MINISTRY OF EDUCATION AND TRAINING
MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT
NATIONAL INSTITUTE OF ANIMAL SCIENCE

HOANG THI THUY

THE ASSOCIATION BETWEEN POLYMORPHISMSOF SOME CADIDATE GENES AND GROWTH PERFORMANCE, BACKFAT THICKNESS AND INTRAMUSCULAR FAT TRAITS IN DUROC PIGS

SUMMARY OF THE PhD THESIS

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Thesis can be found at the library:

1. Library of National Institute of Animal Science

2. National Library of Vietnam

LIST OF SCIENTIFIC PUBLICATIONS RELATED TO THE THESIS

1. Hoang Thi Thuy, Giang Thi Thanh Nhan, Pham Thi Phuong Mai, Tran Thi Thu Thuy, Le Quang Nam, Doan Phuong Thuy, Nguyen Van Hung, Tran Xuan Manh, Doan Van Soan and Pham Doan Lan. 2019. Associations of some candidate genes polymorphisms with growth traits in Duroc pig. Livestock Research for Rural Development, Volume 31(10), October.

2. Hoang Thi Thuy, Giang Thi Thanh Nhan, Pham Thi Phuong Mai, Tran Thi Thu Thuy, Le Quang Nam, Doan Phuong Thuy, Nguyen Van Hung, Tran Xuan Manh, Doan Van Soan and Pham Doan Lan. 2021. The association between polymorphisms of some candidate genes and the growth and backfat thickness of Duroc pig over two generations. Journal of Science and Technology of Livestock. Number 264: 1 - 7.

3. Hoang Thi Thuy, Pham Thu Thao, Giang Thi Thanh Nhan, Nguyen Van Hung, Tran Xuan Manh, Doan Van Soan and Pham Doan Lan. 2021. Polymorphisms of candidate genes and their association with intramuscular fat in Duroc Pig. Journal of Livestock Science and Technology, Institute of Livestock Production. February issue. Vol 120: 90 - 98.

PREFACE

1. The necessary requirements of the thesis

In recent years, the rapid development of molecular genetics hashelped the selection of livestock breedsfaster, more accurate and more efficient.

Many candidate genes related to growth and meat quality had been studied and proposed to be used for selection programs supported by molecular markers such as *MC4R, PIT1, GH, LEP, PIK3C3, FABP3, ADRB3, PLIN2, ACSL4*genes.However, the relationship between gene polymorphisms and traits depended on the characteristics or genetic nature of each pig population at each production facility. Therefore, to apply genes to support selection of livestock breeds for each desired trait, it was necessary to conduct research to evaluate the association of candidate genes on the population which was selected.

Duroc pigs were one of the foreign pig breeds that had the ability to gain weight quickly, good meat quality (soft meat due to fat mixed with lean) and high lean percentage. Therefore, in Vietnam, Duroc pigs were used in lean herd programs, which partly improved the productivity and quality of meat for the pork industry. On the other hand, many studies showed that the weight gain in Duroc pigs in Vietnam was not superior to the weight gain in Duroc pigs of some developed countries. Therefore, how to improve the ability to increase weight, backfat thickness and the quality of meat to promote the pork industry was becoming an important research direction.

In order to had a scientific basis for the application of genetic markers to support the selection and improvement the ability to increase weight and meat quality of Duroc pigs fed at Dabaco Nuclear Pig Breeding Co., Ltd., I researched the topic***"The association between polymorphisms of some candidate genes and growth performance, backfat thickness and intramuscular fat traits in Duroc pigs"*.**

2. The aim of the thesis research

Determine the polymorphisms of *MC4R, PIT1, GH, LEP, PIK3C3* genes and their association with the traits of growth performance, backfat thickness, and reproductive performance in Duroc pigs.

Determine the polymorphism of *ADRB3, ACSL4, FABP3, PLIN2* genes and the association with the intramuscular fatin Duroc pigs.

Select the Duroc pig line in the direction of growth performance using the support from genotype information.

3. Research contents

Content 1: Studying genetic polymorphisms and association of *MC4R, PIT1, GH, LEP* and *PIK3C3* gene polymorphisms with the traits of growth performance, backfat thickness, and reproductive performance in Duroc pigs.

Content 2: Studying genetic polymorphisms and the association of *ADRB3, ACSL4, FABP3* and *PLIN2* gene polymorphisms with the trait of intramuscular fat.

Content 3: Selecting Duroc pigs in the direction of growth performancebased on genotype information.

4. Science and practice meaning of the thesis

The thesis provided information on genotypic frequency, allele frequency and the association with growth performance, backfat thickness, reproductive performance, and intramuscular fat of some candidate genes as *MC4R, PIT1, GH, LEP, PIK3C3, ADRB3, ACSL4, FABP3, PLIN2* on Duroc pigs fed at Dabaco Nuclear Pig Breeding Co., Ltd.

The thesisprovided a scientific basis for the use of some candidate genes to support the selection of Duroc pigs which had highgrowth performance, backfat thickness,and intramuscular fat at Dabaco Nuclear Breeding Pig Co., Ltd.

Articles published in domestic and foreign scientific journals were valuable references in scientific research and training.

5. Thenew contribution of the thesis

The thesis was a systematic study including: polymorphism analysis of candidate genes *MC4R, PIT1, GH, LEP, PIK3C3, ADRB3, ACSL4, FABP3, PLIN2*; Evaluating the association between polymorphisms of these genes with growth performance, backfat thickness, and intramuscular fat; Application to select Duroc pigs with high growth performance based on candidate genes information at Dabaco Nuclear Pig Breeding Co., Ltd.

Provide a scientific basis for orienting the use of molecular markers assisted selection to improve productivity and meat quality in breeding for Duroc pig breed, thereby shortening the selection time and improving the efficiency of livestock production, which met the requirements of high-quality and productive pig production in our country.

Chapter 1. OVERVIEW

With the development of molecular biotechnology, the research on DNA molecular markers had made rapid progress. DNA molecular markers were widely applied in genetic polymorphism studies, which relied on the characteristics of the DNA molecule (diversity, stability and specificity for individuals and species) to apply to breeding, evolutionary research and taxonomy.These methods were superior to traditional phenotype-based selection methods, in particular in reducing selection time and selecting for traits that had low heritability or were difficult to access phenotype or very expensive to judge by phenotype.

Many candidate genes related to growth and meat quality had been studied and proposed to be used for selection programs supported by molecular markers (MAS) such as: PIT1 gene (Feng et al., 2012; Daga et al., 2012; Kim et al., 2014; Al-Khuzai et al., 2018).The *MC4R* gene encoded a protein which was a membrane-bound receptor. This receptor played an important role in controlling food intake, body mass and maintaining stability intracellular energy.The *MC4R* gene was associated with increased weight and back fat thickening (Davoli

et al., 2012; Hirose et al., 2014). The *GH* gene was related to the traits of carcass and growth (Bižienė et al., 2011; Lyubov et al., 2017). The *LEP* gene was important to the food intake control and energy balance.The *LEP* gene was associated with weight gain (Tempfli et al., 2015). The *PIK3C3* gene was related with weight gain during the growing–finishingperiod (Hirose et al., 2011). *FABP3, ADRB3, PLIN2* and *ACSL4* genes which was connected to meat quality traits had the potential to be developed as markers of selection for high-fat pigs (Davoli et al., 2011; Han et al., 2012; Chen et al., 2014; Xue et al., 2015).

Duroc pigs were imported from Canada and Taiwan by Dabaco Nuclear Pig Breeding Co., Ltd. from 2014 - 2018, fed in Tan Chi commune, Tien Du district, Bac Ninh province. Mature boars had a weight of 320 - 350 kg. Mature sows weigh from 250 to 280 kg. Weight gain was from 750 - 800 g per day, backfat thickness was 10 - 12 mm. Weight gain of Duroc pigs fed in Japan reached 873.6 g/day (Suzuki et al., 2005), fed in Spain reached 861 g/day (Rauw et al., 2006).DanFed Company (2014) reported that in Denmark, Duroc boars fed at the productivity control station had an average daily gain of 1,140 g/day, respectively. Thus, the weight gain of Duroc pigs from Canada and Taiwan was not superior to Duroc pigs of other origins and especially much lower than that of some developed countries. Therefore, studying how to select the traits of growth performance, backfat thickness, and intramuscular fat for Duroc pigs was an urgent requirement.

Chapter 2. OBJECTS AND SCOPE OF THE THESIS RESEARCH

2.1. Objects

Duroc pigs were fed at Dabaco Nuclear Pig Breeding Co., Ltd., originating from Taiwan and Canada.

2.2. Time and location

Tine:from December 2016 to December 2020.

Location:

+Dabaco Nuclear Pig Breeding Co, Ltd; Tan Chi commune, Tien Du district, Bac Ninh province.

+ Key Laboratory of Animal Cell Technology, National Institute of Animal Science; Thuy Phuong ward, Bac Tu Liem district, Hanoi city.

2.3. The scope of the thesis research

2.3.1.The evaluation of the growth performance of Duroc pigs

Evaluatethe growth performance of 500 Duroc gilts.

Feeding mode and disease prevention: follow the breeding process of Dabaco Company.

Data collection

Weigh each individual at the beginning and end of the experiment using the Mettler Toledo electronic scale (China).

Growth performance (g/day) was calculated based on the final body weight of each individual and the number of feeding days.

Backfat thickness was measured by the Exago ultrasound machine with an Aloka SSD 500v transducer at the base of the last rib which was 6.5 cm from the vertebral line

on each individual at the same time of the end-weighing method described in the study of Youssao et al., 2002.

Data processing

All analyzes were processed by Minitab 16 software.

2.3.2. Methods for studying candidate gene polymorphisms

+ Study on polymorphism of candidate genes with weight gain and backfat thickness (*MC4R, PIT1, GH, LEP* and *PIK3C3*) was carried out on 02 generations: Generation 1 with 500 Duroc gilts (362 and 138 males); Generation 2 with 188 Duroc gilts (133 females and 55 males).

+ The polymorphism study of candidate genes for intramuscular fat (*ADRB3, ACSL4, FABP3* and *PLIN2*) was performed on 200 Duroc pigs including 118 males and 82 females generated from 23 males and 69 females.

+ Taking samples

Use specialized pliers to cut 2-3 cm of the tail of each pig when the pig was 4 days old. The tail samples were transferred into 1.5 ml tubes containing 90^o ethanol solution. Samples were stored at -20°C before DNA extraction.

+ DNA Extraction

From each tail tissue sample, DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific).

+ PCR reaction

A PCR reaction was prepared with a total volume of 25 µl consisting of 12.5 µM DreamTaq PCR Master Mix 2X (Thermo Fisher Scientific), 0.4 µM each primer and 50 ng DNA.

+ Detection and analysis of PCR products by Electrophoresis

PCR products were checked for quality and size by electrophoresis on 2% agarose gel at 100V for about 30 minutes. Gel electrophoresis was observed under UV light by the fluorescence ethidium bromide.

+ Sequencing of genes

PCR products of candidate genes were cleaned according to the procedure of the PureLink® PCR Purification Kit (Invitrogen).

The sequencing process was carried out in 3 steps: performing the sequencing reaction, cleaning up after the reaction and sequencing on an ABI 3130 machine of AB (Applied Biosystem).

2.3.3.Determining the association of MC4R, PIT1, GH, LEP, PIK3C3 gene polymorphisms with average daily gain, and backfat thickness

Data were handled by Minitab 16 software. General linear model GLM was used to evaluate the association between polymorphisms of genes *PIT1, MC4R, GH, LEP, PIK3C3* with TKL and DML with the model:

$$Yijk = \mu + Gi + SEj + G*SEij + Sk + eijk$$

With:

Yijk was growth performance or backfat thickness;

μ was the population mean;

Gi was the effect of genotype i of each gene (genotype i = GH: genotype AA/GG/AG; LEP: genotype TT/CT; PIK3C3: genotype TT/CC/CT; MC4R: AA/GG/ AG; PIT1: AA/AB/BB);

SEj was the effect of sex j (j = male and female);

G * SEij was the interaction effect between genotype and sex;

Sk was the influence of the sire;

eijk was the random error.

Compare the confidence level between the means using Least Square Mean – LSM with Tukey comparison.

2.3.4.Determining the association of MC4R, PIT1, GH, LEP gene polymorphisms with eproductive performance

Research subjects are: 104 sows; number of litters (445 litters), number of fathers (27 heads) and mothers (73 heads) of sows; The number of litters was shown in Table 2.1.

Table 2.1. Parities of 104 Duroc sows

Parities	1	2	3	4	5	6
Litters	104	85	81	69	56	50

General linear model GLM was used to evaluate the association between polymorphisms of *PIT1, MC4R, GH, LEP* genes with reproductive performance:

$$Yij = \mu + Gi + eij$$

Yij was the phenotypic value of the trait;

μ was the population mean;

Gi was the genotype effect of each gene (genotype i = GH: genotype AA/GG/AG; LEP: genotype TT/CT; MC4R: AA/GG/AG; PIT1: AA/AB/BB);

eij was the random error.

Compare the confidence level between the means using Least Square Mean – LSM using Tukey comparison.

2.3.5. Association between *ADRB3, ACSL4, FABP3* and *PLIN2* gene polymorphisms with intramuscular fat

The study was carried out on 200 Duroc pigs including 118 males and 82 females generated from 23 males and 69 females (follow-up period over a generation).

The loin intramuscular fat of 200 Duroc pigs was measured using an Exago ultrasound machine and an Aloka SSD 500v transducer at the end of the performance test. The ultrasound machine with a flat transducer 12 cm long with a frequency of 3.5 MHz could scan 12.5 cm deep to capture images. The transducer was placed vertically, parallel and about 6-7 cm from the center of the animal's spine at the 10th rib position. From the images obtained by ultrasound, data on backfat thickness could be measured directly on the screen of the ultrasound machine or transferred to a computer and processed by Biosoft Toolbox II for Swine software of Biotronics.In Company. As for the intramuscular fat, it could only be measured through Biosoft software when the image data from the ultrasound machine was

transferred to the computer. Each researched individual was measured and recorded at least 5 images, corresponding to 5 repeated measurements. Then, each image (repeat) would be processed to give parameters of backfat thickness and intramuscular fat. Arithmetic average results of 5 repeated measurements would be used to evaluate and compare these criteria between researched individuals.

General linear model GLM was used to evaluate the association between *FABP3, ADRB3, PLIN2, ACSL4* polymorphisms with intramuscular fataccording to the model:

$$Yijk = \mu + Gi + Sej + Gi*Sj + Sk + eijk$$

With:

Yijk: intramuscular fatwith genotype i and sex j;

μ: the population mean;

Gi: fixed effect of genotype i (i=3, corresponding to 3 genotypes);

Sej: fixed effect of the jth sex (j=2, male or female respectively);

Gi*Sj: cumulative fixed effect of genotype i and sex j;

Sk: influence of the sire;

eijk: random error.

Compare the confidence level between the means using Least Square Mean – LSM using Tukey comparison.

2.3.6. Selection of Duroc pigs for weight gain based on genotype

Step 1: Select Duroc males (20) and females (100) with genotype homozygous for both *MC4R* (AA) and *PIT1* (AA) genes or homozygous for 1 gene and heterozygous for 1 gene from 1000 females and 400 gilt boars. Conduct F0 generation.

Bước 2: Select 60 individuals (50 females + 10 males) carrying both *MC4R*, (AA) and *PIT1* (AA) genotypes with high growth performance from litters of F0 generation to monitor the growth performance in F1 generation. Pair to create F2 generation.

Bước 3.Select sows and boars with high growth performance to access the performance in F2 generation.

* Research targets:

- Pre-test weight (kg);
- Post-test weight (kg);
- Growth performance (g per day);
- Backfat thickness (mm).

* The method of studying the growth performance of Duroc pigs in F1 and F2 which had both *MC4R* (AA) and *PIT* (AA) genotypes were performed as in section 2.3.1.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. GROWTH PERFORMANCE OF DUROC PIGS

Growth performance of 500 Duroc gilts during the period of individual performance testing was presented in Table 3.1.

Table3.1. Results of Duroc pig growth performance monitoring

Traits	Mean ± SE	CV (%)
Birth weight/piglet (kg)	1.55 ± 0.01	16.91
Weaning weight/ piglet (kg)	6.68 ± 0.06	19.34
Pre-test weight/pig (kg)	31.67 ± 0.14	10.16
Post-test weight/pig (kg)	94.71 ± 0.34	8.14
Age to start testing (days)	71.52 ± 0.13	4.05
Age to finish testing (days)	149.29 ± 0.29	4.17
Number of weaning days (days)	23.39 ± 0.09	9.46
The number of test day(days)	77.99 ± 0.28	8.12
Growth performance (g per day)	809.04 ± 4.12	11.39
Backfat thickness (mm)	12.01 ± 0.08	14.41

Growth performance of 500 Duroc gilts during the individual performance testing period showed that: Duroc gilts had birth weight/piglet, weaning weight/piglet of 1.55 kg and 6.68 kg, respectively. Age to start testing was 71.52 days; age to finish testing was 149.29 days; the number of weaning days was 23.39 days; the number of test days was 77.99 days; pre-test weight/pig was 31.67 kg; Post-test weight/pig was 94.71 kg,growth performance was 809.04 g/day, backfat thickness was 12.01 mm.

3.2. GENOLOGY POLYNOLOGIES OF *MC4R, PIT1, GH, LEP AND PIK3C3*

3.2.1.Concentration and purity of the DNA sample

The tail tissue samples of the Duroc pigswere successfully isolated. The electrophoresis image showed that the DNA was concentrated in a clear, bright, unbroken band. After measuring on Nano drop 2000, the DNA samples had high purity with the ratio A260/280 in the range of 1.79 - 2.03 and the total DNA concentration fluctuated in the range of 70-150 µg/µl.

3.2.2. Polymorphism of MC4R, PIT1, GH, LEP and PIK3C3gene segments

With specifically designed primer pairs and normalized PCR reaction conditions. DNA fragments containing polymorphisms on the studied genes (*MC4R, PIT1, GH, LEP* and *PIK3C3*) were specifically cloned. PCR products of candidate genes were cut with specific enzymes. The results show that:

The *MC4R* gene was cutby the *Taq*I enzyme resulting in three different genotypes (AA, AG and GG). Genotype AA had a unique band of 226 bp in size; genotype AG had 3 bands corresponding to size 226 bp, 156 bp and 70 bp; Genotype GG had2 bands with sizes 156 and 70 bp. The results of determining genotype frequencies, allele frequencies of MC4R gene polymorphisms in Duroc pig population showed thatheterozygousAG genotypes predominate

in the 1st and 2nd generation with frequencies of 0.51 and 0.48,correspondingly. The A and G allele frequencies were 0.41 and 0.59, respectively, in both generations.

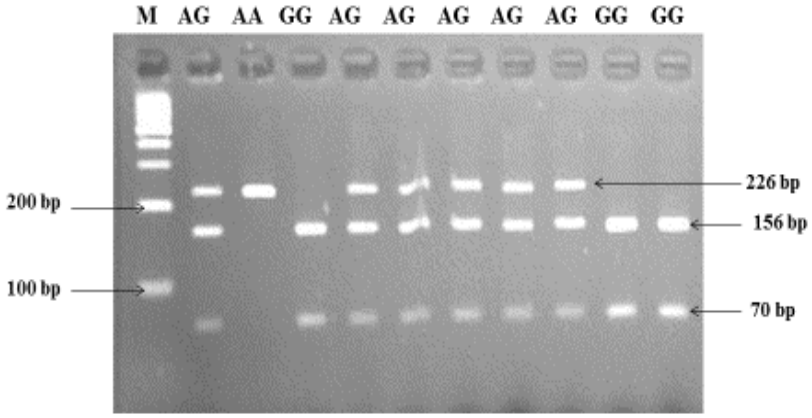
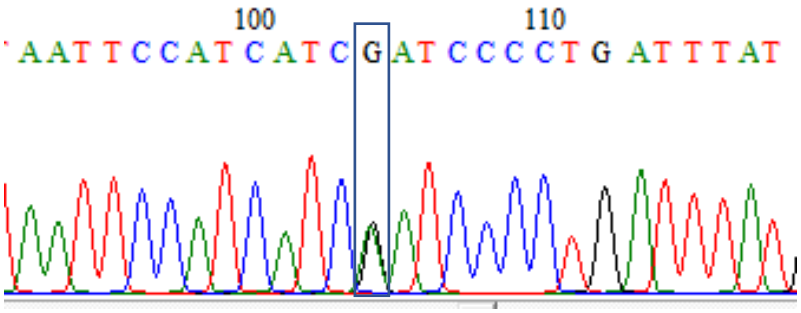


Figure 3.1. Genotyping at *MC4R*polymorphic site by *Taq*I

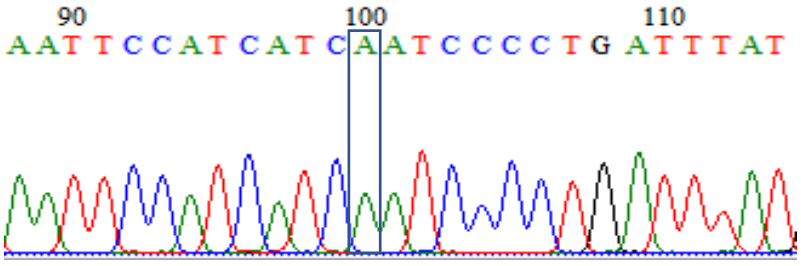
M: Standard DNA scale 100 bp

The sequencing of MC4R gene polymorphisms was shown in Figure 3.2.

GenotypeAG



GenotypeAA



GenotypeGG

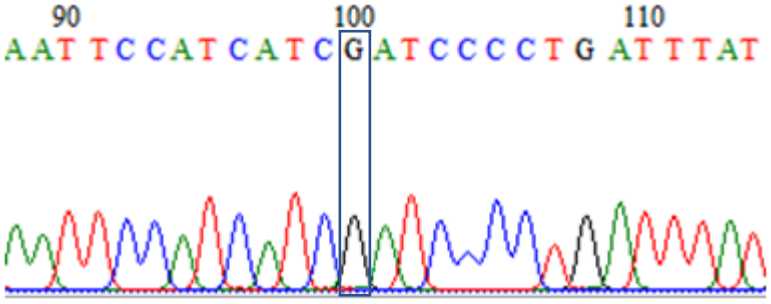


Figure 3.2. Sequencing results of *MC4R* gene polymorphisms

For the *PIT1* gene, the product was cut by *Ras*I enzyme to create three different genotypes AA, AB and BB. Genotype AA had 4 bands with sizes 774 bp, 710 bp, 153 bp and 108 bp, respectively; genotype AB had 6 bands corresponding to size 774 bp, 710 bp, 388 bp, 322 bp, 153 bp and 108 bp; BB genotype had 5 bands with sizes 774 bp, 388 bp, 322 bp, 153 bp and 108 bp, respectively. The results of determining genotype and allele frequencies of *PIT1* gene polymorphism in Duroc pig population showed the highest frequency of heterozygous AB genotypes in both generations, in the 1st and 2nd generation, 0.40; 0.41, respectively. The second highest frequency was genotype AA (0.30) in the 1st generation and 0.32 in the 2nd generation; finally genotype BB was 0.30 in 1st generation and 0.27 in 2nd generation. Frequency of A/B allele in the 1st generation was (0.5A/0.5B); the frequency of the A/B allele in the 2nd generation was 0.53A/0.47B.

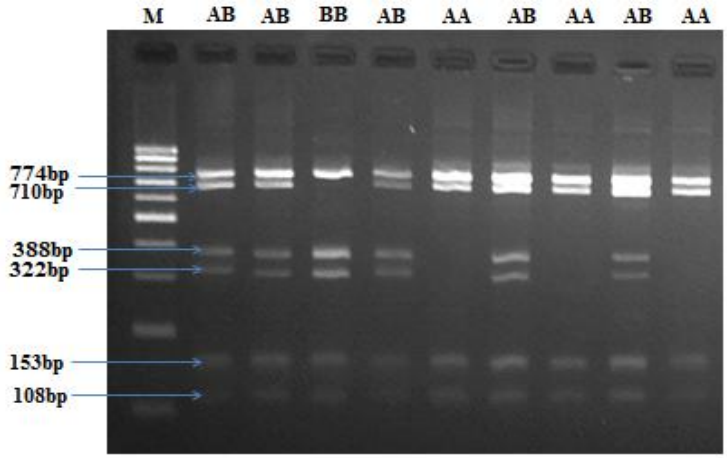


Figure 3.3. Genotyping at *PIT1*polymorphic site by *Ras*I

M: Standard DNA scale 100 bp

The *GH* gene polymorphism was identified using the restriction enzyme *Fok*I. The results of electrophoresis analysis showed that in the studied pig population, there were three genotypes: homozygous AA genotype corresponding to a 605 bp electrophoresis band, homozygous GG genotype corresponding to two electrophoresis bands which were 260 bp and 345 bp and the heterozygous AG genotype for three electrophoresis bands of 605 bp,345 bp and 260 bp, respectively. The results of determining genotype and allele frequencies of *GH* gene polymorphisms in Duroc pig population showed that in the 1st generation, the AA genotype had the lowest rate of 0.15 while the GG genotype was 0.35 and AG genotype had the highest rate of 0.50. The A and G alleles had frequencies of 0.40 and 0.60, correspondingly. In the 2nd generation, the AG genotype had the highest rate of 0.44, followed by the GG genotype (0.41) and the lowest was the AA genotype (0.15). The A and G alleles had frequencies of 0.37 and 0.63, respectively.

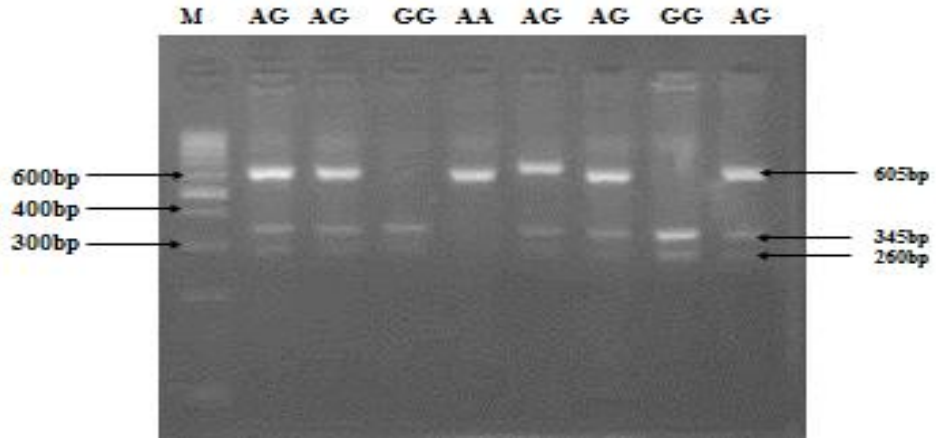
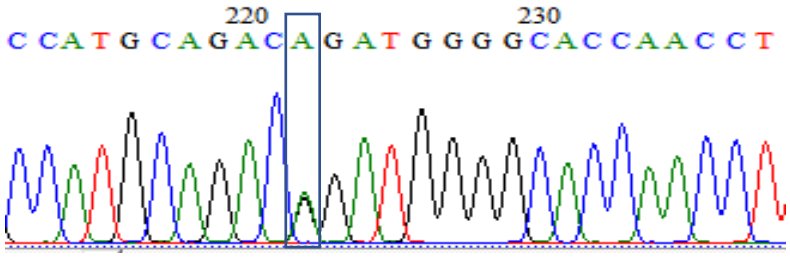


Figure 3.4. Genotyping at *GH* polymorphic site by *Fok*I.

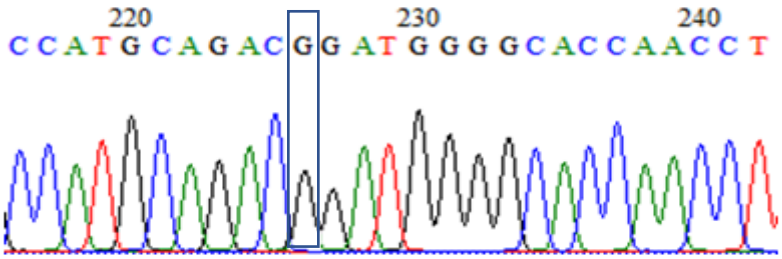
M: Standard DNA scale 100 bp

The sequencing of the *GH* gene polymorphisms was shown in Figure 3.5.

GenotypeAG



GenotypeGG



GenotypeAA

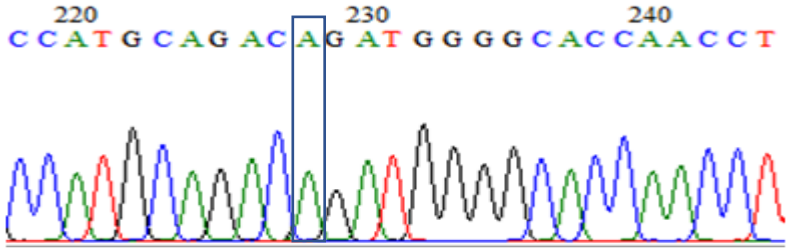


Figure 3.5. Sequencing results of *GH*gene polymorphisms

PCR-RFLP method was used to identify *LEP* gene polymorphisms. The analysis results of the studied population only obtained 2 genotypes. The CT genotype corresponded to 3 electrophoresis bands of 230 bp, 186 bp and 44 bp and the TT genotype corresponded to one 230 bp electrophoresis band. The results of determining the genotype and allele frequencies of the LEP gene polymorphism in the Duroc pig population showed that the T allele was dominant with a frequency of 0.98 in the 1st generation and 0.97 in the 2nd generation.

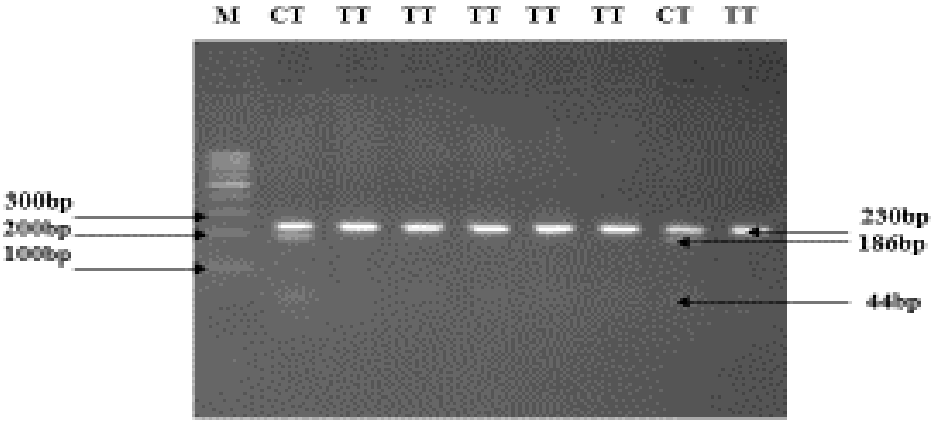
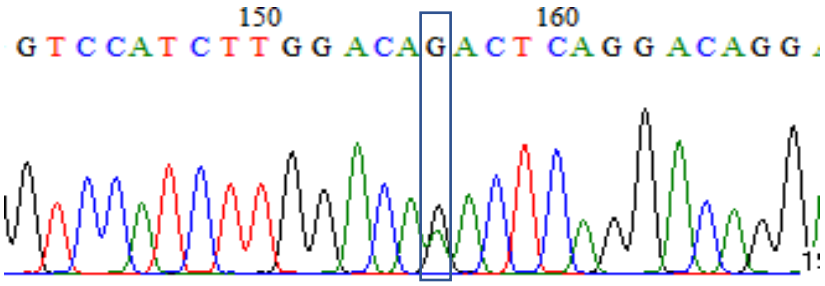


Figure 3.6. Genotyping at *LEP* gene polymorphisms by *Hinf*I

M: Standard DNA scale 100 bp

The sequencing of the *LEP* gene polymorphisms was shown in Figure3.7.

GenotypeTC (reverse direction was genotype AG)



GenotypeTT (reverse direction was genotype AA)

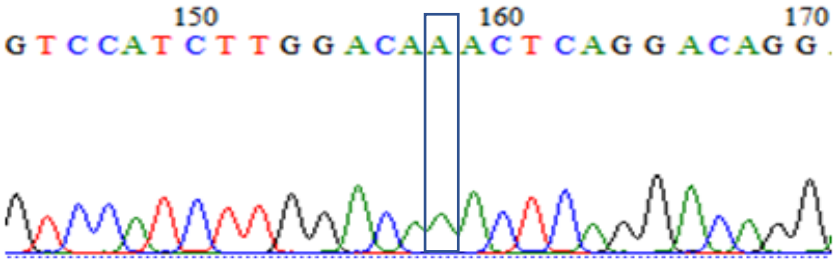


Figure 3.7. Sequencing results of *LEP* gene polymorphisms

When analyzing the C2604T polymorphism on the *PIK3C3* gene fragment belonging to exon 24, chromosome 7 by the restriction enzyme *Hpy*8I, three genotypes were identified including TT genotype corresponding to a 102 bp electrophoresis band, heterozygous CT

genotype corresponding to 3 electrophoresis bands 102 bp, 67 bp and 35 bp and the homozygous CC genotype correspondsing to two bands of 67 bp and 35 bp electrophoresis. The results of determining the genotype and allele frequencies of the *PIK3C3* gene polymorphism in the Duroc pig population showed that the C allele was dominant with a frequency of 0.62.

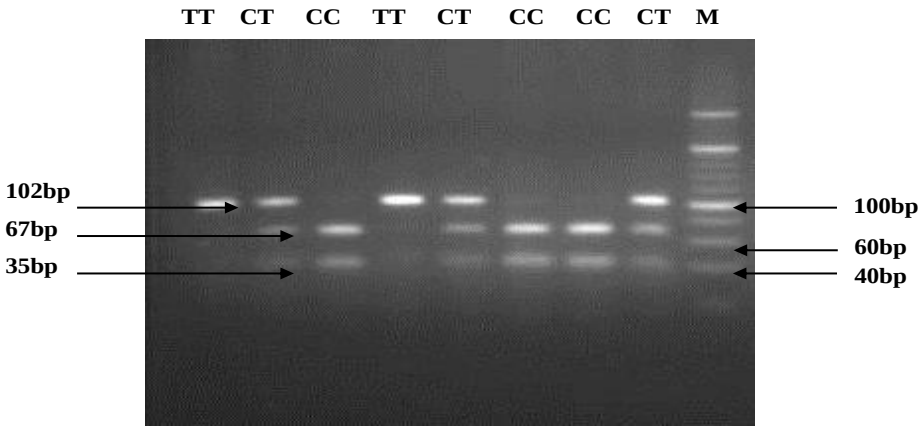
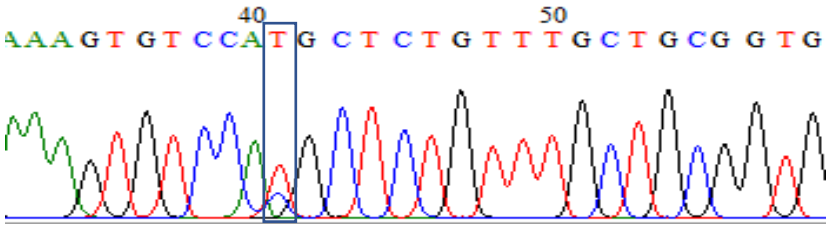


Figure 3.8. Genotyping at *PIK3C3* gene polymorphisms by *Hpy*8I

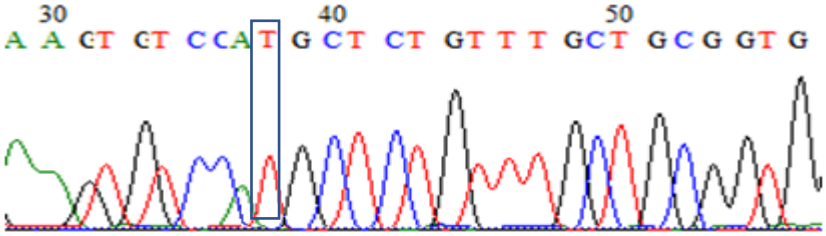
M: Standard DNA scale 50 bp

The sequencing of the *PIK3C3* gene polymorphisms was shown in Figure3.9.

GenotypeCT



GenotypeTT



GenotypeCC

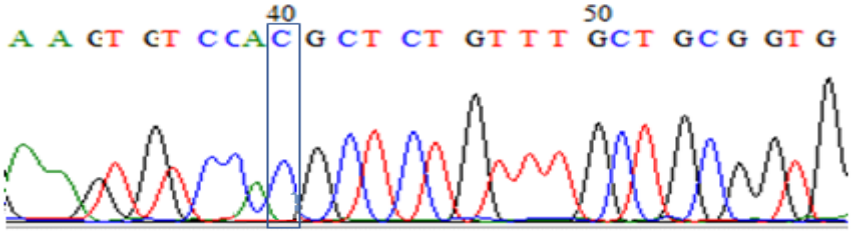


Figure 3.9. Sequencing results of *PIK3C3* gene polymorphisms

3.3. ASSOCIATION OF *MC4R, PIT1, GH, LEP* AND *PIK3C3* GENE POLYNOLOGIES WITH GROWTH PERFORMANCE, BACKFAT THICKNESS

3.3.1. Association of MC4R gene with growth performance and backfat thickness

The influence of factors on the growth performance of Duroc pigs was presented in Table 3.2.

Table 3.2. The association of *MC4R* genotype with growth parameters

Generation	Parameters	AA		AG		GG		p
		n	LSM ± SE	n	LSM±SE	n	LSM±SE	
1	Pre-test weight (kg)	80	31.58 ± 0.44	254	31.71 ± 0.29	166	31.70 ± 0.34	0.96
	Post-test weight (kg)	80	98.61^{a} ± 0.11	254	94.86^{b}±0.69	166	93.15^{b} ± 0.80	0.00
	Growth performance (g per day)	80	**853.26^{a} ± 9.59**	254	820.40^{b} ±6.36	166	790.44^{c} ± 7.31	**0.00**
	Backfat thickness (mm)	80	**12.62^{a} ± 0.29**	254	11.95^{a} ± 0.19	166	11.38^{b} ± 0.22	**0.00**
2	Pre-test weight (kg)	32	31.23^{a} ± 0.52	91	30.00^{ab} ± 0.31	65	29.45^{b} ± 0.35	0.02
	Post-test weight (kg)	32	101.76^{a} ± 1.88	91	94.54^{b} ± 1.15	65	92.47^{b} ± 1.29	0.00
	Growth performance (g per day)	32	**860.31^{a} ±15.91**	91	814.89^{b} ±9.73	65	797.72^{b} ±10.96	**0.00**
	Backfat thickness (mm)	32	**12.85^{a} ± 0.59**	91	11.48^{a} ± 0.36	65	10.04^{b} ± 0.41	**0.00**

In the same indicator, LSM values have different letters, the difference was statistically significant ($p<0.05$)

Genotype *MC4R* was associated with growth performance and backfat thickness ($p<0.05$) in the 1st and 2nd generation. Growth performance in Duroc pigs which had genotypes AA; AG; GG in generation 1 was 853.26, 820.40, 790.44 g/dayrespectively; was highest in pigs carrying genotype AA and lowest in pigs carrying genotype GG. Duroc pigs carrying genotype AA had backfat thickness of 12.62 mm, reaching the highest backfat thickness and the lowest backfat thickness among pigs carrying genotype GG with 11.38 mm. In the 2nd generation, Duroc pigs carrying genotype AA had the highest growth performance (860.31 g/day), backfat thickness (12.85 mm) and the lowest in pigs carrying genotype GG (797.72 g/day; 10.04 mm). In conclusion, *MC4R* gene polymorphism was strongly associated with growth performance and backfat thickness in both generations ($p<0.05$). In which, pigs carrying genotype AA achieved the highest growth performance and backfat thickness in both generations. Generation 1 was 853.3 g/day and 12.62 mm, generation 2 was 860.3 g/day and 12.85 mm.

3.3.2. The association of the PIT1 gene with growth performance and backfat thickness

The results of analysis of the association between polymorphisms and growth parameters of Duroc pigs were shown in Table 3.3.

Table 3.3. The association of *PIT1* genotype with growth parameters

Generation	Parameters	AA		AB		BB		p
		n	LSM ± SE	n	LSM ± SE	n	LSM ± SE	
1	Pre-test weight (kg)	14	31.23^{b}±0.36	202	32.36^{a}±0.29	149	32.15^{ab}±0.37	0.02
	Post-test weight (kg)	14	96.27^{a}± 0.86	202	95.60^{a}±0.69	149	93.36^{b}±0.88	0.02
	Growth performance (g per day)	14	**833.10^{a}±8.00**	202	816.41^{ab}±6.41	149	807.89^{b}±8.20	**0.04**
	Backfat thickness (mm)	14	**12.42^{a}±0.24**	202	11.81^{ab}±0.19	149	11.58^{b}±0.24	**0.01**
2	Pre-test weight (kg)	61	30.23±0.36	78	29.89±0.34	49	29.95±0.42	0.75
	Post-test weight (kg)	61	98.29^{a}±1.22	78	95.55^{a}±1.17	49	89.50^{b}±1.45	0.00
	Growth performance (g per day)	61	**844.70^{a}±10.25**	78	811.62^{b}±9.82	49	782.93^{b}±12.16	**0.00**
	Backfat thickness (mm)	61	**12.37^{a}± 0.40**	78	11.43^{a}±0.38	49	9.62^{b}±0.47	**0.00**

In the same indicator, LSM values have different letters, the difference was statistically significant ($p<0.05$)

Genotype*PIT1* was associated with growth performance and backfat thickness in the 1st and 2nd generation ($p<0.05$). Specifically, the traits of growth performance and backfat thickness in the 1st generation was highest in pigs carrying genotype AA (833.10 g/day; 12.42 mm), then genotype AB (816.41g/day; 11.81 mm) and finally genotype BB (807.89 g/day; 11.58 mm). In the 2nd generation, genotype AA still achieved the highest growth performance and backfat thickness, the lowest was for pigs carrying genotype BB at 844.70 g/day compared with 782.93 g/day; it was +61.77 g/day higher in growth performance; 12.37 mm compared to 9.62 mm, was +2.75 mm higher in backfat thickness. Growth performance and backfat thickness were different between the two genotypes AA and BB in Duroc pig population ($p<0.05$). In conclusion, *PIT1* gene polymorphism was strongly associated with growth performance and backfat thickness in both generations ($p<0.05$). Growth performance and backfat thickness were highest in pigs carrying genotype AA in both generations: Generation 1 was 833.1 g/day; 12.42 mm, the second generation was 844.70 g/day; 12.37 mm.

3.3.3. The association of the GH gene with growth performance and backfat thickness

Growth performance of Duroc pigs according to the *GH* genotype was presented in Table 3.4.

Table 3.4. The association of *GH* genotype with growth parameters

Generation	Parameters	AA		AG		GG		p
		n	LSM±SE	n	LSM±SE	n	LSM±SE	
1	Pre-test weight (kg)	75	32.05±0.47	252	31.75±0.29	173	31.89±0.33	0.82
	Post-test weight (kg)	75	95.73^{ab}±1.10	252	94.23^{b}±0.69	173	96.52^{a}±0.79	0.03
	Growth performance (g per day)	75	818.34^{ab}±10.13	252	809.00^{b}±6.37	173	832.33^{a}±7.27	0.01
	Backfat thickness (mm)	75	**12.57^{a}±0.30**	252	12.02^{ab}±0.19	173	11.48^{b}±0.22	0.00
2	Pre-test weight (kg)	78	30.37±0.34	82	29.49±0.34	30	30.40±0.51	0.10
	Post-test weight (kg)	78	92.31^{b}±1.19	82	95.24^{b}±1.18	30	101.90^{a}±1.78	0.00
	Growth performance (g per day)	78	835.74^{a}±10.15	82	788.50^{b}±10.10	30	**839.93^{a}±15.16**	**0.00**
	Backfat thickness (mm)	78	**12.09^{a}±0.39**	82	11.34^{ab}±0.39	30	9.97^{b}±0.59	**0.01**

In the same indicator, LSM values have different letters, the difference was statistically significant ($p<0.05$)

The *GH* gene polymorphism was associated with growth performance and backfat thickness in the Duroc pig population in both studied generations (p<0.05). Pigs carrying genotype GG had the highest growth performance in the 1st and 2nd generation, respectively, at 832.33 g/day; 839.93 g/day, the lowest was pigs carrying genotype AG 809.00 g/day; 788.50 g/day. Growth performance was different between 2 genotypes GG and AG in Duroc pig population (p<0.05). The *GH* gene polymorphism was closely associated with backfat thickness (p<0.05) in both generations. Backfat thickness was the highest in pigs carrying genotype AA, the lowest in genotype GG in both generations, such as: pigs carrying genotype AA (12.57 mm); AG (12.02 mm); GG (11.48 mm) in the 1st generation. In the 2nd generation, backfat thickness of the genotypesAA; AG; GG were 12.09 mm; 11.34 mm; 9.97 mm. Backfat thickness was different between AA and GG genotypes in Duroc pig population (p<0.05). The *GH* gene polymorphism was strongly associated with growth performance and backfat thickness in both generations (p<0.05). Pigs carrying genotype GG had the highest growth performance in the 1st and 2nd generation, at 832.33 g/day; 839.93 g/day in that order. The highest backfat thickness in pigs carrying genotype AA in generation 1 and 2 was 12.57 mm; 12.09 mm, respectively.

3.3.4. The association of LEP gene with growth performance and backfat thickness

The results of analysis of the association between polymorphisms and growth parameters of Duroc pigs were shown in Table 3.5.

Table 3.5. The association of*LEP* genotype with growth parameters

Generation	Parameters	TT		CT		p
		n	LSM±SE	n	LSM±SE	
1	Pre-test weight (kg)	479	32.40±0.23	21	34.07±0.88	0.06
	Post-test weight (kg)	479	95.09^{b}±0.57	21	100.59^{a}±2.20	0.01
	Growth performance (g per day)	479	817.13^{b}±5.31	21	**870.65^{a}±20.24**	**0.01**
	Backfat thickness (mm)	479	12.92±0.61	21	11.89±0.16	0.09
2	Pre-test weight (kg)	177	29.93±0.26	11	30.78±0.78	0.31
	Post-test weight (kg)	177	94.05^{b}±0.95	11	102.72^{a}±2.87	0.00
	Growth performance (g per day)	177	807.44^{b}±7.75	11	**884.23^{a}±23.42**	**0.00**
	Backfat thickness (mm)	177	11.31±0.31	11	11.40±0.94	0.93

In the same indicator, LSM values have different letters, the difference was statistically significant (p<0.05)

Research on the association of *LEP* gene polymorphism with growth performanceshowed that *LEP* gene polymorphism was associated with growth performance(p<0.05). The results of analysis of the association of LEP gene polymorphism with growth performanceshowed that growth performanceof pigs carrying genotype CT was higher than pigs carrying genotypeTT, respectively 870.65 g/day compared with 817.13 g/day in the 1st generation; 884.23 g/day compared with 807.44 g/dayin the 2nd generation. In this study,the link between *LEP* gene polymorphism and backfat thickness was not found.

In a study by Hirose et al. (2014), the results also showed that there was no significant association between the *LEP* gene polymorphism and the trait of growth performanceas well as backfat thickness (p>0.05). Thus, my research shows that *LEP* gene polymorphism was strongly associated with growth performancein both generations (p<0.05) of Duroc pigs fed at Dabaco Seed Seed Co., Ltd. Pigs carrying the CT genotype had a higher growth performancecompared to pigs carrying the TT genotype, 870.65 g/day and 817.13 g/day in the 1st generation, 884.23 g/day and 807.44g/day in the 2nd generation, respectively. The association was not found between the *LEP* gene polymorphism and back fat in both generations (p>0.05).

3.3.5. The association of PIK3C3 gene with growth performance and backfat thickness

The results of analysis of the association between polymorphisms and growth parameters of Duroc pigs were shown in Table 3.6.

Table 3.6. The association of*PIK3C3* genotype with growth parameters

Parameters	TT (n = 61)	CT (n = 259)	CC (n = 180)	p
	LSM ± SE	LSM ± SE	LSM ± SE	
Pre-test weight (kg)	32.26 ± 0.48	32.14 ± 0.28	32.37 ± 0.32	0.82
Post-test weight (kg)	96.29 ± 1.18	94.65 ± 0.70	95.71 ± 0.9	0.27
Growth performance (g per day)	829.00 ± 10.90	812.72 ± 6.44	822.71 ± 7.32	0.23
Backfat thickness (mm)	11.53 ± 0.33	11.93 ± 0.19	12.04 ± 0.22	0.37

In the same indicator, LSM values have different letters, the difference was statistically significant (p<0.05)

When evaluating the association of the *PIK3C3* gene polymorphism with growth performance and backfat thickness in the 1st generation, the results showed that no association was found between the C2604T polymorphism and growth performance and backfat thickness (p>0.05). Therefore, the *PIK3C3* gene polymorphism was only investigated in the 1st generation, not in the 2nd generation.

3.3.6. Reproductive productivity and the association between MC4R, PIT1, GH, LEP genotypes with reproductive traits

3.3.6.1. Reproductive performance of Duroc sows

The study was conducted on 104 Duroc sows and monitored reproductive performance from the 1st to 6th generation, for a total of 445 litters. The results were shown in Table 3.7.

Table 3.7. Reproductive performance of Duroc sows

Traits	n	Mean ± SE	CV(%)
The litter size (piglet)	445	10.97 ± 0.08	15.71
Alive piglet/litter(piglet)	445	9.96 ± 0.07	15.67
Feeding piglet/litter(piglet)	445	9.49 ± 0.07	15.34
Weaning piglet/litter(piglet)	445	9.23 ± 0.07	16.90
Newborn piglet survival(%)	445	91.15 ± 0.39	8.99
Post - weaning piglet survival(%)	445	97.30 ± 0.39	8.34
Birth weight/litter (kg)	445	14.03 ± 0.12	18.20
Post – weight/litter (kg)	445	62.61 ± 0.56	18.95

The results of Table 3.7 showed that Duroc sows had the litter size, alive piglet per litter, feeding piglet per litter and weaning piglet per litter of Duroc sows were 10.97; 9.96; 9.49 and 9.23, correspondingly. The birth weight per litter, post-weaning weight per litter of Duroc sows in this study were 14.03 kg; 62.61 kg; newborn piglet survival, post - weaning piglet survival were 91.15%, 97.30%, respectively.

3.3.6.2. The association between MC4R, PIT1, GH, LEP genotypes and reproductive traits

The *MC4R, PIT1, GH, LEP* gene polymorphisms were not associated with reproductive performance such as the litter size, alive piglet per litter, feeding piglet per litter and weaning piglet per litter, newborn piglet survival, post - weaning piglet survival and birth weight per litter ($p>0.05$). These gene polymorphismshad no association with weaning weight per litter, except for *MC4R*($p<0.01$).

3.4. *ADRB3, ACSL4, FABP3 AND PLIN2*GENE POLYMORPHISMS

With specifically designed primer pairs and normalized PCR reaction conditions, DNA fragments containing polymorphisms on the studied genes (ADRB3, ACSL4, FABP3 and PLIN2) were specifically cloned. The PCR products of the candidate gene were cut with a specific enzyme. The results showed that:

The PCR product of the ADRB3 gene was cleaved with the TaqI enzyme resulting in three different genotypes (AA, AG and GG). Genotype AA had a unique band of 315 bp in size; genotype AG had 3 bands, in that order 143 bp, 172 bp and 315 bp; Genotype GG had 2 bands with sizes 143 bp and 172 bp, correspondingly. The three genotypes AA, AG and GG had genotype frequencies of 7.0%, 63% and 30%, respectively, with genotype AG having the highest genotype frequencies. The A allele frequency was found to be 0.40 and the G allele frequency was 0.60.

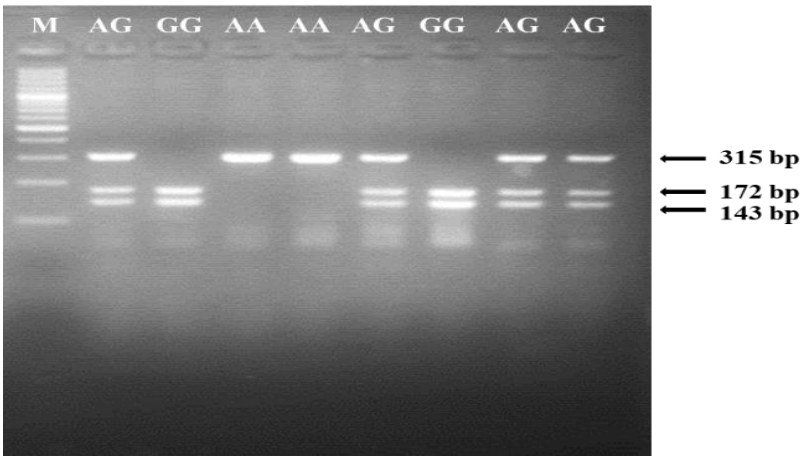
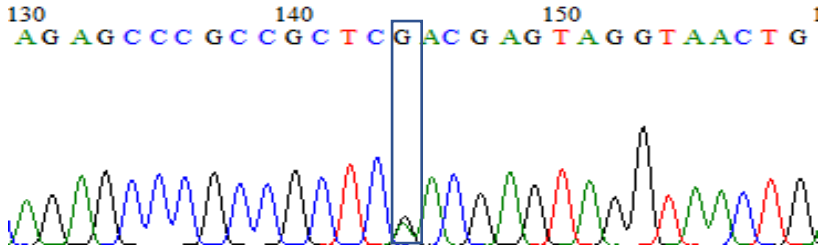


Figure 3.10. Genotyping at *ADRB3*polymorphic site by*Taq*I

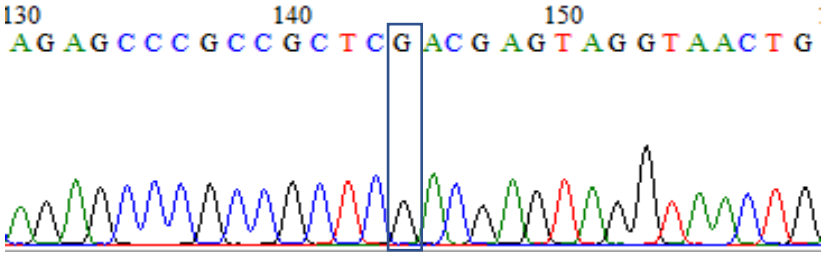
M: Standard DNA scale 100 bp

The sequencing of the *ADRB3* gene polymorphisms was shown in Figure3.11.

GenotypeAG



Genotype GG



GenotypeAA

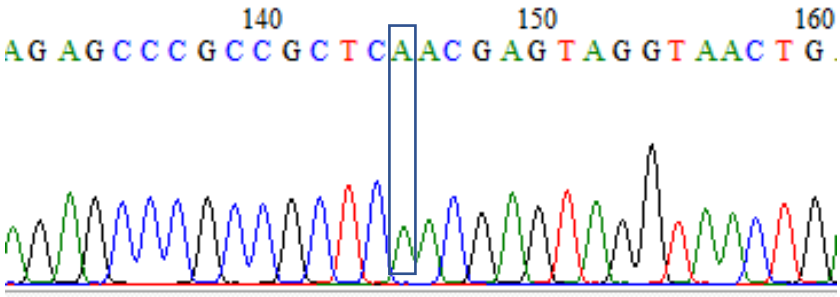


Figure 3.11. Sequencing results of *ADRB3* gene polymorphisms

In the studied Duroc pigs population, the ACSL4 gene segment polymorphism had 2 genotypes: homozygous AA genotype (135 bp and 47 bp) and heterozygous AG genotype(135 bp, 108 bp, 47 bp and 26 bp). In which, genotype AA (92%) had a much higher rate than genotype AG (8.0%). The A and G alleles had frequencies of 0.95 and 0.05, respectively.

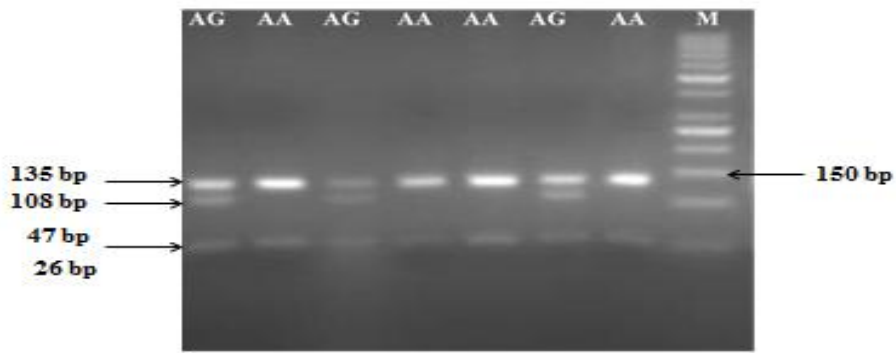
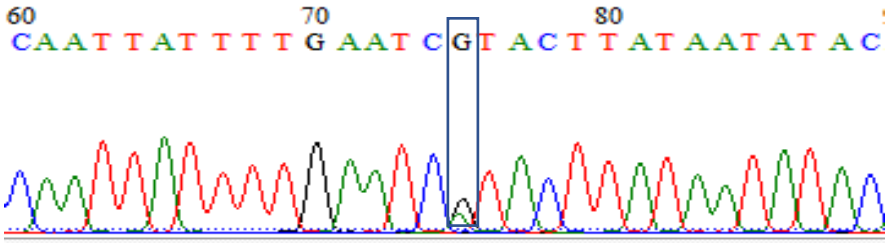


Figure 3.12. Genotyping at *ACSL4* polymorphic site by*Rsa*I

M: Standard DNA scale 100 bp

The sequencing of the *ACSL4* gene polymorphisms was shown in Figure3.13.

GenotypeAG



GenotypeAA

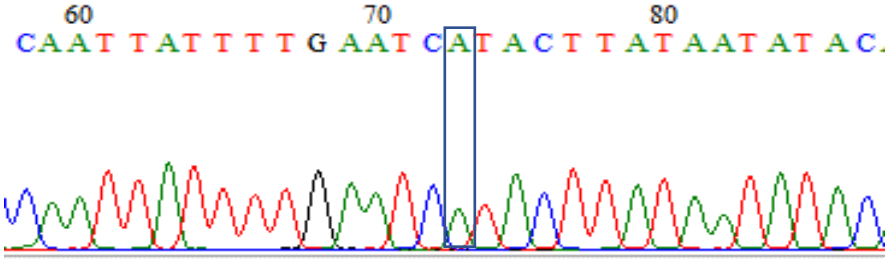


Figure 3.13.Sequencing results of *ACSL4* gene polymorphisms

The observed FABP3 gene fragment polymorphism (*Hinf*I) had two restriction patterns based on the size of the electrophoresis cassettes: genotype CT (6 lines: 339 bp, 231 bp, 172 bp, 98 bp, 59 bp and 25 bp), genotype TT (5 lines: 339 bp, 172 bp, 98 bp, 59 bp and 25 bp). The FABP3 gene at the 5'- UTR c.-314 T>C polymorphism was detected by the restriction enzyme *Hinf*I for the two genotypes TT and CT. Allele C appeared with a very low frequency of 0.04. The T allele appears commonly with a frequency of 0.96.

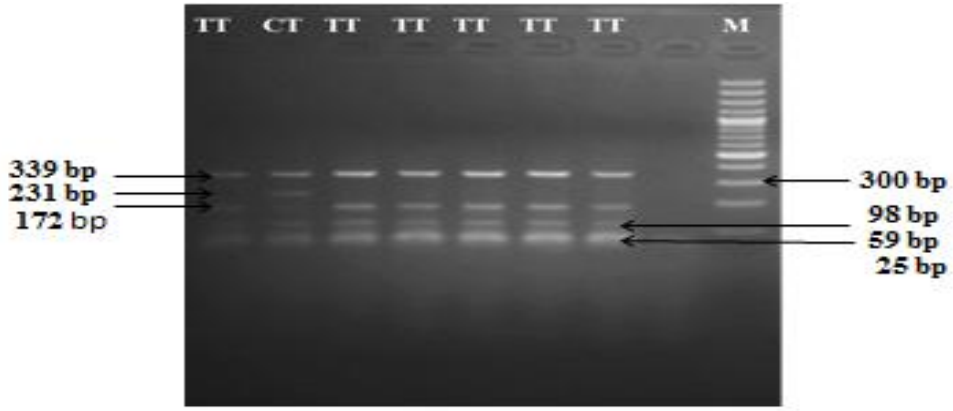
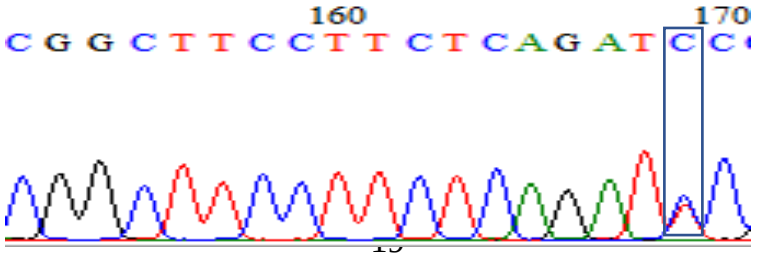


Figure 3.14. Genotyping at *FABP3* polymorphic site by*Hinf*I

M: Standard DNA scale 100 bp

The sequencing of the *FABP3 (Hinf*I*)* gene polymorphisms was shown in Figure3.15.

***Genotype*CT**



***Genotype*TT**

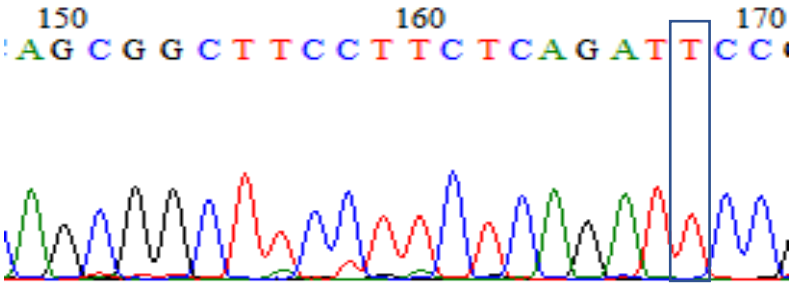


Figure 3.15. Sequencing results of *FABP3 (HinfI)* gene polymorphisms

The PCR product of the *FABP3* gene (*Bsrf*I) was cut by the enzyme *Bsrf*I. Analysis results on the study population only obtained 1 genotype TT corresponding to 1 electrophoresis band of 321 bp. In the studied Duroc pigs population, 100% genotype TT appeared. Therefore, analysis of the association between *FABP3* polymorphisms (*Bsrf*I) and intramuscular fat was not conducted in the next study.

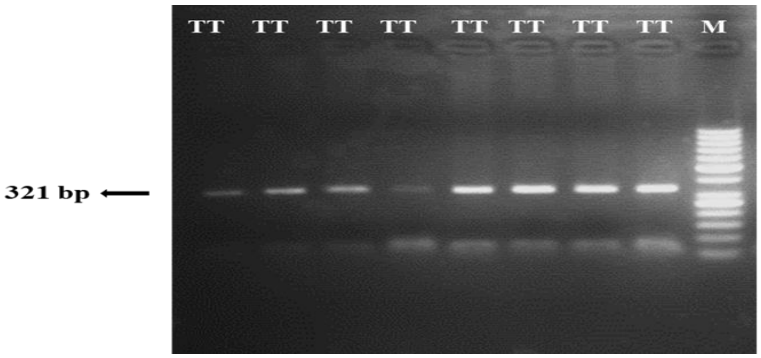


Figure 3.16. Genotyping at *FABP3*polymorphic site by*Bsrf*I

M: Standard DNA scale 50 bp

The sequencing of the *FABP3 (Bsrf*I*)* gene polymorphisms was shown in Figure3.17.

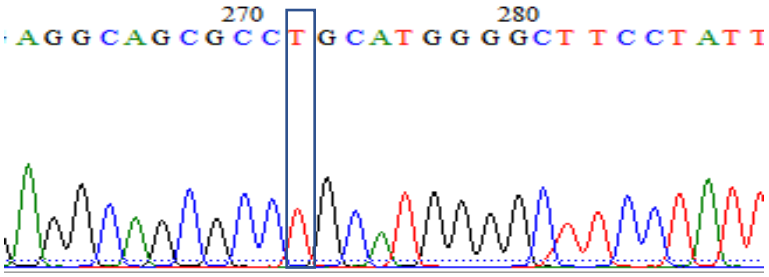
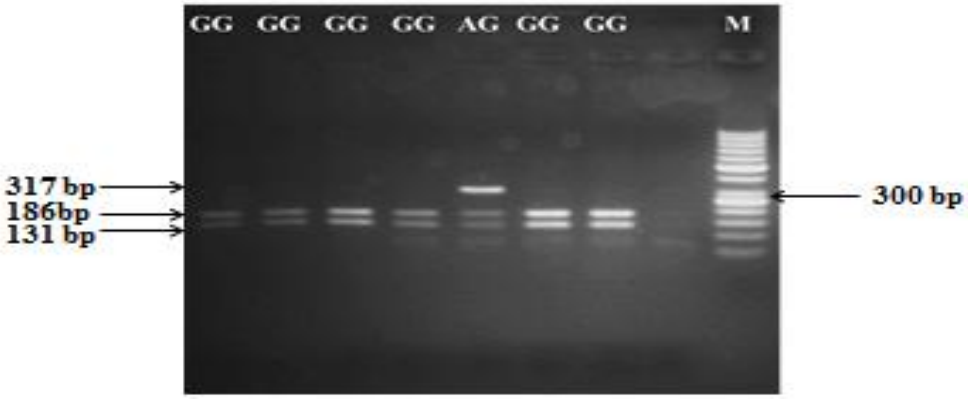


Figure 3.17. Sequencing results of *FABP3 ((Bsrf*I)gene polymorphisms**

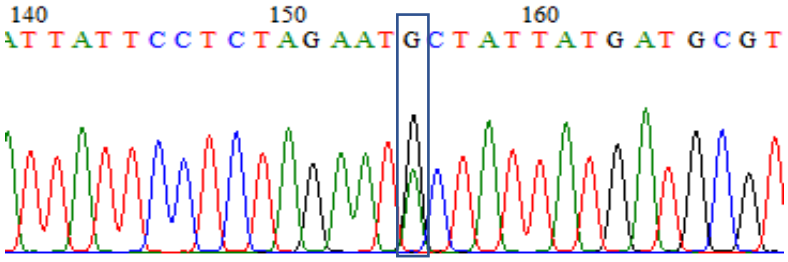
The PCR product of the *PLIN2* gene was cleaved with the enzyme *Mva*1269I. In the study population, we found that there were 2 genotypes AG and GG. Genotype AG had 3 bands, in that order 131 bp, 186 bp, 317 bp; Genotype GG had 2 bands with sizes 131 bp and 186 bp. In the studied Duroc pig population, 2 genotypes AG and GG appeared, in which the GG genotype had a very high frequency of 0.93 compared to the AG genotype frequency of 0.07.



M: Standard DNA scale 50 bp

The sequencing of the *PLIN2*gene polymorphisms was shown in Figure3.19.

GenotypeAG



GenotypeGG

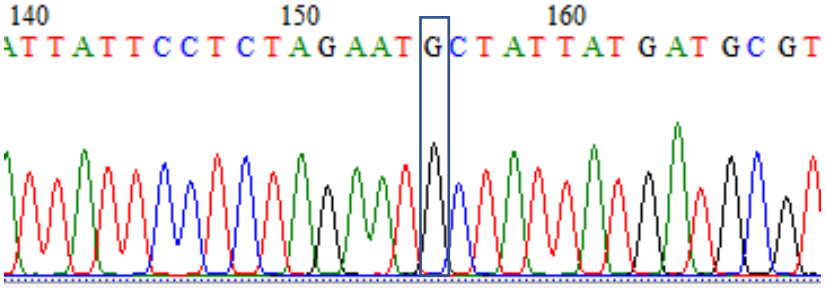


Figure 3.19. Sequencing results of *PLIN2*gene polymorphisms

3.5. THE ASSOCIATION BETWEEN *ACSL4, ADRB3, PLIN2* AND *FABP3* (*Hinf*I) GENE POLYMORPHISM AND INTRAMUSCULAR FAT

3.5.1. The association of the ACSL4 gene with intramuscular fat

The results of the analysis of the association between polymorphisms and intramuscular fat in Duroc pigs were shown in Table 3.8.

Table 3.8. Association of the *ACSL4* gene with intramuscular fat

Gene	Genotype	Intramuscular fat		p
		n	LSM ± SE	
ACSL4	AA	183	2.84 ± 0.05	0.10
	AG	17	2.63 ± 0.13	

The results of Table 3.8 showed that, in this study, the *ACSL4* gene polymorphism was not associated with intramuscular fat (p>0.05). The *ACSL4/Rsa*I gene polymorphism in the

Duroc pig population had 2 genotypes AA and AG with intramuscular fat of 2.84/2.63. Duroc pigs with genotype AA tended to have a higher percentage of carrier fat than pigs with genotype AG.

This was contrary to the statement of Ruść et al. (2011) demonstrated that the *ACSL4/Rsa*I polymorphism was associated with intramuscular fat in the DLY crossbred. Pigs with genotype GG had the highest fat content (2.47%). In the study of Chen et al., 2014, the *ACSL4/Rsa*I polymorphism was found in the Duroc, Landrace, Yorshire and the DLY crossbred, and a link was established between the *ACSL4/Rsa*I gene polymorphism and intramuscular fat. However, the results of the study indicated that this association was only significant in foreign breeds, and the association between the *ACSL4* gene and intramuscular fat was not found in the domestic Chinese breed.

3.5.2. Association of the ADBR3 gene with intramuscular fat

The results of the analysis of the association between polymorphisms and intramuscular fat in Duroc pigs were shown in Table3.9.

Table 3.9. Association of the *ADBR3* gene with intramuscular fat

Gene	Genotype	Intramuscular fat		p
		n	LSM ± SE	
ADBR3	AA	15	2.59 ± 0.16	0.17
	AG	125	2.82 ± 0.05	
	GG	60	2.90 ± 0.07	

The *ADBR3* gene had 03 genotypes AA, AG, GG, in which the pig with genotype GG (2.90) had the highest intramuscular fat , the lowest was the pig with genotype AA (2.59) and the pig with genotype Heterozygous AG genotype for intramuscular fat was 2.82. The results after statistical analysis showed that there was no association between the *ADRB3* gene polymorphism and intramuscular fat ($p>0.05$).

This result was similar to that reported by Cieslak et al. (2009) showed that in different pig herds such as Yorkshire, Landrace, Duroc, Pietrain and Hampshire pigs, there was also no association between the *ADRB3* gene and growth traits such as backfat thickness or visceral fat. Similar results, Wang et al., 2013 studied on commercial pigs Duroc x Shanzhu, the data on the intramuscular fat and fatty acid compositions were collected and studied to find the relationship between these traits with mRNA-level expression of candidate genes *AdPLA, ADRB3, LEPR, MC4R, PPARγ, PPARα, LPL, PEPCK* and *SCD*. The results when studying the *ADRB3*gene showed that this gene did not have any association with intramuscular fat and fatty acid composition in experimental pigs ($p>0.05$).

However, in contrast, Xue et al., 2015 when studying 440 commercial crossbred pigs Shanzhu × Duroc, demonstrated that the *ADRB3* gene has a link to intramuscular fat, in which the heterozygous AG genotype had higher intramuscular fat than that of homozygous AA and GG genotypes ($p<0.05$).

3.5.3. Association of the PLIN2 gene with intramuscular fat

The results of the analysis of the association between polymorphisms and intramuscular fat in Duroc pigs were shown in Table3.10.

Table 3.10. Association of the *PLIN2* gene with intramuscular fat

Gene	Genotype	Intramuscular fat		p
		n	LSM ± SE	
PLIN2	AG	13	2.66 ± 0.14	0.22
	GG	187	2.85 ± 0.05	

The *PLIN2* gene plays a role in regulating the storage and mobilization of lipid droplets. Several studies have been conducted to search for polymorphisms on the *PLIN2* gene. However, the frequency of these polymorphisms was lower in European pig breeds than in Asian pig breeds (Kim et al. 2005).

Our study found that the *PLIN2* gene polymorphism was not associated with intramuscular fat (p>0.05). The studied pig population had 2 genotypes AG and GG, in which genotype GG (2.85) had a higher percentage of intramuscular fat than that of genotype AG (2.66) (table 3.10). This polymorphism was also found in the Duroc, Landrace, Pietrain and Belgian Landrace pig breeds. The results showed that the frequency of the G allele was higher than the frequency of the A allele and the study also indicated that there was no association between the polymorphism at the g.184G/A site and intramuscular fat (Davoli et al., 2011).

3.5.4. Association of the FABP3 (HinfI) gene with intramuscular fat

The results of the analysis of the association between polymorphisms and intramuscular fat in Duroc pigs were shown in Table3.11.

Table 3.11. Association of the *FABP3 (HinfI)* gene with intramuscular fat

Gene	Genotype	Intramuscular fat		p
		n	LSM ± SE	
FABP3	CT	15	2.62 ± 0.05	0.10
(HinfI)	TT	185	2.84 ± 0.14	

FABP3 gene polymorphism (*Hinf*I) had 2 genotypes CT and TT. Pigs carrying the genotype TT tended to had higher intramuscular fat than pigs carrying the genotype CT (2.84 vs. 2.62). However, these two polymorphisms were not associated with intramuscular fat in the studied Duroc population (p>0.05).

Similar results were found in a population of Shanzhu x Duroc economic crossbred pigs, the *FABP3* gene polymorphism determined by the *Hinf*I enzyme also included 2 genotypes TT and CC, and there was no association with intramuscular fat (p>0.05) (Xue et al., 2015).

This result was different from the studies of (Pang et al., 2006; Lee et al., 2010; Li et al., 2010; Han et al., 2012). According to research by Tyra et al., 2011 on 5 pig breeds Duroc, Pietrain, Puławska, Polish Large White (PLW) and Polish Landrace (PL), *FABP3* gene polymorphism was related to intramuscular fat.

Chen et al. (2014) studied 6 pig populations, including 2 native Chinese pig breeds (Yanan and Jinhua), 3 foreign pig breeds (Duroc, Landrace and Yorkshire) and 1 DLY crossbred, single nucleotide polymorphism on *FABP3* gene was detected by the restriction enzyme *Hinf*I, showing that the *FABP3/Hinf*I locus was associated with intramuscular fat of 2/6 studied pig breeds, namely DLY and Yanan.

We found that the gene polymorphisms of the candidate genes in our study had similar results to previous studies. However, in this study, we have not identified a significant association between candidate gene polymorphisms and intramuscular fat. This might be due to the small study population, the genotype diversity of the population was not considerable, in which there were 3 polymorphisms *FABP3/Hinf*I, *PLIN2/Mva1269*I and *ACSL4/Ras*I had two genotypes and polymorphism *FABP3/Bsrf*I had only one genotype resulted in too low frequencies of some alleles in the population to be unsuitable for association assessment.

In this study, intramuscular fat in Duroc pigs was similar to the published study of Le Trong Dai et al. (2014) surveyed on the intramuscular fat in Duroc purebred pigs, which showed that the highest percentage of intramuscular fat in Duroc breed was 2.98%. When increasing the slaughter weight from 95 - 110 kg to 111 - 125 kg, the intramuscular fat increased significantly. At the same time, barrow pigs had a higher intramuscular fat than that of female pigs.

3.6.SELECTION OF DUROC HERDS FOR GROWTH PERFORMANCE BY GENOTYPE

From the results of analysis of the linkage of some candidate genes, the study selected pigs in the direction of growth performance which carried both 2 genotypes AA (*MC4R* gene) and AA (*PIT1*gene). The growth performance of pigs carrying these genotypes was monitored and evaluated through 2 generations.

Growth performance of the 1st and 2nd generation Duroc pigs after being selected to carry genotype AA (*MC4R*) and genotype AA (*PIT1*) was shown in Table 3.26.

Table 3.26. Growth performance of the 1st generation Duroc pigs carrying genotype AA (*MC4R*) and genotype AA (*PIT1*)

Paremens	Generation 1		Generation 2	
	n	Mean ± SE	n	Mean ± SE
Pre-test weight (kg)	60	29.45 ± 0.17	120	29.03 ± 0.15
Post-test weight (kg)	60	97.18 ± 0.16	120	102.24 ± 0.27
Growth performance (g per day)	60	962.37 ± 3.47	120	1015.00 ± 4.28
Backfat thickness (mm)	60	11.00 ± 0.01	120	10.78 ± 0.03

In the same indicator, LSM values have different letters, the difference was statistically significant ($p<0.05$)

The results in Table 3.26 showed that the 1st generation Duroc pigs carrying both two genotypes AA (*MC4R* gene) and AA (*PIT1* gene) have high growth rate with an growth performance of 962.37 g per day. The 1st generation Duroc pigs carrying both two genotypes

AA (*MC4R* gene) and AA (*PIT1* gene) had higher growth performance than the standard for foreign-breed pigs specified in Decision No. 675/QD-CN-BNN (2014) of the Ministry of Agriculture and Rural Development regulating growth performancein in pigs (≥800 g per day). Compared with growth performance of the population before selection (809.04 g/day), the first generation was significantly higher (962.37 g per day).

From the results of the evaluation of the growth performance of Duroc pigs in the 2nd generation , the study selected 120 Duroc pigs (20 males and 100 females) carrying both two genotypes AA of MC4R and AA of PIT1 which had the highest growth rate, with growth performance of 1015.00 g/day.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

1. Candidate genes *MC4R, PIT1, GH, PIK3C3* and *ADRB3 were* highly polymorphic with 3 genotypes identified; *LEP, ACSL4, FABP3* (*Hinf*I) genes have moderate polymorphism with 2 genotypes; *FABP3* (*Bsrf*I) gene was not polymorphic with a single genotype in the population of Duroc pigs fed at Dabaco Nuclear Pig Breeding Co., Ltd.

2. The *MC4R, PIT1, GH* gene polymorphisms were strongly associated with growth performance and backfat thickness in both generations. The *LEP* gene polymorphism was strongly associated with growth performance in both generations but was not associated with backfat thickness. The *PIK3C3* gene polymorphism was not associated with growth performance and backfat thickness.

3. The *MC4R, PIT1, GH,* and *LEP* gene polymorphisms were not associated with reproductive performance. Therefore, the use of these candidate genes in breeding in the direction of growth performance, backfat thickness will not affect reproductive performance.

4. The *ADRB3, ACSL4, FABP3* (*Hinf*I) and *PLIN2* gene polymorphisms were not associated with intramuscular fat in the studied Duroc pig population.

5. Initially, the Duroc pigs carrying the AA genotypes of *MC4R* gene and AA of the *PIT1* gene which growth performance was 962.37 g/day in the 1st generation and 1015.00 g/day in the 2nd generation was selected (100 females and 20 males).

Recommendation

It was recommended to use Duroc pigs with genotype AA of *MC4R* gene, genotype AA of *PIT1* gene, genotype GG of GH gene and genotype CT of LEP gene because of its rapid growth rate and use of genotypes of those genes in Breeding program to improve growth performance in Duroc pigs.