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Association of *FABP3* and *LEPR* Gene Polymorphisms with the Drip Loss Trait of Pork

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Abstract Drip loss is a major parameter for the quality of pork which affects the economic perspective of premium-meat in the world's pork industry. It is clear that the fatty acid binding protein3 (*FABP3*) gene is related to oxidation and glucose utilization in muscles whereas the leptin receptors (*LEPR*) gene is related to energy balance, and both genes affect pork quality. The objective of this study was to analyze the genetic polymorphisms of *FABP3* and *LEPR* genes associated with the drip loss trait of pork. *Longissimus dorsi* muscle samples were taken from a total of 1,114 commercial pigs including purebred Duroc and [(Duroc × Large White) × Landrace] × Duroc. DNA was extracted by the Chelex® method. Drip loss was measured by the bag method based on gravitational technique. The *FABP3* and *LEPR* genes were genotyped by the PCR-RFLP technique. It was found that the *FABP3* gene showed significant association with the drip loss trait. The genotypes GC and CC of [(Duroc × Large White) × Landrace] × Duroc had the lowest drip loss. The *LEPR* gene was also associated with the drip loss trait. The animals of genotype TT had the lowest drip loss in Duroc but the genotype AA had the lowest drip loss in [(Duroc × Large White) × Landrace] × Duroc. Furthermore, the interaction between *FABP3* and *LEPR* significantly affected drip loss. The animals with genotypes GGTT, GCTT and CCTT had the lowest drip loss in Duroc whereas the animals with genotypes GCAA, CCAA, GGAA, CCTT, GCTT, and CCTA had the lowest drip loss in [(Duroc × Large White) × Landrace] × Duroc. These results indicated the importance of *FABP3* and *LEPR* genes to be used for the marker-assisted selection for the improvement of pork quality.

Keywords: Drip loss, fatty acid-binding protein 3*(FABP3)*, leptin receptors (*LEPR*), pork

Introduction

Drip loss is a major parameter that affects the quality of pork from an economic perspective premium-meat in the world's pork industry. Over the last decade, the production of lean meat has progressed rapidly and has largely taken place at the expense of meat quality.

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One serious problem is the drip loss of pork. Improvement of pork quality is very much desired, in terms of drip loss prediction before slaughter. However, traditional selective breeding for drip loss improvement based on recording the trait on slaughtered measurement can not serve the best solution because it is done after slaughter, so the process takes a long time and is costly. Therefore, marker information for this trait can be more beneficial to solve this problem and increase the rate of selection response. A scan of the pig genome has revealed that the drip loss trait of pork is on chromosomes 1, 2, 4, 5, 6, 11, 13, 14, and 18 (De Koning *et al*., 2001; Malek *et al*., 2004; Qu *et al*., 2002; Su *et al*., 2004; Thomsen *et al*., 2004; Jennen *et al*., 2007). With respect to meat quality, some genes on SSC6 are of interest, particularly the fatty acid binding protein3 (*FABP3*) gene (Gerbens *et al*., 1997) and the leptin receptors (*LEPR*) gene (Neuenschwander *et al.,* 1996). The *FABP3* gene influences oxidative capacity of various skeletal muscles (Peeters *et al*., 1989; Vork *et al*., 1991; Veerkamp and Van Moerkerk, 1993). In addition, *LEPR* is a member of the class I cytokine receptor family (Sun *et al.*, 2009; Li *et al.*, 2010). Leptin's specific receptors plays an important role in the regulation of fatness via feed intake, energy expenditure, and energy balance in porcine muscle (Pierzchała *et al*., 2006; Rybarczyk *et al*., 2009). Although *FABP3* and *LEPR* genes are mainly associated with intramuscular fat content (Ovilo *et al.*, 2002; Gerbens *et al*., 1998; Gerbens *et al*., 1999), they tend to be associated with drip loss in pork because of the relationship between oxidation and glucose utilization in the muscle (Schaap *et al*., 1999; Gerbens, 2000). However, studies on the association between polymorphisms of both genes and drip loss trait have not brought conclusive results. In consequence, the objective of this study was to analyze the genetic polymorphisms of *LEPR* and *FABP3* genes on thedrip loss trait in Thai commercial pig breeds.

Materials and methods

Animals

A total of 1,114 hot carcasses of Thai commercial pigs consisting of Duroc (n=419) and [(Duroc × Large White) × Landrace] × Duroc (n=695) breds were cut along the *Longissimus dorsi* muscle tissues between the 9th and the 10th ribs, then placed in a chiller at 4°C and held there for 24 hours

pH and Drip loss measurement

The pH of *Longissimus dorsi* muscle was obtained at 24 hours postmortem using an Orion model 720A pH meter fitted with a Ross sure flow 81-72 electrode (Orion Research, Boston, MA). The bag method based on gravitational technique was used for drip loss measurement. (Honikel, 1998). Meat samples were weighed before (Wd1) and after (Wd2) the 24

hour period where then were hanging in chambers at 4°C. Drip was expressed as the percentage of total weight loss as follow: Drip loss (%) = (Wd1 – Wd2) / Wd1 × 100.

DNA extraction

The DNA samples were extracted from muscle tissues using the Chelex® method as described by Walsh *et al*. (1991).

Genomic analysis

PCRs were performed in a 20 µl reaction mixture containing 50 ng of genomic DNA, 0.4 µl of each primer, 0.5 µl of 2.5 mM dNTP, 0.5 U *Taq* polymerase (Fermentas, USA) and 1 × reaction buffer containing 1.2 µl of 25 mM $MgCl_2$ and 14.40 µl dH_2O. The standard temperature profile was as follows: 3 min at 94 ℃ follow by 35 cycles of 15 s at 94 ℃, 40 s at annealing temperature (Table 1), 30 s at 72 ℃ and a final extention at 72 ℃ for 5 min. The markers of *FABP3* and *LEPR* genes were c.177G>C (Gerbens *et al*. (1997) and c.232T>A (Mackowski *et al*. (2005), respectively. Details about genotyping procedures are given in Table 1.

Table 1. Details of *LEPR* and *FABP3* genes with regard to primers, annealing temperatures, restriction enzymes and references

Genes	Primers	Annealing	Restriction enzymes	Ref.
LEPR	5'-TGCCTGCTGGAATCTCAAAG-3' 5'-TTCCCTGCAATGTTGTCTGC-3'	58 ℃	*Tsp*509I	Mackowski *et al*. (2005)
FABP3	5' -TCAGCCCAAGAGTGAGTTTC-3' 5'-GACCAGTCCCCTTTCCTG-3'	58 ℃	*Hinf*I	Gerbens *et al*. (1997)

The primer selection of *FABP3* segment was based on GenBank accession number (AF164968.1).

Statistical analysis

Genotype frequencies were calculated from direct counting and allele frequencies were estimated from the corresponding genotype frequencies. Effects of *LEPR* and *FABP3* genes on drip loss trait were analyzed using the general linear model of program R (Fox *et al*., 2009) and differences were considered significant at $P<0.05$. The statistic model included fixed effects of marker genotype, breed, sex, and pH as a covariance. Additionally, the interaction effects of both genes were also calculated.

Results

Genotype frequencies of FABP3 and LEPR

The PCR product of *FABP3* marker was 184 bp. The allele C could be digested by *Hinf*I into 144 and 40 bp fragments, whereas allele G could not be digested, so it was 184 bp . In addition, GG, GC and CC genotype frequencies of *FABP3* gene were 0.08, 0.44 and 0.48, respectively, and G and C allele frequencies were 0.30 and 0.70, respectively. The PCR product of *LEPR* marker was 184 bp. The allele A could be digested by *Tsp*509I into 113 and 71 bp fragments, but the allele T still was 184 bp. Genotype frequencies of TT, TA, and AA were 0.11 0.32, and 0.57 respectively, while T and A allele frequencies were 0.27 and 0.73, respectively.

Effect of FABP3

The *FABP3* gene was associated with drip loss in crossbred pigs. However, there was no association in Duroc pigs. The crossbred pigs [(Duroc ×Large White) ×Landrace ×Duroc)] with genotype GC and CC had lower drip loss than genotype GG about 1.93 and 2.04%, respectively,. Genotype GC and CC provided an additive and dominant effect of about 1.02 ±0.36% ($p<0.01$) and -0.91 ±0.44% ($p<0.05$), respectively (Table 2).

Table 2. Association of *FABP3* gene with drip loss trait

Breeds (N=1,114)	Genotypes			Effects	
	GG	GC	CC	additive	dominant
D	8.58±0.67	8.01±0.32	7.33±0.31	0.63±0.36	0.05±0.44
crossbred	8.83±0.70[a]	6.90±0.28[b]	6.79±0.29[b]	1.02±0.36**	-0.91±0.44*

D = Duroc, LW×LR×D = Large White × Landrace × Duroc

Least square mean ± standard error values with different letters in the same row are significantly different (a,b, $p<0.05$)

*$p<0.05$, **$p<0.01$

Effect of LEPR

It was found that *LEPR* gene was associated with drip loss in pigs. In Duroc, the animals with genotype TT had lower drip loss than genotype TA and AA, about 2.16 and 2.51%, respectively. It provided an additive and dominant effect of about 1.25 ±0.32% ($p<0.001$) and 0.91 ±0.44% ($p<0.05$), respectively . In crossbred pigs, the animals with genotype AA had a lower drip loss than genotype TA and TT by about 0.23 and 0.54%, respectively. It provided an additive effect of about 0.77 ±0.21% ($p<0.001$) (Table 3).

Table 3. Association of *LEPR* gene with drip loss trait

Breeds (N=1,114)	Genotypes			Effects	
	TT	TA	AA	additive	dominant
D	5.37±0.62[a]	7.53±0.37[b]	7.88±0.30[b]	1.25±0.32***	0.91±0.44*
crossbred	7.91±0.44[a]	7.60±0.3[a]	7.37±0.26[b]	0.77±0.21***	0.46±0.36

D = Duroc, LW×LR×D = Large White × Landrace × Duroc
Least square mean ± standard error values with different letters in the same row are significantly different (a,b, p<0.05)
*p<0.05, ***p<0.001

Interaction of FABP3 and LEPR

The interaction between *FABP3* and *LEPR* was significantly affected the drip loss trait in both Duroc and crossbred pigs. In Duroc pigs, the haplotypes GGTT, GCTT, and CCTT had higher rates of drip loss than the haplotypes CTAG and CTGG which ranged from 1.64 to 4.59%, respectively. In crossbred pigs, the haplotypes GGAA, GCTT, GCAA, CCTT, CCTA and CCAA had higher rates of drip loss than the haplotypes GGTT, GGTA and GCTA which ranged from 0.04 to 5.06%, respectively (Table 4)

Table 4. Association of interaction between *FABP3* and *LEPR* genes with drip loss trait

Breeds	Genotypes								
	GGTT	GGTA	GGAA	GCTT	GCTA	GCAA	CCTT	CCTA	CCAA
D	4.86±1.99[abc]	8.55±1.01[ab]	9.19±0.96[ab]	4.60±1.12[c]	7.37±0.58[ab]	8.46±0.40[a]	5.73±0.79[c]	7.54±0.46[ab]	7.46±0.41[b]
crossbred	11.38±1.27[a]	9.18±1.39[ac]	7.19±1.19[bc]	7.46±0.77[bc]	7.50±0.49[c]	6.32±0.34[b]	6.91±0.75[bc]	7.30±0.46[bc]	6.49±0.37[bc]

D = Duroc, LW×LR×D = Large White × Landrace × Duroc
Least square mean ± standard error values with different letters in the same row are significantly different (a,b, p<0.05)

Discussion

Low drip loss occurs when animals have lower than normal muscle glycogen levels at the time of slaughter and as a result lactate production is low whereas the reduction in glycolytic substrate availability causes more rapid ATP depletion and allows prolonged activity of proteases, causing meat to be more tender than normal (Dransfield, 1981; Maltin *et al.*, 2003). It can be assumed that the moisture or water retention of meat provides tenderness and it is clear that early postmortem events including rate and extent of pH decline, proteolysis and even protein oxidation are key factors influencing the ability of meat to retain moisture**.** Additionally, much of the water in the muscle is entrapped in structures of the cell, including the intra- and extra-myofibrillar spaces; therefore, key changes in the intracellular architecture of the cell influence the ability of muscle cells to retain water (Huff-Lonergan and Lonergan, 2005). Glucose uptake, oxidative capacity, energy expenditure, and energy balance in porcine muscle relate to AMPK provided by the use of different genetic approaches (Treebak *et al.*, 2006;

Hardie and Sakamoto, 2006). This study indicates that *FABP3* and *LEPR* genes influence the drip loss trait of pork. These results are in the agreement with those of Li *et al.* (2010) who found that the *FABP3* and *LEPR* polymorphisms showed significant association with moisture and tenderness. Thus, both genes may have roles to play in determining drip loss, because *FABP3* stimulates glucose uptake by facilitating AMPKdependent AS160 phosphorylation in skeletal muscle (Kusudo *et al.*, 2011). AMPK is a sensitive indicator of reduced cellular energy status. Consequently, any cellular or metabolic stress that either inhibits ATP synthesis or that accelerates ATP consumption (e.g., contraction of skeletal muscle) causes AMPK activation. It had been known for many years that muscle glycogen phosphorylase and phosphofructokinase (the key enzymes regulating glycogen breakdown and glycolysis, respectively) can also be activated allosterically by a rise in the AMP:ATP ratio. it has generally been assumed that the activation is caused by an increase in the cellular AMP:ATP ratio caused by increased ATP consumption (Grahame *et al.*, 2006). Particularly, *LEPR* may be responsible for the increase in fatty acid oxidation, and hence the increase in energy expenditure, induced by these cytokines. AMPK is a key player in regulation of energy balance not only at the cellular level, but also at the whole body level (Grahame *et al.*, 2006).

Conclusions

The results of this study confirm that the *FABP3* gene showed significant association with the drip loss trait. The genotype GC and CC of crossbred pigs had the lowest drip loss. Moreover, the *LEPR* gene was associated with the drip loss trait. The animals of genotype TT had the lowest drip loss in Duroc but the genotype AA had the lowest drip loss in crossbred pigs. Furthermore, interaction between *FABP3* and *LEPR* significantly affected drip loss. The animals with GGTT, GCTT and CCTT haplotypes had the lowest drip loss in Duroc whereas the animals with GCAA, CCAA, GGAA, CCTT, GCTT, and CCTA haplotypes had the lowest drip loss in crossbred pigs. These results indicated that *FABP3* and *LEPR* genes can be used as the marker-assisted selection for improvement of pork quality.

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