Long-term dietary resveratrol supplementation decreased serum lipids levels, improved intramuscular fat content, and changed the expression of several lipid metabolism-related miRNAs and genes in growing-finishing pigs¹

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ABSTRACT: This study was conducted to evaluate the effects of dietary resveratrol supplementation on growth performance, meat quality, serum lipid profiles, intramuscular fat (IMF) deposition, and the expression levels of several lipid metabolism-related miRNAs and genes in growing-finishing pigs. A total of 36 healthy cross-bred pigs (Duroc × Landrace × Yorkshire) with an average initial BW of 24.67 ± 3.49 kg were randomly divided into two groups and fed either with a basal diet (CON) or basal diet containing 600 mg/kg resveratrol (RES). The trial lasted for 119 d. Resveratrol had no significant effect on growth performance and carcass characteristics. However, the concentrations of serum triglyceride, total cholesterol, low-density lipoprotein cholesterol, and very low-density lipoprotein were lower in RES group than those of CON group

($P < 0.05$). Dietary resveratrol supplementation increased the IMF content in *longissimus dorsi* ($P < 0.05$), up-regulated mRNA abundances of peroxisome proliferator-activated receptor γ , fatty acid synthase, acetyl-CoA carboxylase, and lipoprotein lipase ($P < 0.05$), while downregulated mRNA abundances of carnitine palmitoyl transferase-1, sirtuin 1, and peroxisome proliferator-activated receptor α ($P < 0.05$) in LM. In addition, resveratrol enhanced ($P < 0.05$) the expression of ssc-miR-181a, ssc-miR-370, and ssc-miR-21 and reduced ($P < 0.05$) the expression of ssc-miR-27a in *longissimus dorsi*. These results indicated that dietary resveratrol supplementation significantly improved IMF content and decreased serum lipids levels, which might be related with the changes in ssc-miR-181a, ssc-miR-370, ssc-miR-21, ssc-miR-27a and their downstream genes expression.

Key words: intramuscular fat, lipid metabolism, meat quality, microRNAs, resveratrol

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INTRODUCTION

With the increasing demands for pork quality, how to improve the meat quality has become a research focus. The intramuscular fat (IMF) content, as an important indicator, is used to evaluate

the meat quality with a positive influence on meat quality traits, such as flavor, juiciness, tenderness (Van et al., 2003; Cannata et al., 2010). Hence, how to improve the deposition of IMF has already become a key point of meat quality manipulation.

In order to boost the IMF content fundamentally, it is necessary to clarify the underlying molecular mechanism. In recent years, most of the relevant studies focused on the lipid metabolism-related genes (Zhao et al., 2010; Wang et al., 2012), but failed to consider the relationships between miRNAs and IMF accumulation. MicroRNAs

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are a class of endogenous, ~22 nucleotide length, mature noncoding RNAs that regulate genes expression negatively by binding to the 3'-untranslated region (UTR) of target mRNAs (Bartel et al., 2009). Recent experiments in vitro demonstrated that several miRNAs could influence cholesterol, triglyceride, and fatty acid metabolism indirectly by regulating the expression of lipid-metabolism-related genes. For example, Peng et al., (2013) showed that miR-224 could regulate fatty acid metabolism through acyl-CoA synthetase long-chain family member 4 (ACSL4). Kim et al., (2010) showed that miR-27a could inhibit fat deposition by suppressing the expression of peroxisome proliferator-activated receptor γ (PPAR γ), a key regulatory factor of lipid metabolism. However, the relationship between miRNAs and lipid metabolism is not clear enough until now. Furthermore, most studies on the effects of miRNAs on lipid metabolism have been conducted in vitro, very few studies have investigated whether lipid metabolism-related miRNAs verified in vitro play similar roles in vivo. In the present study, several genes which were key factors verified by previous studies in relation to lipogenesis including PPAR γ , fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), lipoprotein lipase (LPL), and sterol-regulatory element binding protein-1c (SREBP-1c) (Grindflek et al., 2000; Yin et al., 2002; Stoeckman et al., 2002; Smith et al., 2003; Picard et al., 2004), lipid degradation including sirtuin 1 (SIRT1), LIPE, carnitine palmitoyl transferase-1 (CPT-1), peroxisome proliferator-activated receptor α (PPAR α), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) (Mersmann et al., 1998; Picard et al., 2004; DeBerardinis et al., 2006; Rakhshandehroo et al., 2007; Nikolic et al., 2012) and transport of fatty acid including H-FABP (Jurie et al., 2007), were selected. At the same time, a total of six candidate miRNAs which were verified in vitro testing that could regulate the expression of those key lipid metabolism-related genes were selected to investigate their effects on IMF deposition in growing-finishing pigs including ssc-miR-370, ssc-miR-27a, ssc-miR-143-3p, ssc-miR-21, ssc-miR-26a, and ssc-miR-181a. These miRNAs were previously reported to be highly expressed in porcine adipose tissues as well as *longissimus dorsi* (Li et al., 2010).

The IMF content in pigs is regulated by many factors including nutrition, heredity, and environment. Previous reports have revealed that low dietary protein and high dietary energy both can improve IMF content in growing-finishing pigs (Gondret et al., 2002; Teye et al., 2006). In addition, dietary

supplementation with betaine or conjugated linoleic acid can also enhance the IMF content in pigs (Joo et al., 2002; Cordero et al., 2010; Martins et al., 2012). Resveratrol is a nonflavonoid polyphenolic compound containing stilbenes structure and found predominantly in peanuts, giant knotweed, and grapes (Zhang et al., 2007). Its major functions are anti-inflammatory, anticancer, and antioxidation (Holme et al., 2007). Furthermore, it was reported that resveratrol could affect lipid metabolism and regulate preadipocyte differentiation and proliferation by enhancing the activity of SIRT1 (Kulkarni et al., 2015), as well as facilitating its expression (Bai et al., 2008; Shan et al., 2009). Previous report has revealed that dietary supplementation with 300 or 600 mg/kg resveratrol can improve serum lipid profiles and decrease body fat deposition in finishing pigs (Zhang et al., 2015a). However, there was no relevant research about the effects of long-term dietary resveratrol supplementation on lipid metabolism especially IMF metabolism and the expression of several lipid metabolism-related miRNAs in growing-finishing pigs.

The objectives of this research were to investigate the effects of long-term dietary resveratrol supplementation on meat quality and lipid metabolism in a pig model, furthermore, to determine whether lipid metabolism-related miRNAs play important roles in these processes. The results of this study may contribute to our further understanding of the underlying mechanism of miRNAs on IMF deposition and help to clarify the application potential of resveratrol in pig production.

MATERIALS AND METHODS

All experimental procedures were approved by Sichuan Agricultural University Committee of Laboratory Animal Care.

Animal and Experimental Diets

A total of 36 (67 ± 1 d old) healthy crossbred pigs (Duroc $\times$ Landrace $\times$ Yorkshire) with an average initial BW of 24.67 ± 3.49 kg were randomly divided into two groups with six replicate pens (three barrows replicates and three gilts replicates) per group and three pigs per replicate. Pigs were fed either with a basal diet (CON) or basal diet containing 600 mg/kg resveratrol (RES). The resveratrol was purchased from Ci Yuan Biotechnology Co. Ltd. (purity $\geq 99\%$, Xi'an, Shanxi, China). Composition and nutrients levels of basal diet were shown in Table 1. This trial was divided into four phases, with a specific diet for each phase: phase 1

Table 1. Composition and nutrients levels of basal diet (air dry basis, %)

Ingredients, % 25 to 50 kg, 50 to 75 kg, 75 to 100 kg, and 100 to 125 kg				
Corn	76.55	80.30	84.00	88.74
Soybean meal	16.71	15.43	10.87	6.20
Wheat bran			1.00	1.20
Fish meal	2.70			
Soybean oil	1.40	1.40	1.50	1.40
Limestone	0.73	0.70	0.63	0.59
CaHPO ₄	0.47	0.66	0.53	0.43
NaCl	0.30	0.35	0.35	0.35
L-Lysine HCl	0.49	0.51	0.48	0.46
DL-Methionine	0.08	0.07	0.07	0.06
L-Threonine	0.15	0.16	0.15	0.15
L-Tryptophan	0.04	0.04	0.04	0.04
Choline chloride	0.15	0.15	0.15	0.15
Vitamin premix ¹	0.03	0.03	0.03	0.03
Mineral premix ²	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Nutrients levels ³				
DE, Mcal/kg	3.40	3.40	3.40	3.40
CP, %	15.69	13.75	12.13	10.44
Ca, %	0.66	0.59	0.52	0.46
TP, %	0.50	0.46	0.42	0.39
AP, %	0.31	0.27	0.24	0.21
D-Lys, %	1.03	0.90	0.78	0.66
D-Met, %	0.30	0.25	0.23	0.20
D-Met+Cys, %	0.49	0.43	0.39	0.34
D-Thr, %	0.62	0.55	0.49	0.43
D-Trp, %	0.18	0.16	0.14	0.12

¹Vitamin premix provided the following per kilogram of diets: vitamin A, 9,000 IU; vitamin D₃, 3,000 IU; vitamin E, 20 IU; vitamin K₃, 3.0 mg; vitamin B₁, 1.5 mg; vitamin B₂, 4.0 mg; vitamin B₆, 3.0 mg; vitamin B₁₂, 0.02 mg; niacin, 30 mg; pantothenic, 15 mg; folic acid, 0.75 mg; biotin, 0.1 mg.

²Mineral premix provided the following per kilogram of diets, 25 to 50 kg: Fe(FeSO₄•H₂O) 60 mg, Cu(CuSO₄•5H₂O) 4 mg, Mn(MnSO₄•H₂O) 2 mg, Zn(ZnSO₄•H₂O) 60 mg, I(KI) 0.14 mg, Se(Na₂SeO₃) 0.2 mg; 50 to 75 kg: Fe(FeSO₄•H₂O) 50 mg, Cu(CuSO₄•5H₂O) 3.5 mg, Mn(MnSO₄•H₂O) 2 mg, Zn(ZnSO₄•H₂O) 50 mg, I(KI) 0.14 mg, Se(Na₂SeO₃) 0.15 mg; 75 to 125 kg: Fe(FeSO₄•H₂O) 40 mg, Cu(CuSO₄•5H₂O) 3 mg, Mn(MnSO₄•H₂O) 2 mg, Zn(ZnSO₄•H₂O) 50 mg, I(KI) 0.14 mg, Se(Na₂SeO₃) 0.15 mg.

³Nutrients levels were calculated values.

(from 25 to 50 kg BW), phase 2 (from 50 to 75 kg BW), phase 3 (from 75 to 100 kg BW), and phase 4 (from 100 to 125 kg BW). We estimated the duration of each phase and growth performance in growing–finishing pigs according to the criteria recommended by [NRC \(2012\)](#). The phase 1 lasted for 5 wk, and the other three phases lasted for 4 wk, respectively. Pigs were weighed on day 0 and at the end of each phase period. Feed intake was recorded daily for each replicate. The data collected were used to calculate the ADG, ADFI, and the ratio of feed to gain (F/G). During the experimental period, pigs were allowed to access to feed and water ad libitum. When the average BW of pigs was about 80 kg, all pigs were vaccinated with 2 mL of foot-and-mouth disease trivalent vaccine purchased from Jin Yu Biotechnology Co. Ltd. The trial lasted for 119 d.

Sample Collection

At the end of the trial, one pig (close to the average BW of per replicate) from each replicate was selected and bled via *anterior vena cava* after a 12-h overnight fast. After leaving the samples at room temperature for about 30 min, the blood samples were centrifuged at $3,000 \times g$ for 15 min. Then the serum samples were separated and stored at -20°C until analysis.

After bled, these 12 pigs were slaughtered. The left side of the carcass was used to measure carcass characteristics. *Longissimus dorsi* samples between the 5th and 13th ribs were dissected for meat quality measurement, IMF content measurement, and RNA extraction. At the same time, liver samples were collected and frozen quickly in

liquid nitrogen and then stored at -80°C for RNA extraction.

Carcass Data Collection

Carcass weights were recorded within 30 min postslaughter and used to calculate the dressing percentage. The backfat depths of the first rib, last rib, and sixth lumbar vertebrae were measured to calculate the average backfat thickness. The loin muscle area (LMA) was measured between 12th and 13th ribs using a vernier caliper.

Meat Quality Data Collection

The LM samples were cut into five pieces (5 cm thick) between the 5th and 13th ribs. The first piece dissected from 5th to 8th ribs was used for IMF content measurement and RNA extraction. The second piece was stored at 4°C for 24 h and used to measure the shear force. The third piece was used to measure the pH value, meat color, and marbling. The fourth piece and fifth piece were used for measurement of drip loss and cooking loss, respectively.

Muscle pH value was measured within 45 min postslaughter using a pH meter, then the muscle sample was put into refrigerator and stored at 4°C for 24 h. After that, the $\text{pH}^{24\text{h}}$ value was measured. Three points of each muscle sample were selected for measurement. Meat color was determined at 45 min and 24 h postslaughter, respectively, using a Chromameter CR-300 (Minolta, Osaka, Japan). The specific parameters were L^* (lightness), a^* (redness), and b^* (yellowness). Marbling of LM was appraised subjectively according to the NPPC (1991) standards. Drip loss and cooking loss were determined as previously described (Honikel et al., 1986), which were calculated based on weight loss and expressed as the weight change percentage.

The shear force of LM sample was measured using Texture Analyzer (TA-XT Plus, Stable Micro Systems, UK). Briefly, the LM sample was cooked to an internal temperature of 70°C in a thermostatic water bath. After removal from the water bath, LM sample was allowed to cool to 4°C , then four to six 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation from each chop. All data were collected using Texture Expert software version 1.22 (Stable Micro Systems, UK).

Crude Fat Contents of Liver and Longissimus Dorsi

Soxhlet extraction was used to determine the crude fat contents in *longissimus dorsi* and liver, the

results were quantified as the weight percentages of wet muscle and liver tissues.

Liver and Serum Biochemical Indicators

The contents of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein (VLDL) in serum and liver were determined using the kits of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the manufacturer's instructions.

RNA Isolation, Reverse Transcription, and Real-Time PCR

There was no difference in the process of RNA isolation, reverse transcription, and real-time PCR between target miRNAs and genes. Total RNA was isolated from *longissimus dorsi* using Trizol Reagent (TaKaRa, Dalian, China) according to the manufacturer's protocol. The NanoDrop ND-2000 spectrophotometer (NanoDrop, Wilmington, DE) was used to obtain the concentrations of total RNA. The A260/A280 ratio of all samples was between 1.8 and 2.0. cDNA was synthesized from 1 μg of DNase I-treated RNA using a Prime Script TM RT Reagent Kit (TaKaRa, Japan) according to the kit's instructions. It is noteworthy that the reverse transcription primers of lipid metabolism-related miRNAs were specific stem-loop primers and showed in Table 2, the reverse transcription primers of lipid metabolism-related genes were random primers.

Real-time quantitative PCR was performed using a CFX-96 Real-Time PCR detection system (Bio-Rad). U6 was used as the reference gene to calculate the expression levels of target miRNAs, and β -actin was used to normalize the mRNA expression levels of target genes. The PCR reaction included 1 cycle of 95°C for 30 s, 42 cycles of 95°C for 5 s and annealing for 30 s. A melting curve analysis was performed after amplification. Each sample was assayed in triplicate. The amplification primer sequences of target miRNAs are shown in Table 2 and the primer sequences of genes used for real-time PCR are shown in Table 3. All primers were synthesized by Sangon Biotech (Shanghai, China). Relative gene expression data were quantified according to the $2^{-\Delta\Delta\text{Ct}}$ method (Livak et al., 2001).

Statistical Analysis

All experimental data were expressed as means $\pm$ SE and sorted out using excel 2016. Significance analysis between CON group and RES group was

Table 2. Primer sequences of genes used for real-time PCR

Gene	Primer sequence (5' to 3')	Product length, bp	Melting temperature, °C	Accession number
<i>SREBP-1c</i>	F: GCGACGGTGCCTCTGGTAGT R: CGCAAGACGGCGGATTTA	218	88.5	NM_214157.1
<i>FAS</i>	F: AGCCTAACTCCTCGCTGCAAT R: TCCTTGGAACCGTCTGTGTTC	196	88	NM_001099930.1
<i>PPARγ</i>	F: CCAGCATTTCCTCCACTCCACACTA R: GACACAGGCTCCACTTTGATG	124	80	NM_214379.1
<i>ACC</i>	F: AGCAAGGTCGAGACCGAAAG R: TAAGACCACCGGCGGATAGA	169	83	NM_001114269.1
<i>LPL</i>	F: CACATTCACCAGAGGGTC R: TCATGGGAGCACTTCACG	177	84.5	NM_214286.1
<i>PPARα</i>	F: CGACCTGGAAAGCCCGTTAT R: GAGGCTTTGTCCCCACAGAT	279	87.5	NM_001044526.1
<i>PGC-1α</i>	F: GATGTGTCGCCTTCTTGTTTC R: CATCCTTTGGGGTCTTTGAG	93	78.5	NM_213963.2
<i>SIRT1</i>	F: TTGATCTTCTCATTGTTATTGGGTC R: ACTTGGAATTAGTGCTACTGGTCTTA	62	79	NM_001145750.2
<i>CPT-1</i>	F: GACAAGTCCTTCACCTCATCGC R: GGGTTTGGTTTGCCCAGACAG	170	87.5	NM_001007191.1
<i>H-FABP</i>	F: ATGACCAAGCCTACCACA R: AGTTTGCCTCCATCCAGT	170	82	NM_001099931.1
<i>LIPE</i>	F: GCCTTTCCTGCAGACCATCT R: CACTGGTGAAGAGGGAGCTG	104	84.5	NM_214315.3

Table 3. Primer sequences of miRNAs and U6 used for reverse transcription and real-time PCR

miRNA	Primer sequence (5' to 3')	Melting temperature, °C	Accession number
ssc-miR-27a	F: CGGCGGTTACAGTGGCTAAG RT: GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGCGGAA	83.5	MIMAT0002148
ssc-miR-181a	F: CGGCGAACATTCAACGCTGTCCG RT: GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACAACCTCA	82	MIMAT0010191
ssc-miR-143-3p	F: CGCTGAGTTGAGATGAAGCACTG RT: GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGAGCTA	81	MIMAT0013879
ssc-miR-370	F: CCGGCCTGCTGGGGTGGGA RT: GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACACCAGG	85	MIMAT0025373
ssc-miR-26a	F: CGCGCGCGTTCAAGTAATCCAGGA RT: GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACAGCCTA	82	MIMAT0002135
ssc-miR-21	F: CGGCGGTAGCTTATCAGACTGA RT: GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTCAACA	81	MIMAT0002165
Universal reverse primer	R: ATCCAGTGCAGGGTCCGAGG		
U6	F: CTCGCTTCGGCAGCACAA R: AACGCTTCACGAATTTGCGT RT: AACGCTTCACGAATTTGCGT	82	

performed by Independent Samples *t*-test of statistical software SPSS 23.0. In ADG, ADFI, and F/G, the pen was used as the experimental unit, and in carcass and meat quality, IMF content, lipid metabolism-related indicators, lipid metabolism-related genes mRNA and miRNAs expression, the individual pig was considered as the experimental unit. $P < 0.05$ was considered significant and $0.05 \leq P \leq 0.10$ was as a tendency.

RESULTS

Growth Performance and Carcass Characteristics

Compared with CON, dietary supplementation with 600 mg/kg resveratrol had no significant effect on ADG, ADFI, and F/G during the whole growing–finishing period ($P > 0.1$) (Table 4). No significant difference was found in the carcass

Table 4. Effect of dietary resveratrol supplementation on growth performance in growing–finishing pigs¹

Items	CON ²	RES ³	P-value
25 to 50 kg (1 to 5 wk)			
Initial weight, kg	24.70 ± 1.43	24.65 ± 1.45	0.980
Final weight, kg	49.25 ± 2.17	49.65 ± 2.59	0.906
ADG, g	701.22 ± 25.77	714.49 ± 38.58	0.781
ADFI, g	1538.10 ± 64.10	1584.15 ± 87.43	0.680
F/G	2.19 ± 0.02	2.22 ± 0.01	0.365
50 to 75 kg (6 to 9 wk)			
Initial weight, kg	49.25 ± 2.17	49.65 ± 2.59	0.906
Final weight, kg	76.05 ± 2.94	76.20 ± 3.31	0.975
ADG, g	924.43 ± 36.27	915.13 ± 36.48	0.860
ADFI, g	2305.29 ± 109.38	2377.28 ± 124.32	0.673
F/G	2.49 ± 0.04	2.59 ± 0.05	0.110
75 to 100 kg (10 to 13 wk)			
Initial weight, kg	76.05 ± 2.94	76.20 ± 3.31	0.975
Final weight, kg	94.18 ± 3.31	94.62 ± 2.99	0.922
ADG, g	647.32 ± 41.97	658.14 ± 35.14	0.847
ADFI, g	2205.96 ± 121.67	2252.44 ± 92.85	0.768
F/G	3.43 ± 0.11	3.45 ± 0.14	0.921
100 to 125 kg (14 to 17 wk)			
Initial weight, kg	94.18 ± 3.31	94.62 ± 2.99	0.922
Final weight, kg	114.79 ± 3.45	116.09 ± 4.05	0.811
ADG, g	735.99 ± 37.81	766.88 ± 59.22	0.671
ADFI, g	2641.88 ± 97.60	2740.21 ± 160.19	0.612
F/G	3.61 ± 0.12	3.60 ± 0.10	0.974
25 to 125 kg (1 to 17 wk)			
Initial weight, kg	24.70 ± 1.43	24.65 ± 1.45	0.980
Final weight, kg	114.79 ± 3.45	116.09 ± 4.05	0.811
ADG, g	749.24 ± 22.35	760.77 ± 30.59	0.767
ADFI, g	2135.47 ± 79.87	2200.03 ± 92.03	0.608
F/G	2.85 ± 0.03	2.89 ± 0.04	0.419

¹A total of 36 pigs (initial BW, 24.67±3.49 kg) were used in a 119-d study with three pigs per pen and six pens per treatment, the data of ADG, ADFI, and F/G = feed to gain ratio were determined per replicate (pen).

²CON = control group, basal diet.

³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.

Table 5. Effects of dietary resveratrol supplementation on carcass characteristics in growing–finishing pigs¹

Items	CON ²	RES ³	P-value
Dressing percentage, %	74.49 ± 0.58	74.64 ± 0.50	0.849
Carcass length, cm	103.33 ± 1.78	103.33 ± 0.95	1.000
Average backfat thickness, cm	1.79 ± 0.14	1.93 ± 0.18	0.558
Loin muscle area, cm ²	45.82 ± 2.18	42.32 ± 4.47	0.527

¹Values means $n = 6$ for CON and RES groups.

²CON = control group, basal diet.

³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.

characteristics including dressing percentage, carcass length, average backfat thickness, and LMA between the two groups ($P > 0.1$) (Table 5).

Liver and Leaf Lard Weight

Table 6 shows that no significant difference was noted in the weight of liver and leaf lard after

dietary adding 600 mg/kg resveratrol in growing–finishing pigs ($P > 0.1$).

Meat Quality and IMF Content

Dietary resveratrol supplementation had no change in pH, a^* , and L^* values, but significantly decreased the b^*_{24h} value in *longissimus dorsi*

($P < 0.05$). Besides, resveratrol had no effect on drip loss ($P > 0.1$) but resulted in lower cooking loss ($P < 0.05$) (Table 7). Meanwhile, dietary resveratrol supplementation increased the IMF content of *longissimus dorsi* markedly ($P < 0.01$), however, had no significant effect on the lipid content of liver ($P > 0.1$) (Table 8).

Lipid Metabolism-Related Indicators in Liver and Serum

Dietary resveratrol supplementation tended to decrease the liver TC content ($P < 0.1$), but had no significant effect on liver TG content ($P > 0.1$). Meanwhile, resveratrol decreased LDL-C content of liver ($P < 0.05$) (Table 9). Furthermore, resveratrol significantly decreased the levels of serum TG, TC, LDL-C, and VLDL ($P < 0.05$) (Table 10).

Lipid Metabolism-Related Genes mRNA Levels in Longissimus Dorsi

Dietary resveratrol supplementation had no significant effect ($P > 0.1$) on SREBP-1c and H-FABP mRNA expression levels but significantly enhanced PPAR γ , ACC, FAS, and LPL mRNA expression

in *longissimus dorsi* ($P < 0.05$) (Figure 1). At the same time, the LM mRNA abundance of SIRT1, CPT-1, and PPAR α were downregulated in RES group when compared with CON group ($P < 0.05$) (Figure 2).

Lipid Metabolism-Related miRNAs Expression Levels in Longissimus Dorsi

The results of resveratrol on lipid metabolism-related miRNAs expression levels in *longissimus dorsi* were presented in Figure 3. Dietary supplementation with resveratrol increased the expression levels of ssc-miR-181a, ssc-miR-21, and ssc-miR-370 ($P < 0.05$); however, the opposite expression change was observed in ssc-miR-27a ($P < 0.05$). Furthermore, there were no differences for ssc-miR-26a and ssc-miR-143-3p expression levels between the two groups ($P > 0.1$).

DISCUSSION

Zhang et al. (2015) reported that dietary supplementation with 600 mg/kg resveratrol had no effect on growth performance of finishing pigs. Similar to the previous study, the results in our

Table 6. Effects of dietary resveratrol supplementation on the weight of liver and leaf lard in growing-finishing pigs¹

Items	CON ²	RES ³	P-value
Liver weight, kg	1.64 $\pm$ 0.09	1.64 $\pm$ 0.09	1.000
Leaf lard weight, kg	1.54 $\pm$ 0.16	1.52 $\pm$ 0.17	0.921

¹Values means $n = 6$ for CON and RES groups.

²CON = control group, basal diet.

³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.

Table 7. Effects of dietary resveratrol supplementation on meat quality in growing-finishing pigs¹

Items	CON ²	RES ³	P-value
$L^*_{45\text{min}}$	44.03 $\pm$ 0.53	43.84 $\pm$ 0.73	0.837
$a^*_{45\text{min}}$	4.04 $\pm$ 0.37	4.20 $\pm$ 0.40	0.780
$b^*_{45\text{min}}$	2.85 $\pm$ 0.28	2.96 $\pm$ 0.16	0.728
$L^*_{24\text{h}}$	53.84 $\pm$ 1.02	51.85 $\pm$ 1.47	0.316
$a^*_{24\text{h}}$	6.45 $\pm$ 0.45	6.20 $\pm$ 0.45	0.707
$b^*_{24\text{h}}$	4.26 $\pm$ 0.23	3.52 $\pm$ 0.19	0.033
pH ^{45min}	6.36 $\pm$ 0.11	6.43 $\pm$ 0.06	0.555
pH ^{24h}	5.49 $\pm$ 0.06	5.55 $\pm$ 0.02	0.403
Drip loss, %	1.93 $\pm$ 0.08	1.87 $\pm$ 0.04	0.493
Cooking loss, %	36.35 $\pm$ 0.77	33.97 $\pm$ 0.62	0.038
Shear force, kg	4.72 $\pm$ 0.22	4.69 $\pm$ 0.11	0.919
Marbling	1.42 $\pm$ 0.24	1.83 $\pm$ 0.17	0.183

¹Values means $n = 6$ for CON and RES groups.

²CON = control group, basal diet.

³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.

Table 8. Effects of dietary resveratrol supplementation on the crude fat of liver and *longissimus dorsi* in growing–finishing pigs¹

Items	CON ²	RES ³	P-value
Intramuscular fat, %	2.36 ± 0.10	3.04 ± 0.17	0.008
Liver crude fat, %	2.03 ± 0.11	1.90 ± 0.05	0.347

¹Values means *n* = 6 for CON and RES groups.²CON = control group, basal diet.³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.**Table 9.** Effects of dietary resveratrol supplementation on the liver lipid metabolism-related indicators in growing–finishing pigs¹

Items	CON ²	RES ³	P-value
Triglyceride, μmol/g	32.27 ± 2.51	33.72 ± 2.21	0.674
Total cholesterol, μmol/g	7.53 ± 0.36	6.54 ± 0.29	0.059
LDL-C ⁴ , μmol/g	8.45 ± 0.53	6.50 ± 0.57	0.031

¹Values means *n* = 6 for CON and RES groups.²CON = control group, basal diet.³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.⁴LDL-C = low-density lipoprotein cholesterol.**Table 10.** Effects of dietary resveratrol supplementation on serum lipid metabolism-related indicators in growing–finishing pigs¹

Items	CON ²	RES ³	P-value
Triglyceride, mmol/gprot	2.96 ± 0.28	2.16 ± 0.14	0.031
Total cholesterol, mmol/gprot	3.70 ± 0.30	2.89 ± 0.21	0.047
LDL-C ⁴ , mmol/gprot	1.99 ± 0.21	1.33 ± 0.17	0.040
VLDL ⁵ , μg/mL	17.87 ± 1.73	13.39 ± 0.48	0.049

¹Values means *n* = 6 for CON and RES groups.²CON = control group, basal diet.³RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol.⁴LDL-C = low-density lipoprotein cholesterol.⁵VLDL = very low-density lipoprotein.

research indicated that no significant difference was observed in the growth performance of growing–finishing pigs between the two groups. However, [Ahmed et al. \(2013\)](#) showed that ADG and ADFI in *Escherichia coli* and *Salmonella*-challenged piglets were increased significantly by supplementing with 2,000 mg/kg Respig (containing resveratrol). This discrepancy may be due to the oral challenge or difference in the dosage of resveratrol and physiological stage of pigs.

The present study found that dietary supplementation with 600 mg/kg resveratrol reduced the b^*_{24h} value and cooking loss in *longissimus dorsi* [Zhang et al., \(2015\)](#) reported that resveratrol significantly enhanced the a^* value and decreased the cooking loss in *longissimus dorsi* of finishing pigs. The two experiments showed that dietary supplementation

with resveratrol contributed to improve the meat quality to some extent.

It is well known that the serum TG, TC, and LDL-C concentrations are associated with the progression of cardiovascular disease such as atherosclerosis and hyperlipidemia ([Jin et al., 2013](#)). In the current study, dietary resveratrol supplementation resulted in lower contents of serum TG, TC, LDL-C, and VLDL. A previous study also reported that resveratrol decreased the serum TG and TC concentrations in a mouse model ([Qiao et al., 2014](#)). Combined with the current results, those results indicated that resveratrol supplementation could promote serum lipids breakdown and utilization. In recent years, pigs have been used broadly as a research model for human physiological metabolism investigation. The results suggested that

Figure 1. Effects of dietary resveratrol supplementation on mRNA expression levels of lipogenic genes including SREBP-1c, FAS, PPAR γ , LPL, ACC, and H-FABP in LM of growing-finishing pigs. CON = control group, basal diet; RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol. “*” represents a significant difference between CON group and RES group. β -Actin was used to normalize the mRNA expression levels of these genes. Data are presented as means $\pm$ SE ($n = 6$).

Figure 2. Effects of dietary resveratrol supplementation on mRNA expression levels of lipolytic genes including SIRT1, CPT-1, PPAR α , PGC-1 α , and LIPE in LM of growing-finishing pigs. CON = control group, basal diet; RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol. “*” represents a significant difference between CON group and RES group. β -Actin was used to normalize the mRNA expression levels of these genes. Data are presented as means $\pm$ SE ($n = 6$).

resveratrol may be able to prevent the cardiovascular disease and be beneficial to health.

It is known that IMF content is an important indicator for meat quality. This study provided the first evidence that IMF content was increased significantly by resveratrol in a pig model. Intramuscular fat deposition depends on the changes of dynamic balance between lipid anabolism and catabolism (Shi et al., 2009). The lipogenesis, lipolysis, fatty acid transport, and fatty acid oxidation are involved in this process. A lot of genes are involved in lipid anabolism and catabolism. PPAR γ , a key candidate gene of IMF deposition, is able to promote adipose cell differentiation and fat deposition by regulating the expression of lipid metabolism-related genes including FAS, ACC, LPL, and LIPE (Grindflek

Figure 3. Effects of dietary resveratrol supplementation on the expression levels of ssc-miR-181a, ssc-miR-370, ssc-miR-21, ssc-miR-27a, ssc-miR-26a, and ssc-miR-143-3p in LM of growing-finishing pigs. CON = control group, basal diet; RES = resveratrol group, basal diet supplementation with 600 mg/kg resveratrol. “*” represents a significant difference between CON group and RES group. U6 was used as the reference gene to calculate their expression levels. Data are presented as means $\pm$ SE ($n = 6$).

et al., 2000). SIRT1, an upstream regulatory factor, can repress the activity of PPAR γ directly (Picard et al., 2004). SREBP-1c is a transcription factor which can regulate the expression of lipogenic genes such as FAS and ACC (Yin et al., 2002). LIPE and CPT-1 are rate-limiting enzymes of lipolysis responsible for the hydrolysis of triglycerides and transport of fatty acids for β oxidation, respectively (Mersmann et al., 1998; DeBerardinis et al., 2006). PPAR α and PGC-1 α are both the upstream regulatory factors of LIPE and CPT-1 (Rakhshandehroo et al., 2007; Nikolic et al., 2012). In the present study, resveratrol upregulated mRNA expression levels of genes in relation to lipogenesis including FAS, ACC, and PPAR γ in *longissimus dorsi*, and down-regulated CPT-1 and PPAR α mRNA levels. These results indicated that IMF deposition induced by resveratrol might be related to the promotion of intramuscular lipogenic capacity and repression of intramuscular lipolytic capacity during the growing-finishing stages. Furthermore, previous reports showed that resveratrol was a natural agonist of histone deacetylase-SIRT1 which can regulate lipid metabolism indirectly by suppressing the expression of PPAR γ (Lagouge et al., 2006). This research provided the first evidence that dietary resveratrol supplementation decreased significantly the mRNA expression level of SIRT1 in *longissimus dorsi*, and the mRNA abundance of PPAR γ was enhanced simultaneously. These results implied that resveratrol improved the IMF deposition might be associated with SIRT1-PPAR γ pathways and its downstream genes such as FAS and ACC. At the same time, LPL is considered as an important gene determining the transfer of fatty acids

into muscle and adipose tissues. Its main function is to break down the serum TG into glycerol and fatty acids and provide the tissue with raw materials for the synthesis of TG (Goldberg et al., 2009). In this study, resveratrol showed the ability to boost the mRNA expression level of LPL in *longissimus dorsi*, which might partly account for the serum lipid-lowering effects of resveratrol. Therefore, these results suggested that the break down and utilization of serum lipids by LPL may be one of the ways to accumulate the IMF in growing–finishing pigs after adding resveratrol into the diet.

At present, the researches in relation to the underlying mechanism of IMF deposition mostly focused on the expression of lipid metabolism-related genes. However, little information is available on the relationship between miRNAs and IMF metabolism. Therefore, to reveal the possible roles of lipid metabolism-related miRNAs in the process of IMF accumulation, we further measured the expression changes of miRNAs involved in lipid metabolism in *longissimus dorsi* of growing–finishing pigs in the current study. Li et al., (2013) explored the effect of miR-181a on lipid metabolism in vitro using the porcine preadipocyte as a model, the results indicated that the overexpression of miR-181a promoted the deposition of lipid droplet, however, the repression of miR-181a downregulated the lipogenic genes expression. MiR-181a can bind directly to the 3' UTR region of SIRT1 gene and inhibit its expression (Zhou et al., 2012). In this study, we found that the expression level of ssc-miR-181a was enhanced by resveratrol in *longissimus dorsi*, and the expression of SIRT1, the target gene of miR-181a, was decreased simultaneously. Hence, we speculated that ssc-miR-181a possibly played a pivotal role in IMF metabolism by influencing the expression of SIRT1 and its downstream genes.

Many studies have been done to research the influence of miR-27a on lipid metabolism. It has

been reported that miR-27a can inhibit fat deposition by suppressing the expression of PPAR γ directly (Shirasaki et al., 2013). Our present study indicated that resveratrol reduced the expression of miR-27a significantly and increased the mRNA abundance of PPAR γ in *longissimus muscle*. Therefore, one of the regulatory pathways of IMF accretion by resveratrol may be from ssc-miR-27a to PPAR γ and its downstream genes.

Some studies indicated that miR-370 could repress the fatty acid oxidation by binding directly to the 3' UTR region of CPT-1, a key lipolytic gene (Iliopoulos et al., 2010). PPAR α , an upstream regulatory factor of CPT-1, is the target gene of mir-21 (Rakhshandehroo et al., 2007). We found that dietary resveratrol supplementation promoted the expression of ssc-miR-21 and ssc-miR-370 markedly in *longissimus dorsi*. These results suggested that the repression of intramuscular lipolytic capacity may be partly due to the changes of ssc-miR-21 and ssc-miR-370 expression induced by resveratrol. At the same time, there is evidence that miR-143-3p is highly expressed in *longissimus dorsi* in growing–finishing pigs (Chen et al., 2011). Studies in vitro showed that miR-143-3p could promote the synthesis of TG (Yi et al., 2011). Interestingly, when the IMF content was increased by resveratrol, the expression level of ssc-miR-143-3p was not changed. The results indicated that the major role of ssc-miR-143-3p in vivo may be not related with lipid metabolism. In summary, results in the present study indicated that ssc-miR-27a, ssc-miR-181a, ssc-miR-370, and ssc-miR-21 maybe participated in the regulation of resveratrol on IMF metabolism via regulating the expression of lipid metabolism-related target genes in growing–finishing pigs (Figure 4). However, it remains unclear whether resveratrol alters IMF deposition by directly or indirectly changing the expression of miRNAs. Furthermore, because of the limitation of experiment design, the intake dosage of resveratrol was an

Figure 4. Underlying mechanism of dietary resveratrol supplementation on intramuscular fat metabolism in growing–finishing pigs. IMF = intramuscular fat.

average amount. More studies are needed to verify the effects of dietary resveratrol supplementation on growth performance and IMF deposition and to understand the underlying mechanism and pathway of resveratrol improving IMF content via the lipid metabolism-related miRNAs and target genes.

CONCLUSIONS

In conclusion, the results of the present study indicated that dietary resveratrol supplementation had no significant effect on growth performance of growing–finishing pigs, however, which is beneficial for pork quality, serum lipid profiles, and IMF content. The increased IMF induced by resveratrol might be associated with the promotion of intramuscular lipogenic capacity and repression of intramuscular lipolytic capacity. Remarkably, ssc-miR-27a, ssc-miR-181a, ssc-miR-370, and ssc-miR-21 maybe participated in the regulation of resveratrol on IMF metabolism by regulating the expression of lipid metabolism-related target genes in growing–finishing pigs. This study offered a molecular basis understanding the potential mechanisms behind the improved IMF deposition in growing–finishing pigs by resveratrol supplementation.

Conflict of interest statement. None declared.

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