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ORIGINAL ARTICLE

Dietary arginine supplementation enhances antioxidative capacity and improves meat quality of finishing pigs

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Abstract The present study was conducted to test the hypothesis that dietary arginine supplementation may improve meat quality of finishing pigs. Beginning at ~60 kg body weight, pigs were fed a corn- and soybean meal-based diet supplemented with 0, 0.5 or 1% L-arginine until they reached a body weight of ~110 kg. On the last day of the experiment, pigs were food-deprived for 16 h before blood samples were obtained for analysis of amino acids, insulin, and other metabolites. Immediately thereafter, pigs were slaughtered for determination of carcass composition, muscle biochemical parameters, and meat quality. The result showed that arginine did not affect pig growth performance or carcass traits. However, 1% arginine decreased drip loss of pork muscle at 48 h postmortem, while increasing intramuscular fat content ($P < 0.05$). Supplementing 0.5 or 1% arginine to the diet increased arginine concentration and decreased cortisol level in serum, while enhancing antioxidative capacity and glutathione peroxidase activity in serum ($P < 0.05$). Additionally, 1% arginine increased antioxidative capacity in skeletal muscle ($P < 0.05$). Furthermore, 0.5 or 1% arginine decreased the cortisol receptor mRNA level in muscle ($P < 0.05$). Collectively, these results indicate that supplemental arginine improved meat quality and attenuated oxidative stress of finishing pigs.

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Abbreviations

CAT	Catalase
CR	Cortisol receptor
GSH-Px	Glutathione peroxidase
IGF-IR	Insulin-like growth factor-I receptor
MDA	Malondialdehyde
PPARγ	Peroxisome proliferators-activated receptor γ
SOD	Superoxide dismutase
T-AOC	Total antioxidant capacity

Introduction

There is increasing interest in improving pork meat quality among producers and consumers. A number of studies have shown that dietary supplementation with certain nutrients may enhance water-holding capacity, color, and anti-oxidative capacity of pork muscle. These nutrients have antioxidant function and include magnesium, selenium, vitamin E, vitamin C, tryptophan, creatine, and conjugated linoleic acids (Buckley et al. 1995; Swigert et al. 2004). Available evidence shows that oxidative, heat, or slaughter stress can result in the oxidation of fat, protein and metal ions to generate malondialdehyde (MDA), carbonyl protein complexes, aldehydes, ketones, and phenolic compounds (Galli 2007; Fang et al. 2002). These products are known to affect the quality of pork and poultry meats (Lefaucheur et al. 1991; Sandercock et al. 2001). L-Arginine is a nutritionally important amino acid and plays multiple physiologic functions in animals (Jobgen et al. 2006;

Mateo et al. 2007; Wang et al. 2008a; Wu et al. 2007a). One of these functions is to increase antioxidant ability, reduce superoxide release, and ameliorate lipid peroxidation (Galli 2007; Petrovic et al. 2008).

In animals, arginine was metabolized to nitric oxide, proline, glutamine and polyamines with enormous biological importance (Liao et al. 2008; Wu and Morris 1998). For example, physiological levels of these metabolites can attenuate the stress response (Hamasu et al. 2008; Suenaga et al. 2008a, b), enhance the immune function (Li et al. 2007b; Tan et al. 2008a), regulate protein synthesis (Tan et al. 2008b; Yao et al. 2008), and promote wound healing (Flynn et al. 2002). In view of the foregoing, we hypothesized that dietary arginine supplementation may improve meat quality of finishing pigs through increasing anti-oxidative capacity and ameliorating stress. The present study was conducted to test this hypothesis.

Materials and methods

Pigs and diet

A total of 60 Du × (Chang × Da) finishing pigs (male, castrated) of approximately 60 kg body weight (90 days of age) were used in this experiment. All pigs were housed in the animal facilities of the Institute of Animal Science, Guangdong Academy of Agricultural Science. They were allotted randomly to one of three dietary treatments on the basis of body weight in a completely randomized design. Each treatment consisted of five replicates with four pigs per replicate. Pigs in Group 1 (the control) were fed a corn- and soybean meal-based diet (Table 1), whereas pigs in Groups 2 and 3 were fed the basal diet supplemented with 0.5 and 1% L-arginine (>98% purity), respectively (Table 1). L-Alanine was used as isonitrogenous control, as described by Kim and Wu (2004). L-Arginine and L-alanine were purchased from Guangzhou Weijian Medicine and Healthy Foods Import–Export Company (Guangdong, China).

Feeding and slaughter procedure

The experiment was initiated when the pigs weighted ~60 kg (90 days of age) and ended when the pigs reached ~110 kg (170 days of age) body weight. At the beginning and the termination of the experiment, each pig was weighed. The amount of feed consumed was recorded during the experiment to determine average daily gain, average daily feed intake, and the gain to feed ratio. At the end of the experiment, 14 h after the last feeding, two pigs were selected randomly from each replicate to obtain blood samples at 7:00 a.m. via the anterior vena cava using vacuum tubes (containing no anticoagulant), followed immediately by slaughter. Carcasses were weighed and split. The loin eye area and backfat thickness were measured. This study was carried out in accordance with the Chinese guidelines (Science and Technology Ministry of China 2006) for animal welfare and approved by Guangdong Academy of Agricultural Science.

Sample collection and analysis of biochemical parameters

Blood samples were centrifuged for 5 min at 3,000*g*, 4°C, and the serum was stored at −20°C for assays of biochemical parameters. Following slaughter, ~2 cm^3 longissimus muscle (the caudal end of the muscle) was collected immediately and stored at −20°C for the determination of hydroxy radical, MDA, total antioxidant capacity (T-AOC), as well as activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), using commercial kits (Jiancheng Bioengineering Institution, Nanjing, China). Amino acids in serum were analyzed by Amino Acid Analyzer (L-8900, HITACHI, Japan), as described by Kong et al. (2008) and Yin et al. (2008).

Meat quality measurements

Muscle samples were taken at the last thoracic verteba and the pH value was recorded at 45 min, 24 and 48 h postmortem using a pH meter (HI8242C, Beijing Hanna Instruments Science & Technology Co. Ltd., Beijing, China). Drip loss was estimated according to the method described by Carlo et al. (1999). Briefly, three cylindrical muscle cores were taken from each slice with a cork borer (25 mm diameter), weighed, wrapped in a plastic bag, and stored at 4°C. Samples were taken from the container and weighed after stored for 24 and 48 h. Drip loss was estimated by calculating the difference between the initial and the final weight of the muscle sample. Meat color CIE LAB value (L*, relative lightness; a*, relative redness; b*, relative yellowness) was assessed immediately on the fresh surface of meat samples using a colorimeter (CR-410, Minolta Co. Ltd, Suita-shi, Osaka, Japan) according to the method described by Mason et al. (2005). Each sample was a complete cross section of thoraco-lumbar vertebra, about 2-cm thickness. Shear force was determined using an Instron Universal Mechanical Machine (Instron model 4411, Instron Corp., Canton) as follows. Approximately 250 g longissimus muscle sample from each pig was refrigerated overnight at 4°C and then brought to room temperature before

Table 1 Dietary composition and nutrient levels

	Control	Supplementation with 0.5% arginine	Supplementation with 1% arginine
Diet ingredients			
Corn meal	70.82	71.3	71.8
Soybean meal	17.6	17.6	17.6
Wheat bran	3	3	3
Rap meal	4	4	4
Soybean oil	0.2	0.2	0.2
L-Ala	1.84	0.97	0
L-Arg	0	0.5	1
Premix[a]	1	1	1
L-Lysine HCl	0.12	0.12	0.12
Lime powder	1	1.01	1.02
Salt	0.3	0.3	0.29
Dicalcium phosphate	0.3	0.3	0.3
Total	100	100	100
Nutrient levels			
Digestible energy (Mcal/kg)	3.17	3.19	3.20
CP (%)	15.4	15.4	15.4
Lys (%)	0.8	0.8	0.8
Met (%)	0.24	0.24	0.24
Met + Cys (%)	0.50	0.50	0.50
Thr (%)	0.58	0.58	0.58
Trp (%)	0.17	0.17	0.17
Arg (%)	0.96	1.46	1.96
Val (%)	0.70	0.70	0.70
Ca (%)	0.55	0.55	0.55
Total P (%)	0.42	0.42	0.42
Available P (%)	0.19	0.19	0.19
Na (%)	0.15	0.15	0.15
Cl (%)	0.24	0.24	0.24

[a] Providing 1,750 IU/kg vitamin A, 220 IU/kg vitamin D_3, 3 IU/kg vitamin E, 0.55 mg/kg vitamin K_3, 0.25 mg/kg vitamin B_1, 1.0 mg/kg vitamin B_2, 0.7 mg/kg vitamin B_6, 3 μg/kg vitamin B_{12}, 4 mg/kg niacin, 1.6 mg/kg calcium pantothenate, 0.1 mg/kg folic acid, 7 μg/kg biotic, 0.08 g/kg choline chloride, 6.5 mg/kg manganese, 15 mg/kg iron, 15 mg/kg zinc, 1.5 mg/kg copper, 0.07 mg/kg iodine(I_2), 0.03 mg/kg selenium, and 1 g/kg sodium chloride

cooking. The temperature of the water bath was set at 80°C and meat samples were cooked for 1 h to an internal temperature of 70°C, and then cooled to room temperature. About 10 slices of 1 cm × 1.5 cm were cut perpendicular to the fiber orientation of the muscle. The marbling of meat sample (thoraco-lumbar vertebra section) was assessed by a marbling testing standard (NPPC 1991) after being stored at 4°C for 24 h. Intramuscular fat (IMF) content was determined as follows. Approximately 10 g longissimus muscle sample from each pig was cut into pieces and dried in a freezing drier, ground into powder, and extracted for lipids with petroleum ether extract using the Soxtec 2055 fat extraction system (Foss Tecator AB, Box 70, S-26321 Höganäs, Sweden).

Real-time PCR analysis

Total RNA was isolated from fresh longissimus muscle tissue using TRIZOL reagent (Invitrogen Co., USA) according to the manufacturer's instructions. The RNA concentration was determined at 260/280 nm and the cDNA was produced using a commercial kit containing Reverse Transcriptase XL (AMV) and RNAsin (Invitrogen Co., USA). Real-time PCR was performed using an ABI 7500 Mastercycler (Applied Biosystems, USA) with qPCR Mix (TaKaRa, BIOINC, Japan) according to the manufacturers' instructions. The DNA was sequenced by Sangon Technical Co. Ltd. (Shanghai,China). Primers and probes (Table 2) of genes [β-actin, cortisol receptor (CR), leptin, IGF-IR, and PPAR-γ]

Table 2 PCR primers for genes

Gene	Primers	
CR	Forward primer	5′-cctgcccctggtagtcatg-3′
	Reverse primer	5′-agcgtcttgagggctttgtg-3′
PPARγ	Forward primer	5′-ctgaacccagagtctgctgac-3′
	Reverse primer	5′-ggtgatttgtctgtggtctttcc-3′
IGF-IR	Forward primer	5′-acctgcccctggtagtcatg-3′
	Reverse primer	5′-tagcgtcttgagggctttgtg-3′
Leptin	Forward primer	5′-ggctcccgaggtgctgtt-3′
	Reverse primer	5′-gaacctggcccttcgagatc-3′
β-Actin	Forward primer	5′-gacctgaccgactacctcat-3′
	Reverse primer	5′-gccgatggtgatgacctggc-3′

were designed according to the sequences published in GenBank. The real-time PCR cycling conditions used were 95°C for 40 s, followed by 40 cycles at 95°C for 5 s and 58°C for 20 s, then 1 cycle at 72°C for 30 s.

Statistical analysis

Values are expressed as mean ± SEM. Data were analyzed by one-way analysis of variance and the Student–Newman–Keuls multiple comparison test. All statistical analyses were performed using SAS (v6.12, SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was taken to indicate statistical significance.

Results

Growth performance and carcass traits

The effects of dietary arginine supplementation on growth performance and carcass traits of the pigs are summarized in (Table 3). Supplementation with arginine did not affect ($P > 0.05$) average daily gain, average daily feed intake, feed to gain ratio, final body weight, carcass yield, lean meat, percentage of lean tissue, loin eye area, or backfat thickness.

Meat quality

There was no difference in meat quality traits (e.g., pH value and meat color) between the control and arginine-supplemented pigs (Table 4). The drip loss at 48 h post-mortem was lower ($P < 0.05$) in pigs supplemented with 1% arginine when compared with the control pigs, but there was no difference ($P > 0.05$) between the control group and the pigs supplemented with 0.5% arginine. The shear force of meat did not differ ($P > 0.05$) among the three groups of pigs, but had a trend to decrease in

Table 3 Effect of dietary arginine supplementation on growth performance and carcass traits of finishing pigs

Treatment	Dietary supplementation with L-arginine (%)		
	0.0	0.5	1.0
ADG (g/d)	786 ± 3	792 ± 6	764 ± 10
ADFI (kg/d)	3.06 ± 0.13	2.89 ± 0.10	2.98 ± 0.10
F:G	3.90 ± 0.12	3.64 ± 0.18	3.90 ± 0.17
Live weight (kg)	118 ± 2	120 ± 3	121 ± 3
Carcass yield (%)	74.7 ± 0.6	74.1 ± 0.4	74.3 ± 0.4
Lean meat (kg)	26.3 ± 1.4	26.8 ± 0.7	27.2 ± 1.0
Lean percentage (%)	63.8 ± 1.7	65.0 ± 1.5	63.5 ± 1.8
Loin eye area (mm^2)	5375 ± 259	5461 ± 184	5459 ± 87
Backfat depth (cm)			
First rib	3.87 ± 0.35	4.00 ± 0.15	4.10 ± 0.22
Tenth rib	1.99 ± 0.15	2.06 ± 0.22	2.14 ± 0.02
Last rib	1.21 ± 0.16	1.24 ± 0.18	1.29 ± 0.06

Data are mean ± SEM, $n = 20$ for ADG, ADFI and F:G and $n = 10$ for other parameters

ADG average daily gain, *ADFI* average daily feed intake, *F:G* feed to gain ratio

Table 4 Effect of dietary arginine supplementation on meat quality of finishing pigs

Treatment	Dietary supplementation with L-arginine (%)		
	0.0	0.5	1.0
pH			
45 min	6.44 ± 0.07	6.52 ± 0.07	6.61 ± 0.06
24 h	5.57 ± 0.1	5.58 ± 0.07	5.65 ± 0.04
48 h	5.46 ± 0.07	5.46 ± 0.08	5.50 ± 0.05
Drip loss (%)			
24 h	1.21 ± 0.09	1.10 ± 0.10	1.02 ± 0.07
48 h	2.32 ± 0.14^{a}	2.02 ± 0.11^{ab}	1.67 ± 0.14^{b}
L			
0	45.4 ± 1.5	46.2 ± 0.3	46.7 ± 0.9
24 h	53.6 ± 1.0	53.6 ± 0.7	55.3 ± 1.1
48 h	53.8 ± 0.4	54.3 ± 0.7	55.0 ± 0.8
a*			
0	14.9 ± 0.2	16.1 ± 0.9	14.7 ± 0.7
24 h	15.0 ± 0.3	16.1 ± 0.4	14.9 ± 0.5
48 h	15.6 ± 0.3	16.8 ± 0.6	15.0 ± 0.8
b*			
0	3.60 ± 0.42	4.02 ± 0.37	3.67 ± 0.29
24 h	2.86 ± 0.29	3.22 ± 0.41	3.14 ± 0.28
48 h	3.05 ± 0.24	3.59 ± 0.47	3.06 ± 0.31
Shear force (Nf)	47.8 ± 2.7	42.7 ± 1.9	42.8 ± 3.1
Intramuscular fat (%)	1.31 ± 0.14^{b}	1.99 ± 0.18^{a}	1.74 ± 0.17^{a}

Data are mean ± SEM, $n = 10$. Within a row, means with different superscript letters differ ($P < 0.05$)

L lightness, *a** redness, *b** yellowness

Table 5 Effect of dietary arginine supplementation on concentrations of biochemical parameters in blood of finishing pigs

Treatment	Dietary supplementation with L-arginine (%)		
	0.0	0.5	1.0
Cholesterol (mmol/L)	2.10 ± 0.10	2.03 ± 0.09	2.02 ± 0.04
Free fatty acid (mmol/L)	0.27 ± 0.02	0.26 ± 0.03	0.25 ± 0.02
Urea N (mmol/L)	5.40 ± 0.32	5.52 ± 0.28	5.67 ± 0.22
Glucose (mmol/L)	3.73 ± 0.22	4.05 ± 0.30	3.99 ± 0.23
Low density lipoprotein (mmol/L)	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
Triglycerides (mmol/L)	0.46 ± 0.04	0.49 ± 0.03	0.47 ± 0.03
Insulin (μIU/ml)	28.6 ± 2.2	24.0 ± 1.0	27.3 ± 2.5
Phosphokinase(IU/L)	41.3 ± 1.8	35.2 ± 2.6	35.0 ± 3.0
Alkaline phosphatase (IU/L)	86.0 ± 6.8	86.8 ± 6.4	88.6 ± 6.2
Lactate dehydrogenase (IU/L)	494 ± 25	448 ± 15	430 ± 24
Cortisol (ng/ml)	89.4 ± 0.8[a]	56.3 ± 0.6[b]	53.5 ± 0.5[b]

Data are mean ± SEM, $n = 10$. Within a row, means with different superscript letters differ ($P < 0.05$)

arginine-supplemented pigs. The IMF content was higher ($P < 0.05$) in pigs supplemented with 0.5 and 1% arginine than in the control group and did not differ ($P > 0.05$) between the 0.5 and 1% arginine groups (Table 4).

Biochemical parameters in serum

Dietary supplementation with arginine decreased ($P < 0.05$) the concentration of cortisol in serum but did not affect ($P > 0.05$) concentrations of cholesterol, free fatty acid, glucose, urea, low density lipoprotein, triglycerides or insulin in serum (Table 5). The activities of phosphokinase, alkaline phosphatase, and lactate dehydrogenase in serum did not differ ($P > 0.05$) between control and arginine-supplemented pigs. Compared with the control group, supplementing 0.5 and 1% arginine to the basal diet increased ($P < 0.05$) the concentrations of arginine, proline, as well as glutamine plus glutamate in the serum of pigs, but had no effect ($P > 0.05$) on the concentration of lysine in serum (Table 6).

MDA production and enzyme activities

Dietary arginine supplementation did not affect ($P > 0.05$) the concentration of MDA or the activities of SOD and CAT in serum or skeletal muscle of pigs (Table 7). However, supplementation with 1% arginine decreased the level of hydroxy radical in the serum and muscle. Supplementation with 0.5 and 1% arginine increased ($P < 0.05$) the activities of T-AOC and GSH-Px in serum, while supplementation with 1% arginine augmented ($P < 0.05$) T-AOC level in the muscle.

Gene expression

The results of gene sequencing indicated 100% homology of the fragments obtained from the present study with the sequences published in GenBank. The mRNA levels for

Table 6 Effect of dietary arginine supplementation on concentrations of amino acids in serum of finishing pigs

Treatment	Dietary supplementation with L-arginine (%)		
	0.0	0.5	1.0
Arginine	0.20 ± 0.01[b]	0.26 ± 0.03[a]	0.29 ± 0.02[a]
Glutamine	0.51 ± 0.01[b]	0.60 ± 0.02[a]	0.62 ± 0.04[a]
Glutamate	0.22 ± 0.01[b]	0.29 ± 0.01[a]	0.26 ± 0.01[ab]
Proline	0.23 ± 0.02[b]	0.25 ± 0.02[ab]	0.28 ± 0.03[a]
Lysine	0.21 ± 0.03	0.23 ± 0.02	0.24 ± 0.01

Data are mean ± SEM (mmol/L), $n = 10$. Within a row, means with different superscript letters differ ($P < 0.05$)

cortisol receptor (CR), insulin-like growth factor-I receptor (IGF-IR), leptin, and peroxisome proliferators-activated receptor γ (PPARγ) genes in skeletal muscle are summarized in Table 8. The CR mRNA level in the muscle of pigs supplemented with 0.5 and 1% arginine was lower ($P < 0.05$) than that in the control group. The PPARγ mRNA level in the skeletal muscle of pigs supplemented with 1% arginine was higher ($P < 0.05$) than that in the control group and the pigs supplemented with 0.5% arginine. The level of leptin mRNA was not different ($P > 0.05$) among the three groups of pigs.

Discussion

L-Arginine is the physiological substrate of nitric oxide, polyamines, creatine, agmatine, glutamate, and proline with enormous biological importance (Krane 2008; Montanez et al. 2008; Wu and Morris 1998). Increasing evidence indicates that arginine regulates the partitioning of dietary energy in favor of muscle protein accretion and fat reduction in animals (Fu et al. 2005; Wu et al. 2007c, 2008a, b). Additionally, either dietary supplementation

Table 7 Effect of dietary arginine supplementation on antioxidant enzyme activities and MDA content in serum and skeletal muscle of finishing pigs

Treatment	Dietary supplementation with L-arginine (%)		
	0.0	0.5	1.0
Serum			
Hydroxy radical (U/ml)	265 ± 31[a]	211 ± 16[ab]	180 ± 20[b]
MDA (nmol/ml)	3.94 ± 0.28	3.46 ± 0.59	3.52 ± 0.41
T-AOC (U/ml)	2.78 ± 0.24[b]	3.54 ± 0.76[a]	3.98 ± 0.71[a]
SOD (U/ml)	120 ± 8	130 ± 10	133 ± 9
GSH-Px(U/ml)	580 ± 31[b]	664 ± 46[a]	658 ± 27[a]
CAT (U/ml)	563 ± 31	629 ± 34	635 ± 49
Muscle			
Hydroxy radical (U/mg protein)	95.5 ± 1.7[a]	93.8 ± 2.2[a]	79.0 ± 1.6[b]
MDA (nmol/mg protein)	3.34 ± 0.17	2.68 ± 0.22	2.72 ± 0.27
T-AOC (U/mg protein)	1.29 ± 0.10[b]	1.61 ± 0.09[ab]	1.90 ± 0.06[a]
SOD (U/mg protein)	56.3 ± 3.1	62.8 ± 3.0	63.5 ± 4.7
GSH-Px (U/mg protein)	51.3 ± 4.5	62.8 ± 4.1	59.3 ± 2.2
CAT (U/mg protein)	21.5 ± 1.0	24.6 ± 1.3	23.0 ± 1.7

Data are mean ± SEM, $n = 10$. Within a row, means with different superscript letters differ ($P < 0.05$)

Table 8 Effect of dietary arginine supplementation on genes expression in skeletal muscle of finishing pigs

Item	Dietary supplementation with L-arginine (%)		
	0.0	0.5	1.0
CR/β-actin	6.58 ± 0.33[a]	1.78 ± 0.15[b]	1.46 ± 0.12[b]
IGF-IR/β-actin	235 ± 26	376 ± 45	455 ± 47
Leptin/β-actin	2.91 ± 0.34	4.24 ± 0.47	3.89 ± 0.63
PPARγ/β-actin	183 ± 15[b]	263 ± 21[ab]	461 ± 39[a]

Data are mean ± SEM, $n = 10$. Within a row, means with different superscript letters differ ($P < 0.05$)

with L-arginine (Kim and Wu 2004, 2008; Yao et al. 2008) or activation of endogenous arginine synthesis (Frank et al. 2007; Wu et al. 2004b) enhanced average daily weight gain in young pigs, compared with control piglets. Of particular interest, increasing the dietary arginine:lysine ratio increased the growth performance of heat-stressed broilers (Chen et al. 2005). Thus, it is important to determine whether dietary supplementation with arginine could improve the meat quality of pigs.

This study was not designed to determine the effect of dietary arginine supplementation on growth performance in swine. Thus, when body weight was used as the criterion to terminate the experiment, no significant differences in pig growth performance or carcass traits were observed (Table 3). However, when growing-finishing pigs were fed a diet supplemented with 1% L-arginine between 110 and 170 days of age, daily weight gain was enhanced and body fat content reduced, compared with the control group of pigs without arginine supplementation (Tan et al. 2008b). This discrepancy between the present study and the work of Tan et al. (2008b) may be explained by the difference in experimental design, the length of arginine supplementation, and the breed of the pigs used.

Arginine is synthesized from glutamine and proline in most mammals, including pigs (Wu and Knabe 1995; Wu 1997) and humans (Hu et al. 2008a, b). However, increasing extracellular concentrations of arginine regulate the metabolism of protein, glucose and lipids in favor of lean tissue gain and white-fat reduction in animals (Jobgen et al. 2006; Wu et al. 2008a, b). Therefore, as reported by Tan et al. (2008b), we found that supplementing arginine to the diet of growing-finishing pigs decreased early muscle pH decline postmortem and drip losses. This beneficial result can be explained by lower rates of glycogenolysis and glycolysis in skeletal muscle of arginine-treated pigs compared with the control group (Tan et al. 2008b). On the basis of the previous reports that drip loss was positively related with oxidation (Swigert et al. 2004) and that intramuscular administration of zinc metallothionein to preslaughter-stressed pigs improved anti-oxidative status and pork quality (Li et al. 2007a), we surmise that the reduction of muscle drip loss in arginine-supplemented pigs may result from an improved anti-oxidative status in the tissue.

In support of the above proposition, available evidence shows that physiological levels of arginine and nitric oxide have antioxidative function (Wu and Meininger 2008). For example, Wascher et al. (1997) reported that administration of L-arginine diminished superoxide release and copper-induced lipid peroxidation in rats. Recently, Petrovic et al. (2008) demonstrated that L-arginine increased the antioxidative defense system in rats in response to cold acclimation. Importantly, dietary supplementation with arginine decreased the hydroxyl radical level in serum, while increasing T-AOC concentrations in serum and

skeletal muscle as well as the activity of GSH-Px [an antioxidative enzyme (Wu et al. 2004a)] in serum of pigs (Table 7), indicating the enhancement of whole-body antioxidative function. In addition, proline, glutamine, creatine and polyamines were important metabolites of arginine (Hu et al. 2008a, b; Phang et al. 2008; Wu and Morris 1998). Accordingly, dietary supplementation with 1% arginine increased serum concentrations of proline and glutamine + glutamate (Table 6), as well as creatine (He et al. 2008). It is noteworthy that proline attenuates the stress response in the central nervous system of chicks (Hamasu et al. 2008), while reducing inflammatory responses and oxidative stress in mammals (Bassit et al. 2008; Gualano et al. 2008). Similarly, glutamine (Wang et al. 2008b) and polyamines (Rider et al. 2007) enhance anti-oxidative function in cells.

Cortisol, the major circulating glucocorticoid in pigs, regulates protein and amino acid metabolism (Flynn et al. 2008). Another novel and important finding of the present study is that dietary arginine supplementation decreased the concentration of cortisol in serum and the mRNA level of glucocorticoid receptor in skeletal muscle (Table 8). This result suggests a reduction of stress levels in arginine-supplemented pigs, as concluded by other investigators (Han et al. 2008). Similarly, Suenaga et al. (2008a, b) reported that arginine reduced stress and induced sedative effects in chickens.

As reported by Tan et al. (2008b), we found that dietary supplementation with 1% arginine increased IMF (Table 4) but had no effect on serum concentrations of glucose, lipids or insulin (Table 5). Interestingly, the arginine treatment enhanced the PPARγ mRNA level in skeletal muscle (Table 8). This finding can be explained by the fact that PPARγ stimulates fat synthesis in this tissue (Hsu and Ding 2003), which can explain the effect of arginine observed from the current study. In contrast, leptin in muscle has been reported to decrease the activity of acetyl-CoA carboxylase, a key regulatory enzyme in fatty acid synthesis (Steinberg et al. 2003). Consistent with this view, we found that leptin mRNA level in muscle was lowered in arginine-supplemented pigs (Table 8). Clearly, future studies are needed to determine changes of fatty acid synthesis and oxidation, as well as the signaling actions of leptin and nitric oxide [another regulator of lipid metabolism (Jobgen et al. 2006)] in skeletal muscle of arginine-supplemented pigs.

Edmonds and Baker (1987) reported that arginine had an antagonistic effect on lysine absorption, but other investigators did not observe such a phenomenon (Tan et al. 2008a; Yao et al. 2008). This discrepancy may be explained by the differences in the contents of amino acids, including basic amino acids (Wu et al. 2008a, b). Studies with pigs have shown that dietary supplementation with <2% arginine (on dry matter basis) is generally safe and does not result in an antagonism among basic amino acids (Wu et al. 2007b). In support of this view, we found that supplementing 0.5 and 1% arginine to the basal diet increased the concentration of arginine in the serum of pigs but had no effect on that of lysine (Table 6).

In summary, results of the present study demonstrate that dietary arginine supplementation reduced stress levels, enhanced whole-body antioxidative function, and improved the meat quality of finishing pigs. The arginine treatment increased IMF content and reduced muscle drop loss. Further research is required to elucidate the underlying molecular mechanisms.

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