

# Association Analysis of Leptin Gene Single Nucleotide Polymorphisms with Some Production Traits in Pigs (*Sus scrofa* L.)

Diana Rose R. Gonzales<sup>1,\*</sup>, Rainie Rich Chucky S. Yambao<sup>2</sup>, Neilyn O. Villa<sup>1</sup>, Celia B. dela Viña<sup>1</sup>, Maria Genaleen Q. Diaz<sup>1</sup>, and Renato S.A. Vega<sup>3</sup>

<sup>1</sup>Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, College, 4031, Philippines

<sup>2</sup>Dairy Training and Research Institute, <sup>3</sup>Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, 4031, Philippines

\*Author for correspondence; e-mail: drgonzales1@up.edu.ph; Tel.: + 6349536-3368

Funded by the Department of Agriculture (DA) Bureau of Agricultural Research through the DA-Biotech Project Implementing Unit (DA-Biotech PIU); Fund Code: DA-Biotech R1205 (ABR2012 – 070).

**In mammals, obesity, together with other physical traits, is known to be associated with leptin, a hormone encoded by the *LEP* gene and secreted by White adipocytes in response to changes in body weight and energy. By extracting DNA from hair follicles and employing the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique, the associations of *LEP* gene polymorphisms A1112G and T3469C with body length, backfat thickness, adjusted backfat thickness, average daily gain, and weight of 103 sows, and birth weight, weaning weight, and average daily gain of 153 piglets from a Philippine government-accredited breeder farm, were analyzed. Very low frequencies of the AA genotype (0.013–0.039) for A1112G and the CC genotype (0.007–0.097) for T3469C were computed for all sample sows and piglets. Hence, selection against these genotypes can be inferred. For the association analysis, no association was found between any of the *LEP* gene genotypes and the traits in sample sows and piglets. However, non-genetic factors such as breed and parity were shown to interact with the A1112G genotypes and affect weight and average daily gain in sample sows. Landrace and Large White sows with AG and GG genotypes were heavier than those with the AA genotypes. Also, the AG individuals having one to two parities have lower average daily gain than those with already three to four parities. Results of this study can be of help in breeding programs especially in selection programs and improvement of management techniques in farms.**

Key Words: leptin, obesity, PCR-RFLP, pigs, SNP, *Sus scrofa* L.

Abbreviations: ADG – average daily gain, BFT – backfat thickness, PCR-RFLP – Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, SNP – Single Nucleotide Polymorphism

## INTRODUCTION

In livestock, particularly swine, traits such as feed intake, average daily gain (ADG), and birth weight are essential for economic profit in the meat industries. These traits are not only affected by environmental conditions but also by genetic potential (Hermesmeyer et al. 2000). Due to the market demand for low-fat products, obesity in swine is a special concern. Thick fat is undesirable for consumers; it also increases feed costs of production. Hence, breeders aim to increase the production of lean pork (Kulig et al. 2001). Selection practices are known to improve the ADG, backfat thickness (BFT), and lean meat percentage of pig

carcass (Imboonta et al. 2007).

Aside from selection, it is also important to investigate genetic markers that could be useful for testing obesity and meat quality-related features (Kulig et al. 2001). Obesity in mammals, together with other physical growth traits, is known to be genetically associated with leptin (*LEP*), a protein hormone secreted primarily by White adipocytes in response to changes in body weight or energy (Bender et al. 2011).

This *LEP* acts as a signal of satiety to the brain by regulating several neuropeptides involved in appetite control (Bender et al. 2011) thus, controlling body weight and energetic balance (Barb et al. 2001; Peixoto et al.

2006). It was also found to have functions in growth and reproduction (Barb et al. 2001). In swine, LEP is encoded by the *LEP* (also known as *OB*) gene which is located at q13–q21 of chromosome 18 and is composed of three exons and two introns (Neuenschwander et al. 1996). The first exon is a short untranslated sequence, while the second and third exons comprise the amino acid coding regions (Bidwell et al. 1997).

Variants of the *LEP* gene may influence economically important phenotypes in swine as they are associated with obese phenotypes in other mammals such as humans and mice. Various phenotypic associations have already been found with variations in *LEP* gene in humans, cattle, and swine since leptin has a wide range of physiological functions (Van der Lende et al. 2005). However, many of these associations still need confirmation before firm conclusions are made because different studies obtain completely or partially different results.

Genetic polymorphisms in *LEP* gene are possible genetic markers for selection of production traits not only in swine, but also in other livestock such as cattle. Several studies on single nucleotide polymorphisms (SNPs) of *LEP* gene have been conducted to find association/s of these SNPs with production and growth traits in swine. This study aimed to investigate polymorphisms in the *LEP* gene of sows and piglets from a government-accredited farm in Luzon and their possible association with some recorded production traits. Specifically, this study sought to: (1) characterize the sample population using the allelic and genotypic frequencies of each polymorphism; (2) determine if the associations with some production traits, if any, are different across breeds; and (3) evaluate genotypes that produce desirable production traits that may be beneficial for breeding programs. Results of this study may be of great use in improving the production of lean pork and other marketable traits in swine.

## MATERIALS AND METHODS

### Farm Data Retrieval, Sample Collection, and DNA Extraction

The traits considered for the sows were body length (cm), BFT (mm), adjusted BFT (mm) (BFT adjusted to 100 kg), ADG (kg), and weight at the time of data collection (kg). These were measured during the selection of sows. For the piglets, the traits considered were birth weight (kg), weaning weight (kg), and ADG (kg). Weaning of the piglets was done at 30 d of age.

Records of the production traits of sows and piglets were obtained from a commercial breeder farm in the Cavite, Laguna, Batangas, Rizal and Quezon (CALABARZON) area accredited by the Philippine Bureau of Animal Industry where hair samples from 103 sows of Chester White (n = 35), Large White (n = 38), Landrace (n = 20), and F<sub>1</sub> (n = 10) breeds; and 153 piglets of Chester White (n = 46) and Large White (n = 107) sows were collected. The F<sub>1</sub> sows came from a cross between Large White and Landrace swine. The hair samples were kept inside a resealable plastic bag at room temperature until the time of use.

The lower portion of the hair containing the follicle was cut and used for DNA extraction. Approximately ten hair follicles were cut for each sow and 15 for each piglet. DNA was extracted using the SolGent DNA extraction kit (Korea) following the manufacturer's instructions with some modifications on volumes of reagents.

### *LEP* Polymorphisms of Interest and Primer Sequences

The SNPs A1112G and T3469C were chosen based on previous studies in swine. Jiang and Gibson (1999) studied A1112G SNP. The other SNP, T3469C, was analyzed by a number of researches, but inconsistent results were obtained. The primer sequences for both SNPs were adapted from Jiang and Gibson (1999) (Table 1). Amplification protocols of regions containing these SNPs were optimized.

**Table 1.** Primer sequences used to amplify regions in the porcine *LEP* gene that contain A1112G and T3469C SNPs.

Polymorphism	A1112G	T3469C
Primer pair sequences	F: CAACTCACCGTCGCTTTCTTGAT	F: GAGCCAACACTCTCTCGCTGAG
	R: AGGGAAGCGGAGGAGCAAAG	R: GACTCCTGGAAGCTCAGGTTTCTTC
Position/ Location	674 to 1242 / Intron 2	3348 to 3816 / Exon 3
Product size	569 bp	469 bp
Accession No. used	U66254	U66254

### Polymerase Chain Reaction

To amplify the regions containing the two SNPs, 1x PCR Buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP mix, 0.3 μM each of forward and reverse primers, 0.5 U *Taq* polymerase, and 50–100 ng genomic DNA were used for each reaction. The bands of interest were obtained using the protocols that yielded the most intense and consistent results. For A1112G, there was pre-denaturation at 94 °C for 4 min, 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 61 °C, and extension for 30 s at 72 °C, then final extension at 72 °C for 7 min. For T3469C, pre-denaturation was also at 94 °C for 4 min, but there were 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 61 °C, and extension for 30 s at 72 °C, then final extension at 72 °C for 5 min. These conditions were adapted from Jiang and Gibson (1999) but with slight modifications.

For the amplification of the region containing A1112G, some very slightly-stained bands were observed on the gel for some individuals. Only the discrete darkly-staining band that corresponded to the expected PCR product was considered. If very slightly-stained non-specific bands were present, the band of interest was excised from the gel and cleaned up using Eppendorf Perfectprep® Gel Cleanup Kit.

Unlike in A1112G, only one specific band was observed after amplification of the region containing T3469C.

Some products of amplification were sent to MacroGen, Inc. in South Korea for Sanger sequencing to confirm the sequence of interest and to ascertain the presence of A1112G and T3469C SNPs.

### Genotyping

The PCR products containing A1112G and T3469C were digested using *Taq I* and *Hinf I* restriction enzymes, respectively. Afterwards, the fragments were resolved in 2.5% agarose. For A1112G, the A allele does not contain the *Taq I* recognition sequence, thus, individuals with only one band were genotyped as AA. On the other hand, the recognition sequence is present on the G allele, thus, individuals with two bands corresponding to the DNA fragments generated from the cut, were genotyped as GG. The heterozygote AG had three bands. For T3469C, an additional short fragment (4bp) was observed because another restriction site is located at position 3813. The T allele does not have the recognition sequence, while the C allele has. Hence, individuals with two bands were genotyped as TT, while those with three bands were genotyped as CC. The heterozygotes TC were those with four bands.

### Data Analysis

All the analyses were done separately for sows and piglets. To obtain the genotypic and allelic frequencies for each polymorphism, POWERMARKER program (Liu and Muse 2005) was used. On the other hand, to perform association analysis between each polymorphism and production traits for sows, and growth traits for piglets, STATA 12 IC program (StataCorp. 2011) was utilized. The analysis of variance was conducted using the General Linear Model (GLM) with the following statistical model:

$$Y_i = \mu + G_i + B_j + P_k + GB_{ij} + GP_{ik} + BP_{jk} + e \text{ for sows, and}$$

$$Y_i = \mu + G_i + S_j + M_k + F_l + GS_{ij} + e \text{ for piglets}$$

where Y = trait, μ = general mean, G = effect of the *i*<sup>th</sup> genotype, B = effect of the *j*<sup>th</sup> breed, P = effect of the *k*<sup>th</sup> parity, GB = effect of genotype by breed interaction, GP = effect of genotype by parity interaction, BP = effect of breed by parity interaction, S = effect of the *j*<sup>th</sup> sex, M = effect of the *k*<sup>th</sup> breed of mother, F = effect of *l*<sup>th</sup> sire, GS = effect of genotype by sex interaction, and e = random error.

Pairwise mean comparison was performed using Scheffe's method.

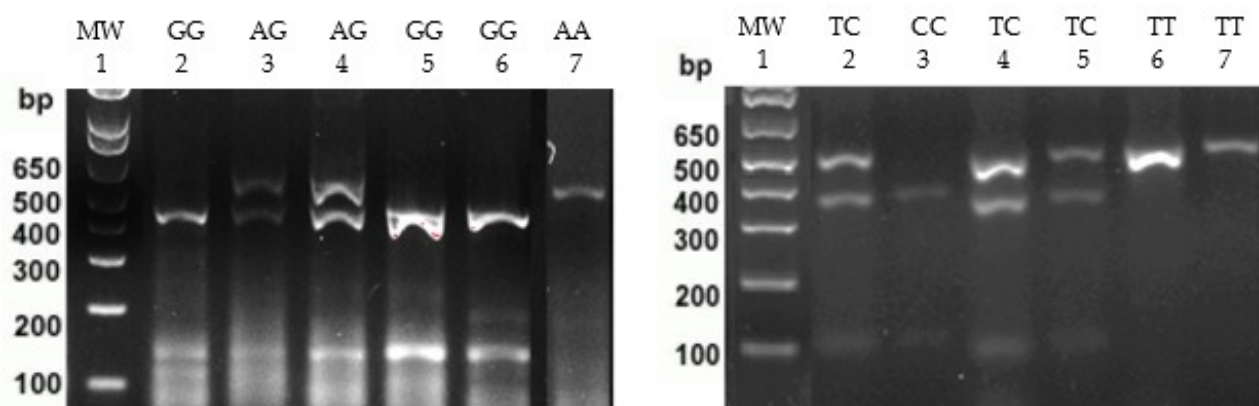
## RESULTS AND DISCUSSION

### LEP Gene Amplification and Genotyping

Hair follicles from 103 sows and 153 piglets were successfully extracted of genomic DNA and genotyped using the PCR-RFLP technique for the A1112G and T3469C polymorphisms. Expected amplification products were obtained after PCR. Genotypes were based on the number and size of fragments each individual showed after restriction enzyme digestion. Figure 1 shows a representation of the bands observed for the two polymorphisms after digestion.

### Characterization of the Samples using Allelic and Genotypic Frequencies

Computations for the allelic and genotypic frequencies were carried out separately for sows and piglets (Table 2). The AA genotype of A1112G and CC genotype of T3469C generally occurred at very low frequencies. From the study of Jiang and Gibson (1999), AA genotype was also observed at very low frequencies as implied by allele A frequencies that range from 0.00 to 0.13 only. The same result of low frequency was obtained for the CC genotype in T3469C in the study of Peixoto et al. (2006) in an F<sub>2</sub> pig population produced by divergent crosses. Another study by Poleszak et al. (2009) in gilts reported no CC genotype. Moreover, TT individuals for T3469C had significantly



**Fig. 1.** Representative photograph of PCR-RFLP patterns of (a) A/G substitution at position 1112: Lane 1 – 1 kb ladder; Lane 7 – AA, 569 bp; Lanes 2, 5, and 6 – GG, 437 + 132 bp; Lanes 3 and 4 – AG, 569 + 437 + 132 bp. (b) T/C substitution at position 3469. Lane 1 – 1 kb ladder; Lanes 6, and 7 – TT, 465 + 4bp; Lane 3 – CC, 347 + 118 + 4bp; Lanes 2, 4, and 5 – TC, 465 + 347 + 118 + 4 bp.

lower BFT and higher average values of lean meat content in carcass compared with the TC individuals. With this result, if lean meat is desired by the breeder, TT individuals for T3469C will be chosen. It can be hypothesized that the low frequency of CC genotype was due to the selection practices of farm owners. It can be inferred that selection against the A and C alleles of A1112G and T3469C, respectively, might be gradually removing the alleles from the population.

Heterozygosity, which is the probability that an individual chosen from the population is heterozygous at a locus (Shete et al. 2000), was also shown in Table 2. It can be observed that heterozygosity values were lower for sows compared with piglets. This trend is expected because between sows and piglets, the former are the ones being selected, not the latter. As shown in the table, there may be selection of sows that favor those with the GG and TT genotypes, and selection against those with AA and CC genotypes for A1112G and T3469C SNPs.

The A1112G SNP is located in an intron, and thus, non-coding. On the other hand, T3469C is located in a coding exon region, but is a synonymous polymorphism and causes no change in the amino acid sequence (Jiang and Gibson 1999) and function of the leptin protein.

Hence, a frequent nucleotide substitution can be tolerated in the population. In his review of studies on isozymes, microsatellites and SNPs, Amos (2010) hypothesized that heterozygosity can have an impact on the rate and distribution of mutations. If natural selection chose where new mutations occur, placing them near existing polymorphisms is favored so as to avoid disruption of areas that work, while adding novelty to regions where variation is tolerated or even beneficial. This hypothesis can also have an implication on the role of *LEP* gene polymorphisms in the overall function of leptin.

**Association Analyses between *LEP* Gene SNPs and Swine Traits**

Statistical analyses using the STATA 12 IC program were conducted to see if the genotypes for *LEP* gene polymorphisms were associated with the production traits of sows and growth traits of the piglets. The level of confidence was set at  $\alpha = 0.05$  such that for p-values less than 0.05, the alternative hypothesis that there is a difference between the average measurements of the traits for the different genotypes is accepted. This means that the trait is associated with the SNP genotype.

Apart from A1112G and T3469C SNPs, combined

**Table 2.** Genotypic and allelic frequencies observed for A1112G and T3469C *LEP* gene polymorphisms among swine (*Sus scrofa* L.) samples.

Sample	A1112G					T3469C				
	Genotypic Frequency			Allele Frequency		Genotypic Frequency			Allele Frequency	
	AA	AG	GG	A	G	TT	TC	CC	T	C
Sows	0.039	0.194	0.767	0.136	0.864	0.680	0.223	0.097	0.791	0.209
Piglets	0.013	0.497	0.490	0.261	0.739	0.484	0.510	0.007	0.261	0.739



genotypes and haplotypes were also tested for possible association with production traits in the sample sows and piglets. Combining the genotypes of A1112G and T3469C was done because the SNPs are contained in one cell, and thus, it is possible that together, the two SNPs can affect the trait of an individual. Additionally, synergistic effect in which the combined effect of two factors is better than either of the two independent factors might apply and might be observed.

Haplotypes were also determined and tested for possible association with production traits. Haplotype is a specific set of SNPs and other genetic variants observed on a single chromosome. The SNPs in a haplotype are physically linked, hence, they have a high tendency of being inherited together (Pierce 2005). Since the two SNPs are both located in the *LEP* gene and are only 2,358 bp apart, they are most likely to be inherited together, hence, they have been considered in the study.

In the linear model used, non-genetic factors such as breed and parity of sows, and sex, breed of mother and sire of piglets were also included as factors that may contribute to each trait. The production traits were quantitative, thus, expression may not be dependent on the genotype alone. The interaction of these non-genetic factors with the genetic factors such as SNP genotype, combined genotype, and haplotype is first determined. When there is no interaction between the non-genetic and the genetic factor, the effect of each factor may be considered, but if there is an interaction between both factors, the individual contribution of each factor cannot be considered.

*Association of LEP gene polymorphisms and production traits in sows.* Results of the association analysis using the linear model showed that only the interaction of A1112G with breed and parity significantly affected weight and ADG, respectively (Table 3). Pairwise comparison was then performed for these traits. Landrace and Large White sows with AG and GG genotypes were significantly heavier than the AA individuals. On the other hand, in sows with AG genotype, ADG was lower for parity 1 and 2, than for parity 3 and 4 (Table 4).

Heavier weight and higher ADG are beneficial to the sows and the suckling piglets since absolute live weight and fatness of sows have a positive influence on their readiness for the next breeding after weaning (Whittemore et al. 1988). Furthermore, production of milk and energetic value at the beginning of lactation is higher if during the management period, the sow accumulated the appropriate reserves as indicated by body weight and BFT (Cozler et al. 1998). The mobilization of these fat reserves maintains a high level of milk production if there is limited feeding of lactating sows (Mullan and Williams 1989). Furthermore, in the review article by Koketsu et al.

**Table 3.** The p-values obtained for the interaction of A1112G, T3469C, A1112G/T3469C, and haplotypes of *LEP* gene polymorphisms with non-genetic factors in sow (*Sus scrofa* L.) samples.

Genetic Factor	Interaction with Breed				
	Body Length (cm)	Backfat Thickness (mm)	Adjusted Backfat Thickness (mm)	Average Daily Gain (kg)	Weight (kg)
T3469C	0.119	0.420	0.706	0.968	0.534
A1112G/T3469C	0.320	0.878	0.686	0.755	0.143
Haplotype	0.754	0.747	0.867	0.952	0.576
Genetic Factor	Interaction with Parity				
	Body Length (cm)	Backfat Thickness (mm)	Adjusted Backfat Thickness (mm)	Average Daily Gain (kg)	Weight (kg)
T3469C	0.638	0.782	0.445	0.964	0.789
A1112G/T3469C	0.948	0.484	0.821	0.178	0.690
Haplotype	0.948	0.462	0.455	0.413	0.822

**Table 4.** Pairwise comparison of the means  $\pm$  standard errors of the weight and average daily gains of sow (*Sus scrofa* L.) samples for A1112G *LEP* gene polymorphism.

Breed	A1112G Genotype		
	AA	AG	GG
Chester White	-	195.717 $\pm$ 16.621 <sup>A</sup>	184.928 $\pm$ 8.220 <sup>A</sup>
F <sub>1</sub>	-	282.267 $\pm$ 34.219 <sup>A</sup>	189.541 $\pm$ 10.129 <sup>A</sup>
Landrace	192.667 $\pm$ 16.544 <sup>B</sup>	203.034 $\pm$ 11.956 <sup>A</sup>	230.870 $\pm$ 11.071 <sup>A</sup>
Large White	174.000 $\pm$ 28.655 <sup>B</sup>	209.451 $\pm$ 10.698 <sup>A</sup>	199.864 $\pm$ 6.580 <sup>A</sup>
Parity	A1112G Genotype		
	AA	AG	GG
A (1 to 2)	-	0.561 $\pm$ 0.009 <sup>A</sup>	0.576 $\pm$ 0.003 <sup>AB</sup>
B (3 to 4)	-	0.599 $\pm$ 0.010 <sup>B</sup>	0.572 $\pm$ 0.007 <sup>AB</sup>
C (>5)	-	0.599 $\pm$ 0.008 <sup>AB</sup>	0.593 $\pm$ 0.005 <sup>AB</sup>

<sup>A</sup>Means within a row sharing a letter are not significantly different at 5% level of significance.

<sup>B</sup>Pairwise comparison of the means cannot be performed for AA genotype across parity classes since there is no individual with such genotype for parity B, and only one individual for parity C.

(2017), parity 1 sows in commercial breeding herds generally do not commonly consume much of their

nutrients and energy stores so that they can grow adequately and reach their mature reproductive performance level. This will, in turn, lead to increase in weight and ADG. These results may then provide bases for selection of sows. Perhaps, selecting Landrace and Large White sows with AG and GG genotypes may be a good choice because they have higher weight, hence, higher energy reserves for gestation and lactation for the next breeding. However, management strategies should be carefully done for the sows, especially for the AG genotypes so that they could maintain these reserves even at their first or second parities and result in higher reproductive performance.

For the rest of the traits in which there was no interaction with non-genetic factors, the association of each genetic factor was considered. But no trait was associated with any single genetic factor (Table 5), similar to the results obtained by Jiang and Gibson in 1999. Body length, weight, and adjusted BFT were associated with parity, with sows of parities 1 and 2 having the least body length and weight but the highest adjusted BFT.

It is also noteworthy that the significant associations

**Table 5.** The p-values obtained for the effect of A1112G, T3469C, A1112G/T3469C, and haplotypes of *LEP* gene polymorphisms on the production traits of sow (*Sus scrofa* L.) samples.

Genetic Factor	Production Trait				
	Body Length (cm)	Back-fat Thickness (mm)	Adjusted Back-fat Thickness (mm)	Average Daily Gain (kg)	Weight (kg)
T3469C	0.229	0.625	0.687	0.911	0.568
A1112G/T3469C	0.646	0.223	0.543	0.898	0.827
Haplotype	0.362	0.827	0.714	0.866	0.381

of the weight and ADG of sows were found with the interaction of non-genetic factors with A1112G, which is present in an intron of *LEP* gene. This result demonstrates the importance of intron in the expression of phenotypes. Introns play different functions especially on gene expression; they can either increase or decrease expression, or sometimes, render no effect. Also, inappropriate intron retention or even a single nucleotide inaccuracy in splicing can likely render the mRNA useless (Rose 2008). Additionally, the chances of conserving an intron increase if it is associated with a function of whatever type (Chorev and Carmel 2012). Alignment of sequences of the region containing A1112G, from

database and from the samples sent for sequencing, showed that the region is conserved. On the other hand, the non-association of T3469C, which is located in exon 3, may be due to the fact that this substitution causes no change in the amino acid sequence in the polypeptide (Jiang and Gibson 1999), hence, there was no effect on the sow production traits. However, the results of various studies showing association of T3469C with different traits suggest that further studies regarding this SNP should be conducted.

*Association of LEP gene polymorphisms and growth traits in piglets.* Results showed that the A1112G and T3469C SNPs did not affect any of the growth traits of the sample piglets (Table 6). No interaction of these SNPs with non-genetic factors was also observed to be associated with any of the traits. A possible reason for this is the fact that the growth traits considered were traits measured before the adult age of swine. Leptin gene expression may be more associated with other traits during the later stages of growth and development when the fat deposit and energy requirements increase, and other factors are present to render the leptin protein to act. A study by Barb et al. (2005) reported that at puberty, the serum leptin levels, hypothalamic leptin receptor mRNA and estrogen-induced leptin gene expression in fat increased with age and adiposity in the swine. Level of dietary energy during gestation, BFT, and feed consumption were positively correlated with milk leptin concentrations in the lactating sow serum. Moreover, in the study of Peixoto et al. (2006), T3469C polymorphism was associated with weight at 21, 42, 63 and 77 d of age, feed intake, ADG, feed conversion, bacon depth and slaughter weight in an experimental population. These studies suggest that leptin gene expression affects traits during the later stages of development of the swine.

For the non-genetic factors alone, sex is not associated

**Table 6.** The p-values obtained for the effect of genetic and non-genetic factors on the production traits of piglet (*Sus scrofa* L.) samples.

Genetic Factor	Production Trait		
	Birth Weight (kg)	Weaning Weight (kg)	Average Daily Gain (kg)
T3469C	0.567	0.361	0.225
A1112G/T3469C	0.482	0.699	0.730
Haplotype	0.970	0.851	0.798
<b>Non-Genetic Factor</b>			
Maternal Breed	0.000	0.196 - 0.500	0.000 - 0.055
Sire	0.000 - 0.160	0.001 - 0.024	0.001 - 0.018

\*p-values for non-genetic factors are given in range since these were from analyses per genetic factor.

with any of the traits, but with the maternal breed and sire. Maternal breed was found to be associated with birth weight and ADG while sire was associated with all the three traits (Table 6). Chester White sows gave birth to piglets with higher birth weight while Large White sows have piglets with higher ADG. These results imply that the piglets of Chester White sows may have grown more slowly than those of the Large White sows. This result is contrary to the information that Chester Whites are said to produce litters that grow out rapidly (Texas A&M University 2001). This result can be explained by the differences in the environment and quality of feed in the United States and the Philippines, and even in other countries. Additionally, the effect of other factors on these growth traits should not be ruled out. In their study, Campos et al. (2012) concluded that other factors such as differences in placental vascularization and efficiency during gestation affected fetal growth even at a given placental size. Also, additional feeding of the sow during late gestation does not increase much the birth weight, and positive effects are not consistent between different studies.

Furthermore, results showed that the Landrace sire had piglets with the highest birth weights, followed by the Pietrain sire. Moreover, the highest weaning weight and ADG of piglets were those of the Pietrain sire. Therefore, if the breeder wants to have piglets with high ADG and weaning weight, he/she may choose a Large White sow and a Pietrain sire.

## CONCLUSION

This study investigated the association of *LEP* gene A1112G and T3469C SNPs and production traits in sows and piglets. Analyses for the possible association of the combination of the two SNPs and the haplotypes were also done. All analyses were based on the linear model that included other non-genetic factors and their interaction with the SNPs. Allelic and genotypic frequencies were computed and the AA and CC genotypes for A1112G and T3469C, respectively, generally occurred at very low frequencies (0.013–0.039 for AA and 0.007–0.097 for CC genotype) for all the sows and piglets. From this result and the result of previous studies that also obtained very low frequency of the CC genotype, it can be inferred that a selection against these genotypes is gradually removing A and C alleles from the population. The computed heterozygosity of the samples also suggests selection in sows. And the selection of sows is not based on their genotypes, which are not readily seen, but rather on the observable phenotypes. These results may suggest association of the *LEP* SNP genotypes

with the phenotypes. However, results of this study showed no statistically significant association of genotype alone on the traits of sows. There may be other factors affecting the traits such as breed and parity. This was shown by the association of the weight of sows with the interaction of A1112G and breed, and ADG with the interaction of A1112G and parity. For the piglets, no association was found between any of the *LEP* gene SNPs and the traits.

The results presented here may be beneficial for breeders, especially the association found between weight and ADG with A1112G by breed and parity interaction in sample sows. Although further studies using higher number of samples and traits are recommended, results of this study will prove to be valuable preliminary findings for the improvement of swine production.

## ACKNOWLEDGMENTS

We are grateful to the Department of Agriculture (DA) Bureau of Agricultural Research through the DA Biotech Project Implementing Unit (DA-Biotech PIU) for supporting and funding this research with a fund code DA-Biotech R1205 (ABR2012 – 070). We would also like to thank Ms. Rachelyn Ann S. Araña for all the help in the statistical analyses.

## REFERENCES CITED

- AMOS W. 2010. Heterozygosity and mutation rate: evidence for an interaction and its implications. *BioEssays* 32: 82–90.
- BARB CR, HAUSMAN GJ, HOUSEKNECHT KL. 2001. Biology of leptin in the pig. *Domest Anim Endocrinol* 21: 297–317.
- BARB CR, HAUSMAN GJ, CZAJA K. 2005. Leptin: A metabolic signal affecting central regulation of reproduction in the pig. *Domest Anim Endocrinol* 29: 186–192.
- BENDER N, ALLEMANN N, MAREK D, VOLLENWEIDER P, WAEBER G, MOOSER V, EGGER M, BOCHUD M. 2011. Association between variants of the leptin receptor gene (*LEPR*) and overweight: A systematic review and an analysis of the CoLaus study. *PLoS ONE* 6(10): 1–12.
- BIDWELL CA, JI S, FRANK GR, CORNELIUS SG, WILLIS GM, SPURLOCK ME. 1997. Cloning and expression of the porcine *obese* gene. *Anim Biotechnol* 8(2): 191–206.
- CAMPOS PH, SILVA BA, DONZELE JL, OLIVEIRA RF, KNOL EF. 2012. Effects of sow nutrition during gestation on within-litter birth weight variation: a review. *Animal* 6(5): 797–806.
- CHOREV M, CARMEL L. 2012. The functions of introns. *Front Genet* 3(55): 1–12.
- COZLER YL, DAVID C, BEAUMAL V, HULIN JC, NEIL M, DOURMAD JY. 1998. Effect of the feeding level during

- rearing on performance of Large White gilts. Part 1: Growth, reproductive performance and feed intake during the first lactation. *Reproduction Nutrition Development, EDP Sciences*, 1998, 38 (4), p. 363–375.
- HERMESMEYER GN, BERGER LL, NASH TG, BRANDT Jr RT. 2000. Effects of energy intake, implantation, and subcutaneous fat end point on feedlot steer performance and carcass composition. *J Anim Sci* 78: 825–831.
- IMBOONTA N, RYDHMER L, TUMWASORN S. 2007. Genetic parameters for reproduction and production traits of Landrace sows in Thailand. *J Anim Sci* 85: 53–59.
- JIANG ZH, GIBSON JP. 1999. Genetic polymorphisms in the leptin gene and their association with fatness in four pig breeds. *Mamm Genome* 10(2): 191–193.
- KOKETSU Y, TANI S, IIDA R. 2017. Factors for improving reproductive performance of sows and herd productivity in commercial breeding herds. *Porcine Health Manag* 3: 1–10.
- KULIG H, GRZESIAK W, SZATKOWSKA I. 2001. Effect of leptin gene polymorphism on growth and carcass traits in pigs. *Arch Tierz Dummerstorf* 44(3): 291–296.
- LIU K, MUSE SV. 2005. PowerMarker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21(9): 2128–2129.
- MULLAN BP, WILLIAMS IH. 1989. The effect of body reserves at farrowing on the reproductive performance of first-litter sows. *Anim Prod* 48(2): 449–457.
- NEUENSCHWANDER S, RETTENBERGER G, MEIJERINK E, JÖRG H, STRANZINGER G. 1996. Partial characterization of porcine obesity gene (OBS) and its localization to chromosome 18 by somatic cell hybrids. *Anim Genet* 27(4): 275–278.
- PEIXOTO JDO, GUIMARÃES SEF, LOPES PS, SOARES MAM, PIRES AV, BARBOSA MVG, TORRES RA, SILVA MDA. 2006. Associations of leptin gene polymorphism with production traits in pigs. *J Anim Breed Genet* 123: 378–383.
- PIERCE BA. 2005. *Genetics: A Conceptual Approach*. 2<sup>nd</sup> ed. New York: W. H. Freeman and Company. 715 p.
- POLESZAK DS, PIETRUSZKA A, KAWECKA M. 2009. Effect of leptin gene polymorphism on fattening and slaughter value of Line 990 gilts. *Acta Vet* 78: 267–272.
- ROSE AB. 2008. Intron-mediated regulation of gene expression. In: Reddy ASH, Golovkin M, editors. *Current Topics in Microbiology and Immunology* 326. Springer-Verlag, Berlin. p. 277–289.
- SHETE S, TIWARI H, ELSTON RC. 2000. On estimating the heterozygosity and polymorphism information content value. *Theor Popul Biol* 57: 265–271.
- STATACORP. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP.
- TEXAS A&M UNIVERSITY. 2001. Breeds of Swine. Instructional Materials Service. *Animal Science* 8394. p. 1–8.
- WHITTEMORE CT, SMITH WC, PHILLIPS P. 1988. Fatness, liveweight, and performance responses of sows to food level in pregnancy. In: YOUNG LG, KING GJ, SHAW J, QUINTON M, WALTON JS, MCMILLAN I. 1999. Interrelationships among age, body weight, backfat and lactation feed intake with reproductive performance and longevity of sows. *Can J Anim Sci* 71: 567–575.
- VAN DER LENDE T, TE PAS MFW, VEERKAMP RF, LIEFERS SC. 2005. Leptin gene polymorphisms and their phenotypic associations. *Vitam Horm* 71: 373–404.