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Effects of dietary inulin supplementation on growth performance, intestinal barrier integrity and microbial populations in weaned pigs

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Abstract

Here, we explored the influences of dietary inulin (INU) supplementation on growth performance and intestinal health in a porcine model. Thirty-two male weaned pigs (with an average body weight of 7·10 (SD 0·20) kg) were randomly assigned to four treatments and fed with a basal diet (BD) or BD containing 2·5, 5·0 and 10·0 g/kg INU. After a 21-d trial, pigs were killed for collection of serum and intestinal tissues. We show that INU supplementation had no significant influence on the growth performance in weaned pigs. INU significantly elevated serum insulin-like growth factor-1 concentration but decreased diamine oxidase concentration ($P < 0·05$). Interestingly, 2·5 and 5·0 g/kg INU supplementation significantly elevated the villus height in jejunum and ileum ($P < 0·05$). Moreover, 2·5 and 5·0 g/kg INU supplementation also elevated the villus height to crypt depth (V:C) in the duodenum and ileum and improved the distribution and abundance of tight-junction protein zonula occludens-1 in duodenum and ileum epithelium. INU supplementation at 10·0 g/kg significantly elevated the sucrase activity in the ileum mucosa ($P < 0·05$). INU supplementation decreased the expression level of TNF-α but elevated the expression level of GLUT 2 and divalent metal transporter 1 in the intestinal mucosa ($P < 0·05$). Moreover, INU increased acetic and butyric acid concentrations in caecum ($P < 0·05$). Importantly, INU elevated the *Lactobacillus* population but decreased the *Escherichia coli* population in the caecum ($P < 0·05$). These results not only indicate a beneficial effect of INU on growth performance and intestinal barrier functions but also offer potential mechanisms behind the dietary fibre-regulated intestinal health.

Key words: **Inulin: Intestinal health: Microflora: Nutrition**

The intestinal epithelial barrier, mainly composed of a single layer of enterocytes and intercellular tight junctions (TJ), is a selective osmotic membrane which not only allows nutrients to enter the circulation from the intestinal lumen but also provides an inherent defence barrier against the entry of pathogens and toxins into the systemic circulation[1,2]. For neonatal mammals, intestinal hypoplasia or disruption of the intestinal epithelial barrier is commonly accompanied by growth retardation and increasing the risk of developing diarrhoea and intestinal infections[3–5]. Therefore, the avenue to improve the intestinal epithelial barrier functions has attracted considerable research interest worldwide.

Inulin (INU) is a group of naturally occurring polysaccharides belonging to a class of dietary fibre known as fructans[6]. The length of fructan chain of INU is ranging from 2 to 60 units, with an average degree of polymerisation of 10[7]. INU can be isolated from a number of fruits and vegetables such as bananas, asparagus, leeks and onions. However, the industrially produced INU is mainly extracted from chicory (Compositae family) and Jerusalem artichoke (*Helianthus tuberosus*), since they are extremely abundant in fructans[8]. As an attractive dietary fibre, INU cannot be hydrolysed by mammal digestive enzymes in the small intestine but at least partially hydrolysed and fermented by intestinal microflora[9].

Previous studies indicated that INU plays a critical role in maintaining the gut health. For instance, INU can improve the intestinal function and gastrointestinal environment in weaned pigs by increasing the number and metabolic activity of beneficial microflora[10]. Previous study indicated that oligosaccharides including the INU can be efficiently utilised by beneficial bacteria such as the *Lactobacillus* and *Bifidobacterium*. However, most harmful bacteria cannot utilise these carbon sources, resulting in inhibition of growth[11,12]. Moreover, fermentation of dietary fibres by intestinal bacteria produces a lot of volatile fatty acids such as the acetate, propionate and butyrate, which can serve as an energy source for enterocytes and protect against various inflammations[13,14]. Previous study also indicated that INU can serve as physical stimuli to promote the intestinal motility and secretion of intestinal fluid in rats[15]. Although, the beneficial effects of INU on intestinal functions have been investigated

Abbreviations: DAO, diamine oxidase; DMT1, divalent metal transporter 1; IGF-1, insulin-like growth factor-1; INU, inulin; TJ, tight junction; ZO-1, zonula occludens-1.

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in a variety of animal species, the molecular mechanisms still remain unclear. The aim of this study was to explore the effects of dietary INU supplementation at different doses on growth performance and intestinal barrier functions in a pig model. The mechanism underlying the INU -regulated intestinal heath has also been partially elucidated.

Materials and methods

All the procedures used in the animal experiment were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (no. 20180715).

Animal care and experimental design

Thirty-two pigs (Duroc × Landrace × Yorkshire), weaned at 21 d (with an average body weight of 7·10 (SD 0·20) kg), were randomly allotted to four dietary treatments (*n* 8). Pigs were fed with a basal diet (control group) or basal diet containing 2·5, 5·0 and 10·0 g/kg INU (99 % purity, kindly provided by Sichuan Junzheng Biofeed Co. Ltd) for 21 d. The experimental diet was formulated on the basis of nutrient requirements established by the National Research Council[16]. The ingredients and nutrient levels of the experimental diets are shown in Table 1. All pigs were housed in individual metabolism cages (0·7 m × 1·5 m) and were given *ad libitum* access to fresh water. The pigs were hand-fed three times per d (08.00, 14.00 and 20.00 hours) in groove feeders to make sure the fresh feed available.

Growth performance determination

At the start and end of the trial, individual pig body weight was recorded before feeding and the daily feed consumption per pig was measured throughout the study. Average daily body weight gain, average daily feed intake and the feed:gain ratio (F:G) were subsequently determined for each group from the data obtained.

Sample collections

At the end of the trial, blood samples were collected by venepuncture at 08.00 hours after 12 h of fasting. Then, the samples were centrifuged at 3500 ***g*** at 4°C for 10 min. After centrifugation, the serum samples were collected and frozen at –20°C until analysed. After blood collection, pigs were euthanised with an intravenous injection of chlorpromazine hydrochloride (3 mg/kg body weight) and then slaughtered by exsanguination protocols. Sections of the duodenum, jejunum and ileum were immediately isolated. Approximately 5 cm segments of the middle of duodenum, jejunum and ileum were gently flushed with ice-cold PBS and then fixed in 4 % paraformaldehyde solution for morphological analyses and immunofluorescence. Finally, the residual duodenal, jejunal and ileal segments were scraped with a scalpel blade, and the mucosa samples were collected and stored at –80°C until analysis.

Serum biochemical analysis

The concentrations of glucose and TAG were measured using available commercial kits according to Nanjing Jiancheng Bioengineering Institute. The levels of insulin, insulin-like growth factor-1 (IGF-1), IgA, IgG, IgM, diamine oxidase (DAO) and D-lactic acid were determined using the ELISA kits that purchased from Jiangsu Jingmei Biological Technology Co. Ltd, and the specific operations were as per the instructions of kits. All the assays were performed in triplicate.

Table 1. Ingredients and nutrient composition of the basal diet

Ingredients	Content (%)	Nutrient composition*	Content (%)
Maize (7·8 % crude protein)	25·880	Digestible energy (Mcal/kg)	3·5121
Extruded maize (7·8 % crude protein)	24·590	Crude protein	21·1276
Soyabean meal (44·2 % crude protein)	10·000	Ca	0·8255
Extruded soyabean	10·300	Available P	0·4285
Fishmeal (62·5 % crude protein)	4·000	Lysine	1·3517
Whey powder (low protein)	7·000	Methionine	0·4158
Soyabean protein concentrate	10·000	Methionine + cysteine	0·6004
Soyabean oil	1·500	Threonine	0·7904
Sucrose	4·000	Tryptophan	0·2242
Limestone	0·700		
Dicalcium phosphate	0·800		
NaCl	0·300		
L-Lysine-HCl (78 %)	0·350		
DL-Methionine	0·150		
L-Threonine (98·5 %)	0·070		
Tryptophan (98 %)	0·070		
Chloride choline	0·100		
Vitamin premix†	0·040		
Mineral premix‡	0·020		
Total	100		

* Values were calculated.
† Vitamin premix provided the following per kg of diets: vitamin A, 2·7 mg; vitamin D_3, 0·075 mg; vitamin E, 20 mg; vitamin K_3, 3·0 mg; vitamin B_1, 1·5 mg; vitamin B_2, 4·0 mg; vitamin B_6, 3·0 mg; vitamin B_{12}, 0·2 mg; niacin, 30 mg; pantothenic acid, 15 mg; folic acid, 0·75 mg; biotin, 0·1 mg.
‡ Mineral premix provided the following per kg of diets, 25–50 kg: Fe ($FeSO_4·H_2O$) 60 mg, Cu ($CuSO_4·5H_2O$) 4 mg, Mn ($MnSO_4·H_2O$) 2 mg, Zn ($ZnSO_4·H_2O$) 60 mg, iodine (KI) 0·14 mg, Se (Na_2SeO_3) 0·2 mg; 50–75 kg: Fe ($FeSO_4·H_2O$) 50 mg, Cu ($CuSO_4·5H_2O$) 3·5 mg, Mn ($MnSO_4·H_2O$) 2 mg, Zn ($ZnSO_4·H_2O$) 50 mg, iodine (KI) 0·14 mg, Se (Na_2SeO_3) 0·15 mg; 75–125 kg: Fe ($FeSO_4·H_2O$) 40 mg, Cu ($CuSO_4·5H_2O$) 3 mg, Mn ($MnSO_4·H_2O$) 2 mg, Zn ($ZnSO_4·H_2O$) 50 mg, iodine (KI) 0·14 mg, Se (Na_2SeO_3) 0·15 mg.

Intestinal morphology analysis

One cm long small intestine (including the duodenum, jejunum and ileum) was dehydrated through a graded series of ethanol and embedded in paraffin. Cross sections of each sample were prepared, stained with haematoxylin–eosin and then sealed by a neutral resin size. The intestinal morphology including villus height and crypt depth was determined by using an image processing and analysis system (Media Cybernetics).

Immunofluorescence analysis

After paraformaldehyde fixations for 72 h, the intestinal tissue samples for immunofluorescence were rinsed in PBS and subsequently transferred to 30 % sucrose solution (dissolved in PBS) and infiltrated overnight. These samples were embedded on the next day in O.C.T. compound (Sakura Finetek Co. Ltd) for frozen tissue specimens. Next, the embedded samples were

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cut into 5 mm thick sections, using a semi-automatic freezing microtome at −20°C and mounted on glass slides. The sections were permeabilised with 0·5 % Triton X-100 in PBS, at room temperature for 10 min. After washing three times with PBS, the sections were blocked with 10 % goat serum in PBS at room temperature for 30 min, followed by incubation overnight at 4°C with rabbit anti-occluding (at 1:100 dilution; Abcam plc.) antibody. After washing with PBS three times, the sections were incubated with a fluorescein isothiocyanate-conjugated goat anti-rabbit IgG secondary antibody (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd) at 37°C for 30 min, followed by counterstaining with 4′, 6-diamidino-2-phenylindole at room temperature for 10 min. Finally, after washing as described above, the sections were sealed with an anti-fluorescence quencher, and zonula occludens-1 (ZO-1) protein distribution was visualised under a laser scanning confocal microscope (FV1000; Olympus Corporation).

Enzyme activity assays

Frozen intestinal mucosa samples were rapidly thawed and then mixed with ice-cold physiological saline at a ratio of 1:9 (w/v). Next, the mixtures were centrifuged at 3000 ***g***, 4°C, for 15 min, to isolate the supernatants. Lactase, sucrase and maltase activities in the supernatant were measured by using available commercial kits according to Nanjing Jiancheng Bioengineering Institute.

Total RNA isolation and reverse transcription

Total RNA was isolated from frozen duodenal, jejunal or ileal samples using TRIzol (Takara Biotechnology Co. Ltd). All the procedures were guided by the manufacturer's manual. Briefly, 100 mg tissues were put into a mortar and grinded with 1 ml TRIzol reagent. The proteins in the grinded samples were precipitated by chloroform. After centrifugation, the supernatant was transferred into a new tube and isopropanol was added and mixed for 10 min. The total RNA has settled by centrifugation. The integrity of RNA was checked by electrophoresis on a 1·5 % agarose gel, and the concentration and quality were verified by UV spectrophotometry using a NanoDrop 2000 (Thermo Fisher Scientific, Inc.). After RNA isolation, 1 μg of total RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit with cDNA Eraser (Takara Biotechnology Co. Ltd). The following conditions were used: 42°C for 2 min, then 37°C for 15 min, followed by 85°C for 5 s.

Analysis of gene expression

Real-time quantitative PCR was performed in an Option Monitor 3 Real-Time PCR Detection System (Bio-Rad) using the SYBR Green Supermix (TaKaRa). Expression levels of *β-actin (housekeeper genes)*, *SGLT1*, *GLUT2*, divalent metal transporter 1 (*DMT1*), *TNF-α*, *IL-6*, *ZO-1*, *Occluding* and *Claudin-1* in the small intestinal were analysed using SYBR Premix Ex Taq II (Tli RNaseH Plus) reagents (TakaRa) and the QuanStudio 6 Flex Real-Time PCR detection system (Applied Biosystems). All primers were commercially synthesised and purified by Sangon Biotech Co. Ltd and are shown in Table 2. The reaction was performed in a volume of 10 μl consisting of 5 μl of SYBR Premix Ex Taq (2×), 1 μl of reverse primers, 1 μl of forward primers, 2 μl of doubled-distilled water and 1 μl of cDNA template. Cycling conditions were as follows: 5°C for 30 s, followed by forty cycles at 95°C for 5 s, 60°C for 34 s, under melt curve conditions at 95°C for 15 s, 60°C for 1 min and then 95°C for 15 s (temperature change velocity 0·5°C/s). The target gene mRNA expression level was calculated using the $2^{-\Delta\Delta Ct}$ method[17]. Each sample was repeated in triplicate.

SCFA assays

The concentrations of main SCFA (acetic acid, propionic acid and butyric acid) were determined by using a gas chromatograph system (VARIAN CP-3800; Varian; Capillary Column 30 m × 0·32 mm × 0·25 μm film thickness) following the previous method[18]. From each sample, 2 g faeces (stored at −20°C) were weighed. Then, 5 ml ddH_2O was added. After vortex, each sample was centrifuged (12 000 ***g***) at 4°C for 10 min. The supernatant (1 ml) was then transferred into an Eppendorf tube (2 ml) and mixed with 0·2 ml metaphosphoric acid. After 30 min incubation at 4°C, the tubes were centrifuged at 4°C for 10 min (12 000 ***g***) and 1 μl of the supernatant was analysed using the GC with a flame ionisation detector and an oven temperature of 100–150°C (N_2 as the carrier gas at 1·8 ml/min)[19].

Table 2. Primer sequences for quantitative real-time PCR

Gene	Primer sequence (5′→3′)	Size (bp)	Accession no.
β-Actin	F: TGGAACGGTGAAGGTGACAGC R: GCTTTTGGGAAGGCAGGGACT	177	XM_003124280.5
Na^+–glucose co-transporter 1	F: AGAAGGGCCCCAAAATGACC R: TGTTCACTACTGTCCGCCAC	96	NM_001164021.1
Facilitated GLUT2	F: GACACGTTTTGGGTGTTCCG R: GAGGCTAGCAGATGCCGTAG	165	NM_21434706.2
Divalent metal transporter 1	F: GCAGGTGGTTGACGTCTGTA R: CACGCCCCCTTTGTAGATGT	100	NM_001128440.1
Zonula occludens-1	F: CAGCCCCCGTACATGGAGA R: GCGCAGACGGTGTTCATAGTT	114	XM_005659811
Occludin	F: CAGGTGCACCCTCCAGATTG R: GGACTTTCAAGAGGCCTGGAT	158	NM_001163647.2
Claudin-1	F: TCTTAGTTGCCACAGCATGG R: CCAGTGAAGAGAGCCTGACC	108	FJ873109.1
TNF-*α*	F: CGTGAAGCTGAAAGACAACCAG R: GATGGTGTGAGTGAGGAAAACG	112	NM_214022.1
IL-6	F: TTCACCTCTCCGGACAAAAC R: TCTGCCAGTACCTCCTTGCT	122	NM_001252429.1

F, forward; R, reverse.

Table 3. Primer and probe sequences used for real-time PCR

Items	Primer and probe sequence (5′–3′)	Anneal temperature (°C)	Product length (bp)
Total bacteria	F: ACTCCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	57·9	200
Lactobacillus	F: GAGGCAGCAGTAGGGAATCTTC R: CAACAGTTACTCTGACACCCGTTCTC P: AAGAAGGGTTTCGGCTCGTAAAACTCTGTT	53·0	126
Bifidobacterium	F: CGCGTCCGGTGTGAAAG R: CTTCCCGATATCTACACATTCCA P: ATTCCACCGTTACACCGGAA	57·9	121
Bacillus	F: GCAACGAGCGCAACCCTTGA R: TCATCCCCACCTTCCTCCGGT P: CGGTTTGTCACCGGCAGTCACCT	53·0	92
Escherichia coli	F: CATGCCGCGTGTATGAAGAA R: CGGGTAACGTCAATGAGCAAA P: AGGTATTAACTTTACTCCCTTCCTC	53·0	96

F, forward; R, reverse; P, probe.

Quantification of intestinal microflora by quantative PCR

Microbial genomic DNA in the caecal digesta was extracted by using the Stool DNA Kit (Omega Bio-Tech) according to the manufacturer's instruction. The microbial real-time quantitative PCR was determined as described previously[20]. All primers and probes for total bacteria, *Escherichia coli*, *Lactobacillus*, *Bifidobacterium* and *Bacillus*[21] (Table 3) were commercially synthesised from TaKaRa Biotechnology (Dalian) Co. Ltd. Briefly, the number of total bacteria was analysed by real-time quantitative PCR using SYBR Premix Ex Taq reagents (TaKaRa Biotechnology (Dalian) Co. Ltd) and CFX-96 real-time PCR detection system (BioRad Laboratories), and the numbers of *Bacillus*, *Lactobacillus*, *E. coli* and *Bifidobacterium* were analysed by real-time quantitative PCR using PrimerScript™ PCR kit (perfect real time; TaKaRa Biotechnology (Dalian) Co. Ltd) and CFX-96 real-time PCR detection system (Bio-Rad Laboratories) as previously described[20]. For the quantification of bacteria in the test samples, specific standard curves were generated by constructing standard plasmids as presented by Chen *et al.*[20]. In addition, bacterial copies were transformed ($\log_{10}$) before statistical analysis.

Statistical analysis

Results were analysed using an one-way ANOVA procedure of SPSS 22.0 (SPSS Inc.) followed by Duncan's test for multi-group comparisons. To determine whether there was a significant linear response to INU, the linear and quadratic procedure was performed. All results were presented as means and total standard errors of the mean. Difference with $P < 0{\cdot}05$ was considered to be significant, and $0{\cdot}05 < P < 0{\cdot}10$ was considered to have a tendency.

Results

Effect of inulin on growth performance in weaned pigs

As shown in Table 4, no significant difference in growth performance was observed among the four groups ($P > 0{\cdot}05$). Interestingly, the average daily feed intake and average daily body weight gain of pigs fed with 2·5 kg/kg INU increased by 12·2 and 20·1 %, respectively. The feed efficiency (F:G) of this group decreased by 8·33 %, as compared with the control group.

Effect of inulin on serum parameters

As shown in Table 5, dietary INU supplementation had no influences on serum concentrations of insulin and D-lactate ($P > 0{\cdot}05$). However, 2·5 g/kg INU supplementation elevated the serum IGF-1 concentration ($P < 0{\cdot}05$) and significantly decreased the serum DAO concentration ($P < 0{\cdot}05$). A higher dose (5·0 and 10·0 g/kg) of INU can also decrease the DAO concentration in the serum ($P < 0{\cdot}05$). Moreover, 10·0 g/kg INU supplementation significantly elevated the serum IgA concentration ($P < 0{\cdot}05$).

Table 4. Effect of dietary inulin (INU) supplementation on growth performance in weaned pigs*
(Mean values with their standard errors; *n* 6 per group)

	Dietary INU (g/kg)										
	CON		2·5		5·0		10·0		*P*		
Items	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA	Linear	Quadratic
Initial weight (kg)	7·10	0·09	7·10	0·12	7·10	0·13	7·10	0·13	1·00	1·00	1·00
Final weight (kg)	12·67	3·95	13·79	3·05	12·83	4·67	12·98	4·89	0·26	0·94	0·74
Average daily feed intake (kg/d)	0·37	0·02	0·42	0·02	0·37	0·02	0·38	0·02	0·30	0·92	0·87
Average daily gain (kg/d)	0·27	0·02	0·32	0·02	0·27	0·02	0·28	0·02	0·26	0·94	0·74
Feed:gain ratio	1·44	0·08	1·32	0·03	1·37	0·02	1·39	0·06	0·37	0·82	0·42

* Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively.

Table 5. Effect of dietary inulin (INU) supplementation on serum metabolites, hormones and immunogloblins in weaned pigs*
(Mean values with their standard errors; *n* 6 per group)

	Dietary INU (g/kg)										
	CON		2·5		5		10		*P*		
Items	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA	Linear	Quadratic
TAG (mmol/l)	0·52	0·05	0·46	0·07	0·45	0·02	0·47	0·07	0·78	0·57	0·59
Glucose (mmol/l)	5·45	0·12	5·93	0·45	5·53	0·53	5·68	0·25	0·82	0·87	0·95
Insulin (mIU/l)	78·32	1·74	86·51	3·90	76·01	4·42	90·60	2·47	0·19	0·06	0·10
Insulin-like growth factor-1 (μg/l)	14·75^{b}	0·37	16·33^{a}	0·67	14·63^{b}	0·27	16·27^{a}	0·25	0·01	0·16	0·35
IgG	202·69	16·36	218·57	15·37	242·86	15·36	216·20	18·89	0·41	0·58	0·27
IgA	25·57	0·65	29·61	0·95	24·27	1·29	31·41	1·35	0·37	0·18	0·42
IgM	18·33	2·21	18·49	1·10	18·89	1·78	18·88	0·83	0·99	0·78	0·95
D-Lactate (μg/ml)	1401·20	141·47	1177·22	279·21	1383·66	182·57	1456·99	395·54	0·89	0·71	0·86
Diamine oxidase (U/l)	356·56^{c}	30·89	164·26^{a}	21·60	253·67^{b}	10·17	144·52^{a}	16·79	<0·01	<0·01	<0·01

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively.

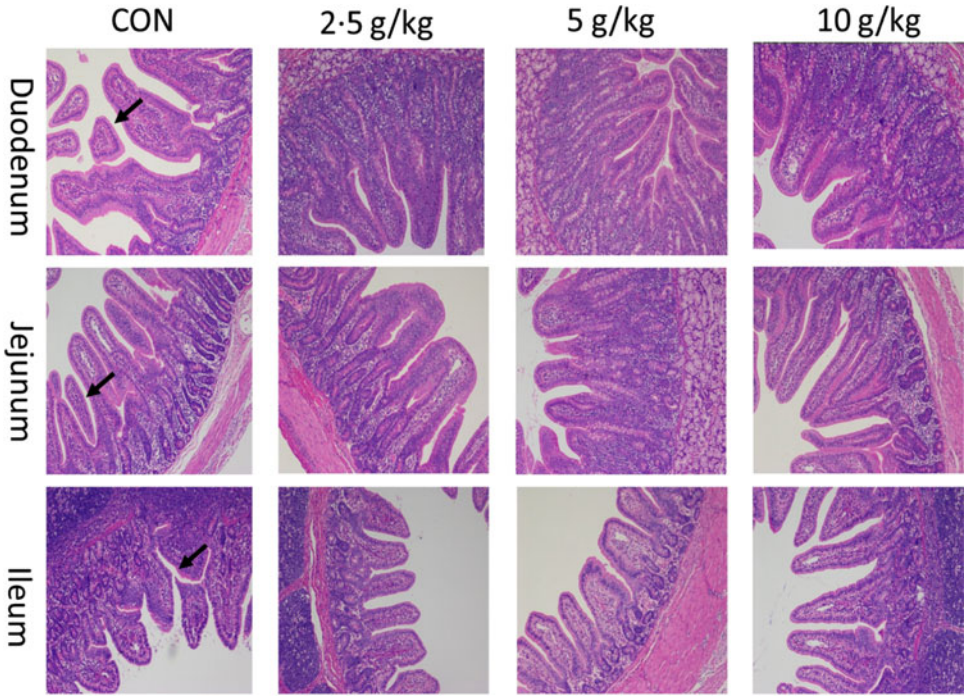

Fig. 1. Effect of inulin (INU) on morphology of the small intestine in weaned pigs (haematoxylin–eosin; ×100). Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively. Black arrows indicate disruption of the intestinal mucosa in the CON group.

Table 6. Effects of dietary inulin (INU) supplementation on intestinal mucosal morphology in weaned pigs*
(Mean values with their standard errors; *n* 6 per group)

	Dietary INU (g/kg)										
	CON		2·5		5		10		*P*		
Items	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA	Linear	Quadratic
Duodenum											
Villus height (μm)	194·67	14·26	271·33	40·33	219·29	9·55	213·96	12·89	0·14	0·92	0·43
Crypt depth (μm)	100·39	3·04	137·81	38·85	81·32	1·96	84·79	5·37	0·23	0·28	0·52
V:C	1·94^{a,b}	0·12	2·18^{c}	0·24	2·57^{c}	0·14	2·54^{b}	0·16	0·05	0·02	0·02
Jejunum											
Villus height (μm)	163·41^{a}	8·08	223·77^{b}	19·89	252·49^{b}	26·47	205·48^{a,b}	4·41	0·02	0·27	0·01
Crypt depth (μm)	83·24	7·89	81·59	6·52	114·04	25·14	80·21	2·96	0·27	0·96	0·36
V:C	2·08^{a}	0·04	2·91^{b}	0·29	2·96^{b}	0·46	2·70^{a,b}	0·06	0·14	0·29	0·08
Ileum											
Villus height (μm)	130·76^{a}	3·93	168·00^{b}	10·44	157·26^{b}	6·55	160·67^{b}	10·70	0·03	0·13	0·06
Crypt depth (μm)	69·31	3·20	68·70	4·65	70·19	4·95	64·01	4·11	0·74	0·37	0·56
V:C	1·85^{a}	0·13	2·58^{b}	0·26	2·40^{b}	0·18	2·63^{b}	0·11	0·036	0·04	0·05

V:C, villus height to crypt depth.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively.

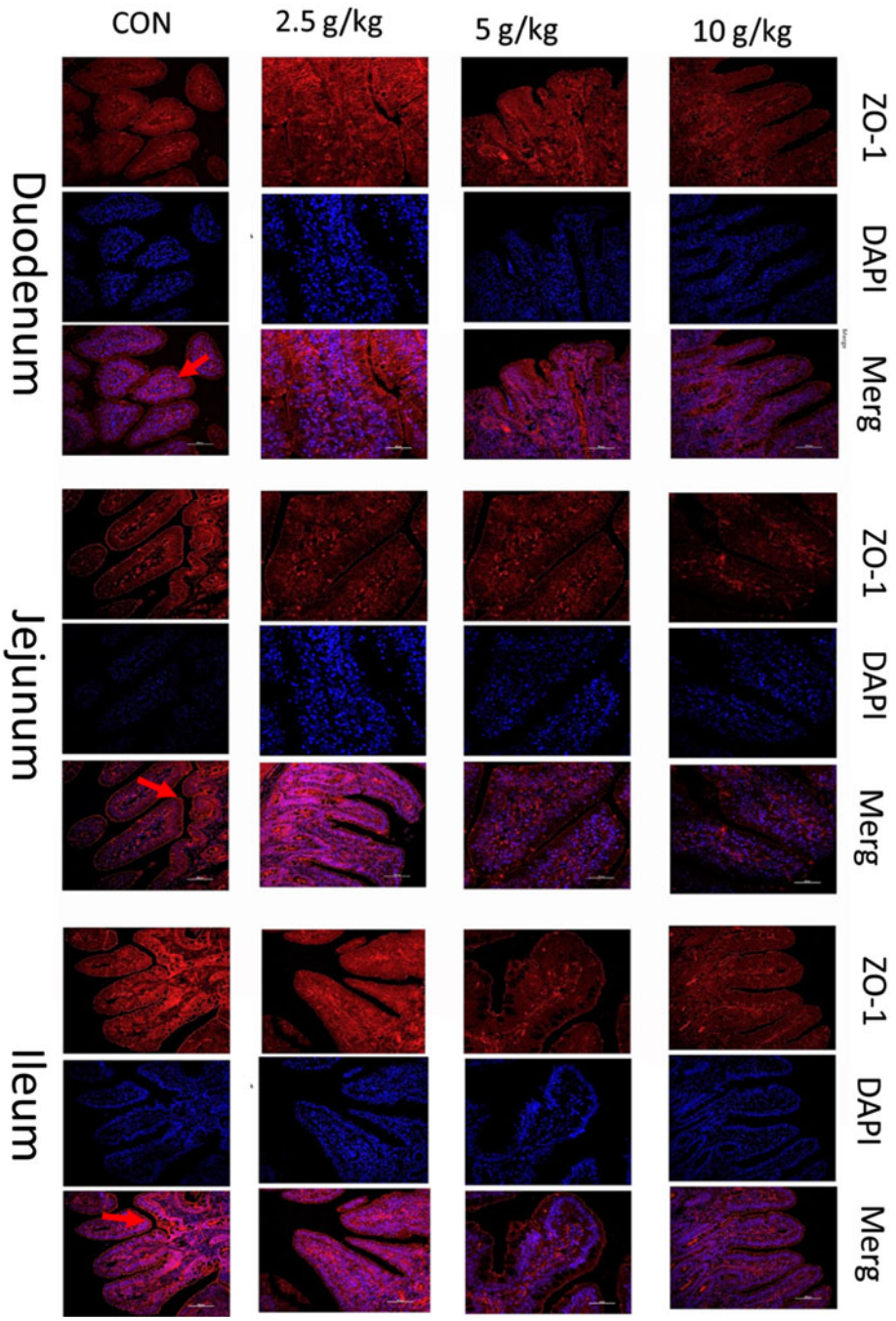

Fig. 2. Effect of inulin (INU) on tight junction distribution. Localisation of zonula occludens-1 (ZO-1) and DAPI (DNA) within the duodenum, jejunum and ileum of weaned pigs was assessed by immunofluorescence. ZO-1 protein (red), DAPI stain (blue), as well as merged ZO-1 protein and DAPI are presented. Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively. Red arrows indicate diffused distribution of ZO-1 protein in the CON group.

Effect of inulin on intestinal morphology and distribution of the tight-junction protein zonula occludens-1

As shown in Fig. 1 and Table 6, 2·5 and 5·0 g/kg INU supplementation significantly elevated the villus height in the jejunum and ileum ($P < 0·05$). In contrast, 10·0 g/kg INU supplementation had no influence on villus height in the jejunum but significantly increased the villus height in the ileum ($P < 0·05$). As compared with the control group, 2·5 and 5·0 g/kg INU supplementation elevated the ratio of villus height:crypt depth (V:C) in the duodenum and ileum ($P < 0·05$). However, there were no significant differences among the INU supplementation groups ($P > 0·05$). Immunofluorescence analysis showed that the staining of the major TJ-related protein ZO-1 in the control group was diffused with little staining at the intercellular TJ region, indicating disruption of the intestinal barrier functions (Fig. 2). However, the ZO-1 protein was highly expressed and localised to the apical intercellular region of the duodenum and ileal epithelium in pigs fed with an INU-containing diet.

Table 7. Effect of dietary inulin (INU) supplementation on enzyme activity in intestinal mucosa*
(Mean values with their standard errors; *n* 6 per group)

	Dietary INU (g/kg)										
	CON		2·5		5		10		*P*		
Items	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA	Linear	Quadratic
Duodenum											
Lactase (U/mg protein)	7·76	1·93	7·34	0·59	7·87	1·14	8·06	1·12	0·98	0·78	0·95
Sucrase (U/mg protein)	49·24	7·81	64·63	5·61	64·84	6·75	49·34	4·35	0·15	0·07	0·07
Maltase (U/mg protein)	26·78	6·83	25·37	3·90	23·82	4·20	26·73	5·72	0·65	0·54	0·48
Jejunum											
Lactase (U/mg protein)	56·47	6·99	54·84	7·43	53·53	3·86	64·25	7·56	0·67	0·35	0·46
Sucrase (U/mg protein)	66·32	8·35	74·38	23·03	75·16	13·54	73·97	20·67	0·99	0·85	0·96
Maltase (U/mg protein)	180·72	21·37	201·34	28·01	203·61	33·75	213·19	11·92	0·82	0·38	0·65
Ileum											
Lactase (U/mg protein)	4·24	0·87	4·10	1·30	4·42	0·83	4·18	0·60	0·99	0·99	0·99
Sucrase (U/mg protein)	2·88[a]	0·31	2·58[a]	0·26	2·59[a]	0·37	6·35[b]	0·88	<0·01	<0·01	<0·01
Maltase (U/mg protein)	28·17	4·25	24·91	1·77	24·07	1·30	29·81	4·50	0·57	0·60	0·36

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively.

Effect of inulin on intestinal mucosa enzyme activity

As shown in Table 7, 2·5 and 5 g/kg INU supplementation increased the sucrase activity in the duodenum mucosa by 31·26 and 31·68 %, respectively ($0·05 < P < 0·10$). INU supplementation at 10·0 g/kg significantly elevated the sucrase activity in the ileum mucosa ($P < 0·05$).

Effect of inulin on the expression of genes related to inflammatory response and barrier functions

As compared with the control group, 2·5 g/kg INU supplementation significantly decreased the expression level of TNF-α in the duodenum and ileum mucosa ($P < 0·05$). But the expression level of IL-6 was not affected by INU supplementation (Fig. 3). Interestingly, INU supplementation altered the expression levels of several critical genes related to intestinal barrier functions (Fig. 4). As compared with the control group, 2·5 g/kg INU supplementation significantly elevated the expression levels of GLU2, ZO-1 and Claudin-1 in the duodenum mucosa ($P < 0·05$). INU supplementation (2·5 g/kg) also elevated the expression level of DMT1 in the jejunum mucosa ($P < 0·05$). Moreover, INU supplementation at a higher dose (5·0 and 10·0 g/kg) significantly elevated the expression levels of ZO-1 and Claudin-1 in the duodenum mucosa ($P < 0·05$) and elevated the expression levels of GLU2 and DMT1 in the jejunum mucosa ($P < 0·05$).

Effect of inulin on intestinal microbial populations and metabolites

As shown in Fig. 5(A), INU supplementation significantly increased the acetic acid concentration in the caecal digesta ($P < 0·05$). INU supplementation at 10·0 g/kg also increased the butyric acid concentration ($P < 0·05$). Interestingly, INU supplementation has resulted in elevated abundance of the *Lactobacillus* population in the caecal digesta ($P < 0·05$). Moreover, 2·5 g/kg INU supplementation significantly decreased the *E. coli* population in the caecum ($P < 0·05$).

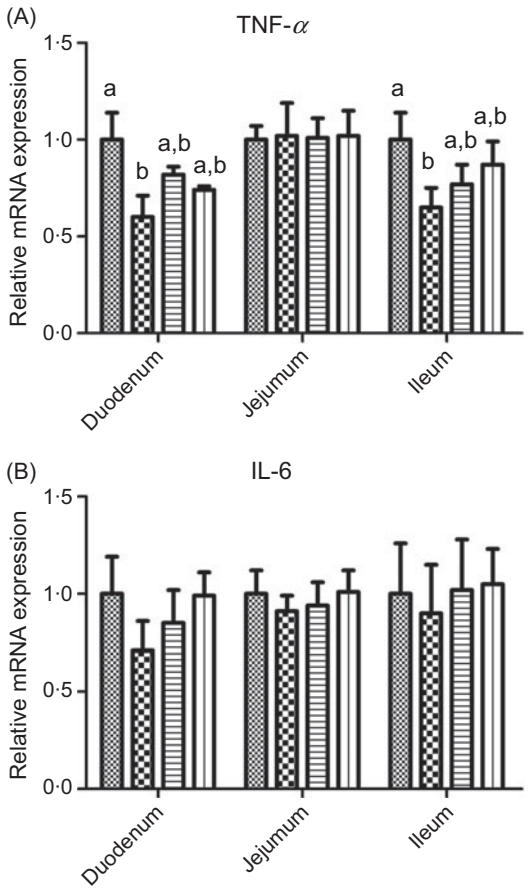

Fig. 3. Effect of inulin (INU) on expression levels of inflammatory cytokines. (A) TNF-α; (B) IL-6. Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively. ▩, CON; ▩, 2·5 g/kg INU; ▭, 5 g/kg INU; ▥, 10 g/kg INU.

Discussion

In recent years, dietary fibres have attracted considerable research interest worldwide since they have been implicated

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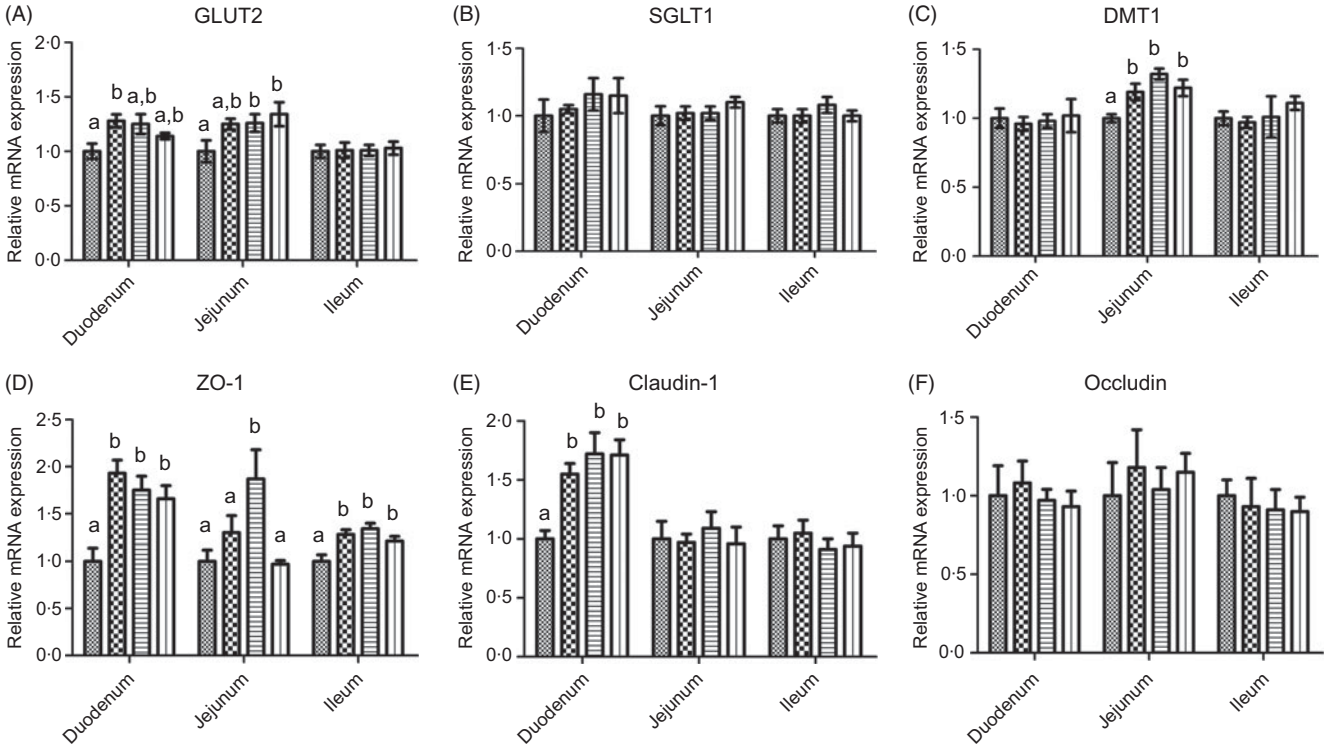

Fig. 4. Effect of inulin (INU) on expression levels of genes related to intestinal barrier functions. (A) GLUT2; (B) Na^+–glucose co-transporter 1 (SGLT1); (C) divalent metal transporter 1 (DMT1); (D) zonula occludens-1 (ZO-1); (E) claudin-1; (F) occludin. Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively. ▩, CON; ▩, 2·5 g/kg INU; ▤, 5 g/kg INU; ▥, 10 g/kg INU.

in regulating the gut health and metabolisms[22]. INU is a soluble dietary fibre extracted from natural plants such as the chicory and Jerusalem artichoke. In the present study, we found that dietary INU supplementation had no significant influence on the growth performance in weaned pigs. This is probably due to the diet formulation, as the INU only accounted for a small portion of the diet, and there were no significant differences in other nutrient levels among the four groups. Moreover, our result is consistent with previous studies on the weaned and growing-finishing pigs[23,24].

Interestingly, INU supplementation with 2·5 and 5·0 g/kg not only elevated the villus height in the jejunum and ileum but also elevated the V:C in the duodenum and ileum. Increasing the intestinal villus height suggested an increased surface area capable of absorption of available nutrients from the intestinal tract[25]. Importantly, increases in the villus height and the ratio of V:C significantly elevated the rate of epithelial turnover[26]. The improved intestinal morphology may be associated with the elevated IGF-1 concentration in the serum, since it has been looked as a critical regulator of organ development and growth[27]. For instance, IGF-1 significantly stimulates cell proliferation and plays an important role in reconstitution of intestinal epithelial integrity after mucosal injury[28]. In the present study, 2·5 g/kg INU supplementation significantly increased IGF-1 concentration in the serum. It is noteworthy that 10·0 g/kg INU supplementation had no significant influence on villus height in the duodenum and jejunum but elevated the villus height and the ratio of V:C in the ileum. To further explore its influence on the integrity of intestinal barrier, we investigated the distribution of the major TJ-related protein ZO-1 by immunofluorescence analysis. The TJ proteins such as claudin-1 and ZO-1 are capable of binding to the cytoskeleton, which not only act as major constituents of the intestinal epithelial barrier but also act as critical regulators of paracellular permeability[29]. We found that the ZO-1 staining of the control group was diffuse with little staining at the intercellular TJ region, suggesting disruption of the TJ. However, the ZO-1 protein was highly expressed and localised to the apical intercellular region of the duodenum and ileal epithelium in pigs fed with an INU-containing diet. The result is consistent with the measurements of the intestinal permeability by using the blood indices. DAO is an intracellular enzyme synthesised by intestinal epithelium and mainly distributed in cytoplasm[30]. Disruption of the intestinal barrier usually leads to releasing of the DAO into the blood circulation[31]. Therefore, the activity of DAO in the blood can serve as one of the circulating markers for monitoring the integrity of intestinal barrier[32]. In the present study, INU supplementation significantly decreased the serum DAO concentration. Both these results indicated that INU supplementation could improve the integrity of intestinal barrier.

The mucosal maltase and sucrase are responsible for the degradation of disaccharides[33]. In the present study, 2·5 and 5·0 g/kg INU supplementation increased the sucrase activity in the duodenum mucosa, while 10 g/kg INU supplementation increased the sucrase activity in the ileum mucosa. We further explored the expression levels of several critical genes involved in nutrient digestion, absorption and intestinal barrier integrity. The GLUT2 and DMT1 are two important transport proteins in

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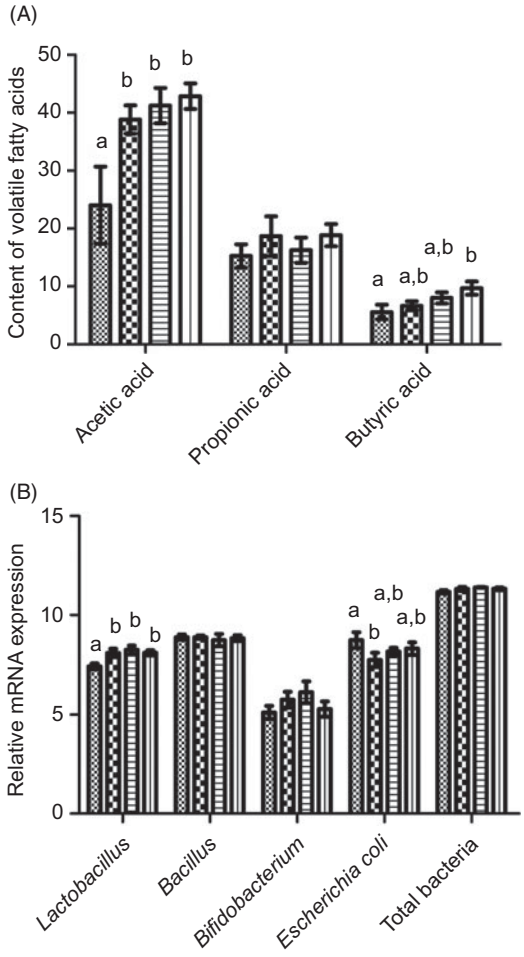

Fig. 5. Effect of inulin (INU) on intestinal microbial population and metabolites. (A) SCFA concentration in the caecum; (B) selected microbial population in the caecum. Pigs were fed with a basal diet (CON) or basal diet supplemented with 2·5, 5·0 and 10·0 g/kg INU, respectively. ▩, CON; ▩, 2·5 g/kg INU; ▤, 5 g/kg INU; ▥, 10 g/kg INU.

the intestinal epithelium that are responsible for glucose and Fe transportation, respectively[34,35]. In the present study, INU supplementation significantly elevated the expression levels of GLUT2 and DMT1 in the Proximal intestinal mucosa, which suggested an improved digestive capacity in pigs after INU ingestion. Our results are also consistent with previous reports that dietary fibres can facilitate the alvine advance rate and significantly improve the alvine absorbing functions[36,37]. TNF-α and IL-6 are two important pro-inflammatory cytokines that play a critical role in regulating the host immunity[38,39]. However, overproduction of pro-inflammatory cytokines may lead to muscle wasting and disruption of intestinal barrier functions[40]. In this study, 2·5 g/kg INU supplementation significantly decreased the expression level of TNF-α in the intestinal mucosa indicating a novel function of the INU in regulating the intestinal inflammatory responses.

INU is mainly catabolised by beneficial bacteria such as the *Lactobacillus* and *Bifidobacterium* and produces various volatile fatty acids (e.g. acetic acid, propionic acid and butyric acid) and organic acids (e.g. succinic acid and pyruvate)[41]. However, harmful bacteria such as *E. coli* and *Salmonella* cannot use the oligofructose[42]. Moreover, the fermented products by beneficial bacteria provide an acidic environment that is important for inhibiting the growth of harmful bacteria[41,42]. The fermented products also play a critical role in maintaining the intestinal health. For instance, the butyric acid can not only serve as an energy source for animals but also promote proliferation and differentiation of the intestinal epithelial cells[13]. In the present study, INU supplementation significantly elevated the concentration of butyric acid in the caecum, which offers a potential mechanism underlying the INU-improved intestinal barrier functions. Additionally, dietary INU supplementation increased the lactobacilli population but decreased the *E. coli* population in the caecum. The result is consistent with previous reports that dietary fibres facilitate the growth of beneficial bacteria such as the lactobacilli and *Bacillus* but inhibit the growth of potential pathogenic bacterial species such as the *E. coli*[9,41–44]. Both these results suggested a beneficial role of dietary fibres in regulating the intestinal microbial ecology and health.

Conclusion

In conclusion, our result suggested a beneficial role of dietary INU supplementation in improving the growth performance and intestinal health in weaned pigs. The mechanisms of action might be closely associated with suppressing of the intestinal inflammatory response, improving of the intestinal morphology and barrier functions, and changes of the microbial fermentation.

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J. H. conceived and designed the experiments. W. W. performed the experiments and wrote the manuscript. D. C., B. Y., X. M., P. Z., J. Y., Z. H., J. L., Y. L. and H. Y. gave constructive comments for the results and discussion of the manuscript. All authors have read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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