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Investigation on the Effect of Two Fat Metabolism Related Pathways on Intramuscular Fat Content in Pigs

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Authors' Contribution
CY and DP performed the experiments, analyzed the data of the study and wrote the paper. CL, AX and PH collected the samples. HO and HY conceived and designed the experiments.

Key words
Pig, Intramuscular fat, Gene expression, PPAR signaling pathway, qRT-PCR array

ABSTRACT

Intramuscular fat (IMF) content has become an important determinant of meat quality for raisers and consumers in modern society. The objective of this work was to investigate the roles of the peroxisome proliferator-activated receptor (PPAR) and fatty acid metabolism signaling pathways in determining the IMF contentin the longissimus dorsi (LD) muscle of pigs. The expression profile of candidate genes involved in the PPAR and fatty acid metabolism signaling pathways were detected in the LD muscle of two pig breeds with different IMF contents (Large White and Min) by a quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) array. Our results showed that three lipid metabolism-related biological processes lipogenesis, fatty acid transport and fatty acid oxidation in these two pathways showed significant differences in activation between Large White and Min pigs. The activation of the PPAR and fatty acid metabolism signaling pathways may play a positive role in reducing IMF content in pigs.

INTRODUCTION

Pork is a major meat source for humans in modern society (Puig-Oliveras *et al*., 2014). Intramuscular fat (IMF), also known as marbling, is an important determinant of pork quality. IMF content is positively correlated with several pork quality traits such as tenderness, juiciness, and flavor (Li *et al*., 2018). Moreover, many studies have indicated that pork quality is significantly improved when the IMF content obviously increases (Hamill *et al*., 2013; Madeira *et al*., 2013). In fact, pork with a suitable IMF content is favored by consumers (Font-i-Furnols *et al*., 2012; Wang *et al*., 2017). Therefore, we should aim to improve the IMF content of pork in the modern pig industry. It is well known that IMF content varies among different pig breeds (Dai *et al*., 2009; Gao *et al*., 2011; Casellas *et al*., 2013; Wu *et al*., 2013; Cui *et al*., 2016; Li *et al*., 2016; Lim *et al*., 2017). For example, the Large White pig, which is a famous commercial lean pig breed worldwide, has very low IMF content in their skeletal muscles. In contrast, the Chinese indigenous pig breeds have higher IMF content and superior quality pork. The Min pig is an excellent indigenous breed raised in the northeastern China (Gao *et al*., 2011) Min pigs breed prolifically and have strong tolerance to extreme environment, such as diseases, cold, crude and poor-quality feed. They also have high IMF content and better meat quality. Compared with Large White, the Min pig has a lower growth rate and lean meat ratio. Thus, these two pig breeds are ideal models for investigating the molecular mechanisms responsible for differences in fat deposition between Chinese indigenous pigs and famous commercial lean pigs.

To the best of our knowledge, the IMF content of pigs depends on the balance between lipogenesis and lipolysis, which includes fatty acid uptake, fat mobilization, fatty acid transport and fatty acid oxidation (Zhao *et al*., 2009; Zhang *et al*., 2015). As a complex porcine trait, IMF content may be affected by multiple genes and metabolic processes. Over the past decades, many studies are focus on the relationship between IMF content and single or multiple candidate genes. In several studies, the expression levels of *PPARA*, *LPL*, *ACSL1, SCD* and *PPARγ* genes are correlated with IMF content (Yang *et al*., 2012; Wang *et al*., 2013, 2016). In fact, these genes are involved in the peroxisome proliferator-activated receptor

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(PPAR) (ssc03320) or fatty acid metabolism (ssc01212) pathways, which are two well-known signaling pathways affecting lipid metabolism (KEGG Pathway Database). The activation of these two signaling pathways is critical to IMF deposition in pigs. However, fewer studies have focused on identifying essential genes related to porcine fat deposition at the whole-pathway level. Fortunately, with the release of porcine genome, we can identify all the genes in one pathway at once, enabling us to study IMF content trait based on whole signaling pathways rather than single genes. Therefore, although there are many factors that could participate in regulating the development of IMF, the transcriptional levels of related genes in these two pathways may partly illustrate the difference in IMF content between these two diverse pig breeds. Consequently, the objective of this study was to detect the expression of all genes involved in the PPAR and fatty acid metabolism signaling pathways in two pig breeds with different IMF contents.

Over the last decade, microarray and next-generation sequencing (NGS) technologies have been widely used in transcriptomic studies of different porcine tissues. For many researchers, the quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) has been widely considered the gold standard for measuring gene expression of a small group of selected genes due to its rapidity, simplicity and low cost (Morales-Prieto *et al*., 2017). In addition, the qRT-PCR array has been used in many studies and is an ideal and reliable tool for analyzing the expression levels of pathway-related genes (Tao *et al*., 2012; Morales-Prieto *et al*., 2017). Thus, in this study, all genes of the PPAR and fatty acid metabolism signaling pathways were detected in Min and Large White pig breeds by a qRT-PCR array. Our results may provide valuable information for elucidating the molecular mechanisms of different IMF contents in pigs.

MATERIALS AND METHODS

Ethics statement

All experiments were performed according to the guidelines of the University Committee on the Use and Care of Animals at Jilin University (approval ID: 201706030).

Animals and sample collection

The experimental pigs were raised under standard conditions at the Institute of Animal Husbandry Research, Heilongjiang Academy of Agricultural Sciences (Harbin, China). Two different breeds: Large White(n=3) and Min (n=3) pigs at an average age of 180 days were chosen randomly and slaughtered in a local abattoir. Samples of longissimus dorsi (LD) muscle between the 10th and 12th ribs were collected and divided into two parts, one part was quickly frozen in liquid nitrogen, and stored at -80°C until they were used in the qRT-PCR array, and another part was stored in 4 °C for determination of the IMF content.

Determination of IMF content

For LD muscles of Large White and Min pigs, the IMF content was determined as crude fat using the Soxhlet extraction method with petroleum ether (Supakankul and Mekchay, 2016) and each sample was repeated three times. In details, the LD samples were dried in an oven at 65 °C until constant weight; and the dried LD samples were cooled to room temperature and ground into powder. About 1g dried LD samples weighed was dried in an oven at 105 °C and then put into a fat package. This sample was extracted with petroleum ether for 6-7 hours, and then refluxed the petroleum ether. The statistical formula used for the determination of IMF content was as follows: IMF content=(W3-W1)/(W2-W1)* 100%, where W3-the weight of fat package+ sample after extraction and drying, W1– the weight of fat package, W2– the weight of fat package + sample after drying.

RNA extraction and qRT-PCR array

A total of six LD samples were used to perform the qRT-PCR array. Total RNA from the LD samples was extracted using TRIzol-A$^+$ Reagent (TIANGEN, Beijing, China), and first-strand cDNA was then synthesized using a BioRT cDNA First Strand Synthesis Kit (Bioer Technology, Hangzhou, China) following the manufacturer's instructions. The primers of genes in the selected pathways (KEGG Pathway Database) are listed in Supplementary Table I. The qRT-PCR array was performed with a Bio Easy SYBR Green I Real Time PCR kit (Bioer Technology) on an iQ™5 real-time PCR detection system (Bio-Rad) according to the manufacturer's instructions (Qiagen).

Bioinformatics and statistical analysis

In this study, the LD muscles of Large White pigs were used as experimental group, the LD muscles of Min pigs were used as control group. Different statistical tools were used for analyzing the data. Graph Pad Prism 6.01 (Graph Pad Software, San Diego, CA, USA) was used for analyzing our results. Student's t-tests were used to compare the control and experimental groups. For all comparisons, *p < 0.05, **p < 0.01, ***p <0.001, ****p < 0.0001, were considered significant difference. An MS-Excel sheet (Supplementary Table II) with macros downloaded from the manufacturer's website (http://www.

sabiosciences.com/pcrarraydataanalysis.php) was used to analyze the qRT-PCR array data based on a protocol (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php) provided by SABiosciences (Qiagen). The $2^{-\Delta CT}$ method was used to calculated the Ct values from the qRT-PCR array data. Five genes (*B2M, GAPDH, HPRT1, ACTB* and *RPL13A*) were used as reference genes. A p value less than 0.05 and $|\log_{\text{Fold Change(FC)}}| \geq 1$ were regarded as the cutoff thresholds for differentially expressed genes(DEGs). TB tools (https://github.com/CJ-Chen/TBtools) was used to construct the heat map of the qRT-PCR array and the Venn diagrams of differentially expressed genes (DEGs). The STRING database was used to predict protein interactions and construct the network for DEGs (Szklarczyk *et al*., 2015). The protein-protein interaction (PPI) network was visualized by Cytoscape (Shannon *et al*., 2003).

RESULTS

IMF content of LD muscles in the large white and min pigs

The IMF content assay indicated that the IMF content of Large White LD muscles was significantly lower than that of Min pig LD muscles (1.693% and 4.963%, respectively, $P < 0.0001$) (Fig. 1). These results indicated that these two pig breeds were fit for identifying the expression of genes in these two signaling pathways associated with different IMF content.

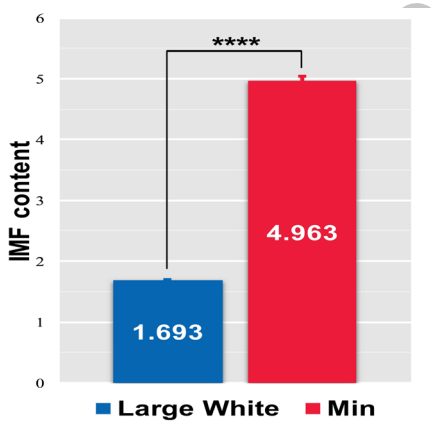

Fig. 1. Detection of IMF content in LD muscles of Large White and Min pigs. Data represent means ± SEM (n=3), ****$P < 0.0001$.

Identification of the PPAR signaling pathway in the LD muscles of the two pig breeds

The PPAR signaling pathway (ssc03320) consists of 69 genes (KEGG Pathway Database). In this study, these genes had their expression detected by the qRT-PCR array. The gene expression profile is significantly different between Large White and Min group, set of genes can be successfully clustered (Fig. 2A). A colored map shows a graphical representation of DEGs in Large White group against Min group, red and green color represent upregulated and downregulated genes respectively (Fig. 2B). Our results showed that 42 out of the 69 examined genes were differentially expressed between the Large White and Min group, with 20 being upregulated and 22 being downregulated in the LD muscle of Large White pigs (Table I). Among the 42 DEGs, some genes related to fatty acid transport (*FAT/CD36, FABP1*, *ACSL1, LPL, and ACSBG2*), fatty acid oxidation (*CPT1A, CPT1B, ACAA1, ACADM* and *ACADL*), and fatty acid biosynthesis (*SCD* and *SCD5*). In addition, we used STRING database and Cytoscape software to construct the PPI network for 42 DEGs. The PPI network contained 40 nodes and 245 edges, the most significant 10 node degree genes (*PPARA, PPARG, ACSL1, CPT1A, CPT1B, FABP1, CD36, LPL, SCD*, and *FABP4*) were selected as hub genes (Fig. 3 and Table II). Moreover, taken into account the results of colored map and PPI network, 12 genes in the PPAR signaling pathway (*PPARA, CD36, FABP1, LPL, ACSL1, CPT1A, CPT1B, ACAA1, ACADL, ACADM, PPARG* and *SCD*) were selected as key hub genes, and their expression patterns are presented in Figure 4, these genes are associated with fatty acid transport, fatty acid oxidation, fatty acid biosynthesis and IMF deposition. Taken together, these results show that the PPAR signaling pathway is more active in the Large White breed than in the Min breed.

GO BP enrichment of DEGs in the PPAR signaling pathway

As shown in Figure 5, the BPs participated by upregulated genes were mainly involved in fatty acid β-oxidation, fatty acid oxidation, lipid oxidation, fatty acid transport and fatty acid catabolic processes. These results indicated that compared with the LD muscle of Min pigs, the LD muscle of Large White pigs consumes more fat during energy metabolism, and this may explain the lower fat deposition in Large White pigs.

Identification of the fatty acid metabolism signaling pathway in the LD muscles of the two pig breeds

The fatty acid metabolism signaling pathway (ssc01212) consists of 47 genes (KEGG Pathway Database). In this study, these genes had their expression detected by the qRT-PCR array. For heatmap, the genes showed significant expression patterns between Large White and Min group, and they successfully clustered into several sets (Fig. 6A). For colored map (Fig. 6B),

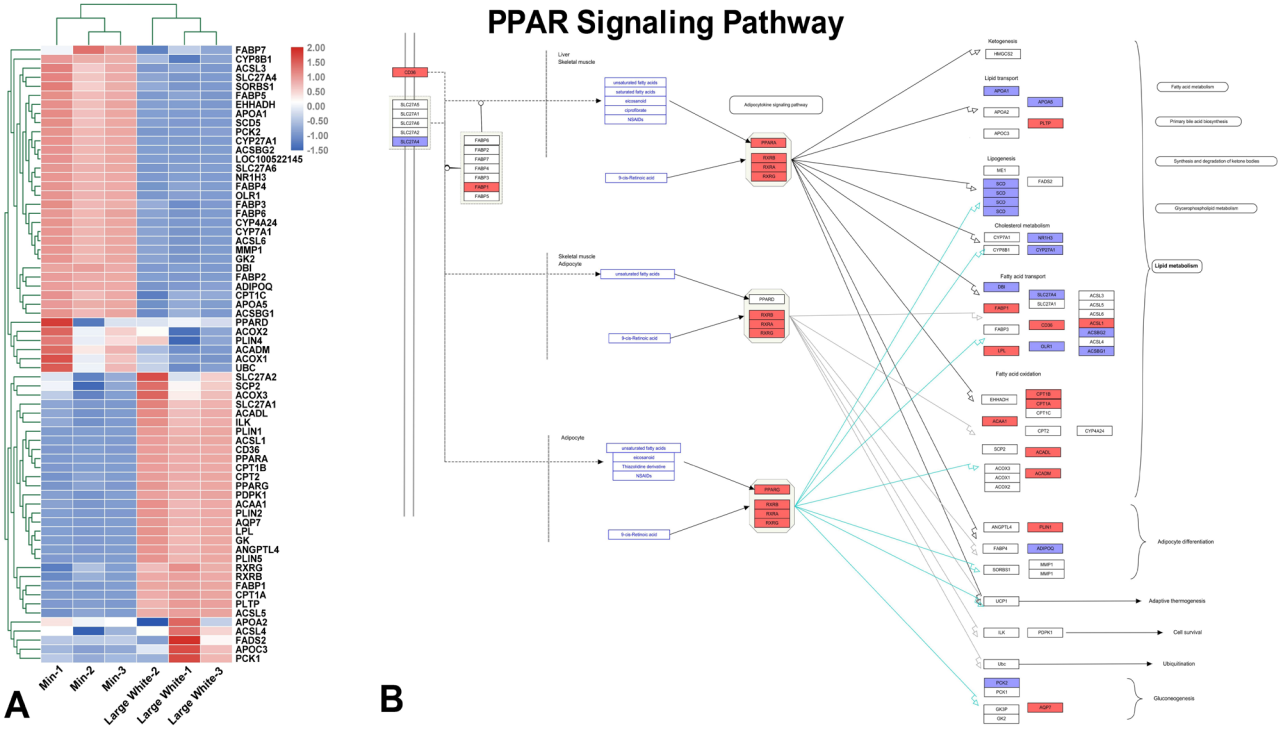

Fig. 2. qRT-PCR array results for the PPAR signaling pathway in two pig breeds. (A) A heatmap of all qRT-PCR array genes in the PPAR signaling pathway. The colors (blue, black, and red) represent the gene expression level in the LD of two pig breeds (Min and Large White). (B) Colored map of the PPAR signaling pathway. Upregulated and downregulated genes are colored in red and blue, respectively.

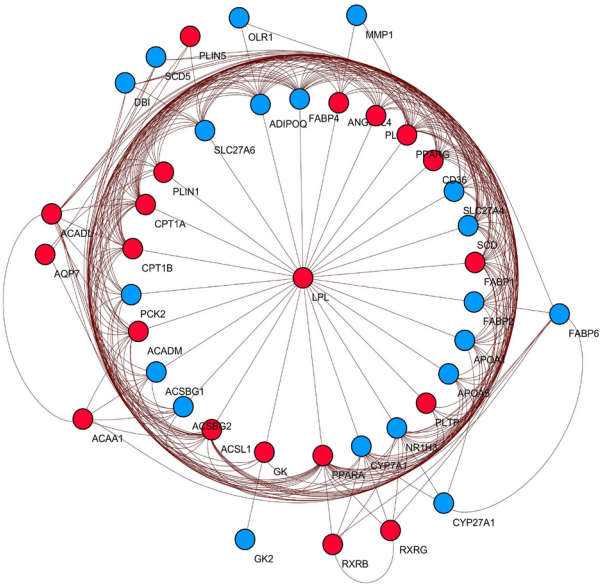

Fig. 3. PPI network for the DEGs in the PPAR signaling pathway. Upregulated and downregulated genes are colored in red and blue, respectively. Node stands for the protein (gene); edge stands for the interaction of proteins(genes).

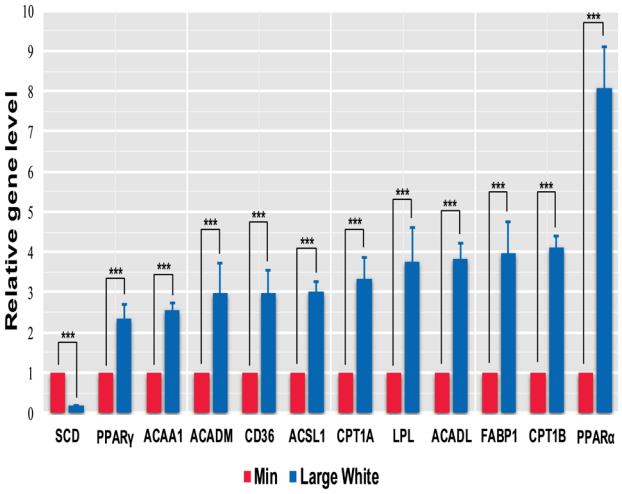

Fig. 4. Comparisons of the expression levels of twelve key hub genes (*PPARA, CD36, FABP1, LPL, ACSL1, CPT1A, CPT1B, ACAA1, ACADL, ACADM, PPARG* and *SCD*) in the PPAR signaling pathway in the two pig breeds. A p value less than 0.05 and $|\log_{\text{Fold Change(FC)}}| \geq 1$ were regarded as the cutoff thresholds for DEGs. All data are shown as means ± SEM (n=3), ***P<0.001.

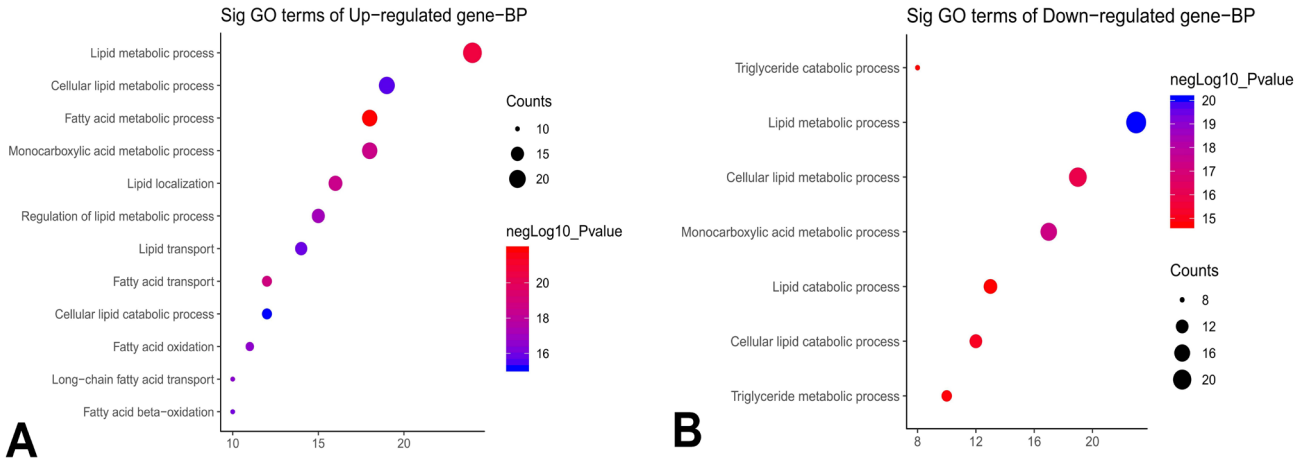

Fig. 5. GO Biological Process (BP) enrichment of upregulated (A) and downregulated (B) genes in the PPAR signaling pathway.

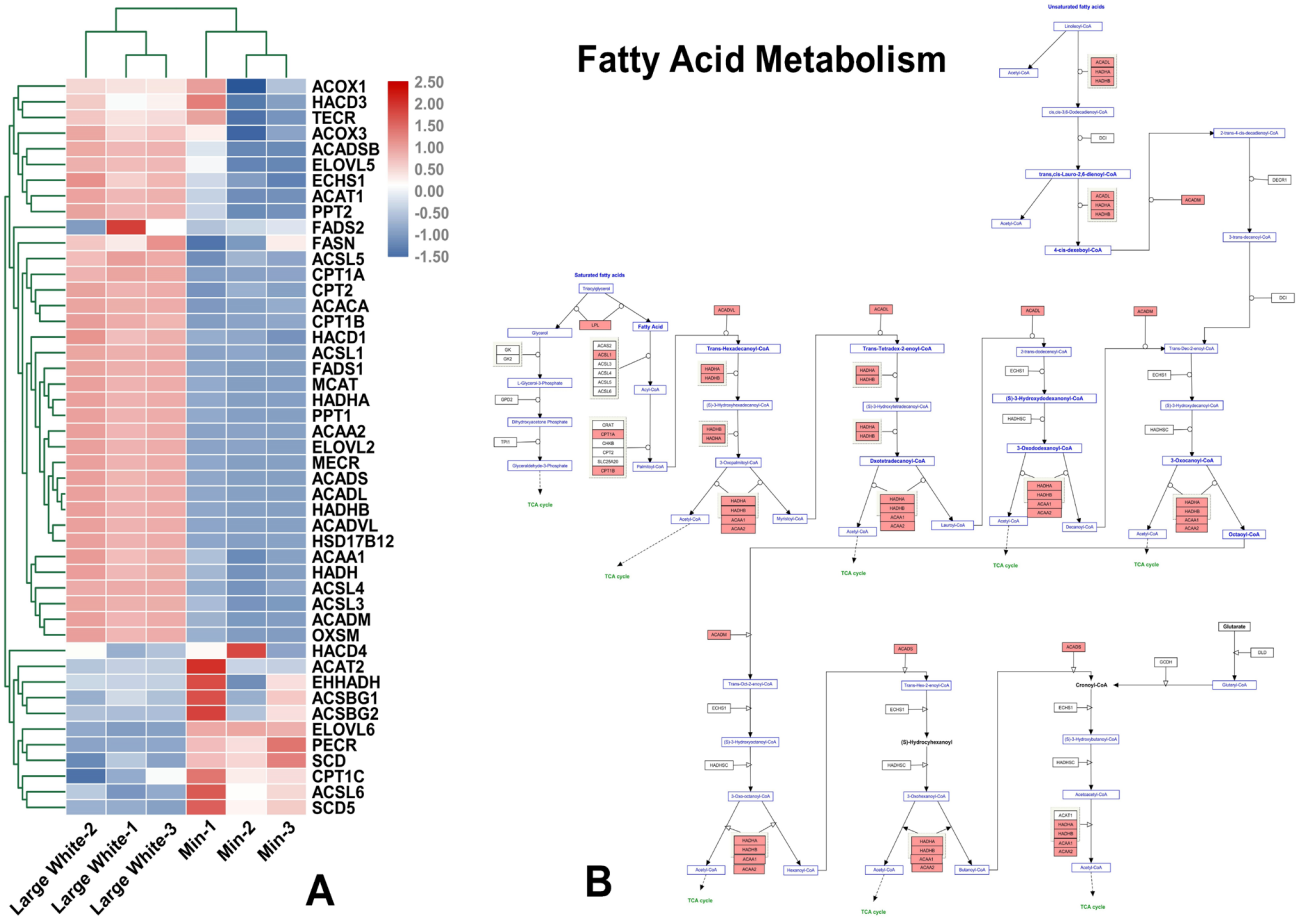

Fig. 6. qRT-PCR array results for the fatty acid metabolism signaling pathway in two pig breeds. (A) A heatmap of all qRT-PCR array genes in the fatty acid metabolism signaling pathway. The colors (blue, black, and red) represent gene expression levels in the LD of two pig breeds (Min, and Large White); (B) Colored map of the fatty acid metabolism signaling pathway. Upregulated and downregulated genes are colored in red and blue, respectively.

Table I. qRT-PCR array results for the PPAR signaling pathway (Large White-Min).

Gene symbol	Fold-change	P-value	Regulation
PPARA	8.084412043	9.72E-06	Up
PLIN5	6.840235862	8.62E-09	Up
ANGPTL4	5.217036652	1.14E-11	Up
GK	5.00157363	7.51E-12	Up
RXRB	4.921137382	1.99E-02	Up
RXRG	4.131238515	1.61E-03	Up
CPT1B	4.096161077	2.79E-06	Up
FABP1	3.961630498	3.89E-10	Up
AQP7	3.919214964	9.43E-11	Up
ACADL	3.843627184	6.38E-09	Up
LPL	3.748782175	7.94E-08	Up
PLIN1	3.542042156	1.87E-07	Up
CPT1A	3.348062077	5.37E-10	Up
PLIN2	3.188133697	2.38E-10	Up
ACSL1	3.007946783	5.34E-09	Up
CD36	2.988137088	5.04E-10	Up
ACADM	2.967591733	2.26E-06	Up
ACAA1	2.56635317	1.60E-13	Up
PPARG	2.348890709	4.72E-06	Up
PLTP	2.02949545	7.26E-10	Up
DBI	-2.05013384	2.67E-08	Down
CYP4A24	-2.12501151	2.04E-13	Down
APOA1	-2.14154421	1.20E-09	Down
MMP1	-2.1442043	1.10E-11	Down
CYP7A1	-2.1442043	6.87E-14	Down
GK2	-2.1442043	1.47E-13	Down
SLC27A4	-2.14516778	3.75E-09	Down
FABP6	-2.15185161	8.05E-09	Down
FABP4	-2.16469078	7.06E-09	Down
CYP27A1	-2.16783714	9.70E-12	Down
SCD	-2.17054507	1.23E-04	Down
ACSBG1	-2.23209128	7.14E-02	Down
OLR1	-2.64415442	1.18E-08	Down
ACSBG2	-2.78828039	7.69E-04	Down
ADIPOQ	-2.79931981	5.91E-05	Down
SCD5	-2.9605646	6.16E-06	Down
FABP2	-3.12676057	1.73E-11	Down
NR1H3	-3.37368493	1.17E-09	Down
PCK2	-3.76994247	2.40E-07	Down
SLC27A6	-4.03289334	4.30E-07	Down
LOC100522145	-4.63203509	5.65E-12	Down
APOA5	-5.63906119	1.58E-03	Down

Table II. The summary for the PPI network of DEGs in the PPAR signaling pathway.

Gene symbol	Degree
LPL	26
PPARA	25
PPARG	25
CPT1A	23
SCD	22
ACSL1	22
FABP1	22
FABP4	22
CPT1B	19
CD36	18
ACADM	17
NR1H3	17
ADIPOQ	17
SLC27A6	16
PLIN2	15
CYP7A1	15
APOA1	14
PCK2	13
ACADL	13
SLC27A4	11
PLIN1	11
FABP2	10
ACSBG1	9
ACSBG2	9
APOA5	9
ANGPTL4	9
FABP6	7
DBI	6
GK	6
PLTP	6
ACAA1	6
SCD5	5
RXRG	5
RXRB	5
CYP27A1	4
PLIN5	3
AQP7	3
MMP1	2
OLR1	2
GK2	1

The top ten degree genes were shown in bold.

upregulated and downregulated genes in the Large White group were in tuitively presented by colored in red and blue, respectively. The results showed compared with Min group, there are 18 genes upregulated and 13 genes downregulated in Large White group (Table III), these including several genes related to fatty acid oxidation (*ACAA2, ACADS, ACADM, ACADL, ACADVL, CPT1A, CPT1B, HADHA,* and *HADHB*), fatty acid transport (*ACSBG2* and *ACSL1*) and fatty acid biosynthesis (*SCD* and *SCD5*). Moreover, PPI networks of 31 DEGs were established by using STRING database and Cytoscape software, which included 31 nodes and 178 edges (Fig. 7 and Table IV). In the PPI networks, the most significant 13 node degree genes (*ACAA2, ACACA, ACADL, ACADM, ACADS, ACSBG2, ACSL1, CPT1A, CPT1B, EHHADH, HADHA, HADHB, and HSD17B12*) were defined as hub genes. Moreover, taken into account the results of colored map and PPI network, 13 genes, 6 genes only in the fatty acid metabolism signaling pathway (*ACACA, ACAA2, ACADS, ACADVL, HADHA* and *HADHB*) and 7 genes (*ACAA1, CPT1A, CPT1B, ACADL, ACADM, ACSL1* and *SCD*) shared by the PPAR and fatty acid metabolism signaling pathways, were selected as key hub genes, and their expression patterns are presented in Figures 8 and 4, respectively. These 13 genes are also involved in fatty acid transport, fatty acid oxidation, fatty acid biosynthesis and IMF deposition. After analyzing these results, we could see that the fatty acid metabolism signaling pathway is more active in the Large White breed than in the Min breed.

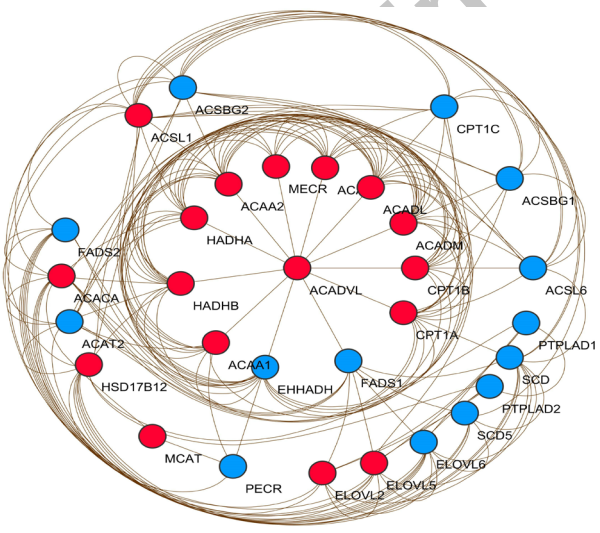

Fig. 7. PPI network for the DEGs in the fatty acid metabolism signaling pathway. Upregulated and downregulated genes are colored in red and blue, respectively. Node stands for the protein (gene); edge stands for the interaction of proteins(genes).

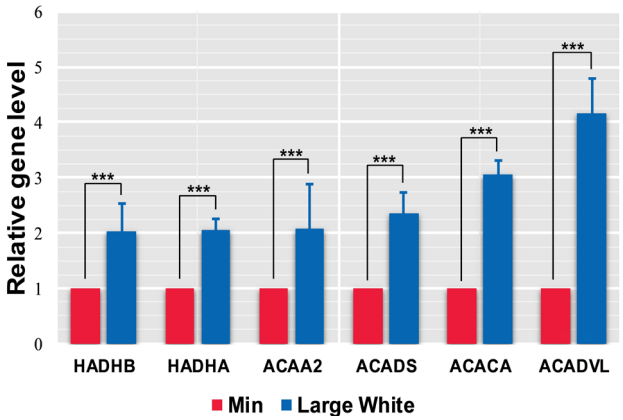

Fig. 8. Comparisons of the expression levels of six key hub genes (*ACACA, ACAA2, ACADS, ACADVL, HADHA* and *HADHB*) in the fatty acid metabolism signaling pathway in the two pig breeds. A p value less than 0.05 and $|\log_{\text{Fold Change(FC)}}| \geq 1$ were regarded as the cutoff thresholds for DEGs. All data are shown as means ± SEM (n=3), ***P<0.001.

GO BP enrichment of DEGs in the fatty acid metabolism signaling pathway

As shown in Figure 9, the BPs participated by upregulated genes were mainly involved in fatty acid β-oxidation, fatty acid oxidation, lipid oxidation and fatty acid catabolic processes. These results indicated that the upregulated genes of the fatty acid metabolism signaling pathway mainly participated in reducing the fat deposition in the LD muscle of Large White pigs.

The DEGs shared by the PPAR and fatty acid metabolism signaling pathways

The PPAR and fatty acid metabolism signaling pathways shared 20 genes (Fig. 10, Table V). Of these 20 shared genes, 10 were differentially expressed in the LD muscle of Large White pigs, with 6 being upregulated and 4 being downregulated (Table V). As shown in Figures 10 and 6 upregulated genes (*ACAA1, ACSL1, ACADM, ACADL, CPT1A, and CPT1B*), which were related to fatty acid oxidation, were shared by these two signaling pathways. These findings suggest that these 6 upregulated genes may play important roles in activating these two signaling pathways and regulating IMF deposition in pigs.

DISCUSSION

In the modern pig breeding industry, IMF content has become an important determinant of meat quality for raisers and consumers. There was significant difference in IMF content between Chinese local pigs and famous commercial lean pigs. As a typical lean-type pig breed, the Large White

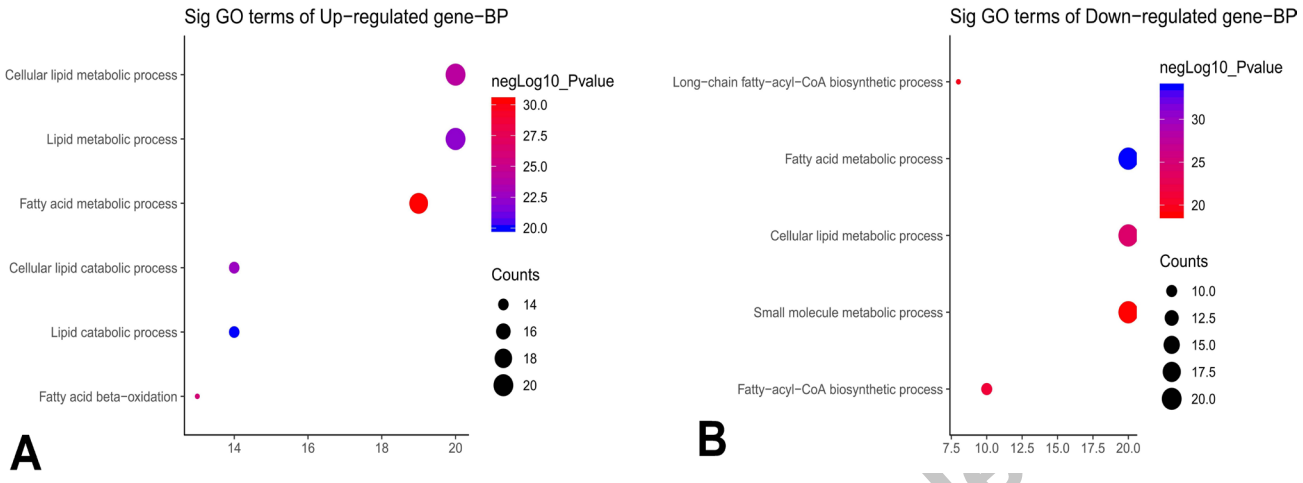

Fig. 9. GO Biological Process (BP) enrichment of upregulated (A) and downregulated (B) genes in the fatty acid metabolism signaling pathway.

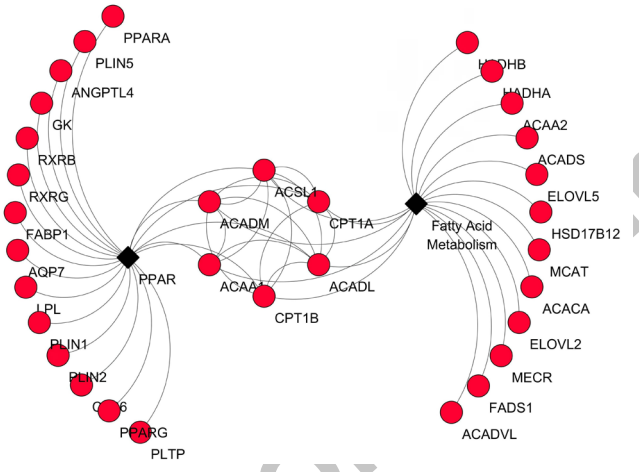

Fig. 10. The upregulated genes shared by the PPAR and fatty acid metabolism signaling pathways.

grows faster and has lower IMF content. By contrast, the Min pig is a well-known Chinese fat-type pig breed and characterized by high intramuscular fat (IMF) content, and in our results, we also found that the Min pig had higher IMF content than that in Large White pig (Fig. 1). Unfortunately, the differences in IMF content of these two pig breeds are not fully understood. The search for candidate genes and signaling pathways associated with IMF deposition is necessary. The IMF deposition is known to be regulated by multiple genes and signaling pathways. At present, many studies have focused on finding candidate genes and potential mechanisms associated with regulation of IMF deposition in pig, few studies focus on the correlation of signaling pathways with IMF content in different pig breeds. Therefore, in the present research, the expression levels of all genes in the PPAR and fatty acid metabolism signaling pathways were detected by a qRT-PCR array. Our research will help cultivate more valuable commodity pig breeds.

PPAR signaling pathway has long been considered important for fatty acid metabolism and meat quality in mammals (He *et al.*, 2013; Wang *et al.*, 2016), genes of which are involved in three biological processes: lipogenesis, fatty acid transport and fatty acid oxidation, that have been reported to associate with IMF deposition. The DEGs we obtained from our experiment are all involved in the three biological processes: (1) fatty acid transport (*FAT/CD36, FABP1*, *FABP3*, *FABP5, ACSL1, ACSBG2, and LPL*) (Bonen *et al.*, 2004; Campbell *et al.*, 2004; Jiang and Li, 2006; Luo *et al.*, 2009; Ellis *et al.*, 2010; Liu *et al.*, 2011; Serao *et al.*, 2011; Widmann *et al.*, 2011; Jeong *et al.*, 2015; Wang *et al.*, 2017), (2) fatty acid oxidation (*CPT1A, CPT1B, ACAA1, ACADM,* and *ACADL*) (Kim *et al.*, 2000; Zha *et al.*, 2005; Wu *et al.*, 2013; Puig-Oliveras *et al.*, 2014; Zhang *et al.*, 2014; Chen *et al.*, 2017; Qiu *et al.*, 2017; Wang *et al.*, 2017), (3) lipogenesis (*SCD*) (Wang *et al.*, 2013, 2015; Ros-Freixedes *et al.*, 2016). Compared with the Min pig group, four fatty acid transport-related genes (*FAT/CD36, FABP1*, *ACSL1, and LPL*) and five fatty acid oxidation-related genes (*CPT1A, CPT1B, ACAA1, ACADM,* and *ACADL*) were highly upregulated, and the lipogenesis-related gene (*SCD*) was downregulated in the Large White pig group (Fig. 4, Table I), which indicated that the ability of transport fatty acids and fatty acid oxidation in Large White was stronger than Min, and the rate of fatty acid synthesis in Large White was weaker than Min. The dynamic balance between fatty acid synthesis and degradation affects the IMF content in pigs, the different ability of fatty acid oxidative degradation and synthesis could contribute to the difference in IMF

content between Large White than Min pig breeds.

Table III. qRT-PCR array results for the fatty acid metabolism signaling pathway (Large White-Min).

Gene symbol	Fold-change	P-value	Regulation
ACADVL	4.1670828	2.00E-08	Up
FADS1	4.14549659	2.01E-07	Up
CPT1B	4.09616108	2.79E-06	Up
ACADL	3.84362718	6.38E-09	Up
MECR	3.65267791	8.44E-08	Up
CPT1A	3.34806208	5.37E-10	Up
ELOVL2	3.20952208	2.99E-09	Up
ACACA	3.05264456	1.26E-03	Up
ACSL1	3.00794678	5.34E-09	Up
ACADM	2.96759173	2.26E-06	Up
MCAT	2.75248718	4.25E-06	Up
ACAA1	2.56635317	1.60E-13	Up
HSD17B12	2.55652762	1.26E-10	Up
ELOVL5	2.4520947	1.41E-03	Up
ACADS	2.36525946	2.93E-11	Up
ACAA2	2.08178922	1.38E-07	Up
HADHA	2.04896505	2.05E-08	Up
HADHB	2.03345962	1.52E-08	Up
FADS2	-2.1272039	1.00E-04	Down
SCD	-2.1705451	1.23E-04	Down
HACD3	-2.1842869	7.22E-07	Down
ACSBG1	-2.2320913	7.14E-02	Down
HACD4	-2.3350926	4.12E-07	Down
ACSL6	-2.4473721	1.98E-08	Down
EHHADH	-2.5648137	1.28E-03	Down
ACSBG2	-2.7882804	7.69E-04	Down
CPT1C	-2.7955525	8.71E-06	Down
ACAT2	-2.9258849	6.10E-05	Down
SCD5	-2.9605646	6.16E-06	Down
ELOVL6	-3.1983219	2.50E-09	Down
PECR	-6.4503496	7.49E-08	Down

Table IV. The summary for the PPI network of DEGs in the fatty acid metabolism signaling pathway.

Gene symbol	Degree
ACSL1	18
ACADL	17
ACADM	17
EHHADH	16
ACAA2	16
HADHB	16
CPT1A	15
ACACA	15
HADHA	14
ACSBG2	13
CPT1B	13
HSD17B12	13
ACADS	13
SCD	12
ELOVL6	12
ACAT2	12
ACADVL	12
ACSL6	11
CPT1C	11
ELOVL5	11
ACAA1	11
FADS1	10
FADS2	10
ACSBG1	9
MECR	8
ELOVL2	8
SCD5	7
PTPLAD2	5
PTPLAD1	5
PECR	4
MCAT	2

The top ten degree genes were shown in bold.

Moreover, as one of the key regulators in the PPAR signaling pathway, the peroxisome proliferator-activated receptor α gene (*PPARα*) plays a critical role in fatty acid oxidation and IMF deposition in pigs and rats (He *et al*., 2013; Wang *et al*., 2016; Zhang *et al*., 2016). In addition, many studies have reported that PPAR*α* regulated lipid metabolism through targeting genes involved in fatty acid uptake (*FABP1, LPL*), fatty acid transport (*FAT/CD36, ACSL1,* and *FABP1*) and fatty acid oxidation (*ACADS,*

ACADM, ACADL, ACADVL, CPT1A, CPT1B and *ACAA1*) (Rakhshandehroo *et al*., 2010; Gessner *et al*., 2015; Xu *et al*., 2015; Zhang *et al*., 2016). Consistent with these findings, *PPARα* was highly expressed in the Large White pig group (Fig. 4, Table I), implying that *PPARα* has a positive role in reducing the IMF content in pigs. Taken together, all these results above make it tempting to suggest that the activation of the PPAR signaling pathway may play positive role in reducing the IMF content in the LD muscle of Large White pigs.

Table V. The Venn diagram summaries for the PPAR and fatty acid metabolism signaling pathways.

The genes shared by the PPAR and fatty acid metabolism signaling pathways
CPT1A, ACSL1, SCD5, ACSL6, ACSL5, ACSL4, ACSL3, CPT1C, CPT1B, FADS2, CPT2, ACADL, ACOX1, SCD, EHHADH, ACSBG1, ACADM, ACOX3, ACAA1, ACSBG2
The DEGs shared by the PPAR and fatty acid metabolism signaling pathways
CPT1A, ACADL, ACSL1, *SCD, ACSBG1,* ***ACADM, ACAA1, CPT1B,*** *ACSBG2, SCD5*

The shared upregulated genes were shown in bold.

At the same time, the fatty acid metabolism signaling pathway also plays an important role in regulating fatty acid metabolism and growth traits in pigs (Yang *et al*., 2012). The biological processes lipogenesis, fatty acid transport and fatty acid oxidation are also critical to this fatty acid metabolism-related signaling pathway, and many genes in this signaling pathway have been widely studied. In our results (Fig. 8, Table III), fatty acid transport-related gene (*ACSL1*) (Ellis *et al*., 2010; Widmann *et al*., 2011), the first step of mitochondrial fatty acid β-oxidation-related genes (*ACADS, ACADM, ACADL,* and *ACADVL*) (Puig-Oliveras *et al*., 2014; Chen *et al*., 2017; Wang *et al*., 2017), the last three steps of mitochondrial fatty acid β-oxidation-related genes (*HADHA* and *HADHB*) (Zha *et al*., 2005), the last step of the mitochondrial fatty acid β-oxidation-related gene (*ACCA2*) (Doi *et al*., 2003; Zha *et al*., 2005), the gene involved in peroxisomal fatty acid oxidation (*ACAA1*) (Wu *et al*., 2013) and the rate-limiting genes of mitochondrial fatty acid β-oxidation (*CPT1A* and *CPT1B*) (Kim *et al*., 2000; Zhang *et al*., 2014; Qiu *et al*., 2017) were all highly expressed in the LD muscle of Large White pigs, and the fatty acid synthesis-related gene (*SCD*) had a low expression level in the Large White group. These results indicated that the Large White pig has stronger ability of fatty acid oxidation, which might lead to fatty acid degradation and low IMF content in the LD muscle of Large White pig breeds. Finally, taking into account the results above, our results indicate that the activation of the fatty acid metabolism signaling pathway may reduce the IMF content in the LD muscle of Large White pigs.

Interestingly, among the upregulated genes in these two signaling pathways, six genes (*ACAA1, ACSL1, ACADM, ACADL, CPT1A, and CPT1B*) (Fig. 10) were shared by them. Notably, acyl-CoA synthetase-1 (*ACSL1*), one member of the long chain acyl-CoA synthetase family (ACSLs), is essential for fatty acid uptake, oxidation and degradation. *ACSL1* is required for the initial step of fatty acid oxidation and specifically directs fatty acids towards mitochondrial β-oxidation (Ellis *et al*., 2010; Widmann *et al*., 2011). Carnitine palmitoyltransferase-1 (*CPT1*) has been regarded as a rate-limiting enzyme of mitochondrial fatty acid β-oxidation and is closely related to fat deposition. In addition, a high expression level of *CPT1* could promote fatty acid decomposition and decrease fat deposition. Additionally, *CPT1A* and *CPT1B*, two common isoforms of *CPT1*, play prominent roles in fatty acid oxidation and lipid accumulation in human, chicken and pigs (Kim *et al*., 2000; Zhang *et al*., 2014; Qiu *et al*., 2017), the decrease in fat deposition are associated with high expression levels of these two genes. Acyl-CoA dehydrogenase medium chainand long chain (*ACADM* and *ACADL*) encode the acyl-CoA dehydrogenases (*MCAD* and *LCAD*) and catalyze the first step of mitochondrial fatty acid β-oxidation (Hashimoto *et al*., 1999; Wang *et al*., 2017). Several researchers have reported that these two genes are closely related to IMF deposition in pigs (Puig-Oliveras *et al*., 2014; Chen *et al*., 2017; Wang *et al*., 2017). Acetyl-CoA acyltransferase (*ACAA1*), also called peroxisomal 3-ketoacyl-CoA thiolase, is encoded by the *ACAA1* gene and involved in peroxisomal fatty acid oxidation (Zha *et al*., 2005). In addition, this gene is also related to the IMF content trait in the LD of pigs (Wu *et al*., 2013). In this study, these six genes were all upregulated in the LD muscle of Large White pigs, which appears to show that these six genes are important for the activation of the PPAR and fatty acid metabolism signaling pathways, and they might be potential candidate genes to identify mechanisms that regulate IMF deposition in pigs.

CONCLUSIONS

In conclusion, our study reports the expression profiles of the PPAR and fatty acid metabolism signaling pathway-related genes in two pig breeds. Although the abundance of mRNA has been determined in our study, it is necessary to investigation DEGs with multi-omics analysis in the subsequent studies. Our results suggest that the activation of these two signaling pathways may play a positive role in reducing IMF content in pigs. These findings may also

provide new insights into the key signaling pathways involved in fat deposition in pigs.

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Statement of conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Supplementary materials

There are two supplementary tables in MS Word associated with this article. Access these online at: http://dx.doi.org/10.17582/journal.pjz/2020.52.....

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