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Effects of *Tremella fuciformis* extract on growth performance, biochemical and immunological parameters of weaned piglets challenged with lipopolysaccharide

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ABSTRACT

Context. In-feed antibiotics are commonly used to improve growth and gut health of weaning pigs. Due to anti-microbial resistance by extensively using antibiotics, however, in-feed antibiotics have been banned in Europe and China. *Tremella fuciformis* is a traditional edible fungus in China. Recent studies have found that *Tremella fuciformis* extract (TFE) has anti-inflammatory, anti-cancer and immune-modulatory functions. Therefore, there is the potential to develop *Tremella fuciformis* as an alternative to antibiotics. **Aims.** The study was performed to explore the effects of TFE on growth performance, and biochemical and immunological parameters of weaned piglets under lipopolysaccharide (LPS) challenge. **Methods.** Forty-eight weaned piglets were assigned into two groups with six pens (four piglets per-pen), receiving a control diet or a control diet with 400 mg/kg TFE (TFE), respectively. After 28 days of the trial, two piglets per pen were selected to be injected with LPS (50 μg/kg of BW) or an equivalent amount of sterile saline. Blood samples were collected at 0 and 3 h after LPS challenge. **Key results.** The results showed that TFE supplementation significantly increased the average daily gain ($P < 0.05$) and decreased the faecal score ($P < 0.05$) during the first week, improved the feed conversion ratio ($P < 0.05$) and BWt gain ($P < 0.05$) during the whole period. Piglets fed the TFE diet had higher plasma levels of white blood cells ($P < 0.05$) than that of piglets fed the control diet diet before the LPS challenge. Regardless of the dietary treatment, the LPS challenge significantly decreased the level of white blood cells, and increased the levels of red blood cells, haemoglobin, haematocrit, total protein, interleukin-1β and tumour necrosis factor-α (all $P < 0.05$). Regardless of the LPS challenge, however, the concentrations of total protein, interleukin-1β and tumour necrosis factor-α were decreased (all $P < 0.05$) in the plasma of piglets fed the TFE diet compared with the control diet diet. **Conclusions.** In summary, the supplementation of TFE in the weaning diet could improve the growth performance and immunity of piglets. **Implication.** TFE could be used as a bioactive substance for improving growth and immune response in pig production.

Keywords: growth, immune, inflammation, lipopolysaccharide, pigs, polysaccharides, *Tremella fuciformis* extract, weaning.

Introduction

Early weaning is one of the most stressful events that induces intestinal and immunological dysfunction, which impairs growth and immune responses (Pluske *et al.* 1997; Smith *et al.* 2010). It has been reported that polysaccharides from Chinese medicinal herbs could improve the growth performance and health status of piglets (Yuan *et al.* 2006; Kang *et al.* 2010).

Tremella fuciformis, classified as the order of the *Tremellales* and the family of the *Tremellaceae*, has been appreciated as an edible mushroom in Asia (Cho *et al.* 2006). The polysaccharides from *T. fuciformis* (TPS) have been regarded as the primary active component. For example, TPS could inhibit cyclophosphamide-induced leucopenia in

mice, and leucocytes increased in a dose-dependent fashion (Jiang *et al.* 2012). Nitric oxide synthase (iNOS), interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α were also upregulated by *Tremella fuciformis* extract (TFE) when cultured in RAW264.7 (Han *et al.* 2015). Meanwhile, TFE suppressed lipopolysaccharide (LPS)-induced production of iNOS and COX-2 in RAW264.7, and oral administration of TFE significantly inhibited LPS-induced production of IL-1β, IL-6 and TNF-α, and expressions of iNOS and COX-2 (Lee *et al.* 2016).

It has been found that the *Tremella fuciformis* polysaccharides was composed of α-D-mannose in the main chain, and β-D-xylose, β-D-gluconic acid and β-D-xylobiose linked to the C-2 of the main chain mannose (Yui *et al.* 1995). Another analysis on *Tremella fuciformis* polysaccharides, named TL04, was composed of (1→2)-and (1→4)-linked-mannose, and (1→3)-linked-glucans (Jin *et al.* 2016). D-Mannose has been proven to have beneficial effects on the immune system and against metabolic syndrome (Hu *et al.* 2016); in particular, D-Mannose could strongly inhibit the attachment of ST-10 *Salmonella typhimurium* to intestinal cells and offer a competitive binding site for this class of bacteria (Hu *et al.* 2016). Although the sugar composition and relative molecular ratios of *T. fuciformis* polysaccharides varied among studies, the bioactive effects are similar, such as anti-inflammatory and immunomodulatory effects (Wu *et al.* 2019). However, data are limited about the effects of *T. fuciformis extract* (TFE) on growth performance, immune response and blood biochemistry of weaned piglets.

Therefore, this study was performed to determine the effects of *Tremella fuciformis* extract on growth performance, and biochemical and immunological parameters of weaned piglets under LPS challenge.

Materials and methods

Preparation of TFE

The TFE was a mixture of *T. fuciformis* spore liquid fermentation broth and carrier wheat bran, which was produced by our laboratory. The preserved strains were streamed and inoculated on potato dextrose agar solid medium in a thermostatic chamber at 25°C for 4 days. A single colony was picked and inoculated in a 500-mL culture flask containing 200 mL of potato dextrose agar medium with an inoculation ring, and cultured in an incubator shaker at 25°C/220 rpm for 4 days to obtain the seed fungus liquid. The seed fungus liquid was inoculated in sterilised potato dextrose agar liquid fermentation medium with 10% inoculation amount and cultured at 25°C/220 rpm for 7 days until the fermentation broth contained up to 5×10^8 *T. fuciformis* spore per mL. The *T. fuciformis* spore liquid fermentation broth was mixed with wheat bran (SICHUAN Giastar Group) in a ratio of 5:1, dried at 60°C in a drying oven, then grounded into powder and screened through a 0.178-mm mesh screen.

Experimental design and animal management

As shown in Fig. 1, a total of 48 piglets (Duroc × Landrace × Yorkshire) weaned at 21 days (BWt 6.40 ± 0.17 kg) were randomly assigned into two groups, with six pens and four piglets per pen at 21 days, receiving a control diet (CON diet) or control diet with 400 mg/kg TFE (TFE diet) for a period of 28 days. Diets were formulated according to the nutrient requirements of swine recommended by the American National Research Council. The feed ingredients (SICHUAN Giastar Group) and nutrient composition of the experimental diets are shown in Table 1. Diets were fed in mash form throughout the experiment. On Day 29, after an overnight fast, two piglets (one challenged with LPS, the other treated with sterile saline) were selected according to their BWs near the average BW of each pen. No diarrhoea or other disease were observed. The selected piglets were intramuscularly injected with LPS (*Escherichia coli* serotype 055: B5, Sigma Chemical) at 50 μg/kg BW, and another piglet was injected with the same amount of sterile saline. The temperature of the feeding room was maintained between 26 and 30°C, and the humidity was maintained between 50 and 60%. The 12 h of light and 12 h of dark were provided in the stall. Piglets had free access to water and feed, and feed intake was recorded daily.

The experimental protocol was approved by the Ethics Committee of Feed Research Institute, Sichuan Agricultural University, China.

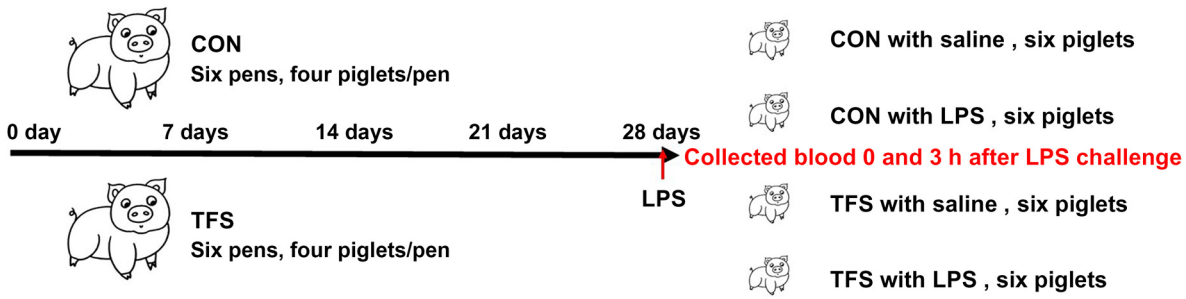

Fig. 1. Diagram of the study design. CON, control diet; TFE, control diet with 400 mg/kg *Tremella fuciformis* extract.

Table 1. Compositions and nutrient levels of the basal diet (air-dried basis).

Ingredients (%)		Nutrient contents (%)	
Corn	21.28	Digestible energy, MJ/kg	14.56
Rice	28	Crude protein	19.76
Extruded soybean, 46% crude protein	7	Total calcium	0.81
Soybean oil	2.5	Available phosphorus	0.59
Sucrose	2	SID Lys	1.36
Glucose	2	SID Met	0.45
Low protein whey powder, 2% crude protein	10	SID Thp	0.36
Dehulled soybean meal, 46% crude protein	17	SID Thr	0.8
Fish meal, 65% crude protein	5	SID SAA	0.74
Spray-dried plasma protein, 78% crude protein	2		
L-Lysine·HCL, 98%	0.4		
DL-Methionine, 98.5%	0.16		
L-Threonine, 98%	0.13		
Choline chloride, 50%	0.16		
$CaCO_3$	0.88		
$CaHPO_4$	0.75		
NaCl	0.4		
Mould inhibitor	0.1		
Mycotoxin absorbent	0.05		
Mineral mixture[A]	0.1		
Vitamin mixture[B]	0.04		
Aureomycin, 15%	0.05		
Total	100		

[A]Mineral premix provided per kg powder diet: Zn, 100 mg; Mn, 4 mg; Fe, 100 mg; Cu, 6 mg; I, 0.2 mg; Se, 0.3 mg.
[B]Vitamin premix provided per kg powder diet: vitamin A, 10 500 IU; vitamin D_3, 3000 IU; vitamin E, 22.5 mg; vitamin K_3, 3 mg; vitamin B_{12}, 0.03 mg; riboflavin, 7.5 mg; niacin, 30 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; thiamin, 3 mg; pyridoxine, 4.5 mg; biotin, 0.12 mg.
SID, standard ileal digestibility; SAA, sulfur-containing amino acid.

Rectum digesta

On Day 28, fresh faeces were collected from two piglets without disease and diarrhoea in each pen. Faecal samples were collected in duplicate into sterile tubes and then stored at −80°C for analysis of volatile fatty acids (VFA).

Blood sample

On Day 29, approximately 10 mL of blood samples were collected via the anterior vena cava puncture at 0 and 3 h after challenge, respectively, then injected into EDTA-Na_2 and sodium heparin tubes, respectively. The vacuum tubes of EDTA-Na_2 were immediately placed on ice for analysis of blood biochemistry. Blood samples with sodium heparin were centrifuged (3000*g*, 15 min, 4°C) to obtain plasma samples, and stored at −80°C for further analysis (Su *et al.* 2020).

Growth performance

Individual piglet BW was measured, then average daily feed intake, average daily gain (ADG) and feed:gain ratio (F:G) were calculated.

Faecal score

Piglets were observed for clinical signs of diarrhoea from Day 1 to Day 28 of the experiment, and a scoring system was applied to indicate the diarrhoea severity. The following scoring system was used: 0, normal; 1, pasty; 2, semi-liquid; and 3, watery. Piglets with a faecal score of ≤1 were considered to not have diarrhoea (Bhandari *et al.* 2008). Scores were evaluated daily for individual pens, and the average faecal score per piglet was calculated.

Haematological and biochemical parameters

Haematological parameters, including white blood cells (WBC), red blood cells (RBC), haemoglobin, haematocrit and platelets (PLT), were analysed by an automatic biochemical analyser (model BC-2800vet, Mindray, Yaan, China). The serum concentrations of complement C3, complement C4, immunoglobulin G (IgG), immunoglobulin M (IgM), C-reactive protein (CRP), total protein (TP), albumin (ALB), alanine aminotransferase (ALT) and aspartate amino transferase (AST) were detected using a Hitachi 7020 Automatic Analyser (Tokyo, Japan) with the assay kits (Sichuan Maker Biotechnology Co. Ltd, Chengdu, China), according to the method of Su *et al.* (2018). Moreover, TNF-α and IL-1β were analysed by a commercially available swine ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

VFA

The VFA concentrations of rectum digesta were measured with a gas chromatographic method according to our previous study (Pena *et al.* 2013). The thawed digesta samples (1 g) were suspended in 2 mL of distilled water in a screw-capped tube, then vortexed and centrifuged at 12 000*g* for 10 min at 4°C. Then, 1 mL of supernatant was transferred to 1.5-mL PE centrifuge tubes and mixed with 0.2 mL of metaphosphoric acid, and incubated at 4°C for 30 min, then the mixture was centrifuged at 12 000*g* for 10 min at 4°C; 1 μL of supernatant was used to analyse VFA by Varian CP-3800 gas chromatograph (Agilent Technologies). A flame ionisation detector was used at an oven temperature of 100–150°C. The polyethylene glycol column was operated with highly purified N_2, as the carrier gas, at 1.8 mL/min. The detectable limit for all VFA was 0.1 mmol/L.

Statistical analyses

The pen was considered as the experimental unit for data about growth performance and VFA. The data about growth performance and VFA was performed using the *t*-test procedure (SAS 9.0). The data were expressed as means ± standard deviations. The data of haematological parameters were analysed using the general linear model procedures of SAS appropriate for a 2 × 2 factorial design (SAS 9.0). The statistical model included the effects of challenge (saline or LPS), diet (CON or TFE) and their interactions. The variability of all the data were expressed as the pooled standard error of the mean. Differences were considered as significant when $P < 0.05$, and a tendency was recognised when $P < 0.10$.

Results

Components of TFE

The TFE contained ~200 mg polysaccharides per gram detected using anthrone colourimetry, as before (Wu *et al.* 2007). The monosaccharide composition of polysaccharides was measured by gas chromatography–mass spectrometry (model 7890a-5975c, Agilent), as detailed in a previous study (Wahjudi *et al.* 2010), which was composed of 0.56% arabinose, 0.86% fucose, 1.31% xylose, 1.36% galactose, 10.10% mannose and 85.50% glucose.

Effect of TFE on growth performance

The effects of TFE on growth performance of weaned piglets are shown in Table 2. TFE supplementation significantly increased ADG ($P = 0.006$) and BW gain ($P = 0.04$) from Day 1 to Day 7, and decreased F:G ($P = 0.03$) during the whole experimental period compared with CON. The ADG of piglets fed the TFE diet tended to increase during Day 8 to Day 14 and the whole experimental period ($P = 0.05$–0.07) compared with piglets fed the CON diet. Piglets fed the TFE diet tended to have higher average daily feed intake than piglets fed the CON diet during Day 1 to Day 7 and Day 15 to Day 21 ($P = 0.07$–0.09).

Effect of TFE on faecal score

In Fig. 2, the faecal score of piglets fed the TFE diet was significantly decreased during Day 1–7 ($P = 0.02$) compared with piglets fed the CON diet, and tended to be decreased during the whole experimental period ($P = 0.08$).

Effects of TFE on VFA

In Fig. 3, there was no significant difference on acetic acid ($P = 0.55$), propionic acid ($P = 0.93$) and butyric acid ($P = 0.88$) in the rectal digesta between piglets fed the TFE and CON diet.

Table 2. Effects of TFE diet on the growth performance of weaned piglets.

Item	CON	TFE	*P*
IBW (kg)	6.40 ± 0.17	6.39 ± 0.17	0.96
FBW (kg)	13.89 ± 0.61	15.02 ± 0.49	0.18
BWG (kg)	7.49 ± 0.59	8.63 ± 0.39	0.04
ADFI (g/day)			
Days 1–7	134 ± 9	163 ± 11	0.07
Days 8–14	288 ± 17	317 ± 18	0.25
Days 15–21	454 ± 34	533 ± 34	0.09
Days 22–28	724 ± 70	761 ± 34	0.65
Days 1–28	400 ± 24	444 ± 22	0.21
ADG (g/day)			
Days 1–7	91 ± 7	124 ± 6	<0.01
Days 8–14	184 ± 12	223 ± 14	0.05
Days 15–21	299 ± 42	350 ± 24	0.31
Days 22–28	496 ± 47	536 ± 23	0.46
Days 1–28	267 ± 21	308 ± 14	0.07
F:G			
Days 1–7	1.49 ± 0.07	1.32 ± 0.07	0.13
Days 8–14	1.59 ± 0.12	1.44 ± 0.07	0.30
Days 14–21	1.62 ± 0.16	1.53 ± 0.05	0.61
Days 21–28	1.47 ± 0.07	1.42 ± 0.05	0.60
Days 1–28	1.54 ± 0.04	1.43 ± 0.02	0.03

Note: values are mean ± standard error, $n = 6$.
IBW, initial BW; FBW, final BW; BWG, BW gain; ADG, average daily gain; ADFI, average daily feed intake; F:G, feed:gain ratio. CON, control diet; TFE, control diet with 400 mg/kg TFE.

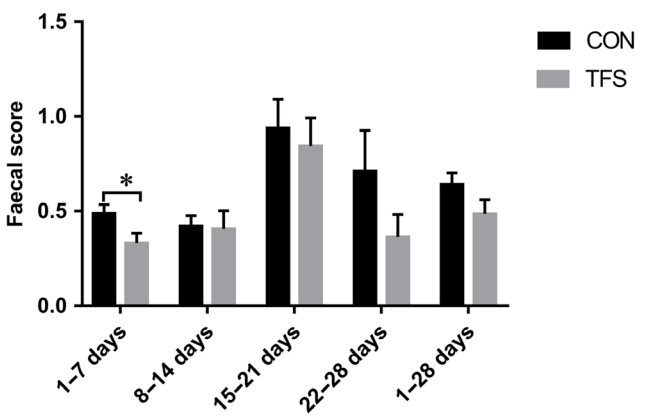

Fig. 2. Effects of TFE diet on the faecal score of weaned piglets. CON, control diet; TFE, control diet with 400 mg/kg TFE. Values are mean ± standard error, $n = 6$. Faecal score: 0, normal; 1, soft feces; 2, mild diarrhoea; and 3, severe diarrhoea.

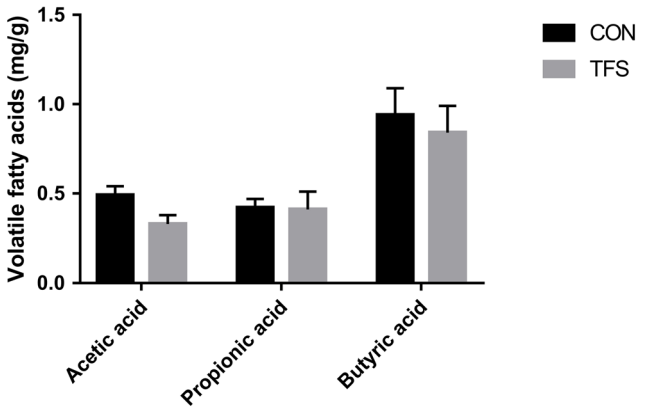

Fig. 3. Effects of TFE diet on the volatile fatty acids concentrations in the rectum digesta of weaned piglets. CON, control diet; TFE, control diet with 400 mg/kg TFE. Values are mean ± standard error, $n = 6$.

Effects of TFE on serum biochemistry

As shown in Table 3, LPS challenge significantly increased the concentrations of IL-1β and TNF-α, and decreased the concentration of immunoglobulin M in plasma (all $P < 0.05$) at 3 h post-challenge. However, piglets fed the TFE diet showed significantly decreased the concentrations of IL-1β ($P = 0.04$) and TNF-α ($P = 0.04$), compared with piglets fed the CON diet. The LPS challenge markedly increased concentrations of TP ($P = 0.04$), ALT ($P = 0.04$) and AST ($P = 0.04$) in the plasma of piglets, compared with piglets without LPS challenge. However, piglets fed the TFE diet had markedly lower concentrations of TP ($P = 0.04$) compared with piglets fed CON diet at 3 h post-challenge. Moreover, LPS challenge × diet interaction for TP was observed ($P = 0.02$).

Effects of TFE on haematological parameters

The effects of TFE on haematological parameters are presented in Table 4. The level of WBC was significantly increased in piglets fed the TFE diet compared with piglets fed the CON diet ($P = 0.02$). At 3 h post-challenge, the level of WBC in piglets challenged with LPS was notably decreased ($P < 0.05$), whereas the levels of RBC ($P = 0.03$),

Table 3. Effects of TFE diet on serum biochemistry after LPS challenge in weaned piglets.

Item		−LPS		+LPS		s.e.m.	*P*		
		CON[2]	TFE[2]	CON	TFE		TFE	LPS	TFE × LPS
C3 (g/L)	0 h	0.028	0.031	0.031	0.030	0.002	0.75	0.63	0.32
	3 h	0.032	0.035	0.30	0.032	0.002	0.25	0.32	0.77
C4 (g/L)	0 h	0.047	0.051	0.040	0.041	0.01	0.79	0.35	0.85
	3 h	0.031	0.044	0.018	0.040	0.01	0.90	0.31	0.71
CRP (mg/L)	0 h	24.26	27.54	24.82	25.62	1.29	0.13	0.60	0.35
	3 h	23.43	27.01	20.96	22.80	2.60	0.28	0.20	0.73
IgG (g/L)	0 h	1.27	1.32	1.16	1.17	0.23	0.91	0.56	0.93
	3 h	1.26	1.24	0.80	0.94	0.26	0.78	0.15	0.81
IgM (g/L)	0 h	0.44	0.54	0.51	0.47	0.13	0.81	0.98	0.49
	3 h	0.48	0.54	0.17	0.26	0.12	0.54	0.02	0.88
IL-1β (ng/L)	0 h	21.54	21.37	20.79	20.29	4.52	0.89	0.69	0.94
	3 h	21.31	19.28	27.85	23.05	2.91	0.04	<0.01	0.36
TNF-α (ng/L)	0 h	139.01	131.55	131.62	129.44	30.90	0.71	0.72	0.84
	3 h	136.56	132.36	179.02	161.48	11.32	0.04	<0.01	0.18
TP (g/L)	0 h	47.62	48.30	46.38	48.01	1.40	0.38	0.56	0.72
	3 h	45.22	45.79	58.49	50.11	1.99	0.04	<0.01	0.02
ALB (U/L)	0 h	28.45	30.04	31.28	30.89	1.40	0.67	0.20	0.49
	3 h	27.32	29.83	27.31	29.09	1.42	0.16	0.81	0.81
ALT (U/L)	0 h	39.64	40.91	40.36	41.85	4.24	0.71	0.81	0.98
	3 h	39.78	41.72	47.21	45.71	4.68	0.88	<0.01	0.17
AST (U/L)	0 h	82.32	109.60	96.98	94.33	15.29	0.44	0.99	0.35
	3 h	99.56	96.66	122.92	130.98	21.63	0.84	0.03	0.66

C3, complement3; C4, complement4; CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin G; IL-1β, interleukine-1 beta; TNF-α, tumour necrosis factor-α; TP, total protein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; −LPS and +LPS, 0 and 50 μg/kg of BW of LPS, respectively; TFE × LPS, interaction effect between CON and TFE; CON, control diet; TFE, control diet with 400 mg/kg TFE; s.e.m., pooled standard error of the means; $n = 6$.

Table 4. Effects of TFE diet on haematological parameters after LPS challenged in weaned piglets.

Item		−LPS		+LPS		s.e.m.	*P*		
		CON	TFE	CON	TFE		TP	LPS	TFE × LPS
WBC (10^9/L)	0 h	20.96	40.30	29.17	37.83	5.28	0.02	0.71	0.33
	3 h	25.32	24.78	6.17	8.00	2.78	0.82	<0.01	0.67
RBC (10^{12}/L)	0 h	5.74	6.10	6.32	6.12	0.22	0.77	0.22	0.22
	3 h	5.93	5.83	7.23	6.24	0.37	0.16	0.03	0.24
HGB (g/L)	0 h	101.00	110.33	116.33	110.50	4.87	0.78	0.16	0.15
	3 h	103.50	105.33	127.67	112.33	6.31	0.30	0.02	0.19
HCT (%)	0 h	35.54	38.43	38.17	37.23	1.43	0.55	0.69	0.21
	3 h	34.98	35.92	43.33	38.37	2.24	0.28	0.03	0.20
MCV (fl)	0 h	62.28	63.10	60.58	61.08	0.12	0.60	0.13	0.90
	3 h	58.98	61.63	60.10	61.75	1.12	0.07	0.60	0.67
MCH (pg)	0 h	17.56	18.00	18.38	18.05	0.41	0.93	0.33	0.37
	3 h	17.38	18.02	34.32	18.03	0.32	0.36	0.32	0.32
MCHC (g/L)	0 h	282.80	286.50	304.67	296.33	4.79	0.11	0.73	0.63
	3 h	295.33	292.83	294.50	292.17	0.16	0.14	0.64	0.36
RDW (%)	0 h	18.30	17.73	18.22	18.45	0.71	0.84	0.64	0.59
	3 h	18.45	17.98	18.13	17.80	0.69	0.56	0.71	0.91
PLT (10^9/L)	0 h	626.00	415.67	521.00	471.83	72.88	0.11	0.84	0.29
	3 h	432.33	447.33	293.50	337.00	65.32	0.66	0.07	0.83
MPV (fl)	0 h	9.32	9.55	9.17	9.30	0.38	0.65	0.59	0.30
	3 h	9.23	9.45	8.93	9.38	0.27	0.23	0.50	0.67
PDW (%)	0 h	17.32	17.05	16.55	17.15	0.26	0.50	0.25	0.12
	3 h	16.42	16.82	16.41	17.07	0.21	0.20	0.56	0.56

WBC, white blood cell; RBC, red blood cell; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width coefficient; PLT, platelets; MPV, mean platelet volume; PDW, platelet distribution width coefficient; −LPS and +LPS, 0 and 50 μg/kg of BW of LPS, respectively; TFE × LPS, interaction effect between CON and TFE; CON, control diet; TFE, control diet with 400 mg/kg TFE; s.e.m., pooled standard error of the means; $n = 6$.

haemoglobin ($P = 0.02$) and haematocrit ($P = 0.03$) were observably increased compared with piglets without LPS challenge. The level of platelets ($P = 0.07$) in piglets challenged with LPS tended to increase compared with piglets without LPS.

Discussion

In the present study, the effects of TFE supplementation on growth performance of weaned piglets were explored. The results showed that TFE supplementation increased ADG, BW gain and the feed conversion ratio of weaned piglets. The polysaccharide or oligosaccharide by TFE, composed of fucose, xylose, galactose and mannose, could be the main bioactive components to enhance the growth performance of piglets. It has been widely reported that mannan oligosaccharides have beneficial effects on growth performance and nutrient digestibility in weanling pigs (LeMieux *et al.* 2003; Zhao *et al.* 2012). A previous study also demonstrated that TFE improved ADG and average daily feed intake in finishing pigs (Ding *et al.* 2012). In addition, birds fed TFE had better growth performance than the non-supplemented birds, but were not significantly different from those fed virginiamycin (Guo *et al.* 2004). As an edible mushroom, the supplementation of *Pleurotus ostreatus* mushroom in the diet of piglets increased feed consumption, gut microbial composition and diversity, as well as short-chain fatty acids synthesis, consequently prevented the occurrence of diarrhoea and increased the growth of piglets (Adams *et al.* 2019). Various bioactive effects had been observed by the polysaccharides in TFE (Kakuta *et al.* 1979), the better growth performance of piglets fed TFE may also be related to the beneficial effect of TFE on alleviating weaning stress.

Weaning stress, particularly occurring in the first week post-weaning, leads to excessive inflammation, in which immune activation could distribute the nutrients from muscular growth into the immune system. Futhermore, weaning stress leads to intestinal damage and being more susceptible to pathogen infection (Hu *et al.* 2015; Wan *et al.* 2016;

Che *et al.* 2017). TFE contains polysaccharides that are responsible for anti-inflammation and immunomodulation (Jiang *et al.* 2012; Hu *et al.* 2016). In agreement with a previous study (Lee *et al.* 2016), we found *T. fuciformis* extract could suppress the inflammatory response via reducing the levels of LPS-induced IL-1β, IL-6 and TNF-α. Similarly, Ruan *et al.* (2018) reported that RAW264.7 cells pre-treated with TFE profoundly inhibited the activation of protein kinase B, p38 mitogen-activated protein kinases and nuclear factor-κB, and attenuated the expression of MCP-1 in macrophages. Meanwhile, TFE also decreased cytokine and reactive oxygen species levels, and attenuated cell inflammation after treatment with LPS. In addition, the lower faecal score of TFE-supplemented piglets in the study further suggested TFE could alleviate the intestinal dysfunction induced by weaning stress.

Haematological parameters are usually common indicators of physical condition in humans and animals (Savran *et al.* 2020). It has been reported that liver injury was associated with the increased concentration of plasma TP (Wang *et al.* 2015). In addition, damage to the hepatic function by LPS can be reflected in the increased concentrations of AST and ALT (Hanley *et al.* 2004; Pan *et al.* 2015). In our study, LPS challenge increased the levels of ALT, AST and TP in serum, but TFE decreased the level of TP when piglets were challenged with LPS, which indicated that TFE might protect hepatic function from LPS challenge. WBC are essential immune cells associated with the induction of inflammation (Li *et al.* 2018). Previous studies reported that the level of WBC was decreased, whereas the levels of RBC and HGB were increased after immune challenge (Reiner *et al.* 2007; Che *et al.* 2011). Consistently, LPS challenge decreased the level of WBC, and increased the levels of RBC and HGB in this study, which could be partly recovered by supplementing TFE. In addition, the current results showed that the level of WBC was markedly increased by the TFE diet, which is similar to previous studies showing that *T. fuciformis* polysaccharides enhanced immunity (Wang *et al.* 1983; Cheung 1996; Reshetnikov *et al.* 2000). The activation of immune cells may be a positive response to inflammation by protecting the host from pathogenic insult (Kauppinen *et al.* 2013).

Conclusions

In conclusion, TFE supplementation in the weaning piglet diet exhibited positive effects on ADG, F:G and BW gain, as well as the potential capacity to enhance immune response.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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