Philippine Journal of Science
147 (2): 293-300, June 2018
ISSN 0031 - 7683
Date Received: 22 May 2017

Leptin (*T3469C*) and Estrogen Receptor (*T1665G*) Gene Polymorphisms and Their Associations to Backfat Thickness and Reproductive Traits of Large White Pigs (*Sus scrofa* L.)

Renato SA. Vega[1], **Ronne Matthews C. Castillo**[2], **Nyka Noelle B. Barrientos**[3], **Mariedel M. Llanes-Autriz**[4], **Byung-Wook Cho**[5], **Celia B. de la Viña**[6], and **Neilyn O. Villa**[6]

[1]Animal Physiology Division, Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños 4031 College, Laguna
[2]UP Rural High School, College of Arts and Sciences, UP Los Baños 4031 College, Laguna
[3]Medical Student, University of the Philippines Manila,
[4]Department of Animal Science, College of Agriculture, Food, Environment and Natural Resources, Cavite State University, Indang, Cavite
[5]Department of Animal Science, College of Natural Resources and Life Sciences, Pusan National University, Miryang 627 – 702, Busan, Republic of Korea
[6]Genetics and Molecular Biology Division, Institute of Biological Sciences, College of Arts and Sciences, UP Los Baños 4031 College, Laguna

The study was conducted to associate polymorphism of the leptin (*LEP*) and the estrogen receptor (*ESR*) genes with backfat thickness and reproductive performance in Large White sows. Nuclear DNA was isolated from hair follicles of 24 (for *LEP*) and 30 (for *ESR*) Large White sows in a commercial breeder farm. Amplification of the T3469C region of *LEP* and T1665G region of *ESR* was accomplished following digestion with *Hinf*I and *Pvu*II restriction enzymes, respectively. Electrophoresis of the *LEP* digestion products revealed genotype frequencies of 0.625 for TT (n=15), 0.25 for TC (6), and 0.125 for CC (3).Meanwhile the *ESR* genotype frequencies for AA, AB, and BB were 0.50 (15), 0.37 (11), and 0.13 (4), respectively. *LEP* polymorphism was not related to backfat thickness at farrowing and 21 days of lactation. Significant genotype associations were observed only for total litter size at birth ($p<0.05$) and total litter size born alive ($p<0.01$). *ESR* polymorphism was not significantly different for backfat thickness, litter size at birth, litter size at weaning, weight at 21 days old, and weaning to estrus interval. The three Large White sows having the CC *LEP* genotype have14 litters size born alive. It is recommended that more Large White CC genotype sows be kept and monitored for further validation of the T3469C *LEP* polymorphism as candidate marker for sow productivity.

Key words: BFT, *ESR*, leptin, reproductive traits, single nucleotide polymorphism

INTRODUCTION

Generally, reproduction traits have low heritable estimates (Robison & Revelle 1973) compared to production traits in all livestock species. Pigs are unique among all mammals because they undergo multiple ovulations and deliver an average of ten piglets in every farrowing. Hence, sows are generally on negative energy balance from farrowing to weaning i.e., they utilize their body energy reserve for the recovery of the brain, uterus, and ovary while nursing an average of ten growing piglets for the period

*Corresponding author: rsvega@up.edu.ph

of 30 days (Flowers 2002).Thus, earlier age of weaning (23 days old) was reported to have significant increase in litters born alive by 0.5 compared to 29 days weaning (Vega et al. 2012).

The pig leptin (*LEP* or *Ob* for obesity) gene in chromosome 18 codes for a 16-kDa protein synthesized by the adipose tissue. Leptin is involved in regulation of feed intake, energy balance, fertility, and immune functions (Chen et al. 2004). Various studies found a polymorphic site in the leptin gene that contains a single nucleotide polymorphism at nucleotide position 3469 where a thymine base is replaced by cytosine, hence the name T3469C. This results to variation in the cutting sites for the *Hinf*I restriction enzyme within the leptin gene. Researches conducted were mostly in relation to economically important production traits in Landrace and Large White pigs (Rybarczyk et al. 2010; Kołodziej et al. 2009; Stepien-Poleszak et al. 2009; Peixoto et al. 2009, 2006; Silveira et al. 2008; Schenkel et al. 2005; Kuliget al. 2001).

The estrogen receptor gene (*ESR*) located at the end of chromosome 1 is a valid candidate gene for reproduction traits because it codes for a steroid-binding hormone receptor that is associated with increasing litter size. Rothschild and co-authors (1996) demonstrated the presence of genetic variations for the *ESR* locus where one allele (B) was considerably associated with increased litter size. The polymorphism of *ESR* gene is described by Ma and co-authors (2012), Rothschild and co-authors (2010), and Ye and co-authors (2009) as involving site T1665G i.e., the substitution of thymine by guanine at nucleotide position 1665; this results in two alleles, namely A and B (Short et al. 1997; Omelka et al. 2005). The B allele is significantly associated with a higher litter size (Rens 2001).

Plasma leptin is significantly related to body fatness in mammals (Suzuki et al. 2009; Vega et al. 2004a, 2004b, 2002; Delavaud et al. 2000; Robert et al. 1998; Takahashi et al. 1996), while estrogen is the female reproductive hormone important for ovulation in livestock. Leptin and estrogen do not have a physiological connection, except that estrogen is a cholesterol-based hormone.

In hot and humid countries like the Philippines, the relationship of body energy reserve and reproductive performance is considered for sustainable sow productivity; however, *LEP* and *ESR* genes are not located on the same chromosome. Therefore, polymorphisms of these genes were separately studied as to their association to backfat thickness, litter size born alive, litter size at weaning, piglets' weight at 21 days old, and the weaning to estrus interval.

MATERIALS AND METHODS

Data Gathering

For *LEP* and *ESR* polymorphisms, 30 and 24 unrelated Large White sows, respectively from a swine accredited breeder farm by the Bureau of Animal Industry (DA-BAI) in Cavite, Philippines were used in this study. The data of the reproduction and production traits such as gestation period, backfat thickness at farrowing (BFTFRW, mm) and at 21 d (BFT21D, mm), litter size at birth (LSB), litter size born alive (LSBA), litter size at weaning (LSW), birth weight (BWT, kg), weight at 21 d old (WT21,kg), and weaning to estrus interval (WEI, d) were provided by the commercial breeder farm based on the regular performance monitoring database. All were bred under similar environmental conditions and management.

Hair follicles of each sow were hand-plucked and placed or stored in airtight resealable plastic bags, and then placed in a refrigerator until DNA extraction. The plastic bags were carefully labeled.

DNA Extraction from Hair Follicles

More or less fifteen hair follicle roots from every sow were cut and placed in a 1.5 mL microcentrifuge tube. One microliter (1 µL) of Proteinase K was added to each tube. Then, 100 µL of lysis buffer was added and mixed using a vortex mixer for 20 s before centrifugation at 10,000 rpm, 25°C for 1 min. The samples were incubated at 55°C overnight.

After incubation, the samples were immersed in 50 µL of protein precipitation solution, vortex-mixed for 10 s, and later placed in an ice bath for 5 min. The samples were centrifuged at 13,000 rpm at 25°C for 3min. The supernatant was transferred to a new 1.5 microcentrifuge tube and the precipitate was discarded. The samples were furthermore added with 150 µL of isopropanol. The tubes were mixed and inverted 30 times, then centrifuged at 13,000 rpm at 25°C for 2 min.

The supernatant was removed, leaving the precipitate in the tube. The precipitate was washed by adding 100 µL of 75% ethanol, inverting again for 30 times and centrifuging at 3,000 rpm at 25°C for 2 min. Afterwards, the liquid was removed and discarded. The washing was done twice. The samples were dried inside the laminar flow cabinet. After the precipitate dried totally, 20 µL of DNA hydration solution was added to each sample. The solution was mixed by pipetting 20 times. Then the samples were incubated at 66°C for one h and were afterwards stored at low temperature of -20°C until use. Two microliters (2 µL) of the sample were subjected to spectrophotometry to determine the quantity and quality of DNA isolated.

PCR Amplification

Polymerase Chain Reaction (PCR) was carried out to amplify the T3469C region of LEP (152bp) and the T1665G region of *ESR* (120 bp) following the protocol of Ding and co-authors (2011) and Short and co-authors (1997), respectively with some modifications and using the pair of primers listed in Table 1.

Table 1. PCR primers and program used to amplify the *leptin* (*LEP* and estrogen receptor (*ESR*) genes.

LEPTIN PRIMERS	F -5' – TGC AGT CTG TCT CCT CGA AA-3'
	R- 5' – CGA TAA TTG GAT CAC ATT TCT G-3'
PROGRAM	↓ 30 cycles ↓ $95_2 \rightarrow 95_1 \rightarrow 55_1 \rightarrow 72_1 \rightarrow 72_5 \rightarrow 12_\infty$
E S R PRIMERS	F - 5'- CCT GTT TTT ACA GTG ACTT TTA CAG AG-3′
	R - 5′-CAC TTC GAG GGT CAG TCC AAT TAG-3′

PCR was performed in a total reaction volume of 20 µL containing 1.5 µL of genomic DNA in 18.5 µL of cocktail mix containing 1.3 µL 10X PCR buffer, 1.3 µL of 15 mM $MgCl_2$, 1.3 µL 2 mM dNTP, 0.74 µL 5 µM Forward Primer, 0.74 µL 5 µM Forward Primer, and 0.21 µL *Taq* polymerase (0.7U). A negative control that contains all the cocktail mix components except DNA was included in each run. The PCR amplification was performed in a G-Storm Thermal Cycler using this PCR profile: initial denaturation at 94°C for 4 min, 31 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 77°C for 1 min, 1 cycle of final extension at 74°C for 8 min, and finally, storage at 4°C.

After amplification, the quality of the amplified product was checked using 2.5% agarose gel electrophoresis for 30 min at 100 volts. The agarose gel was prepared by weighing 1 g agarose mixed in 40 mL new 0.5X TAE buffer. The mixture was heated in a microwave oven until all the agarose was dissolved completely. The solution was cooled down for 5 min at room temperature and poured on a mould. A 12-well comb was inserted. After the gel completely solidified or turned opaque, it was submerged in the electrophoresis tank filled with 0.5X TAE buffer. The 5 µL of the PCR product was placed in each well mixed together with the loading dye, ~1 µL of 10X Blue Juice™. After 30 min of running time, the gel was placed in a staining container with ethidium bromide solution for about 5 s. The gel was then destained in water for 40 min before viewing.

Restriction Fragment Length Polymorphism (RFLP) of PCR Products

***Leptin* (T3469C) Polymorphism**

The cocktail for digestion – consisting of 12.25 µl of sterile H_2O, 2.5 µl 10x buffer, and 0.25 µl *Hinf*I restriction enzyme – was placed in each tube. Ten microliter (10 µl) PCR product was added into each tube and the mixture was incubated in a hot water bath at 37°C for 4 h then 90°C for 20 min. The digested sample was then stored at -20°C.

The samples were subjected to gel electrophoresis using a 2.5% agarose gel at 100 volts for 40 min. Then, the gel was stained in ethidium bromide for 2 s before soaking in the destaining solution for 1 h. The gel was visualized in a UV Illuminator using the program Quantity One.

Leptin gene polymorphism was studied using the SNP T3469C, wherein there is a restriction site at base position 3469 when thymine was replaced by cytosine at that base position. The site of polymorphism is a recognition site for *Hinf*I restriction enzyme. Thus, upon PCR-RFLP, the TT genotype is expected to show a 152 bp uncut band while the CC genotype exhibits two bands (84 and 68 bp). Since SNP is a codominant marker, the TC genotype has all three bands (152, 84, and 68 bp).

***Estrogen Receptor* (T1665G) Polymorphism.** In order to determine the *ESR* genotype of each sow, 10 µL of PCR product was digested with the restriction enzyme *Pvu*II (Vivantis, Malaysia). Digestion was performed at 37°C for 4 h, in a total volume of 25 µL containing 10 µL PCR product, 1X RFLP buffer, and 5 U of the restriction enzyme.

After the restriction enzyme digestion, products were separated on 3% agarose gel. The gel was prepared by mixing 3 g agarose in 100 mL new 0.5x TAE buffer. The mixture was heated in a microwave oven until all the agarose was dissolved completely (~7 min). The solution was cooled down for 5 min at room temperature and poured on the mould with 10-well comb. After the gel completely polymerized, it was submerged in the electrophoresis tank filled with 0.5x TAE buffer. Twenty microliters (20 µL) of the RFLP product was placed in each well mixed together with the loading dye (~1 µL of 10X Blue Juice™) prior to loading. A 50 bp molecular weight ladder was loaded in the first well. After 1 h and 30 min running time, the gel was placed in the container with ethidium bromide solution for about 15 s and transferred in the destaining solution for 40 min before viewing in the gel documentation system.

Genotypes were determined based on the presence of bands of specific sizes. Individuals with only one 120 bp band were identified as AA; those with 65 bp and 55 bp bands were BB; and those with 120 bp, 65bp, and 55 bp bands were AB.

Statistical Analysis

The Large White sows used for the two gene (*LEP* and *ESR*) polymorphisms were not the same and unrelated in this study; hence, analyses were independently done

for each SNP. PowerMarker Version 3.25 (Liu & Muse 2005) was used for marker data analysis. To find out the associations of the *LEP* (T3469C) or *ESR*(T1665G) genotypes to BFTFRW, BFT21D, LSB, LSBA, LSW, BWT, WT21, and WEI, an ANOVA was done to determine the significant differences between the three genotypes for both *LEP* (TT, TC, CC) and *ESR* (AA, AB, BB). If there were significant differences i.e., $p<0.05$, the means were compared using Tukey's T-test.

RESULTS AND DISCUSSION

Neunschwander and co-authors (1996), as cited by Peixoto and co-authors (2006), reported that the leptin gene (*LEP* or *Ob*) in pigs is mapped at chromosome 18 by PCR analysis of somatic cell hybrid, using pig-specific primers. On the other hand, *ESR* is mapped at chromosome 1 and is a candidate gene for fecundity in sows (Buske et al. 2006). Leptin polymorphism studies have been done in association with production traits, while *ESR* polymorphism researches were on reproduction traits. In this study, although the same sows were used for *LEP* and *ESR* polymorphism studies, separate statistical analyses were done. The idea that *LEP* polymorphism was associated to sow productivity performance was due to the fact that sows are on negative energy balance from farrowing to weaning, and the rise in plasma leptin is associated to reproductive traits in mammals (Mantzoros et al. 1997).

Figure 1 shows the bands corresponding to the 465 bp and 120 bp amplicons of the *LEP* and the *ESR* genes, respectively. *Hinf*I digestion of the *LEP* gene revealed three genotypes, namely TT (one band that is 465 bp long), CC (two bands: 118 + 347 bp), and CT (three bands: 118 + 347 + 465 bp) (Figure 2). On the other hand, genotyping of the *ESR* gene upon *Pvu*II digestion showed genotypes AA (one 120 bp band), BB (two bands: 65 + 55 bp), and AB (three bands: 55 + 65 + 120bp) (Figure 3).

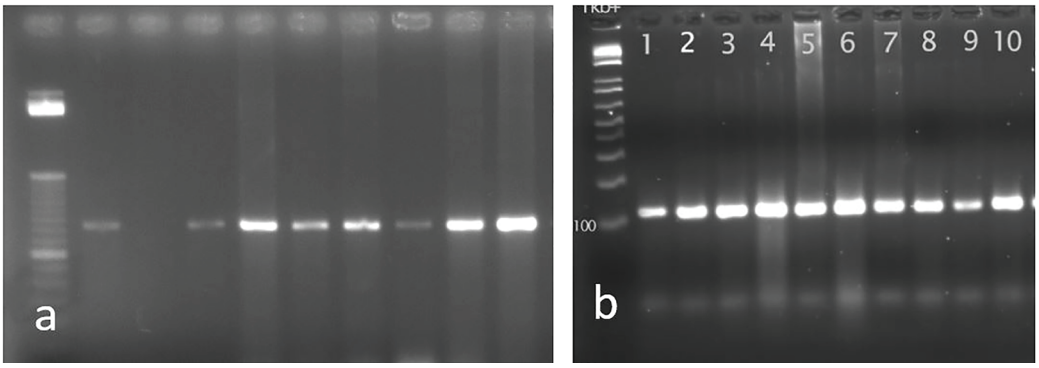


Figure 1. Agarose gel electrophoresis of (a) leptin (*LEP,* 465bp) and estrogen receptor (*ESR,*120 bp) genes amplified through PCR using DNA from sow hair follicles.

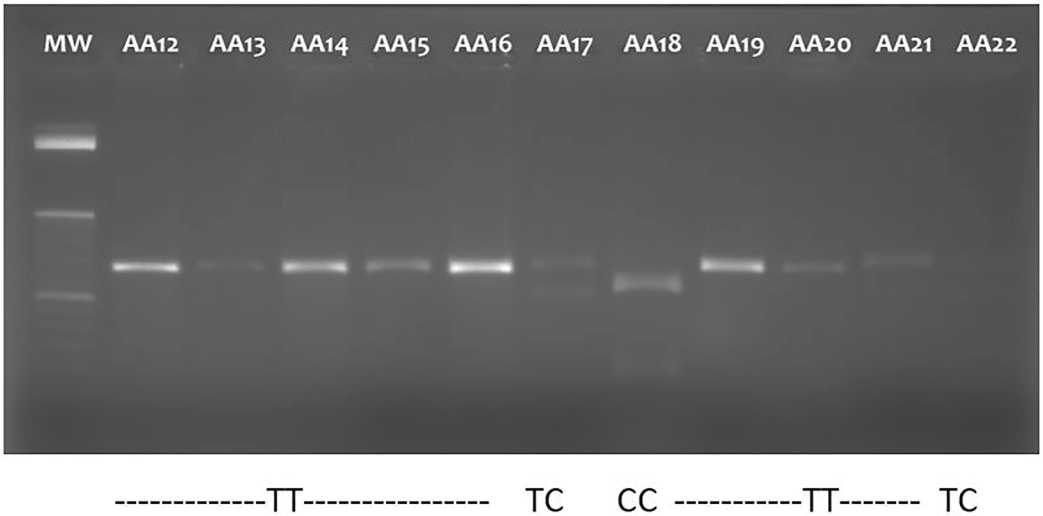
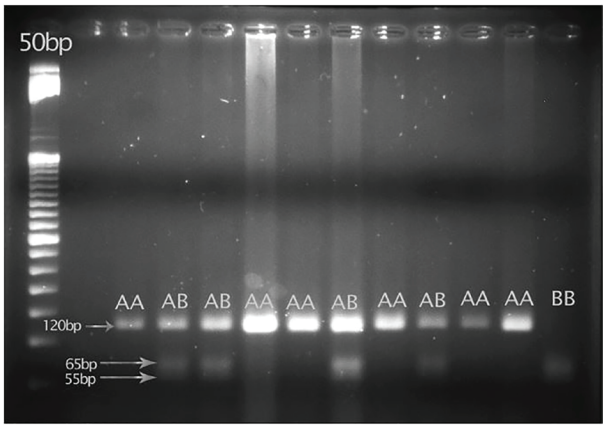


Figure 2. Agarose gel electrophoresis of representative PCR-RFLP products showing *Leptin* T3469C polymorphism in Large White sows. Lane 1: DNA ladder; Lanes 2-6, 9-11: TT (152 bp); Lanes 7 and 12: TC (152 bp + 84 bp + 68 bp); Lane 8: CC (84 bp + 68 bp).



igure 3. Agarose gel electrophoresis of representative PCR-RFLP products showing *ESR* (T1665G) polymorphism in Large White sows. Lane 1- 50 bp ladder; Lanes 2, 5, 6, 8, 10, 11: AA (120 bp); Lanes 3, 4, 7, 9: AB (120 bp + 65 bp + 55 bp); Lane 12: BB (65 bp + 55 bp).

Genotypic Frequencies of *Leptin* and *ESR* Genes

Table 2 presents the frequencies of *LEP* (T3469C) and *ESR* (T1665G) genotypes. Genotype TT showed the highest genotypic frequency of 0.75, while TC and CC both had frequencies of 0.13. A low frequency of CC was also reported by Kulig and co-authors (2001) in their study of the effect of *LEP* (T3469C) locus on several parameters of carcass quality. Peixoto and co-authors (2006) were not able to observe CC in their experimental population of pigs.

Table 2. Frequencies of the *ESR* T1665G and *LEP* T3469C genotypes in Large White sows from an accredited breeder farm in Cavite in 2015.

Genotypes	No. of Sows	Genotypic Frequency
Estrogen Receptor	15	0.50
*Pvu*II		
AA		
AB	11	0.37
BB	4	0.13
Leptin *Hindf*I	18	0.75
TT		
TC	3	0.125
CC	3	0.125

Genotype AA also showed the highest genotypic frequency of 0.50, followed by AB (0.37) then BB (0.13). The trend in the observed genotypic frequencies for *ESR* (T1665G) polymorphism concurs with the studies of Santana and co-authors (2006) and Terman and Kumalska (2012) with Large White sows in Brazil herds having genotypic frequencies of 0.46 (AA), 0.45 (AB), and 0.09 (BB) and Large White Polish herd having 0.56 (AA), 0.25 (AB), and 0.19 (BB).

ESR (T1665G) Gene Polymorphism and Its Association to Test Traits

Table 3 shows the summary of generalized linear model (GLM) analysis using SAS to determine if there is an association between the *ESR* gene SNP and production traits, specifically gestation period, backfat thickness at farrowing, backfat after 21 days, difference of backfat at farrowing and after 21 days, litter size born, litter size born alive, litter size at weaning, birth weight, weight after 21 days, and weaning to estrus interval. The comparatively high p-values (p>0.05) indicate no significant differences among the *ESR* genotypes with respect to the production traits. This means that Large White pigs, regardless of genotype, has the same gestation period, backfat thickness at farrowing, backfat after 21 days, difference of backfat at farrowing and after 21 days, litter size born, litter size born alive, litter size at weaning, birth weight, weight after 21 days, and weaning to estrus interval. This is in contrast with the work of Suwanasopee and Koonawootrittriron (2011), in which p-value was found to be less than 0.05 for litter size and weaning to estrus interval. Several investigations have examined the association of *ESR (*T1665G) on litter size of swine. Santana and co-authors (2006) reported that the A allele had beneficial effect on litter size in Large White breed. Rothschild and co-authors (1996) and Short and co-authors (1997) also detected a statistically significant effect of the polymorphism on litter size in Large White and Yorkshire breeds. The difference in the findings may be due to the fact that the present study was done in the tropics while others were mostly

Table 3. Association of *ESR* (T1665G) polymorphism to sow productivity performances (Mean±SD) in Large White pigs (n=30).

TRAIT	*ESR* T1665GGenotypes		
	AA	AB	BB
Gestation Period (d)	112.23±5.34	113.33±2.01	115.80±1.30
BFTFRW (mm)	18.82±3.71	19.33±3.96	21.40±4.51
BFT21D (mm)	11.58±2.45	11.08±2.54	13.60±0.55
BFTFRW – BFT21D, (mm)	7.23±4.25	8.25±4.37	7.80±4.50
LSB	9.58±2.94	10.00±2.63	10.40±4.35
LSBA	9.47±2.96	10.00±2.63	10.40±4.35
LSW	8.70±1.96	8.83±1.90	8.80±3.03
BWT (kg)	1.57±0.15	1.52±0.22	1.69±0.34
WT21 (kg)	6.76±0.56	6.60±0.52	7.27±0.71
WEI (d)	15.00±19.30	9.41±9.76	10.00±1.75

Means with different letter superscript indicate significant differences at p<0.05.

in temperate environment, thus the presence of varying body energy utilization in the experimental animals. It is generally believed that reproduction traits are highly influenced by environmental factors than genetics. Although large sample population is needed, this may include related (sibs) boars of the sampled sows for future consideration.

LEP (T3469C) Gene Polymorphism and Its Association to Test Traits

The associations of *LEP* (T3469C) polymorphism with gestation period, backfat thickness at farrowing (BFTFRW), at 21 days lactation (BFT21D) and BFT lost, were all not statistically significant between the genotypes (Table 4). Similarities in backfat thickness at farrowing suggest that during pregnancy, the management and nutrition of the breeding herd were sufficient to allow the sows to regain lost body condition from the previous lactation based on their genetic capability. It is common knowledge in commercial farms that a sow reaches her peak of milk production during the 21st day lactation. It is the reason why piglets are being weighed at this time, which represents the milking ability of the sows. The TC sows had the highest backfat, which was higher by 0.63 mm over CC and 1.47 mm over the TT sows. However, there were no significant genotype associations observed for backfat thickness on the 21st day of lactation. Backfat lost by the sows during lactation is synonymous with the body condition indicative of utilized energy by the sow in nurturing her litters (Houdeet al. 2010). This was computed by deducting BFT21D from the BFTFRW. Significant differences in litter size born (LSB) and litter size born alive (LSBA) between TT and CC genotypes was found ($p<0.05$); a 5 piglets per sow per farrowing is a huge mark up. It should be noted that the standard deviation of the three CC genotypes was only 1.6, indicating low variability between sows or high consistency of performance.

Litter size at weaning (LSW), birth weight (BWT), and weaning to estrus interval (WEI) were all comparable between the TT, TC, and CC genotypes (Table 4). From farrowing to weaning, the sow's condition was on a negative energy balance because the practice is gradual feed withdrawal seven days before expected date of farrowing and gradual increase seven days after farrowing. In all genotypes, the backfat loss and backfat thickness (BFT) at 21 days (peak of milk production) were comparable showing that the leptin (T3469C) polymorphism has no relation to sow performance during lactation period.

Surprisingly, the rare CC genotype appeared in three out of 30 LW sows sampled. Rybarczyk and co-authors (2010) studied growth parameters in pigs and found no CC genotype in 126 PIC crossbred (LxLW). Bauer and co-authors (2006) found CC allelic frequency value of only 0.073 (7.3%) with TT and TC having 50% and 42.7%, respectively; further, significant association to average daily gain but not backfat thickness and lean meat was observed. Wierzchowska and co-authors (2012) reported a small percentage of 8% CC genotype out of 173 hybrid sows. Kulig and co-authors (2001) found only 1.5% CC genotype and it was associated to lean meat content and average daily gain. Peixoto and co-authors (2006) found no CC genotype among F_3 pigs. Interestingly in our study, the three leptin CC genotype Large White sows obtained 0.125 genotypic frequency and consistently delivered 14 piglets alive.

As a recommendation, commercial breeder farms must look for and observe Large White breeds having CC (*LEP* T3469C) genotypes in sows and boars. They should continue to monitor the performance of the pigs and their offsprings if their litter size born alive will remain consistently high (14 piglets). However, high quality milk replacement as creep feed must be offered to improve survivability and weaning weight of the piglets post farrowing, especially in our country. Liquid may be preferred over solid milk as creep/ booster feed to minimize piglet mortality.

Table 4. Association of *LEP* (T3469C) polymorphism to sow productivity performance traits (Mean±SD) in Large White pigs (n=24).

TRAIT	*Leptin* T3469C Genotypes		
	TT	TC	CC
Gestation Period (d)	111.93±0.93	112.17±1.48	12.33±2.1
BFTFRW (mm)	18.80±0.95	19.67±1.51	18.67±2.13
BFT21D (mm)	11.40±0.59	13.17±0.94	11.67±2.13
DIFFBFT (mm)	7.4±1.11	6.5±1.76	7.0±2.48
LSB	$8.67±2.49^{B}$	$11.50±1.17^{AB}$	$14.0±1.65^{A}$
LSBA	$8.53±0.69^{B}$	$11.50±1.09^{AB}$	$14.0±1.55^{A}$
LSW	8.13±0.55	9.50±0.86	10.0±1.22
BWT (kg)	1.56±0.04	1.48±0.07	1.64±0.10
WT21 (kg)	6.70±0.18	6.89±0.28	6.70±0.40
WEI (d)	5.87±1.14	10.30±1.81	6.0±2.56

Means with different letter superscript ^{A,B} indicate significant differences at $p<0.05$.

ACKNOWLEDGMENT

The Leptin Project research team would like to extend their gratitude to the DA – Biotech Project Implementation Unit of the Department of Agriculture for the fund provided to accomplish this project, under the leadership of Dr. Vivencio Mamaril; and to the support of the Animal Breeding and Physiology Division, namely Rainie Rich Chucky Yambao, Modesta de los Santos, Filoteo Atendido, Jr., Cenon Cerezo (deceased), and Josh Elisha R. Octura. Gratitude is also extended to Dr. Rita P. Laude, Dr. Angel Lambio, and Dr. Severino S. Capitan (deceased) for their valuable advice.

CONFLICT OF INTEREST

The main author and all co-authors of this paper have no conflict of or competing interest and are not in any way connected in other private institution, company or private piggery farm.

REFERENCES

BAUER M, BABELOVA A, OMELKA R, BAUEROVA M. 2006. Association of *Hinf*I polymorphism in the leptin gene with production traits in White Improved Pig Breed. Slovak J. Anim Sci 39(3): 119-122.

BUSKE B, STERNSTEIN I, BROCKMANN G. 2006. QTL and candidate genes for fecundity in sows. Anim Reprod Sci 95: 167-183

CHEN C, CHANGT, SU H. 2004. Genetic polymorphisms in porcine leptin gene and their association with reproduction and production traits. Australian Journal of Agricultural Research 55(6): 699-704.

DELAVAUD C, BOCQUIER F, CHILLIARD Y, KEISLER DH, GERTLER A, KANN G. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. Journal of Endocrinology 165: 519-526.

DING X, YAN Y, ZANG C, DING Y, YIN Z. 2011. Association of leptin gene polymorphism with litter performance trait in four Chinese Indigenous Pig Breeds. J. of Anim. and Vet. Adv. 10(5): 663-667.

FLOWERS B. 2002. Using reproductive physiology to troubleshoot fertility problems(Part II). *Swine News*. North Carolina State Swine Extension. Vol. 25 No. 8. Retrieved from http://www.ncsu.edu/project/swine_extension/swine_news/2002/sn_v2508.htm on 31 May 2012.

HOUDE AA, MÉTHOT S, MURPHY BD, BORDIGNON V, PALIN MF. 2010. Relationships between backfat thickness and reproductive efficiency of sows: A two-yr trial involving two commercial herds fixing backfat thickness at breeding. Canadian J. of Anim. Sci.90:429-436. 10.4141/CJAS09115

KOŁODZIEJ A, PIETRUSZKA A, JACYNO E, KAWĘCKA M, STĘPIEŃ-POLESZAK D, SOBOCIŃSKA A. 2009. The leptin gene polymorphisms and production traits in boars. Acta Sci. Pol., Zootechnica 8(1-2): 11-18.

KULIG H, GRZESIAK W, SZATKOWSKA I. 2001. Effect of leptin gene polymorphism on carcass traits in pigs. Arch Tierz, Dummerstorf 44(3): 291-296.

LIU K, MUSE S**.** 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Oxford University Press Vol. 21 no. 9.

MA J, LI M, WANG H, LI X. 2012. Genotyping of the porcine ryanodine receptor 1 (*RYR1*) and estrogen receptor 1 (*ESR1*) genes by high resolution melting (HRM) approach. Biotech and Bioprocessing Engineering 17: 1076-79. DOI 10, 1007/s12257-012-0139-6.

MANTZOROS CS, FLIER JS, ROGOL AD. 1997. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J. Clin. Endocrinol. & Metab. 82: 1066-70.

OMELKA R, BAUEROVA M, MLYNEK J, BUCHOVA B, PESKOVICOVA D, BULLA J**.** 2005. Effect of the oestrogen receptor (ESR) gene on reproductive traits of Large White, White Meaty, and Landrace pigs. Czech Journal in Animal Science p. 249-253. *Proteus vulgaris.* (n.d.) Hartmann. Retrieved from http://www.bode-science-center.com/center/relevant-pathogens-from-a-z/proteus-vulgaris.html on 28 Nov 2015.

PEIXOTO JO, FARIA DA, SILVA PV, FONSECA I, LOPES PS, GUIMARAES SEF. 2009. Association between leptin gene single nucleotide polymorphisms and carcass traits in pigs. R. Bras. Zootec 38(2): 271-276

PEIXOTO JO, GUIMARÃES SEF, LOPES PS, SOARES MMA, VIEIRA PA, BARBOSA MVG, TORRES RA, SILVA MA. 2006. Associations of leptin gene polymorphisms with production traits in pigs. Journal of Animal Breeding and Genetics 123(6): 378-383.

RENS B**.** 2001. ESR and PRLR . In Physiological aspects of two major genes for litter size in pigs (p. 3-6). Wageningen,Netherlands: Van Gils.

ROBERT C, PALIN M, COULOMBE N, ROBERGE C, SILVERSIDES FG, BENKEL BF, MCKAY RM, PELLETIER G. 1998. Backfat thickness in pigs is

positively associated with leptin mRNA levels. Can. J. Anim. Sci. 78:473-482.

ROBISON W, REVELLE T. 1973. An Explanation For The Low Heritability Of Litter Size In Swine. Journal of Animal Science 37: 668-675.

ROTHSCHILD M, JACOBSON C, VASKE D, TUGGLE C, WANG L, SHORT T, ECKARDT G, SASAKI S, VINCENT A, MCLAREN D, SOUTHWOOD O, VAN DER STEEN H, MILEHAM A, PLASTOW G**.** 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. PNAS 93: 201-205.

ROTHSCHILD M, STALDER K, DEKKERS J. 2010. Genomic selection shows promise in swine breeding. Iowa State University. Retrieved from National Hog Farmer: http://nationalhogfarmer.com/genetics-reproduction/evaluation-selection/genomic-selection-tools-0426 accessed on 12 Jul 2014.

RYBARCZYK A, KMIEĆ M, GARDZIELEWSKA J, KARAMUCKI T, JAKUBOWSKA G, TERMAN A, POLASIK D. 2010. Effect of carcass meatiness level on meat quality of pigs monomorphic at genes *RYR1* and *LEP.* Polish J of Nutrition Sci. 59(4): 325-328.

SANTANA B, BIASE F, ANTUNES R, BORGES M, FRANCO M, GOULART L.2006. Association of the estrogen receptor gene *Pvu*II restriction polymorphism with expected progeny differences for reproductive and performance traits in swine herds in Brazil. Genetics and Molecular Biology 29: 273-277.

SCHENKEL FS, MILLER SP, YEX, MOORE SS, NKRUMAH JD, LI C, YU J, MANDELL IB, WILTON JW, WILLIAMS JL. 2005. Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. J. of Anim. Sci. 83(9): 2009-20.

SHORT T, ROTHSCHILD M, SOUTHWOOD O, MCLAREN D, DE VRIES A, VAN DER STEEN H, ECKARDT G, TUGGLE C, HELM J, VASKE D, MILEHAM A, PLASTOW G**.** 1997. Effect of the estrogen receptor locus on reproduction and production traits in four Commercial Pig Lines. Journal of Animal Science 75: 3138-42.

SILVEIRA ACP, ANTUNES RC, ALMEDIA JF, BRAGA TF, FREITAS PFA, CESAR ASM, GUIMARAES EC. 2008. *Obese gene* polymorphism in Pietrain and Large White pigs after divergent selection. Genetics and Molecular Research 7(4): 1217-22.

STEPIEN-POLESZAK D, PEITRUSZKA A, KAWEKA M. 2009. Effect of leptin gene polymorphism on slaughter value of line 990 pigs. Acta Vet BRNO 78: 267-272.

SUWANASOPEE T, KOONAWOOTRITTRIRON S. 2011. Genetic markers on reproductive traits in pigs . Thai J Vet Med Supplementary p. 73-76.

SUZUKI K, INOMATA K, KATOH K, KADOWAKI H, SHIBATA T. 2009. Genetic correlations among carcass cross-sectional fat area ratios, production traits, intramuscular fat, and serum leptin concentration in Duroc pigs. J. Anim. Sci. 87:2209-15.

TAKAHASHI M, FUNAHASHI T, SHIMOMURA I, MIYAOKA K, MATSUZAWA Y. 1996. Plasma leptin levels and body fat distribution. Hormone Metabolic Research 28:751-752.

TERMAN A, KUMALSKA M**.** 2012. The Effect of a SNP in *ESR* gene on the reproductive performance traits in Polish Sows. Russian Journal of Genetics 48(12): 1260-63.

VEGA RA, LEE HG, KUWAYAMA H, MATSUNAGA N, HIDARI H. 2002. Age-related changes in plasma leptin from early growing to late finishing stages of castrated Holstein steers: Utilizing multi-species leptin RIA. Asian-Australasian Journal of Animal Science 15(5): 725-31.

VEGA RA, HIDARI H, KUWAYAMA H, SUZUKI M, MANALO DD. 2004a. The relationships of plasma leptin, backfat thickness and TDN intake across finishing stage of Holstein steers. Asian-Australasian Journal of Animal Science 17(3): 330-36.

VEGA RA, HIDARI H, MATSUNAGA N, KUWAYAMA H, MANALO DD, LEE HG, HATA H. 2004b. Plasma leptin and performance of purebred and backcrossed Hereford throughout grazing and feedlot fattening. Asian-Australasian Journal of Animal Science 17(7): 954-959.

VEGA RSA, ESTRELLA CAS, VALERA AMP, CALUD AT, VILLAR EC. 2012. Performance of selected Philippine commercial piggery farms weaning at different ages. Phil J of Vet and Anim Sci. 38(2): 127-133.

WIERZCHOWSKA A, KOŁODZIEJ-SKALSKA A, NAPIERALA D, KAWECKA M, JACYNO M. 2012. The association of leptin gene polymorphism with reproductive usefulness and selected biochemical indicators of Hybrid Sows (Polish Large White x Polish Landrace). Acta Veterinary BRNO 81:333-337.

YE LZ, NIU BY, LUO LF, SHAO GC, MEI SQ, DENG CY, JIANG SW, XIONG YZ, LI FE. 2009. Simultaneous identification and combined genotype effects analysis of pre-implantation protein 3 (PREI3) and estrogen receptor (ESR) gene in Pigs. Livestock Sci 125: 80-83.