Dietary chlorogenic acid improves growth performance of weaned pigs through maintaining antioxidant capacity and intestinal digestion and absorption function

Jiali Chen,^{*} Yan Li,[†] Bing Yu,^{*} Daiwen Chen,^{*} Xiangbing Mao,^{*} Ping Zheng,^{*} Junqiu Luo,^{*} and Jun He^{*,1}

^{*}Institute of Animal Nutrition, Sichuan Agricultural University, and Key Laboratory of Animal Disease-Resistance Nutrition Ministry of Education, Chengdu, Sichuan 611130, People's Republic of China; [†]Sichuan Jun Zheng Bio-Feed Co., Ltd, Chengdu, Sichuan 611730, People's Republic of China

ABSTRACT: Chlorogenic acid (CGA) is a natural phenolic acid, which is an important component of biologically active dietary phenols isolated from various species. Two experiments were conducted to investigate the effects of CGA on growth performance, antioxidant capacity, nutrient digestibility, diarrhea incidence, intestinal digestion and absorption function, and the expression levels of intestinal digestion and absorption-related genes in weaned pigs. In Exp. 1, 200 weaned pigs were randomly allotted to four dietary treatments and fed with a basal diet or a basal diet supplemented with 250, 500, or 1,000 mg/kg CGA, respectively, in a 14-d trial. Pigs on the 1,000 mg/kg CGA-supplemented group had greater ($P < 0.05$) G:F compared with those on the control (CON) group. In Exp. 2, 24 weaned pigs were randomly allotted to two groups and fed with a basal diet (CON group) or a basal diet supplemented with 1,000 mg/kg CGA (the optimum does from Exp. 1; CGA group). After a 14-d trial, 8 pigs per treatment were randomly selected to collect serum and intestinal samples. Compared with the CON group, the ADG, G:F, as well as the apparent total tract digestibility of CP, crude

fat, and ash were increased ($P < 0.05$), whereas the diarrhea incidence was decreased ($P < 0.05$) in the CGA group. Pigs on the CGA group had greater ($P < 0.05$) serum albumin and IGF-1, and lower ($P < 0.05$) serum urea nitrogen than pigs on the CON group. Furthermore, dietary CGA supplementation enhanced ($P < 0.05$) the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in the serum, the activity of maltase in the jejunum and ileum, as well as the activities of sucrase and alkaline phosphatase (AKP) in the jejunum. The mRNA levels of sodium glucose transport protein-1 (SGLT1) and zinc transporter-1 (ZNT1) in the duodenum and the mRNA levels of SGLT1, glucose transporter-2 (GLUT2), and divalent metal transporter-1 (DMT1) in the jejunum were upregulated ($P < 0.05$) in pigs fed the CGA diet. These results suggested that dietary CGA supplementation has the potentials to improve the growth performance and decrease the diarrhea incidence of the weaned pigs, possibly through improving the antioxidant capacity and enhancing the intestinal digestion and absorption function.

Key words: antioxidant capacity, chlorogenic acid, growth performance, intestinal digestion and absorption function, nutrient digestibility, weaned pigs

© The Author(s) 2018. Published by Oxford University Press on behalf of American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2018.96:1108–1118

doi: 10.1093/jas/skx078

INTRODUCTION

Pigs often suffer from nutritional, physiological, and social stresses during the weaning process (Kim et al., 2012). Those stressful events

¹Corresponding author: hejun8067@163.com

Received 25 August 2017.

Accepted March 6, 2018.

usually weaken the digestion and absorption of nutrients and induce oxidative stress in pigs, which are responsible for the growth retardation and diarrhea observed in the first 2 wk after weaning, and subsequently induce great economic loss to the live-stock production (Klues et al., 2010; Zhu et al., 2012). Thus, how to alleviate the negative effects of weaning stress has becoming a major concern for the pig industry. Numerous studies indicated that dietary supplementation with certain nutrients (e.g., glutamine, benzoic acid, and uridine monophosphate) can protect against the detrimental impacts of weaning stress in weaned pigs (Chen et al., 2017; He et al., 2016; Li et al., 2016).

Chlorogenic acid (CGA), a phenolic compound, is found in various species, including coffee, fruits, and vegetables (Bouayed et al., 2007). The beneficial biological activities of CGA are wide-ranging, including anti-oxidation (Ji et al., 2013), anti-inflammation (Ma et al., 2015), antitumor (Rakshit et al., 2010), and antimicrobial (Lou et al., 2011). Collectively, these properties seem to impel CGA to serve as an effective dietary supplement for maintaining the health of animals. Nowadays, CGA has been used as a feed supplement to enhance the antioxidant ability in weaned pigs and sows (Liu et al., 2009; Huang et al., 2015), and to improve the growth performance in weaned rats (Ruan et al., 2014a). However, to the best of our knowledge, the underlying mechanisms responsible for the growth-promoting effect of CGA have not been completely elucidated, especially weaned pigs. Thus, it is speculated that CGA can enhance the growth performance of weaned pigs by improving the antioxidant capacity and enhancing the intestinal digestion and absorption function. This study was conducted to assess this hypothesis.

MATERIALS AND METHODS

Experimental procedures used in this study were approved by the Animal Care and Use Committee of Sichuan Agricultural University.

Experiment 1

Animals and experimental design. Two hundred pigs (Duroc × Landrace × Yorkshire), with an initial average BW of 7.65 ± 0.35 kg and weaned at 24 ± 1 d, were used in a 14-d experiment. At the beginning of the experiment, pigs were randomly allotted to four dietary treatments with 5 replicate pens per treatment and 10 pigs per pen in a randomized complete block design according to initial

BW, sex, and litter. The treatments consisted of basal diet and basal diet supplemented with 250, 500, or 1,000 mg CGA (provided by China Animal Husbandry Industry Co., Ltd, Beijing, China) per kg of feed.

Diets and feeding management. The basal diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (2012), and the ingredient composition and nutrient levels are shown in Table 1. CGA was added to the basal diet at the expense of corn. The pigs were exposed to natural lighting and had free access to water and feed throughout the two experiments. All pigs were housed in a temperature-controlled nursery house, which had fully slatted floors. Pigs had ad libitum access to feed and water through a 1-sided feeder and a stainless steel nipple drinker provided in each pen (1.8×2.5 m). The room temperature was set at 28 ± 1 °C for the first week and gradually decreased to 25 °C by the end of the trial.

Table 1. Ingredients composition and nutrient levels of basal diets (as-fed basis) (Exp. 1 and Exp. 2)

Ingredient	%	Nutrient concentrations ^a	
			%
Corn	28.00	CP	20.36
Extruded corn	28.00	ME/MJ × kg ⁻¹	14.83
Soybean meal	10.00	Ca	0.82
Extruded soybean	7.00	Total P	0.61
Fish meal	5.00	STTD P	0.40
Whey powder	7.00	Lys	1.37
Soybean protein concentrate	8.00	Met	0.45
Soybean oil	2.16	Met + Cys	0.74
Sucrose	2.50	Thr	0.81
Limestone	0.70	Trp	0.21
Dicalcium phosphate	0.45		
Salt	0.30		
L-Lysine HCl	0.28		
DL-Methionine	0.12		
L-Threonine	0.04		
Choline chloride	0.10		
Vitamin premix ^b	0.05		
Mineral premix ^c	0.30		

STTD P = standardized total tract digestible phosphorus.

^aValues were calculated.

^bThe premix provides following per kilogram of diet: Vitamin A: 6,000 IU; Vitamin D₃: 400 IU; Vitamin E: 10 IU; Vitamin K₃: 2 mg; Vitamin B₁: 0.8 mg; Vitamin B₂: 6.4 mg; Vitamin B₆: 2.4 mg; Vitamin B₁₂: 12 µg; folic acid: 0.2 mg; nicotinic acid: 14 mg; D-pantothenic acid: 10 mg.

^cThe premix provides following per kilogram of diet: Fe (as ferrous sulfate): 130 mg; Cu (as copper sulfate): 80 mg; Mn (as manganese sulfate): 60 mg; Zn (zinc sulfate): 120 mg; I (potassium iodide): 0.3 mg; Se (as sodium selenite): 0.35 mg.

Growth performance. The BW of pigs was individually measured after 12-h fasting at the morning of day 1 and day 15 of the feeding trial, and feed intake per pen was collected daily throughout the trial to calculate ADFI, ADG, and G:F.

Experiment 2

Animals and experimental design. Twenty-four pigs (Duroc $\times$ Landrace $\times$ Yorkshire), with an initial average BW of 6.83 ± 0.10 kg and weaned at 24 d, were randomly assigned into two treatments ($n = 12$), consisting of the basal diet (control, CON) or the basal diet supplemented with 1,000 mg/kg CGA. The level of CGA used in Exp. 2 was based on the results of Exp. 1, which showed that 1,000 mg/kg CGA resulted in greater G:F ($P < 0.05$) and ADG than other treatment groups. The experiment lasted for 14 d.

Diets and feeding management. The basal diet was the same as described for Exp. 1. All pigs were obtained from the same farm as in Exp. 1, and housed in individual metabolism cages (0.7×1.5 m) with room temperature maintained at 25–28 °C, and relative humidity controlled at 55–65%. All feeding and experimental procedures used were similar to those previously described for Exp. 1.

Growth performance and diarrhea incidence. The BW of pigs was measured immediately after 12-h fasting at the morning of day 1 and day 15 of the feeding trial, and feed intake of each pig was collected daily throughout the trial to calculate ADFI, ADG, and G:F. The pigs' stool was observed at the same time of each morning by two observers blinded to treatments in the entire experimental period based on the method described by Song et al. (2012). Fresh excreta of each pig was assessed visually with a score from 1 to 5 (1 = normal feces, 2 = moist feces, 3 = mild diarrhea, 4 = severe diarrhea, and 5 = watery diarrhea). The occurrence of diarrhea was defined as production of feces at level 3, 4, or 5. Diarrhea frequency was then calculated as follows: diarrhea frequency (%) = $(A/14 \text{ d}) \times 100$, in which A = total days per pig with diarrhea.

Sample collection and preparation. At the beginning of the trial, representative feed samples of each group were sampled and stored at -20 °C for chemical analysis. From day 11 to day 14 of the experiment, fresh fecal samples were collected immediately after excretion from eight randomly selected pigs in each group. After collection, the daily excreta of each pig was weighted, and 10 mL of a 10% H_2SO_4 solution was added to each 100 g of wet fecal sample, and subsequently stored in a sealed

plastic bag at -20 °C. At the end of the 4-d period, all fecal samples of each pig were thawed at room temperature and mixed thoroughly, and then dried at 65 °C for 48 h, after which they were ground to pass through a 1-mm screen and stored at -20 °C for chemical analyses.

In the morning of day 15, after 12-h fasting, eight pigs with the average BW of each group were randomly selected and blood samples were collected from the anterior vena cava into vacuum tubes without anticoagulant. Blood samples were centrifuged at $3,000 \times g$ for 15 min at 4 °C, and subsequently serum was separated and stored at -20 °C for further analysis. The same 16 pigs were then euthanized by intravenous injection of chlorpromazine hydrochloride (3 mg/kg BW). The abdomen was immediately opened and the small intestine was ligatured into duodenum, jejunum, and ileum according to the anatomical structure characteristic. Then each segment of small intestine was opened longitudinally and washed with cold saline solution. The mucosal samples from the middle of duodenum, jejunum, and ileum were subsequently scraped by glass microscope slides, snap-frozen in liquid nitrogen, and stored at -80 °C for further analysis.

Chemical analyses. Chemical analysis of diet and fecal samples was carried out as follows. DM (method 930.15), CP (method 990.03), crude fat (method 945.16), and ash (method 942.05) were measured according to the procedures described by AOAC (1995). GE was determined using an automatic adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The apparent total tract digestibility (ATTD) of DM, CP, crude fat, ash, and GE was measured using AIA as an endogenous indicator. The AIA in diet and fecal samples was measured as described by the Standards Press of China (2009). The ATTD was calculated using the following formula: $\text{ATTD (\%)} = \{1 - [(A1 \times F2)/(A2 \times F1)]\} \times 100$, in which A1 = the AIA content of the diet, A2 = the AIA content of feces, F1 = the nutrient content of the diet, and F2 = the nutrient content of feces.

Serum sample analysis. Serum albumin (ALB), total protein (TP), and urea nitrogen (UN) were measured using an automatic biochemistry analyzer (Biochemical Analytical Instrument, Beckman CX4, Beckman Coulter Inc., Brea, CA). The levels of serum GH and IGF-1 were determined by the commercial porcine-specific ELISA kits (Beijing Winter Song Boye Biotechnology Co. Ltd, Beijing, China) according to the manufacturer's instructions. The minimum detectable levels were 0.1 and 1.0 ng/

mL for GH and IGF-1, respectively. Serum antioxidant parameters including total antioxidant capacity (**T-AOC**), superoxide dismutase (**SOD**), malondialdehyde (**MDA**), catalase (**CAT**), and glutathione peroxidase (**GSH-Px**) were measured by the commercial kits (Nanjing Jiancheng Institute of Bioengineering, Jiangsu, China) with UV-VIS Spectrophotometer (UV1100, MAPADA, Shanghai, China) according to the manufacturer's instructions.

Digestive and absorptive enzyme activities. For enzyme activity measurement, about 1 g frozen mucosa sample of small intestine was homogenized in ice-cold saline solution (1:9, wt/vol) and then centrifuged at $2,500 \times g$ for 10 min at 4 °C. The supernatant was collected for further analysis. The activities of lactase, sucrose, maltase, and alkaline phosphatase (**AKP**) in each section of small intestine were measured by commercial kits (Nanjing Jiancheng Institute of Bioengineering, Jiangsu, China) according to the manufacturer's instructions. The TP content of the intestinal samples was determined using the Bradford brilliant blue method. All samples were measured in duplicate. Enzyme activities are presented as units (U) per milligram of protein.

RNA extraction and gene expression analysis. Total RNA from duodenum, jejunum, and ileum mucosa was isolated using the Trizol reagent (TaKaTa, Dalian, China) according to the manufacturer's instructions. The concentration and purity of RNA were measured by a spectrophotometer (Beckman Coulter DU800; Beckman Coulter Inc.) at 260 and 280 nm. Ratios of absorption (OD260/OD280 nm) ranged from 1.8 to 2.0 for all samples. The integrity of RNA was detected by formaldehyde gel electrophoresis, and the 28S:18S ribosomal RNA

band ratio was determined as ≥ 1.8 . For each sample, reverse transcription was performed using PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) according to the manufacturer's instructions. All primers were synthesized commercially by Sangon Biotech Limited and are shown in Table 2.

Quantitative real-time PCR was performed to analyze the expression levels of sodium glucose transport protein-1 (**SGLT1**), glucose transporter-2 (**GLUT2**), zinc transporter-1 (**ZNT1**), divalent metal transporter-1 (**DMT1**), and solute carrier family 7 (**SLC_{7A1}**) in duodenum, jejunum, and ileum mucosa using SYBR Premix Ex Taq II (Tli RnaseH Plus) reagents (TaKaRa, Dalian, China) and the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Richmond, CA). Each reaction was performed in a volume of 10 μ L with 5 μ L SYBR Premix Ex Taq (2 $\times$), 1 μ L of each primers, 2 μ L of ddH₂O, and 1 μ L of cDNA. The PCR conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 25 s, and a 72 °C extension step for 5 min. A melting curve analysis was performed after each real-time quantitative PCR assay to verify the specificity of the reactions. The GAPDH gene was chosen as the reference gene to normalize mRNA expression of target genes. The relative expression ratio of target genes relative to the reference gene was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Each sample was repeated in triplicate.

Statistical Analysis

The experimental design of Exp. 1 was a completely randomized block design based on initial

Table 2. Primers used for real-time quantitative PCR^a (Exp. 2)

Gene	Accession no.	Primer sequences ^b (5'–3')	Size, bp
SGLT1	NM_001164021.1	F: GCAACAGCAAAGAGGAGCGTAT R: GCCACAAAACAGGTCATAGGTC	137
GLUT2	NM_001097417.1	F: GACACGTTTTGGGTGTTCCG R: GAGGCTAGCAGATGCCGTAG	149
DMT1	NM_001128440.1	F: GCAGGTGGTTGACGTCTGTA R: CACGCCCCCTTTGTAGATGT	100
ZnT1	NM_001139470.1	F: TGCTCTGCATGCTGTTACTGA R: TGGAAGGAGTCCGAGAGCAT	97
SLC _{7A1}	NM_001012613.1	F: TCTTTGCAGGTCGTTTGGGA R: GGCTGATCACCTGTTGGAGT	137
GAPDH	NM_001206359.1	F: TCGGAGTGAACGGATTTGGC R: TGCCGTGGGTGGAATCATAC	147

^aSGLT1 = sodium glucose transport protein-1; GLUT2 = glucose transporter-2; DMT1 = divalent metal transporter-1; ZNT1 = zinc transporter-1; SLC_{7A1} = solute carrier family 7; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

^bF = forward; R = reverse.

BW, sex, and litter. Data on growth performance in Exp. 1 were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC), with pen as the experimental unit. Statistical differences among groups were determined by Duncan's multiple-range test. For all other indexes (Exp. 2), data were analyzed by *T*-test using the statistical program SAS (SAS Institute Inc., Cary, NC), with the individual pig as the experimental unit. The results of both experiments were expressed as mean and SEM. Statistical significance and a tendency toward difference were considered as $P < 0.05$ and $P < 0.10$, respectively.

RESULTS

Growth Performance (Exp. 1)

The effects of graded levels of CGA on growth performance in pigs are presented in Table 3. There were no significant differences in ADFI and ADG between the pigs fed the CON diet and the CGA diets. However, dietary supplementation with 1,000 mg/kg CGA increased ($P < 0.05$) the G:F of pigs compared with those on the CON group. No significant difference in G:F was observed among the pigs fed the 250 mg/kg CGA-supplemented diet, 500 mg/kg CGA-supplemented diet, and the CON diet.

Growth Performance and Diarrhea Index (Exp. 2)

The results of growth performance and diarrhea index are presented in Table 4. The ADG and G:F of pigs on the CGA group were greater ($P < 0.05$) than those on the CON group. However, no significant difference in ADFI was observed between pigs on the CGA group and the CON group. In addition, lower ($P < 0.05$) diarrhea incidence was observed in pigs fed the CGA-supplemented diet compared with those on the CON diet.

Table 3. Effects of CGA on growth performance in weaned pigs^a (Exp. 1)

Item	Dietary CGA, mg/kg				SEM	P-value
	CON	250	500	1,000		
Initial BW, kg	7.68	7.66	7.64	7.64	0.08	0.989
ADFI, g	337	337	327	350	11	0.567
ADG, g	218	227	218	257	13	0.126
G:F	0.65 ^b	0.67 ^{ab}	0.67 ^{ab}	0.73 ^a	0.02	0.038

^{a,b}Means in the same row with different letter differ ($P < 0.05$). $n = 5$.

^aCON = pigs in the CON group were fed a basal diet.

Table 4. Effects of CGA on growth performance and diarrhea incidence in weaned pigs^a (Exp. 2)

Item	Dietary treatment		SEM	P-value
	CON	CGA		
Initial BW, kg	6.83	6.84	0.06	0.928
ADFI, g	375.39	391.40	24.51	0.649
ADG, g	228.76	273.51	15.41	0.043
G:F	0.60	0.71	0.03	0.002
Diarrhea incidence, %	12.50	5.36	1.56	0.043

^a $n = 12$. CON = pigs in the CON group were fed a basal diet; CGA = pigs in CGA group were fed a basal diet supplemented with 1,000 mg/kg of CGA.

Nutrient Digestibility (Exp. 2)

The results of nutrient digestibility analysis are presented in Table 5. There were no significant differences in the ATTD of DM and GE between pigs on the CGA group and the CON group. However, CGA supplementation increased ($P < 0.05$) the ATTD of CP, crude fat, and ash compared with the CON group.

Serum Biochemical Parameters and Hormone Levels (Exp. 2)

The levels of serum biochemical parameters and hormones in weaned pigs are presented in Table 6. Compared with the CON diet, the serum ALB and IGF-1 were increased ($P < 0.05$), whereas the serum UN was decreased ($P < 0.05$) in pigs fed the CGA-supplemented diet. However, there were no significant differences in serum TP, GLB, or GH between the CGA group and the CON group.

Antioxidant Capacity (Exp. 2)

The activities of SOD, GSH-Px, and CAT in serum were increased ($P < 0.05$) by CGA

Table 5. Effects of CGA on ATTD of nutrients in weaned pigs^a (Exp. 2)

Item, %	Dietary treatment		SEM	P-value
	CON	CGA		
DM	82.38	83.65	0.56	0.133
CP	76.78	82.83	1.14	0.002
GE	83.07	84.84	0.64	0.072
Crude fat	63.22	69.38	1.42	0.009
Ash	47.22	56.06	1.44	0.007

^a $n = 8$. CON = pigs in the CON group were fed a basal diet; CGA = pigs in CGA group were fed a basal diet supplemented with 1,000 mg/kg of CGA.

supplementation (Table 7). Meanwhile, pigs fed CGA diet tended ($P < 0.10$) to have a reduced MDA content compared with those on the CON diet. However, no significant difference in T-AOC activity was observed between pigs on the CGA group and the CON group.

Activities of Enzymes in Small Intestine (Exp. 2)

Table 8 presents the enzyme activities in duodenum, jejunum, and ileum of weaned pigs. In duodenum, the disaccharidase (maltase, lactase, and sucrose) activities and the AKP activity were not significantly affected by dietary treatment. Compared with the CON group, CGA increased ($P < 0.05$) the activities of sucrase, maltase, and AKP in jejunum. Meanwhile, pigs on the CGA group tended ($P < 0.10$) to have an increased jejunal lactase activity than those on the CON group. In addition, the activity of maltase in ileum was increased ($P < 0.05$) by CGA supplementation. However, no significant effects of dietary CGA supplementation were observed on the activities of sucrose, lactase, and AKP in the ileum.

Intestinal Digestion and Absorption-Related Genes Expression (Exp. 2)

The mRNA expression levels of intestinal digestion and absorption-related genes (SGLT1, GLUT2, ZNT1, DMT1, and SLC_{7A1}) in the duodenum, jejunum, and ileum of weaned pigs are presented in Figure 1A, 1B, and 1C, respectively. In the duodenum, greater ($P < 0.05$) mRNA expression levels of SGLT1 and ZNT1 were observed on pigs fed the CGA diet compared with those on the CON diet. Meanwhile, the SGLT1, GLUT2, and DMT1

Table 6. Effects of CGA on serum biochemical parameters and hormone levels in weaned pigs^a (Exp. 2)

Item ^b	Dietary treatment		SEM	P-value
	CON	CGA		
TP, g/L	53.75	59.10	1.91	0.167
ALB, g/L	29.94	34.64	1.24	0.018
GLB, g/L	23.81	24.46	2.41	0.851
UN, mmol/L	3.14	2.62	0.17	0.046
GH, ng/mL	13.20	13.57	0.75	0.729
IGF-1, ng/mL	86.63	103.88	4.81	0.024

^a $n = 8$. CON = pigs in the CON group were fed a basal diet; CGA = pigs in CGA group were fed a basal diet supplemented with 1,000 mg/kg of CGA.

^bTP = total protein; ALB = albumin; GLB = globulin; UN = urea nitrogen; GH = growth hormone; IGF-1 = insulin-like growth factor-1.

Table 7. Effects of CGA on serum antioxidant indicators in weaned pigs^a (Exp. 2)

Item ^b	Dietary treatment		SEM	P-value
	CON	CGA		
SOD, U/mL	58.91	66.39	1.11	0.003
MDA, nmol/mL	4.10	3.51	0.21	0.074
GSH-Px, U/mL	393.48	482.37	6.17	0.001
CAT, U/mL	13.36	17.99	1.10	0.010
T-AOC, U/mL	4.71	5.01	0.50	0.681

^a $n = 8$. CON = pigs in the CON group were fed a basal diet; CGA = pigs in CGA group were fed a basal diet supplemented with 1,000 mg/kg of CGA.

^bSOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; CAT = catalase; T-AOC = total antioxidant capacity.

Table 8. Effects of CGA on enzyme activity of small intestine in weaned pigs^a (Exp. 2)

	Dietary treatment			
Item ^b	CON	CGA	SEM	<i>P</i> -value
Duodenum				
Sucrase, U/ mg protein	25.62	30.58	3.75	0.366
Lactase, U/ mg protein	51.42	55.87	9.68	0.750
Maltase, U/mg protein	224.85	238.76	23.91	0.687
AKP, U/ mg protein	35.91	41.22	5.56	0.511
Jejunum				
Sucrase, U/ mg protein	94.43	127.55	10.26	0.039
Lactase, U/ mg protein	68.07	90.63	7.88	0.062
Maltase, U/mg protein	205.25	266.72	18.25	0.032
AKP, U/ mg protein	17.46	24.03	1.72	0.017
Ileum				
Sucrase, U/ mg protein	64.57	77.53	16.41	0.586
Lactase, U/ mg protein	33.57	46.26	5.95	0.154
Maltase, U/mg protein	198.43	243.23	13.48	0.034
AKP, U/ mg protein	31.42	34.74	2.46	0.357

^a $n = 8$. CON = pigs in the CON group were fed a basal diet; CGA = pigs in CGA group were fed a basal diet supplemented with 1,000 mg/kg of CGA.

^bAKP = alkaline phosphatase.

mRNA expression levels in jejunum were upregulated ($P < 0.05$) by dietary CGA supplementation. In addition, dietary CGA supplementation tended ($P < 0.10$) to increase the GLUT2 mRNA levels in duodenum of pigs. There were no significant differences in ileal digestion and absorption-related gene mRNA levels between the CGA group and the CON group.

DISCUSSION

At weaning, pigs often encounter a serious abrupt change in diet, changing from the digestible and liquid breast milk to a solid dry diet (Campbell et al., 2013). In addition, the digestive tract and endogenous secretion systems of newly weaning pigs are immature (Leonard et al., 2011), which leads to decreased appetite, reduced feed intake, and growth retarded (Wijten et al., 2011; Hu et al., 2013). Therefore, maintaining normal intestinal digestion and absorption function plays key roles in preventing the negative effects of weaning stress on growth performance in weaned pigs. CGA is a naturally polyphenol compound found in various plants, and its beneficial effects in health have been investigated in vitro and in vivo (Johnston et al., 2005; Palócz et al., 2016; Ruan et al., 2016; Zhou et al., 2016b). Thus, CGA has been getting increased attention in animal production.

A number of studies in pigs and aquatic animals have been conducted previously to evaluate the functionality of CGA in improving animal production or health (Zhang and Wen, 2012; Chen et al., 2015; Peng et al., 2015; Zhou, 2015a). Zhou et al. (2015a) reported that dietary olive leaf polyphenol extract (containing CGA) supplementation increased the ADG and decreased the F/G in finishing pigs. Similarly, Huang et al. (2015) found that CGA-supplemented diet (300 mg/kg) in lactation sows increased the birth weight of neonatal pigs. On the contrary, Ruan et al. (2014b) and Wang et al. (2015) found no effects of CGA supplementation on the growth performance in rats and shrimp, respectively. The results of the present study in Exp. 1 showed that the growth performance in pigs fed diets supplemented with 250 or 500 mg/kg CGA was not markedly influenced, but the feed conversion ratio was significantly enhanced in 1,000 mg/kg CGA-supplemented group, which was in accordance with previous studies in weaned rats (Ruan et al., 2014a) and broilers (Chen et al., 2015), suggesting that proper dosage of CGA can increase the bioavailability of dietary nutrients. Another intriguing discovery in the present study is that the

Figure 1. Effects of CGA on mRNA levels of intestinal digestion and absorption-related genes in duodenum (A), jejunum (B), and ileum (C) of weaned pigs (Exp. 2). Values were means for eight piglets. Letters above the bars (a, b) indicate statistical significance ($P < 0.05$) of genes expression between the two treatments. $n = 8$. CON = pigs in the CON group were fed a basal diet; CGA = pigs in CGA group were fed a basal diet supplemented with 1,000 mg/kg of CGA. SGLT1 = sodium glucose transport protein-1; GLUT2 = glucose transporter-2; DMT1 = divalent metal transporter-1; ZNT1 = zinc transporter-1; SLC_{7A1} = solute carrier family 7.

ADG in pigs fed 1,000 mg/kg CGA-supplemented diet was significantly increased in Exp. 2 and numerically increased in Exp. 1. But no significant difference in ADFI was observed in both experiments. It appears, therefore, that CGA could enhance the ADG of weaned pigs by improving the bioavailability of dietary nutrients. The different results of CGA on growth performance of animals may be due to the different sources or dosages of CGA, as well as differences in experimental conditions, feed types, and animal species.

The metabolic status of weaned pigs could be reflected by the biochemistry-related parameters and hormones in serum (Etherton et al., 1986).

Serum ALB and UN are considered as the important indicators of the protein metabolism and AA balance of animals (Holeček, 2002; Zou et al., 2006). Previous investigation showed that the ability of protein synthesis was decreased at the early weaning process of animals, which may induce malnutrition and growth retarded (Zou et al., 2006). In the present work, pigs fed the CGA-supplemented diet had a greater serum ALB concentration, and a lower serum UN concentration compared with the CON group, which was in line with the results of previous study in weaned rats (Ruan et al., 2014a). Therefore, these results indicated that dietary CGA supplementation is helpful in reducing the negative effect of weaning stress on bioavailability of dietary nutrient substances. In mammals, the growth performance is regulated mainly by the brain neuroendocrine axis of GH/IGF-1 (Zhou et al., 2015b). IGFs are integral components of multiple systems, and play important roles in controlling the growth and metabolism of animals (Le Roith et al., 2001). Particularly, IGF-1 is the central hormone in growth regulation. In the present study, the serum IGF-1 concentration of pigs on the CGA-supplemented group was greater than those on the CON group. Taken together, these results indicated that CGA has beneficial effects on promoting the rate of protein synthesis, and on decreasing rate of AA catabolism in weaned pigs, which may partly explain why CGA can promote the feed conversion efficiency of pigs.

Oxidative stress is considered to be the result of an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense capacity of the organism (Pi et al., 2010; Reuter et al., 2010). Without well controlled, oxidative imbalance can damage almost all cellular macromolecules (Yin et al., 2013) and cause irreparable oxidative injury and cell death (Yin et al., 2015). Previous studies have reported that weaning stress could cause oxidative stress by damaging the cellular antioxidant defense system in pigs (Zhu et al., 2012; Yin et al., 2014). The complex systems of enzymatic and nonenzymatic play important roles in protecting the organism against the oxidative damage (Minelli et al., 2009). Previous studies have shown that CGA can prevent H_2O_2 -induced ROS production (Zhou et al., 2016a) and provide even more powerful antioxidant capacity than many other phenolic by scavenging superoxide radicals (Feng et al., 2005). In the present study, GSH-Px, an important component in the enzymatic antioxidant defense systems, was significantly increased in the pigs fed the CGA-supplemented diet, indicating

that lipid hydroperoxides could be reduced by CGA. It is widely recognized that CAT and SOD, two important endogenous antioxidant enzymes, play crucial roles in preventing oxidative damage. In detail, SOD is mainly responsible for scavenging superoxide radicals (Winston and Di Giulio, 1991), whereas CAT plays a key role in elimination of organic hydroxyl radicals (David et al., 2008). In the present study, the activities of CAT and SOD in serum were increased by CGA, which indicated the antioxidant capacity of CGA in weaned pigs. CGA has a special molecular structure, donating hydrogen atoms, and then quickly stabilized by resonance stabilization after oxidizing to their respective phenoxyl radicals. The free hydrogen atoms of CGA can reduce the free radicals and to inhibit oxidation reactions (Liang and Kitts, 2016), which might be the partial reason why CGA possesses the antioxidant activity. In view of the foregoing, it is speculated that CGA can protect weaned pigs against oxidative stress by the improved antioxidant enzyme activities of pigs and the special molecular structure of CGA.

To meet the rapid growth and a high metabolic rate, young animals require a huge amount of nutrients. Reduced digestion and absorption function, characterized by decreased digestion and absorption enzyme activity, will lead to growth retarded, or result in diarrhea. Therefore, a strong activity of digestion and absorption enzyme of pigs is very important in nutrient absorption and healthy growth. Disaccharide enzymes (maltase, lactase, and sucrase), locating on brush-border membranes, play crucial roles in the process of digestion in animals (Wan et al., 2017). The current study indicated that dietary CGA supplementation improved the activity of sucrase in the jejunum and the activity of maltase in the jejunum and ileum, which indicated that CGA has beneficial effects on improving small intestinal digestive function of weaned pigs. In addition, the results of present study showed that dietary CGA supplementation could also improve the activity of AKP in the jejunum of weaned pigs, which is another crucial endogenous enzyme expressed in the brush border, and is considered as an excellent marker enzyme involved in the primary digestive and absorptive processes of the small intestine (Toofanian and Targowski, 1982; Zhang et al., 1996; Zhang et al., 2012). This result further suggested the improved effect of CGA on digestion and absorption function of weaned pigs. Furthermore, the present study verified an improvement in ATTD of nutrients (CP, crude fat, and ash), in accordance with the previous study in Jiong rabbit (Wang,

2013), and a decrease in diarrhea incidence of pigs in the CGA-supplemented group, which probably due to the increased digestion and absorption enzyme activity. Therefore, these combined findings collectively indicated that the increased activities of intestinal digestion and absorption enzymes, as well as the improved apparent nutrient digestibility might be the partial reasons why CGA could improve the feed efficiency and decrease the diarrhea incidence in CGA-supplemented pigs.

Intestinal tract acts as a crucial part in secretions, food digestion, as well as nutrient absorption and metabolism (Yin et al. 2016). To further explain the beneficial effects of CGA on digestion and absorption of small intestine, the present study aimed to investigate whether the CGA supplementation could affect the expression of intestinal nutrient transporters (SGLT1, GLUT2, ZNT1, DMT1, and SLC_{7A1}). SGLT1, an apical intestinal transporter, is responsible for the majority of luminal glucose transport across intestinal epithelium, which is the rate-limiting step for absorption of dietary glucose (Wright et al., 2003; Zhang et al., 2016). GLUT2, a key transporter located at the basolateral membrane of the enterocyte, provides a glucose channel for the metabolic of enterocytes in the intestinal glucose absorption system (Jones et al., 2011; Peng et al., 2015). Therefore, the expression levels of SGLT1 and GLUT2 are closely related to the intestinal digestion and absorption function. In current study, the SGLT1 mRNA expression levels in duodenum and jejunum, and the GLUT2 mRNA expression levels in jejunum were upregulated by CGA. However, previous studies have indicated that CGA inhibited the SGLT1-mediated glucose transport (Welsch et al., 1989; Peng et al., 2015). The discrepancy with other studies may be due to the various sources of CGA, experimental conditions, and animal species. Peng et al. (2015) speculated according to their research that CGA has the ability to maintain normal glucose-uptake rates (Peng et al., 2015). At weaning, pigs were under a serious digestive disorder condition because of the immature digestion and absorption systems, and subsequently induced malnutrition (Wild and Murray, 1992; Kim et al., 2012), which might be partial reason why CGA could improve the expression of SGLT1 in weaned pigs. On the other hand, Peng et al. (2015) reported that high dose of CGA inhibited the upregulated expression of SGLT1 in rats fed high-fat diet while the low dose of CGA was not sufficient to influence the expression of SGLT1. Similarly, other researchers also found the similar effect rules of CGA in Caco-2 cells (Johnston et al.,

2005). Thus, it is speculated from all of the previously mentioned evidences that different dosages of the CGA used in these studies may also one of the possible explanations for the disparity. But the further studies should be conducted to more completely determine the effects of different dosages of CGA on SGLT1-mediated glucose transport in weaned pigs. Therefore, from these results, it is concluded that it is likely that proper dosage of CGA used in weaned pigs could enhance the amount of luminal glucose of transported into the enterocytes by SGLT1 and enhance glucose transportation out of the cell via GLUT2, allowing more energy for growth, which was in accordance with the effect of CGA on growth performance. But the specific mechanisms need further research. Another meaningful new discovery in the present study was that the expression levels of duodenum ZNT1 and jejunum MET1 were upregulated when pigs were fed CGA-supplemented diet. Recent studies indicated that ZNT1 and MET1, which are extensively located at the intestinal mucosa, are served as the important transporters of zinc and iron, respectively (Boudry et al., 2010). Iron and zinc are components of multiple enzymes and proteins. It appears, therefore, that CGA could improve the utilization of minerals in weaned pigs, which was a contributing factor in promoting growth performance by CGA.

In conclusion, the present study provided the first evidence that CGA has potential benefits that serve as an effective feed additive for protecting the weaned pigs against weaning-stress-induced diarrhea and growth retarded. The growth promoting effect of CGA on weaned pigs may be partly attributed to the increased antioxidant capacity and the improved intestinal digestion and absorption function. These results provide a prerequisite foundation for further investigation to assess more of the potential mechanisms responsible for the beneficial effects of CGA on improving growth performance of weaned pigs.

ACKNOWLEDGMENTS

This study was supported by the Special Fund for Agro-Scientific Research in the Public Interest (201403047).

LITERATURE CITED

- AOAC. 1995. Official methods of analysis. 16th ed. Washington (DC): AOAC International.
- Bouayed, J., H. Rammal, A. Dicko, C. Younos, and R. Soulimani. 2007. Chlorogenic acid, a polyphenol from *Prunus domestica* (Mirabelle), with coupled anxiolytic and

- antioxidant effects. *J. Neurol. Sci.* 262:77–84. doi:10.1016/j.jns.2007.06.028
- Boudry, G., E.S. David, V. Douard, I.M. Monteiro, I. Le Huërou-Luron, and R.P. Ferraris. 2010. Role of intestinal transporters in neonatal nutrition: carbohydrates, proteins, lipids, minerals, and vitamins. *J. Pediatr. Gastr. Nut.* 51:380–401. doi:10.1097/MPG.0b013e3181eb5ad6
- Campbell, J.M., J.D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. *J. Anim. Sci. Biotechn.* 4:19. doi:10.1186/2049-1891-4-19
- Chen, Y.M., T. Huang, X.Z. Song, L.J. Xu, B. Liu, and Y.B. Fu. 2015. Effects of dietary eucommia leaves extract on growth performance and immune function of broilers. *Chinese J. Anim. Nutr.* 27:2224–2230.
- Chen, J.L., P. Zheng, C. Zhang, B. Yu, J. He, J. Yu, and D.W. Chen. 2017. Benzoic acid beneficially affects growth performance of weaned pigs which was associated with changes in gut bacterial populations, morphology indices and growth factor gene expression. *J. Anim. Physiol. Anim. Nutr.* 101:1137–1146. doi:10.1111/jpn.12627.
- David, M., V. Munaswamy, R. Halappa, and S.R. Marigoudar. 2008. Impact of sodium cyanide on catalase activity in the freshwater exotic carp, *Cyprinus carpio* (Linnaeus). *Pestic. Biochem. Phys.* 92:15–18. doi:10.1016/j.pestbp.2008.03.013
- Etherton, T.D., J.P. Wiggins, C.S. Chung, C.M. Evock, J.F. Rebhun, and P.E. Walton. 1986. Stimulation of pig growth performance by porcine growth hormone and growth hormone-releasing factor. *J. Anim. Sci.* 63:1389–1399. doi:10.2527/jas1986.6351389x
- Feng, R., Y. Lu, L.L. Bowman, Y. Qian, V. Castranova, and M. Ding. 2005. Inhibition of activator protein-1, NF- κ B, and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. *J. Biol. Chem.* 280:27888–27895. doi:10.1074/jbc.M503347200
- He, L., H. Li, N. Huang, J. Tian, Z. Liu, X. Zhou, K. Yao, T. Li, and Y. Yin. 2016. Effects of alpha-ketoglutarate on glutamine metabolism in piglet enterocytes in vivo and in vitro. *J. Agr. Food Chem.* 64:2668. doi:10.1021/acs.jafc.6b00433
- Holeček, M. 2002. Relation between glutamine, branched-chain amino acids, and protein metabolism. *Nutrition* 18:130–133. doi:10.1016/S0899-9007(01)00767-5
- Hu, C., K. Xiao, Z. Luan, and J. Song. 2013. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. *J. Anim. Sci.* 91:1094–1101. doi:10.2527/jas.2012-5796
- Huang, S.W., J.T. Wei, N. Zhao, X.H. Yang, W. Zhang, and S.Q. Mei. 2015. Effects of chlorogenic acid and vitamin E on reproductive performance and antioxidant capacity of sows. *Chinese J. Anim. Sci.* 51:79–83.
- Ji, L., P. Jiang, B. Lu, Y. Sheng, X. Wang, and Z. Wang. 2013. Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism. *J. Nutr. Biochem.* 24:1911–1919. doi:10.1016/j.jnutbio.2013.05.007
- Johnston, K., P. Sharp, M. Clifford, and L. Morgan. 2005. Dietary polyphenols decrease glucose uptake by human intestinal Caco-2 cells. *FEBS Lett.* 579:1653–1657. doi:10.1016/j.febslet.2004.12.099
- Jones, H.F., R.N. Butler, and D.A. Brooks. 2011. Intestinal fructose transport and malabsorption in humans. *Am. J. Physiol.-Gastr.* 300:G202–G206. doi:10.1152/ajpgi.00457.2010
- Kim, J., C.F. Hansen, B. Mullan, and J. Pluske. 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. *Anim. Feed Sci. Tech.* 173:3–16. doi:10.1016/j.anifeedsci.2011.12.022
- Kluess, J., U. Schoenhusen, W. Souffrant, P. Jones, and B. Miller. 2010. Impact of diet composition on ileal digestibility and small intestinal morphology in early-weaned pigs fitted with a T-cannula. *Animal* 4:586–594. doi:10.1017/S1751731109991455
- Le Roith, D., C. Bondy, S. Yakar, J.-L. Liu, and A. Butler. 2001. The somatomedin hypothesis: 2001. *Endocr. Rev.* 22:53–74. doi:10.1210/er.22.1.53
- Leonard, S., T. Sweeney, B. Bahar, B. Lynch, and J. O'Doherty. 2011. Effects of dietary seaweed extract supplementation in sows and post-weaned pigs on performance, intestinal morphology, intestinal microflora and immune status. *Brit. J. Nutr.* 106:688–699. doi:10.1017/S0007114511000997
- Li, B., H. Zhou, X. Wu, Z. Chen, J. Yao, and Y. Yin. 2016. Effects of dietary supplementation with uridine monophosphate on performance and intestinal morphology of weanling piglets. *J. Anim. Sci.* 94:82–86. doi:10.2527/jas2015-9440
- Liang, N., and D.D. Kitts. 2016. Role of chlorogenic acid in controlling oxidative and inflammatory stress conditions. *Nutrients* 8:16. doi:10.3390/nu8010016
- Liu, Y., Z.S. Wang, and A.G. Zhou. 2009. Effects of hesperidin and chlorogenic acid on performance, antioxidance and immunity of weaned piglets. *Chinese J. Vet. Sci.* 29:1233–1236.
- Livak, K.J., and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 $^{-\Delta\Delta CT}$ method. *Methods* 25:402–408.
- Lou, Z., H. Wang, S. Zhu, C. Ma, and Z. Wang. 2011. Antibacterial activity and mechanism of action of chlorogenic acid. *J. Food Sci.* 76:M398–M403. doi:10.1111/j.1750-3841.2011.02213.x
- Ma, Y., M. Gao, and D. Liu. 2015. Chlorogenic acid improves high fat diet-induced hepatic steatosis and insulin resistance in mice. *Pharm. Res.-Dordr.* 32:1200–1209. doi:10.1007/s11095-014-1526-9
- Minelli, A., I. Bellezza, C. Conte, and Z. Culig. 2009. Oxidative stress-related aging: a role for prostate cancer? *BBA-Rev. Cancer* 1795:83–91. doi:10.1016/j.bbcan.2008.11.001
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Washington (DC): National Academies Press.
- Palócz, O., E. Pászti-Gere, P. Gálfi, and O. Farkas. 2016. Chlorogenic acid combined with *Lactobacillus plantarum* 2142 reduced LPS-induced intestinal inflammation and oxidative stress in IPEC-J2 cells. *PLoS One* 11:e0166642. doi:10.1371/journal.pone.0166642
- Peng, B.J., Z. Qi, Y.L. Zhong, S.H. Xu, and W. Zheng. 2015. Chlorogenic acid maintains glucose homeostasis through modulating the expression of SGLT-1, GLUT-2, and PLG in different intestinal segments of Sprague-Dawley rats fed a high-fat diet. *Biomed. Environ. Sci.* 28:894–903. doi:10.3967/bes2015.123
- Pi, J., Q. Zhang, J. Fu, C.G. Woods, Y. Hou, B.E. Corkey, and M.E. Andersen. 2010. ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function. *Toxicol. Appl. Pharm.* 244:77–83. doi:10.1016/j.taap.2009.05.025
- Rakshit, S., L. Mandal, B.C. Pal, J. Bagchi, N. Biswas, J. Chaudhuri, and T. Mukherjee. 2010. Involvement of ROS in chlorogenic acid-induced apoptosis of Bcr-Abl+ CML cells. *Biochem. Pharmacol.* 80:1662–1675. doi:10.1016/j.bcp.2010.08.013

- Reuter, S., S.C. Gupta, M.M. Chaturvedi, and B.B. Aggarwal. 2010. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Bio. Med.* 49:1603–1616. doi:10.1016/j.freeradbiomed.2010.09.006
- Ruan, Z., S. Liu, Y. Zhou, S. Mi, G. Liu, X. Wu, and G. Wu. 2014a. Chlorogenic acid decreases intestinal permeability and increases expression of intestinal tight junction proteins in weaned rats challenged with LPS. *PLoS One* 9:e97815. doi:10.1016/j.bcp.2010.08.013
- Ruan, Z., S. M. Mi, L.L. Zhou, Y. Zhou, J. Li, W.H. Liu, Z.Y. Deng, and Y.L. Yin. 2016. Chlorogenic acid enhances intestinal barrier by decreasing MLCK expression and promoting dynamic distribution of tight junction proteins in colitic rats. *J. Funct. Foods* 26:698–708. doi:10.1016/j.jff.2016.08.038
- Ruan, Z., Y. Yang, Y. Zhou, Y. Wen, S. Ding, G. Liu, and G. Wu. 2014b. Metabolomic analysis of amino acid and energy metabolism in rats supplemented with chlorogenic acid. *Amino Acids* 46:2219–2229. doi:10.1007/s00726-014-1762-7
- Standards Press of China. 2009. Measurement of the acid insoluble ash in feed. 330 GB/T 23742, Beijing (China).
- Song, M., Y. Liu, J.A. Soares, T.M. Che, O. Osuna, C.W. Maddox, and J.E. Pettigrew. 2012. Dietary clays alleviate diarrhea of weaned pigs. *J. Anim. Sci.* 90:345–360. doi:10.2527/jas.2010-3662
- Toofanian, F., and S. Targowski. 1982. Fetal development and distribution of intestinal alkaline phosphatase in the rabbit. *Res. Vet. Sci.* 32:303–305.
- Wan, J., F. Jiang, Q. Xu, D. Chen, B. Yu, Z. Huang, and J. He. 2017. New insights into the role of chitosan oligosaccharide in enhancing growth performance, antioxidant capacity, immunity and intestinal development of weaned pigs. *RSC Adv.* 7:9669–9679. doi:10.1039/C7RA00142H
- Wang, D.J. 2013. Impact of chlorogenic acid on growth performance, blood biochemical indexes and metallothionein gene expression of Jiong rabbit [MASC dissertation]. Changchun (China): Jilin University.
- Wang, Y., Z. Li, J. Li, Y.F. Duan, J. Niu, J. Wang, and H.Z. Lin. 2015. Effects of dietary chlorogenic acid on growth performance, antioxidant capacity of white shrimp *Litopenaeus vannamei* under normal condition and combined stress of low-salinity and nitrite. *Fish Shellfish Immun.* 43:337–345. doi:10.1016/j.fsi.2015.01.008
- Welsch, C.A., P.A. Lachance, and B.P. Wasserman. 1989. Dietary phenolic compounds: inhibition of Na⁺-dependent d-glucose uptake in rat intestinal brush border membrane vesicles. *J. Nutr.* 119:1698–1704.
- Wijten, P.J., J. van der Meulen, and M.W. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: a review. *Brit. J. Nutr.* 105:967–981. doi:10.1017/S0007114510005660
- Wild, G.E., and D. Murray. 1992. Alterations in quantitative distribution of Na, K-ATPase activity along crypt-villus axis in animal model of malabsorption characterized by hyperproliferative crypt cytokinetics. *Digest. Dis. Sci.* 37:417–425. doi:10.1007/bf01307737
- Winston, G.W., and R.T. Di Giulio. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat. Toxicol.* 19:137–161. doi:10.1016/0166-445X(91)90033-6
- Wright, E.M., M.N.G. Martín, and E. Turk. 2003. Intestinal absorption in health and disease—sugars. *Best Pract. Res. Cl. Ga.* 17:943–956. doi:10.1016/S1521-6918(03)00107-0
- Yin, J., J.L. Duan, Z.J. Cui, W.K. Ren, T.J. Li, and Y.L. Yin. 2015. Hydrogen peroxide-induced oxidative stress activates NF- κ B and Nrf2/Keap1 signals and triggers autophagy in piglets. *RSC Adv.* 5:15479. doi:10.1039/C4RA13557A
- Yin, J., W.K. Ren, X.S. Wu, G. Yang, J. Wang, T.J. Li, J.N. Ding, L.C. Cai, and D.D. Su. 2013. Oxidative stress-mediated signaling pathways: a review. *J. Food Agric. Environ.* 11:132–139.
- Yin, J., W.K. Ren, G. Yang, J.L. Duan, X.G. Huang, R.J. Fang, C.Y. Li, T.J. Li, Y.L. Yin, Y.Q. Hou, et al. 2016. l-Cysteine metabolism and its nutritional implications. *Mol. Nutr. Food Res.* 60:134–146. doi:10.1002/mnfr.201500031
- Yin, J., M. M. Wu, H. Xiao, W.K. Ren, J.L. Duan, G. Yang, and Y.L. Yin. 2014. Development of an antioxidant system after early weaning in piglets. *J. Anim. Sci.* 92:612–619. doi:10.2527/jas.2013-6986
- Zhang, C., and A. Wen. 2012. Different of chlorogenic acid doses on growth performance, non-specific immunity and antioxidant functions of *Cyprinus carpio* var. *J. Sichuan Agric. Univ.* 30:92–97.
- Zhang, X., F. Cao, Z. Sun, W. Yu, L. Zhao, G. Wang, and T. Wang. 2012. Effect of feeding *Aspergillus niger*-fermented *Ginkgo biloba*-leaves on growth, small intestinal structure and function of broiler chicks. *Livest. Sci.* 147:170–180. doi:10.1016/j.livsci.2012.04.018
- Zhang, Y., J. Shao, Q. Xie, and D.H. Alpers. 1996. Immunolocalization of alkaline phosphatase and surfactant-like particle proteins in rat duodenum during fat absorption. *Gastroenterology* 110:478–488. doi:10.1053/gast.1996.v110.pm8566595
- Zhang, S., Q. Yang, M. Ren, S. Qiao, P. He, D. Li, and X. Zeng. 2016. Effects of isoleucine on glucose uptake through the enhancement of muscular membrane concentrations of GLUT1 and GLUT4 and intestinal membrane concentrations of Na⁺/glucose co-transporter 1 (SGLT-1) and GLUT2. *Brit. J. Nutr.* 116:593–602. doi:10.1017/S0007114516002439
- Zhou, Y. 2015a. Effect of polyphenols extract from *eucommia ulmoides* olive leaf on meat quality and chlorogenic acid ameliorate liver-intestine injury [PhD dissertation]. Nanchang (China): Nanchang University.
- Zhou, Y., Z. Ruan, L.L. Zhou, X.G. Shu, X.H. Sun, S.M. Mi, Y.H. Yang, and Y.L. Yin. 2016b. Chlorogenic acid ameliorates endotoxin-induced liver injury by promoting mitochondrial oxidative phosphorylation. *Biochem. Biophys. Res. Co.* 469:1083–1089. doi:10.1016/j.bbrc.2015.12.094
- Zhou, Y., X. Wei, Z. Zi, B. Zou, S. Xia, N. Lu, and D. Xia. 2015b. Potassium diformate influences gene expression of GH/IGF-I axis and glucose homeostasis in weaning piglets. *Livest. Sci.* 172:85–90. doi:10.1016/j.livsci.2014.12.003
- Zhou, Y., L. Zhou, Z. Ruan, S. Mi, M. Jiang, X. Li, and Y. Yin. 2016a. Chlorogenic acid ameliorates intestinal mitochondrial injury by increasing antioxidant effects and activity of respiratory complexes. *Biosci. Biotech. Bioch.* 80:962–971. doi:10.1080/09168451.2015.1127130
- Zhu, L., K. Zhao, X. Chen, and J. Xu. 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. *J. Anim. Sci.* 90:2581–2589. doi:10.2527/jas.2011-4444
- Zou, X., G. Zheng, X. Fang, and J. Jiang. 2006. Effects of glutamine on growth performance of weanling piglets. *Czech J. Anim. Sci.* 51:444–448. doi:10.17221/3963-CJAS