	27	11.33±0.48a	9.92±0.47a	8.92±0.29ab	14.50±0.66ab	56.24±2.32abc
	AB	19	10.36±0.58b	10.00±0.56a	9.47±0.34ab	13.75±0.24b	64.61±2.76ab
YL	AA	23	10.69±0.53ab	10.69±0.51a	8.50±0.31ab	14.15±0.30b	59.93±2.51abc
	AB	169	10.42±0.19ab	10.42±0.19a	8.52±0.11ab	15.26±0.57ab	57.11±0.92abc
	BB	108	10.65±0.24ab	10.65±0.23a	8.57±0.14ab	15.20±0.59ab	57.17±1.16abc
Yorkshire	AA	31	11.12±0.45a	9.67±0.44ab	8.38±0.27ab	15.00±1.60ab	69.70±8.52a
	AB	29	11.62±0.47a	9.55±0.46ab	8.86±0.28ab	15.08±0.61ab	33.01±4.26d

Least squares means in a column with different letters are statistically different (p = 0.01), ^sTNB = Total Number of Born, NBA = Number Born Alive, NWP = Number of Weaned Piglets, LWB = Litter Weight at Birth, LWW = Litter Weight at Weaning

Table 4: Least squares means and standard errors for the Prolactin Receptor (PRLR) genotype effects on reproductive traits^{sd} of sows by Parity Number (PN)

PN	Genotype	n	TNB	NBA	NWP	LWB	LWW
1	AA	20	10.25±0.75b	9.52±0.78a	8.40±0.45a	14.85±1.01a	53.77±3.42a
	AB	70	8.85±0.52b	8.38±0.59a	7.75±0.32a	12.80±0.71a	48.08±2.40a
	BB	45	10.40±0.70a	9.70±0.75a	8.00±0.43a	14.23±0.95a	48.04±3.22a
≥2	AA	63	11.07±0.33a	9.62±0.31a	7.95±0.21a	15.16±0.39a	51.84±1.72a
	AB	155	11.23±0.27a	9.74±0.26a	8.32±0.17a	15.20±0.32a	54.32±1.41a
	BB	67	11.05±0.36a	9.58±0.34a	8.40±0.23a	15.18±0.43a	54.65±1.87a

Least squares means in a column with different letters are statistically different (p = 0.05), by parity number. ^sTNB = Total Number of Born, NBA = Number Born Alive, NWP = Number of Weaned Piglets, LWB = Litter Weight at Birth, LWW = Litter Weight at Weaning

Table 5: Additive (a) effects of favorable allele and dominance (d) effects estimated for reproductive traits^{sd} of sows by genetic group and in general

Genetic group	n	TNB	NBA	NWP	LWB	LWW	
Duroc	14	a	-1.18±1.66	-0.09±1.12	0.53±1.15	1.58±2.03	8.15±7.25
		d	-4.66±1.83**	-3.83±0.96**	-4.33±0.83**	-4.27±2.54	-21.46±8.11**
Landrace	46	a	0.97±0.97	-0.07±0.94	-0.55±0.65	0.07±1.10	-8.37±4.51*
		d	-0.97±0.97	0.07±0.94	0.55±0.65	-0.07±1.10	8.37±4.51*
YL	300	a	-0.11±0.22	-0.09±0.22	-0.04±0.11	-0.08±0.29	0.73±1.03
		d	-0.22±0.26	-0.23±0.27	-0.04±0.14	-0.46±0.35	-0.55±1.24
Yorkshire	60	a	-0.49±0.74	0.13±0.68	-0.48±0.56	0.05±0.93	-2.06±4.05
		d	0.49±0.74	-0.13±0.68	0.48±0.56	-0.05±0.93	2.06±4.05
General	420	a	-2.26±0.95**	-0.29±0.18	0.05±0.11	0.42±0.23*	-0.58±0.91
		d	-2.67±0.98**	-0.21±0.24	-0.05±0.15	-0.56±0.31*	0.29±1.24

**p<0.01, *p = 0.07, ^sTNB = Total Number of Born, NBA = Number Born Alive, NWP = Number of Weaned Piglets, LWB = Litter Weight at Birth, LWW = Litter Weight at Weaning

substitution of A for B allele in Landrace group resulted in -8.37 kg (p = 0.07) while the value for heterozygosis was de 8.37 kg (p = 0.07). There was no significance (p>0.05) in additive and dominance effects for LWW in YL and Yorkshire. Also, for TNB, NBA, NWP, LWB and LWW traits in Duroc group, the additive effect was not different from zero (p>0.05). For Duroc genetic group, dominance effects were important (p<0.01) for TNB (-4.66 piglets), NBA (-3.83 piglets), NWP (-4.33 piglets) and LWW (-21.46 kg). Drogemuller *et al.* (2001) reported effects of the A allele ranged from 0.2 piglets per litter difference between homozygotes in Large White to more than one piglet in a Landrace population (Southwood *et al.*, 1999). (Vincent *et al.*, 1998) found inconsistent the mode of additive gene action for allele A on NBA trait with estimates fluctuating from -0.33 to +0.47 piglets per litter. In this study the range of additive effects for NBA was from -0.07 to -0.29 piglets. Furthermore, for Vincent *et al.* (1998) was not obvious whether PRLR is a major gene for

litter size or it is only a linked marker to a gene determining the effect. Thus, the PRLR gene is located to some distance of the unknown quantitative trait locus, associations between the candidate gene and trait may vary between populations, or families. This may be a possible reason for the lack of significant PRLR effects (Drogemuller *et al.*, 2001) or maybe the observed variation among genetic groups could be due only to sampling strategies. One possible reason for the lack of effect in the current study is that different linkage disequilibrium existed in the genetic groups.

CONCLUSION

Our study revealed the existence of genetic polymorphism in pigs for the Prolactin Receptor gene (PRLR), with a frequency for A and B alleles of 0.46 and 0.54, respectively. The BB genotype was not found in Landrace and Yorkshire. There were differences

in genotypic frequencies between genetic groups, resulting in non-Hardy-Weinberg equilibrium. Effects of interaction between genetic group x PRLR genotypes were observed. Duroc had the highest values to TNB in homozygosis genotypes followed by Yorkshire and Landrace genetic group with differences between AA vs AB genotypes in Landrace. YL showed the best performance to NBA trait without differences between genotypes. AA genotype in Duroc showed the best performance to NWP but not different of genotypes in L, YL and L genetic groups. No differences were detected between BB and AB genotypes for LWB in YL followed by Yorkshire and Duroc. Besides, among AA genotypes of Yorkshire and Duroc, not differences for LWW was detected, being similar the performance as AB genotypes in Landrace genetic group. In YL group, no differences were detected between genotypes for LWW variable.

A significant effect of the PRLR genotype on TNB was found for the first parity litter data. In second and later sow parities data, no differences between genotypes was observed for reproductive traits. Independently of genetic group, the additive and dominance effects of alleles for PRLR gene were in TNB and LWB traits, with additive effect in TNB of 2.26 piglets and 0.42 kg for LWB. In the analysis by genetic group, Landrace showed additive effects for LWW values with 8.37 kg. Dominance effects in Duroc for TNB, NBA, NWP and LWW were estimated in -4.66, -3.83, -4.33 and -21.46, respectively.

The results suggest a major study of the polymorphism in the PRLR gene and its effects on reproductive traits in order to include the gen information in selection programs.

REFERENCES

- Clevenger, C.V., D.O. Freier and J.B. Kline, 1998. Prolactin receptor signal transduction in cells of the immune system. *J. Endocrinol.*, 157: 187-197. PMID: 9659281.
- Drogemuller, C., H. Hamann and O. Distl, 2001. Candidate gene markers for litter size in different German pig lines. *J. Anim. Sci.*, 79: 2565-2570. PMID: 11721835.
- Falconer, D.S. and T.F.C. Mackay, 1996. Introduction to Quantitative Genetics. 4th Edn. Addison Wesley Limited, Edinburg Gate, Harlow Essex, U.K., pp: 325. ISBN-13: 978-0582243026.
- Goliasova, E. and J. Wolf, 2004. Impact of the ESR gene on litter size and production traits in Czech Large White pigs. *Anim. Gen.*, 35: 293-297. PMID: 15265068.
- Hernandez, S.H., C. Lemus Flores, R. Alonso Morales and J.G. Herrera Haro, 2006. Effect of candidate genes on reproductive traits of sows. *Rev. Científica, FCV-LUZ* 6: 648-654.
- Herrera, H.J.G. and A.S. Barreras, 2005. Statistical Analysis of Livestock Experiments. Manual of Procedures. Colegio de Postgraduados, pp: 216. ISBN: 968-839-300-2.
- Isler, B.J., K.M. Irwin, M.F. Rothschild and G.J. Evans, 2000. Association between the prolactin receptor gene and reproductive components in swine. In: Proceedings of the 27th International Conference on Animal Genetics, July 22-26. Minneapolis, MN, USA, pp: 67.
- Johnson, R.K., M.K. Nielsen and D.S. Casey, 1999. Responses in ovulation rate, embryonal survival and litter traits in swine to 14 generations of selection to increase litter size. *J. Anim. Sci.*, 77: 541-557. PMID: 10229349.
- Kelly, P.A., J. Djiane, M.C. Postel-Vinay and M. Edery, 1991. The prolactin/growth hormone receptor family. *Endocr. Rev.*, 12: 235-251. PMID: 1935820.
- Kmiec, M. and A. Terman, 2004. Polymorphism in the PRLR/*Alul* gene and its effect on litter size in Large White sows. *Anim. Sci. Papers Rep.*, 22: 523-527.
- Kmiec, M. and A. Terman, 2006. Associations between the prolactin receptor gene polymorphism and reproductive traits of boars. *J. Applied Gen.*, 47: 139-141. PMID: 16682755.
- Korwin-Kossakowska, A., M. Kamyczek, D. Cieslak, M. Pierzchała and J. Kurył, 2003. Candidate gene markers for reproductive traits in polish 990 pig line. *J. Anim. Breed. Genet.*, 120: 181-191. DOI: 10.1046/j.1439-0388.2003.00386.x.
- Kuehl, R.O., 1999. Design of Experiments: Statistical Principles of Research Design and Analysis. 2nd Edn. Duxbury Press, pp: 688. ISBN-13: 978-0534368340.
- Li, Y., J.R. Molina, J. Klindt, D.J. Bolt and L.L. Anderson, 1989. Prolactin maintains relaxin and progesterone secretion by aging corpora lutea after hypophysial stalk transection or hypophysectomy in the pig. *Endocrinology*, 124: 1294-1304. PMID: 2917512.
- Linville, R.C., D. Pomp, R.K. Johnson and M.F. Rothschild, 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. *J. Anim. Sci.*, 79: 60-67. PMID: 11204716.
- Pope, W.F., 1994. Embryonic Mortality in Swine. In: Zavy, M.T. and R.D. Geisert (Ed.). Embryonic Mortality in Domestic Species. CRC Press, Boca Raton, FL., pp: 53-77. ISBN 0-8493-7634-3.
- Putnová, L., A. Knoll, J. Dvorak and S. Cepica, 2002. A new *HpaII* PCR-RFLP within the porcine prolactin receptor (PRLR) gene and study of its effect on litter size and number of teats. *J. Anim. Breed Genet.* 119: 57-63. DOI: 10.1046/j.1439-0388.2002.00316.x.

- Rothschild, M.F., A.L. Vincent, C.K. Tuggle, G. Evans, T.H. Short and O.I. Southwood *et al.*, 1998. A mutation in the prolactin receptor gene is associated with increased litter size in pigs. *Anim. Genet.*, 29: 60-74.
- Rothschild, M.F., 1996. Genetics and reproduction in the pig. *Anim. Reprod. Sci.*, 42: 143-151. DOI: 10.1016/0378-4320(96)01486-8.
- Rothschild, M.F. and M. Soller, 1997. Candidate gene analysis to detect traits of economic importance in domestic livestock. *Probe*, 8: 13-22.
- Southwood, O.I., H.A.M. van der Steen, A.J. Mileham and D. Cuthbert-Heavens, 1995. Evaluation of the oestrogen Receptor (ESR) gene in Meishan synthetic and Large White pigs. In: *Proceeding of 46th Annual Meeting of the European Association Animal Production*, Prague: 4-7 September, pp: 4.
- Spiess, E.B., 1989. *Genes in Populations*. 2nd Edn. John Wiley and Sons. New York, pp: 774. ISBN-13: 9780471849735.
- Terman, A., 2005. Effect of the Polymorphism of Prolactin Receptor (PRLR) and Leptin (LEP) genes on litter size in Polish pigs. *J. Anim. Breed. Genet.*, 122: 400-404. DOI: 10.1111/j.1439-0388.2005.00547.x.
- Van Rens, B.T.T.M. and T. Van Der Lende, 2002. Litter size and piglet traits of gilts with different prolactin receptor genotypes. *Theriogenology*, 57: 883-893. PMID: 11991391.
- Van Rens, B.T.T.M., G.J. Evans and T. Van der Lende, 2003. Components of litter size in gilts with different prolactin receptor genotypes. *Theriogenology*, 59: 915-926. PMID: 12517393.
- Vincent, A.L., L. Wang, C.K. Tuggle, A. Robic and M.F. Rothschild, 1997. Prolactin receptor maps to pig chromosome 16. *Mamm Genome*, 8: 793-794. PMID: 9321486.
- Vincent, A.L., G. Evans, T.H. Short, O.I. Southwood, G.S. Plastow, C.K. Tuggle and M.F. Rothschild, 1998. The prolactin receptor gene is associated with increased litter size in pigs. In: *Proceeding of the 6th World Cong. Genet. Applied Livest. Production*, Armidale, Australia, pp: 15-18.
- Young, K.H., R.R. Kraeling and F.W. Bazer, 1989. Effects of prolactin on conceptus survival and uterine secretory activity in pigs. *J. Reprod. Fertil.*, 86: 713-722. PMID: 2760896.