# GENOTYPE AND ALLELE FREQUENCIES OF HEART FATTY ACID BINDING PROTEIN (HFABP), LEPTIN RECEPTOR (LEPR) AND INSULIN-LIKE GROWTH FACTOR 2 (IGF2) GENES OF SELECTED PHILIPPINE NATIVE PIG HERDS IN LUZON AND VISAYAS

Ester Flores[1], Emmanuel Bacual[1] and Sherwin Matias[1]

## ABSTRACT

**Nucleus herds of Native Pigs (NP) at various locations in the Philippines implement a breeding program for food security and genetic conservation. The market niche of NP is lechon due to its unique fat composition. Genes associated with fat deposition and meat quality can be screened to identify individual NP carrying favorable alleles of these genes for selection to complement the breeding program. This study was conducted to determine the genotype and allele frequencies of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) marker of Heart Fatty Acid Binding Protein (HFABP) and Leptin Receptor (LEPR) genes and Mutagenically-Separated Polymerase Chain Reaction (MS-PCR) marker of Insulin-like Growth Factor2 (IGF2) gene. The HFABP gene has three RFLP markers hence, there are at most, 6 favorable alleles possible. Out of 543 screened individuals, 18.5%, 42% and 39.5% carry two (HL2), three (HL3) and four (HL4) favorable alleles, respectively. For LEPR, 38%, 45.6% and 16.1% were of AA (favorable), AB and BB genotypes. For IGF2-in7, the favorable genotype CC was 3.3% followed by 19.5% CG and 77.2% GG. Results suggest good potential for marbling and reduction in backfat depth through selection. The availability of genetic tests can hasten the identification of individuals carrying favorable genotypes.**

Keywords: marbling gene, native pig, back fat, HFABP, LEPR

## INTRODUCTION

Native pigs are known to be adaptable and resilient. Furthermore, its meat has a unique taste and texture that is preferred and paid a premium for by Filipino consumers according to Baguio (2017). This could be due to back fat and intramuscular fat deposition and the high frequency of gene markers for these traits could lend support. Intramuscular fat or marbling influences tenderness, juiciness, flavor and texture (Eikelenboom *et al*., 1996). Demand for native pigs is high, especially in the lechon market. Several native pig R&D stations have been established as potential sources of genetically improved breeding

[1]Philippine Carabao Center National Headquarters and Genepool, Science City of Muñoz, Nueva Ecija (email: esterflrs@gmail.com).

animals for smallholder and potential commercial native pig raisers that supply the lechon market. The breeding goal was to improve growth (pre-wean ADG) and reproductive performance. The carcass traits of the native pig that consumers pay premium on are not included in the breeding objective. In order to make genetic improvements, the traits need to be measured and selected. Tenderness, juiciness, marbling and fat deposition are just some of the phenotype or meat quality characteristics that are difficult to measure or are measured late in life as a trait. At the same time, fat deposition traits negatively affect loin muscle weight or lean meat content (Peñagaricano *et al*., 2015) and perhaps growth rate (Chen *et al*, 2002) hence, a balance to selection is needed. To be able to measure the loin eye area (LEA) and intramuscular fat deposition (IMF), the animal needs to be slaughtered. Fortunately, the use of real-time ultrasound scans does away with slaughter but still, measurements for IMF and LEA are normally done at around the finishing period. Preliminary selection of replacement animals is usually done at weaning and later at about four (4) months. While the selection of individuals with good performance for these traits to become parents of the next generation can be done, deliberate mating of individuals heterozygous for gene markers of interest to produce homozygous offspring to become breeders in the next generation can be planned as well. This will increase the frequency of favorable genotypes in a population and hasten response to selection. The use of genetic markers to identify individuals carrying favorable alleles of genes associated with production traits becomes very attractive in this situation. Genetic markers have been identified that influence backfat thickness, intramuscular fat deposition/ marbling as well carcass lean yield and could be potential use in marker-assisted selection. Thus, with the availability of genetic screening protocol, the inclusion of these traits in the breeding objective could be explored in the future.

There are several genes that influence fat deposition and meat quality in pigs such as the HFABP gene which maps to chromosome 6 in pigs (SSC6). It plays an essential role in long-chain fatty acid uptake and metabolic homeostasis as reported in previous studies (Li *et al*., 2006). Gerbens *et al*., in a series of studies (1997; 1999; 2000), showed the relationship of *MspI*, *HaeIII* and *HinfI* polymorphism of the HFABP gene with intramuscular fat content or fat deposition.

Insulin-like growth factor 2 (IGF2) gene, on the other hand, is a major candidate gene affecting muscle mass or muscle growth in pigs (Liu and Mathur, 2003). Previous studies have shown that IGF2 has been recently found to increase lean yield by ~2.7% in the Pietrain breed of pig (Buys, 2003). The IGF2-in3-G3072A is a causative mutation for paternally expressed quantitative trait loci that has an effect on muscle growth and backfat thickness according to Vykoukalová *et al*. (2006). Furthermore, the same authors reported the linkage disequilibrium between IGF2-in3-G3072A and IGF2-in7-G162C (IGF2-NciI) mutations in four breeds and the associations between these polymorphisms to growth performance and meat quality. Their study showed a significant difference in backfat depth and lean meat content among the different genotypes.

The leptin receptor (LEPR) gene is a high-affinity acceptor and regulator of leptin, an important protein linking nutrition, peripheral metabolism, and reproduction that was discovered by Zhang *et al*. (1997) during cloning studies. In a study by Perez-Montarelo *et al*. (2012), the LEPR gene is considered a genetic marker associated with body composition, growth rate, or obesity in some swine breeds.

Breeding animals cannot be slaughtered for carcass traits to be evaluated. With the advent of new technology and research, the meat quality of native pigs can now be further

improved. Ultrasound technology has long been used to assess the meat quality traits in several livestock species including beef cattle and swine (Wolcott, 1996). Aside from measuring backfat depth and loin eye area, real-time ultrasound scans can be used to predict intramuscular fat (IMF) content in live pigs (Maignel *et al*., 2015). The genetic and phenotypic correlation between chemical intramuscular fat content and ultrasound intramuscular fat score were estimated to be 0.75 and 0.76, respectively (Jung *et al*., 2015). The use of real-time ultrasound scans to determine the extent of IMF or marbling on a finished pig prior to slaughter is very useful for marketing but not for the selection of future breeding boars/gilts as animals need to be evaluated before the finishing period. Fortunately, the use of other ultrasound scan measures such as backfat thickness, muscle depth (MD) and loin eye area (LEA) is a major determinant of fat deposition and carcass yield, or more specifically, carcass lean (Sather *et al.*, 1996). These measures could be used to validate marker effect. As replacement animals need to be selected at an early age, the use of DNA markers to screen for individual animals carrying favorable alleles of HFABP and other genes influencing carcass traits becomes very attractive as a selection tool especially if combined with performance records such as the body weight of individual animals. Therefore, this study was done to look into the potential use of DNA markers of genes influencing carcass traits for selection in native pigs. Specifically, to determine the genotype and allele frequencies of the DNA markers for the HFABP, LEPR and IGF2 genes in the selected native herds in Luzon and Visayas. The selection of animals with high genetic potential for marbling, thinner back fat, bigger loin eye area, or muscle depth can be executed at an earlier stage of an animal's life without sacrificing the animal. Improvement of meat quality traits is necessary to provide a competitive market that will eventually increase the level of income of our farming families.

## MATERIALS AND METHODS

A total of 543 native pigs from nine (9) different herds located in Luzon (8 herds) and Visayas (1 herd) were sampled for DNA extraction. For most herds, sampling was on all female animals and current breeding boars. Blood collected in EDTA tubes and hair samples were transported on ice from the collection site to PCC National Genepool and Headquarters and were stored at 4-10°C storage until processed in the laboratory.

Blood samples were processed for genomic DNA extraction using a commercial DNA extraction kit, Wizard Genomic DNA Purification Kit (Promega, USA), following the manufacturer's protocol. The extracted DNA was quantified using the Nanodrop Spectrophotometer, and the samples that met the required DNA concentration (about >50ng/uL) were used for genotyping. Further, extracted DNA was placed in a 1.5 microcentrifuge tube with a proper label and stored in the refrigerator at 4°C temperature.

Native pigs were sampled and genotyped for HFABP, IGF2-in7-G162C and LEPR genes. The target region for each gene was optimized using the pair of primers from published journals; HFABP (Pang *et al*,. 2006), IGF2-in7-G162C (Buys, 2003), LEPR (Perez-Montarelo *et al*., 2012). PCR products were loaded onto 2% agarose gels in 1x TAE buffer at 100V for 40 minutes. The gel was stained with GelRed™ nucleic acid gel stain and photographed under blue/UV light. Primers that produced a distinct single band are considered optimized.

To determine the genotypes through Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method, optimized PCR products are subjected to

restriction enzyme digestion for 1-4 hours. The digested PCR products were loaded onto 3-4% gels of low EEO and LM sieve agarose in 1x TAE buffer at 100V for 40-50 minutes of electrophoresis before gel documentation. Bands visible in agarose gel were scored directly. For the HFABP gene, there are three mutation sites and these were identified using the *HaeIII*, *HinfI* and *MspI* restriction enzymes. Thus, there are six genotypes after digestion, (*HaeII* - DD, Dd, dd, *HinfI* - HH, Hh, hh, *MspI* - AA, Aa, aa) for the three markers and 27 possible haplotypes. The 27 possible haplotype combinations can be grouped into the number of favorable alleles from six (6) to zero (0) categorized as HH6, HL5, HL4, HL3, HL2, HL1 and LL0 respectively. For the LEPR gene, the PCR products were digested using *MspI* restriction enzyme.

For the Insulin-like growth factor 2 (IGF2) gene, the mutation in intron 7 of the gene (IGF2-in7-G162C marker) was identified and scored through Mutagenically-separated Polymerase Chain Reaction (MS-PCR) method. This was done by using three primers for amplifying different DNA fragments with different genotypes hence, bands are scored directly in the gel after PCR. Genotype frequency is calculated by dividing the number of genotypes by the total number of samples. For example, if N=100, AA=25, AB=55, BB=20, the genotype frequencies are: AA=25/100, AB=55/100 BB=20/100.The allele frequency is calculated based on $p^2 + 2pq + q^2 = 1$ given as: A= (AA*2+1*AB)/200, B = (AB*1+BB*2)/200.

Real-time ultrasound scan measurements of selected traits associated with meat quality and carcass yield were done on 44 native pigs aged 3 - 8 months from a single herd. Live animal measurements of muscle depth, rib fat depth and intramuscular fat is normally taken between the 10th – 13th rib parallel to the spine, 5cm. off the midline by real-time scanning using an ultrasound machine (Exago® portable ultrasound Diagnostic Equipment, Eco Control Medical (ECM), Angoulême France) equipped with a 120mm 3.0 MHz linear-array transducer. To obtain the loin eye (longissimus dorsi muscle) area measurement (LEA), a cross-sectional scanning is necessary. The transducer with an offset pad was placed parallel between the 12th and 13th ribs. The boundary of the eye muscle was outlined/traced for area measurement (Figure 1A). The cross-sectional rib fat depth is taken approximately 5 cm. off the midline or the first 1/3 section of the loin (Figure 1B). Fixed machine settings were maintained for all animals. Measurement was reported at the nearest millimeter based on ICAR guidelines. Values were automatically calculated by the ultrasound machine. Three images were taken per animal and the mean values were calculated. Preliminary analysis of average ultrasound scan measurements of animals according to genotype for the IGF2-in7-G162C, LEPR and HFABP gene markers was done. Separation of means was done by Tukey HSD using JMP® 13.2.1 software (2016 SAS Institute Inc.) where the age of the animal at weighing/ measurement, sex of the animal and gene marker genotype as fixed effects are included in the model for standard least squares analysis under the Fit Model platform of JMP® 13.2.1 software (2016 SAS Institute Inc.). A separate analysis was done for each gene marker.

## RESULTS AND DISCUSSION

The agarose gel electrophoresis of MS-PCR product of IGF2-in7-G162C gene marker showing the GG genotype with an expected band size of 72bp and CG genotype with an expected band size of 72bp and 92bp in native pigs is shown in Figure 2. The CC genotype although not represented in Figure 2 is expected to have a single 92 bp band. This

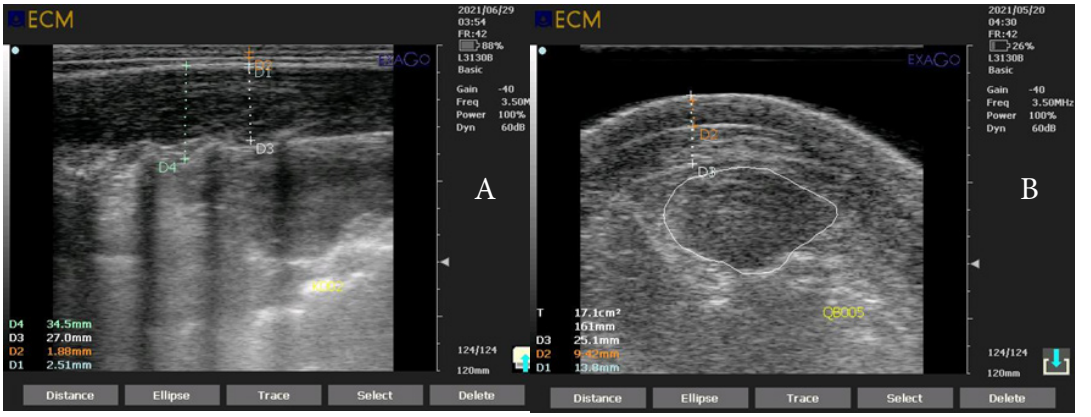


Figure 1. Illustration of scan measurement taken longitudinally from a native pig using the Exago® ultrasound machine (A) between the 10th – 13th rib 5cm from the midline for muscle depth (MD), (B) between the 12th-13th rib cross sectional for the loin eye area (Longissimus dorsi muscle) and rib fat depth.

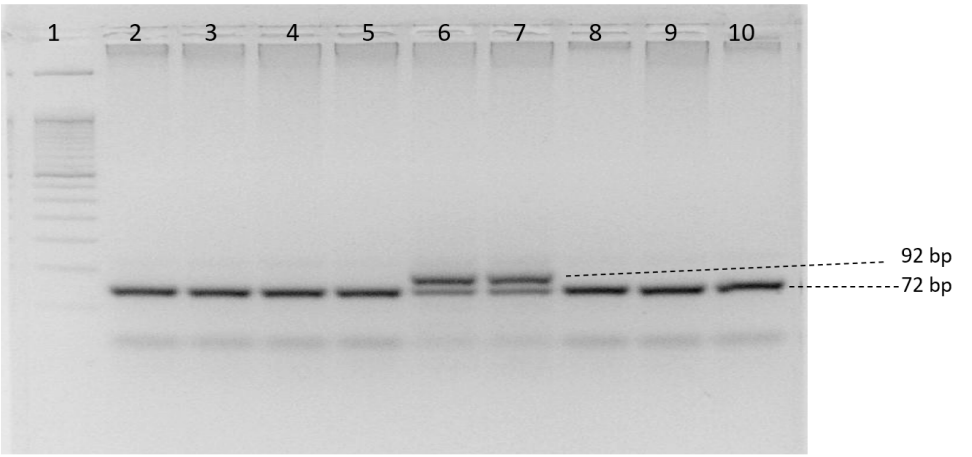


Figure 2. Agarose gel electrophoresis of MS-PCR product of the IGF2-in7-G162C gene marker in native pigs. Genotype GG – Lane 2, 3, 4, 5, 8, 9, 10. Genotype CG – Lane 6, 7. Lane 1 - Ladder.

is similar to the expected band size in a commercial pig.

The Insulin-like growth factor 2 (IGF2) gene has been reported and shown to affect carcass yield and leanness. Vykoukalová *et al*. (2006) reported the associations between the polymorphisms in the gene and growth and meat performance of the Large White breed. The study showed a significant difference in backfat depth and lean meat content with the Large White breed. Thus, pigs carrying the favorable genotype CC of the IGF2-in7-G162C marker are expected to have on average, thinner backfat. Table 1 shows that the overall frequency of the favorable genotype CC is only 3.3% overall. This is not surprising given that backfat depth is not included in the breeding objective of native pigs hence, no selection pressure has been applied to reduce the thickness. The pattern is the same for all herds sampled in the different regions. Region VIII had the highest favorable genotype and allele frequencies at 18.4% and 32.7%, respectively. While the CC genotype might be low overall, there is an

Table 1. Genotype and allele frequencies of Insulin-like Growth Factor 2-intron 7 (IGF2-in7-G162C) marker of Philippine native pigs from various herds in different regions.

| Region | Genotype | | | | | | Grand Total | Allele | |
|---|---|---|---|---|---|---|---|---|---|
| | CC | | CG | | GG | | | C* | G |
| | n | % | n | % | n | % | | % | % |
| CAR | 0 | 0.0 | 9 | 22.5 | 31 | 77.5 | 40 | 11.3 | 88.8 |
| Reg II | 0 | 0.0 | 2 | 1.5 | 128 | 98.5 | 130 | 0.8 | 99.2 |
| Reg III | 3 | 2.8 | 36 | 33.0 | 70 | 64.2 | 109 | 19.3 | 80.7 |
| Reg IV | 5 | 2.6 | 41 | 21.6 | 144 | 75.8 | 190 | 13.4 | 86.6 |
| Reg VIII | 9 | 18.4 | 14 | 28.6 | 26 | 53.1 | 49 | 32.7 | 67.3 |
| Total | 17 | 3.3 | 102 | 19.7 | 399 | 77.0 | 518 | 13.1 | 86.9 |

*Favorable genotype/Allele

opportunity to increase the frequency in the various herds by mating heterozygous individuals.

The agarose gel electrophoresis of PCR-RFLP product for the LEPR gene shows the different bands for AA, AB and BB genotypes in native (A) and commercial (B) pigs having similar banding patterns (Figure 3) indicating the methodology for screening the gene marker applied on commercial pigs works for the native pig as well. The gel sample for the commercial pig was from the previous DOST-PCAARRD project on commercial pigs entitled: Private-Public Partnership in the Application of Animal Genomics to Increase Productivity and Improve Efficiency of the Philippine Swine Industry utilizing the same.

Previous studies have established that LEPR gene polymorphism provides a useful tool to improve carcass/meat quality in pigs. This was supported by a study by Perez-Montarelo *et al*. (2012), wherein they reported that the LEPR gene is considered a genetic marker associated with body composition, growth rate, or obesity in some swine breeds. This can

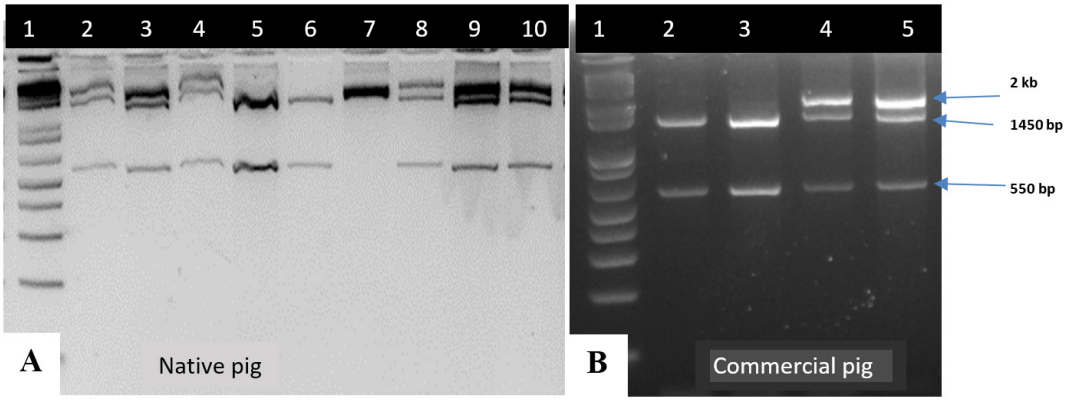


Figure 3. Agarose gel electrophoresis of PCR-RFLP product for the LEPR gene of (A) native pig and (B) commercial pig. For (A): Lane 1– 1kb ladder, AB genotype – Lane 2,3,4, 8, 9,10. BB genotype – Lane 5,6. AA Genotype – Lane 7. For (B): BB genotype Lane 2,3. AB genotype – Lane 4,5.

be seen indirectly in backfat thickness measurements with an animal carrying the favorable allele B to have thinner or smaller measurements. In Table 2, the genotype frequencies were 38%, 45.8% and 16.2% for the AA, AB and BB genotypes, respectively. However, the trend is the opposite for the herds in Region IV wherein the BB genotype has a higher frequency than the AA genotype. This could be a breed effect and should be investigated further.

The IGF2 and LEPR genes are involved in fat deposition and the current study suggests or even validates the observation that the native pig generally has a low frequency of the genotype associated with thinner backfat and this could be because no selection pressure is being applied to the population to reduce the backfat thickness or rib fat depth. Producing a native pig that grows fast and thinner backfat can be done through selection in the future. There is an opportunity to increase the frequency of the favorable genotype through marker-assisted selection in combination with real-time ultrasound scan measures. The fact that there are animals carrying favorable genotypes and heterozygotes suggests the frequencies can increase with a carefully crafted mating plan.

Figures 4, 5 and 6 show the gel electrophoresis of the *MspI, HaeIII* and *HinfI* PCR-RFLP marker of the HFABP gene. The results shown for commercial pigs were from the previous research project on commercial pigs. For *MspI* PCR-RFLP, Figure 3 only presents the AA genotype. For the Aa genotype, expected bands are 816bp, 750bp and 66bp whereas for the aa genotype only the 816bp band is expected.

The agarose gel electrophoresis of *HaeIII* PCR-RFLP in Figure 5 represents the three possible genotypes for the said marker although the favorable dd genotype is shown only in the native pig sample. Both the commercial and native pig samples have very similar banding patterns and sizes. Figure 6 shows the agarose gel electrophoresis of *HinfI* PCR-RFLP for the native pig respectively.

Table 3, 4 and 5 shows the genotype and allele frequencies for the three PCR-RFLP markers of the HFABP gene associated with fat deposition and intramuscular fat in commercial pigs. On average, the favorable genotype aa of the *MspI* RFLP marker makes up only 0.6% of the animal sampled. The *HaeIII* and *HinfI* RFLP markers on the other hand had the favorable genotype having higher frequencies at 69.2% and 59.5% respectively.

The three PR-RFLP markers of the HFABP gene can be grouped or classified according to the number of favorable alleles which can be zero (0) to six (6) and Table 6 shows

Table 2. Genotype and allele frequencies of Leptin Receptor (LEPR) gene marker of Philippine native pigs from various herds in different regions.

| Region | Genotype | | | | | | Grand Total | Allele | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | | AB | | BB | | | A | B |
| | n | % | n | % | n | % | | % | % |
| CAR | 17 | 50.0 | 13 | 38.2 | 4 | 11.8 | 34 | 69.1 | 30.9 |
| Reg II | 84 | 65.1 | 43 | 33.3 | 2 | 1.6 | 129 | 81.8 | 18.2 |
| Reg III | 43 | 39.8 | 55 | 50.9 | 10 | 9.3 | 108 | 65.3 | 34.7 |
| Reg IV | 31 | 14.0 | 121 | 54.8 | 69 | 31.2 | 221 | 41.4 | 58.6 |
| Reg VIII | 29 | 64.4 | 14 | 31.1 | 2 | 4.4 | 45 | 80.0 | 20.0 |
| Total | 204 | 38.0 | 246 | 45.8 | 87 | 16.2 | 537 | 60.9 | 39.1 |

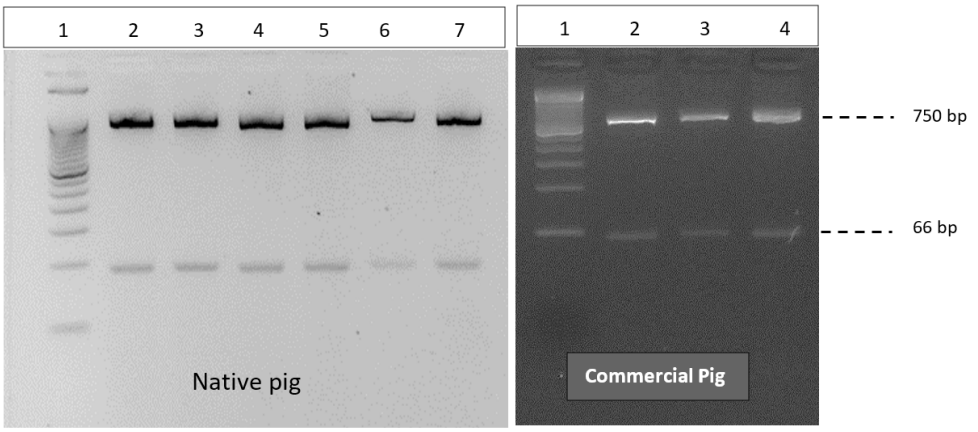


Figure 4. Agarose gel electrophoresis of *MspI* PCR-RFLP for the heart fatty acid binding protein (HFABP) gene of native and commercial pig. For native pig: Lane 1 – 100bp Ladder, Lane 2 to 7 – AA genotype. For commercial pig: Lane 1 – 100bp Ladder, Lane 2 to 4 – AA genotype.

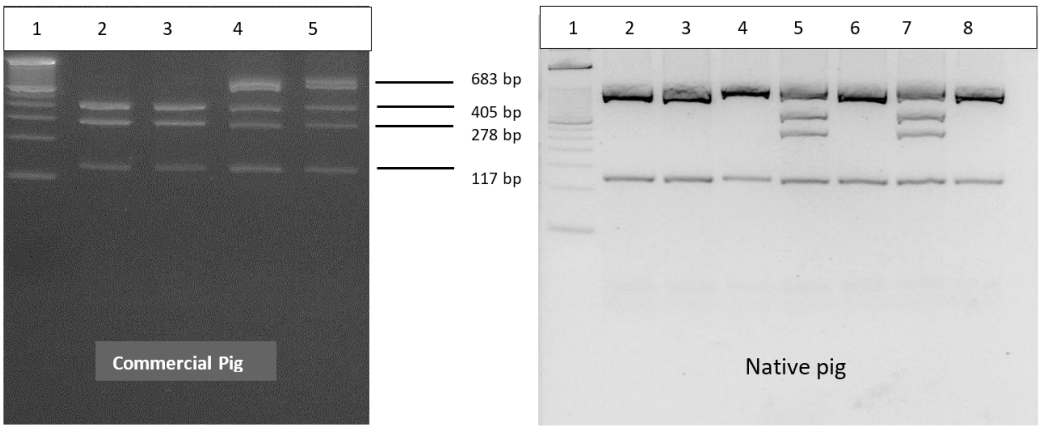


Figure 5. Agarose gel electrophoresis of *HaeIII* PCR-RFLP for the heart fatty acid binding protein (HFABP) gene of native and commercial pig. For commercial pig: Lane 1 – 100bp Ladder, Lane 2 to 3 – DD genotype, Lane 4 to 5 – Dd genotype. For native pig: Lane 1 – 100bp ladder, Lane 2, 3, 4, 6, 8 – dd genotype. Lane 5, 7 – Dd genotype.

the proportion of animals sampled according to the number of favorable alleles. All animals sampled were classified only to HL2, HL3 and HL4. The highest number fall under HL3 at 42% followed by HL4 at 39%. Region VIII had the highest proportion of HL4 animals genotyped at 62.7% and the lowest was Region II at 29.4.

The proportion of native pigs sampled according to the number of favorable alleles of the HFABP gene was compared to the results obtained from a previous DOST-PCAARRD project on commercial pigs entitled: Private-Public Partnership in the Application of Anir al Genomics to Increase Productivity and Improve Efficiency of the Philippine Swine Industry and is shown in Table 7. For the commercial breed, the result was obtained predominantly from Pietrain, Duroc and Pietrain-Duroc cross. Some Berkshire pigs were also genotyped during that time. It is not surprising to see in the commercial breed that the highest

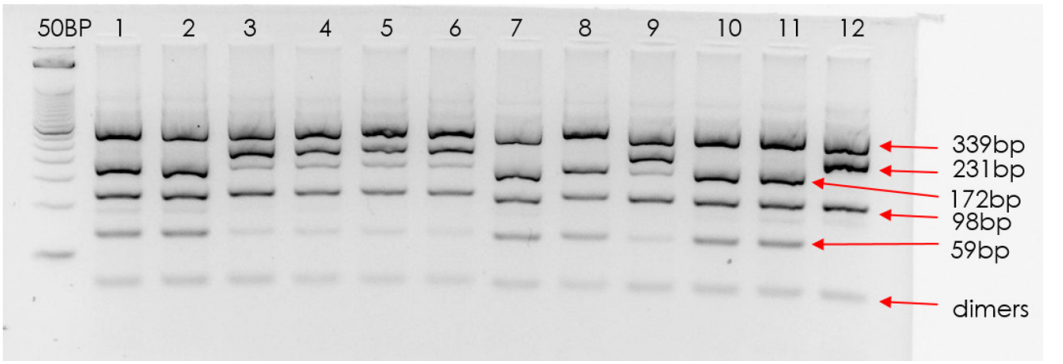


Figure 6. Agarose gel electrophoresis of *HinfI* PCR-RFLP for the heart fatty acid binding protein (HFABP) gene of native pig. Lane 1,2,7,8,10,11 – HH genotype. Lane 3,4,5,6, 9 – Hh genotype. Lane 12 – hh genotype. 50BP - Ladder.

Table 3. Genotype and allele frequencies of Heart-Fatty Acid Binding Protein (HFABP) *MspI* Restriction Fragment Length Polymorphism (RFLP) marker of Philippine native pigs from various herds in different regions.

| Region | Genotype | | | | | | Grand Total | Allele | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | | Aa | | aa* | | | A | a* |
| | n | % | n | % | n | % | | % | % |
| CAR | 37 | 94.9 | 1 | 2.6 | 1 | 2.6 | 39 | 96.2 | 3.8 |
| Reg II | 200 | 97.1 | 5 | 2.4 | 1 | 0.5 | 206 | 98.3 | 1.7 |
| Reg III | 104 | 100.0 | 0 | 0.0 | 0 | 0.0 | 104 | 100.0 | 0.0 |
| Reg IV | 198 | 89.2 | 22 | 9.9 | 2 | 0.9 | 222 | 94.1 | 5.9 |
| Reg VIII | 51 | 100.0 | 0 | 0.0 | 0 | 0.0 | 51 | 100.0 | 0.0 |
| Total | 590 | 94.9 | 28 | 4.5 | 4 | 0.6 | 622 | 97.1 | 2.9 |

*Favorable genotype/allele

Table 4. Genotype and allele frequencies of Heart-Fatty Acid Binding Protein (HFABP) *HinfI* Restriction Fragment Length Polymorphism (RFLP) marker of Philippine native pigs from various herds.

| Region | Genotype | | | | | | Grand Total | Allele | |
|---|---|---|---|---|---|---|---|---|---|
| | HH* | | Hh | | hh | | | H* | h |
| | n | % | n | % | n | % | | % | % |
| CAR | 28 | 71.8 | 1 1 | 28.2 | 0 | 0.0 | 39 | 85.9 | 14.1 |
| Reg II | 100 | 48.1 | 91 | 43.8 | 17 | 8.2 | 208 | 70.0 | 30.0 |
| Reg III | 60 | 57.1 | 35 | 33.3 | 10 | 9.5 | 105 | 73.8 | 26.2 |
| Reg IV | 144 | 67.9 | 53 | 25.0 | 15 | 7.1 | 212 | 80.4 | 19.6 |
| Reg VIII | 34 | 66.7 | 17 | 33.3 | 0 | 0.0 | 51 | 83.3 | 16.7 |
| Total | 366 | 59.5 | 207 | 33.7 | 42 | 6.8 | 615 | 76.3 | 23.7 |

*Favorable genotype/allele

Table 5. Genotype and allele frequencies of Heart-Fatty Acid Binding Protein (HFABP) *HaeIII* Restriction Fragment Length Polymorphism (RFLP) marker of Philippine native pigs from various herds.

| Region | Genotype | | | | | | Grand Total | Allele | |
|---|---|---|---|---|---|---|---|---|---|
| | DD | | Dd | | dd* | | | D | d* |
| | n | % | n | % | n | % | | % | % |
| CAR | 2 | 5.1 | 4 | 10.3 | 33 | 84.6 | 39 | 10.3 | 89.7 |
| Reg II | 7 | 3.4 | 54 | 26.5 | 143 | 70.1 | 204 | 16.7 | 83.3 |
| Reg III | 6 | 5.9 | 21 | 20.8 | 74 | 73.3 | 101 | 16.3 | 83.7 |
| Reg IV | 20 | 9.0 | 74 | 33.5 | 127 | 57.5 | 221 | 25.8 | 74.2 |
| Reg VIII | 0 | 0.0 | 2 | 3.9 | 49 | 96.1 | 51 | 2.0 | 98.0 |
| Total | 35 | 5.7 | 155 | 25.2 | 426 | 69.2 | 616 | 18.3 | 81.7 |

*Favorable genotype/allele

Table 6. Proportion of native pigs sampled from the various collaborating herds in various Regions based on the number of favorable alleles for the Heart-Fatty Acid Binding Protein (HFABP) *HaeIII*, *HinfI* and *MspI* Restriction Fragment Length Polymorphism (RFLP) marker.

| Region | Genotype | | | | | | Grand Total |
|---|---|---|---|---|---|---|---|
| | 2 | | 3 | | 4 | | |
| | n | % | n | % | n | % | |
| CAR | 2 | 5.1 | 13 | 33.3 | 24 | 61.5 | 39 |
| Reg II | 44 | 21.6 | 100 | 49.0 | 60 | 29.4 | 204 |
| Reg III | 21 | 20.2 | 47 | 45.2 | 36 | 34.6 | 104 |
| Reg IV | 50 | 22.6 | 83 | 37.6 | 88 | 39.8 | 221 |
| Reg VIII | 0 | 0.0 | 19 | 37.3 | 32 | 62.7 | 51 |
| Total | 117 | 18.9 | 262 | 42.3 | 240 | 38.8 | 619 |

Table 7. Comparison of the proportion of animals in each haplotype group according to the number of favorable alleles for the for the Heart-Fatty Acid Binding Protein (HFABP) *HaeIII*, *HinfI* and *MspI* Restriction Fragment Length Polymorphism (RFLP) markers for the different breed type of pigs.

| Breed Type | Low | | | Med-High | | | High |
|---|---|---|---|---|---|---|---|
| | LL0 | LL1 | LL2 | HL3 | HL4 | HL5 | HH6 |
| Commercial* | 1.1 | 1.1 | 62.6 | 21.2 | 14 | 0.0 | 0.0 |
| Kurobuta* | 0.0 | 0.0 | 4.5 | 27.3 | 50 | 18.2 | 0.0 |
| Native | 0.0 | 0.0 | 18.9 | 42.3 | 39 | 0.0 | 0.0 |

*Data from previous research project entitled: Private-Public Partnership in the Application of Animal Genomics to Increase Productivity and Improve Efficiency of the Philippine Swine Industry.

proportion were those with 2 favorable alleles only (HL2). The native pig can be compared closely with the results on the Berkshire breed, known to have very high marbling potential and thick backfat. Some animals had the HL5 haplotype group. This was not seen in the native and other commercial breeds. Nevertheless, it can be seen in Table 7 that the native pig very closely tracks with the Berkshire even without selection. Similarly, the Kadon pig native to Thailand is well appreciated by the local people due to its better texture and flavor (Vasupen *et al*., 2008). The study by the same authors revealed Kadon loin meat contained an average of 8.7% fat whereas Liu and Mathur (2003) report Western Canadian pigs' average intramuscular fat (IMF) to 3%. IMF is positively correlated with juiciness, tenderness and flavor (Liu and Mathur, 2003). Thus, the juiciness, tenderness and flavorful taste of the native pig lechon may be due to very good intramuscular fat deposition. However, the genotyping results suggest good marbling potential only. Marbling is a combination of genetics and feeding management in order to bring about genetic potential. It might be good to validate this genetic potential by looking into a suitable feeding regimen to maximize the expression of marbling potential in future studies.

The result of HFABP genotyping suggests very good potential for improving marbling in the native pig population but the *MspI* PCR-RFLP marker had very low frequency for the favorable genotype aa. A careful mating plan can be drawn up to increase the a allele in the population and the frequencies of the other favorable alleles as well. Figure 7 illustrates a way to increase the number of favorable alleles to five (HL5) by pairing a sow with favorable allele H to a boar that only has the h allele.

Preliminary results of ultrasound scan measurement of rib fat depth, muscle depth and loin eye area is done on 44 native pigs from a single herd in Region IV are shown in Table 8. Least squares means and standard error of the scan measurements per gene marker genotype are given. For the IGF2 gene that was reported to be associated with lean carcass, higher carcass yield and thinner backfat, the favorable genotype CC had the highest LEA and measurement followed by CG and the least, the GG genotype. While there was no significant difference between genotypes, the pattern was already evident. There were only 2 animals with CC genotype hence, the standard error was large. With a higher number of samples, it is likely, a significant difference will be observed. The effect was also consistent looking at the rib fat depth or backfat as the favorable genotype CC had the smallest value. The initial result appears to be consistent with the reports of studies done in commercial Large White pigs (Vykoukalová *et al*., 2006).

As for the LEPR gene, there was a significant difference in backfat depth at the 10th – 13th rib between genotypes. Two measurements were taken, the first, Backfat A, takes only the first layer while Backfat B measures the first and second layers of fat at the same site. The result suggests the favorable genotype to be AA genotype. Other studies reported

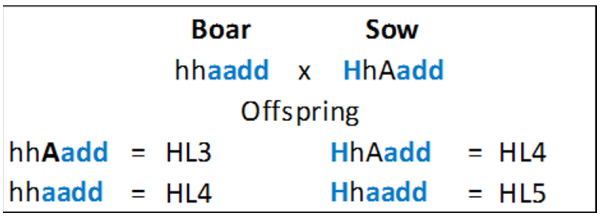


Figure 7. Possible genotype combinations of offspring from mating of parents with different haplotype for heart fatty acid binding protein (HFABP) restriction fragment length polymorphism (RFLP) markers.

Table 8. Least squares mean estimate of the different ultrasound scan measures taken at two different sites according to genotype of the IGF2, LEPR and HFABP gene markers in native pigs.

| Gene | | n | 12th - 13th Rib | | | | 10th - 13th Rib | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ribfat Depth (RF), mm | | Loin Eye Area (LEA), cm2 | | Muscle Depth (MD), mm | | Backfat A (RF), mm | | Backfat B (RF), mm | |
| | | | LS-Mean | SE | LS-Mean | SE | LS-Mean | SE | LS-Mean | SE | LS-Mean | SE |
| **IGF2** | | | | | | | | | | | | |
| Genotype | CC | 2 | 3.7 | 1.4 | 17.3 | 3.8 | 38.8 | 6.7 | 5.98 | 1.69 | 10.2 | 3.2 |
| | CG | 11 | 6.0 | 0.7 | 16.6 | 2.1 | 38.1 | 3.7 | 8.43 | 0.93 | 13.9 | 1.8 |
| | GG | 17 | 6.2 | 0.6 | 12.8 | 1.7 | 30.1 | 3.0 | 8.22 | 0.75 | 13.0 | 1.4 |
| **LEPR** | | | | | | | | | | | | |
| Genotype | AA | 6 | 4.2 | 0.9 | 16.5 | 2.6 | 34.4 | 4.5 | 5.4$^{a}$ | 1.1 | 7.9$^{a}$ | 2.2 |
| | AB | 18 | 4.9 | 0.9 | 14.8 | 2.5 | 32.3 | 4.4 | 7.2$^{ab}$ | 1.1 | 12.6$^{ab}$ | 2.1 |
| | BB | 5 | 6.9 | 1.0 | 15.5 | 2.7 | 40.4 | 4.8 | 10.0$^{b}$ | 1.2 | 16.5$^{b}$ | 2.3 |
| **HFABP *HinfI* RFLP** | | | | | | | | | | | | |
| Genotype | hh | 4 | 5.9 | 1.2 | 12.7 | 3.4 | 28.0 | 6.0 | 8.8 | 1.5 | 16.6 | 2.9 |
| | Hh | 6 | 6.1 | 0.8 | 15.8 | 2.3 | 39.1 | 4.1 | 8.0 | 1.0 | 10.3 | 2.0 |
| | HH | 19 | 3.9 | 0.8 | 18.4 | 2.1 | 39.9 | 3.7 | 5.9 | 0.9 | 10.1 | 1.8 |
| **HFABP *MspI* RFLP** | | | | | | | | | | | | |
| Genotype | aa | | - | | - | | - | | - | | - | |
| | Aa | 3 | 3.3$^{a}$ | 1.4 | 11.3 | 3.9 | 30.4 | 6.9 | 6.3 | 1.7 | 10.5 | 3.3 |
| | AA | 25 | 7.3$^{b}$ | 0.8 | 19.9 | 2.4 | 40.9 | 4.1 | 8.8 | 1.0 | 14.2 | 2.0 |
| **HFABP *HaeIII* RFLP** | | | | | | | | | | | | |
| Genotype | dd | 23 | 4.7 | 1.0 | 11.0$^{a}$ | 2.8 | 31.9 | 4.9 | 7.4 | 1.2 | 10.2 | 2.4 |
| | Dd | 5 | 5.9 | 0.9 | 20.2$^{b}$ | 2.6 | 39.5 | 4.5 | 7.7 | 1.1 | 14.5 | 2.2 |
| | DD | | - | | - | | - | | - | | - | |

For each gene marker, genotype means in rows with different superscripts within a column are significantly different, $P< 0.05$.

IGF2 – Insulin-like growth factor 2 gene, LEPR –Leptin receptor gene, HFABP –heart fatty acid binding protein gene, Backfat A - measurement of the first layer of fat next to the loin eye area, Backfat B measures the first and second layer of fat at the same site.

that the pigs with BB genotype have significantly thinner backfat but these were breed-specific (Chen *et al*., 2004) or the restriction enzyme used were different (Terman, 2005). The herd from where this animal came also had higher allele B frequency contrary to other herds (Table 2). The preliminary result from this study suggests a breed-specific effect for the LEPR gene. Further studies should include more animals from the same herd and additional herds of a different genetic group to be scanned to validate results.

The HFABP *HaeIII* PCR-RFLP also had significant differences between genotypes with the favorable genotype dd having smaller mean LEA and muscle depth measures. Higher fat deposition normally would also equate to less lean muscle yield and the result is consistent with this. On the other hand, all backfat depth measures taken were lesser for animals with dd genotype even though there were no significant differences in the mean. The same pattern could be observed with the genotype means for the HFABP *MspI* PCR-RFLP marker where the Aa genotype had a significantly smaller mean rib fat depth taken between the 12th and 13th rib than the AA genotype. This preliminary result supports the finding of Newcom *et al*. (2005) where the IMF content can be improved without substantial effect on backfat thickness. Nevertheless, the results are preliminary and further studies should be done especially the combined effects and interaction among genes when the data set has grown.

Future studies should look into the effect of selecting for thinner backfat on other traits such as litter size, growth rate (ADG) and carcass lean yield. Genetic correlations between traits should be estimated. Fertility measures, such as the number of piglets born alive and age at first service in pigs, have been reported to be influenced by backfat thickness (Roongsitthichai and Tummaruk, 2014) hence, this is consistent with the breeding objective of the various native pig herds. Knowing which genes influences this trait is useful in complementing conventional selection strategy. The other trait that is important for the native pigs is IMF content which is positively correlated with backfat thickness (Liu and Mathur, 2003). However, too thick backfat would also decrease lean yield which could affect the quality of lechon end product (all fat and not much meat). With the use of HFABP gene markers as a complementary tool for selection, it is possible to increase IMF content in pigs without a substantial effect on backfat thickness. Thus, understanding further the effect of the three genes on fat deposition in native pigs should be done in the future as a guide to balancing response to selection when developing a separate multi-trait index for boars and sows.

The genotyping result for the LEPR and IGF2 gene markers suggests these markers could be used in native pigs for improving backfat thickness and increased lean meat yield. This was validated by ultrasound scan measures of LEA/MD and backfat thickness that showed the expected pattern and significant difference in mean values between genotypes for LEA/MD and backfat, respectively. The frequency of favorable genotypes was generally low thus, marker-assisted selection could potentially be effective. On the other hand, the frequency of favorable alleles for the HFABP gene was also generally high suggesting good marbling potential. A feeding/finishing trial of growing native pigs should validate this observation. While results are preliminary, there was also enough variation in ultrasound scan measurements of native pigs such that careful selection at an early age with the use of real-time ultrasound measures combined with marker-assisted selection can be explored. On average, animals with higher LEA/MD also had thinner backfat thickness and possibly less IMF thus, selecting for the latter traits might have an unfavorable effect on fertility and should be taken into consideration in the future.

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