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Association among Leptin Receptor Gene (*LEP-R*) with Carcass Weight and Dorsal Fat in a Commercial Type Swine Population from Mexico

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Abstract: The goal of the present study was to determine by molecular methods the existence of an association among alleles of the Leptin Receptor gene (*LEP-R*) and dorsal fat in pigs, taking as a fixed effect the genotype for the ryanodyn receptor gene (*Ryr-1*). Genotyping for both genes was achieved by Polymersase Chain Reaction-restriction Fragment Longitude Polymorphism (PCR-RFLP) using specific PCR primers and restriction enzymes. Polymorphisms of LEP-R were determined by amplification of a 2 kilobase (kb) pair DNA fragment and digestion with restriction enzyme Hpa II whereas for *Ryr-1* gene the PCR product is a 659 base pair (bp) fragment followed by digestion with enzyme Bis HKA I. A total 192 animals were analyzed (F1) not including the parental 4 males and 26 females. For *Ryr-1* gene it was found a distribution of 75.7% dominant homozygous (normal), 22.6% heterozygous (normal carrier) and 1.7% recessive homozygous (affected). Allele frequencies were 0.87 N (normal) and 0.13 n (mutant). For *LEP-R* gene the results indicate 3.2% AA, 29.5% for AB and 67.2% for BB. Allele frequencies were 0.18 A (negative for RFLP), 0.82 B (positive for RFLP). Statistical analyses indicate that LEP-R genotype had an influence on dorsal fat deposition in the studied population.

Key words: Ryanodin receptor, leptin receptor, production traits, swine, genotype, Mexico

INTRODUCTION

Current trends in swine meat production include high carcass yield and low body fat content. Recent research advances, mostly in mice have indicated the influence of leptine on fat deposition (Chen *et al*., 2004). Other studies on swine have shown that animals with a high concentration of leptine in the blood serum have a more elevated measure of body fat (Tartaglia, 1997). With regard to genetic variants of the Leptine Receptor gene (*LEP-R*), a correlation among different alleles and body fat has also been reported. This gene is located at position 6q3.3-q6.5 of the porcine chromosome map, close to the ryanodin receptor gene (*Ryr-1*) (Tartaglia, 1997). This last gene is regarded as a major gene and it is considered to have a fixed effect for analysis of genetic variants of *LEP-R* gene (Fujii *et al*., 1991; Leach *et al*., 1996). On the other hand, *Ryr-1* gene also known as *halothane* gene is for itself of great importance for the swine industry since, homozygous animals for the mutated form when confronted to environmental stress or subjected to halothane show severe symptoms that render their meat useless and can cause even death. For this reason, the mutation causing the halothane effect it is also called Porcine Stress Syndrome (PSS) (MacLennan *et al*., 1990; Cechova *et al*., 2007).

For selection purposes, it would be ideal to have animals with a high carcass yield along with a low dorsal fat content however, some swine breeds showing this phenotype have been found to have very high frequencies of the Ryr-1 mutation. This association was almost certainly created when selection for production traits also selected the Ryr-1 mutation due to the physical linkage among both genes. This is true mainly for the Large white and Pietrain breeds which are very often used for production purposes by crossing with other pure

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breeds such as Duroc, Landrace and Yorkshire (Laville *et al*., 2009; Monin *et al*., 1999; Fujii *et al*., 1991; Otsu *et al*., 1991).

Both genetic traits for dorsal fat thickness and percentage of lean meat have high heredabilities (0.50) which encourages selection schemes for the achievement of goals directed to obtain animals with low fat content. Therefore, studies aimed to find correlations among allelic variants of genes with these productions traits such as the ones analyzed in the present study have an important role for the porcine industry (Oliver *et al*., 2000). In Mexico, previous studies have shown a high frequency (25%) of the PSS mutation in the swine population (Riojas-Valdes *et al*., 2005; Davalos-Aranda *et al*., 2010) whereas this frequency is lower (2.7%) in countries such as the USA due to the existence of programs aimed to the identification of carrier animals by molecular methods and their exclusion from breeding purposes (Ritter *et al*., 2008; Rempel *et al*., 1993).

The role of leptine in fat deposition was demonstrated in 1997 using ob/ob and db/db mice which presented genetic obesity with an autosomic recessive inheritance on the other hand, the hormonal function of leptine is manifested as a reduction of 10% of body weight when a 53% reduction of hormone concentration in blood is presented. Furthermore, an increase of 10% body weight results from the 300% elevation of circulating leptine (Chen *et al*., 2004; Tartaglia, 1997).

Leptine is coded by the *LEP* gene and joins to its specific receptor, LEP-R which belongs to the type 1 cytoquines family receptors. It has two different forms (short and long) and has a nucleotide sequence conservation of 85% with rodents, 88% in humans and 92% in cattle (Tartaglia, 1997). The two forms of the gene can be determined by Restriction Fragment Length Polymorphism (RFLP) analysis which produces three different genotypes; AA, a 2 kb fragment; AB, 2 kb, 1450 and 550 bp fragments and BB, 1450 and 550 bp fragments. The B allele indicates the presence of a restriction site for the enzyme Hpa II (positive for RFLP) whereas the A allele lacks the site. The B allele has been regarded as a favorable marker for dorsal fat thickness in the sense that animals with that allele have a smaller dorsal fat layer (Fujii *et al*., 1991).

The objective of the present study was to analyze the role of allelic variation of *LEP-R* gene in the final weight and dorsal fat thickness of commercial type (crossed) pigs taking the genotype for *Ryr-1* gene as a fixed factor.

MATERIALS AND METHODS

A total of 222 animals were included in the study, 4 males and 26 females as parental generation, plus 192 offspring (F1). Both the phenotype and genotype traits were measured and analyzed only in the F1. Parents were of the Duroc, Pietrain, Yorkshire and Landrace breeds, located in a farm from the metropolitan area of Monterrey, Mexico. Final weight was determined with a scale and carcass dorsal thickness with a ruler (mm), taken the measurement between the 11 and 12th ribs at the back of the carcass. For the genetic analysis, DNA was extracted from blood using the standard phenol-chloroform technique and both DNA isolation, PCR and restriction products were visualized by standard 2% agarose gel electrophoresis always using a molecular weight marker and in the case of PCR amplifications, a negative control. Amplifications were done in a heated-lid thermocycler (M.J. Research PTC-100 R.T.). Genotyping of the *Ryr-1* gene was based in the protocol of Fujii *et al*. (1991). The expected PCR product is a 659 bp fragment which includes the mutation causing Porcine Stress Syndrome (PSS). PCR primer were as follows: F-5' TTC AGT TTG CCA CAG GTC CTA CCA 3'; R-5' ATT CAC CGG AGT GGA AGT CTC TGA AG 3'. PCR conditions were; 1.5 mM $MgCl_2$, buffer 1X, 0.2 mM dNTP's, 2 pmol primers, 0.025 U polymerase Taq, 30 ng genomic DNA and ultrapure water for a final volume of 50 μL. PCR reaction was set to initial denaturing at 94°C, 3 min; 34 cycles of denaturing at 94°C for 1 min, annealing at 53°C for 2 min, extension at 72°C for 3 min; final extension at 72°C for 5 min. Restriction analysis for the *Ryr-1* gene was achieved using enzyme Bis HKA I (Fujii *et al*., 1991) adding 2 μL 10X buffer, 2 μL 10X BSA, 10 U μL^{-1} enzyme, 7.2 μL PCR product and molecular grade water for a total volume of 20 μL. Expected genotypes are; NN (normal): 2 products of 135 and 524 bp; Nn (carrier): 4 products of 135, 166, 358 and 524 bp; nn (affected): 3 products of 135, 358 and 166 bp.

PCR analysis for *LEP-R* gene was performed according to the protocol of Stratil *et al*. (1988) which amplifies a 2 kb fragment of intron 4. Primer pairs were: F-5'GGA AGG CAT TTG TTT CAG CAG TAA 3'; R-5'CAA GTC CTC TTT CAT CCA GCA CTG 3'. PCR conditions were as follows; 1.5 mM $MgCl_2$, 1X buffer, 0.2 mM dNTP's, 2 pmol primers, 0.025 U polymerase Taq, 30 ng genomic DNA and molecular grade water for a final volume of 50 μL using an initial denaturing step of 95°C for 2 min, 32 cycles of denaturing at 94°C, 1 min, annealing at 52°C, 1 min, extension at 72°C, 2 min and a final extension at 72°C, 10 min. RFLP, in the case of *LEP-R* gene was performed with the enzyme Hpa II; this enzyme recognizes three specific polymorphisms; AA (2 kb), BB (1450 and 550 bp) and AB (2 kb, 1450 and 550 bp). To a reaction mix containing 8.5 μL PCR product was added 1 μL buffer L, 8 U μL^{-1} enzyme and water for a final 10 μL volume. Digestion for both genes was at 37°C for 4 h. Allelic and genotype frequencies were calculated using Newton's binomial equation:

$$(p+q)^2 = p^2+2pq+q^2$$

Where:

p = The normal or dominant allele

q = The mutant or recessive allele

For the *LEP-R* gene, the A allele (negative for RFLP) was considered as the normal gene. Statistical analysis was performed using variance analysis to determine the calculated F in a Student's t-test and by obtaining the Minimum Significant Difference (MSD) in an average weight analysis by genotype.

RESULTS AND DISCUSSION

For Ryr-1, genotype frequencies were 75.7% for NN (homozygous dominant), 22.6% for Nn (heterozygous) and 1.7% for nn (homozygous recessive). Allelic frequencies were 0.87 for N and 0.13 for n. For *LEP-R* gene, genotype frequencies were 3.2% for AA (homozygous, negative for RFLP), 29.5% for AB (heterozygous) and 67.2% for BB homozygous, positive for RFLP). Allele frequencies were 0.18 for A and 0.82 for B (Table 1). The total of 192 animals (F1) was divided in 6 groups according to their genotype combination for both genes (*Ryr-1* and *LEP-R*) (Table 2). Although, a variance analysis among genotype combination and both final weight and carcass dorsal fat was performed, no significative difference was found.

Overall, concerning the body weight trait, results showed that group 5, animals negative for *Ryr-1* gene (NN) and positive for LEP-R (BB) had the higher final weight whereas animals positive for both Ryr-1 and LEP-R (nn/BB) had the lowest final weight. With regard to the character for carcass dorsal fat also no significative differences were found. Again, group 5 (genotype NN/BB) had the highest fat deposition whereas genotype nn/BB had the lowest.

Table 1: Allele and genotype frequencies of *Ryr-1* and *LEP-R* genes

Genes	Allele		Genotype		
	----------Frequencies----------				
Ryr-1	N	n	NN	Nn	nn
	0.87	0.13	0.757	0.226	0.017
LEP-R	A	B	AA	AB	BB
	0.18	0.82	0.032	0.295	0.672

Table 2: No. of animals and average dorsal fat thickness according to genotype for *Ryr-1* and *LEP-R* genes

Groups	Genotype	No. of animals	Mean difference for fat thickness
1	NN/AA	5	0.853
2	Nn/AB	12	2.651
3	NN/AB	51	9.101
4	Nn/BB	32	6.415
5	NN/BB	89	16.120
6	nn/BB	3	0.516
Total	-	192	-

Results obtained for *Ryr-1* gene (24.3% animals with the mutant allele) are in agreement with previous results in the same area and were also reported in others researchers on porcine stress syndrome and insulin like growth factor (Riojas-Valdes *et al*., 2005; Davalos-Aranda *et al*., 2010). Genetic and allelic frequencies of *Ryr-1* gene found in the present study are higher than results reported in other countries, ranging from 2.7-12.2% (Ritter *et al*., 2008; Lee *et al*., 2002). A relationship among Ryr-1 genotype with low dorsal fat thickness previously reported was also confirmed in the present study (Leach *et al*., 1996) as well with better weight gains in the heterozygous (Cechova *et al*., 2007). With regard to LEP-R genotypes, the finding of higher frequencies of allele B has also been reported in previous studies (Kennes *et al*., 2001). The B is regarded as the positive allele for fat thickness (Tartaglia, 1997; Lin *et al*., 2001). The relationship among LEP-R alleles with fat deposition found in the present study is in agreement with these reports, confirming the role of allele B and genotype BB in higher body weight.

CONCLUSION

In this study, the best final weights were found in animals with a negative genotype for *Ryr-1* gene in association with the positive allele for the *LEP-R* gene but these animals also had the highest fat thickness. On the other hand, animals positive for both genes take longer to reach their best final weight (do not have a desirable final weight) but have the lowest fat thickness and animals Ryr-1 negative and heterozygous for *LEP-R* gene (nn/AB) had both a good final weight and fat thickness.

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