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Effects of fulvic acid on growth performance and meat quality in growing-finishing pigs

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ABSTRACT

An experiment was conducted to evaluate the effects of dietary fulvic acid (FA) on growth performance and meat quality in growing-finishing pigs. Two hundred and sixteen (30.0 ± 2.5 kg body weight) crossbred (Landrace × Yorkshire) castrated male pigs were randomly allotted to 6 dietary treatments with 6 replicates (pens) per treatment and 6 pigs per pen. The basal diet was supplemented with different levels of FA (0.0%, 0.1%, 0.2%, 0.4%, 0.6%, and 0.8%). Pigs were fed diets based on a 2-phase feeding program (phase 1 for 45 d, and phase 2 for 42 d). The results from the entire experimental period showed pigs fed diets supplemented with FA increased average daily gain (ADG) linearly and quadratically ($P<0.05$) during phase 1 and the whole feeding period, respectively, whereas gain to feed ratio (G:F) was increased quadratically ($P<0.05$). Dietary supplementation of FA did not affect ADG and average daily feed intake during phase 2, but G:F was increased quadratically ($P<0.05$). The slaughter weight and hot carcass weight of pigs fed diets supplemented with FA increased linearly and quadratically ($P<0.05$). The backfat thickness was reduced ($P<0.05$) quadratically in response to dietary FA supplementation. Feeding diets supplemented with FA reduced pH in muscle (24 h) linearly ($P<0.05$) and malonaldehyde (MDA) quadratically ($P<0.05$), while a^* value, b^* value, and marbling increased linearly and quadratically ($P<0.05$). The results of this study indicated that supplementation of diets with FA is an effective way to increase growth performance, reduce backfat thickness, and improve meat quality in growing-finishing pig. Broken-line regression analysis indicated that the optimum dietary FA supplementation to increase G:F and reduce backfat thickness and MDA under the current experimental conditions was 0.48–0.79%.

1. Introduction

Fulvic acid (FA) is a type of humic acid (HA) that is formed by the decomposition and transformation of plant and animal residues and microbial materials (Oades, 1988; Janos, 2003). The FA is obtained from peat and different types of coal such as weathered coal and lignite. Compared to other HA, the FA is soluble in both acid and alkali solutions, is lower in molecular weight, and has a greater biological activity. It also contains more oxygen-containing functional groups compared to other HA (Stevenson, 1994; Yong, 2001; Zhang et al., 2011). The FA can absorb strongly metal ions and organic compounds between humic substances and mineral oxides as natural sorbents (Chiou et al., 2000; Saito et al., 2005), and it can participate in redox reactions and increase metabolism. Many studies have indicated that utilizing FA in agriculture can accelerate seed germination and improve rhizome growth (Nardi et al., 2002; Zancani et al., 2009; Zancani et al., 2011), stimulate oxygen transport, accelerate respiration, promote the efficient utilization of nutrients by the plant, and improve land salinization (Anjum et al., 2011; Visser, 1988). These findings have prompted scientists' interests in the specific properties of FA and its possible benefits as a substitute for the antibiotics used to improve the growth and health of animals.

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In recent years, many reports have indicated that FA can form a film on the mucus epithelium of the gastrointestinal tract and protect against infections and toxins, thus, ensuring improved utilization of nutrients in animal feed (Islam et al., 2005; Trckova et al., 2005). Also, the FA has antimicrobial and anti-inflammatory properties (Van Rensburg et al., 2001). It has been shown that FA added to animal feed increased growth rate and product quality, and provided better resistance to viral infections (Banaszkiewicz and Drobnik, 1994; Karaoglu et al., 2004; Kucukersan et al., 2005; McMurphy et al., 2009; Písaříková et al., 2010) to improve feed conversion ratio and decrease the cost of feed to get greater profit in animal production (An et al., 2009; Gu et al., 2001; Wu et al., 2009).

Studies focusing on the applications of HA, FA, and the weathered coal have been conducted extensively in China since the beginning of the 1980s. However, the use of FA as a dietary supplement for pigs has not been well-studied, particularly in growing-finishing period. Therefore, the objective of this research was to test the effects of different levels of FA supplemented diets on growth performance, carcass traits and meat quality to explore the effective dose of dietary FA supplementation in growing-finishing pigs.

2. Materials and methods

2.1. Fulvic acid

The FA used in the current experiment was extracted from weathered coal obtained (Yongye Nongfeng Bio-tech. Co. Ltd., Inner Mongolia, China). The analyses of FA were conducted following the methods reported by Klymenko et al. (2010). Two liters of 0.1 M NaOH solution was poured over 400 g of air-dried weathered coal, and the mixture was stirred for 24 h. The dark supernatant containing humic and fulvic acids was acidified to pH 2.0 with concentrated sulfuric acid to separate the humic acid fraction. Filtration was used to separate the FA solution. Then, 4 M NaOH solution was added to the FA-containing extract to achieve a pH of 7. The resulting solution was used as a preparation of FA. The measured effective content of FA was 12.05%.

2.2. Experimental design, animals, and diets

A total of 216 crossbred (Landrace × Yorkshire) castrated male pigs with an average initial BW of 30.0 ± 2.5 kg were used in the current experiment. At the beginning of the experiment, pigs were assigned to 6 dietary treatments on the basis of initial BW. There were 6 pens per treatment and 6 pigs per pen. The dietary treatments included the following: basal control diet supplemented with 0.0%, 0.1%, 0.2%, 0.4%, 0.6%, or 0.8% FA. The pigs were allowed ad libitum access to feed and water throughout the experiment. All of the experimental diets were formulated to meet or exceed the recommended requirements of the NRC (1998) for a 2-phase feeding program (Table 1). Pigs were fed phase 1 and 2 diets for 45 and 42 d, respectively, until they reached a BW of 90 kg. All procedures involving pigs were approved by the Animal Care and Use Committee of Heilongjiang Province, China.

Table 1
Formula and chemical composition of experiment diet (as-fed basis).[a]

Item	Phase 1	Phase 2
Ingredients (%)		
Maize	63.80	65.00
Soybean meal (46% CP)	23.30	17.20
Wheat bran	10.00	15.00
Monocalcium phosphate	0.50	0.40
Limestone	1.00	1.00
Salt	0.30	0.30
Premix[b]	1.00	1.00
Choline chloride (50%)	0.10	0.10
Total	100.00	100.00
Chemical composition[c]		
ME (MJ/kg)	13.59	13.54
Crude protein (%)	16.40	14.52
Ca (%)	0.56	0.52
P (%)	0.22	0.19
Lys (%)	0.85	0.72

[a] A 2-phase feeding program was used. The duration of each phase differed depending on the growth rate of the pigs.
[b] Supplied per kilogram diet: vitamin A, 8000 IU; vitamin D_3, 2000 IU; vitamin E, 30 IU; vitamin K_3, 1.5 mg; vitamin B_1, 1.6 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 12 μg; niacin, 20 mg; d-pantothenic acid, 15 mg; Zn (ZnO), 80 mg; Fe ($FeSO_4 \cdot 7H_2O$), 100 mg; Cu ($CuSO_4 \cdot 5H_2O$), 20 mg; Mn ($MnSO_4 \cdot H_2O$), 25 mg; I (KI), 0.3 mg; and Se ($NaSeO_3 \cdot 5H_2O$), 0.2 mg.
[c] Calculated values.

2.3. Sampling and measurements

Individual BW and feed consumption per pen were measured after 45 and 42 d to monitor the average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). At the end of the experiment, 1 pig from each pen was selected randomly and a total of 36 pigs were transported to the abattoir for slaughter. The carcasses were placed in a conventional chiller at 4 °C. After a 24 h chill period, longissimus muscle (LM) samples, including lean and fat, were obtained from the left sides of the carcasses between the 10th and 11th ribs. At the same time, abdominal fat (leaf fat) was stripped from the left side of the carcass and weighted. Backfat thickness was measured at the first, 10th, and last ribs, and average was recorded. The longissimus muscle area (LMA) was measured by taking a digital image of the exposed surface of the muscle area at the 10th rib. The images were standardized using a fixed camera and 2 rulers, and the LMA was calculated using imaging software (Pomar et al., 2001).

Before the meat quality was evaluated, the meat samples were thawed at ambient temperature. The pH of LM was measured at 24 h post-mortem by a portable pH-Meter (pH-Meter, EL2; Mettler-Toledo, Shanghai, China). The color measurement of lightness (L^*), redness (a^*), and yellowness (b^*) values were determined (Minolta Chroma Meter, CR-301; Konica Minolta, Tokyo, Japan). Marbling score data were obtained according to published pork quality standards (NPPC, 2000). Drip loss was measured using the plastic bag and approximately 20 g of meat samples at 4 °C for 24 h to determine drip loss percentage as described by Honikel (1998). Cooking loss was determined by calculating the weight loss during cooking. Meat samples were cooked in a water bath until a temperature of 75 °C was achieved and then cooled at room temperature. Shear force values were determined (C-LM3-type Digital Muscle-Shear Apparatus; Northeast Agricutural Universtiy, Beijing, China). Malonaldehyde (MDA) contents were determined using a kit (Jiancheng Biological Engineering Research Institute of Nanjing; Nanjing, China).

2.4. Statistical analysis

Data were analyzed by a one-way ANOVA using the GLM procedure of the SAS (9.1; SAS Inst. Inc., Cary, NC, US) with the pen being the experimental unit. The linear, quadratic, and cubic effects of fulvic acid on growth performance, carcass traits, and meat quality were assessed. The procedures described by Robbins et al. (2006) were used to estimate the optimum FA supplementation. Probability values less than 0.05 were considered significant.

3. Results

3.1. Growth performance

During phase 1, ADG increased linearly and quadratically ($P<0.05$), while G:F increased quadratically ($P<0.05$) as the levels of supplemented FA increased (Table 2). The dietary treatments did not affect ADFI. During phase 2, dietary supplementation of FA did not affect ADG and ADFI, but G:F increased quadratically ($P<0.05$) with the increasing levels of supplemental FA. Throughout the experiment period, ADG increased linearly and quadratically ($P<0.05$), while G:F increased quadratically ($P<0.05$) with the increasing levels of supplemented FA.

3.2. Carcass traits and meat quality

Dietary supplementation of FA increased slaughter weight linearly ($P<0.05$) and hot carcass weight both linearly and quadratically ($P<0.05$) as FA increased (Table 3). Pigs responded quadratically ($P<0.05$) to FA supplementation in backfat thickness. There were no differences in dressing percentage, leaf fat percentage, and LMA among treatment groups.

With increasing levels of FA supplementation, $pH_{24\ h}$ in pigs reduced linearly ($P<0.05$), a^* and b^* values, and marbling scores increased linearly and quadratically ($P<0.05$), whereas MDA reduced quadratically ($P<0.05$; Table 4). The addition of FA did not affect L^* value, shear force, drip loss, or cooking loss.

The optimum dietary supplementation of FA were estimated to be 0.79%, 0.53%, 0.50%, and 0.48% for ADG, G:F, backfat thickness, and MDA, respectively, based upon the quadratic broken-line method (Table 5). The estimates with the linear broken-line model predicted much lower than those estimated with the quadratic model.

4. Discussion

The FA or HA preparation fed as a supplement to piglets had a beneficial effect on their growth and general health status (Kunavue and Lien, 2012; Vucskits et al., 2010; Wu et al., 2009) and reduce a mortality rate (Fuchs et al., 1995; Roost et al., 1990). Literatures indicated that growing-finishing pigs fed diets with HA substances had greater ADG and G:F, but ADFI was not affect (Dänicke et al., 2012; Ji et al., 2006; Long et al., 1994; Yang et al., 1998; Yao et al., 2004; Wang et al., 2008). It is thought that this improvement is due to the effect of HA on the metabolism of proteins and carbohydrates by microbes (Huck et al., 1991), resulting in a direct antibacterial or antiviral effects, which should result in improved growth performance. Kim et al. (2004) and Morales et al. (2011) speculated that ingestion, fixation in some specific organ or fluid, and elimination of mineral ions by vertebrates are dynamic processes that are greatly accelerated when supplemented with FA, which may explain the effect on weight gain in livestock. The current study was consistent with previous studies, and there were improvements in

Table 2
Growth performance of pigs fed diets supplemented with fulvic acid.[a]

Item[b]	Fulvic acid (%)						SEM[c]	*P*-value		
	0.0	0.1	0.2	0.4	0.6	0.8		Linear	Quadratic	Cubic
ADG (kg)										
Phase 1	0.65	0.68	0.70	0.70	0.71	0.74	0.03	< 0.01	0.04	0.06
Phase 2	0.82	0.84	0.85	0.85	0.89	0.85	0.02	0.12	0.11	0.26
Overall	0.73	0.75	0.77	0.77	0.80	0.79	0.02	< 0.01	0.02	0.11
ADFI (kg)										
Phase 1	1.72	1.73	1.75	1.78	1.74	1.86	0.06	0.07	0.17	0.19
Phase 2	2.68	2.69	2.65	2.65	2.74	2.72	0.06	0.15	0.29	0.42
Overall	2.20	2.21	2.20	2.21	2.24	2.31	0.04	0.07	0.13	0.26
G:F										
Phase 1	0.38	0.39	0.40	0.40	0.41	0.40	0.01	0.11	0.01	0.08
Phase 2	0.30	0.31	0.31	0.31	0.32	0.31	0.01	0.30	0.03	0.09
Overall	0.33	0.34	0.35	0.35	0.36	0.35	< 0.01	0.15	0.02	0.11

[a] ADG = average daily gain, ADFI = average daily feed intake, and G:F = gain to feed. Fulvic acid was obtained commercially (Yongye Nongfeng Bio-tech Co. Ltd., Inner Mongolia, China).

[b] Phase 1 for 45 d and Phase 2 for 42 d.

[c] Pooled standard error of the mean.

Table 3
Carcass traits of pigs fed diets supplemented with fulvic acid.[a]

Item[b]	Fulvic acid (%)						SEM[c]	P-value		
	0.0	0.1	0.2	0.4	0.6	0.8		Linear	Quadratic	Cubic
Slaughter weight (kg)	97.0	97.4	98.3	100.7	99.7	103.5	2.2	0.03	0.09	0.19
Hot carcass weight (kg)	67.8	68.2	70.1	71.3	70.8	73.6	1.7	0.02	0.04	0.14
Dressing percentage (%)	69.9	70.0	71.3	70.7	71.1	71.1	0.7	0.11	0.22	0.42
Backfat thickness (cm)	2.2	2.1	1.9	1.9	1.9	2.0	0.1	0.15	0.02	0.17
Leaf fat (%)	1.5	1.4	1.4	1.4	1.4	1.4	0.2	0.55	0.82	0.93
LMA (cm^2)	37.9	41.5	42.1	41.7	41.7	42.9	2.3	0.12	0.23	0.56

[a] LMA=longissimus muscle area. Fulvic acid was obtained commercially (Yongye Nongfeng Bio-tech Co. Ltd., Inner Mongolia, China).
[b] Backfat thickness=average of first rib, 10th rid and last rib carcasses backfat thickness.
[c] Pooled standard error of the mean.

Table 4
Meat quality of pigs fed diets supplemented with fulvic acid.[a]

Item[b]	Fulvic acid (%)						SEM[c]	P-value		
	0.0	0.1	0.2	0.4	0.6	0.8		Linear	Quadratic	Cubic
$pH_{24\ h}$	5.6	5.6	5.6	5.6	5.6	5.6	0.1	<0.01	0.06	0.21
L^*	47.5	48.1	48.8	48.3	48.4	49.0	0.5	0.10	0.30	0.10
a^*	13.6	13.7	14.1	14.6	14.8	15.2	0.3	0.00	<0.01	0.26
b^*	4.3	4.4	4.5	4.6	4.6	4.6	0.4	0.02	0.01	0.75
Marbling[5]	2.3	2.4	2.7	2.9	2.9	3.1	0.2	<0.01	<0.01	0.47
Shear force (kg)	3.8	3.6	3.7	3.6	3.7	3.7	0.1	0.38	0.35	0.41
Drip loss (%)	1.1	1.0	1.0	1.0	1.0	1.1	0.2	0.48	0.11	0.32
Cooking loss (%)	34.1	34.0	33.8	33.3	33.6	33.9	1.0	0.48	0.06	0.22
MDA (nmol/mg protein)	3.2	3.1	2.5	2.3	2.6	2.6	0.2	0.25	0.04	0.19

[a] $pH_{24\ h}$=pH determined at 24 h post-mortem; and MDA=malonaldehyde. Fulvic acid was obtained commercially (Yongye Nongfeng Bio-tech Co. Ltd., Inner Mongolia, China).
[b] L^*, a^*, and b^*=measured in triplicate (Minolta Chroma Meter, CR 301; Minolta, Tokyo, Japan); marbling=based on a 1–5 scale (NPPC, 2000); and shear force=determined using a digital shear apparatus (C-LM3; Northeast Agricutural Universtiy, Beijing, China).
[c] Pooled standard error of the mean.

ADG and G:F of finishing-growing pigs with increasing FA supplementation. However, Schuhmacher and Gropp (2000) and Trckova et al. (2006) reported pigs fed diets supplemented with FA had no effect on growth performance. The reason for inconsistency on the growth performance is not clear, and further investigation is needed.

Fulvic acid can improve nutrient absorption and accelerate digestive functions that can regulate growth and change the metabolism to enhance animal carcass traits. Kocabagli et al. (2002) and Ozturk et al. (2012) demonstrated that the body and carcass weights increased linearly with adding levels of HA substances in the broiler diet. But, many research reported that feeding supplemental FA or HA substances had no effect on slaughter weight, hot carcass weight, dressing percentage, or leaf fat percentage (Avci et al., 2007; Gong et al., 2011). Ji et al. (2006) and Wang et al. (2008) reported a reduction in backfat thickness in finishing pigs fed diets with 0.5% HA substances (27.8% or 16.8% FA, respectively). The effect supplementation with FA on carcass traits in the current study is consistent with previous reports. Wang et al. (2008) speculated that the effects of FA may be caused by the redistribution of proteins and lipids to improve carcass traits, which also may be associated with some biological active substances in FA (Trckova et al., 2005).

Animal muscle pH is neutral before slaughter, but that pH declines after slaughter because glycolysis causes lactic acid accumulation in muscles. The pH value reflects meat hydrolysis to affect drip loss and cooking loss (Hofmann, 1994). The drip loss and cooking loss volumes affect the flesh color and shear force to change tenderness of fresh meat (Huff-Lonergan et al., 2002). Van der Wal et al. (1988) reported that a greater drip loss leads to reduced tenderness, and greater drip loss and shear force may have an adverse effect on the water-holding capacity and tenderness of meat (Wang et al., 2012). The water-holding capacity is related to the lipid peroxides contents in the muscle (Macit et al., 2003; Schaefer et al., 1995). Lipid peroxidation is a natural phenomenon involved in the loss of unsaturated lipids by peroxidation, thus, causing lipid degradation and membrane disruption. The MDA is a terminal product of lipid peroxidation, thus, the content of MDA can be used to estimate the extent of lipid peroxidation (Del-Rio et al., 2005). Beer et al. (2000) reported that the FA has a stimulatory effect on the spontaneous contractile activity of smooth muscle tissues. Ozturk et al. (2012) demonstrated that meat L^* value was increased with increasing levels (0.05%, 0.1%, and 1.5%) of HA substances supplementation. The current study showed that pigs fed different levels of FA supplementation

Table 5
Estimation of optimum supplementation of pig diets with fulvic acid.[a]

Item[b]	Estimated optimum (%)[c]	95% confidence interval (%)[d]	*P*-value	R^2
Quadratic broken-line analysis				
ADG (kg)	0.79 ± 0.18	0.54–1.13	0.002	0.833
G:F (kg/kg)	0.53 ± 0.07	0.33–0.76	0.031	0.834
Backfat thickness (cm)	0.50 ± 0.13	0.22–0.93	0.004	0.922
MDA (nmol/mg protein)	0.48 ± 0.15	0.32–1.04	0.042	0.833
Linear broken-line analysis				
ADG (kg)	0.25 ± 0.20	0.14–0.65	0.400	0.816
G:F (kg/kg)	0.25 ± 0.20	0.14–0.65	0.390	0.443
Backfat thickness (cm)	0.24 ± 0.16	0.16–0.56	0.258	0.438
MDA (nmol/mg protein)	0.24 ± 0.14	0.14–0.52	0.198	0.310

[a] ADG = average daily gain; G:F = gain to feed ratio; and MDA = malonaldehyde.
[b] ADG and G:F = based on the entire feeding period.
[c] Quadratic broken-line model: $Y=L+U\times(R-x)\times(R-x)$, where L is the ordinate, R is the abscissa of the breakpoint, and the value $R-x$ is zero at values of $x>R$; linear broken-line model: $Y=L+U\times(R-x)$, where L is the ordinate, R is the abscissa of the breakpoint, and the value $R-x$ is zero at values of $x>R$.
[d] 95% confidence interval of the dietary supplemented with fulvic acid.

had a linear or quadratic effect on MDA content, a^* and b^* values, and marbling scores. These finding were consistent with the previous studies of FA supplementation in finishing pigs (Ji et al., 2006; Wang et al., 2008). It appears that FA may be associated with meat color, but the precise mechanism underlying this effect remains unclear. Berg (2001) and Zhang et al. (2002) found that suitable nutritional levels of Fe or Cu may influence pork color. Aiken et al. (1985) and Wang et al. (2010) reported that HA substances contain minute quantities of several minerals, including Fe, Mn, and Cu. This may be a reason for changes in meat color. The effect of tenderness, drip loss, and cooking loss were hardly found in pigs fed diets supplemented with FA. There were no linear, quadratic, or cubic effect with increasing level of FA supplementation in the current study. However, Gong et al. (2011) found HA substances had a passive effect, regarding water retention capacity in broilers. The inconsistent results may be attributed mostly to the composition of different HA substance preparations and levels, as well as the different species and ages of animals used in different studies.

5. Conclusion

Based on the results of this study, it is concluded that dietary supplemental FA can improve growth performance, carcass traits, and meat quality in growing-finishing pigs. The broken-line analysis indicated that the optimum level of supplementation for FA is 0.48–0.79% for a improvement in G:F and reductions of backfat thickness and MDA content.

Conflict of interest statement

None of the authors of the above manuscript has declared any conflict of interest statement.

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