

# Genotypic Effects of Insulin-like Growth Factor Binding Protein 2 on Fat-free Lean Traits in Crossbred between Duroc and Pietrain Pigs

## อิทธิพลของรูปแบบยีนอินซูลินไลค์โกรทแฟกเตอร์ไบน์ดิงโปรตีน 2 ต่อลักษณะเนื้อแดงปราศจากไขมันในสุกรลูกผสมระหว่างดูร์โรคและเพียเทรน

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**บทคัดย่อ:** อินซูลินไลค์โกรทแฟกเตอร์ไบน์ดิงโปรตีน 2 (ไออีเอฟบีพี 2) นับว่าเป็นยีนที่มีอิทธิพลต่อลักษณะสำคัญทางเศรษฐกิจในสุกร สำหรับการศึกษานี้ข้อมูลลักษณะเนื้อแดงปราศจากไขมันที่อายุต่าง ๆ จำนวน 11 ลักษณะถูกเก็บรวบรวมจากสุกรที่มีชีวิตจำนวน 408 ตัว โดยเป็นสุกรลูกผสมดูร์โรคเจซี่ x เพียเทรนในรุ่น F<sub>2</sub> ลักษณะที่ศึกษาประกอบด้วย ปริมาณเนื้อแดงปราศจากไขมัน เปอร์เซ็นต์เนื้อแดงปราศจากไขมัน ที่อายุ 10, 13, 16, 19 และ 22 สัปดาห์ และอัตราการเพิ่มขึ้นของเนื้อแดงปราศจากไขมันจากอายุ 10 ถึง 22 สัปดาห์ ไออีเอฟบีพี 2 สามารถจำแนกได้เป็น 3 จีโนไทป์ ได้แก่ AA, AB และ BB โดยเทคนิค PCR-RFLP ใช้เอ็นไซม์ตัดจำเพาะ *MspI* แบบหุ่นทางสถิติที่ใช้เพื่อศึกษาความสัมพันธ์ระหว่างไออีเอฟบีพี 2 จีโนไทป์และลักษณะเนื้อแดงปราศจากไขมัน ประกอบด้วยอิทธิพลคงที่ของไออีเอฟบีพี 2 จีโนไทป์ และเพศ และอิทธิพลร่วมของกลุ่มการคลอด ครอก คอกขุนภายในกลุ่มการคลอด และค่าคาดเคลื่อนที่เหลือ ผลการศึกษาพบว่าไออีเอฟบีพี 2 จีโนไทป์มีความสัมพันธ์ ( $P < 0.05$ ) กับลักษณะดังกล่าว โดยสุกรที่มีจีโนไทป์ BB มีลักษณะเนื้อแดงปราศจากไขมันที่อายุ 10, 13 และ 16 สัปดาห์ มากกว่าสุกรที่มีจีโนไทป์ AB ไม่แตกต่างอย่างมีนัยสำคัญทางสถิติ ( $P > 0.05$ ) ที่อายุ 19 และ 22 สัปดาห์ นอกจากนี้ยังพบว่าสุกรมีจีโนไทป์ BB มีเปอร์เซ็นต์เนื้อแดงปราศจากไขมันที่อายุ 13, 16, 19 และ 22 สัปดาห์ ตลอดจนอัตราการเพิ่มขึ้นของเนื้อแดงปราศจากไขมันจาก 10 ถึง 22 สัปดาห์ มากกว่า ( $P < 0.05$ ) ผลจากการศึกษานี้แสดงให้เห็นความสัมพันธ์ของไออีเอฟบีพี 2 จีโนไทป์กับลักษณะสมรรถภาพการผลิตในสุกรลูกผสมรุ่น F<sub>2</sub> และควรมีการศึกษาเพิ่มเติมในสุกรพันธุ์อื่นและสุกรฝูงอื่นเพื่อการนำไปใช้ประโยชน์

**คำสำคัญ:** ไออีเอฟบีพี 2 เนื้อแดงปราศจากไขมัน สุกร

**Abstract:** Insulin-like growth factor binding protein 2 (IGFBP 2) was selected as a candidate gene for economically important traits in pigs. For this study, 11 phenotypic data of fat-free lean at several ages were collected from 408 live- $F_2$  pigs obtained from Duroc × Pietrain MSU resource population. The study traits included fat-free lean, fat-free lean percent at ages 10, 13, 16, 19 and 22 weeks as well as fat-free lean gain from 10 to 22 weeks. Three IGFBP 2 genotypes (AA, AB and BB) were determined using *MspI* PCR-RFLP. The statistical model, which included the fixed effects of IGFBP 2 genotypes and sex, as well as the random effects of the farrowing group, litter and finishing pen nested within the farrowing group and residual error, was used to determine potential associations of the IGFBP 2 genotypes with fat-free lean traits. The results indicated a significant effect ( $P < 0.05$ ) of the IGFBP 2 genotypes on these traits. Pigs with the BB genotype had more favorable ( $P < 0.05$ ) fat-free lean at 10, 13 and 16 weeks of age than pigs with the AB genotype, but there was non-significant effect ( $P > 0.05$ ) of the IGFBP 2 genotypes on fat-free lean at 19 and 22 weeks of age. Furthermore, pigs with the BB genotype had most favorable ( $P < 0.05$ ) fat-free lean percent at 13, 16, 19 and 22 weeks of age as well as fat-free lean gain from 10 to 22 weeks. The results of this study indicated associations between the IGFBP 2 genotypes and fat-free lean traits in  $F_2$  crossbred pigs, and the validation of these results in additional breeds and pig populations is warranted.

**Keywords:** IGFBP 2, fat-free lean, pig

## Introduction

The characteristics of the pig positively affect profitability include a high growth rate, a low feed conversion ratio, and leanness of the carcass. Therefore, pig breeding programs have traditionally focused on growth rate and leanness as major objectives in selection (Chen *et al.*, 2002; Hammond and Leitch, 1998; Schinckel and de Lange, 1996). Today's hog market is based on weight and percent lean, thus pig producers who select or purchase pigs that grow faster and have more efficient lean deposition will have higher-value return. However, simultaneous improvement in leanness and growth rate is difficult to achieve because the genetic correlation between the two traits is generally unfavorable (Chen *et al.*, 2002; McPhee *et al.*, 1988). Marker assisted selection (MAS) might be a useful tool to improve selection efficiency for economically important traits in the pigs by

measuring early in their life to accurately predict those traits (Dekkers and Hospital, 2002).

Therefore, MAS would aid in selection by allowing producers to reduce production cost and time. Candidate gene identification is one of the important procedures in the MAS program (Hayes and Goddard, 2003). Therefore, IGFBP 2 has been selected for further investigation into its potential. Insulin-like growth factor binding protein 2 (IGFBP 2) is a growth-associated gene that belongs to the IGF family. The IGFBP consists of six homologous proteins (IGFBP 1 to IGFBP 6) with high binding affinity for IGF-I and IGF-II (Baxter, 2000; Rechler, 1993).

The function of IGFBPs included binding to IGF-I and IGF-II as carriers, protecting the IGFs from proteolysis to prolong their half-life, delivering them to their target tissue, and acting as modulators of IGFs availability and activity (Clemmons, 1998; Cohick, 1998; Hoeflich *et al.*, 1999). The porcine

IGFBP 2 gene contains 27 kb located on Chromosome 15 and contains four exons (Mote and Rothschild, 2006; Song *et al.*, 1996; Wang *et al.*, 2008). Previous studies have considered IGFBP 2 as a candidate gene for growth, carcass and body composition traits in farm animals (Li *et al.*, 2006; Pagan *et al.*, 2001). However, few studies have reported the IGFBP 2 gene in pigs (Mote and Rothschild, 2006; Wang *et al.*, 2007). Therefore, this study is aimed to determine the association of IGFBP 2 gene polymorphisms with production performance in a Duroc × Pietrain F<sub>2</sub> pig population.

## Materials and Methods

The Animal protocols were approved by the Michigan State University Institutional Animal Care and Use Committee (AUF#09/03-114-00). The experimental animals were treated and kept in accordance with accepted standards for the humane treatment of animals.

### 1. Experimental population and management

The population in this study was previously described by Prasongsook *et al.* (2015). In brief, the pig population was closely studied at Michigan State University. The founder population consisted of Duroc sires and Pietrain dams, known as the F<sub>0</sub> generation. The first progeny (F<sub>1</sub>) were subsequently intercrossed to produce the second progeny (F<sub>2</sub>). F<sub>2</sub> pigs were designated for the experiment in finishing pens. The feed was formulated to meet the nutritional requirements of the National Research Council (NRC, 1988) using commercial corn-soybean diets provided *ad libitum*. Comprehensive information on the development and husbandry management of this population was previously reported by Edwards *et al.* (2008).

### 2. Phenotypic data collections

Eleven fat-free lean phenotypic traits from 408 live-F<sub>2</sub> pigs were analyzed for this study. Body weight was measured at 10, 13, 16, 19 and 22 weeks of age, and, at the same time, the 10<sup>th</sup> rib backfat thickness and 10<sup>th</sup> rib *Longissimus* muscle area were estimated by B-mode ultrasound (Pie Medical 200SLC, Classic Medical Supply, Inc., Tequesta, FL, USA.). Fat-free lean and fat-free lean percent at 10, 13, 16, 19 and 22 weeks of age, and fat-free lean gain from 10 to 22 weeks were calculated from the equation according to the National Pork Producers Council guidelines (National Pork Producer Council [NPPC], 2000). The equations are represented as follows:

Fat-free lean (lbs)

$$= [0.833 \times \text{sex of pig (barrow = 1, gilt = 2)} \\ - [16.498 \times 10^{\text{th}} \text{ rib backfat thickness, inches}] \\ + [5.425 \times 10^{\text{th}} \text{ rib } \textit{Longissimus} \text{ muscle area, inches}^2] \\ + [0.291 \times \text{live weight, lbs}] \\ - [0.534] \quad \text{_____ (1)}$$

Then convert pound of fat-free lean to kilogram of fat-free lean by

Fat-free lean (kg)

$$= 0.454 \times \text{fat-free lean (lbs)} \quad \text{_____ (2)}$$

Fat-free lean percent (%)

$$= \frac{[\text{fat-free lean (kg)} / \text{live weight (kg)}] \times 100}{0.74} \quad \text{_____ (3)}$$

Fat-free lean gain from 10 to 22 wk (g/day)

$$= \frac{[22 \text{ wk fat-free lean (g)} - 10 \text{ wk fat-free lean (g)}]}{\text{days from 10 to 22 wk (day)}} \quad \text{_____ (4)}$$

### 3. Genotypic data collections

The blood collection and polymerase chain reaction (PCR) procedures followed the method outlined in our previous study (Prasongsook *et al.*, 2015). Briefly, the PCR reaction targeted the IGFBP 2 intron 2 region to amplify a 245 bp fragment of the

porcine IGFBP 2 gene (GenBank Accession No. BV727778). The PCR mixtures and conditions were as previously described (Prasongsook *et al.*, 2015), with an initial denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, and extension at 72 °C for 1 minute over 30 cycles. Finally, the PCR reaction concluded with a final elongation step at 72 °C for 10 minutes. The restriction enzyme digestion of the PCR product was performed using 20 U of the *MspI* restriction enzyme, which recognizes the site CCGG. Digestion was incubated overnight at 37 °C. The digested products were electrophoresed on a 2.0% agarose gel at 95 volts for 1.5 hours in Tris-borate-EDTA (TBE) buffer. The individual PCR-RFLP fragment sizes were visualized using ethidium bromide and band patterns were analyzed under ultraviolet light. According to the results of previous study, the IGFBP 2 genotypes were classified into AA, AB, and BB. The AA genotype showed a 245 bp fragment (no *MspI* site), the BB genotype showed two fragments of 190 bp and 55 bp, and the AB genotype showed a combination of 245 bp, 190 bp, and 55 bp.

#### 4. Allele and genotype frequency analysis

Allele and genotype frequencies of the IGFBP 2 polymorphisms were estimated by the FREQ procedure, and Hardy-Weinberg equilibrium (HWE) was tested by  $\chi^2$  of SAS 9.0 (Statistical Analysis System [SAS], 2003).

#### 5. Statistical analysis

A mixed effects model was used to analyze the IGFBP 2 data obtained by using the MIXED procedure of SAS 9.0 (SAS, 2003) to estimate least square means (LSMeans) to compare the study traits between the fixed effects using an F-test followed by a significant t-test for these effects. The statistical analysis model is represented as follows:

$$Y_{ijklmn} = \mu + genotype_j + sex_k + farrowing\ group_l + litter_m + finishing\ pen\ (farrowing\ group)_n + e_{ijklmn}$$

Where;  $Y_{ijklmn}$  = observations of the trait measured on animal  $i$  within genotype  $j$ , sex  $k$ ,  $\mu$  = overall mean,  $genotype_j$  = the fixed effect of genotype;  $j, j = AA, AB, BB$ ,  $sex_k$  = the fixed effect of sex;  $k, k = 0$  female,  $k = 1$  male,  $farrowing\ group_l$  = the random effect of farrowing group;  $l, l = 1$  to 11 with the mean of 0 and the variance of  $\sigma_f^2$ ,  $litter_m$  = the random effect of litter with the mean of 0 and the variance of  $\sigma_l^2$ ,  $finishing\ pen\ (farrowing\ group)_n$  = the random effect of the finishing pen nested within the farrowing group;  $n, n = 1, 2, \dots, 8$  modified open front building,  $n = 9, 10, \dots, 34$  test station building with the mean of 0 and the variance of  $\sigma_p^2$ ,  $e_{ijklmn}$  = the residual effect with the mean of 0 and the variance of  $\sigma_e^2$

## Results and Discussion

### Allele and genotype frequency

According to the results of previous study by Prasongsook *et al.* (2015), the genotypic and allelic frequencies of IGFBP 2 are shown in Figure 1 and genotype frequencies were in Hardy-Weinberg equilibrium ( $\chi^2 = 0.20$ ;  $P = 0.90$ , respectively)

### Effect of IGFBP 2 genotypes on fat-free lean

The study of candidate genes is a primary method for determining whether specific genes are related to economically important traits in farm animals (Li *et al.*, 2003). Table 1 presents the LSMeans for production performance traits by IGFBP 2 genotype groups. This study found significant effects ( $P < 0.05$ ) of IGFBP 2 genotypes on fat-free lean at several ages. Estimating fat-free lean using serial live weight and real-time ultrasonic

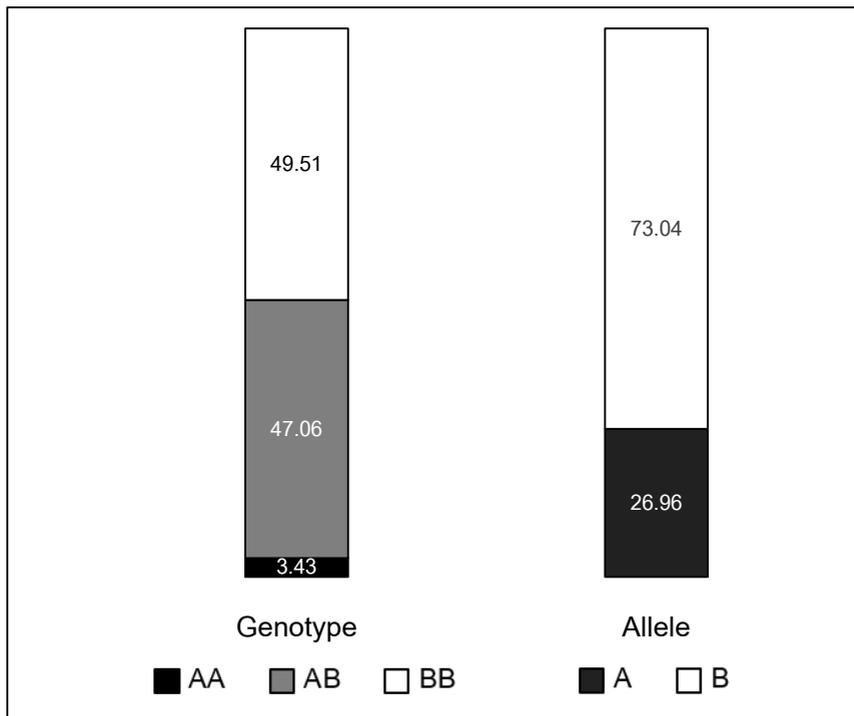


Figure 1. Genotype and allele frequencies of IGFBP 2 in the F<sub>2</sub> pig population, Adapted from Prasongsook *et al.*, (2015)

measurements (NPPC, 2000; Schinckel, 1994) is a simpler and more economical method for predicting protein accretion rates in live pigs for commercial herds (Schinckel *et al.*, 1996).

In the current study, pigs with the BB genotype had greater ( $P<0.05$ ) fat-free lean at 10, 13 and 16 weeks of age than pigs with the AB genotype. However, the fat-free lean did not differ significantly ( $P>0.05$ ) among genotype groups at 19 and 20 weeks of age, which is close to the market age. This indicated that the differences in fat-free lean among IGFBP 2 genotype groups at younger age will not correlate with fat-free lean at the market age. Nonetheless, this information could help the pig producers optimize their feeding and management strategies.

Furthermore, significant effects of the IGFBP 2 genotypes on fat-free lean percent were found ( $P<0.05$ ). Pigs that contained the BB genotype had greater ( $P<0.05$ ) fat-free lean percent at 13, 16, 19 and 22 weeks of age than those pigs with the AA or AB genotypes. Lean tissue growth in the pigs is closely related to feed efficiency and carcass quality (Schinckel, 1994).

Moreover, lean tissue growth gain is closely related to the body protein deposition, which is a primary determinant of dietary amino acid and energy requirements (NRC, 1998; Schinckel *et al.*, 2002). To minimize feeding costs and optimize carcass value, it is thus in the pork producers' interest to optimize the carcass lean content in slaughter pigs. In the current study, fat-

Table 1. Least square means±standard errors for fat-free lean traits of F<sub>2</sub> pigs separated by genotypes

Traits	N	Genotypes			P-value
		AA	AB	BB	
10 wk fat-free lean, kg	408	9.16±0.46 <sup>ab</sup>	9.37±0.29 <sup>b</sup>	9.78±0.27 <sup>a</sup>	0.003
13 wk fat-free lean, kg	408	14.93±0.81 <sup>ab</sup>	15.28±0.40 <sup>b</sup>	16.00±0.36 <sup>a</sup>	0.010
16 wk fat-free lean, kg	408	23.46±0.94 <sup>ab</sup>	23.16±0.40 <sup>b</sup>	23.95±0.33 <sup>a</sup>	0.010
19 wk fat-free lean, kg	408	29.98±1.22	30.04±0.43	30.69±0.32	0.260
22 wk fat-free lean, kg	408	39.18±1.46	38.48±0.48	38.30±0.33	0.790
10 wk fat-free lean percent, %	408	39.04±1.62	37.41±0.58	37.18±0.44	0.590
13 wk fat-free lean percent, %	408	36.96±0.69 <sup>b</sup>	37.63±0.34 <sup>b</sup>	38.30±0.30 <sup>a</sup>	0.030
16 wk fat-free lean percent, %	408	38.28±0.66 <sup>ab</sup>	37.98±0.28 <sup>b</sup>	38.61±0.23 <sup>a</sup>	0.030
19 wk fat-free lean percent, %	408	37.03±0.70 <sup>b</sup>	37.68±0.34 <sup>b</sup>	38.19±0.30 <sup>a</sup>	0.030
22 wk fat-free lean percent, %	408	50.67±0.89 <sup>b</sup>	50.64±0.35 <sup>b</sup>	52.00±0.27 <sup>a</sup>	0.030
Fat-free lean gain, g/day	408	342.05±14.82 <sup>b</sup>	352.60±8.06 <sup>b</sup>	369.90±7.38 <sup>a</sup>	0.030

<sup>a, b</sup> Least square means within the same row with different superscripts differ ( $P < 0.05$ )

free lean gain ranges from about 342 to 370 g/day, consistent with previous studies that reported a fat-free lean gain varies between 200 to 450 g/day (de Lange *et al.*, 2001; NRC, 1998).

The results indicated that pigs with the BB genotype had a more favorable ( $P < 0.05$ ) fat-free lean gain ( $369.90 \pm 7.38$  g/day) compared to pigs with the AA genotype ( $342.05 \pm 14.82$  g/day) and pigs with the AB genotype ( $352.60 \pm 8.06$  g/day). Specifically, pigs with the BB genotype had a fat-free lean gain that was 27.85 g/day higher than pigs with the AA genotype and 17.30 g/day higher than pigs with the AB genotype.

### Conclusion

IGFBP 2 genotype effects were found for fat-free lean at 10, 13 and 16 weeks of age, fat-free lean percent at 13, 16, 19 and 22 weeks of age, and

fat-free lean gain from 10 to 22 weeks of age. However, there was no significant effect of the IGFBP 2 genotypes on fat-free lean at 19 and 22 weeks of age. Pigs with the BB genotype exhibited more desirable fat-free lean at 10, 13 and 16 weeks of age than pigs with the AB genotype. Furthermore, pigs with the BB genotype had the most favorable fat-free lean percent at 13, 16, 19 and 22 weeks of age and the highest fat-free lean gain from 10 to 22 weeks. Additional genetic improvement using MAS and targeting genes with significant effects like those associated with the IGFBP 2 locus, could substantially enhance the efficiency of pig production. To advance these findings, it is essential to evaluate associations between IGFBP 2 polymorphisms and production performance traits across different breeds and pig populations and conduct further functional studies to define the effects of IGFBP 2 at a molecular level.

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