Dietary supplementation with arginine and glutamic acid enhances key lipogenic gene expression in growing pigs¹

C. J. Hu,*† Q. Y. Jiang,† T. Zhang,* Y. L. Yin,*†² F. N. Li,* J. Y. Su,* G. Y. Wu,‡ and X. F. Kong*²

*Key Laboratory of Agro-Ecological Processes in Subtropical Region, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan 410125, China; †College of Animal Science, South China Agricultural University, Guangzhou, Guangdong 510642, China; and ‡Department of Animal Science, Texas A&M University, College Station 77843-2471

ABSTRACT: Our previous study showed dietary supplementation with Arg and Glu increased intramuscular fat deposition and decreased back fat thickness in pigs, suggesting that the genes involved in lipid metabolism might be regulated differently in muscle and s.c. adipose (SA) tissues. Sixty Duroc × Large White × Landrace pigs with an average initial BW of 77.1 ± 1.3 kg were randomly assigned to 1 of 5 treatment groups (castrated male to female ratio = 1:1). Pigs in the control group were fed a basic diet, and those in experimental groups were fed the basic diet supplemented with 2.05% L-alanine (isonitrogenous group), 1.00% L-arginine (Arg group), 1.00% glutamic acid + 1.44% L-alanine (Glu group), or 1.00% L-arginine + 1.00% glutamic acid (Arg+Glu group). Fatty acid percentages and mRNA expression levels of the genes involved in lipid metabolism in muscle and SA tissues were examined. The per-

centages of C14:0 and C16:0 in the SA tissue of Glu group pigs and C14:0 in the longissimus dorsi (LD) muscle of Glu and Arg+Glu groups decreased ($P < 0.05$) compared to the basic diet group. The Arg+Glu group showed the highest ($P < 0.05$) hormone-sensitive lipase expression level in SA tissue and higher ($P < 0.05$) mRNA levels of *PPAR γ* in the LD muscle than the basic diet and isonitrogenous groups. Additionally, the mRNA level of fatty acid synthase in the Arg+Glu group was more upregulated ($P < 0.05$) than that of the Arg group. An increase in the mRNA level of *PPAR γ* in the biceps femoris muscle was also observed in the Arg+Glu group ($P < 0.05$) compared with the basic diet and isonitrogenous groups. Collectively, these findings suggest that dietary supplementation with Arg and Glu upregulates the expression of genes involved in adipogenesis in muscle tissues and lipolysis in SA tissues.

Key words: arginine, fatty acids, glutamic acid, growing pigs, lipid metabolism

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INTRODUCTION

Pork, the most consumed meat, is an important source of fat for humans. Fat and fatty acids contribute to various aspects of pork meat quality and nutritional value. Intramuscular fat (IMF) content greatly affects the eating quality of pork meat (DeVol et al.,

1988), and fatty acids within the muscle are important for meat flavor and nutritional value (Wood et al., 2004). However, excessive consumption of SFA is associated with a high risk of cardiovascular disease in humans (Kromhout, 1992). Therefore, the pig industry should follow nutritional approaches that maintain appropriate IMF and fatty acid contents in pork, providing superior nutritional value for humans.

The AA arginine and glutamic acid are involved in the lipid metabolism of pigs (Hu et al., 2015; Kong et al., 2015). Previous studies have shown that Arg regulates lipid metabolism in a tissue-specific manner (Wu et al., 2014). Tan et al. (2009, 2011) showed that dietary supplementation with 1% Arg increased IMF deposition by upregulating the mRNA levels

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²Corresponding authors: yinyulong@isa.ac.cn; nnkxf@isa.ac.cn
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of fatty acid synthase, thereby regulating fatty acid composition in the muscle. Dietary supplementation with monosodium glutamate modified fat deposition and fatty acid profiles and differentially regulated the expression of genes related to lipid metabolism in muscle and adipose tissues (Kong et al., 2015).

Our previous study showed that dietary supplementation with both Arg and Glu increased IMF deposition and decreased back fat thickness in pigs (Hu et al., 2017), suggesting that adipogenesis and lipolysis might be regulated in a tissue-specific manner by dietary supplementation with these AA. Therefore, the present study aimed to test the hypothesis that dietary Arg and Glu supplementation differentially regulate the expression of lipid metabolism genes in muscle and s.c. adipose (SA) tissues.

MATERIALS AND METHODS

The experimental design and procedures presented in this study were reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science.

Animals and Experimental Treatments

Sixty Duroc × Large White × Landrace pigs were fed a corn and soybean meal-based diet (Table 1) meeting the NRC (2012) requirements for growing pigs. After being fed the same diet for 1 wk, animals with an average initial BW of 77.1 ± 1.3 kg were randomly assigned to 1 of 5 treatment groups (12 pigs/group, castrated male to female ratio = 1:1). Pigs in the control group were fed the basic diet, whereas those in experimental groups were fed the basic diet supplemented with 2.05% L-Alanine (isonitrogenous group), 1.00% L-arginine (Arg group), 1.00% glutamic acid + 1.44% L-alanine (Glu group), or 1.00% L-arginine + 1.00% glutamic acid (Arg+Glu group). The AA used to supplement diets were obtained from Wuxi Jinghai Amino Acid Co., Ltd. (Wuxi, China).

Pigs were housed in cages (3.5 × 5.0 m) equipped with feed intake recording equipment (Henan Heshun Automation Equipment Co., Ltd., Zhengzhou, China). The space provided by the equipment allowed 1 pig at a time to have ad libitum access to the feed. Pigs were labeled with an individual electronic ear marker and had 24 h access to feed and water. The housing conditions were controlled by an air-conditioning system.

Sample Collection

The diet was removed 12 h before slaughter, at the end of the feeding trial. Blood samples were collected

Table 1. Composition and nutrient levels of the experimental diets (as-fed basis)¹

Item	Basic diet group	Isonitrogenous group	Arg group	Glu group	Arg+Glu group
Ingredient, %					
Corn	76.3	76.3	76.3	76.3	76.3
Soybean meal	16.5	16.5	16.5	16.5	16.5
Soybean oil	0.56	0.56	0.56	0.56	0.56
Corn starch	2.44	0.39	1.44	—	0.44
CaHPO ₄	0.65	0.65	0.65	0.65	0.65
Calcium carbonate	1.08	1.08	1.08	1.08	1.08
Salt	0.43	0.43	0.43	0.43	0.43
Lys	0.16	0.16	0.16	0.16	0.16
Thr	0.08	0.08	0.08	0.08	0.08
Try	0.05	0.05	0.05	0.05	0.05
Premix ²	1.75	1.75	1.75	1.75	1.75
Ala	—	2.05	—	1.44	—
Arg	—	—	1.00	—	1.00
Glu	—	—	—	1.00	1.00
Chemical composition ³					
DE, MJ/kg	14.26	14.17	14.21	14.15	14.17
CP, %	13.04	14.88	14.88	14.88	15.31
CF, %	2.42	2.42	2.42	2.42	2.42
EE, %	5.94	5.94	5.94	5.94	5.94
Ca, %	0.62	0.62	0.62	0.62	0.62
Total P, %	0.42	0.42	0.42	0.42	0.42
AP, %	0.20	0.20	0.20	0.20	0.20
AA composition, ³ %					
Asp + Asn	1.73	1.73	1.73	1.73	1.73
Thr	0.79	0.79	0.79	0.79	0.79
Ser	0.80	0.80	0.80	0.80	0.80
Glu + Gln	3.06	3.06	3.06	4.05	4.05
Gly	0.95	0.95	0.95	0.95	0.95
Ala	1.09	3.14	1.09	2.53	1.09
Val	0.83	0.83	0.83	0.83	0.83
Met	0.24	0.24	0.24	0.24	0.24
Ile	0.79	0.79	0.79	0.79	0.79
Leu	1.50	1.50	1.50	1.50	1.50
Tyr	0.48	0.48	0.48	0.48	0.48
Phe	0.77	0.77	0.77	0.77	0.77
His	0.62	0.62	0.62	0.62	0.62
Lys	0.85	0.85	0.85	0.85	0.85
Arg	1.01	1.01	2.01	1.01	2.01
Pro	1.56	1.56	1.56	1.56	1.56
Cys	0.28	0.28	0.28	0.28	0.28

¹Isonitrogenous group = basic diet supplemented with 2.05% Ala; Arg group = basic diet supplemented with 1.00% Arg; Glu group = basic diet supplemented with 1.00% Glu + 1.44% Ala; and Arg+Glu group = basic diet supplemented with 1.00% Arg + 1.00% Glu.

²Premix provided for 1 kg of complete diet: Cu as copper sulfate, 10 mg; Fe as iron sulfate, 100 mg; Se as sodium selenite, 0.30 mg; Zn as zinc oxide, 100 mg; Mn as manganese oxide, 10 mg; vitamin D₃, 386 IU; vitamin A as retinyl acetate, 3,086 IU; vitamin E as D-α-tocopherol, 15.4 IU; vitamin K as menadione sodium bisulfate, 2.3 mg; vitamin B₂, 3.9 mg; calcium pantothenate, 15.4 mg; niacin, 23 mg; and vitamin B₁₂, 15.4 mg.

³Analyzed values in the basal diet group and nutrient contents in the other groups were calculated according to their contents in the basic diet group.

Table 2. Primers used for real-time PCR

Gene	Name	Primer	Sequences (5' to 3')	Accession No.	Size, bp
<i>ACC</i>	Acetyl-coA carboxylase	Forward	TTCCAGGCACAGTCCTTAGG	NM_001114269	161
		Reverse	TCATCCAACACGAGCTCAGT		
<i>CPT-1</i>	Carnitine palmitoyltransferase I	Forward	CTGCCTGACCTATGAAGCCT	NM_001007191	221
		Reverse	AGAAGAGGTGCCTGTCGATC		
<i>FAS</i>	Fatty acid synthase	Forward	CTGCTGAAGCCTAACTCCTCG	NM_001099930	242
		Reverse	TGCTCCTGCACGTCTCCC		
<i>FATP-1</i>	Fatty acid transport protein 1	Forward	GGAGTAGAGGGCAAAGCAGG	NM_001083931	208
		Reverse	AGGTCTGGCGTGGGTCAAA G		
<i>FABP-4</i>	Fatty acid transport protein 4	Forward	TGGAACTTGTCTCCAGTG	NM_001002817	147
		Reverse	GGTACTTTCTGATCTAATGGTG		
<i>HSL</i>	Hormone sensitive lipase	Forward	GCAGCATCTTCTTCCGCACA	NM_214315	195
		Reverse	AGCCCTTGCGTAGAGTGACA		
<i>LPL</i>	Lipoprotein lipase	Forward	CTCGTGCTCAGATGCCCTAC	NM_214286	148
		Reverse	GGCAGGGTGAAAGGGATGTT		
<i>PPARγ</i>	Peroxisome proliferator activated receptor γ	Forward	AGGGCCAAGGATTCATGACA	XM_013981982	248
		Reverse	GTGGTTCAACTTGAGCTGCA		
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	Forward	AAGGAGTAAGAGCCCCTGGA	NM_001206359	140
		Reverse	TCTGGGATGGAAACTGGAA		

from the jugular vein using 10-mL centrifuge tubes containing sodium heparin and centrifuged at $3,000 \times g$ and 4°C for 15 min to recover the plasma. These samples were then stored at -20°C until biochemical analysis. After collection of blood samples, pigs were sacrificed using electrical stunning (120 V, 200 Hz) and exsanguination. The longissimus dorsi (**LD**) muscle (with s.c. fat removed) and SA tissues between the sixth and seventh ribs and the biceps femoris (**BF**) muscle were immediately collected from the right side of the carcass and stored at -20°C for fatty acid composition analysis or snap-frozen in liquid nitrogen (approximately 10 g of each tissue) and then stored at -80°C for mRNA analysis.

Plasma Analysis

Total cholesterol (**TC**), triglycerides, low-density lipoprotein cholesterol (**LDL-C**), high-density lipoprotein cholesterol (**HDL-C**), lipase, and apolipoprotein A1 in the plasma were measured using the biochemical analytical instrument TBA-120FR (Toshiba, Otawara-shi, Japan) and respective commercial assay kits (Yonghe-Yangguang Science and Technology Co., Ltd., Changsha, China).

Fatty Acid Percentage Analysis

After s.c. fat and fascia were removed, muscle and SA tissue samples were sliced, vacuum freeze-dried, and then ground into powder. Fatty acid percentages in muscle and SA tissues were analyzed by gas chromatography, according to the method of Li et al. (2015). Total lipids were extracted from tissue sam-

ples using the chloroform:methanol (1:1, vol/vol) procedure. Fatty acid methyl esters were prepared using KOH/methanol and analyzed using an Agilent 6890N gas chromatographer equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA) and a CP-Sil 88 fused silica open tube capillary column (100 m $\times$ 0.25 nm; Chrompack, Palo Alto, CA). The gas chromatography conditions were as follows: oven temperature was set at 45°C for 4 min, increased to 175°C at $13^{\circ}\text{C}/\text{min}$, held at 175°C for 27 min, increased from 175°C to 215°C at $4^{\circ}\text{C}/\text{min}$, and held at 215°C for 35 min; injector and detector temperatures were maintained at 250°C . The carrier gas (hydrogen) flow rate was 30 mL/min. Individual fatty acid peaks were identified by comparing their retention times with those of their respective standards (Sigma Chemicals, Saint Louis, MO), and the fatty acid composition was calculated as a percentage of the total fatty acids.

RNA Extraction and Complementary DNA Synthesis

Total RNA was extracted from LD, BF, and SA tissues (approximately 50 mg of each) using TRIzol reagent (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. A NanoDrop ND-1000 (Thermo Fisher, Wilmington, DE) was used to measure the 260:280 nm ratio of each sample, which was between 1.8 and 2.0, and RNA integrity was determined using 1% agarose gel electrophoresis. All samples examined in this study were complete RNA samples, as agarose gels clearly showed the 5S, 18S, and 28S rRNA bands. Complementary DNA was synthesized from 1 μg RNA in a 20- μL reaction volume using

Table 3. Effects of dietary supplementation with arginine and glutamic acid on plasma concentrations of metabolites

Item	Dietary treatment ¹					SEM	P-value
	Basic diet group	Isonitrogenous group	Arg group	Glu group	Arg+Glu group		
Apolipoprotein A1, g/L	0.11 ± 0.004	0.10 ± 0.002	0.10 ± 0.003	0.10 ± 0.005	0.10 ± 0.004	0.002	0.85
Cholesterol, mmol/L	1.91 ± 0.12	1.86 ± 0.04	1.82 ± 0.06	1.85 ± 0.09	1.58 ± 0.08	0.04	0.06
High-density lipoprotein cholesterol, mmol/L	0.62 ± 0.05	0.57 ± 0.02	0.56 ± 0.02	0.61 ± 0.04	0.56 ± 0.05	0.02	0.69
Low-density lipoprotein cholesterol, mmol/L	1.16 ± 0.08	1.17 ± 0.04	1.14 ± 0.06	1.13 ± 0.07	0.93 ± 0.06	0.03	0.20
Lipase, U/L	10.96 ± 0.39	11.99 ± 1.16	10.74 ± 0.25	12.20 ± 1.41	12.98 ± 1.39	0.50	0.66
Triglyceride, mmol/L	0.58 ± 0.07	0.60 ± 0.06	0.50 ± 0.05	0.57 ± 0.05	0.54 ± 0.05	0.03	0.84

¹Isonitrogenous group = basic diet supplemented with 2.05% Ala; Arg group = basic diet supplemented with 1.00% Arg; Glu group = basic diet supplemented with 1.00% Glu + 1.44% Ala; and Arg+Glu group = basic diet supplemented with 1.00% Arg + 1.00% Glu.

a PrimeScript first-strand cDNA synthesis kit (Takara, Osaka, Japan) according to the manufacturer's instructions and stored at -80°C until further analysis.

Real-Time PCR Analysis

Primers targeting the genes selected for PCR analyses were designed using Primer 3 (<http://bioinfo.ut.ee/primer3-0.4.0/>) and are listed in Table 2. Polymerase chain reaction was performed to test the specificity of primers for the selected genes and to verify the amplified products. Total reaction volumes (10 µL) comprised 2 µL of cDNA template solution, 5 µL of SYBR Green PCR master mix (Thermo Fisher Scientific, Waltham, MA), 2.2 µL of water, and 0.4 µL of each primer. The relative expression of each target gene was determined using real-time (RT) PCR with an ABI 7900HT system (Applied Biosystems, Carlsbad, CA) and 3 replicates per biological sample. The RT-PCR program included a 10-min incubation at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C and annealing and extension for 20 s at 60°C. A melting curve program (60°C–99°C with a heating rate of 0.1°C/s) and fluorescence measurements were performed to generate melting curves for each sample, check primer specificity, and ensure the purity of PCR products. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used to normalize the mRNA levels of the selected genes. The relative expression ratio of mRNA was calculated according to the following formula (Liu et al., 2015): $R = 2^{-\Delta\Delta Ct(\text{sample-control})}$, where $\Delta\Delta Ct(\text{sample-control}) = (Ct_{\text{target gene}} - Ct_{\text{GAPDH}})_{\text{treated}} - (Ct_{\text{target gene}} - Ct_{\text{GAPDH}})_{\text{control}}$.

Statistical Analysis

Data were statistically analyzed using a 1-way ANOVA in SPSS 17.0 (SPSS Inc., Chicago, IL). Levene's test for homogeneity of variance was used, followed by Duncan's multiple-range test (in the case of variance homogeneity) or Tamhane's T2 test (in the

case of variance heterogeneity). Differences between means were considered statistically significant at $P < 0.05$, with a trend toward significance at $0.05 \leq P < 0.10$.

RESULTS

Plasma Analysis

As shown in Table 3, the plasma concentration of TC in the Arg+Glu group showed a decreasing trend ($P = 0.06$) compared to the basic diet group. Dietary Arg or Glu supplementation did not affect ($P > 0.05$) the plasma concentrations of triglycerides, HDL-C, LDL-C, lipase, or apolipoprotein A1.

Fatty Acid Percentages

The fatty acid percentages in the SA tissue of growing pigs (Table 4) were greatly affected by dietary Glu supplementation. The percentages of SFAs, including C14:0 and C16:0, decreased more in the Glu group ($P < 0.05$) than in the basic diet group. Moreover, the PUFA:SFA ratio in the Glu group increased ($P < 0.05$) relative to the basic diet group. Supplementation with Arg or Arg+Glu did not affect ($P > 0.05$) fatty acid percentages in the SA tissue. The percentage of C14:0 increased ($P < 0.05$) more in the Arg+Glu group than in the Glu group.

The fatty acid percentages in the LD muscle of growing pigs subject to different dietary treatments are shown in Table 5. Consistent with the changes observed in the SA tissue, the percentages of C14:0 decreased ($P < 0.05$) in the Glu and Arg+Glu groups compared with that in the basic diet group.

In the BF muscle, fatty acid percentages presented a pattern different from that observed in the LD muscle (Table 6): no significant differences ($P > 0.05$) were observed in the percentages of C14:0 and C16:0 between Glu and basic diet groups. Supplementation with Arg or Glu did not affect ($P > 0.05$) fatty acid percentages in the BF muscle.

Table 4. Effects of dietary supplementation with arginine and glutamic acid on fatty acid percentages (g/100 g total fatty acids) of s.c. adipose tissue in growing pigs

Item	Dietary treatment ¹					SEM	P-value
	Basic diet group	Isonitrogenous group	Arg group	Glu group	Arg+Glu group		
C14:0	1.36 ± 0.03 ^a	1.28 ± 0.03 ^{a,b}	1.32 ± 0.05 ^a	1.20 ± 0.03 ^b	1.32 ± 0.03 ^a	0.02	0.03
C16:0	24.90 ± 0.51 ^a	23.71 ± 0.23 ^{a,b}	24.22 ± 0.56 ^{a,b}	22.97 ± 0.49 ^b	23.61 ± 0.35 ^{a,b}	0.20	0.04
C16:1	1.89 ± 0.14	1.73 ± 0.08	2.06 ± 0.15	1.66 ± 0.06	1.88 ± 0.11	0.05	0.15
C17:0	0.52 ± 0.04	0.47 ± 0.05	0.45 ± 0.03	0.50 ± 0.04	0.47 ± 0.04	0.02	0.78
C18:0	13.85 ± 0.86	13.20 ± 0.30	12.64 ± 0.49	12.75 ± 0.35	12.75 ± 0.32	0.18	0.19
C18:1n-9	36.13 ± 0.14	36.67 ± 0.76	37.58 ± 0.79	36.38 ± 0.90	37.82 ± 0.63	0.35	0.45
C18:2n-6	19.42 ± 0.87	20.87 ± 0.79	19.79 ± 0.98	22.44 ± 1.21	20.13 ± 0.63	0.41	0.17
C18:3n-6	0.08 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.005	0.47
C20:0	0.28 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.02	0.29 ± 0.02	0.006	0.99
C20:1	1.18 ± 0.06	1.28 ± 0.06	1.16 ± 0.06	1.32 ± 0.08	1.22 ± 0.05	0.03	0.37
C20:3n-6	0.13 ± 0.007	0.13 ± 0.007	0.12 ± 0.007	0.15 ± 0.009	0.14 ± 0.007	0.003	0.25
C20:4n-6	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01	0.30 ± 0.02	0.28 ± 0.01	0.007	0.46
PUFA:SFA ²	0.49 ± 0.03 ^b	0.56 ± 0.03 ^{a,b}	0.53 ± 0.03 ^b	0.63 ± 0.05 ^a	0.54 ± 0.02 ^{a,b}	0.02	0.04

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$). Data are expressed as means ± SEM; $n = 12$.

¹Isonitrogenous group = basic diet supplemented with 2.05% Ala; Arg group = basic diet supplemented with 1.00% Arg; Glu group = basic diet supplemented with 1.00% Glu + 1.44% Ala; and Arg+Glu group = basic diet supplemented with 1.00% Arg + 1.00% Glu.

²PUFA = 18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6. SFA = C14:0 + C16:0 + C17:0 + C18:0 + C20:0.

Expression of Fat Metabolism-Related Genes

The mRNA levels of fat metabolism-related genes in the SA, LD muscle, and BF muscle tissues of growing pigs under different dietary treatments are shown in Fig. 1A, 1B, and 1C, respectively. In the SA tissue, no significant differences ($P > 0.05$) were observed in the mRNA levels of *PPAR γ* , acetyl-coA carboxylase (*ACC*), carnitine palmitoyltransferase I (*CPT-1*), fatty acid synthase (*FAS*), and lipoprotein lipase (*LPL*) among the 5 dietary groups. The Arg+Glu group presented the highest ($P < 0.05$) mRNA level of hormone-sensitive lipase (*HSL*). The mRNA level of the fatty acid transport protein 1 (*FATP-1*) was more upregulated ($P < 0.05$) in the Arg+Glu group than that in the basic diet and Glu groups. The mRNA level of *FATP-4* was more downregulated ($P < 0.05$) in the Arg, Glu, and Arg+Glu groups compared with that of the isonitrogenous group.

In the LD muscle tissue, the Arg+Glu-supplemented pigs presented higher ($P < 0.05$) mRNA levels of *PPAR γ* than pigs in the basic diet or isonitrogenous groups. Additionally, the mRNA level of *FAS* in the Arg+Glu group was more upregulated ($P < 0.05$) than that in the Glu group, whereas the mRNA level of *HSL* decreased more ($P < 0.05$) in the Arg+Glu group than in the Arg group. The mRNA level of *FATP-4* in Arg and Arg+Glu groups was more downregulated ($P < 0.05$) than that of the basic diet group.

In the BF muscle tissue, no significant differences ($P > 0.05$) were observed in the mRNA levels of *HSL*, *LPL*, *ACC*, *FAS*, *FATP-1*, and *CPT-1* among the 5 dietary groups. However, the mRNA level of *PPAR γ* in the Arg+Glu group was more upregulated ($P < 0.05$)

than that of the basic diet or isonitrogenous groups. The mRNA level of *FATP-4* was more downregulated ($P < 0.05$) in the Arg, Glu, and Arg+Glu groups compared with that of the basic diet group.

DISCUSSION

Fat content and fatty acid composition play an important role in the quality of pork meat (Wood et al., 2008). Our previous study showed dietary supplementation with both Arg and Glu increased IMF levels and decreased average back fat thickness in growing pigs (Hu et al., 2017). Therefore, the present study investigated whether the effects of Arg and Glu on the lipid metabolism of growing pigs were mediated via the tissue expression of specific genes, leading to an increase in IMF content and a decrease in average back fat thickness. The results showed that dietary supplementation with Arg+Glu decreased the percentages of C14:0 in the LD muscle tissue and upregulated the relative mRNA level of *HSL* in the SA tissue, as well as that of *PPAR γ* in the LD and BF muscles. These findings indicate that fatty acid percentages improved and the expression levels of lipid metabolism-related genes in porcine skeletal muscle and SA tissues were differentially regulated, which could lead to an increase in IMF content and decrease in back fat thickness.

Plasma TC levels reflect the nutritional status of animals and are positively associated with adiposity (Teixeira et al., 2001). In the present study, dietary supplementation with arginine or glutamic acid had no effect on the plasma TC concentration, which was consistent

Table 5. Effects of dietary supplementation with arginine and glutamic acid on fatty acid percentages (g/100 g total fatty acids) of longissimus dorsi muscle in growing pigs

Item	Dietary treatment ¹					SEM	P-value
	Basic diet group	Isonitrogenous group	Arg group	Glu group	Arg + Glu group		
C14:0	1.45 ± 0.05 ^a	1.34 ± 0.04 ^{a,b}	1.38 ± 0.05 ^{a,b}	1.25 ± 0.05 ^b	1.29 ± 0.03 ^b	0.02	0.03
C16:0	26.55 ± 0.48	25.13 ± 0.40	25.25 ± 0.57	24.69 ± 0.61	24.62 ± 0.45	0.23	0.06
C16:1	2.67 ± 0.47	2.81 ± 0.11	2.82 ± 0.37	2.45 ± 0.30	2.77 ± 0.30	0.14	0.92
C17:0	0.28 ± 0.02	0.32 ± 0.02	0.28 ± 0.02	0.36 ± 0.03	0.31 ± 0.02	0.01	0.08
C18:0	13.62 ± 0.28	12.97 ± 0.39	12.84 ± 0.42	13.10 ± 0.31	12.57 ± 0.29	0.15	0.29
C18:1n-9	41.68 ± 0.65	40.72 ± 0.77	41.50 ± 1.03	39.71 ± 0.98	41.41 ± 0.82	0.38	0.48
C18:2n-6	11.18 ± 0.94	14.03 ± 0.75	13.23 ± 1.14	15.43 ± 1.32	14.00 ± 0.91	0.48	0.08
C18:3n-6	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.005	0.86
C20:0	0.21 ± 0.01	0.19 ± 0.02	0.17 ± 0.02	0.21 ± 0.02	0.20 ± 0.01	0.01	0.54
C20:1	0.34 ± 0.08	0.46 ± 0.09	0.39 ± 0.10	0.50 ± 0.11	0.35 ± 0.10	0.04	0.78
C20:3n-6	0.25 ± 0.03	0.26 ± 0.02	0.22 ± 0.04	0.27 ± 0.04	0.29 ± 0.03	0.02	0.66
C20:4n-6	1.76 ± 0.27	1.86 ± 0.29	1.79 ± 0.35	2.00 ± 0.34	2.08 ± 0.28	0.13	0.94
PUFA:SFA ²	0.32 ± 0.03	0.41 ± 0.03	0.39 ± 0.04	0.46 ± 0.05	0.43 ± 0.03	0.02	0.17

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$). Data are expressed as means ± SEM; $n = 12$.

¹Isonitrogenous group = basic diet supplemented with 2.05% Ala; Arg group = basic diet supplemented with 1.00% Arg; Glu group = basic diet supplemented with 1.00% Glu + 1.44% Ala; and Arg+Glu group = basic diet supplemented with 1.00% Arg + 1.00% Glu.

²PUFA = 18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6. SFA = C14:0 + C16:0 + C17:0 + C18:0 + C20:0.

with the results of previous studies (Tan et al., 2009; Ren et al., 2011). In contrast, a decreasing trend of plasma TC concentration was observed with Arg+Glu dietary supplementation, which was accompanied by a decrease in the average back fat thickness (Hu et al., 2017), a key index to evaluate the fat content of pigs. These findings indicate that dietary supplementation with Arg+Glu has a beneficial effect on the health of pigs.

Fatty acids in the muscle play an important role in flavor, nutritional value, and oxidative stability of meat (Wood et al., 2004; Montaña et al., 2016). Therefore, improving fatty acid composition has a vital role in pro-

ducing high-quality pork meat with a superior nutritional value for human consumption. Montoya et al. (2002) reported that mono- and polyunsaturated fat diets were healthier for humans, and it has been suggested that the ratio of PUFA:SFA should be above 0.4 (Wood et al., 2004). In the present study, dietary glutamic acid supplementation significantly decreased the SFA percentages of palmitic acid (C16:0) and myristic acid (C14:0) in SA tissue and C14:0 in LD muscle tissue. Excessive SFA consumption increases the risk of cardiovascular disease and causes inflammation and insulin resistance in the muscle (Kromhout, 1992; Kennedy et al., 2009).

Table 6. Effects of dietary supplementation with arginine and glutamic acid on fatty acid percentages (g/100 g total fatty acids) of biceps femoris muscles in growing pigs

Item	Dietary treatment ¹					SEM	P-value
	Basic diet group	Isonitrogenous group	Arg group	Glu group	Arg+Glu group		
C14:0	1.33 ± 0.04	1.29 ± 0.03	1.32 ± 0.05	1.25 ± 0.04	1.20 ± 0.03	0.02	0.13
C16:0	24.47 ± 0.42	23.66 ± 0.33	23.86 ± 0.46	23.69 ± 0.35	23.11 ± 0.38	0.18	0.19
C16:1	3.28 ± 0.12	2.91 ± 0.10	3.20 ± 0.17	2.83 ± 0.14	2.88 ± 0.10	0.06	0.07
C17:0	0.31 ± 0.02	0.31 ± 0.02	0.27 ± 0.02	0.35 ± 0.03	0.31 ± 0.02	0.01	0.20
C18:0	12.28 ± 0.32	11.73 ± 0.30	11.91 ± 0.43	12.34 ± 0.25	11.91 ± 0.22	0.14	0.57
C18:1n-9	42.63 ± 0.48	41.88 ± 0.97	43.13 ± 1.06	40.20 ± 1.05	42.00 ± 0.87	0.41	0.25
C18:2n-6	12.50 ± 0.61	15.12 ± 0.87	13.28 ± 1.03	15.54 ± 1.18	15.10 ± 0.73	0.42	0.09
C18:3n-6	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.004	0.73
C20:0	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.007	0.93
C20:1	0.33 ± 0.06	0.55 ± 0.09	0.41 ± 0.10	0.46 ± 0.12	0.47 ± 0.07	0.04	0.51
C20:3n-6	0.31 ± 0.03	0.28 ± 0.04	0.23 ± 0.05	0.36 ± 0.04	0.34 ± 0.03	0.02	0.19
C20:4n-6	2.33 ± 0.31	2.07 ± 0.41	2.18 ± 0.37	2.75 ± 0.42	2.65 ± 0.33	0.16	0.64
PUFA:SFA ²	0.40 ± 0.03	0.48 ± 0.04	0.43 ± 0.04	0.50 ± 0.04	0.48 ± 0.03	0.02	0.28

¹Isonitrogenous group = basic diet supplemented with 2.05% Ala; Arg group = basic diet supplemented with 1.00% Arg; Glu group = basic diet supplemented with 1.00% Glu + 1.44% Ala; and Arg+Glu group = basic diet supplemented with 1.00% Arg + 1.00% Glu.

²PUFA = 18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6. SFA = C14:0 + C16:0 + C17:0 + C18:0 + C20:0.

Figure 1. Effects of dietary supplementation with arginine and glutamic acid on relative mRNA expression levels of fat metabolism genes in (A) the s.c. adipose tissues, (B) longissimus dorsi muscle, and (C) biceps femoris muscle of the growing pigs. The mRNA expression levels of *PPAR γ* , acetyl-coA carboxylase (*ACC*), fatty acid synthase (*FAS*), lipoprotein lipase (*LPL*), hormone-sensitive lipase (*HSL*), fatty acid transport protein 1 (*FATP-1*), *FATP-4*, and carnitine palmitoyltransferase 1 (*CPT-1*) were normalized using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as an internal control. ^{a-c}Mean values with different letters were considered to be significantly different ($P < 0.05$). Data are expressed as means $\pm$ SEM; $n = 12$.

However, this effect was not consistent with that reported by Kong et al. (2015), who showed that dietary glutamic acid supplementation increased the percentage of C16:0 in the LD muscle. Further studies are needed to clarify the effects of dietary glutamic acid supplementation on the fatty acid composition of pig muscle. Another important finding is that dietary Arg+Glu supplementation decreased the percentages of C14:0 in the LD muscle tissue of growing pigs. Additionally, an increase in linoleic acid (C18:2n-6) content was observed in both LD and BF muscles with Arg+Glu dietary supplementation. Linoleic acid, an essential fatty acid, has a potential anti-inflammatory and immunomodulatory

role in humans (Pischon et al., 2003; Kalogeropoulos et al., 2010). These findings indicate that dietary Arg+Glu supplementation may be a novel and effective means to produce high-quality pork meat.

Lipogenesis and lipolysis are major factors affecting fat accumulation in tissues. Dietary supplementation with AA can modify the IMF content and fatty acid composition of muscles by altering fat metabolism gene expression (Duan et al., 2016). Our previous study showed that Arg+Glu supplementation increased fat accumulation in the skeletal muscle of growing pigs and decreased it in s.c. tissue (Hu et al., 2017). Therefore, the mRNA levels of genes associated with lipid metabolism

(including *FAS*, *ACC*, *HSL*, *LPL*, and *PPAR γ*) were examined in the present study. The enzyme *FAS* catalyzes the synthesis of C16:0 from acetyl-CoA and malonyl-CoA (Wakil, 1989), and *ACC* is the key enzyme for malonyl-CoA biosynthesis (Alberts and Vagelos, 1968). The enzyme *LPL* catalyzes the hydrolysis of triacylglycerol and facilitates the entry of fatty acids into peripheral and white adipose tissues (Goldberg, 1996). In the present study, dietary arginine or glutamic acid supplementation showed no effects on the mRNA levels of *PPAR γ* , *ACC*, *FAS*, and *LPL*. However, these results are not consistent with previous findings in which dietary arginine supplementation increased the mRNA level of *HSL* and reduced the mRNA levels of *ACC α* and *LPL* in SA tissue (Tan et al., 2011) and increased the mRNA level of *FAS* in LD muscle of growing pigs (Madeira et al., 2014). Additionally, Kong et al. (2015) reported that dietary supplementation with monosodium glutamate increased IMF deposition by upregulating the mRNA level of *ACC* in LD muscle. These differences could possibly be explained by the variations in the experimental basic diet. The enzyme *HSL* catalyzes the hydrolysis of triacylglycerols in adipose tissue (Enevoldsen et al., 2001). In the present study, dietary supplementation with Arg+Glu upregulated the mRNA level of *HSL* in SA tissue, which may contribute to the decrease in the average back fat thickness observed in our previous study (Hu et al., 2017). In *HSL*-deficient animals, there was a marked increase in the plasma TC level (Haemmerle et al., 2002). Interestingly, in the present study, dietary supplementation with Arg+Glu decreased the plasma TC level but increased the mRNA level of *HSL* in SA tissue. Furthermore, pigs supplemented with both AA showed an increase in the mRNA level of *FAS* relative to that of the Glu group and a decrease in the mRNA level of *HSL* relative to that of the Arg group in LD muscle. The enzyme *FAS* catalyzes the de novo synthesis of C16:0 from acetyl-CoA and malonyl-CoA (Wakil, 1989); although dietary Arg+Glu supplementation increased the mRNA level of *FAS* relative to that of the Glu group, there was no change in the percentage of C16:0 in the LD muscle between the Glu and Arg+Glu groups. The enzyme *PPAR γ* is highly expressed in white adipose tissue and plays a key role in fat accumulation. Some target genes involved in fat metabolism, such as *LPL* and *HSL*, are regulated by *PPAR γ* (Laplante et al., 2003). Dietary Arg+Glu supplementation upregulated the mRNA level of *PPAR γ* in the LD and BF muscles. Collectively, these findings suggest that dietary Arg+Glu supplementation enhances the expression of genes involved in lipogenesis in skeletal muscle and lipolysis in SA tissues. The FATP family consists of 6 membrane proteins that facilitate the uptake of long- and very long chain fatty acids. Both FATP-1 and FATP-4 are believed

to be the main FATP in adipose tissue (Lobo et al., 2007). Overexpression of FATP-1 results in enhanced glucose oxidation, whereas FATP-1 knockdown leads to a decrease in the tricarboxylic acid cycle activity (Guitart et al., 2009; Wiczer and Bernlohr, 2009). The mRNA level of FATP-4 was positively correlated with obesity and insulin resistance levels (Gertow et al., 2004). In the present study, dietary Arg+Glu supplementation upregulated the mRNA level of *FATP-1* in the SA tissue but not in LD or BF tissue, which might contribute to increased catabolism in SA tissue, leading to a reduction in back fat thickness (Hu et al., 2017).

In conclusion, dietary supplementation with both arginine and glutamic acid modified fatty acid composition, upregulated the mRNA levels of genes involved in fat synthesis in muscles, and promoted lipolytic gene expression in SA tissues. These findings will facilitate our understanding of the molecular mechanisms of lipid metabolism in growing pigs and provide basic knowledge for producing high-quality pork meat for human consumption on the basis of nutritional approaches.

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