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Effect of *Tremella fuciformis* ferment substance on the growth performance and lipid metabolism of finishing pigs*

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ABSTRACT

An experiment was conducted to study the effects of *Tremella fuciformis* ferment substance (TFS) supplementation in diets on the growth performance, fat deposition, blood lipids, and mRNA expression of lipid storage-related enzymes in the liver and adipose tissue of finishing pigs. Twenty eight-week-old pigs (10.03±0.59 kg) were randomly allocated to two dietary treatments: maize-soyabean meal-based diets with (TFS group) and without TFS (control group). The results of the experiment indicate that compared with the control group, the TFS diet led to a significant increase in the average daily gain and feed conversion ratio of pigs weighing 10 to 100 kg. However, the average backfat depth and leaf lard weight were not affected by TFS. Ingestion of TFS specifically decreased the serum triglyceride and glucose concentrations, but did not change the levels of total cholesterol and free fatty acids in the serum. The mRNA expressions of fatty acid synthase (FAS) and acetyl CoA carboxylase (ACCα) in the liver were down-regulated by dietary TFS. Conversely, the gene expressions of FAS and ACCα in the adipose tissue increased. The mRNA level of carnitine palmityl transferase-I (CPT-I) in adipose tissue was also increased by TFS. These results suggest that the addition of TFS at a dose of 4 g kg^{-1} improves growth performance and lowers lipid metabolism in finishing pigs.

KEY WORDS: *Tremella fuciformis* ferment substance, growth performance, fat deposition, blood lipids, fatty acid synthase, acetyl CoA carboxylase, pigs

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INTRODUCTION

Considerable attention has recently been focused on the testing of the potency of growth promotants in altering lipid metabolism. This concern has been triggered by the suggestion of the WHO that excess fat deposition is undesirable in the human body and can possibly result in fatal diseases, such as atherosclerosis. Nowadays, consumers are well aware of this fact and prefer lean meat. In pig production, some researchers have paid attention to the effects of prebiotics (as an alternative to feed antibiotics) on the blood lipid content and fat deposition of pigs. Grela et al. (2001) reported that backfat thickness over the shoulder and leaf fat weight were significantly reduced in growing-finishing pigs fed with diets supplemented with mannanoligosaccharide (5 g kg^{-1} of diet). Zhou et al. (2007) reported that a dose of 5 g kg^{-1} chitosan added to the diet decreased the serum total cholesterol level and average backfat thickness of finishing pigs. These findings indicate that some prebiotics can decrease the serum lipids levels and fat deposition of pigs. The mechanisms of lipid lowering in response to prebiotics (fructooligosaccharide and inulin) have been proposed in rats and humans. The triacylglycerol-lowering action of oligofructose is due to a reduction in *de novo* fatty acid synthesis in the liver through the inhibition of lipogenic enzymes,namely, acetyl-CoA carboxylase (ACCα) and fatty acid synthase (FAS) (Delzenne and Kok, 1999; Letexier et al., 2003; Kang et al., 2006).

For centuries, natural medicinal products from fungi have been used as feed additives for farm animals in China. These natural medicinal products show many bioactivities, such as antimicrobial action, immune enhancement (Wang et al., 1998) and blood lipid reduction (Shen and Chen, 1989). Therefore, they are now recognized to possess prebiotic effects. Shen and Chen (1989) reported that *Tremella fuciformis* spore polysaccharide lowered the levels of total cholesterol, free cholesterol and triglyceride in the serum of rats with hyperlipidaemia. In weanling pigs, TFS with polysaccharides as the most important components improved growth performance, and 4 g kg^{-1} was the optimal inclusion level (Zou, 2006; Wu, 2007; Fu et al., 2009). However, the effects of TFS as an alternative of feed antibiotics on the performance, blood lipid and fat deposition in finishing pigs have not been studied well to date. Whether TFS affects the mRNA levels of lipid storage-related enzymes in the liver and adipose tissue of finishing pigs also remains unknown.

The present experiment was conducted to examine the effects of TFS on the growth performance, carcass traits, blood lipid, and mRNA levels of FAS, ACCα, hormone-sensitive lipase (HSL) and carnitine palmityl transferase-I (CPT-I) in the liver and subcutaneous fat tissue of finishing pigs.

MATERIAL AND METHODS

Animals and diets

After feeding 20 weanling (4-week old, 6.83±0.52 kg) DLY (Landrace × Yorkshire × Duroc) piglets, a non-antibiotic diet for 4 weeks (10.03±0.59 kg), they were randomly divided into 2 dietary treatment groups with 10 replicate pens per treatment and 1 pig per pen: 1. control diets containing no antibiotic (control group), and 2.4 g kg^{-1} *Tremella fuciformis* ferment substance (TFS) added to the control diets (TFS group). All diets were formulated according to NRC (1998) requirements for pigs. The compositions of the diets are shown in Table 1. All pigs had free access to water and feed. Feed intake and body weights were recorded every two weeks. The environmental temperature in the building for piglets

Table 1. Formulation and calculated nutrient content of basal diets, air-dry basis

Item	10-20 kg	20-50 kg	50-80 kg	80-100 kg
Ingredients, %				
yellow maize	62.65	76.21	82.46	86.74
wheat bran	2.00	2.00	2.00	2.00
fish meal	5.00	2.00	1.00	1.00
soyabean meal	22.00	17.00	12.00	8.00
whey powder	5.00	-	-	-
soyabean oil	1.00	-	-	-
calcium carbonate	0.90	1.05	0.91	0.95
dicalcium phosphate	0.10	0.50	0.47	0.23
L-lysine-HCl	0.35	0.26	0.23	0.19
DL-methionine	0.08	0.01	-	-
L-threonine	0.05	0.03	0.02	-
salt	0.20	0.30	0.30	0.30
choline chloride	0.10	0.10	0.08	0.06
vitamin premix[1]	0.05	0.04	0.03	0.03
sweetening agent	0.02	-	-	-
mineral premix[2]	0.50	0.50	0.50	0.50
Calculated nutrient content, %[3]				
crude protein	18.98	15.57	13.32	11.94
digestible energy, MJ/kg	14.02	13.77	13.81	13.84
TID lysine	1.17	0.83	0.66	0.55
TID methionine + TID cysteine	0.64	0.47	0.40	0.37
TID threonine	0.70	0.52	0.43	0.36
TID tryptophan	0.20	0.15	0.12	0.11
Ca	0.70	0.66	0.55	0.50
non-phytate P	0.32	0.25	0.21	0.16

[1] content per kg of premix: IU: vit. A 10 000 000; vit. D_3 2 000 000; vit. E 25 000; mg: vit. B_{12} 30; riboflavin 16 000; niacin 35 000; pantothenic acid 25 000; menadione 5 000; [2] content per kg of diet: mg: Cu (as copper sulphate) 5; Fe (as ferrous sulphate) 80; Mn (as manganese oxide) 3; (zinc as zinc oxide) 80; Se (as sodium selenite) 0.25; I (as potassium iodide) 0.14; [3] calculated values based on feed composition and the true ileal digestibility (TID) data of NRC (1998)

(10-20 kg) and growing pigs (20-50 kg) ranged from 22 to 28°C, and that for finishing pigs (50-100 kg) ranged from 15 to 22°C. The trial was conducted in the pig farm of Sichuan Agricultural University (Ya'an, China), and all experimental procedures and housing were approved by the Sichuan Province Committee on Laboratory Animal Care.

TFS preparation

The TFS was provided by Sichuan Habio Bioengineering Co., Ltd., Sichuan (China). After the *T. fuciformis* berk spore was fermented, the fermenting liquor was mixed with a carrier (bran), and then the mixture was dried and ground into powder. The powder was TFS, which contained 5-6×10^8 *T. fuciformis* berk spore per gram and 56.7% polysaccharides.

Sample collection

Each pig was removed from the test, bled, and slaughtered at approximately 100 kg liveweight. Blood was taken from the overnight-fasted pig *via* the anterior vena cava, and then serum was prepared by centrifuging the whole blood at 1500 *g* for 15 min at 4°C. The liver and subcutaneous fat in the back were rinsed in ice-cold phosphate buffer solution (pH 7.2), minced with scissors, frozen in liquid nitrogen, and stored at -80°C prior to analysis.

Body fat and carcass quality data collection

After slaughter, the carcass weights were recorded, and backfat opposite the first rib, last rib and last lumbar vertebra were measured to calculate the average backfat thickness. The cross-section of the *Longissimus* muscle (LM) in the right side of each carcass was determined using a compensating planimeter. Leaf lard weight was also recorded.

Measurement of serum lipid and glucose

The concentrations of triglyceride, total cholesterol and glucose in the serum samples were analysed by SHIMADZU CL8000 Clinical Chemistry Analyser using colorimetric method (enzyme) following the manufacturer's instructions (NingBo RuiYuan Biotechnology Co., Ltd., Zhejiang, China). Serum free fatty acid concentrations were measured by a spectrophotometer (148-4 ZDSYS-04) using colorimetric method (chemistry) following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

RNA isolation and quantitative Real-Time PCR

RNA samples were isolated from the frozen subcutaneous fat and liver (200 mg) from four pigs in each group using the RNAgents total RNA isolation system (Takara, Dalian, China). cDNA was synthesized using a reverse transcription kit (Takara, Dalian, China) from 1 μg total RNA. PCRs were performed with the Bio-rad Chromo 4 Real-Time PCR Detection System instrument and software (Opticon Monitor 3). The quantifications of mRNA were performed by reverse transcriptase-polymerase chain reaction (RT-PCR), as described in the RT-PCR system kit (Takara, Dalian, China). The primer sequences for the target and housekeeping genes (β-actin) are listed in Table 2.

Table 2. Primer sequences of genes

Gene	Primer (5'-3')	GenBank Accession number	Product size bp
FAS	F: CTACATCGAGTGCATCAGACAGG R: GAACAGGAAGAGGCTGTGGTT	EF589048	147
ACCα	F: GGTGATGGTCTATATCCCTCCTC R: GATTTCTACGGTCCCTTCTGGT	NM_001114269	147
HSL	F: GTGAAGGACAGGACAGTGAGG R: GAGGTAAGGCTCGTGGGATTT	AY686758.1	169
CPT-I	F: AAAGTCTGGGCTATCTGTGTCC R: GGCTGTATTCCTCGTCATCCA	NM_001007191	159
β-actin	F: TCGCACTTCATGATCGAGTTG R: CGACGGCCAGGTCATCAC	AY550069	138

FAS - fatty acid synthetase; ACCα - acetyl-CoA carboxylase α; HSL - hormone-sensitive lipase; CPT-I - carnitine palmityl transferase -I; F - forward primer; R - reverse primer

The RT-PCR assays were performed in 25 μl reactions containing gene-specific primers (100 nM), SYBR green, DNA, and water. The PCR conditions were 10 s at 95°C, followed by 40 cycles of two-step PCR denaturation at 95°C for 5 s and annealing extension at the annealing temperature of each primer for 30 s. Melt curves were analysed to confirm the specific amplification. The relative amount of the target genes' mRNA was normalized to β-actin mRNA levels. The data were analysed according to the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Data were expressed as means ± standard deviations (SD). The statistical significance of difference was analysed by independent-sample t-tests (SPSS 13.0 for windows; SPSS Inc., Chicago, Ill, USA). Statistical significance was set at $P<0.05$.

RESULTS

Under the condition that the pigs were slaughtered at the same liveweight, the incorporation of 4 g kg^{-1} TFS into the basal ration resulted in a 12% increase in ADG ($P<0.05$) and significant decreases in the days of experiment and feed intake/gain (F/G) ($P<0.05$). However, the difference in feed intake of the pigs in the two treatments was not significant ($P>0.05$) (Table 3).

Table 3. Effects of TFS on the growth performance of pigs

Item	Control group	TFS group
Initial body weight, kg	10.06 ± 0.67	10.00 ± 0.53
Final body weight, kg	100.34 ± 1.40	100.34 ± 2.30
Days of experiment	148 ± 10	133 ± 11[a]
ADG[1], g	612 ± 41	684 ± 63[a]
ADFI[2], g	1781 ± 123	1885 ± 130
F/G[3]	2.91 ± 0.10	2.76 ± 0.12[a]

[a] significantly different compared with the control group at $P<0.05$ by independent-sample t-tests; [1]ADG - average daily gain; [2]ADFI - average daily feed intake; [3]F/G - feed intake/gain

TFS had no effects on the dressing percentage, average backfat depth and leaf lard weight. However, the LM area was significantly ($P<0.05$) increased by the addition of TFS (Table 4).

Table 4. Effects of TFS on the carcass quality of pigs

Item	Control group	TFS group
Dressing percentage, %	71.79 ± 2.36	71.11 ± 2.31
Average backfat depth, cm	2.60 ± 0.26	2. 68 ± 0.17
Longissimus muscle area, cm^2	30.95 ± 3.15	34.34 ± 1.37[a]
Leaf lard weight, kg	1.46 ± 0.19	1.45 ± 0.15

[a] significantly different compared with the control group at $P<0.05$ by independent-sample t-tests

TFS significantly decreased the serum TG and glucose contents. However, there were no significant differences ($P>0.05$) in the serum TC and FFA contents between the control and TFS groups (Table 5).

Table 5. Effects of TFS on the serum lipids and glucose of pigs

Item	Control group	TFS group
TG[1], mmol/l	0.47 ± 0.12	0.39 ± 0.11[a]
TC[2], mmol/l	2.73 ± 0.48	2.80 ± 0.23
FFA[3], μmol/l	213.31 ± 46.77	233.94 ± 40.30
Glucose, mmol/l	5.56 ± 0.44	4.91 ± 0.69[a]

[a] significantly different compared with the control group at $P<0.05$ by independent-sample t-tests
[1]TG - triglycerides; [2]TC - total cholesterol; [3]FFA - free fatty acids

In the liver, TFS significantly ($P<0.01$) decreased the mRNA levels of FAS and ACCα, but its effects on these genes in the subcutaneous fat were opposite, with significant increases in the mRNA levels of FAS ($P<0.01$) and ACCα ($P<0.05$). The expressions of HSL and CPT-I in the liver were not significantly affected by TFS. However, the mRNA level of CPT-I in subcutaneous fat increased ($P<0.01$) (Table 6).

Table 6. Effects of TFS on the mRNA levels of FAS, ACCα, HSL and CPT-I in the liver and subcutaneous fat tissue of pigs

Item	Liver		Subcutaneous fat	
	control group	TFS group	control group	TFS group
FAS[1]	26.10 ± 6.03	10.77 ± 1.93[A]	56.61 ± 3.71	159.99 ± 44.44[A]
ACCα[2]	9.19 ± 2.51	4.44 ± 0.88[a]	15.30 ± 0.88	21.00 ± 1.93[A]
HSL[3]	0.03 ± 0.01	0.02 ± 0.01	3.76 ± 0.29	4.61 ± 0.81
CPT-I[4]	0.36 ± 0.07	0.27 ± 0.07	0.06 ± 0.01	0.17 ± 0.01[A]

[a] significantly different compared with the control group at $P<0.05$ by independent-sample t-tests
[A] significantly different compared with the control group at $P<0.01$ by independent-sample t-tests
[1] FAS - fatty acid synthetase; [2]ACCα - acetyl-CoA carboxylase α; [3]HSL - hormone-sensitive lipase; [4]CPT-I - carnitine palmityl transferase-I

DISCUSSION

In the present study, TFS had positive effects on the ADG and feed conversion of the pigs. Similar findings were reported in studies when TFS was added to weaned pig diets (Zou, 2006; Wu, 2007; Fu et al., 2009), and when *T. fuciformis* polysaccharide extract (Guo et al., 2004) and medicinal fungi fermentation product (Zhang et al., 2005) were added to poultry diets. The improved ADG observed in the present study was not completely due to the increased feed intake but more likely due to the improved feed conversion ratio.

Compared with the control group, TFS decreased the serum TG content in pigs, similar to the report of Hou et al. (2008). The results indicated that *Tremella polysaccharides* inhibited the absorption of lipids in the alimentary tract and decreased the levels of TG in the blood of rats fed with high-fat diets. Similarly, Liu et al. (2009) showed that fermented products of *Tremella aurantialba*, orally administered to hyperglycaemic mice for seven days, the TG in the serum was reduced, but the serum TC did not remarkably change. These results agree with the findings of studies on rats, which showed the net effect of non-digestible oligosaccharides on triacylglycerol and the moderate reduction in cholesterol concentration (Delzenne et al., 1993; Daubioul et al., 2000).

In the current study, TFS decreased the serum TG content and mRNA levels

of lipogenic enzymes (FAS and ACCα) in the liver of pigs. These findings are consistent with reports on reduced hepatic lipogenesis when oligofructose, levan and inulin were added to the diets of rats and humans (Delzenne and Kok, 1999; Letexier et al., 2003; Kang et al., 2006). This reduced lipogenic rate possibly plays a role in the triacylglycerol-lowering action of TFS. Contrary to the liver, TFS increased the mRNA levels of FAS and ACCα in subcutaneous fat tissue. Similarly, FAS activity was increased in the white adipose tissue but was decreased in the liver by oligofructose supplementation in rats (Agheli et al., 1998). However, Letexier et al. (2003) reported that the addition of inulin to a moderately high carbohydrate diet in humans reduced FAS, ACC1 and SREBP-1c mRNA concentrations in the liver, but had no effect on the expressions of these genes in adipose tissue. Adipose tissue lipogenesis was inversely correlated with hepatic lipogenesis, assessed either by measuring the mRNA levels of FAS (Shillabeer et al., 1992) or by using stable isotopes *in vivo* (Delzenne et al., 2001). They suggested that the regulation of FAS mRNA level in adipose tissue may depend on substrate availability rather than on plasma hormonal concentrations, and thus may differ from hepatic regulation (Agheli et al., 1998).

The increase in mRNA levels of FAS and ACCα in the adipose tissue of TFS-fed pigs cannot explain the absence of change in average backfat depth and leaf lard weight of these pigs. An alternative reason might be the regulation found in adipose tissue, which was simply a feedback mechanism or a compensatory response to the variations in plasma lipid levels (Agheli et al., 1998). Therefore, the changes in the expression of lipogenic genes were not consistent with the alteration of fat deposition in adipose tissue. The increased fatty acid mobilization from adipose tissue by lipolysis can be another reason. In the present study, the mRNA level of CPT-I was significantly increased by TFS, although the expression level of CPT-I was lower than those of FAS and ACCα. These results suggest that increased lipolysis may partly neutralize increased lipogenesis. However, the mRNA expression of HSL was not significantly affected by TFS, which was consistent with the results when levan was added to the diets of rats and when short-chain fructooligosaccharides were fed to dogs (Kang et al., 2006; Respondek et al., 2008).

The mechanism of action of TFS on lipid metabolism is not well known. The modifications of glucose and insulin concentrations, which control the hepatic expression of lipogenic genes, may be one of the reasons, as demonstrated during the administration of inulin-type fructans and oligofructose in rats (Kok et al., 1996; Delzenne and Kok, 1999). In the present study, the glucose concentration in the serum was decreased by TFS. The result was in full agreement with a previous study on hyperglycaemic mice (Liu and Yu, 2009). The fermentation end products, the short-chain fatty acids (SCFAs), in the caeco-colon can also be involved in the mechanism of action of TFS on lipid metabolism. The effect of TFS on SCFA

concentrations in blood was not determined in the current research. However, according to an *in vitro* study, *T. fuciformis* extract was rapidly degraded and became highly fermentable with an increase in the total SCFA production (Guo et al., 2003). SCFAs, such as acetate and propionate, are end products of the bacterial fermentation of TFS, which reach the liver through the portal vein. Acetate is a lipogenic substrate, and propionate is an effective inhibitor of lipid synthesis in isolated hepatocytes (Wright et al., 1990; Demigne et al., 1995; Daubioul et al., 2002).

CONCLUSIONS

The addition of *Tremella fuciformis* ferment substance (TFS) to the diets of pigs improved growth performance, lowered plasma triglycerides and glucose concentrations, decreased the mRNA levels of fatty acid synthetase (FAS) and acetyl-CoA-carboxylase (ACCα) in the liver, and increased the expressions of FAS, ACCα and carnitine palmityl transferase-I in subcutaneous fat tissue. These results support the hypothesis that TFS has a lowering effect on the lipid metabolism in finishing pigs.

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