MINISTRY OF EDUCATION AND TRAINING

MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT

NATIONAL INSTITUTE OF ANIMAL SCIENCE

NGUYEN VAN HOP

SELECTION INCREASES THE INTRAMUSCULAR CONTENT OF DUROC PIG BY COMBINING THE BLUP METHOD AND *H-FABP* GENE

BRIEF INFOMATION OF PhD THESIS

Major: Animal genetics and breeding
Code number: 9.62.01.08

HA NOI, 2023

The work was completed at: National Institute of Animal Science

Supervisors:

1. PhD. Nguyen Huu Tinh

2. Assoc. Prof. PhD. Ngo Thi Kim Cuc

Reviewer 1: Assoc. Prof. PhD. Nguyen Manh Ha

Reviewer 2: Assoc. Prof. PhD. Do Duc Luc

Reviewer 3: PhD. Pham Thi Kim Dung

The dissertation will be defended at the National thesis council at: National Institute of Animal Science

Time: …h date ….. Month …… year of 2023

The dissertation can be found at:

1. Library of NIAS

2. National library

LIST OF RELEVANT SCIENTIFIC PUBLICATIONS OF THE THESIS

1. Nguyen Van Hop, Nguyen Huu Tinh, Ngo Thi Kim Cuc, and Do The Anh (2023). Additive and dominant genetic effects of *H-FABP* Polymorphisms on intramuscular, backfat thickness, loin depth, and days to 100 kg traits in Duroc pigs. Journal of Animal Husbandry Sciences and Technics, No 288 (05.2023): 12-21.

2. Nguyen Van Hop, Nguyen Huu Tinh, Ngo Thi Kim Cuc, and Do The Anh (2023). Heritability and genetic trend of intramuscular fat, backfat thickness, and days to 100kg traits on Duroc pigs. Journal of Animal Husbandry Sciences and Technics, No 288 (05.2023): 21-28.

INTRODUCTION

1.1. The necessity of thesis

The best linear unbiased prediction (BLUP) is a statistical method used to improve reproductive and growth productivity in pigs. However, the performance improvement affected the fat content, thereby reducing the tenderness, juiciness, or flavor of pork (Purslow, 2017). Research showed that Duroc pigs are used to improve commercial pig performance without affecting meat firmness and reducing muscle fat levels (Tomović et al., 2016; Diao et al., 2018). Furthermore, this breed was used in the D(LY)/D(YL) hybrid formula due to its adaptability good feed conversion efficiency, and high meat quality (Diao et al., 2018; Ding et al., 2018).

The intramuscular fat content in pigs is highly heritable and correlated with other traits. The heritability of this trait ranged from 0.31-0.69 (Jiao et al., 2014; Ishii et al., 2018; Willson et al., 2020). The intramuscular fat content is correlated with a strong positive genetic with backfat thickness (Solanes et al., 2009; Schwab et al., 2009a) but it has been a negative genetic correlation with the lean meat percentage (Ros Freixedes, 2014; Ishii et al., 2018; Mikule, 2020). Therefore, in pig breeding programs, it was necessary considered selectively balance the intramuscular fat content and backfat thickness.

Recently, the Heart Fatty Acid-Binding Protein (*H-FABP*) gene with 3 polymorphisms *Hinf*I, *Msp*I, and *Hae*III has been used as a candidate gene in the selection and improvement of intramuscular fat content and pork quality (Gerbens et al., 1997; Lee et al., 2010; Kováčik et al., 2011, and Trakovická et al., 2016). In order to shorten the selection time to improve the intramuscular fat content in pigs without increasing back fat, we carried out the thesis: "*Selection increases the intramuscular content of Duroc pig by combining the BLUP method and H-FABP gene.*"

1.2. Objectives of study

(1) To evaluated the heritability and genetic correlation of intramuscular fat trait with back at thickness and day of 100 kg in Duroc pigs.

(2) To determine the frequency of genotype and the influence of *H-FABP* genotypes on the intramuscular fat content, backfat thickness, loin depth, and day of 100 kg in Duroc pigs.

(3) Advanced selective evaluation enhances intramuscular fat content in Duroc pigs.

(4) Evaluation of the effects of Duroc sire with different percentages of intramuscular fat content on growth and some meat quality of commercial pork.

1.3. The novelty of the study

The study combined the BLUP method and *H-FABP* gene to improve the intramuscular fat content but did not affect the backfat thickness in Duroc pigs in Vietnam:

(1) The study evaluated the interaction between three polymorphisms of the *H-FABP* gene (*Msp*I, *Hae*III, and *Hinf*I), thereby selecting three genotypes (AADDHH, AaDDHH, and AADdHH) that positively affected the intramuscular fat content;

(2) To selected Vietnamese Duroc pigs with high intramuscular fat content (3.26±0.32%), at the same time improved days of 100 kg (148.3±16.3 days) and still maintained backfat thickness (11.6±1.1 mm).

1.4. The academic and practical contributions of the thesis

1.4.1. Academic contributions

(1) The study estimated the genetic correlation between intramuscular fat content with the traits of back fat thickness and days to 100 kg, which served as a basis for selecting the best selection method to improve meat quality in Duroc pigs.

(2) This research has been provided some molecular information at three locations of *H-FABP* gene polymorphisms (*Msp*I, *Hae*III, and *Hinf*I) and their polymorphism association with intramuscular fat content, back fat thickness, loin depth, and days to 100 kg, which were a reference basis for further studies.

(3) The results of this study have supplemented material for teaching and research in the pig breeding program in Vietnam.

1.4.2. Practical contributions

(1) The study selected the Vietnamese Duroc pigs with the intramuscular fat content(3.26±0.32%) and simultaneously improved age at 100 kg (148.3±16.3 days) and maintain backfat thickness.

(2) Research has contributed to improving the quality of pork to meet consumer demand.

CHAPTER 1. REVIEW OF LITERATURE

1.1. Physiological basis of the intramuscular fat trait in pork

Intramuscular fat (IMF) is fat that is located inside and interspersed between muscle fiber cells. Most of the fat is accumulated in the final stages of the growth period. Phospholipids and triacylglycerols were the two main components of IMF (Poklukar et al., 2020). IMF in pork had an effect on the tenderness, juiciness, and flavor of meat (Cho et al., 2015; Gong et al., 2019). Currently, besides reproductive and growth performance, and meat quality the IMF has been used in pig breeding programs. Therefore, breeders need to evaluate the heritability of this trait in order to increase selection efficiency for swine lines with high IMF.

1.2. Inheritance ability of the intramuscular fat trait

Previous research results showed that the IMF trait in Duroc pigs had a high heritability (h2) and varied from 0.31 to 0.69 (Solanes et al., 2009, Ros-Freixedes et al., 2013, Ishii et al., 2018, Willson et al., 2020). The genetic correlation between IMF and the loin area in the carcass was between 0.3 and -0.34 (Solanes et al., 2009, Ishii et al., 2018). The strong correlation between IMF and backfat thickness (BF) ranged from 0.40 to 0.64 (Solanes et al. 2009; Reixach and Estany, 2010; Schwab et al., 2009b; Ros-Freixedes et al., 2013). Besides, the correlation between IMF and the loss of cooking, hardness of meat, and the number of dorsal vertebrae were also negative (-0.53; -0.37

and -0.61). The results indicated that improving the IMF will be increased the juiciness and tenderness of meat indirectly (Ishii et al., 2018; Willson et al., 2020; Mikule, 2020).

1.3 Molecular basis of intramuscular trait

In parallel with the development of biotechnology, the quantitative trait loci (QTL) affecting the IMF in pigs were discovered. In which, a number of genes associated with IMF have been identified and used such as *H-FABP*, A-FABP, ADRP, LERP, MC4R, ADD1 (SREBP1), and MYOD. According to Gerbens et al. (1999), *H-FABP* gene polymorphisms also affected BF in purebred Duroc pigs but these effected also manifest independently.

1.4 The approaches in improving intramuscular in pigs

1.4.1. Quantitative genetic method

Because the heritability of the IMF trait was high (range 0.31 - 0.69), direct selection programs based on the phenotypic value of the IMF trait could yield the desired selection response. However, genetic correlation was not expected between IMF and some other meat quality traits such as BF. In addition, the degree of accuracy of currently used IMF measurement and identification methods should be considered in select programs.

1.4.2 Molecular genetic method

Marker genes could provide new solutions for improving IMF and pork quality traits (Balnikov et al., 2021; Tinh et al., 2021; Balatsky et al., 2022; Wang et al. ., 2022). The *H-FABP* gene was a member of the FABP family that played an important role in fatty acid transport in muscle by binding and regulating fat in the muscle. In pigs, this gene is located on chromosome 6. The *H-FABP* gene with 3 polymorphisms *Hinf*I, *Msp*I, and *Hae*III has been identified for many pig populations (Chen et al., 2014). This gene is currently used as a candidate gene to improve IMF and pork quality (Gerbens et al., 2000; Kováčik et al., 2011; Trakovická et al., 2016).

1.5. Effect of pig breed on intramuscular trait

The IMF in pork depended on many different factors such as breed, sex, nutrition, and feeding, in which breed played a very important role (Benítez et al., 2019; Huang et al., 2020; Kim et al., 2020; Mikule, 2020). Among the industrially raised pig breeds, the Duroc pig had the highest IMF with the exception of some indigenous pig populations (Casellas et al., 2013, Tomović et al., 2016, Diao et al., 2018, Meadus et al., 2018). Moreover, Duroc pigs are widely used in pig production because of their adaptability, good feed conversion ability, and high meat quality (Diao et al., 2018). This breed was used as a terminal sire to produce commercial pork DLY/DLY based on excellent performance in growth traits and feed conversion efficiency (Ding et al., 2018). Therefore, to improve IMF in commercial pork, it is needed to focus on research on the Duroc pig breed.

CHAPTER 2. MATERIALS AND METHODS

2.1. Time and location of study

The study was carried out from 2016 to 2021 at Binh Thang Pig Research and Development Center and the Department of Biotechnology and Microbiology - Institute of Animal Science for Southern Vietnam.

2.2. Material of study

- The duroc pig population which originated from the United States, Canada, Denmark, and Taiwan, were raised at the Binh Thang Pig Research and Development Center (including 12-15 sires and 90-100 sows/year).

- Data of individual performance test (IPT) recorded from 2011 to 2016 that were collected from 1779 heads (in which 693 males and 1086 females) of Duroc pigs - Nuclear herd consists of 30-32 sows and 5-6 males in each generation were selected.

- In the 3rd generation, 3 Duroc sires with different intramuscular fat content were chosen and mated with 9 LY/YL pigs and tested the yield of 90 individuals of progeny.

2.3. Contents of the study

2.3.1. Content 1: To estimated the heritability and genetic correlation of the intramuscular fat trait with day to 100 kg and backfat thickness in Duroc pig.

2.3.2 Content 2: Analysis of *H-FABP* gene polymorphism associated with traits of intramuscular fat, days to 100 kg, backfat thickness, and loin depth in Duroc pig.

2.3.3. Content 3: Evaluation and selection of nuclear Duroc herds based on the terminal sire index (TSI) combined with the *H-FABP* genotype.

2.3.4 Content 4: Investigate meat yield and intramuscular fat content in commercial pigs that are generated from Duroc sires with intramuscular fat content.

2.4. Methodology of research

2.4.1. Data collection

IPT data on boars and gilts Duroc pigs were collected from 2011 to 2016, the number of sires and sows in this period were 96 and 486 in this period. The number of progeny IPT was 1779 (693 young boars and 1086 gilts) heads. The data collected included: intramuscular fat, days to 100 kg, backfat thickness, and loin depth.

2.4.2. Individual performance test

2.4.2.1. Individual performance test

- In the starting generation, from the Duroc pig herd, for each litter 2 young boars and 4 gilts are selected for IPT. A total of 1,032 gilts with complete pedigrees were selected for ITP.

- In the 1st, 2nd and 3rd generations, ITP is performed similarly to the starting generation.

* ***Methods of individual performance test***

- IPT of male and female gilts: According to Vietnam Standard Number 3897-84, some contents have been changed to be suitable for the current pig breeding program.

- The indicators of IMF, BF, and LD were measured at the end of IPT finish at position P2 by ultrasound technique on Aloka SSD equipment, IMF was estimated using Biosoft Toolbox software (USA).

2.4.2.2. Method of data adjust

Day of 100 kg (D100) adjusted according to the recommendations of the American Federation of Pig Breeds Improvement (NSIF, 2002): $D100_{ĐC} = T_{TT} + [(P_{100} - P_{TT})(T_{TT} - a)/P_{TT}]$.

2.4.3. Blood sample collection and analysis of the H-FABP gene

2.4.3.1. Blood sample collection

704 individuals and 50 samples/generation from 1st to 3rd generation were collected blood sample. Blood samples were put into tubes with anticoagulants and stored at -4^{o}C degree.

2.4.3.2. DNA extraction of blood samples

Blood extraction were followed the instructions of the Bioline kit.

2.4.3.3. PCR-RFLP procedure

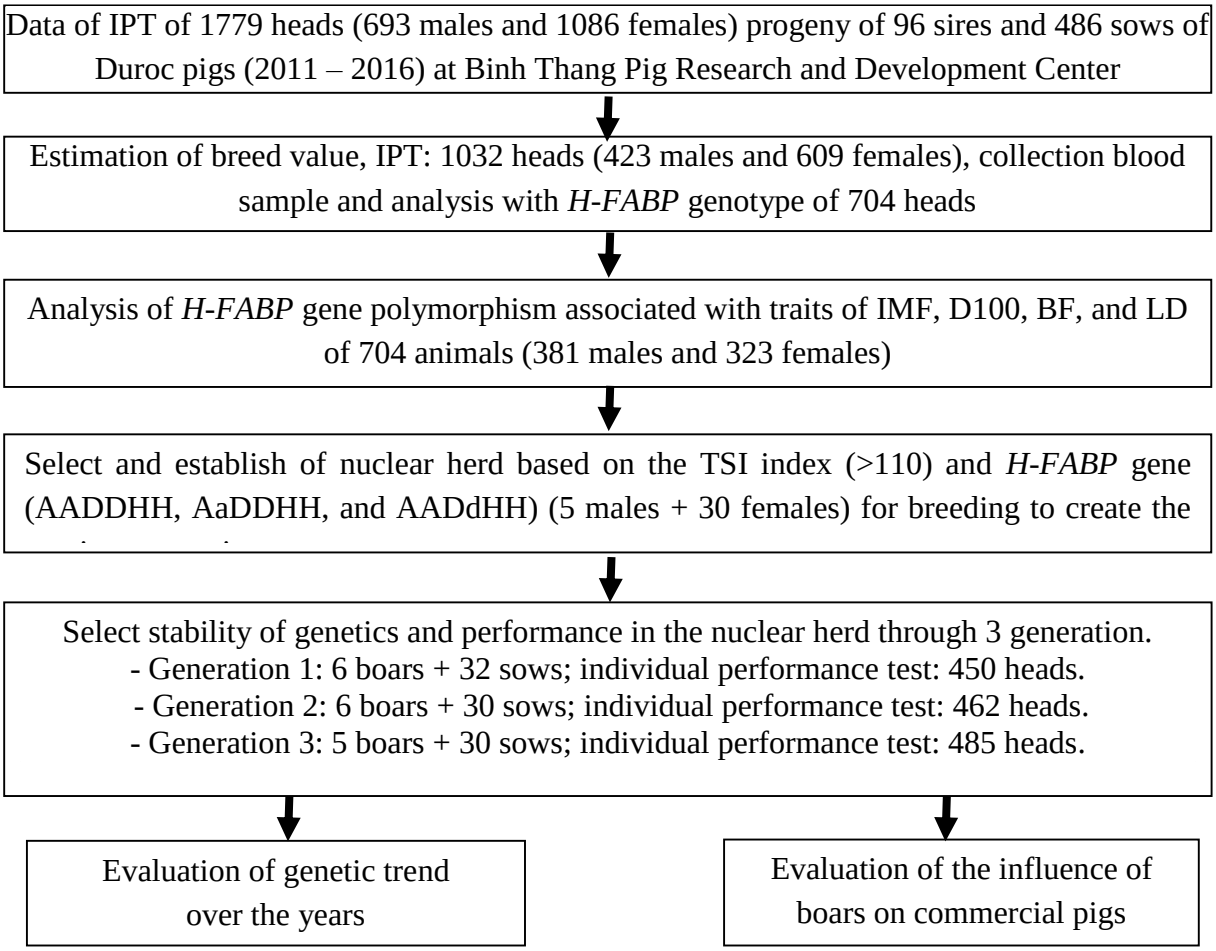
Perform PCR reactions with primer pairs was performed according to Gerbens et al. (1997) to clone the *H-FABP* gene fragment. The sequence of primer pairs was as followed:

- *Hinf*I: 5-GGACCCAAGATGCCTACGCCG-3’; 5’-CTGCATCTTTGACCAA GAGG-3’ (*Genbank X.98558.1*); *Msp*I, *Hae*III: 5’-ATTGCTTCGGTGTGTT TG AG-3’; 5’-TCAGGAATGGGAGTTATTGG-3’ (*Genbank X.98558.1*).

PCR products are checked by electrophoresis and observed under an ultraviolet lamp. Then, it is incubated with *Msp*I, *Hae*III, and *Hinf*I restriction-cutting enzymes. The cutting products were electrophoresed on 2% agarose gel and the results were read.

2.4.4. The method for setting up the nuclear herd

The method for setting up the nuclear herd are carried out according to the scheme 2.1:



2.4.5. Commercial pig examined methods

2.4.5.1 Experiment design

The experiment was designed with three treatments (3 groups), each group consisted of one Duroc sire with IMF of 3.1, 3.3 and 3.5% respectively, mated with three YL sows had an average IMF of 2.3% (from 2.2-2.5%). Thirty commercial pigs/group were conducted.

2.4.5.2. Nursing care

Commercial pigs' individual performance tests are raised according to the Binh Thang Pig Research and Development Center process.

2.4.5.3. Data collection

- Data collected includes: D100; BF; LD; IMF.

- Lean meat percentage (LMP) was estimated using the formula (Kyriazakis and Whittemore, 2006): LMP(%) = 59 - 0.9 x BF + 0.2 x LD (- 0.9 and 0.2 were coefficients; Backfat thickness and loin depth were located at P2 (10^{th} rib), (mm).

2.5. Statistical analysis

2.5.1. Estimation of genetic parameters and estimated breeding values for the traits of intramuscular, backfat thickness, and age days to 100 kg.

Genetic parameters were determined by the REML method and EBV were estimated by the BLUP method from VCE6 (Groeneveld, 2010) and PEST (Groeneveld, 2006) software.

For the trait of D100, BF, and IMF, the following genetic statistical model were used: $Y_{ijklm} = \mu + \alpha_i + \beta_j + HYS_k + a_l + e_{ijklm}$ *(where, y_{ijklm}: is the phenotypic value of the trait; μ: is the mean phenotypic value of the herd; α_i is the effect of type of housing (closed, open); β_j: is the effect of gender; HYS_k is the effect of herd x year x month; a_l is the additive genetic effect of the individual; e_{ijklm} is the random error).*

2.5.2. H-FABP gene polymorphism

2.5.2.1 Frequency determination of H-FABP genotype

The frequency of genotypic occurrence of survey individuals were calculated based on the Hardy-Weinberg law as follows: p = (2AA + Aa)/2n. *Where, p is the frequency of allele normal (A); q is the frequency allele mutation (a); n is the total number of individuals analyzed; AA is number of individuals with the dominant homozygous genotype; Aa is the number of individuals with heterozygous genotype.*

Check whether the *H-FABP* genotype frequency distribution is balanced according to the Hardy-Weiberg law by Chi-square test. (χ^2).

- The expected heterozygous frequency (He) was calculated by the formula of Nei (1978):

$$He = 1 - \sum_{i=1}^{n} p_i^2$$

p_i is the i^{th} allele frequency

- The polymorphism coefficient (PIC) was calculated according to Botstein et al (1980)

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

n: number of alleles; p_i is the i^{th} allele frequency

2.5.2.2. Determining the polymorphic association between H-FABP genotypes with the traits of intramuscular, backfat thickness, and age days to 100 kg.

The association of *H-FABP* genes polymorphism at 3 sites of cutting enzyme *Hae*III, *Msp*I, *Hinf*I to D100, BF, LD, and IMF traits were determined as follows:

Model 1: The association of each *Hae*III, *Msp*I, and *Hinf*I polymorphic site to the ratio of IMF, D100, BF, and LD were determined with the following linear model: $\mathbf{Y_{ij} = \mu + G_i + e_{ij}}$. *Where, Y_{ijkl}: is the genotypic value of the parameters (IMF, BF, LD, and D100), μ is the average of the observed breed herd, Gi is the influence of H-FABP genotype (with i = AA), Aa, aa with Msp*I *polymorphism; i = DD, Dd, dd with Hae*III *polymorphism; with i = HH, Hh, hh with HinfI polymorphism), e_{ij} = random error.*

Model 2: The association of 2 polymorphic sites combined from 3 polymorphic sites *Hae*III, *Msp*I, *Hinf*I, including *Hae*III-*Msp*I, *Hae*III-*Hinf*I, and *Msp*I-*Hinf*I, to the ratio of IMF, D100, BF, and LD were determined by model the same as model 1. Where Gi is the combined effect of 2 from 3 polymorphic sites (i=9).

Model 3: The association of 3 combined polymorphism sites (*Hae*III, *Msp*I, and *Hinf*I) to the ratio of IMF, D100, BF, and LD were determined by model the same as model 1. Where Gi is the genotype linked effect between the three polymorphic sites (i=16).

2.5.2.3 Estimation of additive and dominant trait effects

The additive effect (a) and dominant (d) for each trait was estimated using the GLM of the SAS 9.1 software. The values (1, 0,−1) and (0, 1, 0) were used to estimate the dominant traits and the additive effect of genotypes AA/DD/HH, Aa/Dd/Hh and genotypes aa/ dd/hh (Óvilo et al., 2002).

2.5.3. Estimation of the Genetic predisposition

Genetic propensity for traits D100, IMF, BF, and TSI index were evaluated based on the variation of mean values of breed (BV) and values of the TSI index by birth year of Duroc pigs from 2012 to 2021. Therefore, the trend of variation in mean values of breed for traits IMF, BF, D100 and TSI index have been shown in the graphs.

The mean annual genetic progress of each trait in Duroc pigs from 2012 to 2021 was evaluated through a linear regression analysis of the mean breed value. The mean value of TSI index of the group of individuals by year of birth was calculated by Scatter on Excel with the following model: y = bx + a. *Where, y is the mean breed value of the studies trait of a group of individuals born in the same year; a is constant; x is year of birth of a group individuals; b is regression coefficient*

2.5.4. Comparison of meat performance parameters of commercial crossbred pigs

Age reach 100 kg; Backfat thickness; Loin depth; Intramuscular and lean percentage of crossbreed combinations were compared by GLM model of SAS 9.1 software according to the following statistical model: $\mathbf{Y_{ijk} = \mu + D_i + TB_j + (D_i*TB_j)_{ij} + e_{ijk}}$ (*In which, Yijk is the phenotypic value of the observe traits (IMF, BF, LD, and D100), μ is the average commercial pig herd, Di is the influence of the boar (with i = D31, D33, and D35); TBj is the effect of sex (j = male, female); eijk = random error. Di*TBj: interaction between boar and sex)*

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Heritability and genetic correlation of intramuscular fat trait with growth and backfat thickness in Duroc pig herd

3.1.1 The influence of permanent factors in the statistical model for genetic analysis of yield traits

Table 3.1: Effects of permanent factors on studied traits in Duroc pigs

Traits	House	Sex	HYS
Day of 100 kg (day)	***	*	*
Backfat thickness (mm)	**	**	*
Loin depth (mm)	*	**	*
Intramuscular content (%)	**	ns	*

*Note: ***: P<0,001; **: P<0,01; *: P<0,05; ns:* non-significant*; HYS are effect of herd x year x month*

The permanent influence factors in the model of genetic statistical analysis are presented in Table 3.1. The results showed that the IMF ratio are not effected (P>0.05) but the D100 (P<0.05) and BF and LD traits (P<0.01) in Duroc pigs are effected. Meanwhile, the factors of herd breed, year of birth, and season (HYS) had been effected the observed traits (P<0.05).

3.1.2 Composition of variance and heritability of intramuscular fat, back fat thickness, and days to 100 kg traits

Table 3.2: Composition of variance and heritability of the traits of intramuscular fat (IMF), backfat thickness (BF) and age at 100 kg (D100)

Parameters	IMF **(n=1779)**	**BF (n=1779)**	**D100 (n=1779)**
Additive genetic effects (σ^2_A)	0.0765	10.9846	86.8428
Environmental effects (σ^2_E)	0.0543	11.0263	141.6813
Phenotypic variance (σ^2_P)	0.1308	22.0109	228.5241
Heritability (h^2±SE)	0.58±0.03	0.50±0.08	0.38±0.07

The components of variance and heritability of selected traits are demonstrated in Table 3.2. The results showed that the heritability of the trait of IMF and BF were high (0.58 and 0.50), while of the trait D100 was at medium level (0.38). Thus, the studied population of Duroc pigs had quite large genetic variation, especially in the trait of BF.

3.1.3. Genetic correlations between intramuscular fat and backfat thickness and age at 100 kg

Table 3.3: Genetic, environmental (r±SE) and phenotypic correlations between intramuscular fat, backfat thickness, and day to 100 kg traits

Pair of traits	**(r_A±SE)**	**(r_E±SE)**	**(r_P±SE)**
IMF – BF	0.63±0.20	0.13±0.07	0.62
IMF – D100	0.34±0.16	0.26±0.05	0.48
BF – D100	0.26±0.03	0.19±0.08	0.35

r_A: Genetic correlation, r_E: Environmental correlation, r_P: Phenotypic correlation

In general, genetic correlation, environmental correlation and phenotypic correlation among the three studied traits were all positive (Table 3.3). However, the level of correlation between these three components were different. The environmental correlation were loose between the IMF and BF and D100 traits (0.13- 0.26). Meanwhile, there was a strong genetic correlation between the ratio of IMF and BF traits (0.63). From the results of this study, it was necessary to select the balance between IMF and BF so that NAC could be maintained at a high level for Duroc pigs.

3.1.4. Estimated breeding value of selected traits on Duroc pig at the first generation

The results of EBV assessment of IMF, BF and D100 traits are listed in table 3.4. In trait D100, EBV varied widely from -8.88 to 6.30 (days) in males and -8.91 to 6.51 (days) in females. Mean EBV in males and females were -1.29 and -1.20 (day). Similarly, for the BF trait, EBV ranged from -2.36 and -2.61 to 1.12 and 1.02 mm in

males and females. This result showed that the variation between the maximum and minimum values of EBV in these two traits was very large. In addition, the mean EBV in both D100 and BF was negative, indicating the ability to select good growing individuals and high NAC. The EBV in the IMF trait was very large, some individuals had the IMF trait EBV approaching 2%. However, the average EBV of this trait in the population was positive in both males and females (0.13 and 0.12%). Thus, to improve the IMF ratio in Duroc pigs was not much of a problem, but it could affect other traits, especially BF, because these two traits were closely correlated as discuss about it in the upper part.

Table 3.4: *Estimated breeding value and terminal sire index at the first generation*

Traits	Number of boars (n=402)			Number of gilts (n=630)		
	Mean±SD	Min	Max	Mean±SD	Min	Max
D100 (day)	-1.29±3.13	-8.88	6.30	-1.20±2.53	-8.91	6.51
BF (mm)	-0.61±2.46	-2.36	1.12	-0.80±2.57	-2.61	1.02
IMF (%)	0.13±2.33	1.77	-1.52	0.12±2.50	1.88	-1.65
TSI	110.89±97.21	179.16	41.68	109.94±91.15	174.39	45.49

3.2. The *H-FABP* genes polymorphism associated with the intramuscular content, days to 100 kg, backfat thickness, and loin depth in Duroc pigs.

*3.2.1. H-FABP genotype at three polymorphism sites Hae*III*, Msp*I*, HinfI*

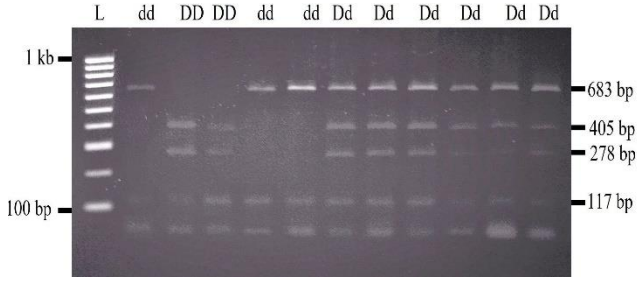


Figure 3.1: PCR-RFLP genotypes of *H-FABP*/*Hae*III

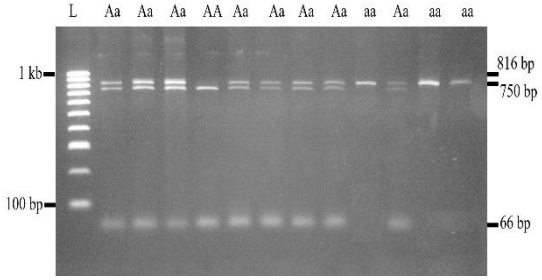


Figure 3.2: PCR-RFLP genotypes of *H-FABP*/*Msp*I

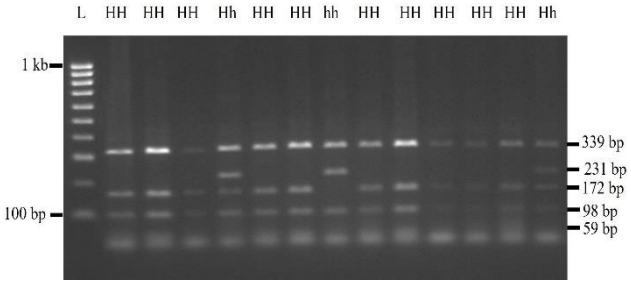


Figure 3.3: PCR-RFLP genotypes of *H-FABP*/*Hinf*I

The results of PCR-RFLP analysis of *H-FABP* gene polymorphisms at the polymorphism positions identified by *Hae*III, *Msp*I, and *Hinf*I showed that all genotypes DD, Dd, dd; AA, Aa, aa; HH, Hh, hh are appeared in the Duroc herds (Figures 3.1, 3.2, and 3.3). So, this gene may be evaluated and applied in pig breeding programs.

*3.2.2. Genotype frequencies of H-FABP at three polymorphism sites Hae*III*, Msp*I*, HinfI*

The genotype frequencies of *H-FABP* at three polymorphism sites *Hae*III, *Msp*I, and *Hinf*I in Duroc pigs are presented in Tables 3.5, 3.6, and 3.7. Overall, the frequency distribution of three *H-FABP* genotypes at the three sites of *Hae*III, *Msp*I, and *Hinf*I

polymorphisms in the surveyed breeding herd were different in observed value compare with the expected genotype frequency. Statistically, the investigated χ^2 values were higher than the theoretical χ^2 values (3.84). Thus, the frequency distribution of *H-FABP* genotypes at these 3 positions polymorphisms in the surveyed pigs was not balanced state according to the Hardy-Weinberg equilibrium. In other words, maybe some factors changed the genetic structure of this Duroc pig herd for the *H-FABP* genotype.

Table 3.5: Genotypic and allele frequencies of the *H-FABP*/*Hae*III gene polymorphism in Duroc pigs.

Items	Genotypes			Allele		He	PIC	χ^2 (3.841)	P
	DD	Dd	dd	D	d				
Number of sample	199	376	129						
Observed	0.283	0.534	0.183	0.550	0.450				
Expected	0.302	0.495	0.203			0.495	0.373	4.377	<0.05

For the frequency of the *H-FABP* genotype at the *Hae*III polymorphism site, the results of table 3.5 showed that the frequencies of alleles D and d surveyed as a whole herd were roughly equivalent (0.550 and 0.450). However, genotypes DD, Dd, and dd had a relatively large difference (Dd/54.3 %; DD/28.3% and dd/18.3%).

Table 3.6: Genotypic and allele frequencies of the *H-FABP*/*Msp*I gene polymorphism in Duroc pigs.

Items	Genotypes			Allele		He	PIC	χ^2 (3.841)	P
	AA	Aa	aa	A	a				
Number of sample	159	389	156						
Observed	0.226	0.553	0.222	0.502	0.498				
Expected	0.252	0.500	0.248			0.500	0.375	7.781	<0.01

For the frequency of the *H-FABP* genotype at the *Msp*I polymorphism site (Table 3.6). Alleles A and a were nearly equal in observed frequency in the surveyed pig herd (0.502 and 0.498), and the heterozygous genotype Aa (0.553) also had a much higher frequency of observations than the two homozygous genotypes AA (0.226) and aa (0,222).

Table 3.7: Genotypic and allele frequencies of the *H-FABP*/*Hinf*I gene polymorphism in Duroc pigs.

Items	Genotypes			Allele		He	PIC	χ^2 (3.841)	P
	HH	Hh	hh	H	h				
Number of sample	539	118	47						
Observed	0.766	0.168	0.067	0.849	0.151				
Expected	0.721	0.256	0.023			0.256	0.223	83.66	<0.001

For the frequency of the *H-FABP* genotype at the *Hinf*I polymorphism site (Table 3.7), the results showed that the frequency of the H allele is very high (0.849) compared to the h allele (0,151). Besides, the frequency of the HH genotype is higher (0.766) compared with Hh and hh genotypes (0.168 and 0.067). When combining *H-FABP* genotypes at all three polymorphism sites *Hae*III, *Msp*I, and *Hinf*I (table 3.8), The

results showed that a total of 25 out of 27 combined genotypes were present at all three polymorphic sites in the survey Duroc pig herd. When compared with some other studies, it can be seen that this study had a very high number of combinations of genes. Specifically, the study of Pang et al. (2006) found 9 genotype combinations, while Chen et al. (2014) identified only 8 genotypes and Uemoto et al. (2007) identified 10 genotype combinations.

Table 3.8: Frequencies of the *H-FABP* combine between three polymorphisms

TT	Genotypes	n	Ratio (%)	TT	Genotypes	n	Ratio (%)
1	AADDHH	19	2.6989	14	AaDdHh	56	7.9545
2	AADdHH	20	2.8409	15	AaddHh	2	0.2841
3	AAddHH	67	9.517	16	aaDDHh	8	1.1364
4	AaDDHH	36	5.1136	17	aaDdHh	1	0.142
5	AaDdHH	236	33.523	18	aaddHh	1	0.142
6	AaddHH	17	2.4148	19	AADDhh	2	0.2841
7	aaDDHH	126	17.898	20	AADdhh	2	0.2841
8	aaDdHH	12	1.7045	21	AAddhh	5	0.7102
9	aaddHH	6	0.8523	22	AaDDhh	1	0.142
10	AADDHh	1	0.142	23	AaDdhh	33	4.6875
11	AADdHh	14	1.9886	24	Aaddhh	2	0.2841
12	AAddHh	29	4.1193	25	aaDdhh	2	0.2841
13	AaDDHh	6	0.8523				
		Total				**704**	**100**

3.2.3. Association of the H-FABP genotypes and additive and dominance effects with the studied traits

3.2.3.1. Effects of H-FABP genotypes with research traits

Table 3.9. Effect of *H-FABP* genes at three *Hae*III, *Msp*I, and *Hinf*I polymorphic sites on studied traits

Parameters	***Hae*III**	***Msp*I**	***HinfI***
Day of 100 kg (day)	ns	**	ns
Backfat thickness (mm)	*	***	*
Loin depth (mm)	*	*	*
Intramuscular content (%)	**	**	**

****: P<0,001; **: P<0,01; *: P<0,05; ns:* non-significant.

The results of table 3.9 showed the influence of *H-FABP* genotypes on different traits at three polymorphic positions. The *H-FABP*/*Hae*III genotypes did not affect D100, BF traits (P>0.05), but effected LD and IMF traits (P<0.05). The *H-FABP*/*Msp*I genotypes effected all traits (P<0.05). Meanwhile, *H-FABP*/*Hinf*I genotypes affected all traits in this study with significant levels (P<0.5), especially IMF trait (P<0.01).

*3.2.3.2. Association of the H-FABP/Hae*III *genotypes and additive and dominance effects with the research traits*

The *H-FABP*/*Hae*III gene affected all traits except the D100 trait (table 3.10). The additive effect of DD genotype was very significant on the IMF trait (P<0.001).

IMF in DD genotype was higher than dd and Dd genotypes (0.75 and 0.45%) whereas BF of DD genotype was higher than genotypes Dd and dd by 1.08 and 0.88 mm, respectively. However, the effect of DD genotype was better than the Dd and dd genotypes on the LD trait. Thus, selection for the Dd genotype can reduced BF, while selection for the DD genotype ha beenthe potential improved the IMF but increased BF.

Table 3.10. Association of the *H-FABP*/*Hae*III genotypes and additive and dominance effects with the research traits (Mean±SE)

Items	DD (n=199)	Dd (n=376)	dd (n=129)	a	d
D100 (day)	152.1±6.6	151.9±6.4	151.0±5.6	-2.54±0.9	-0.19±0.6
BF (mm)	11.98^{a}±0.9	10.9^{b}±1.1	11.1^{b}±1.1	1.11***±0.15	-0.4***± 0.10
LD (mm)	$60.3^{a\pm}$2.9	$58.8^{b\pm}$2.9	$59.3^{b\pm}$2.8	1.70***±0.42	-0.59^{*}±0.29
IMF (%)	$3.37^{a\pm}$0.3	$2.92^{b\pm}$0.30	$2.62^{c\pm}$0.35	1.02^{***} ±0.04	0.05±0.03

****: P<0,001; **: P<0,01 Average values in each row with different superscripts is significantly different (P<0.05).*

*3.2.3.3. Association of the H-FABP/ Msp*I *genotypes and additive and dominance effects with the research traits*

The IMF of the aa genotype was higher than Aa and AA genotypes (0.26 and 0.31%). The additive effect (+0.45) and the dominance effect (-0.16) (table 3.11) were contributed to this result. The aa genotype can be used to improve IMF in Duroc pigs. However, if this genotype was selected, the BF and LD also increased (11.8 and 60.1 mm). In contrast, the AA genotype had a positive effected on growth when D100 in this genotype was the lowest (149.5 days).

Table 3.11. Association of the *H-FABP*/*Msp*I genotypes and additive and dominance effects with the research traits (Mean±SE)

Items	**AA (n=159)**	**Aa (n=389)**	**aa (n=156)**	**a**	**d**
D100 (day)	149.5^{b}±4.3	152.2^{a}±6.5	153.2^{a}±6.9	-4.65^{***}±0.91	0.90±0.63
BF (mm)	11.3^{b}±1.1	10.98^{c}±1.1	11.8^{a}±0.9	0.33^{*}±0.15	-0.30±0.10
LD (mm)	59.9^{a}±2.2	58.8^{b}±2.9	60.1^{a}±3.2	0.91*±0.42	-0.65^{*}±0.29
IMF (%)	2.96^{b}±0.47	2.91^{b}±0.32	3.22^{a}±0.28	0.45***± 0.04	-0.16***± 0.02

****: P<0,001; **: P<0,01 Average values in each row with different superscripts is significantly different (P<0.05).*

3.2.3.4. Association of the H-FABP HinfI genotypes and additive and dominance effects with the research traits

Table 3.12 showed that the dominant effected action the T100 trait at a significant level (P<0.05), while the additive effected did not affect this trait (P>0.05). The hh genotype has been T100 lowest (149.0 days) compared with the two genotypes HH and Hh (152.5 and 150.8 days). Similarly, the Hh genotype has a favorable effect on the BF trait, and the BF of genotype Hh was lower by 0.5 and 0.6 mm compared to HH and hh genotypes, respectively. Dominant influence trait will be increase the IMF (P<0.001). Therefore, selecting the HH genotype, the IMF in pigs is significantly improved compared with the 2 genotypes Hh and hh (3.05% versus 2.78 and 2.82%).

Table 3.12. Association of the *H-FABP*/*HinfI* genotypes and additive and dominance effected with the research traits (Mean±SE)

Items	HH (n=539)	Hh (n=118)	hh (n=47)	a	d
D100 (day)	152.5^{a}±6.1	149.0^{b}±6.2	150.8^{a}±6.7	1.64±0.94	-2.19^{*}±0.73
BF (mm)	11.3^{a}±1.1	10.8^{b}±1.1	11.4^{a} ±1.1	-0.38^{*}±0.16	-0.5***± 0.1
LD (mm)	59.3^{ab}±2.9	59.8^{a}±2.7	58.6^{b} ±2.9	0.27±0.44	0.80^{*}±0.34
IMF (%)	3.05^{a}±0.38	2.78^{b}±0.34	2.82^{b} ±0.32	0.13^{**}±0.04	-0.13^{***}±0.03

****: P<0,001; **: P<0,01 Average values in each row with different superscripts is significantly different (P<0.05).*

The hh genotype was LD the lowest (58.6 mm) and was the highest in the Hh genotype (59.8 mm). This association was further confirmed when the dominant effect of this trait reached 0.8 mm (P < 0.05). Similarly, in the IMF trait, Hh and hh genotypes have beenthe lowest effected on the IMF trait (2.78 and 2.82%), while the HH genotype has a good effected on this trait (3.05%). This result was due to the superior effect of reducing the IMF by 0.13% and this effect was very significant (P<0.001). Therefore, the HH genotype was likely to improved IMF in Duroc pigs in this study.

*3.2.4. Association of H-FABP genotypes combined Hae***III***, MspI, and HinfI polymorphic sites with research traits*

*3.2.4.1. Effect of H-FABP genotypes combined Hae*III*, MspI, and HinfI polymorphic sites with research traits*

Table 3.13. Effect of *H-FABP* genotypes combined *Hae*III, *Msp*I, and *Hinf*I polymorphic sites with research traits

Items	*Hae*III-*Msp*I	*Hae*III- *Hinf*I	*Msp*I- *Hinf*I
Day of 100 kg (day)	**	***	*
Backfat thickness (mm)	*	*	*
Loin depth (mm)	*	*	*
Intramuscular content (%)	*	*	*

****: P<0.001; **: P<0.01 *: P<0.05;*

Table 3.13 showed that combining genotypes between 2/3 polymorphic positions, there were a significant effect (P<0.05 and P<0.001) on the studied traits. Specifically, the combination of *H-FABP*/*Hae*III and *H-FABP*/*Msp*I genes was significant with the D100 trait (P<0.01), BF, LD, and IMF traits (P<0.05). Similarly, the combined genotype of *H-FABP*/*Hae*III and *H-FABP*/*Hinf*I genes had a significant effected on the D100 trait (P<0.001) and the other three traits (P<0.05). The significant effect (P<0.05) of the combined genotype of *H-FABP*/*Msp*I and *H-FABP*/*Hinf*I genes was significant in all four studied traits (D100, BF, LD, and IMF).

3.2.4.2. Association of the *H-FABP* genotypes combined *Hae*III and *Hinf*I polymorphic sites with research traits

A total of 9 *H-FABP* genotypes combine 2 polymorphisms *Hae*III and *Hinf*I which have been different effected on the IMF trait (table 3.14). The two genotypes

DDHH and DDhh have been had the highest IMF (3.41 and 3.27%) and higher than the average of the Duroc pig herd (2.99%). The other genotypes all have been had IMF lower than the average of the herd and have not recommended for selection to improve the IMF.

Table 3.14: Association of the *H-FABP* genotypes combined *Hae*III and *Hinf*I polymorphic sites with research traits (Mean±SE)

Genotypes	*n*	D100 (days)	BF (mm)	LD (mm)	IMF (%)
DDHH	181	152.4^{b}±6.4	12.1^{a}±0.8	60.3^{abc}±2.9	3.41^{a}±0.28
DDhh	3	158.5^{a}±9.3	11.9^{ab}±1.6	59.4^{bcd}±2.5	3.27^{a}±0.42
DDHh	15	147.2^{cd}±6.0	11.2^{bc}±0.9	61.0^{ab}±2.6	2.95^{b}±0.16
DdHh	71	150.8^{bc}±6.4	10.9^{cd}±1.1	60.3^{abc}±2.7	2.94^{b}±0.27
DdHH	268	152.4^{b}±6.3	10.9^{cd}±1.0	58.5^{cd}±2.8	2.92^{b}±0.25
Ddhh	37	149.9^{cd}±6.6	11.2^{bc}±1.0	57.9^{d}±2.8	2.87^{bc}±0.23
ddHH	90	152.8^{b}±5.1	11.2^{bc}±1.1	59.6^{abcd}±2.9	2.73^{c}±0.31
ddHh	32	145.8^{d}±3.8	10.4^{d}±0.9	58.1^{d}±1.9	2.36^{d}±0.18
ddhh	7	152.0^{b}±5.0	12.3^{a}±1.0	61.8^{a}±1.8	2.36^{d}±0.21
Average		**151.8±6.3**	**11.3±1.1**	**59.3±2.9**	**2.99±0.39**

Average values in each row with different superscripts is significantly different (P<0.05).

3.2.4.3. Association of the *H-FABP* genotypes combined *Hae*III, *Msp*I polymorphic sites with research traits

Table 3.15: Association of the *H-FABP* genotypes combined *Hae*III, *Msp*I polymorphic sites with research traits (Mean±SE)

Genotype	n	D100 (days)	BF (mm)	LD (mm)	IMF (%)
AADD	22	149.8^{cd}±3.9	12.1^{ab}±0.9	61.1^{a}±1.5	3.51^{a}±0.38
AaDD	43	147.9^{d}±5.9	12.2^{a}±0.9	60.2^{ab}±2.0	3.49^{a}±0.32
AADd	36	148.3^{d}±4.4	11.4^{dc}±1.1	59.3^{bc}±1.2	3.39^{ab}±0.20
aaDD	134	153.8^{b}±6.4	11.9^{abc}±0.8	60.3^{ab}±3.3	3.31^{b}±0.26
AaDd	325	152.4^{bc}±6.3	10.8^{e}±1.0	58.8^{bc}±3.0	2.87^{c}±0.21
aaDd	15	149.0^{d}±9.7	11.6^{bcd}±1.2	58.4^{c}±3.3	2.81^{cd}±0.20
AAdd	101	149.8^{cd}±4.3	11.1^{ed}±1.1	59.9^{abc}±2.6	2.69^{d}±0.31
aadd	7	149.5^{cd}±4.0	11.5^{dc}±0.9	59.9^{abc}±1.1	2.34^{e}±0.15
Aadd	21	157.2^{a}±7.5	10.7^{e}±1.1	56.7^{d}±2.8	2.33^{e}±0.20
Average		**151.8±6.3**	**11.3±1.1**	**59.3±2.9**	**2.99±0.39**

Average values in each row with different superscripts is significantly different (P<0.05).

In Table 3.15, when analyzing *H-FABP* genotypes combining two polymorphisms *Msp*I and *Hae*III, the results showed that there were 4 genotypes with higher IMF than the average of Duroc pigs, including AADD, AaDD, AADd, and aaDD at 3.51, 3.49, 3.39, and 3.31% respectively. All other genotypes had IMF lower than the average of the whole herd. The results of this study showed that selecting for IMF ratio improvement based on the combined genotype of *H-FABP*/ *H-FABP*/*Msp*I and *H-FABP*/*Hae*III can improved growth rate, but it did not improve DML.

3.2.4.4. Association of the H-FABP genotypes combined MspI and HinfI polymorphic sites with research traits

In Table 3.16, when analyzing the combination of *H-FABP*/*Msp*I and *H-FABP*/*Hinf*I gene, only two genotypes aaHH and AAHH are given higher IMF than the whole herd average, 3.26 and 3.10% respectively. However, these two genotypes had relatively high DML. Furthermore, the T100 of the aaHH genotype is highest compared with the other genotypes. Therefore, it is been necessary to consider the TSI index before using this genotype in breeding.

Table 3.16. Association of the *H-FABP* **genotypes** combined *Msp*I and *Hinf*I polymorphic sites with research traits (Mean±SE)

Genotype	*n*	D100 (days)	BF (mm)	LD (mm)	IMF (%)
aaHH	144	153.5^{a}±6.4	11.9^{ab}±0.9	60.1^{abc}±3.2	3.26^{a}±0.34
AAHH	106	149.5^{abc}±4.0	11.5^{abc}±1.1	60.4^{abc}±2.1	3.10^{ab}±0.39
AaHH	289	153.0^{ab}±6.4	11.0^{bc}±1.1	58.5^{bc}±2.9	2.93^{bc}±0.35
aaHh	10	147.1^{c}±10.6	11.4^{abc}±0.8	61.1^{a}±3.0	2.87^{cd}±0.24
AaHh	64	149.8^{abc}±6.2	10.9^{c}±1.2	60.7^{ab}±2.7	2.84^{cd}±0.20
Aahh	36	149.6^{abc}±7.2	11.2^{bc}±1.1	58.1^{c}±2.6	2.83^{cd}±0.26
AAhh	9	154.0^{a}±1.2	12.2^{a}±1.0	61.7^{a}±1.4	2.81^{cd}±0.55
AAHh	44	148.3^{bc}±4.7	10.6^{c}±1.0	58.2^{bc}±1.8	2.67^{d}±0.48
Average		**151.8±6.3**	**11.3±1.1**	**59.3±2.9**	**2.99±0.39**

Average values in each row with different superscripts is significantly different (P<0.05).

3.2.4.5. Association of the *H-FABP* genotypes combined *Msp*I and *Hinf*I polymorphic sites with research traits

Table 3.17: Association of the *H-FABP* genotypes combined *Hae*III, *Msp*I, and *Hinf*I polymorphic sites with research traits (Mean±SE)

Genotype	n	D100 (days)	BF (mm)	LD (mm)	IMF (%)
AaDDHH	36	146.7^{cdef}±4.3	12.3^{ab}±0.8	60.3^{abcd}±1.8	3.60^{a}±0.19
AADDHH	19	149.2^{defg}±3.9	12.4^{a}±0.7	61.3^{abc}±1.4	3.53^{a}±0.40
AADdHH	20	144.7^{def}±1.0	11.5^{cd}±1.0	59.3^{cde}±1.0	3.46^{ab}±0.20
aaDDHH	126	154.5^{b}±6.0	11.9^{abc}±0.8	60.1^{abcde}±3.3	3.34^{b}±0.25
AADdHh	14	152.8^{bc}±2.4	11.2^{cdef}±1.1	59.0^{cde}±1.3	3.31^{b}±0.14
aaDDHh	8	143.3^{g}±0.7	11.4^{cde}±0.8	61.9^{ab}±2.2	2.95^{c}±0.19
AaDDHh	6	151.3^{bc}±6.7	11.3^{cde}±1.0	60.2^{abcde}±2.7	2.92^{c}±0.12
AaDdHH	236	153.4^{bc}±6.0	10.8^{def}±1.0	58.4^{de}±2.9	2.86^{c}±0.20
AAddHH	67	151.1^{bcd}±3.4	11.3^{cde}±1.0	60.6^{abcd}±2.3	2.87^{c}±0.20
AaDdhh	33	149.3^{cdef}±6.6	11.1^{cdef}±1.0	58.0^{e}±2.6	2.86^{c}±0.22
AaDdHh	56	149.9^{cde}±6.1	10.9^{def}±1.2	60.8^{abc}±2.8	2.85^{c}±0.20
aaDdHH	12	145.2^{fg}±4.3	11.6^{bcd}±1.4	59.5^{cde}±2.5	2.84^{c}±0.21
AAddhh	5	154.8^{b}±1.1	12.6^{a}±0.7	62.2^{a}±1.7	2.38^{d}±0.22
AAddHh	29	146.0^{efg}±3.9	10.4^{f}±1.0	57.9^{e}±1.9	2.35^{d}±0.18
AaddHH	17	160.5^{a}±3.4	10.7^{ef}±1.0	55.9^{f}±2.4	2.32^{d}±0.20
aaddHH	6	149.7^{cde}±4.4	11.6^{bcd}±1.0	59.8^{bcde}±1.1	2.32^{d}±0.15
Average		**151.8±6.3**	**11.3±1.1**	**59.3±2.9**	**2.99±0.39**

Average values in each row with different superscripts is significantly different (P<0.05).

The influence of genotypes combining the *Hae*III, *Msp*I and *Hinf*I polymorphism sites of the *H-FABP* gene was not been the same as for each polymorphism site or each pair of polymorphisms (table 3.17). A total of 5/16 genotypes had IMF higher than the average of the herd, these genotypes are arranged in order from high to low as follows: AaDDHH (3.6%), AADDHH (3.53%), AADdHH (3.46%), aaDDHH (3.34%), and AADdHh (3.31%) Besides, individuals with these genotypes had a large proportion in the Duroc pig herd (215 individuals). Therefore, these genotypes were need to consider selectively improving the IMF in Duroc pigs in this study. However, other selective traits were need to be consider, especially D100 and BF traits.

3.3. Results of selection of Duroc pig

3.3.1 Results of selection of the starting generation nuclear Duroc pig

Table 3.18: Results of selection combining TSI index and *H-FABP* gene in the starting generation nuclear Duroc pig

Genotype	Selected sires		Selected sows	
	n	TSI	n	TSI
AADDHH	0	-	5	148.6
AADdHH	2	152.5	10	150.4
AaDDHH	3	150.7	15	149.3
Average	**5**	**151.4**	**30**	**149.6**

TSI: Terminal sire index

A total of 5 males and 30 females were selected for this study by combining the TSI index and the *H-FABP* genotype (table 3.18). The nuclear Duroc herd consists of 5 individuals that have been selected with AADdHH and AaDDHH genotypes, while in the nuclear female herd, besides two AADdHH and AaDDHH genotypes, AADDHH genotype were used to select. All three genotypes had the best effect on IMF as discussed above. Besides, the TSI index was been also very high, an average of 151.4 in the male herd and 149.6 in the female nucleus were selected.

Table 3.19: Some performance indicators of the starting generation nuclear Duroc pig (Mean±SD)

Items	Population		Selected herd	
	Boars	Gilts	Boars	Gilts
n	402	630	5	30
D100 (day)	151.7±20.5	151.9±23.4	147.5±7.4	147.8±10.2
BF (mm)	11.2±3.6	11.3±3.8	11.9±0.9	11.7±1.4
LD (mm)	59.4±16.1	59.3±16.1	60.9±3.0	60.7±4.0
IMF (%)	3.01±0.7	2.98±0.7	3.51±0.5	3.45±0.4

Average values in each row with different superscripts is significantly different (P<0.05).

In Table 3.19, the results of the selected nuclear Duroc herd showed that there was not significant difference between males and females for all four traits studied in both the population herd (unselected) as well as the selected herd. Traits in unselected

herds were as followed: T100 was 151.7 and 151.9 days; DML was 11.2 and 11.3 mm; DT was 59.3 and 59.4mm; IMF was 3.01 and 2.98% in males and females respectively. By selection method combining the TSI index and *H-FABP* genotype, the growth and meat quality of the nuclear Duroc herd were significantly higher than the unselected herd, especially for IMF and D100 traits. Compared with the unselected herd, the IMF was 0.5% higher in males and 0.47% in females; D100 is decreased by 4.2 days in males and 4.1 days in females; LD was 1.5 mm higher in males and 1.4 mm in females.

3.3.2. Performance of selected Duroc pig through 3 generations

In general, the studied traits are improved markedly over three generations except for BF. After three generations, D100 has been decreased by 3.5 days, LD has been increased by 2.2 mm, and especially IMF has been increased by 0.27% in Duroc pigs. In the 1st, 2nd, and 3rd generations D100, LD, and IMF were been higher than the start generation ($P<0.05$) while the DML trait was not improved.

Table 3.20 also showed that the coefficients of variation (CV) among individuals is tended to decrease gradually from the start generation to the 3rd generation for all four studied traits: D100, BF, LD, and IMF. The IMF is decreased from 13.0% in start generation to 9.8% in the 3rd; The D100 has been reduced from 14.8% in start generation to 11.0% in the 3rd. Similarly, BF and LD were had CV values of 12.4 and 10.3% respectively in start generation decreasing to 9.5 and 8.6% in the 3rd. Thus, after 3 generations of selecting Duroc pigs, the studied traits all had CV not exceeding 11.0% in the 3rd generation. In other words, in the study, the Duroc pig herd was being become more and more stable.

Table 3.20: Days to 100 kg, backfat thickness, loin depth, and intramuscular fat content of selected Duroc pig through 3 generations

Items	SG (n=1032)		G1 (n=1450)		G2 (n=462)		G3 (n=485)	
	Mean±SD	CV(%)	Mean±SD	CV(%)	Mean±SD	CV(%)	Mean±SD	CV(%)
D100 (day)	151.8^{a}±22.5	14.8	148.7^{b}±19.2	12.9	148.5^{b}±15.9	10.7	148.3^{b}±16.3	11.0
BF (mm)	11.3±1.4	12.4	11.7±1.2	10.3	11.5±1.3	11.3	11.6±1.1	9.5
LD (mm)	59.3^{b}±6.1	10.3	60.1^{ab}±5.5	9.2	60.4^{a}±5.3	8.8	61.5^{a}±5.2	8.6
IMF (%)	2.99^{b}±0.39	13.0	3.23^{a}±0.35	10.8	3.24^{a}±0.34	10.5	3.26^{a}±0.32	9.8

Average values in each row with different superscripts is significantly different (P<0,05); SG, G1, G2 và G3: Start, 1, 2, and 3 generations

3.3.3. Genetic stability of selected traits over three generations

The heritability of the IMF trait was decreased from the start generation (0.58) to the 3rd generation (0.50) (table 3.21). This showed that the variation between individuals in the selective Duroc herd of the IMF trait was increasingly narrowing both genetically and phenotypically after 3 generations of selection. However, the heritability of this trait is still classified as highly heritable. Thus, the selective program is applied in the study was effective for the IMF in Duroc herds.

Table 3.21: Variance components and heritability of intramuscular fat trait in Duroc pigs through three generations

Parameters	SG	G1	G2	G3
Additive genetic effects (σ^2_A)	0.0765	0.0637	0.0598	0.0539
Environmental effects (σ^2_E)	0.0543	0.0524	0.0528	0.0532
Phenotypic variance (σ^2_P)	0.1308	0.1161	0.1126	0.1071
Heritability (h^2±SE)	0.58±0.04	0.55±0.05	0.53±0.06	0.50±0.08

SG, G1, G2 và G3: Start, 1, 2, and 3 generations

Table 3.22: Variance components and heritability of backfat thickness trait in Duroc pigs through three generations

Parameters	SG	G1	G2	G3
Additive genetic effects (σ^2_A)	10.9846	11.8675	11.7624	11.4571
Environmental effects (σ^2_E)	11.0263	11.0791	11.0568	11.1023
Phenotypic variance (σ^2_P)	22.0109	22.9466	22.8192	22.5594
Heritability (h^2±SE)	0.50±0.01	0.52±0.02	0.52±0.08	0.51±0.05

SG, G1, G2 và G3: Start, 1, 2, and 3 generations

The heritability of the BF trait was almost unchanged through 3 generations and remained high (table 3.22). This result showed that there was not improved in BF in the selected herd. For the D100 trait, the results in table 3.23 showed that the heritability of this trait in the 3rd generation was 0.34 and lower than in the start generation (0.38). It can be seen that over three generations of selection for this trait has been gradually narrowed the genetic and phenotypic variation among individuals in the Duroc pig herd.

Table 3.23: Variance components and heritability of days to 100 kg trait in Duroc pigs through three generations

Parameters	SG	G1	G2	G3
Additive genetic effects (σ^2_A)	86.8428	83.3756	76.3612	75.2688
Environmental effects (σ^2_E)	141.6810	140.9880	141.9899	142.5347
Phenotypic variance (σ^2_P)	228.5241	224.3635	218.3511	217.8035
Heritability (h^2±SE)	0.38±0.11	0.37±0.05	0.34±0.06	0.34±0.05

Note: SG, G1, G2 và G3: Start, 1, 2, and 3 generations

Thus, after three generations of selection, the two traits IMF and D100 were relatively stable in genetics and phenotypic value. Particularly for the BF trait, it is been necessary to continue to select based on the TSI index to gradually improve the phenotypic value of this trait in the next generations.

3.3.4. Genetic trend of selected traits in Duroc pig

3.3.4.1. Genetic trend of intramuscular fat trait

The genetic trend of the IMF trait was tend to increased gradually from 2012-2021 (Figure 3.4). However, the EBV of the traits was tend to increased and decreased uniformly over the years. From 2012 to 2013, the genetic trend of IMF trait was decreased and was started to increase in the 2014-2015 period. In the 2016 - 2021 period,

the rate of genetic improvement of IMF trait was been fast and gradually stable. Specifically, in the 2016 - 2017 period, EBV was increased by 0.2%, in the period from 2018 - 2021 EBV was been always stable at a high level (0.36 - 0.37%). To obtain this result, the study wasused a combination of both the BLUP selection method and combined with *H-FABP* genotype.

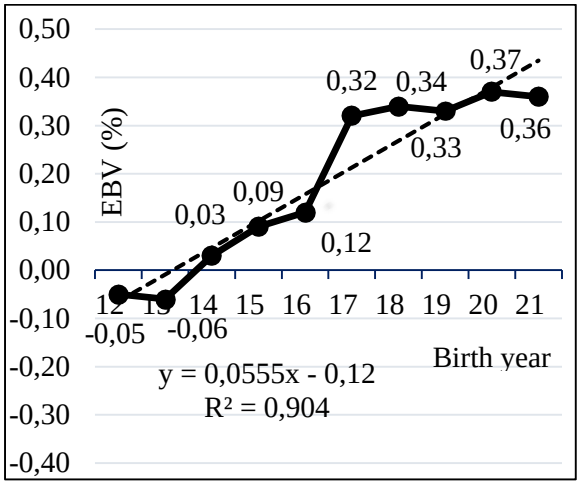


Fig 3.4: *Genetic trend of intramuscular fat trait*

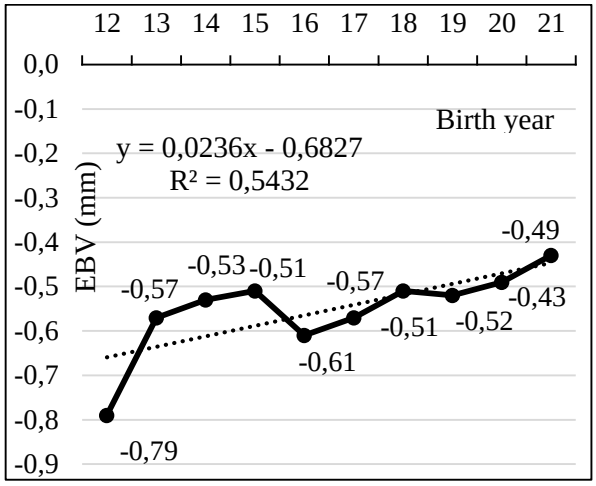


Fig 3.5: *Genetic trend of backfat thickness trait*

3.3.4.2. Genetic trend of backfat thickness trait

Figure 3.5. showed that before 2013 the EBV of the BF trait was very good (-0.79 mm). At the next stage, the efficiency of this trait selection was tends to decrease as EBV increases gradually (from -0.79 to -0.57). In the next stages, the EBV of this trait has been insignificant changed and was relatively stable, but EBV was been always negative. The reason for this result was that from 2014 to 2021, the IMF trait was interested and selected. When selecting for IMF, BF will tend to increased because these two traits are strongly positively correlated (Solanes et al., 2009; Schwab et al., 2009a).

3.3.4.3. Genetic trend of days to 100kg

The genetic trend for the D00 trait is shown in Figure 3.6. In general, EBV from 2012 to 2021 was not followed a certain rule. From 2012 to 2015, the selection efficiency for this trait was not been high. However, the down ward trend (2015 - 2017), or in other words the ability to reduce the number of days reaching D100 was been effective. In the fourth stage, the D100 trait EBVwas followed a horizontal line. This result was possible because at this stage D100 not a preferred trait for selection.

3.3.4.4. Genetic trend of TSI index

For the TSI index, figure 3.7 showed that the coefficient of the positive regression equation (2.8695) and the coefficient of determination (R^2) was high (0.8071), indicating that the selectivity index has been increased over the years. The TSI index has been increased and stabilized in the period from 2017 to 2021 (121.99-126.19), the average TSI index was reached 125.05 in the whole period of selection.

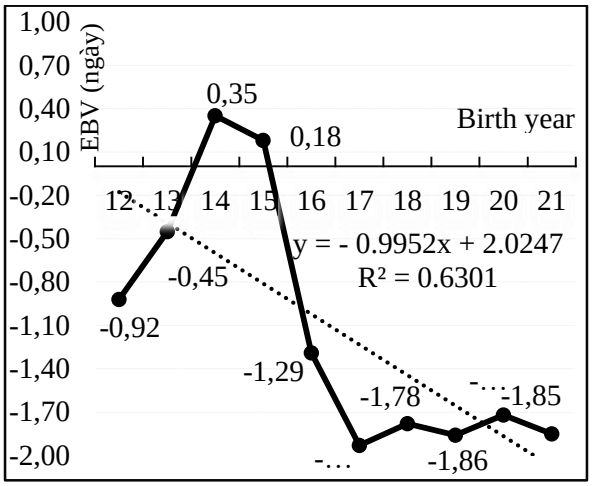


Fig 3.4: *Genetic trend of days to 100kg*

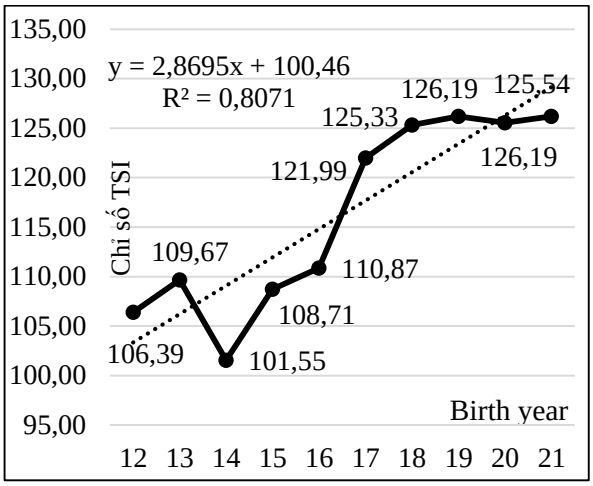


Fig 3.5: *Genetic trend of TSI index*

In summary, selected traits always have been had a positive or negative genetic correlation, so when the target of selection were focused on a certain trait, it can affected other traits in a positive or negative way. In this study, selective targeting were focused on improving IMF in Duroc pigs. Therefore, the growth and BF in this pig herd were more or less affected by the selection of genotypes with high IMF. Therefore, it is been necessary to continue, maintain, and select based on the TSI index with T100, BF, and IMF traits in the next generations so that genetic advancement of all three traits can be achieved simultaneously.

3.4. Effect of Duroc boars with different intramuscular content on commercial pig

3.4.1. The influence levels of Duroc boars with different intramuscular content and sex on commercial pig

The results of table 3.24 showed that boars with different IMF were affected D100, BF, LD, IMF, and LMP in commercial pigs to different levels. Specifically, Duroc boars significantly were affected D100 and LD traits (P<0.05) and were very significant in BF, IMF, and LMP (P<0.001). Sex only wasaffected LMP trait (P<0.05) and was not affected most other traits (P>0.05). Besides, table 3.24 showed that there was not interaction between boar and sex on all traits in commercial pigs (P>0.05). On the other hand, there was not difference between the commercial male and females in this study.

Table 3.24: The influence levels of Duroc boars with different intramuscular content and sex on commercial pig

Items	Boars	Sex	Boar x Sex
Day of 100 kg (day)	*	ns	ns
Backfat thickness (mm)	***	ns	ns
Loin depth (mm)	**	ns	ns
Intramuscular content (%)	***	ns	ns
Lean percentage (%)	***	*	ns

(*): P<0.05; (**): P<0.01; (***): P<0.001; (ns) non-significant.

3.4.2. Effect of Duroc boars with different intramuscular content on commercial pig

For the MG trait, the results of table 3.25 showed that the boar is played an important role in improving the IMF in commercial pigs. The IMF is increased from D31 boar to D35 boar (2.83 - 3.12%). The IMF in commercial pigs in D35 boar was been higher than that of D33 and D31 0.18% and 0.29%, respectively. For BF, boars with high IMF will be increased BF in commercial pigs. The BF of the offspring of the D35 boar was been higher than that of the D31 and D33 boar offspring by 0.60 and 0.93 mm, respectively.

The results of table 3.25 showed that the D100 of commercial pigs aretends to increased gradually according to the IMF of boar. The commercial pigs of D31 boar were the fastest growing (147.1 days) and wereslowest in the commercial pigs of D35 boar (150.0 days). In contrast, LD is tended to decreased gradually from commercial pigs of D31 boar to D35 boar and the average decrease was 0.415 mm. LD values were lowest in males that were born from D33 boar (60.7 mm) and in females that were born from D35 boar (60.6 mm). Besides, there were a big difference between males and females generated from D33 males.

Table 3.25: Effect of Duroc boars with different intramuscular content on commercial pig (Mean ±SE)

Items	D31	D33	D35
Day of 100 kg (day)	147.1^{b}±0.58	148.1^{ab}±0.47	150.0^{a}±0.5
Backfat thickness (mm)	10.67^{c}±0.11	11.0^{b}±0.08	11.6^{a}±0.11
Loin depth (mm)	61.5^{a}±0.18	61.2^{ab}±0.2	60.7^{b}±0.2
Intramuscular content (%)	2.83^{c}±0.03	2.94^{b}±0.03	3.12^{a} ±0.01
Lean percentage (%)	61.8^{a}±0.1	61.1^{b}±0.1	60.3^{c}±0.1

Average values in each row with different superscripts is significantly different (P<0,05)

Table 3.26: Effect of Duroc boars with different intramuscular content and sex on commercial pig (Mean ±SE)

Items	D31		D33		D35	
	Male	Female	Male	Female	Male	Female
n	15	15	15	15	15	15
W_B (kg)	28.4±2.2	28.2±2.1	28.2±2.2	28.5±1.7	28.1±2.2	28.3±2.2
W_F (kg)	108.2±3.1	107.8±2.7	107.6 ±3.5	106.7±2.5	107.2±3.8	106.5 ±2.9
D100 (days)	146.9±1.2	147.3±1.6	147.3 ±2.1	147.5±1.8	148.9±1.3	149.0 ±1.1
BF (mm)	10.8^{bc}±0.6	10.6^{c} ±0.5	11.0^{b} ±0.3	10.9^{bc}±0.4	11.7^{a} ±0.6	11.5^{a} ±0.2
LD (mm)	61.6^{a} ±0.5	61.5^{ab}±0.5	60.7^{c} ±0.5	61.4^{ab} ±0.5	60.8^{bc}±0.7	60.6^{c} ±0.5
IMF (%)	2.86^{c}±0.07	2.80^{c}±0.06	2.98^{b}±0.14	2.90^{bc}±0.07	3.14^{a} ±0.08	3.06^{a}±0.10
LMP (%)	61.6^{ab}±0.1	62.00^{a}±0.1	61.1^{bc}±0.1	61.3^{bc}±0.1	60.6^{e}±0.2	60.8^{de}±0.2

D31, D33, D35: Duroc boar with intramuscular content 3.1; 3.3 và 3.5% respectively; Average values in each row with different superscripts is significantly different (P<0,05); n: number of animal; W_B: Begin weight (kg); W_F: Finish weight (kg); LMP (%): lean meat percentage.

While LD decreased, BF gradually increased in commercial pigs which were born from Duroc boars with different IMF. As a result, the group of commercial pigs of

the D35 boar had the lowest LMP among the surveyed 3 boars. Thus, LMP in commercial pigs was affected by boars with different IMF, in which LMP wa lowest in D35 boar (60.3%) and highest LMP in D31 boar (61.8%). When evaluating the influence of sex in the same experimental group, table 3.26 showed that IMF in castrated males was always slightly higher than in females, but these differences was not statistically significant (P>0, 05).

When comparing 3 boars (D31, D33, D35) of the same sex, the IMF gradually was increased from D31 male to D35 male. For males, the IMF increased gradually from 2.86% in D31 boar to 2.98% in D33 boar and 3.14% in D35 boar. Similarly in females, IMF gradually increased from 2.80% in D31 boar to 2.90% in D33 boar and 3.06% in D35 boar, all of these differences being statistically significant (P< 0.05). However, when the IMF increased, there was also an increased in DML from D31 boar to D35 boar, but there was only a statistically significant difference (P<0.05) when comparing group 3 boars with the other boar. At the same time, BF was always higher in males than in females, but the difference was not statistically significant.

In summary, in the present study, the use of Duroc boar with different IMF did not affect the growth and LD in commercial pigs but had a very clear effect on the IMF. At the same time, using Duroc males with high IMF will be increased BF and reduced LMP.

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

(1) In the Duroc pig in this study, intramuscular fat content and back fat thickness had high heritability (0.58 and 0.50) and were closely correlated with each other (0.63). The estimated breed values for the intramuscular fat content, back fat thickness, and days to 100 kg with large inter-individual variability provide a good opportunity to selectively improve these traits.

2) The *H-FABP* genotypes appeared fully at the three *Hae*III, *Msp*I, and *Hinf*I polymorphisms and were all associated with selected traits, including the intramuscular fat content and back fat thickness, and the days to 100 kg in the Duroc pig herd. Three *H-FABP* genotypes incorporating three polymorphisms, including AADDHH, AaDDHH, and AADdHH with the best effect on the intramuscular fat content were selected in the nuclear Duroc herd.

3) After three generations of selection combining terminal sire index (TSI) with *H-FABP* genotypes, nuclear Duroc pigs were selected with markedly improved selection traits, especially for intramuscular fat content (up from 2.99% to 3.26%). In addition, all three selection traits have begun to stabilize genetically and show a positive genetic predisposition, especially for the intramuscular fat content that achieves genetic progress of 0.047%/year.

4) In the commercial crossbreed system, when using Duroc boar with a higher intramuscular fat content, the better the intramuscular fat content in the commercial pig was improved, while at the same time, the days to 100 kg was not affected, but increased back fat thickness and reduced lean meat percentage.

4.2. Recommendations

It is necessary to continue to apply the selective method combining terminal sire index (TSI) with *H-FABP* genotype for this nuclear Duroc pigs herd in the next generations, in order to simultaneously improve the intramuscular fat content, and growth, and limit the effects effect on backfat thickness in Duroc pigs.