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The effect of dietary garcinol supplementation on oxidative stability, muscle postmortem glycolysis and meat quality in pigs

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ABSTRACT

The objective of this study was to evaluate the effects of dietary garcinol (0, 200, 400 and 600 mg/kg) on the growth performance, meat quality, postmortem glycolysis and antioxidative capacity of finishing pigs. Dietary garcinol increased pigs' average daily gain, pH_{24h}, a* and myoglobin content of *longissimus dorsi* (LM) ($P < 0.05$), and decreased feed/gain ratio, the L^*_{24h}, glycolytic potential, drip loss, shear force, and backfat depth ($P < 0.05$). The glutathione peroxidase (GPx), catalase (CAT) and total antioxidative capacity (T-AOC) were significantly increased by garcinol ($P < 0.05$), while the activity of lactate dehydrogenase (LDH) and malonaldehyde (MDA) content were decreased ($P < 0.05$). Moreover, garcinol decreased the p300/CBP-associated factor (PCAF) activity, the acetylation level and activities of glycolysis enzymes phosphoglycerate kinase 1 (PGK1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3 (PFKFB3) ($P < 0.05$). The results of this study showed that garcinol decreased postmortem glycolysis, and this may be due to the mechanism of decreasing glycolytic enzyme acetylation induced by PCAF. The present study indicates that garcinol can facilitate the growth performance of pigs and improve pork quality by changing postmortem glycolysis and antioxidative capacity.

1. Introduction

Muscle postmortem glycolysis and oxidative stability are widely believed to be two important indicators of meat quality (Briskey, 1964; Monin & Sellier, 1985; Rossi et al., 2013). Postmortem extended glycolysis drives hydrogen and lactate accumulation in muscle and results in a fairly consistent ultimate pH and adverse meat quality, even across different species. It is widely believed an ultimate pH of pork of approximately 5.5–5.7 has the most acceptable quality (Mullen & Troy, 2005). In contrast, while meat with an abnormally low ultimate pH caused by extended glycolysis, closer to the isoelectric point of the myofibrillar proteins (pH 5.1–5.2), has a lower water holding capacity, paler color, lower protein extractability and poor processing yield. Meanwhile, lower ultimate pH is associated with paler color of meat (Enfält, Lundström, Hansson, Johansen, & Nyström, 1997; Joo, Kauffman, Kim, & Park, 1999). In addition, lipid peroxidation is considered to be another destructive factor that has adverse impact on meat quality including flavor and color (Asghar, 1988). The primary and secondary metabolites of oxidative reactions in muscles have serious adverse effects on the quality of meat, among which short-chain aldehydes and ketones may lead to the loss of meat color and the reduction of nutritional value (Asghar, 1988).

Recently, numerous studies have focused on improving the meat quality of pigs through dietary supplementation with antioxidative nutrients, such as resveratrol, oregano oil, and selenium (Simitzis, Symeon, Charismiadou, Bizelis, & Deligeorgis, 2010; Zhan, Wang, Zhao, Li, & Xu, 2007; Zhang et al., 2015). Garcinol is the major component of the *Garcinia indica* (*G. indica*) fruit rind, extensively used as a traditional treatment for gastric ailments and skin irritation (Liu et al., 2015). Previous studies *in vitro* showed that garcinol has potential antioxidative and anti-inflammatory effects (Hong et al., 2007; Liao, Sang, Liang, Ho, & Lin, 2004). Additionally, studies in rodent models showed the capability of garcinol to treat different oxidative and inflammatory situations (Tanaka et al., 2000; Yoshida et al., 2005). Furthermore, garcinol is regarded as an extremely potent natural inhibitor of p300/CBP - associating factor (PCAF) (Balasubramanyam et al., 2004), which has been observed to activate the glycolysis pathway through acetylation of the glycolytic enzymes phosphoglycerate kinase 1 (PGK1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3 (PFKFB3) (Wang, Yao, Shao, Zheng, & Huang, 2018; Li et al., 2018); therefore, garcinol may have a predictable potential ability to attenuate muscle postmortem glycolysis in addition to promoting antioxidative stability.

To the best of our knowledge, there are no relevant studies about

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testing whether garcinol treatment could promote the growth performance of finishing pigs and pork quality and, if so, whether postmortem glycolysis and oxidative stability can be altered by garcinol, a possible mediator of the process. It is hypothesized that supplementing pigs' diets with garcinol may improve meat quality by influencing muscle postmortem glycolysis and oxidative stability, as well as promoting the growth performance of finishing pigs.

2. Materials and methods

2.1. Animals and experimental design

This experiment was approved by the Animal Care and Use Committee of College of Animal Sciences and Technology, Huazhong Agricultural University, and was in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The animal handling protocol permit number is HZAUSW-2017-0006. Eighty barrows (Duroc × Landrace × Yorkshire; average body weight = 79.4 kg) were used in this study. The pigs were randomly allotted to four dietary treatments (five replicates (pens) with four pigs per pen), control diet (basal diet), basal diet + 200 mg garcinol, basal diet + 400 mg garcinol, and basal diet + 600 mg garcinol per kg of feed (Table 1). The experimental diet was formulated to meet the 75–100 kg finishing pigs nutrient requirements (NRC, 2012). The garcinol purchased from Xin Lu Biotechnology Company (Xi'an, China), was extracted from dried fruit rind of *Garcinia indica* with a purity of 98.1%, as measured by HPLC. The experiment lasted 52 days, and all pigs were free to eat feed and drink water.

2.2. Slaughter process and sample collection

After 52 days of treatments, the pigs were moved to a commercial slaughter room and electrically stunned and then slaughtered according to standard commercial procedures. The live body weight and hot carcass weight were immediately recorded for the dressing percentage calculation. The first and last rib as well as the last lumbar spine backfat thickness of each pig were measured, and the average value was taken. The 10th rib *M. longissimus dorsi* (LM) area was measured, and an approximately 2-cm-thick 10th rib LM sample was collected for RNA isolation. The LM samples anterior to the 13th rib from the left side carcass were then collected and kept at 2–4 °C for further analysis of meat quality parameters. Approximately 200 g of right side LM sample of the carcass was collected for muscle chemical composition measurement.

Table 1
Feed ingredients and nutrient content of basal diets.

Ingredients, %	Content	Nutrient level[a]	Content
Corn	77.50	Digestible energy, MJ/kg	15.48
Soybean meal	16.50	Crude protein, %	12.76
Soybean oil	2.00	Ca, %	0.44
$CaCO_3$	0.72	Total P, %	0.45
$CaHPO_4{\cdot}2H_2O$	0.50	Available P, %	0.23
L-Lysine, 50%	0.10	Total lysine, %	0.72
Choline chloride	0.10	True digestible lysine, %	0.58
Salt	0.30	Total Met + Cys, %	0.44
Corn starch	0.15	Total Thr, %	0.55
Vitamin and mineral premix[b]	0.33		

[a] All data were calculated values.
[b] Provided the following (per kilogram of complete diet): 100 mg of Fe (as ferrous sulfate); 15 mg of Cu (as copper sulfate); 120 mg of Zn (as zinc sulfate); 40 mg of Mn (as manganese sulfate); 0.3 mg of Se (as Na_2SeO_3); 0.25 mg of I (as KI); 13,500 IU of vitamin A; 2250 IU of vitamin D_3; 24 IU of vitamin E; 6.2 mg of riboflavin; 25 mg of nicotinic acid; 15 mg of pantothenic acid; 1.2 mg of vitamin B 12; 0.15 mg of biotin.

2.3. Growth performance

The average daily feed intake (ADFI) and average daily gain (ADG) were measured every 5 d by measuring feed disappearance and weighing the pigs until the experiment was completed. The feed/gain ratio (F/G) was calculated.

2.4. Meat quality analysis

2.4.1. Meat color measurement

The 3.0-cm-thick LM samples of the carcass were used for meat color measurement. The color of the meat sample was measured after 45 min and 24 h postmortem. According to Hayes, Kenny, Ward, and Kerry (2007), the meat sample was cut up and exposed to the air for 20 min followed by analyses of the meat color which were performed using a chromameter (CR-300, Minolta Camera, Osaka, Japan). The instrument was calibrated on the CIE LAB color space system using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras). The colorimeter had D65 illuminant, the standard observer position 10° and a 1 cm diameter aperture. The mean of three measurements was used for further statistical analysis. The lightness, redness, and yellowness (L*, a* and b*, respectively) of meat samples were recorded and calculated.

2.4.2. Meat pH measurement

The 3.0-cm-thick LM samples (45 min and 24 h) postmortem of the carcass was used for pH measurement. The pH values were measured using a portable pH meter (HANNA Instruments, Cluj l Napoca, Romania). Each muscle was measured in three different positions, and the mean value was taken.

2.4.3. Cook loss measurement

The 4.0-cm-thick LM samples of the left carcass side were used for cook loss measurement. According to Beattie, Bell, Borggaard, and Moss (2008), approximately 50 g of meat sample was taken, sealed in a plastic bag, and heated in a constant temperature water bath with 80 °C water until the center temperature of the meat reached 70 °C, and then the meat sample was removed and dried, cooled to room temperature and reweighed.

2.4.4. Drip loss percentage measurement

The dripping loss was measured after 24 h at 4 °C. Referring to the method of Honikel, Kim, Hamm, & Roncales (1986), meat samples of about 50 g were weighed and suspended in a foam box, and put into a 4 °C cold storage for 24 h of natural water dripping, and then weighed again to calculate the difference.

2.4.5. Shear force measurement

The 5.0-cm-thick LM samples of the left carcass side were used for shear force measurement. According to Yuan et al. (2012), the muscle samples were packaged in polyethylene bags at 48 h postmortem and then cooked to an internal temperature of 70 °C in a water bath. After cooling to 4 °C, the samples were cut parallel to the longitudinal orientation of the myofibers and then the shear force was measured using a texture analyzer (Northeast Agricultural University, Harbin, China) with a 15-kg load transducer, a crosshead speed of 200 mm/min, and a shearing action similar to a Warner-Bratzler shear device. The repeated measurement times of samples were 3 times, the shear force values of 3 meat samples were recorded, and the average was calculated.

2.5. Muscle chemical analysis and malondialdehyde (MDA) content measurement

The contents of moisture, crude protein and ash in muscle samples were measured according to AOAC methods (Cunniff, 1996). The intramuscular fat content (IMF) of the sample was measured according to

a previous publication (Cunniff, 1996). The myoglobin content was analyzed using a spectrophotometer (Mepda Instrument Co. Ltd., Shanghai, China) according to a previous publication (Serrano, García, Valencia, Lázaro, & Górriz, 2013). The glycogen, lactate, glucose and glucose-6-phosphate (G6P) contents of LM samples were measured using commercial kits (Nanjing Jiancheng Bioengineering Company, Jiangsu, China) according to the procedures. Glycolytic potential (GP) was calculated according to a previous publication as GP = 2 (glycogen content + glucose content + glucose-6-phosphate content) + lactate content (Monin & Sellier, 1985). Approximately 0.5 g of frozen sample was used for malondialdehyde (MDA) content measurement using commercial kits according to the procedures (Nanjing Jiancheng Bioengineering Company, Jiangsu, China).

2.6. RNA extraction, transcription and real-time PCR

The LM total RNA was isolated (postmortem 45 min) using TRIzol Reagent (TaKaRa Biotechnology, Dalian, China) following the manufacturers' protocol. The total RNA purity and quantity were analyzed by using a spectrophotometer (Mepda Instrument Co., Ltd., Shanghai, China) at 260 and 280 nm. Subsequent PCRs were performed using the 260/280 ratios at 1.9–2.0. The synthesis of cDNA of total RNA was performed using the PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) according to the manufacturer's protocol. The primers for mRNA expression are shown in Table 2. The primers of 18S RNA, MyHCI, MyHCIIa, MyHCIIx, and MyHCIIb, LDH, GAPDH and PGK1 were used as previously described (Wimmers et al., 2008; Wang et al., 2015; Kwasiborski, Rocha, & Terlouw, 2009). The primers for PFKFB3 were designed using Primer 5.0 software. Quantitative real-time PCR was performed using PrimeScript™RT Master Mix (TakaRa, Dalian, China) and the Step One Plus™ real-time PCR system according to a previous publication (Pfaffl, 2001).

2.7. Enzyme activity and cross sectional area (CSA) determination

Approximately 0.5 g of frozen sample was used to measure antioxidative enzyme, glycolytic enzyme and PCAF activity. The activity of LDH was measured as previously described (Kaloustian, Stolzenbach, Everse, & Kaplan, 1969). The activities of the antioxidative enzymes T-SOD, GPx, CAT and T-AOC and the glycolytic enzymes PFKFB3, GAPDH and PGK were measured by using commercial kits (Nanjing Jiancheng Bioengineering company, Jiangsu, China) following the manufacturer's procedures using a spectrophotometer (Mepda Instrument Co., Ltd., Shanghai, China). The enzyme activities of PCAF were determined using an Activity Assay Kit (Sigma, USA). The cross-sectional area (CSA, μm^2) was measured after the HE staining method and image processing software according to Zhang et al. (2015).

Table 2
Primers used for real-time quantitative PCR.

Item	Accession no.	Primer sequence (5′–3′)	Length, bp
MyHCI	AB053226	F:AAGGGCTTGAACGAGGAGTAGA R:TTATTCTGCTTCCTCCAAAGGG	115
MyHCIIa	AB025260	F: GCTGAGCGAGCTGAAATCC R: ACTGAGACACCAGAGCTTCT	137
MyHCIIx	AB025262	F: AGAAGATCAACTGAGTGAACT R: AGAGCTGAGAAACTAACGTG	149
MyHCIIb	AB025261	F: ATGAAGAGGAACCACATTA R: TTATTGCCTCAGTAGCTTG	166
LDH	NM_001113287	F: ACAGTGCTGACACTCTGTGG R: CTGGGAGCCCACATTCACAT	179
PGK1	AY677198	F: GGTGGAATGGCCTTTACCTT R: GCCAATCTTGGCATTCTCAT	186
GAPDH	NM_001206359.1	F: GGAGCGAGATCCCGCCAACA R: ACATGGGGGCATCGGCAGAA	158
PFKFB3	XM_021065031.1	F: CCGCATCGTGTACTACCTGAT R: CGTCTGGATGGTGCTCTTCA	223
18S RNA	AF102857	F:GAGCGAAAGCATTTGCCAAG R:GGCATCGTTTATGGTCGGAAC	101

MyHC = myosin heavy chain; LDH = lactate dehydrogenase; PGK1 = phosphoglycerate kinase 1; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; PFKFB3 = 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase-3.

2.8. Western blotting and immunoprecipitation

The *longissimus dorsi* muscle lysates were homogenized on ice in RIPA lysis buffer (Upstate; Temecula, CA) containing protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO) and phosphathase inhibitor cocktail 1 (Sigma–Aldrich, St. Louis, MO). After centrifugation at 4 °C and 14,000*g*, the supernatants were collected for the assay. The primary antibodies used were: anti-PGK1, anti-GAPDH and anti-PFKFB3 antibodies (1:1000 dilution; Abcam) and anti-acetyl-lysine antibody (1:1000 dilution; Abcam). For Western Blotting, the lysates were then resolved by SDS-PAGE and immunoblotted with the indicated antibodies. Membranes processed with the antibodies of interest were treated with Restore™ Plus Western Blot Stripping Buffer (Pierce, Rockford, IL) for 1 h or overnight and then exposed to anti-β-actin (Sigma–Aldrich, St. Louis, MO) to assess the equal loading.

For the immunoprecipitation (IP), lysate was centrifuged at 12,000*g* for 20 min. Aliquots of protein (1 mg) were incubated with 5 μl of respective antibodies for 3–4 h at 4 °C followed by a 1-h incubation with Protein A Sepharose beads (Upstate; Temecula, CA). Immunocomplexes were washed five times with NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA and 0.5% NP-40) before being resolved by SDS-PAGE and immunoblotted with the indicated antibodies according to the manufacturer's specification (Upstate IP protocol).

To assess the acetylation of PGK1, GAPDH and PFKFB3 in muscle lysate, protein extracts were immunoprecipitated with either anti-PGK1, anti-GAPDH, anti-PFKFB3 antibody or IgG as a control. Immunoprecipitates were separated by SDS-PAGE and immunoblotted using an anti-acetyl-lysine antibody or anti-PGK1, anti-PGK1, anti-GAPDH, anti-PFKFB3 antibody to detect lysine acetylation levels, respectively.

2.9. Statistical analysis

All the results from the experiment were analyzed by using one-way ANOVA, performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, US). Each pen was regarded as the experimental unit and within each pen, four pigs were replicates. The treatments, panelists, and the interaction between them were assigned as fixed terms, and the samples and sessions as random effects. The results in the tables are shown as with the means + standard error of mean (SEM), and other figure results are presented as the means + standard error (SE). Means were considered to be significantly different at $P < 0.05$.

3. Results

3.1. Growth performance

As shown in Table 3, dietary garcinol supplementation increased ADG and decreased F/G compared with the control group ($P < 0.05$), while it had no effect on ADFI. ADG and F/G in 200 mg/kg garcinol were not significantly different compared to the 400 mg/kg garcinol groups, but ADG in the 200 and 400 mg/kg garcinol groups were less than those in the 600 mg/kg group ($P < 0.05$).

3.2. Meat quality and carcass characteristics

As shown in Tables 4 and 5, dietary garcinol had no effect on LA, dressing percentage, body weight and carcass weight or backfat depth of the last rib ($P > 0.05$), while garcinol reduced the backfat depths of

Table 3
Effect of dietary garcinol supplementation on growth performance of finishing pigs.

Item	Con	200	400	600	SEM
ADFI (g/day)	2748.3	2777.6	2753.4	2748.1	1.45
ADG (g/day)	880.2^{c}	902.3^{b}	918.8^{b}	934.4^{a}	1.35
F/G (g/g)	3.12^{a}	3.07^{b}	2.99^{b}	2.94^{b}	0.214

Means with different superscript letters are significantly different ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group; ADFI, average daily feed intake; ADG, average daily gain; and F/G, feed/gain ratio. n = 20.

Table 4
Effect of dietary garcinol supplementation on carcass characteristics of finishing pigs.

Item	Con	200	400	600	SEM
Final weight, kg	119.6	118.1	121.2	119.3	1.45
Carcass weight, kg	90.2	89.6	91.6	90.9	1.35
LA, cm^2	57.4	57.5	58.2	58.7	0.012
Dressing, %	75.4	75.8	75.6	76.1	0.214
Backfat thickness, cm					
First rib	4.48^{a}	3.98^{b}	3.85^{b}	3.79^{c}	0.018
Last rib	2.21	2.24	2.25	2.27	0.237
Last lumbar vertebra	2.78^{a}	2.47^{b}	2.39^{b}	2.24^{c}	0.895
Average backfat	3.16^{a}	2.89^{b}	2.83^{b}	2.76^{c}	1.87

Means with different superscript letters are significantly different ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group; LA = *M. longissimus dorsi* area. n = 20.

Table 5
Effect of dietary garcinol supplementation on meat quality of finishing pigs.

Item	Con	200	400	600	SEM
pH_{45min}	6.56	6.61	6.67	6.69	0.014
pH_{24h}	5.54^{c}	5.61^{b}	5.71^{b}	5.78^{a}	0.018
Color parameters					
L^*_{45min}	45.2	45.9	46.8	47.2	0.123
a^*_{45min}	5.03^{c}	6.27^{b}	6.21^{b}	6.34^{a}	0.521
b^*_{45min}	3.71	3.72	3.76	3.81	0.18
L^*_{24h}	55.4^{a}	53.1^{b}	52.7^{b}	52.5^{b}	0.664
a^*_{24h}	6.30^{b}	7.51^{a}	7.54^{a}	7.62^{a}	0.025
b^*_{24h}	5.63^{b}	5.72^{a}	5.84^{a}	5.89^{a}	0.134
Shear force, kg	4.67^{a}	4.32^{b}	4.21^{b}	4.17^{c}	0.178
Drip loss, %	2.01^{a}	1.68^{b}	1.54^{b}	1.49^{c}	0.034
Cook loss, %	33.8	32.8	32.3	32.2	0.046

Means with different superscript letters are significantly different ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group. n = 20.

first rib and last lumbar vertebra as well as the average backfat depths ($P < 0.05$). Additionally, dietary garcinol increased pH_{24h} and a*, b^*_{24h} ($P < 0.05$) and decreased L^*_{24h}, drip loss, and shear force ($P < 0.05$) (Table 5), while the garcinol group showed no difference on pH_{45min}, L^*_{45min}, b^*_{45min}, or cook loss compared to the control group. There were no differences in carcass and meat quality parameters between the 200 and 400 mg/kg garcinol groups, but the values of these groups were less than those of the 600 mg/kg group ($P < 0.05$).

3.3. Muscle glycolytic potential, chemical composition, LDH activity and mRNA level

There was no effect ($P > 0.05$) of garcinol on the content of glycogen, moisture, crude protein, IMF and LDH mRNA, but garcinol supplementation increased ($P < 0.05$) myoglobin content and significantly decreased the content of muscle glucose + glucose-6-P, lactate and glycolytic potential ($P < 0.05$) (Table 6), as well as LDH activity (Fig. 1). The muscle myoglobin values in groups of 200 and 400 mg/kg garcinol were lower than those of the 600 mg/kg group ($P < 0.05$), and the glucose + glucose-6-P, glycolytic potential and lactate values in groups of 200 and 400 mg/kg garcinol were higher than those of the 600 mg/kg group ($P < 0.05$) (Table 6).

Table 6
Effect of dietary resveratrol supplementation on *M. longissimus dorsi* glycolytic potential and chemical composition of finishing pigs.

Item	Con	200	400	600	SEM
Moisture, %	70.1	71.4	70.5	71.0	0.21
Crude protein, %	21.3	21.6	21.8	22.3	1.92
IMF, %	3.68	3.25	3.21	3.17	0.41
Myoglobin, mg/g	1.64^{c}	2.04^{b}	2.11^{b}	2.20^{a}	0.327
Glycogen, μmol/g	8.87	8.81	8.72	8.64	0.241
Glucose + glucose-6-P, μmol/g	5.31^{a}	4.15^{b}	4.10^{b}	4.07^{c}	0.052
Glycolytic potential, μmol/g	92.4^{a}	88.2^{b}	84.5^{b}	81.7^{c}	1.48
Lactate, μmol/g	76.2^{a}	64.2^{b}	60.1^{b}	54.2^{c}	0.124

Means with different superscript letters are significantly different ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group; IMF = intramuscular fat content. n = 20.

3.4. Acetylation, mRNA and activity levels of glycolytic enzyme

Dietary garcinol supplementation decreased the activity of PCAF at 200, 400 and 600 mg/kg ($P < 0.05$). In addition, dietary garcinol supplementation decreased the acetylation levels of the glycolytic enzymes PGK1, GAPDH, and PFKFB3 (Fig. 2). Moreover, dietary garcinol supplementation decreased ($P < 0.05$) the activities of PGK1, GAPDH, PFKFB3, and LDH (Fig. 2); however, there were no differences in PGK1, GAPDH and PFKFB3 mRNA levels among the groups ($P > 0.05$).

3.5. MyHC mRNA and myofiber cross-sectional area

There were no differences between the groups on muscle MyHCI, MyHCIIx, and MyHCIIb mRNA levels and myofiber cross-sectional area ($P > 0.05$). However, dietary garcinol supplementation increased ($P < 0.05$) MyHCIIa mRNA levels compared to the control group (Fig. 3), and no difference was observed for MyHCIIa mRNA between the 200 and 400 mg/kg garcinol groups, but the values obtained for these groups were lower than those of the 600 mg/kg group ($P < 0.05$).

3.6. Muscle antioxidative enzyme activities

Garcinol increased ($P < 0.05$) T-AOC, CAT and GPx enzyme activity (Table 7) and decreased T-SOD activity and MDA content (Table 7). Also, no differences in MDA content were observed among the three groups (200, 400, and 600 mg/kg). No differences were observed in the antioxidative enzyme activity between the 200 and 400 mg/kg groups (Table 7), but these differences were significantly different from those of the 600 mg/kg group.

4. Discussion

Garcinol was first isolated from *Garcinia indica* (*G. indica*, also known as kokum) in the Western Ghats of India in the 1980s (Krishnamurthy, Lewis, & Ravindranath, 1981). Numerous studies have revealed the effect of garcinol on antioxidant, anti-inflammatory (Krishnamurthy et al., 1981; Panda, Ashar, & Srinath, 2012), anti-glycation (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000), and anti-obesity (Lee, Teng, Kalyanam, Ho, & Pan, 2019). Due to these effects, garcinol may be useful for improving the growth performance and meat quality of finishing pigs.

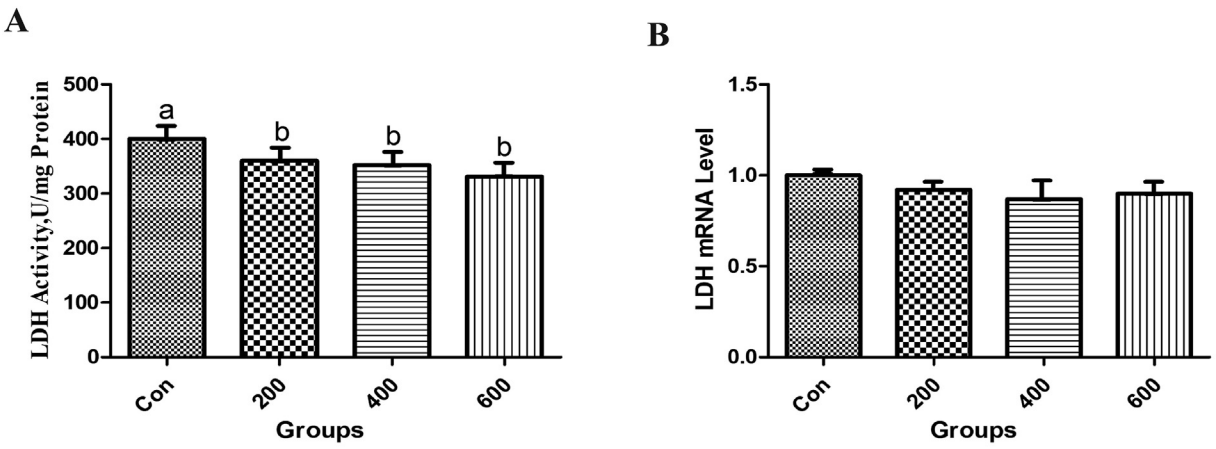

Fig. 1. Effect of dietary garcinol supplementation on lactate dehydrogenase (LDH) mRNA level (A) and enzyme activity (B) in *M. longissimus dorsi* of finishing pigs. Values with no common superscripts means significant difference ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group, n = 20.

The study in mice to evaluate the safety profile of garcinol showed that garcinol and its metabolites can be detected in almost all organs including muscle and liver. This result suggested that garcinol could be absorbed and metabolized in many organs (Majeed et al., 2018). According to their research, garcinol did not show any adverse effects at a high single dose of 2000 mg/kg in an acute safety study of Wistar rats, at a highest dose of 100 mg/kg/d for 28 d in a repeated dose oral toxicity study. A 90-d repeated dose oral toxicity and reproductive/developmental toxicity study including a histopathological examination showed that there were no treatment-related changes in growth performance, feed intake, and hematological and biochemical variables induced by garcinol, all indicating that garcinol is not harmful, even at very high dosages (Majeed et al., 2018). So far, to the best of our knowledge, there are no relevant studies about garcinol treatment on pigs. The present study did not find any adverse effects of dietary garcinol on the growth of pigs, this is consistent with the mouse model study.

The results of this study showed that the growth performance of finishing pigs was improved by dietary garcinol. This improvement may be due to the effect of garcinol on gut health and the microenvironment. Previous studies in mice showed that garcinol has a bactericidal effect and changes the gut microbiota composition (Lee et al., 2019). Although the study was conducted on mice, it is still speculative that garcinol may improve the gut microenvironment and intestinal absorption and utilization of nutrients in pigs. For the meat industry, superabundant adipose tissue of carcass lipids at market weight may result in feed waste and reduce consumer acceptability for pork. Recently, garcinol has been shown to reduce the adipogenesis *in vitro* or modulating gut microbiota composition *in vivo* (Hsu, Lin, Ho, & Yen, 2012; Lee et al., 2019). The present study found that dietary garcinol significantly decreased the backfat thickness of pigs, which suggests that garcinol may improve carcass characteristics by reducing subcutaneous fat deposition. In addition, the results of this study on meat quality parameters showed that the a*, pH_{24h}, and contents of myoglobin in LM were improved by dietary garcinol supplementation, while the shear force, L^*_{24h}, and drip loss of meat were reduced. Previous studies have shown that there is a positive correlation between LM drip loss and lactate content (Klont et al., 2001; Lebret et al., 2006). In addition, increased ultimate pH has a positive impact on the tenderness of meat (Huff-Lonergan, Lonergan, & Vaske, 2000). The presented results showed that dietary garcinol decreased lactate content and increased pH_{24h} in muscles, which may partly account for the effect of dietary garcinol supplementation on meat quality traits.

It is also suggested that energy metabolic substrate in the muscle can affect many aspects of meat quality (Lebret et al., 2006). Moreover, energy metabolism-related enzymes may be related to muscle characteristics and meat quality, such as LDH. On one hand, it is often used as a muscle anaerobic glycolytic index to determine the metabolic muscle type. On the other hand, to some extent, it reflects the lactate production in the muscle, as muscle lactate accumulation depends on the competition between the cytosolic enzyme LDH and the mitochondria for the pyruvate derived from glycolysis (Guo et al., 2011). In addition, it is believed that high ultimate pH is closely related to a decrease in muscle glycolytic potential slaughter, this can result in greater water-holding capacity of meat. On the contrary low ultimate pH is produced by a net increase in glycolytic flux (Lefaucheur et al., 2011). Therefore, this study next tested the effect of dietary garcinol on the glycolytic potential or muscle postmortem glycolysis of finishing pigs. It is well-known that under anaerobic conditions, the increase of glycolytic flux causes significant lactate and H^+ accumulation, and there is a negative correlation between muscle ultimate pH and net lactate accumulation ($R^2 = 0.59$) especially if plotted over the entire postmortem period (Matarneh, England, Scheffler, Oliver, & Gerrard, 2015). The results of this study showed that dietary garcinol decreased lactate and muscle glycolytic potential, decreased the activity of LDH, but had no effect on the LDH mRNA level. These results show that dietary garcinol decreases glucose utilization and lactate production in muscles, which could be the main reason for the improvement in ultimate pH and postmortem meat quality. In addition, previous research showed that energy metabolism-related enzymes, such as LDH, and the glycolytic enzymes, such as PGK1 and GAPDH, may affect meat quality, as they are directly related to postmortem glycolysis (Kastenschmidt, Hoekstra, & Briskey, 1968). The results of the present study show that dietary garcinol supplementation decreases the activity of the glycolytic enzymes PGK1, PFKFB3 and GAPDH, as well as their acetylation levels, but has no effect on their mRNA levels. This finding suggests that garcinol would not affect postmortem glycolysis by changing the expression of these enzymes but affect their activity. For decades, epigenetic research has shown that lysine acetylation can affect cellular processes, including glycolysis, by regulating enzyme activity. In addition, it has been shown in different animal models that PCAF can serve as an activator of the glycolytic pathway and can increase glucose utilization by directly acetylating PGK1, PFKFB3 and GAPDH (Wang et al., 2018; Li et al., 2018). In such cases, it could be postulated that PCAF may be a main regulator of postmortem glycolysis. In this study, dietary garcinol supplementation reduced PCAF activity, which is consistent with previous research, as it is a natural inhibitor of PCAF (de Jong et al., 2017). Moreover, increasing dietary garcinol supplementation decreased the acetylation level of the glycolytic enzymes PGK1, PFKFB3 and GAPDH, which indicates that a possible mechanism

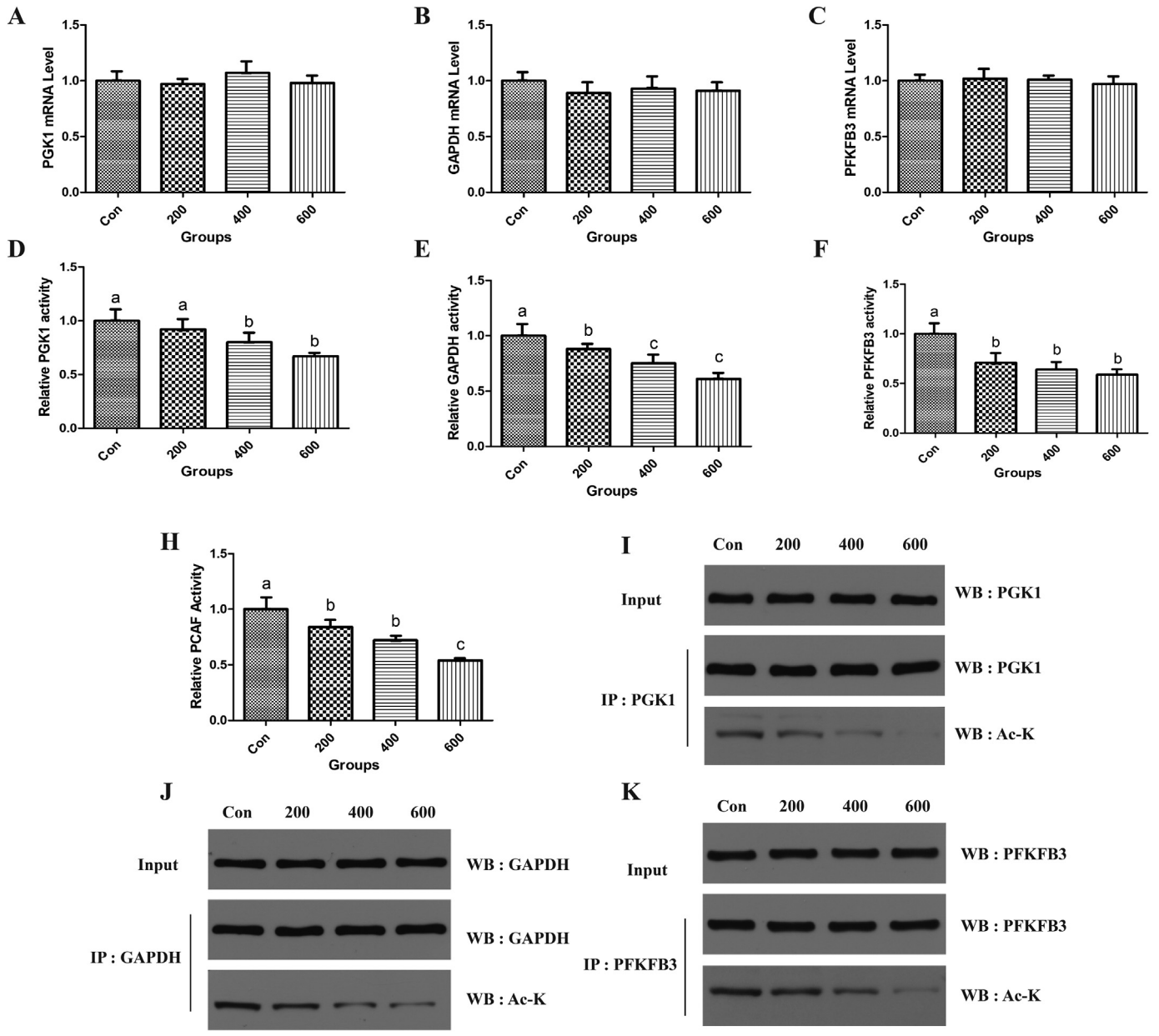

Fig. 2. Effect of dietary garcinol supplementation on acetylation, mRNA and activity levels of glycolytic enzyme of finishing pigs. (A–C) glycolytic enzyme mRNA levels; (D–F) glycolytic enzyme activity levels; (H) PCAF activity; (I–K) glycolytic enzyme acetylation levels. Values with no common superscripts means significant difference ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group, n = 20.

for decreasing postmortem glycolysis may involve in the inhibition of PCAF.

In addition, muscle fiber characteristics were also detected in this study. Slow oxidative type I, fast oxidative type IIA, and fast glycolytic types IIX and IIB are the only four fiber types in adult pig skeletal muscle (Schiaffino & Reggiani, 1996). These fiber types are expressed by myosin heavy chain (MyHC) isoform genes I, IIa, IIx, and IIb, respectively (Lefaucheur, Ecolan, Plantard, & Gueguen, 2002). Previous studies indicated that pork with a low percentage of type IIA and IIB fibers had high L* and redness (a*), respectively, and pork containing high L* and low a* and has a high value in drip loss. Therefore, changes in muscle fiber characteristics result in changes in meat quality. As type IIA belongs to oxidative/fast muscle fiber and type IIB belongs to the glycolytic/fast fiber (Ryu & Kim, 2005), in the present study, the results showed that dietary supplementation with garcinol had no effect on the muscle fiber characteristics but increased the MyHCIIa mRNA level, suggesting that dietary garcinol supplementation may increase the oxidative potential or decrease the glycolytic potential. This finding is consistent with the results of this study regarding glycolytic potential. In addition, as the rank order of myoglobin content in muscle fibers is I > IIA > IIX > IIB (Lefaucheur, 2010), the increase of MyHCIIa mRNA level may also help to explain the role of dietary garcinol in increasing the content of myoglobin.

It is well-known that natural dietary antioxidants can improve meat quality by improving the antioxidative status of finishing pigs (Jiang et al., 2009). For example, resveratrol (Zhang et al., 2015), selenium (Zhan et al., 2007) and arginine (Ma et al., 2010) in pig diets can improve the antioxidative status and scavenge reactive oxygen species (ROS), thereby improving the growth performance and meat quality of finishing pigs. Previous studies *in vivo* indicated that garcinol can eliminate free radicals and shows protective antioxidative effects (Yamaguchi, Saito, Ariga, Yoshimura, & Nakazawa, 2000). It was also reported that dietary garcinol supplementation decreased blood MDA content and ROS in mouse liver, while increasing GPx activity (Jing

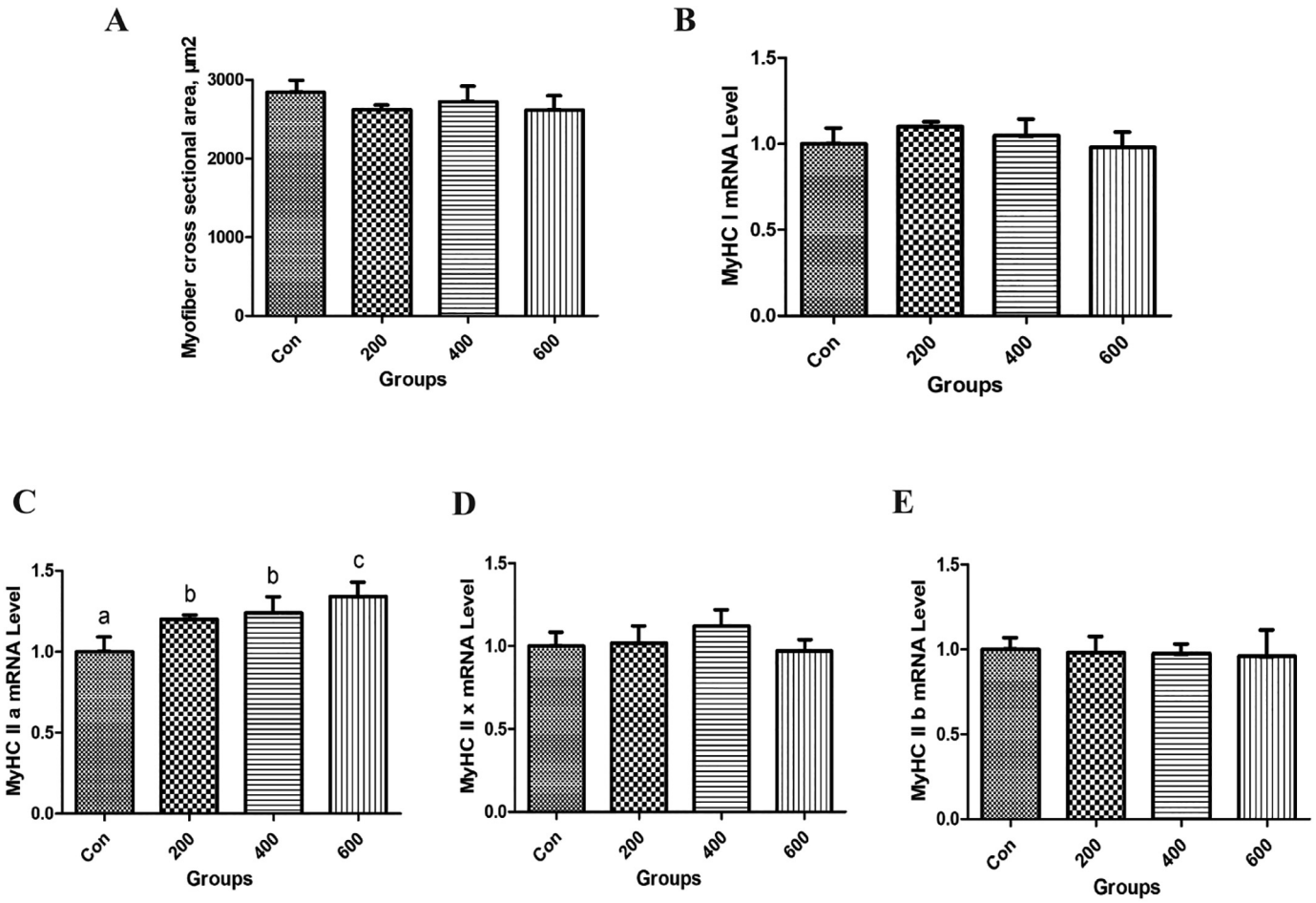

Fig. 3. Effect of dietary garcinol supplementation on MyHC isoform gene mRNA level and myofiber cross-sectional area of finishing pigs. (A) myofiber cross-sectional area; (B–E) MyHC isoform gene. Values with no common superscripts means significant difference ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group; 600 = 600 mg/kg garcinol group, MyHC = myosinheavy chain, n = 20.

Table 7
Effect of dietary garcinol supplementation on *M. longissimus dors*i antioxidative enzyme activities and MDA content of finishing pigs.

Item	Con	200	400	600	SEM
MDA, nmol/mg protein	0.20^{a}	0.15^{b}	0.14^{b}	0.11^{b}	0.0015
GPx, U/mg protein	62.4^{c}	68.5^{b}	71.3^{b}	72.8^{a}	4.12
CAT, U/mg protein	110.2^{c}	115.2^{b}	117.6^{b}	121.4^{a}	2.78
T-AOC, U/mg protein	1.28^{c}	1.72^{b}	1.89^{b}	1.94^{a}	0.054
T-SOD, U/mg protein	19.6^{a}	17.2^{b}	17.0^{b}	16.5^{c}	0.124

Means with different superscript letters are significantly different ($P < 0.05$). Con = control group; 200 = 200 mg/kg garcinol group; 400 = 400 mg/kg garcinol group;600 = 600 mg/kg garcinol group. MDA = malonaldehyde; GPx = glutathione peroxidase; CAT = catalase; T-AOC = total antioxidative capacity; T-SOD = total superoxide dismutase. n = 20.

et al., 2014). Consistent with previous studies, in this study, it was found that dietary garcinol supplementation can beneficially increase antioxidative enzyme activity in muscle, indicating an improved antioxidative capacity. Moreover, dietary garcinol supplementation decreased the MDA content, which is the secondary product of lipid oxidation, suggesting a decrease in lipid peroxidation. It was speculated that the improved pork quality under garcinol treatment in this study may be partly attributable to the improved antioxidative status. Oxidative deterioration of muscle can lead to the production of hydroperoxides and other oxygenated compounds that may adversely affect the overall quality of products by causing loss of color and nutritive value. The present study shows that the oxidative stability of muscle was improved. This finding may be due to the antioxidant function and ROS scavenging properties of garcinol.

Nevertheless, this study has several limitations. Due to the artificial limitation of dietary garcinol supplementation level, it was difficult to determine the best dosages and feeding length of garcinol, as many antioxidative additive levels show quadratic interaction with meat quality and pig performance. A previous study indicated that garcinol did not show any adverse effects at a high single dose of 2000 mg/kg in an acute safety study of Wistar rats (Majeed et al., 2018). In the present study, it was observed that dietary supplementation with 600 mg/kg garcinol had a better effect on meat quality than the 200 and 400 mg/kg garcinol groups. It is possible that increasing the garcinol supplementation level (*e.g.* 800 or 1000 mg/kg) may further increase the beneficial effects. In addition, the mechanism of meat quality in response to garcinol treatment is superficial. Further research should focus on solving these problems.

5. Conclusions

The present study suggest that garcinol supplementation in pigs diet can improve the growth performance and meat quality of finishing pigs. In addition, this study provides the first evidence in pigs that garcinol increases antioxidative capacity without affecting muscle fiber characteristics parameters, while decreasing postmortem glycolysis; this evidence may be valuable for revealing the mechanisms of garcinol treatment on improving growth performance of pigs and pork quality.

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Ethical statement

The animal studies were approved by the Animal Care and Use Committee of College of Animal Sciences and Technology, Huazhong Agricultural University and performed in accordance with relevant regulations and guidelines.

Declaration of competing interest

The authors declare no conflict of interest.

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