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Association of ESR1, FUT1, LEP and PRLR Genes with Some Productive Traits of Yorkshire-Landrace Sows in Mexico

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Abstract: The association of ESR1, FUT1, LEP and PRLR genes with Number of Piglets Born alice (NBP) Number of piglets Weaned Per litter (NWP), Weight of the litter at Weaning Adjusted to 21 days (WWA21) and Reproductive Value of the Sow (RVS) was investigated. The forty-eight Yorkshire×Landrace sows were grouped into two production levels: high production and low production. The association was determined through analyses of variance. In addition, genetic frequencies, measures of diversity and additive and dominance effects were estimated. The B allele of the ESR1 gene, the A allele of the FUT1 gene and the A allele of the PRLR gene were associated with NBP in the low production group of sows. In addition, the G allele of the gene FUT1 and the A allele of PRLR gene were associated with WWA21 and NWP, respectively, in the low production group. In the group of high production sows, the B allele of PRLR gene was associated with NBP. The effect of the C allele of LEP gene increased NBP (1.14), NWP (0.93) and RVS (5.38). These markers can be of particular importance in breeding programs aiming to improve reproduction traits and litter weight.

Key words: Litter size, litter weight, pigs, reproductive value, genetic markers, LEP

INTRODUCTION

The selection of sows to increase profitability, using QTL genes related to reproductive and productive traits is more efficient than the genetic selection using conventional procedures or using one molecular markers at a time (Goncalves *et al*., 2008). Litter size is a very important and easy to measure trait which is often included in scientific research. It is commonly measured as the Total number of Piglets Born (TBP) or the Number of Piglets Born alive (NBP) per litter (Kumalska and Terman, 2017). Several genes are involved in the phenotypic expression of reproductive traits. The PvuII polymorphism of the Estrogen Receptor gene 1 (ESR1) is known to affect total number of pigs born and number of piglets born alive at farrowing (Ye *et al*., 2009). The M307 polymorphism of the FUT1 gene encodes the alpha 1-2 focusyltransferase enzyme that reduces the epithelial adhesion of *Escherichia coli* F18 in weaning pigs, causing that animals of AA genotype to be resistant (Kim *et al*., 2013). This gene has been associated with litter size (Buske *et al*., 2006) and litter weight at weaning (Bao *et al*., 2011a, b). Leptin is a hormone that is associated with the regulation of metabolism and reproduction (Smolinska *et al*., 2009). Leptin is encoded by the LEP gene of which one of its most studied polymorphisms consists of the change of a single cytosine base by thymine at nucleotide number 72 of the chain (T3469C) recognized by the HinfI enzyme (Chao *et al*., 2012). The PRLR gene encodes the specific receptor for prolactin, located on chromo some 16 in the 16q1.4 region (Thuy *et al*., 2006). This gene is significantly associated litter size at farrowing (Drogemuller *et al*., 2001). The identification of favorable polymorphisms for traits of economic importance could be useful as selection criteria for breeding stock in

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populations of Yorkshire-Landrace sows in Mexico. The objective of this study was to identify genetic differences between groups of sows of low and high levels of production through four loci of recognized influence on reproductive traits.

MATERIALS AND METHODS

Animals and traits: The forty-eight breeding sows of the Yorkshire-Landrace type with two or more farrowings were sampled from a breeding stock farm with 300 sows. Based on production levels, 24 females were selected on high levels of production (based on litter size and weight) and 24 females were selected on low levels of production. To identify the sows with high or low production an analysis of average K conglomerates was carried out. The variables included were the Number of Piglets Born alive (NBP) Number of Piglets Weaned per litter (NWP) litter Weight Adjusted to 21 days (WWA21) and the Reproductive Value of the Sow (RVS).

Genotyping: DNA was extracted from blood samples from the sows using the Kit, Quick-DNA™ Universal Kit (Zymo Research, Orange, CA, USA). For PCR-RFLP genotyping, the PCR Kit, Thermo Scientific™ DreamTaq™ PCR Kit (Takara Bio, Inc., Kusatsu, Shiga, JAP) and restriction enzymes of the New England Biolabs™ brand (New England Biolabs, Inc., Ipswich, MA, USA) in a TECHNE™ TC-5000 thermocycler (Techne Inc. Burlington, NJ, USA) were used. The amplification and PCR-RFLP protocols as well as the oligonucleotide primers of each gene were taken from the following researchers; Gene ESR1 (Short *et al*., 1997), FUT1 (Buske *et al*., 2006), LEP (Jiang and John, 1999) and PRLR (Thuy *et al*., 2006).

Analysis of genetic data: Genetic analyses were carried out using the POPGENE Software (Version 1.32, POPGENE, 2000). For each locus, the allelic frequencies, genetic diversity, Fixation index (Fis) and genetic differences between the high and low production groups of sows were determined.

Statistical analyses: The variables analyzed were NBP, NWP, WWA21 and RVS. RVS is an index generated by the PigCHAMP program according to Equation:

$$RVS = 100+\left(\left(N\times h^2\right)/\left(1+(N-1)\times r_e\right)\times(PIS-100)\right)$$

Where:

N = The farrowing number
h^2 = The heritability (0.20)
r_e = The repeatability (0.25)
PIS = The productivity index of the sow

Obtained as; PIS = 100+6.5 (NBP-NBP mean)+1.0 (WWA21-WWA21 mean). To determine the significant association of genotype and level of production with the traits evaluated, the linear model used was:

$$Y_{ij} = \mu + PL\times G_i + e_{ij}$$

Where:

Y_{ij} = The variable of interest
μ = The general average of thevariable
$PL\times G_i$ = The combination of level of production and genotype for each gene
e_{ij} = The random error term

Additive and dominance effects: The additive and dominance effects were estimated by regression procedures as described by Oliver *et al*. (2002). The regression analysis was carried out for each trait, under a fixed effects model which included the additive and dominance effects. To estimate the additive effect by trait the values of 1, 0, -1 were assigned to the homozygous dominant, heterozygous and homozygous recessive genotypes, respectively, for each of the ESR1, FUT1, LEP and PRLR genes. To estimate the dominance effect, values of 0, 1 and 0 were used (0 for the homozygotes and 1 for the heterozygote). All analyzes were carried out through the (SPSS., 2011).

RESULTS AND DISCUSSION

No differences were found in the allelic frequencies between the high and low production groups of sows for ESR1, FUT1 and PRLR genes (Table 1). However, differences were found in the allelic frequencies for the LEP gene ($p<0.05$). In the low production group of sows, the frequency of the leptin T allele was low and no animals with TT genotype were found. The results of the fixation index were negative in both groups, except for FUT1 gene which was positive and close to zero. Negative values indicate an excess of heterozygotes, possibly due to the absence of selection on the traits under the influence of those genes (Table 2). With respect to the results by genotype and production level of the

Table 1: Allele frequencies of the analyzed polymorphisms of studied genes in sows with high or low production

	SHP		SLP	
Genes	Allele1	Allele 2	Allele 1	Allele 2
ESR1	0.625 (A)	0.375 (B)	0.625 (A)	0.375 (B)
FUT1	0.271 (A)	0.729 (G)	0.292 (A)	0.708 (G)
LEP*	0.604*(C)	0.396* (T)	0.792*(C)	0.208* (T)
PRLR	0.312 (A)	0.687 (B)	0.312 (A)	0.687 (B)

SHP: Sows with High Productions levels, SLP: Sows with high Productions Levels, Alleles in parentheses, *denoted significance difference *$p<0.05$, X^2

Table 2: Genetic diversity of the analyzed polymorphisms of studied genes in sows with high or low production

Genes	Ho	He	Fis
Sows with high productions levels			
ESR1	0.67	0.47	-0.42
FUT1	0.38	0.40	0.05
LEP	0.54	0.48	-0.13
PRLR	0.46	0.43	-0.07
Sows with low productions levels			
ESR1	0.58	0.47	-0.24
FUT1	0.42	0.41	-0.01
LEP	0.42	0.33	-0.26
PRLR	0.54	0.43	-0.26

Ho: Observed Heterozygosity; He: Expected Heterozygosity, Fis: Fixation index, $p>0.05$, X^2

Table 3: The results of the association study of ESR1, FUT1, LEP and PRLR genotypes with productive traits in sows with high or low production levels

Genes/PN	Traits	----------Genotypes----------			SEM	p<
ESR1		**AA**	**AB**	**BB**		
H	NBP	13.61^{a}	12.83^{a}	14^{a}		
L		8.96^{b}	9.03^{b}	10.05^{ab}	1.05	0.0001
H	NWP	11.46^{a}	10.78^{a}	11^{a}		
L		8.1^{b}	7.46^{b}	8.1^{b}	0.70	0.0001
H	WWA21	73.73^{a}	73.88^{a}	71.10^{a}		
L		51.96^{b}	55.19^{b}	51.15^{b}	3.61	0.0001
H	RVS	114.6^{a}	111.21^{a}	110.4^{a}		
L		91.44^{b}	93.96^{b}	89.6^{b}	2.62	0.0001
FUT1		**AA**	**AG**	**GG**		
H	NBP	15.85^{a}	12.6^{ab}	13.4^{ab}		
L		8.35^{c}	10.28^{bc}	8.23^{c}	0.84	0.0001
H	NWP	10.65^{ab}	11.16^{a}	10.92^{a}		
L		8.75^{abc}	8.25^{bc}	7.12^{c}	0.61	0.0001
H	WWA21	65.05^{bc}	70.66^{ab}	77.17^{a}		
L		55.2^{cd}	56.42^{cd}	51.34^{d}	2.87	0.0001
H	RVS	112.9^{a}	110.84^{a}	112.96^{a}		
L		92.90^{b}	94.22^{b}	91.52^{b}	2.38	0.0001
LEP		**CC**	**CT**	**TT**		
H	NBP	12.4^{a}	13.73^{a}	12.3^{a}		
L		9.19^{b}	8.95^{b}		0.74	0.0001
H	NWP	10.85^{a}	10.99^{a}	11.3^{a}		
L		7.75^{b}	7.69^{b}		0.52	0.0001
H	WWA21	74.01^{a}	74.45^{a}	69.73^{a}		
L		54.54^{b}	52.71^{b}		2.6	0.0001
H	RVS	109.75^{a}	113.72^{a}	111.83^{a}		
L		93.59^{b}	91.59^{b}		1.89	0.0001
PRLR		**AA**	**AB**	**BB**		
H	NBP	10.7^{bc}	12.96^{ab}	13.69^{a}		
L		12.7^{abc}	8.91^{c}	8.97^{c}	0.96	0.0001
H	NWP	10.3^{a}	11.31^{a}	10.78^{a}		
L		9.4^{ab}	8.09^{b}	7.09^{b}	0.66	0.0001
H	WWA21	72.55^{a}	71.83^{a}	75.82^{a}		
L		50.6^{b}	56.11^{b}	51.07^{b}	3.41	0.0001
H	RVS	106.6^{a}	112.81^{a}	112.53^{a}		
L		90.10^{b}	94.46^{b}	90.81^{b}	2.53	0.0001

$^{a-d}$Different letters denote significance difference between genotypes or between sows with different levels of production, PN: Production levels, SEM: Standard Error of the Mean, p: probability value, H: High production sows, L: Low production sows, NBP: No. of Piglets Born alive, NWP: No. of Piglets Weaned per l; WWA21: Weight of the litter at Weaning Adjusted to 21 days; RVS: Reproductive Value of the Sow

sows, the estimated means for the traits studied, showed significant differences (Table 3). For the ESR1 gene, it was found that in the group of sows of high and low production, the three genotypes behave similar for the four traits studied; however, a higher value was found for

Table 4: Additive and dominant effects of ESR1, FUT1, LEP and PRLR genes for some productive traits of sows

Variables	NBP	p<	NWP	p<	WWA21	p<	RVS	p<
Additive								
ESR1	0.02	0.98	-0.37	0.51	0.44	0.89	-0.97	0.74
FUT1	-0.67	0.30	-0.40	0.41	2.02	0.47	0.17	0.95
LEP	1.14	0.10*	0.93	0.07*	3.70	0.21	5.38	0.04**
PRLR	-0.37	0.60	0.51	0.33	-0.09	0.98	0.10	0.97
Dominance								
ESR1	-0.12	0.89	-0.34	0.60	3.76	0.31	1.87	0.58
FUT1	0.46	0.59	0.45	0.48	-0.97	0.79	-0.60	0.86
LEP	1.06	0.20	0.39	0.54	2.40	0.50	3.15	0.33
PRLR	-0.67	0.43	0.42	0.51	-0.87	0.81	0.82	0.80

NBP: No of Piglets Born alive, NWP: No of Piglets Weaned per l; WWA21: Weight of the litter at Weaning Adjusted to 21 days; RVS: Reproductive Value of Sow, p: probability value, *, **: significant values

BB genotype for NBP in the low production group. For the FUT1 gene and in the low production group, differences were found between genotypes for NBP associated with allele A and WWA21 trait associated with the G allele with no difference for any trait in the low production group. The results for the LEP gene in both production groups showed no differences between genotypes for any trait. For the PRLR gene, the high production level group showed differences only for NBP with higher means for BB genotype. In the group of low production sows, differences were observed for NBP and NWP with higher means for AA genotypes. The additive and dominance effects of each SNPs for all sows (Table 4) did not show significant effects for three of the four loci studied. Significant additive effects were found for the C allele in LEP gene for NBP (+1.14;<0.10), NWP(+0.93;<0.07) and RVS (+5.38; $p<0.05$).

In this study, the additive effect of the genes (ESR1, FUT1, LEP and PRLR) and their association with the evaluated traitsdid not show clear trends between sows of high or low level of production. These results, like others previously found in other breeds of pigs, suggest that different genes or combinations of genes could influence reproductive and productive traits in a different manner depending on the population studied. If the majority of the genes that affect reproductive and productive traits were the same in each population and in each breed, it would be expected homogeneous heritability estimates and high values of association (Munoz *et al*., 2010). Expected heterozygosity values close to 0.50 (Table 2) suggest that the genotypic frequencies are in equilibrium and the absence of selection for reproductive traits here explored.

The A allele of ESR1 gene had higher frequency in the high and low level of production sows (Table 1). The lack of differences in the allelic frequencies between sows of high and low production, indicates the absence of selection towards any allele, independently, of the association reports of this genotype with litter size. The association analysis (Table 3) showed that the BB

genotype gave the highest value for NBP (14 piglets) in the high and low production group being the B allele, the one that favored the highest NBP. This result agree to that obtained by Mencik *et al*. (2019). However, there are publications that disagree and suggest the A allele as the improver allele (Goliasova and Wolf, 2004) whereas others mention no differences between A and B alleles (Dall'Olio *et al*., 2011). In a study of the association of NBP with the ESR1 gene (Balatsky *et al*., 2012), found the BB genotype as the improver gene with a mean of 12.47 piglets. In addition, Horogh *et al*. (2005) reported that the BB genotype with 11.36 pigs born as the most suitable genotype for this trait. Mencik *et al*. (2019) further mention, that second-parity sows with the BB genotype had a significantly higher number ($p<0.05$) of weaned piglets compared to the AB genotype.

Comparing the results of additive and dominance effects of this study with previous reports for NBP (Table 4), they are similar to those of Munoz *et al*. (2007) who obtained an additive value for BB genotype of +0.04 and a dominance value of -0.14. Goliasova and Wolf (2004) obtained an additive value of -0.055 and a dominance value of +0.071. Mencik *et al*. (2019) obtained additive and dominance values of +0.20 and +0.07 for NBP, compared to values, here, obtained of 0.02 and -0.12. For WWA21, the same researchers obtained additive and dominance effects of 0.01 and -0.02 while in this study, an additive effect of -0.37 and a dominance effect of -0.34 were found. In consequence, the additive and dominance effects for WWA21 seem to decline in both studies. Here, additivity and dominance for WWA21 were positive but not significant, contrary to Goliasova and Wolf (2004) results who estimated an additive effect of -0.35 and a dominance effect of +1.49 kg in litters with AB and BB genotypes. The explanation of these discrepancies between researchers could be due to the fact that the polymorphism of ESR1 gene is related to other markers that do not cause the mutation (Noguera *et al*., 2003). The usefulness of the PvuII polymorphism of ESR1 gene in marker assisted selection for the prolificacy of the pig has been a subject of debate. The great variation among the studies has been attributed to the linkage disequilibrium plus mutation, epistatic and epigenetic interaction. As a consequence, the use of ESR1 gene mutation for selection should be evaluated in each pig population before it is applied in a genetic program (Braglia *et al*., 2006).

The G allele of FUT1 gene was the most frequent for high and low level of production sows (Table 1). The frequencies of both alleles agree to those published by Syrovnev (2014) with values of 0.29 for A and 0.71 for G alleles. Geraci *et al*. (2019) on the other hand, reported values of 0.11 for A and 0.88 for G alleles. In addition, the observed and expected proportions of heterozygous were similar between the two production groups (Table 2). For the association model (Table 3), the A allele was related to high NBP and the G allele with high WWA21. Bao *et al*. (2011a, b) found also that the average growth and development in piglets with the AA genotype was high. Bao *et al*. (2011a, b) state that the weaning weight of the litter at day 35 of AA and AG genotypes was significantly greater than for the BB genotype.

Conversely, Hernandez-Lopez *et al*. observed that organisms with the G allele improved NBP. Horak *et al*. (2005) found a significantly lower NBP and NWP in sows with AA genotype. On the other hand, GG homozygotes exceeded the total number of piglets born and that the additive and dominance effects were significant (Table 4) with negative additive effects for NBP and NWP and negative dominance effects for WWA21 and RVS. The lack of effects of the FUT1 polymorphisms has been previously described. A study of association in Italian pigs of the large white breed, determined that the genotypes of the FUT1 gene did not present any effect on productive traits such as average daily gain (Geraci *et al*., 2019). Asimilar situation to what happened with WWA21 in this study.

Significant differences in allele frequencies were found ($p<0.05$) between groups of high and low production sows for LEP gene (Table 1) where the sows of low production level showed a higher frequency of the C allele which may be due to the selection pressure applied for breeding companies for greater weight gain. The frequency of the C allele was greater in the high and low production groups (0.604 and 0.792, respectively), a different situation from what was previously published where it is mentioned that the CC genotype tends to be less frequent in Landrace pigs (Amills *et al*., 2008). Other researchers had found a lower frequency of the C allele (0.1-0.11) in the large white breed (Villalba *et al*. 2009; Hunyadi-Bagi *et al*., 2016). The means estimated with the linear model for the LEP gene (Table 3) showed no production differences between genotypes for any trait but a decrease was observed for all traits in the low production group; contrary to what was described by Perez-Montarelo *et al*. (2012) where the T allele was positively associated with body weight gain.

The CC genotype of LEP gene (Table 4) showed a positive effect on NBP, NWP and RVS (+1.14, +0.93 and +5.38, respectively). Polymorphisms of LEP gene in the literature have been associated with food consumption, daily weight gain, feed conversion, bacon depth and slaughter weight (Chao *et al*., 2012). However, there are several research mentioning that the LEP gene

does not contribute directly to the genetic variability of carcass traits, production, growth, daily weight gain, fatness and backfat (Hirose *et al*., 2014). Discrepancies between studies could be related to the specific presence and frequency of this SNP in each breed (Balatsky *et al*., 2012).

The allelic frequencies of the A allele of PRLR gene performed similar to the other genes studied, probably associated to the lack of selection to a specific allele. The expected heterozygosity (0.439) of the gene PRLR for the combined data of high and low production level sows was different to that of 0.65 obtained by Ziolkowska *et al*. (2010). It was found a positive association of the B allele of the PRLR gene with NBP in the group of high production sows (Table 3) but it behaved different in the low production group where the A allele was associated with NBP and NWP. This disagree with Hernandez-Lopez *et al*. who found that the NBP mean was better for the BB genotype. Terman *et al*. (2016) showed that the A allele of PRLR gene had a significant influence on reproductive traits in crossbreddinglines of pigs. However, the literature also mentions cases where there was no effect of PRLR alleles on reproductive traits (Hunyadi-Bagi *et al*., 2016). The uneven effect of the polymorphisms on traits associated with reproduction, between high and low production groups of sows can be explained by different selection strategies and the influence of pleiotropic effects (Drogemuller *et al*., 2001).

Additive and dominance effects in PRLR gene showed no statistically significant effects for any of the four traits studied. The effects of additivity (-0.37) and dominance (-0.67), here, obtained (Table 4) for NBP were negative as those reported (-0038 and -0638) by Linville *et al*. (2001). Conversely, Vincent *et al*. (1998) found positive additive (+0.16) and dominance (+0.55) effects of B allele for NBP; a result that was corroborated by Drogemuller *et al*. (2001). The additive effect of A allele of PRLR gene was negative for WWA21 but positive for NWP. Contradictory reports of the effects of genetic markers associated with reproduction are common in the literature (Sabev, 2019).

CONCLUSION

All polymorphisms of ESR1, FUT1, LEP and PRLR genes in high and low-productive sows were successfully genotyped. The allele frequencies were similar among groups with the exception of LEP gene where the C allele showed greater frequency in the sows with low production level. The observed and expected heterozygosity were similar among groups of sows and had mostly negative Fis. In the low production group of sows, the effect of the B allele of ESR1 gene and A allele of FUT1 gene were associated with NBP, B allele of FUT1 gene with WWA21 and A allele of PRLR gene with NBP and NWP. In the high production group, B allele of PRLR gene was associated with NBP and C allele of LEP gene showed positive additive effect for NBP, NWP and RVS. Other research have reported different effects of these genes in several traits; therefore, it is advisable to perform preliminary studies to confirm the positive effect of a gene before being apply it in a genetic selection program.

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