Short-chain fructo-oligosaccharides enhances intestinal barrier function by attenuating mucosa inflammation and altering colonic microbiota composition of weaning piglets

Wangsheng Zhaoa, Meng Yuanb, Pengcheng Li, Honglin Yan, Hongfu Zhang and Jingbo Liua,b

School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, China; State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

ABSTRACT
Short-chain fructo-oligosaccharides (scFOS) exert beneficial effects on growth performance and immune function of piglets. However, the effects of scFOS on intestinal integrity of piglets remain to be understood. This study was conducted to investigate the protective effects of scFOS on the intestinal barrier function of piglets. A total of 16 castrated male piglets weaned at 28 d were randomly allocated into 1 of 2 dietary treatments (n = 8): a control diet (CON) or a scFOS-supplemented diet (4 g/kg scFOS substituted the same amount of maize in CON). The experiment lasted 28 d. The average daily scFOS intake of treated group was 1.94 g. Results showed that scFOS consumption increased average daily gain and decreased feed to gain ratio and diarrhoea index (*p* < .05). scFOS-fed piglets had lower serum D-lactate, interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α) concentrations than CON-fed piglets (*p* < .05). scFOS consumption increased intestinal expression of zona occludens 1 (ZO1), occluding (OCLN) and claudin 1 (CLDN1) (*p* < .05). scFOS supplementation down-regulated IL-1β expression in jejunal mucosa and IL-6 expression in ileal mucosa (*p* < .05). scFOS-fed piglets had higher acetic acid level in colonic chyme than their counterparts (*p* < .05). scFOS consumption increased relative abundances of Bacteroidetes, Lactobacillus spp., Prevotella spp. and Bifidobacterium spp. in colonic chyme (*p* < .05). In conclusion, post-weaning scFOS consumption improved performance and intestinal integrity of piglets by suppressing mucosal inflammation, increasing production of short-chain fatty acids and modulating microbiota composition in the hindgut.

HIGHLIGHTS
- Dietary supplementation of 4 g/kg short-chain fructo-oligosaccharides (scFOS) improved growth performance of weaning piglets.
- Dietary scFOS inclusion enhanced gut integrity and ameliorated intestinal inflammatory response.
- scFOS consumption resulted in increased short-chain fatty acids production and altered microbiota composition in the hindgut of piglets.

Introduction
During the weaning transition, piglets encounter numerous stresses that may result in imbalanced intestinal homeostasis, increased occurrence of diarrhoea and reduced growth performance (Pluske et al. 1997; Kick et al. 2012; Zhang et al. 2016). Weaning-induced impaired intestinal epithelial barrier, characterised by increased intestinal permeability, is one of the primary causes for severe diarrhoea and growth retardation of nursery piglets (Smith et al. 2010). Antibiotics and zinc oxide have been widely used to overcome weaning-associated disorders in piglets. The growth-promoting effect of antibiotics and zinc oxide likely benefits from the improvement of gut functions and the adjustment of gut microbiota (Smith et al. 2002; Xu et al. 2018). However, the overuse of antibiotics and zinc oxide has caused severe antibiotic resistance problem and environmental contamination (Erik and Knudsen 2001; Vahjen et al. 2015). Therefore, consistent efforts have been made to seek natural substituents of antibiotics and zinc oxide to improve the growth performance of weaning piglets. An
approach to overcome impaired growth and health of piglets during the weaning period is by administration of prebiotics in the feed (Samal and Behura 2015).

Fructo-oligosaccharides (FOS) have been recommended as a prebiotic compound which has been defined as a non-digestible ingredient that benefits the host’s health by selectively stimulating the growth of beneficial bacteria and increasing production of short-chain fatty acids (SCFAs) (Mikkelsen and Jensen 2004). Currently, the commercial FOS are produced either from sucrose by the action of fructosyltransferases or from inulin by enzymatic degradation (Ganaie et al. 2014; Mutanda et al. 2014). Inulin-type FOS, consisting of long-chain FOS (lcFOS) of which the degree of polymerisation (DP) is larger than 5, are mainly degraded by bacteria in the distal colon, whereas FOS produced from sucrose are short-chain FOS (scFOS) with lower DP (DP < 5) and are mainly fermented in the proximal colon (Crittenden and Playne 1996; Meyer and Stasse-Wolthuis 2009). The effects of inulin-type FOS on growth performance of piglets have been extensively studied and were inconclusive. Some reports have shown that lcFOS ingestion improves growth performance of piglets (Oli et al. 1998; Kołowska et al. 2016), whereas other reports have found little or no effect (Orban et al. 1997; Mikkelsen et al. 2003). Shim et al. (2005) contended that scFOS may be more beneficial than lcFOS. Maternal scFOS consumption has been shown to improve the growth performance and promote the maturation of the immune system of suckling piglets (Le Bourgot et al. 2014; Schokker et al. 2018). Le Bourgot et al. (2017) reported that maternal scFOS consumption increased intestinal cytokines secretion, goblet cell number and butyrate concentration in weaning pigs. However, little is known about the effects of post-weaning ingestion of scFOS on the intestinal barrier function of weaning piglets. Intestinal microbiota has been shown to play a critical role in regulating intestinal barrier function (Cani et al. 2008) and impaired intestinal barrier could be restored by faecal microbiota transplantation (Hu, Ma, et al. 2018; Hu, Wang, et al. 2018). Previous studies showed that scFOS promoted the colonisation of beneficial bacteria in piglets and mice (Shen et al. 2010; Mao et al. 2018). Hence, we hypothesised that scFOS may improve the intestinal barrier function of piglets after weaning. The purpose of this study was to test this hypothesis and to elucidate the underlying mechanisms.

Materials and methods

This experiment was conducted in accordance with the Chinese guidelines for animal welfare and all experimental procedures were approved by the Ethics Committee of Southwest University of Science and Technology (Mianyang, Sichuan, China) under permit number DKX-1020150040.

Animals and treatments

Sixteen healthy castrated male piglets, which are Duroc × (Landrace × Yorkshire) commercial hybrid pigs, were selected from a high-sanitary-status breeder farm of New Hope Group (Mianyang, Sichuan, China) (Liu et al. 2019). Piglets were weaned at 28 d of age and brought to the animal experimental facilities at the Swine Research Unit of Southwest University of Science and Technology. Selected piglets with an initial body weight of 7.03 ± 0.14 kg were randomly allocated to two dietary treatments (n = 8 piglets per treatment) for 28 d. Piglets were fed either a maize-soybean basal diet (control, CON) or a scFOS-containing diet (scFOS was added to the basal diet at 4 g/kg as fed basis at the expense of the same amount of maize, scFOS). Table 1 shows the composition of the basal diet which was formulated to meet the nutritional requirement for 7–11 kg piglets recommended by the National Research Council (2012). The dosage

| Ingredients | Content (%) | Nutrient level a | Content |
|-------------|-------------|------------------|---------|
| Maize       | 30.83       | DE / (MJ/kg)     | 15.09   |
| Extruded maize | 24.00      | CP (%)           | 20.88   |
| Extruded soybean | 6.00       | Ca (%)           | 0.80    |
| Dehulled soybean meal (44% CP) | 10.00 | AP (%)           | 0.41    |
| Soybean protein concentrate | 10.00 | SID Lys (%)      | 1.35    |
| Whey powder | 5.00        | SID Met (%)      | 0.46    |
| Fish meal   | 5.00        | SID Thr (%)      | 0.80    |
| Soybean oil | 1.80        | SID Tep (%)      | 0.22    |
| Sucrose     | 3.00        | -                | -       |
| Glucose     | 2.00        | -                | -       |
| Salt        | 0.30        | -                | -       |
| Limestone   | 0.72        | -                | -       |
| CaHPO4      | 0.52        | -                | -       |
| Chloride choline | 0.10  | -                | -       |
| Vitamin premix b | 0.05  | -                | -       |
| Mineral premix b | 0.30  | -                | -       |
| DL-Met (98.5%) | 0.13       | -                | -       |
| L-Lys-HCl (78%) | 0.18      | -                | -       |
| L-Thr (98.5%) | 0.07       | -                | -       |
| Total       | 100.00      | -                | -       |

aThe vitamin premix provided the following per kg of the diet: retinyl acetate, 0.95 mg; cholecalciferol, 0.02 mg; DL-α-tocopheryl acetate, 28 mg; menadione, 2 mg; riboflavin, 1 mg; pyridoxine, 3 mg; vitamin B12, 48 μg; D-pantothenic acid, 30 mg; folic acid, 2.0 mg; biotin, 1 mg.

bThe mineral premix provided the following per kg of the diet: Fe (ferrous sulphate), 100 mg; Cu (copper sulphate), 6 mg; Mn (manganese oxide), 4 mg; Zn (zinc sulphate), 100 mg; I (potassium iodate), 0.14 mg; Se (sodium selenite), 0.35 mg.

Nutrient levels were calculated values.

DE: Digestible energy; CP: Crude protein; Ca: Calcium; AP: Available phosphorus; SID Lys: Standard ileal digestible lysine; SID Met: Standard ileal digestible methionine; SID Thr: Standard ileal digestible threonine; SID Trp: Standard ileal digestible tryptophan.
of scFOS used in this study was based on a previous study showing that the addition of 4 g/kg scFOS to diet significantly improved growth performance of piglets (Xu et al. 2005). The scFOS (Meioligo-P) was provided by Meiji Seika Kaisha, Ltd (Tokyo, Japan) and comprised 6.5% fructosyl fructosyl nystose (GF5), 43.4% 1F-β-fructofuranosyl nystose (GF4), 40.9% nystose (GF3), 7.1% 1-kestose (GF2) and 2.1% glucose and fructose. All piglets were individually housed in stainless-steel metabolic cages equipped with a nipple drinker and a feeder (Liu, Xue, et al. 2018) and were allowed ad libitum access to feed and water throughout the experiment. The piglets had no access to probiotics and antibiotics during the whole trial.

**Performance measurement and sample collection**

Feed intake of each piglet was recorded daily to determine the average daily feed intake (ADFI) during the 28-d trial and piglets were weighed at the start and the end of the experiment to determine the average daily weight gain (ADG). Feed to gain ratio of each piglet during the entire trial was calculated by dividing ADFI by ADG. Faeces consistency of each piglet was evaluated every morning and evening along the trial by a trained observer who was blind to the treatments as previously described (Diao et al. 2018; Liu, Yan, et al. 2018). In brief, the scoring system adopted in this study was as follows: 0 = normal, firm faeces, 1 = possible slight diarrhoea, soft, formed faeces, 2 = moderate diarrhoea, definite unformed, starchy faeces, 3 = very watery and frothy diarrhoea, and severe fluid faeces. Diarrhoea index of each piglet was calculated as the summation of faeces scores during the whole trial/total observational days. Scores during the whole trial/total observational days. At the end of the feeding trial, after an overnight fasting, blood samples of piglets were collected from the precaval vein into tubes without anticoagulant (5 mL) containing 125 mg tiletamine and 125 mg zolazepam, was dissolved in 5 mL of sterilised saline to a final concentration of 50 mg/mL just before use), followed by exsanguination. Immediately post-mortem, mucosal scrapings from jejunum and ileum were collected, flash-frozen in liquid nitrogen and stored at −80°C until RNA extraction and the determination of SCFAs.

**Intestinal barrier function determination**

Diamine oxidase (DAO) activity in serum was determined using spectrophotometry as described by Hou et al. (2011). The reaction mixture, containing 0.5 mL of undiluted serum sample, 3 mL of phosphate buffer (0.2 M, pH 7.2), 0.1 mL of horseradish peroxidase solution (0.004%), 0.1 mL of o-dianisidine-methanol solution (0.5% of o-dianisidine in methanol) and 0.1 mL of substrate solution (0.175% of cadaverine dihydrochloride), was incubated for 30 min at 37°C and the absorbance at 436 nm was measured to indicate DAO activity. The results were presented as units per litre (U/L) of serum. Undiluted serum samples were used to measure D-lactate and endotoxin levels by adopting commercial ELISA kits purchased from Beijing Luyuan Byrd biological technology Co., Ltd. (Beijing, China) with a microplate reader, according to the manufacturer’s instructions. The D-lactate and endotoxin concentrations were expressed as microgram per millilitre (μg/mL) and Endotoxin Units per millilitre (EU/ml) of serum, respectively. All procedures were performed in duplicate.

**Serum cytokines analyses**

Concentrations of serum inflammatory cytokines, interleukin 1β (IL-1β), interleukin 6 (IL-6), tumour necrosis factor-alpha (TNF-α) and interleukin 10 (IL-10), were measured with commercial available kits (Porcine IL-1β Quantikine ELISA kit, Porcine Quantikine IL-6 ELISA kit, Porcine TNF-α Quantikine ELISA kit and Porcine IL-10 Quantikine ELISA kit; R&D system Inc., Minneapolis, MN), according to the manufacturer’s instructions. All measurements were done by using undiluted serum samples. The optical density was measured spectrophotometrically at a wavelength of 450 nm with the wavelength correction at 540 nm. The sample cytokine concentration was determined by comparing the optical density of the sample to a standard curve of the corresponding cytokine. The cytokines concentrations were presented as picogram per millilitre (pg/mL) of serum. All samples, standards and controls were assayed in duplicate. Sample cytokines concentrations were only accepted if the standard curve correlation coefficient (r) reached 0.99 and the CV of each sample was under 20%.
Measurement of mRNA expression of intestinal tight junction protein and cytokines

Total RNA from jejunal and ileal mucosa was extracted using Bio-Rad Aurum Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad Laboratories, Hercules, CA). Before synthesising the cDNA, RNA quality control was performed. The purity (OD 260/280, ranging from 1.9 to 2.2, and OD 260/230, ranging from 2.0 to 2.2) and concentration of total RNA (ranging from 150 to 1200 ng/μL) were measured using the Nanodrop ND-1000 (Nanodrop Technologies, Thermo Scientific, Wilmington, DE). An amount of 1 μg of RNA was loaded on a 2% agarose gel to verify the integrity by visually evaluating the 28S and 18S ribosomal RNA loaded on a 2% agarose gel to verify the integrity by visually evaluating the 28S and 18S ribosomal RNA bands. A minus-reverse transcription (–RT) control PCR was performed using primer for tyrosine 3-mono-oxygenase/tryptophan 5-monoxygenase activation protein (YWHAZ) to verify the absence of genomic DNA in RNA samples. The thermal cycling conditions of –RT control PCR were as follows: an initial denaturation step at 95 °C for 14 min 40 s, then 40 cycles of amplification (denaturation at 95 °C for 20 s, annealing at 60 °C for 20 s and extension at 72 °C for 40 s), followed by storing at 15 °C. The DNA-free RNA (1 μg) was converted into cDNA by using the ImProm-II cDNA synthesis kit (Promega, Madison, WI). The targeted genes in this study were designed with Primer3Plus based on the certain exon-exon boundaries of published gene sequences of pigs on the NCBI database.

All the primers were designed according to the sequence which can be reached by corresponding accession number and can only amplify the isoform listed above.

ACTB: β-actin; TOP2B: topoisomerase II beta; TBP: TATA box binding protein; YWHAZ: tyrosine 3-mono-oxygenase/tryptophan 5-monoxygenase activation protein; IL-1β: interleukin 1 beta; IL-6: interleukin 6; TNF-α: tumour necrosis factor alpha; IL-10: interleukin 10; ZO1: zona occludens 1; ZO2: zona occludens 2; OCLN: occludin; CLDN1: claudin 1; CLDN2: claudin 2.

Table 2. Nucleotide sequences of primers used to measure targeted genes.

| Gene symbols | Nucleotide sequence of primers (5’-3’ | Accession No. | Annealing temperature |
|--------------|--------------------------------------|---------------|-----------------------|
| ACTB         | F: TCTGGCACCACACACCTCCTCT          | XM_003124280.3 | 60                    |
|              | R: TGACTCTGGGTCATCTTCCTCAC         |               |                       |
| TOP2B        | F: AACTGGGATGATGACTTTGTCTG         | NM_00258386.1 | 60                    |
|              | R: TTGGAAACACTTGTCGTTCTCGC         |               |                       |
| TBP          | F: GATGGACGTTCGTTAGGG              | DQ178129      | 59                    |
|              | R: AGCAGCACAGTACGAGCAA             |               |                       |
| YWHAZ        | F: ATGCAACCAACACATCTCTAC           | DQ178130      | 60                    |
|              | R: GCATTATAGCGTGCTCGTTC            |               |                       |
| IL-1β        | F: ACGTGCAATGATGACTTTGT            | NM_214055.1   | 58                    |
|              | R: AGAGCCCTTCAGCTGTGAGG            |               |                       |
| IL-6         | F: TTCACTCTCCGAGAAAC              | NM_214399.1   | 60                    |
|              | R: TCTGCCAGACTCTCTCTGCT            |               |                       |
| TNF-α        | F: CATGATCCGAGACTGG                | NM_214022.1   | 62                    |
|              | R: CTGAAATGCGAGTGGGC              |               |                       |
| IL-10        | F: TAATGGCGAAGGCCAGAGGT            | NM_214041.1   | 60                    |
|              | R: GGCCTTCCTTCTGTTTCAC            |               |                       |
| ZO1          | F: ATCTCAGAAGGAGGACGAGG            | NM_214041.1   | 60                    |
|              | R: GCCCTTCAGAAACGTCGAGA            |               |                       |
| ZO2          | F: CACCATAGCCAGAAGAAATGGAGA        | XM_003480423.3| 61                    |
|              | R: AAGGTTCTCGGGGGGTCCTCTCT         |               |                       |
| OCLN         | F: CATGGCTGCTCTGCTGCTATGTGG        | NM_001426267.2| 65                    |
|              | R: ACCATCAACCCAGATACAGCTCA         |               |                       |
| CLDN1        | F: TATGACCCCATAGACCTCGG            | NM_002144039.1| 59                    |
|              | R: GCAGCAAGAAGGAGGACC             |               |                       |
| CLDN2        | F: TTTCCTCTTGCTTCTGCTGTA          | NM_001161638.1| 62                    |
|              | R: CACTCTTGGCTTTGGGGTTG            |               |                       |

All the primers were designed according to the sequence which can be reached by corresponding accession number and can only amplify the isoform listed above.
Colonic SCFAs profile determination

About 0.5 g of thawed colonic chyme was suspended in 1.5 mL of sterile distilled water in a screw-capped tube. After 30-min stewing and centrifugation at 5000 rpm for 10 min, 200 µL of metaphosphoric acid (25%, w/v) and 23.3 µL of crotonic acid (21 mM) were added to 1 mL of supernatant, followed by 30-min stewing and 10-min centrifugation at 10,000 rpm. Then 300 µL of supernatant was mixed with 900 µL of methanol and the resulting mixture was centrifuged at 10,000 rpm for 10 min. After that, the supernatant was filtered with a 0.22 µm filter membrane. The filtrate was transferred into a sterile tube and stored at 4 °C until further analysis. The acetic acid, propionic acid and butyric acid were measured with a gas chromatograph (VARIAN CP-3800, Varian, CA) equipped with a flame ionisation detector and a capillary column (25 mm × 0.32 mm, 0.3-µm film thickness; Varian Inc., Palo Alto, CA). Helium was used as the carrier gas and isobutyric acid was used as an internal standard. A standard SCFAs mixture containing acetate, propionate and butyrate was used for the calculation and the results were expressed as µmol/g of sample. Each sample was analysed in triplicate.

Measurement of selected bacteria abundances in colonic digesta

The DNA extraction was carried out following the instructions of commercial Stool DNA isolation Kit (Omega BioTek, Norcross, GA, USA). The concentration and quality of total DNA were assessed using NanoDrop ND-1000 (Nanodrop Technologies, Thermo Scientific, Wilmington, DE) and agarose gel electrophoresis (Yan, Zhang, et al. 2019). The abundances of selected bacteria in colonic chyme were measured according to the methods described by Bergström et al. (2012). The abundances of Universal (total bacteria), Phylum Firmicutes, Phylum Bacteroidetes, Prevotella spp., Bacteroides spp., Lactobacillus spp., Roseburia spp., Ruminococcus spp. and Bifidobacterium spp. were determined in this study. Primers sequences were cited from Bergström et al. (2012) and were listed in Table 3. The RT-qPCR was performed using the KAPA SYBR® FAST qPCR Kit (Kapa Biosystems, Inc., Wilmington, MA). Each reaction consisted of 5.5 µL 2x KAPA SYBR FAST qPCR Kit Master Mix, 0.4 µL forward primer (10 µM), 0.4 µL reverse primer (10 µM), 2.7 µL Milli-Q water and 2 µL DNA template in a total volume of 11 µL. The thermal cycling conditions were as follows: an initial denaturation and enzyme activation step at 50 °C for 2 min and 95 °C for 10 min, then 40 cycles of denaturation/annealing and data acquisition (95 °C for 15 s, 1 min at 60 °C), and melt curve analysis from 70 to 90°C with 0.5°C increment every 5 s. Following the thermocycling programme, the raw fluorescence data recorded by the SDS software were exported to LinregPCR software. The LinregPCR software was used to perform baseline corrections and calculate the average PCR efficiency for each amplicon. The initial quantities (N0) for each amplicon were calculated based on the corresponding threshold and average PCR efficiency. The relative abundance of the specific microbe was calculated as N0, specific/N0, universal (Yan, Cao, et al. 2019).

Table 3. Primer sequences used to analyse abundances of selected microbes.

| Microbes                  | Nucleotide sequence of primers (5’-3’)                          |
|--------------------------|-----------------------------------------------------------------|
| Universal                | F: ACTCTACGGGAGGCGAGCAGT R: TATTTGCGGCTGTCGAGCAC                 |
| Firmicutes               | F: TGAAGCCTCAAGGAAATTGACG R: ACCATGCACACCTGTCC                   |
| Bacteroidetes            | F: GGAACATGCGTTTAATTCGATGAT R: AGCTGACGAGAAACACATGACG           |
| Lactobacillus spp.       | F: AGCATGAGGGAACTCTCCA R: CACGCTCAACATAGGGG                    |
| Prevotella spp.          | F: CACCAAGGCGAGCAGTCA R: GGATAACCGCTGGACCT                     |
| Bacteroides spp.         | F: CGATGGAGATGGGTTCTGAGGA R: GCTGGCAGCGGAGGGTCGA                |
| Ruminococcus spp.        | F: GAGTGAAATGAGGTAACGGAATTCC R: GCCGACTTCGCCAGGTT                |
| Roseburia spp.           | F: TACGCTAGGAAAACTGTCG R: CGGACCCGAAGGCAAT                     |
| Bifidobacterium spp.     | F: GCGTCTTAAACACATGCAAGTC R: CAACCGTTTTTCAGGAGCTATT            |

Statistical analysis

Piglet was considered as the experimental unit for all analyses (n = 8 per treatment) and all data were expressed as mean± standard deviation. After determination of normality, data fit a normal distribution were tested for significance with the Student’s t-test (PROC TTTEST, SAS version 9.1, SAS Institute, Cary, NC) and non-normal data were tested for significance with Mann–Whitney U test (PROC NPAR1WAY, SAS version 9.1, SAS Institute, Cary, NC). Statistical significance was set at p < .05, whereas .05 ≤ p ≤ .10 was considered a trend towards significance.

Results

Growth performance and diarrhoea index

The growth performance and diarrhoea index of the two treatments are listed in Table 4. Piglets fed the diet containing scFOS exhibited higher final body weight and average ADG than those fed CON (p < .05). scFOS
concentrations than those fed CON (饮食有显著较低的IL-1)
activity in serum of scFOS-fed piglets compared to
Table 5. There was a tendency towards lower DAO
concentrations of weaning piglets.

| Items            | Control group | scFOS group | p Value |
|------------------|---------------|-------------|---------|
| Initial body weight, kg | 7.02 ± 0.16   | 7.03 ± 0.13 | .84     |
| Final body weight, kg  | 14.99 ± 0.41  | 15.52 ± 0.49 | <.05   |
| Average daily feed intake, g | 477.50 ± 16.66 | 486.11 ± 22.50 | .40     |
| Average daily weight gain, g  | 284.82 ± 9.81 | 302.98 ± 13.08 | <.05   |
| Feed to gain ratio   | 1.68 ± 0.03   | 1.60 ± 0.02  | <.05   |
| Diarrhoea index      | 0.24 ± 0.04   | 0.19 ± 0.02  | <.05   |

scFOS: short-chain fructo-oligosaccharides.

Table 5. Effects of short-chain fructo-oligosaccharides on serum intestinal permeability biomarkers and cytokines concentrations of weaning piglets.

| Items            | Control group | scFOS group | p Value |
|------------------|---------------|-------------|---------|
| Diamine oxidase, U/L | 8.71 ± 1.54   | 7.38 ± 0.94 | .06     |
| D-lactate, µg/mL  | 23.14 ± 1.50  | 20.47 ± 1.69 | <.05   |
| Endotoxin, EU/mL | 195.55 ± 13.56 | 201.38 ± 12.11 | .88     |
| IL-1, pg/mL      | 179.02 ± 12.27 | 162.10 ± 14.18 | <.05   |
| IL-6, pg/mL      | 45.36 ± 4.81  | 31.90 ± 5.06 | <.05   |
| TNF-α, pg/mL     | 113.59 ± 7.09 | 114.87 ± 11.24 | .79     |
| TNF-α/mg content | 218.77 ± 21.49 | 186.04 ± 23.44 | <.05   |

scFOS: short-chain fructo-oligosaccharides; EU: endotoxin units; IL-1β: interleukin 1 beta; IL-6: interleukin 6; IL-10: interleukin 10; TNF-α: tumour necrosis factor alpha.

Intestinal barrier function and serum cytokines concentrations

Dietary scFOS supplementation significantly decreased serum D-lactate concentration (p <.05) as shown in Table 5. There was a tendency towards lower DAO activity in serum of scFOS-fed piglets compared to CON-fed piglets (p=.06). Piglets fed scFOS-containing diet had significant lower IL-1β, IL-6 and TNF-α concentrations than those fed CON (p <.05).

mRNA expression of intestinal tight junction proteins

The mRNA expression of tight junction proteins in jejunal and ileal mucosa is shown in Table 6. Dietary scFOS supplementation significantly up-regulated mRNA expression of ZO1, OCLN and CLDN1 in jejunal and ileal mucosa of piglets (p <.05).

mRNA expression of intestinal cytokines

Table 7 reveals that the expression of IL-1β (p <.05) was significantly down-regulated and the mRNA abundance of IL-6 (p=.10) tended to decrease in jejunal mucosa of scFOS-fed piglets, as compared to CON-fed piglets.

Table 6. Effects of short-chain fructo-oligosaccharides on mRNA expression of tight junction proteins in jejunum and ileum of weaning piglets.

| Genes  | Control group | scFOS group | p Value |
|--------|---------------|-------------|---------|
| ZO1    | 1.00 ± 0.20   | 1.51 ± 0.16  | <.05   |
| ZO2    | 1.00 ± 0.40   | 1.09 ± 0.29  | .62    |
| OCLN   | 1.00 ± 0.30   | 1.37 ± 0.35  | <.05   |
| CLDN1  | 1.00 ± 0.35   | 1.81 ± 0.30  | <.05   |
| CLDN2  | 1.00 ± 0.26   | 1.10 ± 0.18  | .39    |
| CLDN3  | 1.00 ± 0.25   | 1.44 ± 0.19  | <.05   |
| CLDN4  | 1.00 ± 0.17   | 1.20 ± 0.31  | .13    |

scFOS: short-chain fructo-oligosaccharides; ZO1: zona occludens 1; ZO2: zona occludens 2; OCLN: occludin; CLDN1: claudin 1; CLDN2: claudin 2.

There was a trend towards higher butyric acid content in colonic chyme of scFOS-fed piglets than those fed CON (p <.05). There was a trend towards higher acetic acid concentration in colonic chyme than those fed CON (p <.05). There was a trend towards higher butyric acid content in colonic chyme.
of pigs fed scFOS than their counterparts (p=.06). As shown in Table 9, scFOS consumption significantly increased relative abundances of Phylum Bacteroidetes, Lactobacillus, Prevotella spp., and Bifidobacterium spp. in colonic chyme of piglets (p < .05).

Table 9. Effects of short-chain fructo-oligosaccharides on relative abundance of selected bacteria in colonic chyme of weaning piglets.

| Microbes (%) | Control group | scFOS group | p Value |
|--------------|---------------|-------------|---------|
| Firmicutes   | 40.72 ± 8.08  | 40.48 ± 5.29 | .95     |
| Bacteroidetes| 41.77 ± 4.98  | 49.16 ± 4.33 | <.05    |
| Lactobacillus| 1.80 ± 0.62   | 3.96 ± 1.07  | <.05    |
| Prevotella   | 20.36 ± 4.94  | 27.02 ± 6.44 | <.05    |
| Bacteroides  | 0.37 ± 0.26   | 0.46 ± 0.10  | <.1     |
| Ruminococcus | 0.26 ± 0.13   | 0.26 ± 0.13  | <.1     |
| Roseburia    | 0.21 ± 0.09   | 0.26 ± 0.13  | <.1     |
| Bifidobacterium | 0.12 ± 0.06 | 0.90 ± 0.44  | <.05    |

scFOS: short-chain fructo-oligosaccharides.

Discussion

The inclusion of non-digestible oligosaccharides in the diet of weaning piglets has been shown to mitigate weaning-induced diarrhoea and disruption of growth performance (Rozeboom et al. 2005; Liu et al. 2008). However, previous studies obtained discordant results regarding the effects of FOS supplementation on growth performance of piglets. Some studies have shown that FOS consumption significantly improved growth performance and intestinal morphology of weaning piglets (Oli et al. 1998; Xu et al. 2005). Conversely, others contended that dietary FOS inclusion has no effect on growth performance of weaning piglets (Orban et al. 1997; Mikkelsen et al. 2003). The contradictory findings may stem from the different type and dosage of FOS used between previous studies. Previous studies showed that the growth performance, immune function or intestinal morphology of piglets were improved after consumption of scFOS (Xu et al. 2005; Le Bourgot et al. 2017; Schokker et al. 2018). Consistently, in this study, piglets fed the diet containing scFOS increased average ADG and decreased feed to gain ratio of piglets. Diarrhoea is the most common clinical symptom after weaning (Xu et al. 2018). In this study, we provided evidence of a significant decrease in diarrhoea index in scFOS-fed piglets, which indicated that the addition of scFOS to the diet might ameliorate weaning-induced intestinal dysfunction (Cao et al. 2018).

The integrity of the intestinal barrier has been shown to play a critical role in preventing pathogens, toxic or allergenic materials entering the body through the intestine (Liu et al. 2012). The intestinal epithelial barrier, the first line of defence against a harmful environment within intestinal lumen, is mainly formed by a layer of epithelial cells connected by tight junction proteins (Pi et al. 2014). In this study, intestinal permeability was evaluated by measuring DAO activity, D-lactate and endotoxin concentrations in serum as well as expression of tight junction proteins in the small intestine. DAO, an intracellular enzyme primarily synthesised in intestinal epithelia of mammalian, will be released into the blood when the intestinal epithelial barrier is injured (Nieto et al. 2000). D-lactate and endotoxin are derived from intestinal bacteria and will be released into the blood when the intestinal barrier is damaged (Cani et al. 2008; Chen et al. 2017). Therefore, blood DAO activity, D-lactate and endotoxin concentrations could be regarded as circulating markers to evaluate intestinal barrier function (Chen et al. 2017). The results of this study showed that dietary scFOS supplementation decreased serum DAO activity and D-lactate concentration, indicating that scFOS attenuated intestinal permeability of weaning piglets. However, dietary FOS inclusion impaired intestinal barrier function of rodents (Ten Bruggencate et al. 2005; Rodenburg et al. 2008). In addition, FOS consumption had no effect on intestinal permeability of healthy men (Ten Bruggencate et al. 2006). The inconsistent findings between previous studies and this study might be attributed to the differences in species, the degree of polymerisation of tested FOS and diet composition, as distinct dietary protein sources could differentially modulate the effects of FOS on gut fermentation and microbiota (Bai et al. 2016). Tight junction proteins, such as claudin families, occludin and ZO1s, anchor to the actin-based cytoskeleton to form a selective permeable barrier, termed tight junction permeability barrier (Wong and Gumbiner 1997). The expression of tight junction proteins is important for maintaining the intestinal epithelial barrier (Anderson and Van Itallie 1995). Cao et al. (2018) reported that weaning impaired intestinal integrity by down-regulating the expression of ZO1, OCLN and CLDN1 in jejunal mucosa of piglets. In this study, scFOS consumption increased mRNA expression of ZO1, OCLN and CLDN1 in both jejunal and ileal mucosa of weaning piglets, which was consistent with the results of the previous study in neonatal piglets (Le Bourgot et al. 2017). Collectively, post-weaning consumption of scFOS attenuated intestinal permeability of piglets by enhancing the intestinal barrier function.

Weaning-associated inflammatory status has been associated with impaired intestinal barrier function.
Both in vivo and in vitro studies showed that the increased secretion of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, disrupted the intestinal mucosal integrity (McKay and Baird 1999; Pié et al. 2004). To further investigate the mechanism through which scFOS improved intestinal barrier function, the serum cytokines concentration and intestinal cytokines expression were determined. In this study, post-weaning scFOS consumption decreased serum IL-1β, IL-6, and TNF-α concentrations and down-regulated expression of IL-1β and/or IL-6 in the small intestine, which was similar with the previous study showing that maternal scFOS consumption promoted the maturation of the intestinal immune system of suckling piglets (Le Bourgot et al. 2014). Additionally, the results of this study showed that dietary scFOS supplementation tended to increase the expression of IL-10 in the small intestine of weaning piglets, which was consistent with the previous study demonstrating that non-digestible polysaccharides could increase the intestinal expression of anti-inflammatory cytokine, IL-10 (Hu, Ma, et al. 2018; Hu, Wang, et al. 2018). Thus, dietary scFOS supplementation may alleviate the weaning-induced impaired intestinal barrier function by suppressing the production of pro-inflammatory cytokines and facilitating the secretion of anti-inflammatory cytokine.

scFOS are considered to confer beneficial effects to host health by selectively stimulating the growth of beneficial bacteria (Le Bourgot et al. 2017; Mao et al. 2018). Intestinal microbiota has been shown to play a critical role in regulating intestinal integrity of weaning piglets (Hu, Ma, et al. 2018; Hu, Wang, et al. 2018). Therefore, we further investigated the effects of post-weaning scFOS consumption on colonic SCFAs profile and microbiota composition. In this study, dietary scFOS supplementation increased colonic acetic acid and butyric acid concentrations, which was similar with the previous study showing that maternal scFOS consumption increased production of total SCFAs or butyric acid in suckling or weaning piglets, respectively (Le Bourgot et al. 2017). Likewise, Shim et al. (2005) also showed that dietary scFOS inclusion significantly increased butyric acid and isobutyric acid production of weaning piglets. SCFAs are important for intestinal health, as indicated by improved gut barrier function and accelerated intestinal growth in SCFAs-infused or fibre-treated piglets (Diao et al. 2017; Cheng et al. 2018). Acetic acid has been shown to be an anti-inflammatory substance to maintain intestinal homeostasis and butyric acid is considered to enhance intestinal barrier function by increasing the expression of tight junctions (Huang et al. 2015). It can be inferred that the beneficial effects of scFOS consumption on the intestinal barrier function of piglets might be associated with increased production of acetic and butyric acid in the hindgut. It is believed that gut microbiota plays a causal role in FOS-induced beneficial effects on host intestinal barrier function (Mao et al. 2018). Previous studies showed that scFOS consumption increased the abundance of Bifidobacterium spp. in piglets and mice (Shen et al. 2010; Mao et al. 2018). In agreement, the results of this study revealed that dietary scFOS supplementation increased colonic Bifidobacterium spp. abundance. However, inconsistent results showed that dietary scFOS had no effect on the number of culturable bifidobacteria (Houdijk et al. 2002; Mikkelsen et al. 2003). This discrepancy mainly due to the different methods (culture-dependent methods versus culture-independent methods) used for the determination of microbial abundances between studies. In this study, scFOS consumption increased relative abundance of Lactobacillus spp., which was in line with the previous study showing an increased number of lactobacilli in piglets treated with scFOS (Oli et al. 1998). An increase in gut bifidobacterial and lactobacilli is considered beneficial for host health because bifidobacteria and lactobacilli have been shown to prevent diarrhoea and promote intestinal integrity (Gibson and Roberfroid 1995; Sawicki et al. 2017). Prevotella spp. participates in energy extraction by degradation of dietary non-digestible carbohydrates into SCFAs and is of importance for maintaining intestinal homeostasis. Increased abundance of Prevotella spp. has been associated with improved intestinal integrity (Xue et al. 2018). Previous study seeking the link between dietary patterns and gut microbial enterotypes showed that Prevotella enterotype was strongly associated with carbohydrates consumption (Wu et al. 2011). Similarly, in this study, scFOS ingestion increased the abundance of Prevotella spp. in piglets, which might explain the increased luminal SCFAs production in scFOS-fed piglets. Thus, these observations may imply that post-weaning scFOS consumption could shape the microbiota community of piglets to a healthier pattern, which may be associated with enhanced intestinal integrity.

Conclusions
Collectively, post-weaning scFOS consumption resulted in improved growth performance and enhanced intestinal barrier function of piglets, which might be
associated with attenuated intestinal inflammatory response, increased production of SCFAs and beneficially-modified microbiota composition in the hindgut.

**Disclosure statement**

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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