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Differential expression of lipid metabolism-related genes and myosin heavy chain isoform genes in pig muscle tissue leading to different meat quality

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The aim of this study was to investigate the variations in meat quality, lipid metabolism-related genes, myosin heavy chain (MyHC) isoform genes and peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) gene mRNA expressions in longissimus dorsi *muscle (LM) of two different pig breeds. Six Rongchang and six Landrace barrows were slaughtered at 161 days of age. Subsequently, meat quality traits and gene expression levels in LM were observed. Results showed that Rongchang pigs not only exhibited greater pH, CIE* a $*_{24\,h}$ *and intramuscular fat content but also exhibited lower body weight, carcass weight, dressing percentage, LM area and CIE* b $*_{24\,h}$ *compared with Landrace pigs (*$P < 0.05$*). Meanwhile, the mRNA expression levels of the lipogenesis (peroxisome proliferator-activated receptor gamma, acetyl-CoA carboxylase and fatty acid synthase) and fatty acid uptake (lipoprotein lipase)-related genes were greater in the Rongchang (*$P < 0.05$*), whereas the lipolysis (adipose triglyceride lipase and hormone sensitive lipase) and fatty acid oxidation (carnitine palmitoyltransferase-1B)-related genes were better expressed in the Landrace. Moreover, compared with the Landrace, the mRNA expression levels of MyHCI, MyHCIIa and MyHCIIx were greater, whereas the mRNA expression levels of MyHCIIb were lower in the Rongchang pigs (*$P < 0.05$*). In addition, the mRNA expression levels of PGC-1α were greater in Rongchang pigs than in the Landrace (*$P < 0.05$*), which can partly explain the differences in MyHC isoform gene expressions between Rongchang and Landrace pigs. Although the small number of samples does not allow to obtain a definitive conclusion, we can suggest that Rongchang pigs possess better meat quality, and the underlying molecular mechanisms responsible for the better meat quality in fatty pigs may be partly due to the higher mRNA expression levels of lipogenesis and fatty acid uptake-related genes, as well as the oxidative and intermediate muscle fibers, and due to the lower mRNA expression levels of lipolysis and fatty acid oxidation-related genes, as well as the glycolytic muscle fibers.*

Keywords: Rongchang pigs, meat quality, myosin heavy chain, lipid metabolism, PGC-1α

Implications

Different pig breeds show obvious differences in meat quality traits, but little is known about the molecular mechanisms responsible for the differences. Based on the results obtained from such studies, meat quality could be manipulated in order to produce high-quality pork.

Introduction

It is well-known that different pig breeds show obvious differences in meat quality traits (Hu *et al.*, 2008; Miao *et al.*, 2009; Guo *et al.*, 2011; Ruusunen *et al.*, 2012; Shen *et al.*, 2014); however, up to now, little is known about the molecular mechanisms responsible for the differences. Usually, the differences in meat quality traits are smaller between pig breeds that are commonly used for commercial production, because the goals of pig breeding, such as fast growth and leanness of the carcass, have been similar for a long time (Ruusunen *et al.*, 2012). However, the Chinese local and Western pig breeds do not have similar breeding processes, and they have many different characteristics. The Rongchang pigs, one of the most important local fatty pig breeds of the southwest area of China, exhibit earlier sexual maturity, higher fat deposits, poorer feed efficiency and lower growth rate and lean meat content compared with Western pig breeds, but the sensory quality of its meat is superior.

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The Landrace pigs, one of the Western lean pig breeds, have been intensively selected for a thin subcutaneous fat layer and high muscularity in the carcass, which has led to deterioration in meat quality (Lefaucheur *et al*., 2002; Shan *et al*., 2009). Therefore, the underlying molecular mechanisms responsible for the differences in meat quality traits between pig breeds can be investigated using these two pig breeds as the ideal comparison model.

Intramuscular fat (IMF) content is an important determinant factor for meat quality traits (Fernandez *et al*., 1999a). In general, the Chinese local fatty pigs have higher IMF content than Western lean pigs (Young, 1992; Hu *et al*., 2008; Miao *et al*., 2009; Zhao *et al*., 2009; Guo *et al*., 2011), but the knowledge about the molecular mechanisms is limited. The extent of fat deposition in skeletal muscles depends on the balance between synthesis and degradation of triglycerides (TG), which includes TG synthesis, fat mobilization, fatty acid transport as well as fatty acid oxidation (Zhao *et al*., 2009). Therefore, the expression levels of lipid metabolism-related genes in muscle tissue could theoretically contribute to the difference in IMF content, which lead to different meat quality between pig breeds. Moreover, skeletal muscle comprises four types of myofibers, which are encoded by four different myosin heavy chain (MyHC) isoform genes (MyHCI, MyHCIIa, MyHCIIx and MyHCIIb) and have different biochemical and biophysical characteristics such as oxidative and glycolytic capacities, contraction speed, cross-sectional area, glycogen content and TG content (Schiaffino and Reggiani, 1996; Lefaucheur *et al*., 2002; Lefaucheur, 2010). Thus, the compositions of myofiber types are considered as important influencing factors for meat quality (Franck *et al*., 2007). However, to the best of our knowledge, there are no published articles that have investigated the variations in myofiber-type compositions between Rongchang and Landrace pigs. Therefore, the objective of the present study was to compare the differences in meat quality traits and the relative mRNA expression levels of lipid metabolism-related genes and MyHC isoform genes in the *Longissimus dorsi* muscle (LM) of Landrace and Rongchang pigs, and consequently provide some valuable information for understanding the possible molecular mechanisms responsible for meat quality trait differences between fatty and lean pig breeds.

Material and methods

The study was approved by the Animal Care Advisory Committee of Sichuan Agricultural University.

Animals and sample collection

Six Rongchang (originating from six litters) and six Landrace (originating from six litters) young boars of 42 days of age were chosen for the experiment. The feeding experiment lasted for 112 days after 7 days of adaptation period. The pigs were housed in separate pens according to breed and were reared in the same environmental conditions. In order to eliminate the possible influence of different diets on the test results, the Rongchang pigs and Landrace pigs were provided *ad libitum* access to the same basal diet and water before being slaughtered. The basal diets were formulated mainly based on corn, soybean meal and wheat bran, and were formulated to meet or exceed the NRC (2012) recommendations for the nutrient requirements of the different growth phases. The ingredients and nutrient levels of the basal experimental diets are shown in Table 1. At the end of the feeding experiment, all pigs (161 days of age) were electrically stunned and exsanguinated after fasting for 12 h. Within 10 min *postmortem*, LM samples from the 6th to the 10th rib and from the 10th to the 11th rib of the left-side carcass were collected for meat quality measurements (samples were stored at 4°C for further analysis) and RNA isolation (samples were snap-frozen in liquid nitrogen and then stored at −80°C until further analysis), respectively.

Table 1 *Ingredients and nutrients of the experimental diets (as-fed basis)*

Item	42 to 84 days	84 to 126 days	126 to 161 days
Ingredients (g/kg)			
Corn	533.0	592.0	645.0
Soybean meal	224.8	241.0	183.6
Fish meal	50.0	0.0	0.0
Wheat bran	100.0	90.0	97.1
Sucrose	20.0	0.0	0.0
Soybean oil	55.0	55.0	55.0
L-Lys HCl	0.0	0.3	0.2
Limestone	9.0	9.9	9.6
$CaHPO_4$	0.8	4.4	2.1
Premix[1]	7.4	7.4	7.4
Nutrients[2]			
DE (MJ/kg)	14.68	14.68	14.68
CP (g/kg)	186.0	165.0	145.0
Ca (g/kg)	6.6	5.8	5.0
P (g/kg)	5.4	4.7	4.2
Lys (g/kg)	10.3	8.5	7.0

DE = digestible energy.
[1]Provided the following (per kilogram of complete diet): 100 mg of Fe (as ferrous sulfate); 15 mg of Cu (as copper sulfate); 120 mg of Zn (as zinc sulfate); 40 mg of Mn (as manganese sulfate); 0.3 mg of Se (as Na_2SeO_3); 0.25 mg of I (as KI); 13 500 IU of vitamin A; 2250 IU of vitamin D3; 24 IU of vitamin E; 6.2 mg of riboflavin; 25 mg of nicotinic acid; 15 mg of pantothenic acid; 1.2 mg of vitamin B12; 0.15 mg of biotin; 1 g of choline chloride; 3 g of salt.
[2]All data were calculated values.

Carcass traits and meat quality

Hot carcass weight was recorded and then divided by slaughter weight to obtain dressing percentage. Backfat thickness at the first rib, last rib and last lumbar vertebrae of the left-side carcass was measured and subsequently the average backfat thickness was calculated. The *longissimus dorsi* muscle area (LA) at the 10th rib of the right-side carcass was measured according to the unified standard. Meat color was measured in triplicate using a Minolta Chromameter CR-300 (CR-300, Minolta Camera Co., Tokyo, Japan) on a freshly cut surface of the LM sample between the 9th and 10th

rib at 45 min and 24 h *postmortem*. The pH-value was measured in triplicate, using a handheld pH meter (pH-STAR, SFK-Technology, Copenhagen, Denmark) equipped with a glass electrode, at the center of the LM sample between the eighth and ninth rib at 45 min and 24 h *postmortem*. IMF content was determined on a sample of LM between the seventh and eighth rib by petroleum ether extraction as previously described (Fortin *et al.*, 2005). Shearing force was determined on a sample of LM between the sixth and seventh rib, as previously described (Zeng *et al.*, 2012), using a Tensipresser (TTP-50BXII, Taketomo Electric Corp., Tokyo, Japan).

RNA isolation and reverse transcription

Total RNA was isolated from LM using Trizol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. The purity and concentration of total RNA were measured by a spectrophotometer (Beckman Coulter, DU800, Fullerton, CA, USA) at 260 and 280 nm. Ratios of absorption (260/280 nm) of all samples were between 1.8 and 2.0. The integrity of RNA was detected by electrophoresis in 1.3% agarose-formaldehyde gel. For each sample, 2 μg of total RNA was used to synthesize the cDNA using a PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa) according to the instructions of the manufacturer.

Real-time PCR

The primers were synthesized by TaKaRa Biotechnology (TaKaRa) and are presented in Supplementary Table S1. Real-time PCR was performed using SYBR® Premix Ex Taq II (Tli RNaseH Plus) reagents (TaKaRa) and a CFX-96 Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA). Each 25 μl PCR mixture contained 12.5 μl SYBR® Premix Ex Taq II (Tli RNaseH Plus), 1 μl forward primers (10 μM), 1 μl reverse primers (10 μM), 8.5 μl double-distilled water and 2 μl cDNA (the concentration of cDNA was adjusted to the same by ddH_2O). Cycling conditions were as follows: 95°C for 10 s, followed by 40 cycles at 95°C for 5 s and 60°C for 25 s and a 72°C extension step for 15 s. A melting curve was generated following each real-time quantitative PCR assay to check and verify the specificity and purity of the PCR product. The standard curve of each gene was run in triplicate to obtain reliable amplification efficiency (E). The correlation coefficients (R^2) of all standard curves were >0.99, and the amplification efficiency (E) was between 90% and 110%. The β-actin gene was used as the reference gene to normalize the mRNA expression amount of target genes according to a previous report by Zhao *et al.* (2009) and our unpublished data, which showed that the mRNA synthesis of this gene is stable and secure in muscles of various pig breeds. The relative amount of mRNA of a gene was calculated as previously described (Pfaffl, 2001). In brief, the relative expression ratio (R) of a target gene was calculated based on the following equation: $R = (E_{\text{target}})^{\Delta Ct\ \text{target}}/(E_{\text{reference}})^{\Delta Ct\ \text{reference}}$. The $\Delta C_{t\text{target}}$ value was the C_t deviation of $C_{t\text{Landrace}} - C_{t\text{Rongchang}}$ of the target gene transcript. The $\Delta C_{t\text{reference}}$ value was the C_t deviation of $C_{t\text{Landrace}} - C_{t\text{Rongchang}}$ of the reference gene transcript. The final data of each sample used for statistical analysis were calculated as the fold change relative to the mean R-value of the Landrace group, which was arbitrarily defined as 1. All experimental sample analyses were repeated in triplicate.

Statistical analysis

Each pig was considered as an experimental unit. Results for gene mRNA level in figures are presented as mean and s.d., and other results in the table are presented as mean and r.s.d.

Comparisons between breed groups were made by a simple t-test using SPSS 18.0 for Windows statistical software package (SPSS, Chicago, IL, USA). The level of statistical significance was set at $P < 0.05$.

Results

Carcass traits and meat quality traits between breeds

Carcass traits and meat quality traits in the Landrace and Rongchang pigs are shown in Table 2. Compared with Landrace pigs, the Rongchang pigs not only exhibited greater pH, CIE $a^*_{24\,h}$ and IMF but also exhibited lower BW, carcass weight, dressing percentage, LA and CIE $b^*_{24\,h}$ ($P < 0.05$). In addition, Rongchang pigs tended to exhibit lower CIE $L^*_{24\,h}$ compared with Landrace pigs ($P = 0.061$). Other meat quality traits had no significant differences between the two pig breeds ($P > 0.05$).

Relative mRNA expression levels of lipid metabolism-related genes in LM between breeds

The relative mRNA expression levels of lipid metabolism-related genes in LM of the Landrace and Rongchang pigs are shown in Figure 1. Compared with Landrace pigs, the relative mRNA expression levels of the lipogenesis (PPARγ, FAS and ACC) and fatty acid uptake-related genes (LPL) in LM were greater in Rongchang pigs, whereas the relative mRNA

Table 2 *Characteristics of carcass traits and meat quality traits in the Landrace and Rongchang pigs*[1]

Item	Landrace	Rongchang	r.s.d.	Significance
BW (kg)	98.57	57.40	6.37	<0.001
Carcass weight (kg)	72.38	37.31	4.99	<0.001
Dressing percentage (%)	73.42	64.97	1.94	<0.001
LA (cm^2)	51.64	8.44	3.76	<0.001
Backfat thickness (cm)	2.22	2.51	0.25	0.150
CIE $L^*_{45\,min}$	44.57	44.55	2.61	0.981
CIE $a^*_{45\,min}$	5.94	6.80	2.00	0.427
CIE $b^*_{45\,min}$	3.54	3.62	1.28	0.925
$pH_{45\,min}$	6.54	6.80	0.09	0.009
CIE $L^*_{24\,h}$	53.44	51.91	1.06	0.061
CIE $a^*_{24\,h}$	7.25	9.68	1.43	0.003
CIE $b^*_{24\,h}$	7.65	4.17	0.67	0.002
$pH_{24\,h}$	5.51	5.76	0.03	0.004
Shearing force (kg/cm^2)	5.39	5.44	0.39	0.754
IMF (%)	2.35	3.12	0.15	<0.001

LA = *longissimus dorsi* muscle area; IMF = intramuscular fat content.
[1]$n = 6$.

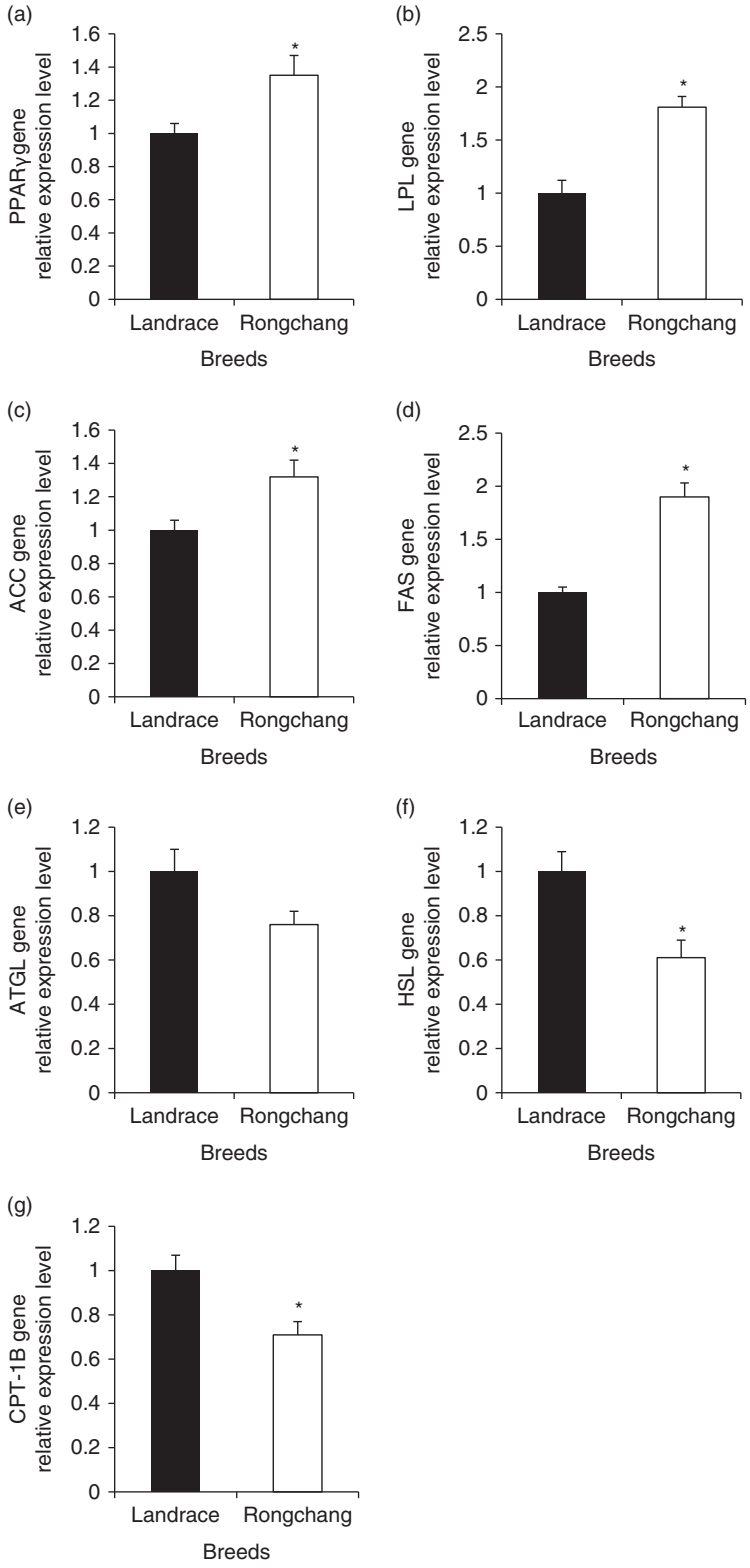

Figure 1 Relative mRNA expression levels of lipid metabolism related genes in *longissimus dorsi* muscle (LM) of the Landrace and Rongchang pigs. Each column represents the mean of six pigs ± s.d. $^*P < 0.05$.

expression levels of the lipolysis (HSL) and fatty acid oxidation (CPT-1B)-related genes were lower in Rongchang pigs ($P < 0.05$). In addition, Rongchang pigs tended to exhibit lower relative mRNA expression levels of adipose triglyceride lipase (ATGL) compared with Landrace pigs ($P = 0.053$).

Relative mRNA expression levels of MyHC isoform genes in LM between breeds

The relative mRNA expression levels of MyHC *isoform genes* in LM of the Landrace and Rongchang pigs are shown in Figure 2. Compared with Landrace pigs, the relative mRNA

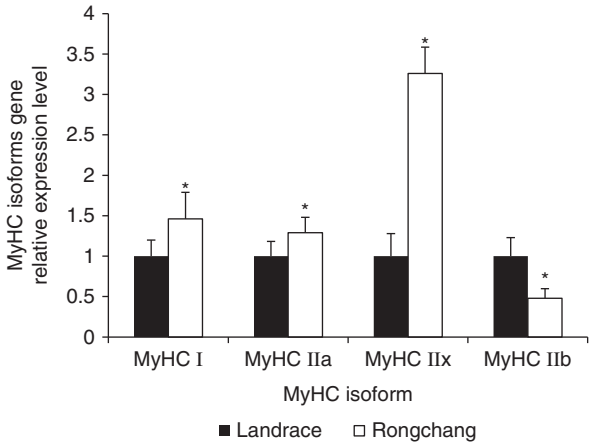

Figure 2 Relative mRNA expression levels of myosin heavy chain (MyHC) isoform genes in *longissimus dorsi* muscle (LM) of the Landrace and Rongchang pigs. Each column represents the mean of six pigs ± s.d. $^*P < 0.05$.

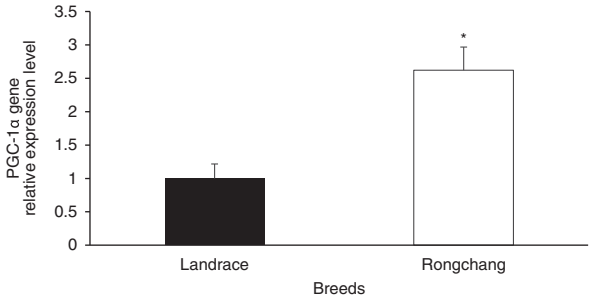

Figure 3 Relative mRNA expression levels of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) in *longissimus dorsi* muscle (LM) of the Landrace and Rongchang pigs. Each column represents the mean of six pigs ± s.d. $^*P < 0.05$.

expression levels of oxidative fiber type (MyHCI and MyHCIIa) and intermediate fiber type (MyHCIIx) were greater in Rongchang pigs ($P < 0.05$), whereas the relative mRNA expression levels of glycolytic fiber type (MyHCIIb) were lower in Rongchang pigs ($P < 0.05$).

Relative mRNA expression levels of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) in LM between breeds

The relative mRNA expression levels of PGC-1α in LM of the Landrace and Rongchang pigs are shown in Figure 3. Compared with Landrace pigs, the relative mRNA expression levels of PGC-1α were greater in Rongchang pigs ($P < 0.05$).

Discussion

It is well-known that different pig breeds show obvious differences in meat quality traits (Hu *et al*., 2008; Miao *et al*., 2009; Guo *et al*., 2011; Ruusunen *et al*., 2012; Shen *et al*., 2014); however, up to now, little is known about the molecular mechanisms responsible for the differences. Usually, the differences in meat quality traits are smaller between Western modern pig breeds because of the long-time similar breeding aims such as fast growth and leanness of the carcass (Ruusunen *et al*., 2012).The Rongchang pig is one of the local fatty-type pig breeds of the southwest area of China with a white coat color phenotype (Lai *et al*., 2007), and is especially noted for its early sexual maturity, increased IMF content and high meat quality as other Chinese local pigs. Landrace pigs, a Western pig breed, have been intensively selected for their thin subcutaneous fat layer and high muscularity in the carcass, which has led to deterioration in meat quality (Lefaucheur *et al*., 2002; Shan *et al*., 2009). Therefore, these two pig breeds can be used as an ideal comparison study model to investigate the possible mechanisms responsible for the differences in meat quality traits between pig breeds.

Carcass characteristics and meat quality

In the present study, Rongchang pigs had lower carcass weight, LA and dressing percentage compared with the Landrace. These results are in accordance with previous reports, which have compared those traits of the Jinhua pigs, a Chinese local fatty-type pig breed, with the Landrace at same age (Miao *et al*., 2009; Guo *et al*., 2011), and may be attributed to the lower muscle growth potential of Rongchang pigs compared with the Landrace.

It is well-known that pork meat quality traits are affected by the genotype of the pig. For example, the Jinhua pigs (Miao *et al*., 2009; Guo *et al*., 2011) and Laiwu pigs (Hu *et al*., 2008) exhibited better meat quality traits when compared with the Landrace and Duroc pigs, respectively. Moreover, Ruusunen *et al.* (2012) and Shen *et al.* (2014) also found differences in meat quality traits between different genotype pigs. In the present study, we have compared meat quality traits in the Rongchang with the Landrace at 161 days of age. Consistent with previous results on different genotype pigs (Miao *et al*., 2009; Guo *et al*., 2011; Shen *et al*., 2014), our results showed that Rongchang pigs exhibited better meat quality compared with the Landrace at the same age.

Lipid metabolism-related gene expressions in LM

IMF content is an important determinant of many aspects of meat quality characteristics (Fernandez *et al*., 1999a and 1999b) – for example, meat color, shearing force, etc. Generally, an increase in IMF content is mainly due to an increase in TG content, which has been demonstrated by previous research in pigs (Bergeron *et al*., 2007). It has been reported that the content of IMF depends on the balance between lipogenesis and lipolysis metabolic pathways, which include fatty acid uptake from circulating lipids, *de novo* lipogenesis and lipid mobilization, as well as fatty acid oxidation (Zhao *et al*., 2009). Therefore, although there are many determinants for IMF content, the expression levels of those lipid metabolic pathways-related genes in muscle tissue could partly explain the difference in IMF contents between pig breeds. In the present study, Rongchang pigs had higher IMF content compared with the Landrace, which is in accordance with previous reports that have compared the IMF content between other Chinese local fatty pigs and western

commercial pigs (Young, 1992; Miao *et al*., 2009; Zhao *et al*., 2009; Guo *et al*., 2011). Therefore, as stated above, one could assume that, compared with the Landrace, Rongchang pigs have higher fatty acid uptake and/or *de novo* lipogenesis capacity, whereas they have lower lipid mobilization and/or fatty acid oxidation capacity in LM. Therefore, in the present study, in order to shed light onto the reasons that can, at least in part, explain why Rongchang pigs exhibit higher IMF than the Landrace, the relative mRNA expression levels of those key lipid metabolism pathways-related genes were assessed. In order to assess the fatty acid uptake capacity of muscle tissues from circulating TG, the mRNA expression levels of LPL were measured. LPL functions as a 'metabolic gatekeeper' and is considered as a key lipase impacting the partitioning of circulating lipids among tissues (Greenwood, 1985; Zechner, 1997). Indeed, previous studies have reported that LPL can hydrolyze circulating lipoproteins for skeletal muscle lipid biosynthesis (Tan *et al*., 2011), and LPL correlates well with the uptake of fatty acids derived from circulating TG in skeletal muscles (Ellis *et al*., 1994; Levak-Frank *et al*., 1995). Our present results showed that Rongchang pigs had higher LPL expression levels compared with the Landrace. These data indicated that the higher IMF content in LM of Rongchang pigs compared with the Landrace could be partly mediated by a higher fatty acid uptake capacity. Concerning lipid synthesis, we have assessed the relative mRNA expression levels of PPARγ, FAS and ACC. PPARs consisting of three isotypes – namely, PPARα, PPARβ and PPARγ – belong to the nuclear receptor family of ligand-activated transcriptional factors, and play an important role in fatty acid metabolism (Schoonjans *et al*., 1996). In particular, PPARγ plays an important role in fat accumulation by inducing the expressions of lipid metabolism-related genes such as LPL (Tontonoz and Spiegelman, 2008) and FAS (Schadinger *et al*., 2005). It is well-known that FAS and ACC play a central role in the *de novo* lipogenesis in mammals. ACC catalyses the carboxylation of acetyl-CoA to malonyl-CoA, and is considered to be a rate-limiting enzyme of lipogenesis in animals (Numa *et al*., 1970; Munday, 2002). FAS catalyses all the reaction steps in the synthesis of palmitate from acetyl-CoA and malonyl-CoA in the presence of NADPH (Munoz *et al*., 2003). Our present results showed that the relative mRNA expression levels of PPARγ, FAS and ACC were greater in Rongchang pigs than in the Landrace. Therefore, we can speculate that Rongchang pigs have a higher capacity of lipogenesis, and therefore they exhibit higher IMF content compared with the Landrace breed. With regard to lipid mobilization and fatty acid oxidation, we have assessed the relative mRNA expression levels of ATGL, HSL and CPT-1B, respectively. ATGL and HSL are known to be the major enzymes for TG catabolism in adipose tissue. ATGL, a newly identified lipase, is the rate-limiting enzyme in hormone-induced lipolysis, and hydrolyses the first ester bond of the stored TG into non-esterified free fatty acids and diacylglycerol (Zimmermann *et al*., 2004). Further, diacylglycerol is hydrolyzed by activated HSL, which cleaves the fatty acid from diacylglycerol for exportation and oxidation (Mersmann, 1998; Carmen and Victor, 2006) and has a greater substrate affinity for diacylglycerol than TG (Haemmerle *et al*., 2002). CPT-1B is highly expressed in muscles and is involved in regulating mitochondrial fatty acid oxidation, which facilitates lipid mobilization (Lee *et al*., 2006). Our results showed that the relative mRNA expression levels of ATGL, HSL and CPT-1B in LM were lower in Rongchang pigs than in the Landrace. These data indicated that the higher IMF content in LM of Rongchang pigs than the Landrace could be, at least in part, mediated by the lower lipid mobilization and fatty acid oxidation capacity.

MyHC isoform genes and PGC-1α expression in LM

Eight isoforms of MyHC encoded by a separate gene are found in the skeletal muscles of mammals (Weiss *et al*., 1999; Shrager *et al*., 2000). In postnatal pigs, skeletal muscle consists of four MyHC isoforms: MyHCI, MyHCIIa, MyHCIIx and MyHCIIb, with different biochemical and biophysical characteristics such as oxidative and glycolytic capacities, contraction speed, cross-sectional area and TG content (Schiaffino and Reggiani, 1996; Lefaucheur *et al*., 2002; Lefaucheur, 2010). Muscle fibers are dynamic structures and exhibit high plasticity, and under normal physiological conditions transition among four MyHC isoforms follows an obligatory pathway: MyHCI $\leftrightarrow$ MyHCIIa $\leftrightarrow$ MyHCIIx $\leftrightarrow$ MyHCIIb (Schiaffino and Reggiani, 1994; Pette and Staron, 2000). In general, oxidative metabolism decreases in the rank order MyHCI $\rightarrow$ MyHCIIa $\rightarrow$ MyHCIIx $\rightarrow$ MyHCIIb (Lefaucheur *et al*., 2002), and these metabolic changes are consistent with the transition of MyHC profile toward a faster type (Lefaucheur *et al*., 2004). It is well-known that intensive selection for faster muscle growth in Western modern pigs has resulted in a shift in muscle fiber toward a higher glycolytic and lower oxidative metabolism (Lefaucheur *et al*., 2004; Hu *et al*., 2008; Guo *et al*., 2011), which is associated with leaner pigs, better feed efficiency and higher growth rate, but gives rise to a deterioration in meat quality (Lefaucheur *et al*., 2002; Shan *et al*., 2009). Previous studies have reported that, compared with the Landrace (Guo *et al*., 2011), Duroc (Hu *et al*., 2008) and Large White (Lefaucheur *et al*., 2004), respectively, the mRNA expression levels of MyHCIIa and MyHCIIx were greater in Jinhua, Laiwu and Meishan pigs, whereas the mRNA expression levels of MyHCIIb were lower. Those results may be related to the variation of meat quality between pig breeds (Hu *et al*., 2008; Guo *et al*., 2011), because the composition of skeletal muscle fibers is one of the important factors in determining meat quality (Chang *et al*., 2003). As mentioned above, one can assume that Rongchang pigs have better meat quality traits compared with Landrace pigs due to differences in the type and composition of myofibers. As we expected, compared with the Landrace, the mRNA expression levels of MyHCI, MyHCIIa and MyHCIIx were greater, whereas the mRNA expression levels of MyHCIIb were lower in Rongchang pigs. Moreover, these results were further confirmed by the higher mRNA expression levels of PGC-1α in Rongchang pigs, because it is widely known that PGC-1α can induce myofiber-type transitions from fast to slow (Lin *et al*., 2002; Ueda *et al*., 2005; Yamaguchi *et al*., 2010). It is

reported that MyHCIIb exhibits higher glycolytic metabolism capacity and glycogen content than other MyHC isoforms (Lefaucheur, 2010), and mainly uses glycogen and glucose as fuel through the glycolytic pathway. It is also reported that pigs carrying the mutated halothane gene, which is harmful to meat quality, exhibit greater proportions of MyHCIIb (Depreux *et al.*, 2000). Therefore, it is not difficult to understand that greater MyHCIIb expression will lead to faster *postmortem* pH decrease and lower ultimate pH as presented in our results. Moreover, Ruusunen *et al.* (2012) reported that the rate and the extent of the *postmortem* pH decrease and the ultimate pH affect meat color, and a fast *postmortem* pH decrease usually increases the lightness on the meat surface. Therefore, the greater CIE $L^*_{24\,h}$ in the Landrace than in Rongchang pigs may be due to the lower ultimate pH value. Myoglobin is the principle protein responsible for meat color, especially for CIE a^* values. Lefaucheur (2010) reported that MyHCIIb has lower myoglobin content than other MyHC isoforms. Moreover, type I muscle fibers are positively correlated with CIE a^* value ($r = 0.44$; Mancini and Hunt, 2005). Therefore, the greater CIE a^* value in Rongchang pigs compared with Landrace pigs could be attributed to a lower MyHCIIb expression as well as greater MyHCI, MyHCIIa and MyHCIIx expressions, as presented in this study.

Conclusion

The findings of this study indicated that the mRNA expression patterns of muscle lipid metabolism-related genes and MyHC isoform genes were different between the Rongchang and Landrance pigs. The possible reason of the higher meat quality in Rongchang pigs than the Landrace appeared to be that Rongchang pigs possessed a higher capacity of lipogenesis and fatty acid uptake, along with greater mRNA expression levels of type I, type IIa, type IIx myofibers, although they possessed a lower capacity of fat mobilization and fatty acid oxidation, along with lower mRNA expression levels of typeIIb myofibers. These results may provide valuable information for understanding the possible molecular mechanisms responsible for the differences in meat quality traits between different pig breeds. Therefore, based on this information, it could be possible to manipulate meat quality forming process to produce high-quality pork. However, the sample used in the present study is too small and does not allow to obtain a definitive conclusion; therefore, further research needs to be carried out on a larger sample.

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Supplementary material

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