Effects of dietary supplementation of fulvic acid on lipid metabolism of finishing pigs

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Effects of dietary supplementation of fulvic acid on lipid metabolism of finishing pigs[1]

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ABSTRACT: The experiment was conducted to investigate the effects of dietary supplementation of fulvic acid on lipid metabolism of finishing pigs. One hundred eighty crossbred barrows (Landrace × Yorkshire, 60 ± 2.5 kg) were randomly allotted to 5 dietary treatments (36 pigs/treatment) and fed a basal diet supplemented with 0, 0.2%, 0.4%, 0.6%, and 0.8% fulvic acid for 42 d. Thirty pigs (6 pigs/treatment) were slaughtered at the end of the experiment. Blood samples and adipose tissue were collected for determination of blood parameters and lipid metabolic enzymes. The results showed that compared with the control group, dietary supplementation of 0.2%, 0.4%, and 0.6% fulvic acid significantly reduced mean backfat thickness of pigs ($P < 0.05$). The serum concentrations of low-density lipoprotein, leptin, growth hormone, insulin, and triiodothyronine were significantly increased by adding fulvic acid in diets ($P < 0.05$). With the raised concentration of dietary fulvic acid, hormone sensitive lipase (HSL) activity was significantly increased ($P < 0.05$), and lipoprotein lipase (LPL) activity was significantly decreased ($P < 0.05$) in adipose tissue. In conclusion, dietary supplementation of fulvic acid reduced the mean backfat thickness of pigs. This change related to the increased activity of HSL and the decreased activity of LPL in adipose tissue.

Keywords: blood parameter, finishing pig, fulvic acid, hormone, lipid metabolism

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INTRODUCTION

Humic substances (**HS**) are very common organic materials that can be found in lignite, turf, soil, water, etc. (Andersen et al., 2005; Rashid and King, 1969). They are produced from the organic materials of dead plants and animal tissues by microbial activity (Herzig et al., 2001). The HS have been used as an antidiarrheal, analgesic, immunostimulatory, and antimicrobial agent in veterinary practices in Europe (Huck et al., 1991). In addition, HS can improve the economy and ecology of animal production by increasing growth rate and improving feed efficiency and immunity, as well as diminishing the risk of disease (Islam et al., 2005; Tohid et al., 2010; Vucskits et al., 2010).

The two most active fractions of HS are humic acid (**HA**) and fulvic acid (**FA**). Fulvic acid can be extracted from HS and contains many reactive functional groups, including carboxyls, hydroxyls, carbonyls, phenols, quinones, and semiquinones (Aiken et al., 1985). These reactive groups make FA a candidate for both metal chelating and antioxidant activity (Glynn, 1995; Plaza et al., 2005). The FA is soluble in both acid and alkali solutions. Fulvic acid also has lower molecular weight and greater biological activity compared with other HS. Many reports indicated that the FA formed a film on the mucus epithelium of the gastrointestinal tract, protected against infections and toxins, and improved utilization of nutrients in animal feed (Islam et al., 2005; Trckova et al., 2005). Kunavue and Lien (2012) reported that dietary supplementation with FA improved feed efficiency and immunity as well. Recently, the positive effect of FA on meat quality in growing-finishing pig has been reported by Bai et al. (2013).

Using FA as an alternative antimicrobial feed additive in animal production is still in its infancy. The finishing stage is the most important stage of lipid metabolism. However, application of FA in finishing pig diets has not been well studied. Therefore, this research was conducted to investigate the effects of FA on lipid metabolism in finishing pigs.

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MATERIALS AND METHODS

The protocols used in this experiment were approved by the Northeast Agricultural University Institutional Animal Care and Use Committee.

Animals and Treatments

One hundred eighty crossbred barrows (Landrace × Yorkshire) with an average initial BW of 60 ± 2.5 kg were randomly allotted to 5 dietary treatments. Each treatment consisted of 6 pens with 6 pigs per pen. Treatments included a basal diet, or the basal diet supplemented with 0.2%, 0.4%, 0.6%, or 0.8% FA (Inner Mongolia Yongye Nongfeng Biotechnology Co. Ltd, Hohhot, China). The basal diet was formulated to meet or exceed the nutrient requirements of finishing pigs (NRC, 2012) as shown in Table 1. The feeding experiment lasted for 42 d following a 1 wk adaptation period. Each pen was equipped with a dry feeder. The feed and water were provided ad libitum throughout the trial period.

Sample Collection

At the end of the study, 30 pigs (6 pigs/treatment) weighing approximately 90 kg were selected. Blood samples were obtained by anterior vena cava puncture using anticoagulant-free vacuum tubes. After samples were kept at 37°C for 30 min and centrifuged at 3,000×g for 15 min, serum were collected and stored at −20°C for subsequent analysis. Pigs were slaughtered following procedures of GB/T 17236–2008 at the abattoir of Henan province. The pigs were stunned by electrical shock, exsanguinated, dehaired, and eviscerated. The head was removed, and the carcass was split longitudinally. Adipose tissue samples were taken from the dorsal subcutaneous depot at the 10th rib of the carcass, flash frozen using liquid N_2, and stored at −70°C.

Serum Analysis

The concentrations of triglyceride (**TG**), high-density lipoprotein (**HDL**), glucose (**GLU**), and low-density lipoprotein (**LDL**) were measured with corresponding commercial kits (Nanjing Jiancheng Biochemical Reagent Co., Nanjing, China) following the recommended procedures. The concentrations of insulin (**INS**), growth hormone (**GH**), triiodothyronine (**T3**), thyroxine (**T4**), and leptin were analyzed using commercially available ELISA kits (R&D SYSTEMS®, Minneapolis, MN) in a microplate reader (TECAN 8500; Tecan Group Ltd., Männedorf, Switzerland).

Table 1. Composition and nutrient levels of basal diets (as-fed basis)[1]

Item	Basal diet
Ingredient, %	
Corn	65.00
Soybean meal	17.20
Wheat bran	15.00
$CaHPO_4$	0.40
Limestone	1.00
Salt	0.30
Premix[2]	1.00
Choline chloride (50%)	0.10
Total	100.00
Nutritional composition,[3] %	
Digestible energy (MJ/kg)	3.24
Crude protein	14.82
Crude fat	3.20
Crude fibre	3.07
Nitrogen Free Extract	60.10
Calcium	0.52
Phosphorus	0.19
Lysine	0.72
Methionine	0.23

[1]Basal diet formulated according to feeding standard of the NRC.

[2]The premix provides following for per kg diet: vitamin A, 8,000 IU; vitamin D_3, 2,000 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; Se ($NaSeO_3 \cdot 5H_2O$), 0.2 mg.

[3]Nutrient levels were calculated values.

Enzyme Assays

The subcutaneous adipose tissues homogenates (10%) were prepared in chilled normal saline for enzyme analysis. The activities of fatty acid synthase (**FAS**), lipoprotein lipase (**LPL**), and hormone-sensitive lipase (**HSL**) in subcutaneous adipose tissue were measured with ELISA kits (R&D SYSTEMS, Minneapolis, MN) in a microplate reader (TECAN 8500; Tecan Group Ltd., Männedorf, Switzerland).

Statistical Analysis

The data were tested by ANOVA between the treatment groups and control group with Statistical Packages for Social Science 18.0 (SPSS 18.0) software. Duncan's multiple-range, linear, and quadratic tests were performed. The results were expressed as arithmetic means and standard error of means (**SEM**), and differences were considered significant when $P < 0.05$.

Table 2. Effect of fulvic acid (FA) levels on carcass characteristics of finishing pigs

	FA (%)						*P*-value		
Item	0	0.2	0.4	0.6	0.8	SEM	Duncan	linear	Quadratic
No. of pigs	6	6	6	6	6				
Mean back fat thickness, cm	2.21[a]	1.85[b]	1.82[b]	1.90[b]	2.04[ab]	0.04	0.01	0.65	0.08
Leaf lard percentage, %	1.52	1.40	1.42	1.43	1.41	0.01	0.07	0.30	0.40

[a–c]Means in the same row with different superscripts differ ($P < 0.05$).

RESULTS

Carcass Characteristics

The effects of FA on carcass traits are shown in Table 2. Dietary supplementation of 0.2%, 0.4%, and 0.6% FA significantly reduced mean backfat thickness ($P < 0.05$). There was no difference in leaf lard percentage among dietary treatment ($P > 0.05$).

Blood Metabolites

The effects of FA on serum metabolites are presented in Table 3. Diets with 0.2% and 0.4% FA significantly increased serum concentrations of LDL ($P < 0.05$) compared with the control group. There was no linear relationship with the increase of dietary FA between the treatment groups. The serum concentrations of GLU, TG, and HDL were not significantly affected by adding FA to the diet.

Endocrine Parameters in Serum

The effects of FA supplementation on endocrine parameters are shown in Table 4. Compared with the control group, the concentrations of GH were significantly increased with supplementation of 0.2%, 0.4%, and 0.6% FA in diets ($P < 0.05$). In addition, the concentrations of INS and T3 were significantly increased with dietary supplementation of 0.2% and 0.4% FA ($P < 0.05$). The concentrations of leptin were significantly increased in FA groups ($P < 0.05$) without linear relationships, while the concentrations of T4 were significantly decreased in these FA groups ($P < 0.05$).

Activities of Lipid Metabolism Enzymes

The effects of FA supplementation on the activities of lipid metabolism enzymes in the subcutaneous adipose tissue are presented in Table 5. The HSL activity in FA-fed pigs was significantly higher than the control ($P < 0.05$). There was a quadratic relationship within the treatment groups. The FAS activity was similar among dietary treatments ($P > 0.05$). The activity of LPL was significantly decreased with the addition of FA ($P < 0.05$) compared with the control group, but there was no linear relationship among dietary treatments.

DISCUSSION

Dietary supplementation of 0.2%, 0.4%, and 0.6% FA significantly reduced the mean backfat thickness of pigs. In agreement with our results, Wang et al. (2008) reported that inclusion of either 5% or 10% HS significantly decreased backfat thickness ($P < 0.05$). In the present experiment, leaf lard percentage (leaf fat weight) tended to decrease with the increased concentrations of FA. Similarly, Ozturk et al. (2012) found that dietary supplementation with 1.5 HS reduced abdominal fat rate of broilers compared to 0 HS. Besides, FA is abundant and cheap. El-Husseiny et al. (2008) indicated that dietary supplementation of HS achieved economical efficiency better than the control or those that received antibiotic growth promoter.

Fat deposition is determined by a complex balance between lipogenic and lipolytic enzymes. The FAS is considered to be the key factor in regulating the synthesis of fatty acids in lipid metabolism, and it catalyzes the last step in the lipogenic pathway. HSL is regarded as an important enzyme catalyzing the hydrolysis of TG

Table 3. Effect of fulvic acid (FA) levels on blood metabolites of finishing pigs[1]

	FA(%)						*P*-value		
Item	0	0.2	0.4	0.6	0.8	SEM	Duncan	Linear	Quadratic
No. of pigs	6	6	6	6	6				
GLU, mmol/L	5.74	5.40	5.34	5.53	5.69	0.06	0.30	0.97	0.09
TG, mmol/L	0.33	0.34	0.35	0.34	0.35	0.01	0.85	0.14	0.33
HDL, mmol/L	1.49	1.50	1.48	1.51	1.47	0.01	0.57	0.62	0.73
LDL, mmol/L	1.33[c]	1.47[ab]	1.45[ab]	1.40[bc]	1.35[c]	0.02	0.01	0.90	0.18

[1]Abbreviations: GLU = glucose, TG = triglyceride, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

[a–c]Means in the same row with different superscripts differ ($P < 0.05$).

Table 4. Effect of fulvic acid (FA) levels on endocrine parameters of finishing pigs[1]

	FA(%)						*P*-value		
Item	0	0.2	0.4	0.6	0.8	SEM	Duncan	Linear	Quadratic
No. of pigs	6	6	6	6	6				
GH, ng/mL	3.04^{b}	3.27^{a}	3.29^{a}	3.25^{a}	3.15^{b}	0.03	0.05	0.62	0.06
INS, mIU/L	34.97^{c}	38.38^{a}	37.17^{ab}	35.40^{bc}	36.77^{abc}	0.38	$P < 0.01$	0.91	0.80
Leptin, ng/mL	6.94^{c}	8.20^{ab}	8.53^{a}	7.98^{b}	8.47^{a}	0.12	$P < 0.01$	0.19	0.26
T4, ng/mL	91.90^{a}	77.83^{c}	77.91^{c}	86.55^{b}	84.87^{b}	1.17	0.04	0.85	0.43
T3, ng/mL	1.84^{c}	2.02^{ab}	2.08^{a}	1.93^{abc}	1.87^{bc}	0.03	$P < 0.01$	0.93	0.16

[1]Abbreviations: GH = growth hormone, INS = insulin, T3 = thyroid hormone, T4 = thyroxine.

$^{a–c}$Means in the same row with different superscripts differ ($P < 0.05$).

in adipose tissue and adjusting the disposal of TG in the body. LPL is described as the "metabolic gatekeeper" (Zechner, 1997). The LPL is a critical determinant of plasma TG clearance, which influences uptake performance of tissue to fatty acids. The activity of LPL can be measured for the extent of fat deposition and determine the bulk of fat in the cell (Zechner, 1997). FAS, HSL, and LPL are abundant in adipose and other tissues containing lipid. Therefore, the activities of these enzymes clearly indicate the changes in lipid metabolism. In this study, HSL activity was significantly higher in the FA groups than the control group, but FAS activity was not affected by dietary treatments. The results suggested that FA increased lipolysis without affecting fatty acid synthesis. Meanwhile, the activity of LPL was significantly decreased in the FA groups compared to the control group. Adipose tissue relies on plasma TG as an important source of fatty acids for subsequent storage. Utilization of plasma TG is dependent on LPL. Therefore, uptake of TG by adipose tissue was decreased, and backfat thickness was significantly decreased in FA-fed finishing pigs. Besides, the serum LDL concentration was also significantly higher in the FA groups than the control group. The result suggested that FA exerts a significant effect on the transport of TG in serum. Plasma TG is packaged into the TG-rich lipoproteins chylomicrons and LDL. Thus, the concentrations of TG and LDL tended to increase in serum with the decreased activity of LPL in adipose tissue, but the change of TG was not significant. Similar to our result, Yalçın et al. (2006) and Avci et al. (2007) reported that serum TG concentration was not affected by the supplementation of HS.

Lipid metabolism is a complex metabolic process and is regulated by a variety of hormones. GH increases fatty acid mobilization (Goodman and Knobil, 1961) and reduces carcass lipid accretion (Evock-Clover et al., 1992). There is a negative correlation between the serum concentrations of GH and the backfat thickness in finishing pigs (Althen and Gerrits, 1976). Furthermore, GH has the capability of quickly increasing plasma GLU and INS in pigs (Gopinath and Etherton, 1989). The INS regulates lipid metabolism by affecting enzyme activity (Farese et al., 1991). INS can enhance both the activity and mRNA expression of LPL in adipose tissue and can stimulate changes in LPL mRNA stability (Raynolds et al., 1990). Leptin could inhibit fat synthesis in fat cells, increase the expression of HSL, and regulate LPL activity in adipose tissue. On the other hand, leptin could also promote mitochondrial uptake and oxidation of fatty acids to promote fat decomposition (Sarmiento et al., 1997). Thyroid hormone has an effect on lipid metabolism in the synthesis, mobilization, and decomposition of lipids. Thyroid hormone can promote degradation and inhibit oxidation of LDL by increasing the number and activity of LDL receptors on the cell membrane. Besides, thyroid hormone can activate HSL, promoting the mobilization of fat and the oxidation of fatty acids (Costantini et al., 1998). Thus far, there is a gap in the information about the effect of FA on lipid metabolism associated with hormones. To determine whether FA stimulates changes of hormones in finishing pigs, serum hormones were measured in this study.

The results show that the concentrations of GH and leptin in serum are significantly increased in FA-fed pigs

Table 5. Effect of fulvic acid (FA) levels lipogenic and lipolytic enzymes of finishing pigs[1]

	FA(%)						*P*-value		
Item	0	0.2	0.4	0.6	0.8	SEM	Duncan	Linear	Quadratic
No. of pigs	6	6	6	6	6				
FAS, nmol/mL	1.11	1.14	1.07	1.08	1.05	0.01	0.31	0.10	0.33
LPL, IU/mL	2.56^{a}	2.24^{bc}	2.16^{bc}	2.19^{bc}	2.13^{c}	0.04	$P < 0.01$	0.09	0.09
HSL, IU/mL	1.27^{c}	1.46^{a}	1.49^{a}	1.48^{a}	1.41^{ab}	0.02	$P < 0.01$	0.36	0.04

[1]Abbreviations: FAS = fatty acid synthase, LPL = lipoprotein lipase, HSL = hormone-sensitive lipase.

$^{a–c}$Means in the same row with different superscripts differ ($P < 0.05$).

compared with pigs fed the control diet. Moreover, the levels of T3 and INS in serum were also markedly increased after adding FA to diets. However, the T4 concentration was significantly decreased in diets with FA. The results indicated that the effect of FA on lipid metabolism in pigs may be due to the regulation of hormones.

As an anabolic hormone, INS could promote the uptake of GLU in tissues. The serum INS concentration is increased because GH decreases INS sensitivity in adipocyte (Louveau and Gondret, 2004). Many studies indicate that the serum INS level is promoted by GHRH and GH treatment in pigs (Klindt et al., 1995). Leptin specifically weakens the effect of INS on adipose tissue (Margetic et al., 2002). In the present experiment, the serum INS concentration was significantly increased with supplemental FA. Interestingly, the fat deposition was not enhanced. That may be explained by increased concentrations of GH and leptin in serum. Meanwhile, leptin increased HSL activity and decreased LPL activity in adipose tissue. Thus, fat deposition was markedly reduced in adipose tissue mass in FA-fed finishing pigs. However, the concentration of FAS was not affected in this study. This finding may be due to decreased activities of other lipogenesis enzymes caused by hormones. In general, thyroid hormones and leptin predominantly regulate energy mechanisms in animal bodies. Leptin promotes energy consumption of brown adipose tissue and muscle and reduces the energy stored in white adipose tissue (Baile et al., 2000). Thyroid hormones increase the number of mitochondria and the activity of metabolic enzyme in muscle (Winder, 1979). Therefore, dietary FA might increase the energy consumption of brown adipose tissue and muscle and decrease the energy store in white adipose tissue by increasing the concentrations of thyroid hormones and leptin in serum. Based on these results, supplemental dietary FA can significantly decrease the thickness of backfat in finishing pigs by regulating hormones levels.

Conclusion

The current study showed that dietary supplementation with FA decreased backfat thickness of finishing pigs. The reduction of backfat thickness by FA was related to the increased activity of HSL and the decreased activity of LPL in adipose tissue. Considering the rich resources and low price, dietary supplementation of FA can benefit the pig industry by reducing backfat thickness.

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