Developmental patterns of *FASN* and *LIPE* mRNA expression in adipose tissue of growing Jinhua and Landrace gilts

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ABSTRACT: The present study was aimed to investigate the developmental patterns of *FASN* (fatty acid synthase) and *LIPE* (lipase, hormone-sensitive) mRNA in adipose tissue in pigs of different breeds and the relation with carcass fat content. Subcutaneous adipose tissue was sampled and total RNA was extracted to determine *FASN* and *LIPE* mRNA levels by semi-quantitative RT-PCR. The results showed that the *FASN* mRNA level increased with age ($P < 0.05$) and Jinhua gilts expressed higher *FASN* mRNA compared with Landrace gilts at 80 and 125 days of age ($P < 0.05$). In addition, Jinhua gilts expressed lower *LIPE* mRNA compared with Landrace gilts at 80 days of age ($P < 0.01$). Furthermore, the ratio of *FASN*/*LIPE* mRNA had a similar model in the two breeds, and was higher in Jinhua gilts than that in Landrace gilts at 80 and 125 days of age ($P < 0.05$). The *FASN* mRNA level was positively related to carcass fat content in Jinhua and Landrace gilts ($r = 0.802$, $P = 0.01$; $r = 0.734$, $P = 0.02$; respectively), and the ratio of *FASN*/*LIPE* expression exhibited significantly positively related carcass fat content ($r = 0.804$, $P = 0.01$; $r = 0.749$, $P = 0.02$; respectively).

Keywords: gilt; carcass fat content; *FASN*; *LIPE*; gene expression; RT-PCR

The Jinhua pig is one of the most important Chinese local breeds characterized by low growth rate and high adiposity (Xu, 1994), while Landrace pigs are known by high growth rate, but low fat deposition. Therefore, these two breeds of pigs can serve as ideal animal models for studying adipose tissue deposition in pigs. Fat deposition in adipose tissue represents a balance between synthesis and degradation (Chilliard, 1993). *FASN* (fatty acid synthase), which is one of the key enzymes in the conversion of acetyl-CoA and malonyl-CoA to triglycerol, plays an important role in *de novo* lipogenesis in mammals (Semenkovich, 1997; Yan et al., 2002; Smith et al., 2003). *FASN* in pig adipose tissues is extremely positively correlated with the weight of carcass fat (Xiong et al., 2001). *LIPE* (lipase, hormone-sensitive) is the key enzyme catalyzing the hydrolysis of stored triglycerol in adipose tissue into free fatty acid and glycerol, and one of the most important factors for controlling the lipolysis and fat accumulation in animals (Haemmerle et al., 2003; Kazala et al., 2003). Although it is clear that *FASN* and *LIPE* are involved in the regulation of fat deposition by balancing the lipogenesis and lipolysis, reports about the developmental changes of *FASN* and *LIPE* expression in adipose tissue of Jinhua pigs and reports about the breed differences between Jinhua and Landrace pigs are missing. Therefore, Jinhua and Landrace pigs with significant differences in fat deposition were used in the present study to investigate the developmental changes of *FASN*

and *LIPE* expression levels in adipose tissue and their effects on the adipose tissue deposition.

MATERIAL AND METHODS

Animals

This study was approved by the Institutional Animal Care and Use Committee of Zhejiang University. Purebred Jinhua and Landrace piglets (9 gilts of each breed, weaned at 28 days of age) were used in this experiment. Gilts were allocated to two groups according to breed. The feeding experiment lasted 90 days after a 7-day adaptation period. Jinhua and Landrace gilts were reared in the same conditions. All pigs had *ad libitum* access to an experimental diet and water via nipple drinkers. The experimental diets were formulated to meet the NRC (1998) nutrient requirements. Three gilts of each breed were slaughtered at 35, 80 and 125 days of age. The subcutaneous adipose tissue was quickly dissected and frozen in liquid nitrogen, then stored at –70°C until extraction for the total RNA. Left half-carcasses without head, legs and guts (except kidney) were weighed. Adipose tissue in the left half-carcass was dissected and weighed; the carcass fat content was calculated. All the animal experiments were done according to the guidelines of the National Institute of Animal Health (P.R. China) for animal experiments.

Primer design

Primer sequences for *FASN*, *LIPE* and *ACTB* encoding for β-actin were designed using Primer 5.0 software (Lalitha, 2000) on the basis of known sequences deposited in GeneBank (Table 1). The mRNA of *ACTB* was used as an internal standard for the determination of mRNA targeted levels.

Total RNA extraction

Total RNA of subcutaneous adipose tissue was isolated using TRIzol Reagent (Gibco BRL) as described by the manufacturer (Sigma, USA). The extracted RNA was dissolved in DEPC-treated water. The concentration and purity of RNA were checked using a spectrophotometer at 260/280 nm.

cDNA cloning

The synthesis of first strand cDNA was performed using Reverse Transcription System Kit (First Strand cDNA-synthesis Kit, Promega, USA) as described by the manufacturer with oligo-dT primer and using approximately 1 μg of total RNA as template.

Polymerase chain reaction (PCR)

The reverse-transcribed cDNA was amplified with *Taq* DNA polymerase (Promega, USA) by a PCR in a thermocycler (Gene Amp PCR System 9600, Pharmacia, Japan) using paired sense and antisense primers (Table 1). The PCR conditions for *FASN* were as follows: denaturation at 94°C for 2 min, followed by 31 cycles of amplification at 94°C for 50 s, 57°C for 50 s, and 72°C for 1 min, and followed by final extension cycle at 72°C for 10 min. The PCR conditions for *LIPE* were as follows: denaturation at 94°C for 2 min, followed by 31 cycles of amplification at 94°C for 50 s, 59°C for 50 s, and 72°C for 1 min, and followed by final extension cycle at 72°C for 10 min. The PCR conditions for *ACTB* were the same as those for *FASN*.

DNA sequencing and sequence analysis

The PCR products were electrophoresed in 1% agarose gel and selected electrophoresis bands

Table 1. Parameters of gene-specific primers for *FASN*, *LIPE* and *ACTB* genes

Target genes	Accession number	Primer sequence	Product size (bp)
FASN	EF589048	F: 5'-ACCGCCTCTGCAGTGTCTATTAC-3' R: 5'-ACGCCGCCCGAGCCCGAGTG-3'	358
LIPE	AY686758	F: 5'-CCCCGGGCCCGTCCTCGTC-3' R: 5'-GTTCCCGCCTGCGCTGTCTCCTG-3'	397
ACTB	DQ845171	F: 5'-CCGCACCACTGGCATTGTCAT-3' R: 5'-CAGCACCGTGTTGGCGTAGAGG-3'	460

were purified using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). The purified amplified products were directly ligated into pUCm-T Vector (Promega, USA) and transformed into *E. coli* JM109. Plasmids were then isolated and purified for DNA sequencing using Wizard Miniprep Kit (Promega, USA) and analysed on ABI PRISM 310 Genetic Analyser (Perkin Elmer, USA). The sequences of amplified fragments were aligned using the BLAST sequence analysis program (http://www.ncbi.nlm.nih.gov//BLAST) with the corresponding reported sequences according to which the gene-specific primers were designed. Amplified DNA fragments were 99% identical to the known sequences of *FASN*, *LIPE* and *ACTB* in GenBank. The results indicated that the amplified cDNA fragments of the three genes were gene-specific products.

mRNA expression analysis

The expression of pig *FASN* and *LIPE* mRNA was determined by semi-quantitative RT-PCR (Kousteni et al., 1999) using the housekeeping gene *ACTB* as internal control. The PCR products were electrophoresed on 1% agarose gel. Electrophoresis band intensities of the PCR products were quantified by NIH Image Version 1.62 software (Pharmacia, Japan).

Statistical analyses

All data were analyzed by the ANOVA procedure of SPSS 11.5. Comparison among age groups with the same breed uses one-way analysis of variance with age as the main effect. Differences among means were tested by Duncan's comparisons. Comparison between breed groups with the same age was determined by *t*-test. Bivariate correlations were used to evaluate the correlation between carcass fat content and gene expression level. All data are presented as mean ± SEM.

RESULTS

Carcass fat deposition

As shown in Figure 1, the carcass fat percentage showed an age-dependent pattern. It was low at 35 days of age, followed by a significant stepwise increase in the two breeds from 35 to 125 days of age. Jinhua gilts had a higher carcass fat percentage than Landrace gilts during the whole experimental period ($P < 0.05$).

Developmental patterns of *FASN* and *LIPE* mRNA expression

As shown in Figure 2, the developmental changes in *FASN* mRNA expression of adipose tissue in growing Jinhua and Landrace gilts were evaluated by semi-quantitative RT-PCR. The *FASN* mRNA expression level in subcutaneous adipose tissue showed an age-dependent pattern. It was low at 35 days of age, followed by a significant stepwise increase with age in the two breeds ($P < 0.05$). In addition, the *FASN* mRNA expression was higher in Jinhua gilts than that in Landrace gilts at 80 and 125 days of age ($P < 0.05$).

As shown in Figure 3, the *LIPE* mRNA expression in subcutaneous adipose tissue did not dif-

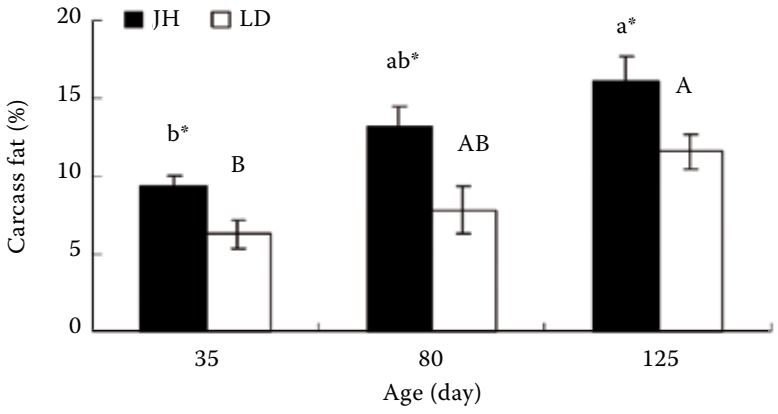

Figure 1. Developmental changes in carcass fat content in growing Jinhua and Landrace gilts
LD = Landrace gilts; JH = Jinhua gilts
means without a common letter (small letters for Jinhua pigs and capital letters for Landraces) differ significantly between ages, $P < 0.05$
Single asterisk *indicates significant differences ($P < 0.05$) between breeds at the same age

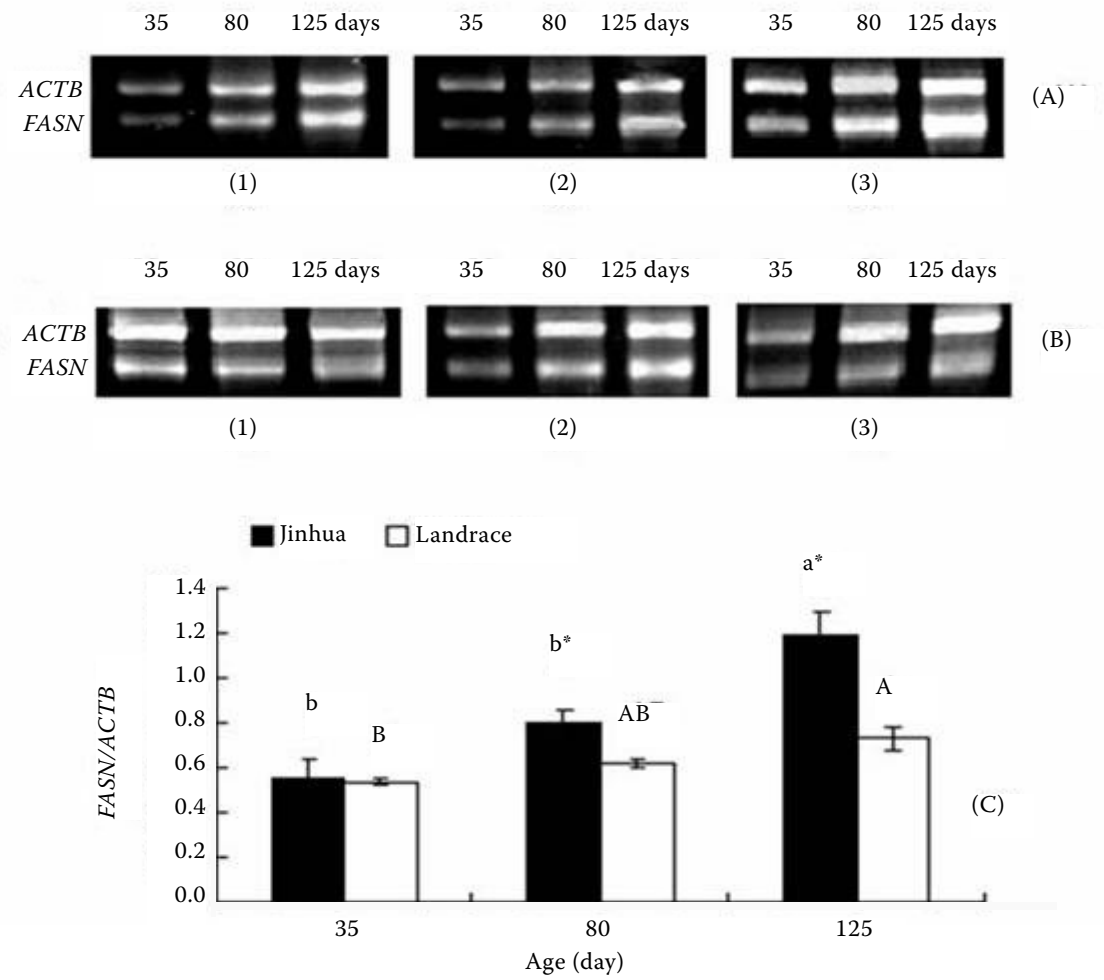

Figure 2. Expression of *FASN* mRNA in adipose tissue of pigs from 35 to 125 days of age; (A) electrophoresis of RT-PCR products for *FASN* and *ACTB* genes in the subcutaneous adipose tissue of Jinhua gilts; (B) electrophoresis of RT-PCR products for *FASN* and *ACTB* genes in the subcutaneous adipose tissue of Landrace gilts; (1), (2), (3): products from the first pig, second pig and third pig in each age group, respectively; (C) the developmental patterns of *FASN* mRNA in Jinhua and Landrace gilts, significant differences between ages ($P < 0.05$) are designated by letters and means without common superscript (capital letters for Landrace gilts and small letters for Jinhua gilts); single asterisk *indicates significant differences ($P < 0.05$) between breeds at the same age

fer from 35 to 125 days of age in the two breeds ($P > 0.05$). Whereas, the *LIPE* mRNA expression level in Jinhua gilts was lower than that in Landrace gilts at 80 days of age ($P < 0.01$).

Developmental patterns of *FASN* and *LIPE* mRNA expression

As shown in Figure 4, the ratio of *FASN* to *LIPE* mRNA in subcutaneous adipose tissue showed an age-dependent pattern. It was low at 35 days of age, followed by a significant increase with age in the two breeds ($P < 0.05$). The *FASN*/*LIPE* mRNA expression was higher in Jinhua gilts than that in Landrace gilts at 80 and 125 days of age ($P < 0.05$).

The relationship between *FASN* and *LIPE* mRNA expression and carcass fat content

The relationship between the expression of the two genes and carcass fat content was analyzed by bivariate correlations. As shown in Table 2, the *FASN* expression was positively correlated with carcass fat content in Jinhua gilts (*FASN*/carcass fat content: $r = 0.802$, $P = 0.01$). A similar result was observed in Landrace gilts (*FASN*/carcass fat

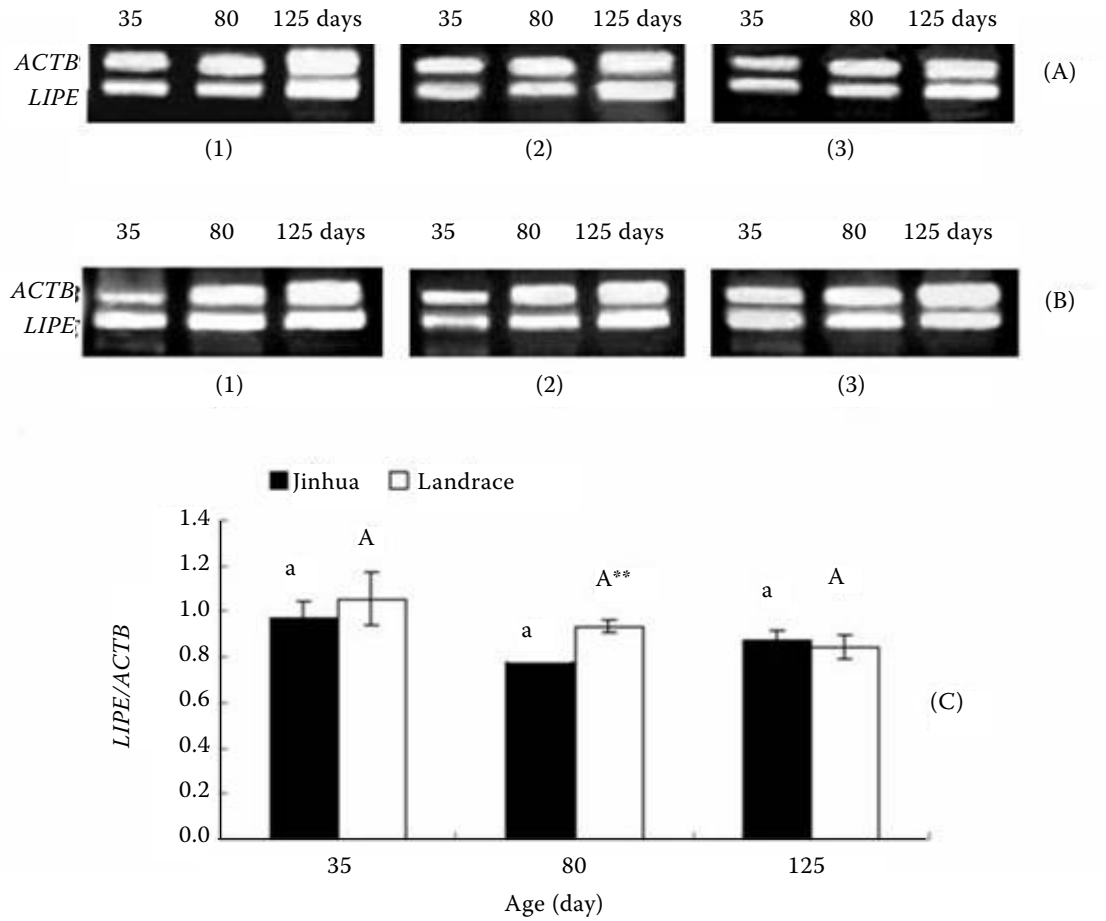

Figure 3. The expression of *LIPE* mRNA in adipose tissue of pigs from 35 to 125 days of age; (A) electrophoresis of RT-PCR products for *LIPE* and *ACTB* genes in the subcutaneous adipose tissue of Jinhua gilts; (B) electrophoresis of RT-PCR products for *LIPE* and *ACTB* genes in the subcutaneous adipose tissue of Landrace gilts; (1), (2), (3): products from the first pig, second pig and third pig in each age group respectively; (C) the developmental patterns of *LIPE* mRNA in Jinhua and Landrace gilts, significant differences between ages ($P < 0.05$) are designated by letters and means without common superscript (capital letters for Landrace gilts and small letters for Jinhua gilts); double asterisk **indicates extreme differences ($P < 0.01$) between breeds at the same age

content: $r = 0.734$, $P = 0.02$). Furthermore, the *FASN* per *LIPE* mRNA and carcass fat content showed a significantly positive relationship in the two breeds (in Jinhua gilts: $r = 0.804$, $P = 0.01$; in Landrace gilts: $r = 0.749$, $P = 0.02$), whereas the mRNA level of *LIPE* had no significant rela-

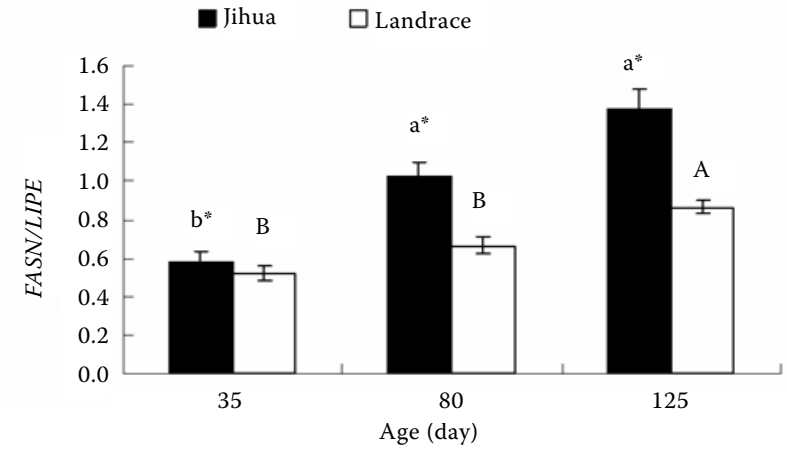

Figure 4. The developmental patterns of *FASN*/*LIPE* mRNA levels in adipose tissue of growing Jinhua and Landrace gilts, significant differences between ages ($P < 0.05$) are designated by letters and means without common superscript (capital letters for Landrace gilts and small letters for Jinhua gilts); single star *indicate significant differences ($P < 0.05$), between breeds at the same age

Table 2. Correlations between *FASN* and *LIPE* mRNA expression levels and carcass fat content in growing Jinhua and Landrace gilts

	Breed	*FASN* mRNA levels	*LIPE* mRNA levels	*FASN*/*LIPE*
Carcass fat content (%)	JH	$r = 0.802$ ($P = 0.01$)	$r = -0.226$ ($P = 0.56$)	$r = 0.804$ ($P = 0.01$)
	LD	$r = 0.734$ ($P = 0.02$)	$r = -0.443$ ($P = 0.24$)	$r = 0.749$ ($P = 0.02$)

JH = Jinhua gilts; LD = Landrace gilts

tion with carcass fat content in the two breeds ($P > 0.05$).

DISCUSSION

There are many factors affecting fat deposition in adipose tissue such as breed, age and nutrition (Mersmann et al., 1973; Yang, 2005). Jinhua gilts contained more carcass fat compared with Landrace gilts during growth. These results are in agreement with previous reports which indicate that carcass fat content displayed breed differences (Wanger et al., 1999). This difference may be attributed to different phenotypes in the fat deposition of Jinhua and Landrace gilts (Jinhua gilts had a greater ability of fat deposition). The higher lipid accretion in Jinhua pigs is probably related to an increase in extra energy available for lipid synthesis. Similar results were reported when Meishan (White et al., 1995), Iberian pigs (Morales et al., 2003) or Creole pigs (Renaudeau and Mourot, 2007) were compared to conventional lean pigs. In addition, carcass fat content increased with age in the two breeds. These results are in accordance with the results of some studies which indicate that carcass fat content displayed age-dependent patterns (Carr et al., 1978; Friesen et al., 1994; D'Souza et al., 2004; Correa et al., 2006).

Fat accumulation in adipose tissue represents a balance between synthesis and degradation (Chilliard, 1993). In pigs, the majority of lipids in the body is derived from *de novo* fatty acid synthesis (O'Hea and Leveille, 1969) and adipose tissue is considered to be the primary site of *de novo* fatty acid synthesis (O'Hea and Leveille, 1969; Bauman, 1976). *FASN* and *LIPE* play the rate-limiting role in the homeostasis of accumulation and metabolism of triglycerides in adipose tissue, which include both the fatty acid synthesis (lipogenesis) and fat degradation (lipolysis). The *FASN* is highly expressed in pig adipose tissue and to a much lesser extent in liver. In this study, a positive correlation between the *FASN* mRNA level and carcass fat content was observed in Jinhua and Landrace gilts. These results are in accordance with previous reports. The increased *FASN* expression could lead to obesity through the accumulation of triglycerides in humans (Semenkovich, 1997). In pigs, there was a significant correlation between the *FASN* potential enzyme activity and its mRNA expression level, the decreased *FASN* expression reduced the capacity of adipose tissue to synthesize lipids and subsequently led to decreased fat accumulation (Huang et al., 2008). However, there was no obvious relationship between the *FASN* expression and intramuscular fat (IMF) content in the porcine longissimus muscle (Chen et al., 2004) and there was a negative correlation between the *FASN* mRNA level and IMF content in Kazak sheep (Qiao et al., 2007). It suggested that the *FASN* function as an enzyme of fat storage has a diverse influence in subcutaneous adipose tissue and intramuscular fat tissue. In this study, the *FASN* mRNA level showed an age-dependent pattern, which increased with age in the two breeds. These results are in accordance with previous studies which indicate that adipose tissue may have different activity of related enzymes at different growth periods (Shan et al., 2006). All Jinhua and Landrace gilts were studied in the period of growth in which adipose tissue may be in the process of differentiation, which could cause developmental patterns of *FASN* expression levels in adipose tissue. In addition, Jinhua gilts had higher *FASN* expression levels compared with Landrace gilts, suggesting the differential expression levels of *FASN* in different breeds of pigs.

LIPE is an adipocyte enzyme that cleaves fatty acids from intracellular triacylglycerol (Mersmann, 1998), and an increase in *LIPE* could depress the accumulation of triglycerol in adipose cells (Thompson et al., 1993; Sztalryd et al., 1995). In

the present study, there was no obvious relationship between *LIPE* expression and carcass fat content in the two breeds, but there was a significant positive relationship between carcass fat content and the ratio of *FASN* to *LIPE* mRNA. The results confirmed that fat accumulation in adipose tissue represents a balance between synthesis and degradation (Chilliard, 1993). There was no significant correlation between IMF content and *LIPE* mRNA expression, whereas the ratio of *FASN* to *LIPE* mRNA was found to be positively correlated with IMF content (Chen et al., 2004). However, there was a negative relationship between *LIPE* expression or the *FASN*/*LIPE* mRNA and IMF content in Kazak sheep (Qiao et al., 2007). This discrepancy may be caused by different characteristics of fat deposition in the species. In this study, Jinhua gilts had lower *LIPE* expression compared with Landrace gilts, which implies that in the course of fat deposition pigs may accumulate body fat through a depression of lipid utilization and therefore Jinhua gilts need lower *LIPE* expression due to a decrease in lipid utilization. In addition, Jinhua gilts had higher the *FASN*/*LIPE* mRNA compared with Landrace gilts. This may be explained by the model difference in carcass fat accumulation in different pig breeds.

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