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Effects of Heart Fatty Acid-binding Protein Genotype on Intramuscular Fat Content in Duroc Pigs Selected for Meat Production and Meat Quality Traits

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ABSTRACT : Using multi-trait animal model BLUP, selection was conducted over seven generations for growth rate (DG), real-time ultrasound loin-eye muscle area (LEA), backfat thickness (BF), and intramuscular fat content (IMF) to develop a new line of purebred Duroc pigs with enhanced meat production and meat quality. This study was intended to investigate the relationship between restriction fragment length polymorphism (RFLP) of a heart fatty acid-binding protein (H-FABP) gene and intramuscular fat content (IMF) of this Duroc purebred population. The present experiment examined the RFLP of 499 slaughtered pigs. The DNA was separated from the blood or ear tissue of the pigs, which were slaughtered at 105 kg of body weight. Intramuscular fat content of the longissimus muscle was measured using chemical analysis. A significant difference was detected in the breeding value of IMF among the H-FABP PCR RFLP genotypes. The AA genotype has a significantly larger positive effect on the IMF breeding value than do the Aa and aa genotypes for the *Msp*I RFLP. In addition, the DD genotype has a significantly greater positive effect on IMF breeding value than the Dd and dd genotypes for the *Hae*III RFLP. For the *Hin*fI RFLP, the hh genotype has a significantly larger positive effect on IMF breeding value than the HH genotype. Multiple regression analysis was performed using the IMF breeding values as the dependent variable and the three H-FABP genotypes as independent variables. Results revealed that the contribution of the genotypes to variation in IMF breeding values was approximately 40%. These results demonstrated that H-FABP RFLPs affect IMF in this Duroc population. (**Key Words :** Duroc Pigs, H-FABP, Intramuscular Fat, RFLP)

INTRODUCTION

Meat quality traits, in addition to meat production traits, have become important traits for selection in pig breeding. Selection for meat production and meat quality (intramuscular fat content: IMF) traits was conducted using multi-trait animal model BLUP in Duroc pigs over seven generations (Suzuki et al., 2005). The average breeding value of IMF at the seventh generation (1.20%) was greater than the initially desired gain (0.7%). To improve meat quality traits such as IMF, via traditional methods of selection, it would be necessary to slaughter numerous pigs and accurately measure IMF. In contrast, DNA markers such as the heart fatty acid-binding protein (H-FABP) gene may be useful as markers to select for IMF content (Gerbens et al., 1999). This gene codes for the protein related to intracellular transport of fatty acids in skeletal muscle and plays an important role in lipid metabolism regulation. The different frequencies of restriction fragment length polymorphisms (RFLPs) produced by digestion with restriction enzymes *Msp*I, *Hae*III, and *Hin*fI among breeds (Gerbens et al., 1997) and polymorphisms at this locus have been associated with fatness traits in Duroc pigs (Gerbens et al., 1999). To date, a significant correlation between H-FABP gene polymorphisms and intramuscular fat content has been reported for an unselected Duroc population (Gerbens et al., 1999), a Meishan×Large white cross population (Gerbens et al., 2000), a Large white×Landrace cross population (Gerbens et al., 2001), and a Chinese native pig breed and four western pig breeds (Zeng et al., 2005). On the other hand, Nechtelberger et al. (2001) reported that no significant relationship exists between the

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H-FABP gene polymorphisms and the intramuscular fat content of the Austrian Pietrain, Large White, and Landrace breeds. Therefore, this study is intended to examine the relationship between RFLPs of the H-FABP gene and the IMF breeding value of Duroc pigs.

MATERIALS AND METHODS

Experimental animals

Duroc pigs used in this experiment were of a line that has been selected through seven generations from 1995-2001 at the Miyagi Prefecture Animal Industry Experiment Station. Selection criteria traits were daily gain from 30 kg to 105 kg body weight (DG), loin-eye muscle area (LEA) and backfat thickness (BF) at 105 kg body weight measured using ultrasound technology, and intramuscular fat content (IMF) measured in slaughtered sib pigs. The average population size of each generation was 15.6 boars and 44.5 gilts. Gilts farrowed only once and boars were retained for use for one 4-6 wk breeding period. Thereby, a new generation was obtained each year. Pigs were weaned at 4 wk. At 8 wk, one or two male piglets (total 50 piglets) and two to four female piglets (total 100 piglets) from each litter were selected as candidates for boars and gilts mainly based on body weight at 8 wk. In all, about 80 piglets comprising mainly boars and some gilts from each litter were selected for full-sib testing in each generation. This first stage of selection was conducted within litters. Boars for full-sib tests were subsequently castrated. Performance tests began when body weight reached 30 kg and ended at 105 kg. Therefore, DG was from 30 kg to 105 kg body weight. Backfat thickness (BF) and LEA of 105 kg animals were measured on the left side at the half position of length from the root of the ear to the root of the tail using an ultrasound (B-mode) color scanning scope (SR-100; Kaijo Corp., Tokyo, Japan). Computer software was used to determine LEA. Pigs were provided *ad libitum* access to a commercial diet (15% crude protein, 78% total digestible nutrients, 0.76% lysine content on a dry matter basis) during the testing periods from 30 kg to 105 kg of live weight. Pigs had free access to water. Boars were reared individually in performance testing pens. Gilts and barrows were reared in growing pens with group feeding in a concrete-floored building with eight pigs per pen, which allowed 1.2 m^2 of floor space for each pig.

Selection method

Details of the selection method were described by Suzuki et al. (2005). Objectives of this selection were to produce a Duroc line to be used as terminal sire to improve meat production and meat quality traits. Subsequently, these Duroc boars will be supplied to pork producers as commercial terminal sires. Because of the limited accommodation ability of facilities, selection was conducted without a control line. First and second generations of selection were performed using an index selection method based on relative desired gains (Yamada et al., 1975). Traits that we selected for were DG, LEA, BF, and IMF. Genetic and phenotypic parameters used to derive selection criteria were obtained, respectively, from performance test data of the first and second generations. Respective means of DG, LEA, BF, and IMF at the first generation were 865 g, 36.1 cm^2, 2.34 cm, and 4.3%. Relative desired gains were established respectively as 135 g, 3.9 cm^2, -0.54 cm, and 0.7% for DG, LEA, BF, and IMF. Consequently, the selection index equation was I = 0.038DG+1.38EM-15.10BF+12.63IMF-56.68. Selection was made within sire families for boars and within-litters for gilts at the first generation to avoid rapid loss of genetic diversity from the population. Breeding values of DG, LEA, BF, and IMF were estimated from the third generation onwards by multiple-trait, animal model BLUP. Breeding values were calculated using the PEST3.1 program (Groeneveld, 1990) after estimating genetic parameters using the VCE4.25 program (Neumaier and Groeneveld, 1998) with models including generation and sex as fixed effects and random effects of individual additive genetic effect and error. Relative economic weights of selection traits were calculated from the relative desired gains, which were established for DG, LEA, BF, and IMF from performance test data of the first generation, as described previously. Aggregate breeding values were calculated by multiplying the relative economic weights by the estimated breeding value of each trait; then the selection was executed. Relative economic weights of selection traits were calculated from the relative desired gain as follows. The selection index where the breeding goal is predetermined as intended genetic gains was proposed by Yamada et al. (1975) as

$$Q = G'Rb$$
$$b = (G'R)^{-1}Q \quad (1)$$

$$Pb = RGa$$
$$a = (RG)^{-1}Pb \quad (2)$$

From (1) and (2), the economic weight can be found assuming the desired gains index as:

$$a = (RG)^{-1}Pb = (RG)^{-1}P(G'R)^{-1}Q$$

where a is the economic weight, P represents the phenotypic variance, covariance matrix, G is the genetic variance, covariance matrix, R is the numerator relationship matrix, b is the weighting coefficient vector, and Q is the desired gain vector.

The relative desired gains of DG, EM, BF, and IMF were established from performance test data of the first generation, as described before. However, the breeding goals changed to 1,000 g, 40 cm^2, 2.0 cm, and 5.0% respectively for DG, EM, BF, and IMF. Therefore relative desired gain were 135 g, 3.9 cm^2, -0.34cm and 0.7%, respectively. The reason is that improvement of the intramuscular fat cannot be expected when the backfat thickness is thinned too much. When the IMF breeding goal is assumed as 6%, the weight to IMF becomes too high. Therefore, the respective economic weights were assumed to be 0.076, -0.391, -10.850, and 3.753 for DG, EM, BF, and IMF. However, the genetic parameters estimated at the third generation differed from those of the fifth and seventh generations. The relative economic weights obtained using these parameters were also different. Then, the relative economic weights obtained at the third generation were used for the third and fourth generation and those at the fifth generation were used for the fifth generation and afterwards. The aggregate breeding values were calculated by multiplying the relative economic weights by the estimated breeding value of each trait; then selection was executed. About 15 boars and 50 gilts were selected at each generation. Inbreeding coefficients for individual pigs were computed for each generation. Based on inbreeding information, all mating was planned to minimize the rate of increase in inbreeding.

Meat quality measurements

Although 543 full-sib (barrows and gilts) pigs were slaughtered in this experiment, DNA was preserved for only 499 pigs (367 barrows, 132 gilts). The pigs in the first to seventh generations numbered respectively 51, 86, 70, 80, 73, 69, and 70. All animals were slaughtered at 105 kg body weight using manual low voltage (200 V) electrical stunning 24 h after feed removal with free access to water. Processed dressed carcasses were placed in a refrigerator as soon as possible and stored at 4°C for 24 h. Subsequently, for measuring meat quality in the longissimus muscle, a 7-10 cm long piece of the loin (two thoracic vertebrae sections above the last rib) was taken from the left half of each pig carcass. Then the chops were moved to a laboratory to measure meat quality traits. External loin adipose tissue was removed. Two minced loin meat samples of approximately 20 g were analyzed using the Soxhlet method to determine IMF.

RFLP screening

The DNA was isolated from the blood of animals in the first and second generations and from ear tissue of animals in the third to seventh generations using a commercial kit. The PCR conditions and restriction digestions with *Msp*I, *Hae*III, and *Hin*fI were identical to those described by Gerbens et al. (1997).

Statistical analyses

Effects of the three H-FABP RFLP genotype classes on the mean breeding value of IMF were estimated as follows. First, breeding values for the IMF of 499 pigs were predicted using the PEST3.1 program (Groeneveld, 1990) and genetic parameters estimated for the entire selection population with the VCE4 program (Neumaier and Groeneveld, 1998). The mathematical model included fixed effects of generation and sex and the random effects of individual additive genetic effects and error. Secondly, these breeding values were analyzed using the GLM procedures of SAS (SAS Institute Inc., Cary, NC). The mathematical model included the fixed effect of each H-FABP RFLP polymorphism. A multiplex test was performed using Tukey's studentized range test.

The following multiple regression analysis was done to reveal the contribution of the H-FABP genotypes to the variance of the breeding values of IMF. First, two kinds of breeding values concerning IMF were estimated to elucidate the effect of H-FABP genotype on the IMF breeding value. One of them (BV1) was estimated considering the generation, sex, and genotypes of H-FABP as the fixed effects. The (BV2) was estimated considering only the generation and sex as fixed effects. The difference (BV3) between the two breeding values was inferred to be the effect of the H-FABP genotype. Then, multiple regression analysis was conducted assuming BV3 for the three H-FABP RFLP genotype classes as independent variables and BV2 as the dependent variable.

RESULTS

Selection for DG and real-time ultrasound measurement of LEA, BF, and IMF was conducted using multi-trait animal model BLUP in Duroc pigs over seven generations (Suzuki et al., 2005). The desired gains for DG, LEA, and BF were not achieved (average expected breeding value for DG, LEM and BF were 122.5 g, 1.73 cm^2, and 0.02 cm), but the average breeding value for IMF at the seventh generation (1.20%) became greater than the initially desired gain (0.7%). The mean IMF level reached approximately 5.0%.

Effect of genotype

Table 1 shows the means of IMF breeding values for all three H-FABP RFLPs. A significant difference ($p<0.05$) was found for the IMF breeding values among H-FABP genotypes. The AA genotype has a significantly larger positive effect on the IMF breeding value than do the Aa and aa genotypes *for the Msp*I RFLP. In addition, the DD genotype has a significantly greater positive effect on IMF breeding values than the Dd and dd genotypes for the

Table 1. Comparison of IMF breeding values for three H-FABP RFLPs

*Msp*I				*Hae*III				*Hinf*I			
Genotype	N[a]	PV[b]	BV[c]	Genotype	N	PV	BV	Genotype	N	PV	BV
AA	207	4.50[d]	0.66[d]	DD	177	4.61[d]	0.73[d]	HH	253	4.13[d]	0.31[d]
Aa	242	4.12[e]	0.37[e]	Dd	258	4.11[e]	0.38[e]	Hh	216	4.40[de]	0.64[de]
Aa	50	4.08[e]	0.34[e]	dd	64	3.99[e]	0.28[e]	hh	30	4.62[e]	0.84[e]

[a] Number of animal genotyped. [b] PV: Phenotypic value for intramuscular fat.
[c] BV: Breeding value for intramuscular fat.
[d-e] Means within the same column with different letters are significantly different at $p<0.05$.

Table 2. Comparison of phenotypic and breeding value of intramuscular fat percentage among haprotype of H-FABP RFLPs

	AA/DD/HH	AA/DD/Hh	AA/DD/hh	AA/Dd/HH	AA/Dd/Hh	AA/dd/HH	Aa/Dd/HH	Aa/Dd/Hh	Aa/dd/HH	Aa/dd/HH
N[a]	48	99	30	15	13	2	126	104	12	50
PV[b]	4.43[d]	4.70[d]	4.62[d]	4.07[d]	3.77[d]	2.62[e]	4.08[d]	4.19[d]	3.82[d]	4.08[d]
BV[c]	0.47[de]	0.82[d]	0.84[d]	0.27[de]	0.31[de]	-0.46[f]	0.28[de]	0.52[de]	0.15[e]	0.34[de]

[a] Number of animal genotyped. [b] PV: Phenotypic value for intramuscular fat.
[c] BV: Breeding value for intramuscular fat.
[d-f] Means within the same row with different letters are significantly different at $p<0.05$.

Table 3. Effect of H-FABP RFLP on the breeding value for intramuscular fat

Dependent variable	Independent variable	R^{2a}
IMF breeding value	*Msp*I BV[b], *Hae*III BV, *Hinf*I BV	0.394
IMF breeding value	*Msp*I BV, *Hae*III BV,	0.389

[a] R^2: Coefficient of determination for multiple regression.
[b] BV: Breeding value.

*Hae*III RFLP. For the *Hinf*I RFLP, the hh genotype has a significantly larger positive effect on IMF breeding values than the HH genotype. The AA/DD/Hh and AA/DD/hh genotypes had the highest breeding value of all genotypes (Table 2).

Table 3 shows multiple regression analysis results. The independent variables were IMF breeding values of the three H-FABP RFLPs, whereas the dependent variable was the IMF breeding value. The highest contribution rate as measured by the coefficient of determination was 0.394, when the independent variables were the breeding values of the three H-FABP RFLPs. Multiple regression analyses were also performed without the *Hinf*I RFLP, because the partial regression coefficient of the *Hinf*I RFLP breeding value was not statistically significant and the contribution rate without the *Hinf*I RFLP was 0.389. These results confirm that approximately 40% of the variation in the breeding values for IMF is explained using the *Msp*I and *Hae*III polymorphisms.

DISCUSSION

Although the effects of H-FABP genotypes on the IMF breeding values in the present Duroc population were significant, the effects of the H-FABP genotypes were completely opposite to those reported by Gerbens et al. (1999) and Zeng et al. (2005). Present results indicate that AA, DD, and hh genotypes have the largest effects on IMF; but Gerbens and coworkers concluded that the aa, dd, and HH genotypes showed the greatest effects. Moreover, the aa/dd/HH genotype showed the greatest effect in their report, but the AA/DD/hh and AA/DD/Hh genotypes showed the highest values in our results. The different genetic backgrounds of our population and their population might explain the different results, because we performed genotyping based on their reported method. Ovilo et al. (2002) reported that, in an F_2 cross population involving Iberian and Landrace, the DD genotype had a significantly greater positive effect on IMF than the dd genotype, a result concurs with ours. Other studies have reported that RFLPs of the H-FABP gene are associated with fatness traits in Meishan crossbred pigs (Gerbens et al., 2000) and Duroc×Landrace pigs (Grindflek et al., 2000). On the other hand, no significant influence of H-FABP RFLP on IMF was detected in Austrian breeds of pigs (Nechtelberger et al., 2001).

Present results show the association between RFLPs of the H-FABP gene and the IMF breeding value. Nevertheless, Gerbens et al. (2001) reported that differences in mRNA and protein expression levels of H-FABP genotypes were unable to explain IMF variation in crossbred pigs. They argued that these negative results might reflect limitations of assays or inappropriate sampling times. In addition, Ovilo et al. (2002) attempted to locate the H-FABP gene on the porcine microsatellite map and sought quantitative trait loci (QTL) in that map. They analyzed the effect of the candidate gene polymorphism. The H-FABP polymorphism showed a significant effect on IMF when an animal model was fitted. However, these associations were not observed when the candidate gene effect was included in a QTL regression analysis. Ovilo and coworkers stated that this dependence on the analytical model and the quoted differences in the results obtained in other experiments suggest that the polymorphism responsible for the effect found is not the PCR-RFLP analyzed, but another close

polymorphism linked in different phases depending on the population.

Oikawa et al. (2002) statistically inferred that a major gene exists in this Duroc population. Then, we examined the H-FABP genes as a candidate of major gene. As a result, we obtained the result that the effect of the H-FABP gene was large to explain approximately 40 percent of the variance of breeding value. However, the change in IMF due to selection was not able to relate to the change in the H-FABP gene. Now, we are executing the QTL analysis of the sixth chromosome for IMF of this Duroc population. The QTL analysis might be able to be confirmed whether the H-FABP gene is a major gene or the effect of H-FABP gene on IMF might be the result of linkage disequilibrium between the H-HABP gene and QTL.

IMPLICATIONS

The H-FABP genotypes explained approximately 40% of the variation in the IMF breeding values. Therefore, it seems that preliminary selection for intramuscular fat content, using H-FABP genotypes as marker may be effective. Nevertheless, it is necessary to examine which H-FABP RFLP genotype (AA and aa genotypes for *Msp*I RFLP, DD and dd genotypes for the *Hae*III RFLP) have the good effect for the intramuscular fat accumulation beforehand because the effect of H-FABP RFLP genotype on intramuscular fat accumulation is thought to be different according to the pig breed or population.

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