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Non Ruminant Nutrition

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Comparative effects of dietary supplementations with sodium butyrate, medium-chain fatty acids, and n-3 polyunsaturated fatty acids in late pregnancy and lactation on the reproductive performance of sows and growth performance of suckling piglets

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Abstract

This study was conducted to compare the effects of adding sodium butyrate (**SB**), medium-chain fatty acids (**MCFAs**), or n-3 polyunsaturated fatty acids (**n-3 PUFAs**) to the diet of sows during late gestation and lactation on the reproductive performance of sows and the growth performance and intestinal health of suckling piglets. Twenty-four sows (Landrace × Large-White hybrid; third parity; 200 ± 15 kg) were randomly assigned to receive 1 of 4 diets: basal diet (control group), basal diet + 1 g SB/kg (SB group), basal diet + 7.75 g MCFA/kg (MCFA group), or basal diet + 68.2 g n-3 PUFA/kg (n-3 PUFA group). The experiment began on day 85 of gestation and ended day 22 of lactation. Colostrum samples were collected from each sow. After the experiment, blood and tissue samples were collected from 1 randomly selected piglet. The results showed that the weaning-to-estrus interval of sows in the SB, MCFA, and n-3 PUFA groups was shorter than that of sows in the control group ($P < 0.05$). The incidence of diarrhea in suckling piglets in the SB, MCFA, and n-3 PUFA groups was lower than that of piglets in the control group ($P < 0.05$). The fat, protein, IgA, IgG, and IgM concentration in colostrum from sows increased following dietary supplementation with SB, MCFA, or n-3 PUFA ($P < 0.05$). Comparison with the control group, the mRNA expression of claudin-1, zona occludens 1, and interleukin-10 increased in the jejunum mucosa of suckling piglets in the SB, MCFA, and n-3 PUFA groups, while that of TLR4 decreased ($P < 0.05$). Compared with the control group, the Chao1 and ACE indexes of microbial flora in the colon contents of piglets in the SB, MCFA, and MCFA groups increased ($P < 0.05$), while the relative abundance of *Firmicutes*, *Actinobacteria*, and *Synergistetes* decreased at the phylum level ($P < 0.05$). In conclusion, during late pregnancy and lactation, dietary SB supplementation had a greater effect on intestinal health and caused a greater decrease in preweaning mortality of suckling piglets than did dietary MCFA or n-3 PUFA supplementation; dietary MCFA supplementation shortened the weaning-to-estrus interval of sows to a greater extent than did dietary SB or n-3 PUFA supplementation; and dietary n-3 PUFA supplementation increased the fat and protein content in the colostrum to the greatest extent.

Key words: medium-chain fatty acid, n-3 polyunsaturated fatty acid, sodium butyrate, sow, suckling piglet

Introduction

The reproductive performance of sows and growth performance of newborn piglets are fundamental to the development of pig farming. Three-quarters of fetal weight is gained in the last quarter of pregnancy, during which sufficient energy is required to meet the nutritional needs of sows. Sows with inadequate nutrient intake during lactation will utilize body fat and protein to meet the nutritional needs of breastfeeding, resulting in weight loss, a prolonged weaning-to-estrus interval or loss of estrus, and shortened productive life.

Recently, studies have found that fatty acids act as a source of energy, and have several unique roles, such as metabolic regulation, antibacterial activity, and anti-inflammatory effects (Liu, 2016). Several studies have reported that the addition of fatty acids to the daily feed rations of sows during late pregnancy and/or lactation can reduce body weight loss during lactation, shorten the weaning-to-estrus interval, and increase the fat content of milk. Furthermore, the type and amount of fat in daily feed rations also affect the immunoglobulin and fatty acid composition in milk and can improve the survival rate and daily weight gain of newborn piglets when ingested through the breast milk (Lauridsen et al., 2004; Shen et al., 2015; Jin et al., 2017). Butyric acid is a short-chain fatty acid; as a new feed additive, its sodium salt, i.e., sodium butyrate (**SB**), can significantly enhance the growth performance of piglets by improving the morphology of the small intestine, promoting the proliferation of beneficial bacteria, and boosting the immune system (Huang et al., 2015). Medium-chain fatty acids (**MCFAs**) are saturated fatty acids with 6- to 12-carbon chains. Medium-chain fatty acids can provide the body with a rapid supply of energy because they have good stability and low energetic value, and can be rapidly digested, absorbed, and oxidatively metabolized in the body (Zentek et al., 2011). Azain 1993) reported that the addition of 10% medium-chain triglycerides (**MCTs**) to the daily feed rations of sows from late pregnancy to 7-d postpartum can improve the MCFA content in milk and the survival rate of piglets weighing < 900 g. n-3 polyunsaturated fatty acids (**n-3 PUFAs**), including α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, are long-chain fatty acids containing more than 2 double bonds and 16- to 22-carbon chains. They play important roles in growth and development, as well as enhancing the immune system in animals. A study carried out by McAfee et al. (2016) revealed that the addition of 1% fish oil to the daily feed rations of sows during late pregnancy and lactation can promote the growth of newborn piglets and reduce weaning stress.

Numerous studies have investigated the effects of SCFA, MCFA, and n-3 PUFA on the reproductive performance of sows or growth performance of piglets; however, studies comparing the functions of SB, MCFA, and n-3 PUFA on sow and piglet performance are limited. In the present study, we compared the effects of dietary SB, MCFA, and n-3 PUFA supplementation during late pregnancy and lactation on the reproductive performance of sows and growth performance of piglets, to provide a theoretical reference for the reasonable use of SB, MCFA, and n-3 PUFA in sow reproduction.

Materials and Methods

Animal Use and Care

All experimental procedures were approved by the Southwest University Animal Care and Use Committee.

Twenty-four tested sows (Landrace × Large-White hybrid; third parity; 200 ± 15 kg) were provided by the Sichuan Giastar Group Co., Ltd. Sows were individually housed in gestation crates (0.60 by 2.15 m) with partially slatted floors until day 110 of gestation when they were transferred to individual farrowing crates (1.20 by 2.15 m) with a cast iron sow floor and plastic creep floor.

Experimental Diets and Design

Twenty-four sows were randomly assigned to 1 of 4 diets: basal diet (control group), basal diet + 1 g coated SB/kg (SB group), basal diet + 7.75 g coated MCFA/kg (MCFA group), and basal diet + 68.2 g coated n-3 PUFA/kg (n-3 PUFA group). Each dietary treatment included 6 replicates with 1 sow per replicate. Of note, sows were selected based on their reproductive performance at the second parity in order to prevent a false lack of effect. The basic daily feed ration (Table 1) for sows during late pregnancy and lactation was formulated according to NRC (1998). Coated SBs, MCFAs, and long-chain n-3 PUFAs used in the present study were provided by Singao Agribusiness Development Co., Ltd. (Xiamen, Fujian, China). Sodium butyrate is the active ingredient of coated SB, accounting for more than 98% of its content; the active ingredient of coated MCFA is MCT, which accounts for 70.0% of its content; the main active ingredients of coated n-3 PUFA include α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, which account for 20% of its content. The experiment began on day 85 of gestation and ended on day 21 of lactation. During the test period from day 85 of gestation

Table 1. Ingredients and composition of basal diets (air-dry basis)

	Stages	
	Late gestation	Lactation
Ingredients (%)		
Corn	50.04	47.65
Barley	17.40	18.00
Soybean meal	17.20	19.00
Expanded soybean	6.00	6.00
Soybean oil	2.70	2.70
Fish meal	2.00	2.00
Limestone	1.60	1.60
$CaHPO_4$	1.40	1.40
NaCl	0.40	0.40
Lys	0.26	0.25
Premix[1]	1.00	1.00
Total	100.00	100.00
Composition[2] (%)		
DE (Mcal/kg)	3.39	3.42
CP	15.40	15.90
EE	5.00	5.10
Ash	5.80	5.90
CF	3.90	3.50
Ca	1.07	1.20
Total P	0.63	0.74
Available P	0.50	0.59
Lys	1.14	1.17

[1]The premix provided the following per kilogram of the diet for late gestation: Cu 5 mg, I 0.15 mg, Fe 83 mg, Mn 20 mg, Zn 128 mg, VA 13,400 IU, VD_3 2,800 IU, choline chloride 1,000 mg, VE 22.4 mg, VK_3 3 mg. The premix provided the following per kilogram of the diet for lactation: Cu 15 mg, I 0.13 mg, Fe 82 mg, Mn 20 mg, Zn 128 mg, VA 10,000 IU, VD_3 2,000 IU, VE 30 mg, and VK_3 1.5 mg.
[2]DE is calculated value and others are measured values.

until farrowing, each sow was provided 2.70 kg/d diet. On the day of farrowing, each sow was provided 1.5 kg diet; the amount was then being successively increased (2 kg/d on days 1 and 2 of lactation; 4 kg/d on days 3 to 7 of lactation; 5.5 kg/d from days 8 to 14; 6.0 kg/d from days 15 to 21). The feed was divided into 2 equal portions, provided at 0800 and 1600 h. Water was provided from nipple drinker systems throughout the whole feeding period.

Recording and Sample Collection

The daily food intake of sows was recorded throughout the lactation period. The total born litter size, number of piglets born alive, number of piglets stillborn, individual birth weight, and litter birth weight were recorded. To confirm that a piglet is stillborn, animals that appear to be stillborn should be dissected to determine whether their lungs float; this is because stillborn individuals (intrapartum) look the same as their living littermates, but do not breath (Mota-Rojas et al., 2006). The feed intake of each sow during lactation was recorded daily and average daily feed intake was calculated. Within 24 h of farrowing, the litter size of these sows was standardized to 11 piglets per litter within the same dietary treatment group to eliminate the effect of litter size on milk production. Piglets removed and added to sows were randomly selected. The piglets were weaned at 22 d of age, after which the sows were transferred to the breeding facility. Estrous was detected with a boar once daily, and the interval from weaning to first estrus was recorded.

Colostrum samples (20 mL) were collected from the functional glands of each sow within 2 h of the first piglet's birth before suckling after 20 IU of oxytocin injection (Qilu Limited Company, Shandong, China). Colostrum samples (10 mL) were centrifuged at 4 °C and 3,000 × *g* for 20 min, and the supernatant was harvested and stored at −80 °C for analysis of immunoglobulin A (**IgA**), IgG, and IgM. The other 10 mL sample was used to analyze colostrum composition.

The weights of suckling piglets were recorded in the morning at day 22 of lactation. During the lactation period, the fecal consistency score of piglets was determined daily according to the following criteria: 5, normal, feces firm and well formed; 3 to 4, soft consistency, feces soft and formed; 1 to 2, fluid feces, usually yellowish; and 0, feces watery and projectile. When the fecal consistency was scored at 0, 1, or 2, the piglets were considered to have diarrhea. The incidence of diarrhea in piglets (%) was determined as: number of cumulative piglets with diarrhea/(total number of suckling piglets × number of breastfeeding days) × 100%. The preweaning mortality of piglets was recorded, whereby the survival rate of piglets at weaning (%) = number of weaned piglets per litter/the adjusted litter size × 100%.

On day 22 after delivery, 1 piglet from each litter was randomly selected for slaughter. Before slaughter, a 5-mL blood sample was collected via jugular puncture into a 10-mL tube treated with sodium heparin, and centrifuged at 3,000 × *g* and 4 °C for 20 min. Plasma was harvested and stored at −20 °C. Then, piglets were anaesthetized with an intravenous injection of sodium pentobarbital (50 mg/kg BW) and exsanguinated by severing the carotid artery and jugular vein. The abdominal cavity was opened, and the viscera were removed. Samples of the jejunum mucosa (about 4 cm^2 size) were taken from the midpoint of the jejunum and placed in a 4% formalin solution. Approximately 20 g of digesta (wet weight) was taken from the caecum. The mucosa of 20 cm jejunum and colon were collected and immediately frozen in liquid nitrogen and stored at −80 °C.

Chemical Analysis

The composition of colostrum, including fat, nonfat solids, protein, and lactose, was analyzed with an automatic milk analyzer (MilkoScan FT120; Foss Electric A/S, Hillerød, Denmark). The concentrations of IgA, IgG, and IgM in the supernatant and plasma were determined using porcine-specific commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). Intra- and inter-assay coefficients of variations (CVs) were 3.0 and 4.51% for IgA, 2.76 and 4.45% for IgG, and 3.2 and 4.80% for IgM, respectively. The levels of plasma total protein, albumin, globulin, glucose, urinary nitrogen, triglycerides, free fatty acids, cholesterol, high-, low-, and very low-density lipoprotein, and the activities of plasma glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase were determined using porcine-specific commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute). Intra- and inter-assay CVs were: total protein, 1.7 and 3.52%; albumin, 1.9 and 3.25%; globulin, 3.5 and 5.6%; glucose, 1.1 and 3.7%; urinary nitrogen, 2.0 and 1.7%; triglycerides, 1.82 and 1.9%; free fatty acids, 5.0 and 8.23%; cholesterol, 0.8 and 1.1%; high-density lipoprotein, 4.7 and 5.1%; low-density lipoprotein, 2.6 and 3.7%; very low-density lipoprotein, 8 and 4.6%; glutamic oxaloacetic transaminase, 1.8 and 4.0%; glutamic pyruvic transaminase, 2.0 and 3.91%; alkaline phosphatase, 2.4 and 3.8%, respectively.

The morphology of jejunum mucosa samples was analyzed as described by Sun et al. (2013). The jejunum villus height and crypt depth were measured under a microscope with 40× combined magnification. At least 10 well-oriented intact villi, and the associated crypt depth of each section, were measured in each piglet.

Tissue samples were homogenized using 10 mL TRIzol (Invitrogen, Carlsbad, CA) and a mechanical tissue disruptor. Then, nucleic acid was extracted with 2 mL of chloroform and RNA was precipitated with ethanol. Total RNA was further purified and concentrated (PureLink RNA Mini Kit; Invitrogen), and the concentration and quality were determined by measuring absorbance at 260 and 280 nm using a NanoDrop spectrophotometer, respectively (Thermo Fisher Scientific Inc., Waltham, MA). All RNA samples contained a 260:280 nm ratio >1.8. First-strand complementary DNA (cDNA) was synthesized using a RevertAide First-Strand cDNA Synthesis Kit (K1622; Fermentas Inc., Burlington, Ontario, Canada). To quantify the mRNA levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH; a housekeeping gene), tight junction proteins, and cytokines, real-time PCR was performed using cDNA. Primer sequences for all genes are listed in Table 2. Primer specificity was tested with a BLAST analysis against the genomic NCBI database. Real-time PCR was performed using the SYBR Green method on the ABI 7900 Sequence Detection System (Applied Biosystems, Grand Island, NY). Analyses were performed in triplicate, and mean values were calculated. Data were collected and analyzed using the "fit point" option of the LightCycler software (version 3.5; Idaho Technology Inc., Salt Lake, UT). A calibration curve was generated via amplification of serially diluted cDNA using the fit point option of the software for target genes and using GAPDH gene as an internal reference. The fluorescence was determined within the geometric region of the semi-log view of the amplification plot. The relative expression of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

The mucosa was placed in chilled lysis buffer (1× Tris-buffered saline, 1.5% Triton X-100, 0.5% deoxycholic acid sodium

Table 2. The sequences of primers

Gene[1]	Sequence number	Product length, bp	Primer sequences, 5′→3′	T_m value
ZO-1	XM_003353439.1	169	F: GAGGATGGTCACACCGTGGT R: GGAGGATGCTGTTGTCTCGG	60 °C
Occludin	NM_001163647.2	105	F: TGGGTTAAAAACGTGTCGGC R: CACTTTCCCGTTGGACGAGT	60 °C
Claudin-1	NM_001161635.1	155	F: ACCCCAGTCAATGCCAGATA R: GGCGAAGGTTTTGGATAGG	58 °C
TLR4	AB232527	113	F: CAGATAAGCGAGGCCGTCATT R: TTGCAGCCCACAAAAAGCA	55 °C
MγD88	AB292176.1	148	F: GATGGTAGCGGTTGTCTCTGAT R: GATGCTGGGGAACTCTTTCTTC	60 °C
NF-κB p65	EU399817.1	133	F: CAGCCCTATCCCTTTACG R: GCCACAGCCTGAGCAA	60 °C
TNF-α	NM_214022.1	168	F: CCACGCTCTTCTGCCTACTGC R: GCTGTCCCTCGGCTTTGAC	61 °C
IL-6	NM_9405217033	146	F: TCAGTCCAGTCGCCTTCT R: CCTTTGGCATCTTCTTCC	56 °C
IL-10	NM_214041.1	136	F: CACTGCTCTATTGCCTGATCTTCC R: AAACTCTTCACTGGGCCGAAG	56 °C
IL-1β	NM_9405217038	165	F: CAAGGAAGTGATGGCTAA R: ACCAAGGTCCAGGTTTT	54 °C
GAPDH	AF017079.1	178	F: ACATCAAGAAGGTGGTGAAG R: ATTGTCGTACCAGGAAATGAG	60 °C

[1]ZO-1, zonula occludens-1; TLR4, toll-like receptor 4; MγD88, myeloid differentiation factor 88; NF-κB p65, nuclear factor-kappa B p65; TNF-α, tumor necrosis factor; IL-6, interleukin-6; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

salt, 0.1% SDS, protease inhibitor cocktail, and 1 mM PMSF). Homogenate was placed on ice for 25 min, and then centrifuged at 12,000 × *g* at 4 °C for 25 min. The supernatant was collected and protein expression was analyzed by western blotting. Primary antibodies used in the experiment included anti-β-actin (ab8226; Abcam, Shanghai, China), anti-toll-like receptor 4 (**TLR4**; ab22048; Abcam), and anti-occludin (ab31721; Abcam). Secondary anti-rabbit (#7074; Cell Signaling Technology) or anti-mouse (#7076; Cell Signaling Technology) IgG horseradish peroxidase-conjugated antibody was used for detection and diluted to 1:2000.

Total bacterial DNA from approximately 0.50 g of caecum content was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Bacterial community diversity and composition were determined in each caecum sample by high-throughput sequencing of microbial 16S ribosomal DNA genes. The extracted DNA was amplified using the 515F/860R universal prokaryote primer set (forward primer 515F, 5′-GTGCCAGCMGCCGCGGTAA-3′; reverse primer 806R, 5′-GGACTACHVGGGTWTCTAAT-3′) targeting the V4 hypervariable regions of bacterial 16S rRNA genes. A unique 5-8-base error-correcting barcode for each sample was added to the end of 515F, allowing sample multiplexing during sequencing. The PCR assay was performed in an ABI Gene Amp 9700 thermocycler under the following conditions: initial denaturation at 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The PCR products were visualized on 1.5% (w/v) agarose gels to check for primer dimers and contaminant bands; amplicons with those issues were excluded. Paired-end sequencing was performed using the Illumina HiSeq2500 Platform (Novogene, Beijing, China). Raw 16S data sequences were obtained, and then screened and assembled using the QIIME and FLASH software packages. UPARSE was used to analyze these effective sequences and determine operational taxonomic units (**OTUs**). Reads were clustered to OTUs using uclust (Edgar, 2010) with a sequence identity threshold of 97%. Subsequently, high-quality sequences were compared against the Ribosomal Database Project classifier program to assign taxonomy (v.2.20) (Wang et al., 2007) at a 90% confidence threshold. Sequences were aligned and phylogenetic trees were obtained through uclust and FastTree, respectively. Chimeric sequences were removed with ChimeraSlayer (Haas et al., 2011). Singletons and OTUs below 0.005% were removed, as recommended by Bokulich et al. (2013). Alpha-diversity analysis including Shannon, ACE, Chao1, and Simpson was performed by Mothur software package (ver. 1.32.0) (Schloss et al., 2009).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Data were subjected to 1-way analysis of variance (ANOVA) in a randomized complete block design. Differences between treatment means were determined by Tukey's multiple comparison tests. Results are reported as means ± standard error of the mean (SEM), and $P < 0.05$ was considered statistical significance. For data of reproductive performance, a power analysis was performed if the difference did not reach a significance of 0.05. When the power value > 0.75, a nonsignificant effect was considered to be the true absence of a treatment response.

Results

As shown in Table 3, the feed intake of sows in SB and MCFA groups was higher than that in the control group ($P < 0.05$); however, the feed intake of sows in n-3 PUFA group was lower ($P < 0.05$). The weaning-to-estrus intervals of sows in the SB, MCFA, and n-3 PUFA groups were lower than that of sows in the control group ($P < 0.05$). There were no differences in the

Table 3. Effects of supplementing sow diet with SB, MCFA, or n-3 PUFA during late pregnancy and lactation on the reproductive performance of sows and growth performance of piglets until weaning at 22 d of age

	Treatments[1]					
	CON	SB	MCFA	n-3 PUFA	SEM	*P*-value
Daily feed intake per sow, kg/d	6.46[b]	7.58[a]	7.33[a]	5.75[c]	0.22	0.010
Daily digestable energy intake per sow, Mcal/d	22.1[b]	25.9[a]	25.4[a]	22.4[b]	0.61	<0.001
Reproductive performance of sows						
Number of piglets born per litter	14.2	15.0	14.6	14.8	0.50	0.695
Number of piglets born alive per litter	13.4	14.2	13.6	13.8	0.51	0.685
Number of piglets stillborn per litter	0.83	0.83	1.00	1.00	0.25	0.932
Born alive rate, %	94.7	94.5	94.5	93.7	1.69	0.892
Weight of piglets at birth, kg	1.44	1.47	1.52	1.43	0.08	0.850
Litter weight of piglets at birth, kg	20.4	23.1	21.6	21.0	1.40	0.563
Weaning-estrus interval, d	6.60[a]	5.40[b]	4.00[c]	5.40[b]	0.54	0.030
Growth performance of piglets						
Survival rate of piglets at weaning, %	82.0[b]	91.3[a]	87.5[ab]	85.0[ab]	2.17	0.045
Weight of piglets at weaning, kg	5.36	5.65	5.51	5.73	1.32	0.761
Daily body weight gain of piglets, g/d	186	198	190	204	8.27	0.302
Incidence of diarrhea in piglets, %	20.6[a]	12.2[d]	14.5[c]	17.3[b]	1.21	<0.001

[1]CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids. Data are presented as mean ± SEM (n = 6).
[a–c]Values in the same row with different small letter superscripts mean significant difference (P < 0.05).

numbers of piglets born, born alive, or stillborn per litter, the born alive rate, the weight of piglets at birth, or litter weight of piglets at birth among the 4 groups (P > 0.05). Of note, for the reproductive performance that did not reach as significance of 5%, all power values exceeded 0.75.

The survival rate of piglets at weaning in the SB group was higher than that of piglets in the control group (P < 0.05). There were no significant differences in the weight of piglets at weaning among the 4 groups (P > 0.05). Compared with the control group, the daily body weight gain of piglets in n-3 PUFA group tended to be higher (P = 0.095). The incidence of diarrhea in suckling piglets decreased following dietary supplementation with SB, MCFA, or n-3 PUFA (P < 0.05).

The composition of colostrum is presented in Table 4. Supplementing the sows' diet with SB, MCFA, or n-3 PUFA increased the contents of fat, protein, IgA, IgG, and IgM in their colostrum (P < 0.05). The content of solids-not-fat in colostrum of sows receiving n-3 PUFA was higher than that of sows in the control and SB groups (P < 0.05). There was no difference in the lactose content of colostrum from sows among the 4 groups (P > 0.05).

Plasma biochemical indexes for suckling piglets are presented in Table 5. Compared with the control group, the addition of SB to sow diets increased the content of plasma total protein, urinary nitrogen, triglycerides, free fatty acids, high-density lipoprotein, globulin, IgA, IgG, and IgM, and the activity of plasma alkaline phosphatase, and decreased the content of plasma cholesterol (P < 0.05). Compared with suckling piglets in the control group, the content of plasma glucose, urinary nitrogen, free fatty acids, high-density lipoprotein, IgG, and IgM, and the activities of plasma glutamic pyruvic transaminase and alkaline phosphatase were increased by MCFA supplementation. Conversely, the plasma albumin/globulin ratio, activity of plasma glutamic oxaloacetic transaminase, and content of plasma triglycerides and cholesterol decreased when compared with the control group (P < 0.05). Increased content of plasma urinary nitrogen, free fatty acids, high-density lipoprotein, globulin, and IgG, increased activities of plasma alkaline phosphatase, and decreased plasma albumin/globulin ratio and content of plasma albumin, triglycerides, and cholesterol were observed in piglets in the n-3 PUFA group when compared with the control group (P < 0.05).

Table 4. Effects of supplementing sow diet with SB, MCFA, or n-3 PUFA on the colostrum composition of sows

	Treatments[1]					
	CON	SB	MCFA	n-3 PUFA	SEM	*P*-value
Fat, %	3.67[d]	4.09[c]	4.47[b]	5.07[a]	0.20	<0.001
Protein, %	12.3[c]	16.9[b]	15.3[b]	19.2[a]	0.63	<0.001
Lactose, %	2.89	3.26	3.30	2.61	0.21	0.135
Solids-not-fat, %	23.1[b]	22.6[b]	24.5[ab]	26.6[a]	0.86	0.027
IgA, μg/mL	5.80[c]	18.5[a]	6.70[b]	6.98[b]	0.71	<0.001
IgG, μg/mL	45.1[c]	169[a]	81.3[b]	98.0[b]	6.71	<0.001
IgM, μg/mL	45.1[c]	165[a]	61.2[b]	56.7[b]	5.65	<0.001

[1]CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids. Data are presented as mean ± SEM (n = 6).
[a–c]Values in the same row with different small letter superscripts mean significant difference (P < 0.05).

As shown in Fig. 1, there were no differences in jejunum villus height or villus height/crypt depth ratio among the 4 groups (P > 0.05); the jejunum crypt depth of suckling piglets in the SB, MCFA, and n-3 PUFA groups was lower than that of suckling piglets in the control group (P < 0.05).

As shown in Table 6, compared with the control group, the expression of claudin-1, occludin, and zonula occludens-1 (ZO-1) mRNA was higher in the colon mucosa of suckling piglets fed diets supplemented with SB, MCFA, or n-3 PUFA (P < 0.05). Compared with control diet, supplementation with SB, MCFA, or n-3 PUFA decreased the mRNA expressions of TLR4, myeloid differentiation factor 88 (**MγD88**), interleukin-1β (**IL-1β**), and tumor necrosis factor (**TNF-α**) (P < 0.05), and increased the expression of IL-10 in the colon mucosa of suckling piglets (P < 0.05). The mRNA expression of NF-κB p65 in the colon

Table 5. Effects of supplementing sow diet with SB, MCFA, or n-3 PUFA during late pregnancy and lactation on the plasma biochemical indexes of suckling piglets at 22 d of age

	Treatments[1]					
	CON	SB	MCFA	n-3 PUFA	SEM	*P*-value
Total protein, g/L	56.1^{b}	66.7^{a}	57.7^{b}	58.9^{b}	1.16	<0.001
Glucose, mmol/L	2.24^{b}	2.56^{b}	3.49^{a}	2.31^{b}	0.12	0.020
Plasma urinary nitrogen, mmol/L	2.20^{b}	2.91^{a}	3.17^{a}	2.84^{a}	0.10	<0.001
Glutamic oxaloacetic transaminase, U/L	69.0^{a}	68.4^{a}	46.8^{b}	64.3^{a}	3.03	<0.001
Glutamic pyruvic transaminase, U/L	44.0^{b}	39.6^{b}	63.9^{a}	43.7^{b}	2.26	<0.001
Alkaline phosphatase, U/L	411^{d}	594^{c}	734^{a}	689^{b}	13.1	<0.001
Triglycerides, mmol/L	0.64^{b}	0.71^{a}	0.37^{c}	0.34^{c}	0.02	<0.001
Free fatty acids, μmol/L	200^{d}	283^{c}	450^{b}	855^{a}	17.9	<0.001
Cholesterol, mmol/L	4.22^{a}	3.15^{b}	2.37^{c}	1.79^{d}	0.09	<0.001
High-density lipoprotein, mmol/L	1.14^{c}	1.80^{a}	2.11^{a}	1.36^{b}	0.06	<0.001
Low-density lipoprotein, mmol/L	0.88	0.90	0.96	0.98	0.03	0.060
Very low-density lipoprotein, mmol/L	0.87	0.90	1.05	0.88	0.02	0.081
Albumin, g/L	22.8^{a}	21.0^{a}	22.3^{a}	17.3^{b}	0.65	<0.001
Globulin, g/L	33.2^{b}	45.8^{a}	35.5^{b}	41.7^{a}	1.29	<0.001
Albumin/globulin	0.70^{a}	0.46^{c}	0.63^{b}	0.42^{c}	0.03	<0.001
IgA, μg/mL	8.85^{b}	12.5^{a}	9.04^{b}	10.3^{ab}	0.72	<0.001
IgG, μg/mL	43.5^{c}	91.6^{a}	69.2^{b}	90.8^{a}	3.73	<0.001
IgM, μg/mL	72.4^{c}	104^{a}	84.7^{b}	78.1^{bc}	4.60	0.002

[1]CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids. Data are presented as mean ± SEM (n = 6).
$^{a-c}$Values in the same row with different letter superscripts mean significant difference ($P < 0.05$).

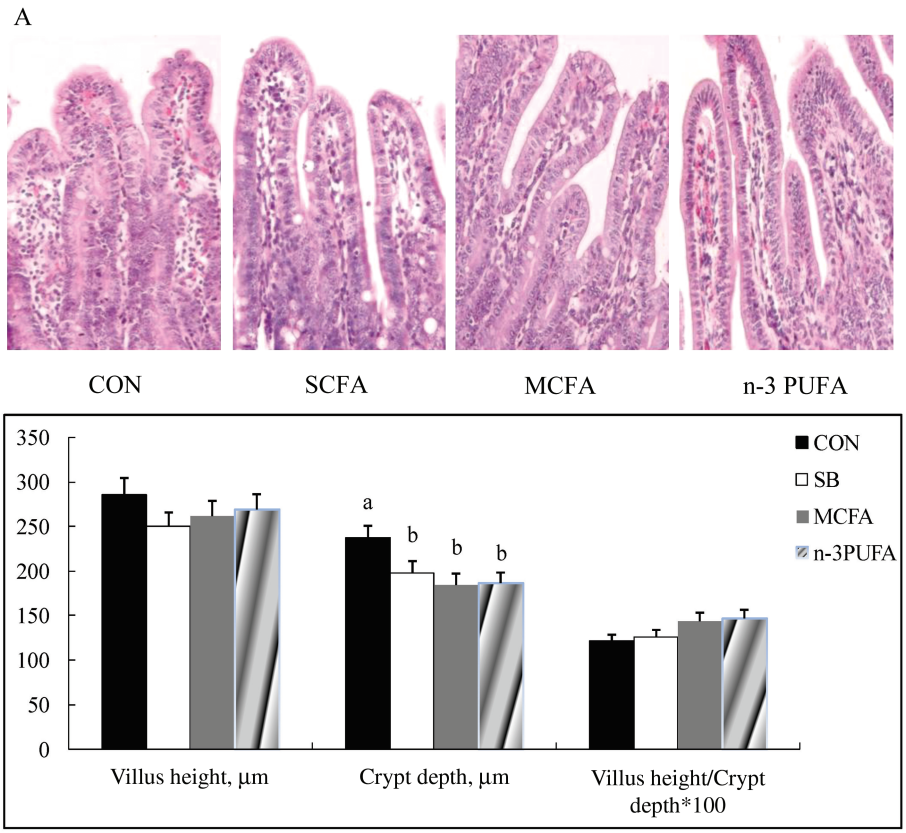

Figure 1. The effects of supplementing sow diet with SB, MCFA, or n-3 PUFA on the morphology of the jejunum mucosa of suckling piglets at 22 d of age. All data are mean ± SD (n = 6); ^{a,b}values with different letter superscripts within the same index mean significant difference ($P < 0.05$). CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids.

mucosa of suckling piglets in the n-3 PUFA group was lower than that of piglets in the control, SB, and MCFA groups ($P < 0.05$). The relative protein expression of TLR4 in the jejunum mucosa of suckling piglets from the SB, MCFA, and n-3 PUFA groups was lower while that of occludin was higher compared the control group ($P < 0.05$) (Fig. 2).

Overall, there were 1,157, 1,635, 1,769, and 1,822 OTUs in the caecum digesta of piglets in the control, SB, MCFA, and n-3 PUFA groups, respectively, while there were 326, 303, 366, and 270 unique OTUs, respectively. The Chao1 and ACE indexes in the caecum digesta of piglets in the SB, MCFA, and n-3 PUFA groups were higher than those of piglets in the control group ($P < 0.05$) (Table 7). There were no differences in the Simpson and Shannon indexes for the caecum digesta of piglets among the 4 groups ($P < 0.05$).

As shown in Table 7, 9 microbial communities were identified in the caecum content of piglets with a richness of more than 0.1% at the phylum level, including *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochaetes*, *Tenericutes*, *Verrucomicrobia*, *Deferribacteres*, and *Synergistetes*, with *Firmicutes* being the most abundant. Supplementing the sow diet with SB, MCFA, or n-3 PUFA increased the relative richness of *Firmicutes*, *Actinobacteria*, and *Synergistetes* in the caecum content of suckling piglets ($P < 0.05$). The relative richness of *Deferribacteres* in the caecum content of suckling piglets in the SB group, *Verrucomicrobia* in the n-3 PUFA group, and *Tenericutes* in the MCFA group were also higher than those of piglets in the control group ($P < 0.05$). The addition of SB, MCFA, and n-3 PUFA reduced the relative richness of *Bacteroidetes* and *Actinobacteria* in the caecum content of suckling piglets ($P < 0.05$).

Table 6. Effects of supplementing sow diet with SB, MCFA, or n-3 PUFA on the mRNA expressions of tight junction proteins and inflammatory cytokines in the colon mucosa of suckling piglets at 22 d of age

	Treatments[1]					
Items[2]	CON	SB	MCFA	n-3 PUFA	SEM	P-value
Tight junction proteins						
Claudin-1	1.20[b]	4.61[a]	4.27[a]	4.90[a]	0.21	<0.001
Occludin	1.06	1.31	1.22	1.20	0.12	0.340
ZO-1	1.05[d]	2.09[a]	1.37[c]	1.55[b]	0.07	<0.001
Cytokines						
TLR4	0.98[a]	0.42[b]	0.71[c]	0.43[b]	0.03	<0.001
MγD88	1.00[a]	0.53[b]	0.65[b]	0.60[b]	0.05	0.002
NF-κB p65	1.13[a]	0.94[a]	1.22[a]	0.41[b]	0.05	<0.001
IL-6	0.94	1.25	1.11	1.03	0.06	0.150
IL-10	1.04[d]	5.57[a]	1.42[c]	2.11[b]	0.17	<0.001
IL-1β	1.02[a]	0.57[b]	0.69[b]	0.61[b]	0.02	<0.001
TNF-α	0.90[a]	0.25[b]	0.23[b]	0.27[b]	0.04	<0.001

[1]CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids. Data are presented as mean ± SEM ($n = 6$).
[2]ZO-1, zonula occludens-1; TLR4, toll-like receptor 4; MγD88, myeloid differentiation factor 88; NF-κB p65, nuclear factor-kappa B p65; IL-6, interleukin-6; TNF-α, tumor necrosis factor.
[a–c]Values in the same row with different letter superscripts mean significant difference ($P < 0.05$).

Discussion

Intake and Reproductive Performance of Sows

Previous studies have shown that the feed intake of sows fed a diet supplemented with fatty acids was related to several factors. First is the dose of fatty acids in the sow diet. Jang et al. (2017) reported that supplementing sows' diet with 1% SB increased feed intake during late pregnancy and lactation, whereas supplementing the diet with 8% fish or coconut oil decreased feed intake by lactating sows but increased the total energy intake (Lauridsen and Danielsen, 2004). Gatlin et al. (2002) also observed that supplementing the diet with 10% MCTs decreased the feed intake of lactating sows. In this study, the feed intake of sows fed diets supplemented with SB and MCFA increased, whereas that of sows fed a diet supplemented with n-3 PUFA decreased compared with that of the control group. In addition, the average daily digestible energy intake of sows in the SB and MCFA groups (25.9 and 25.4 Mcal/d,

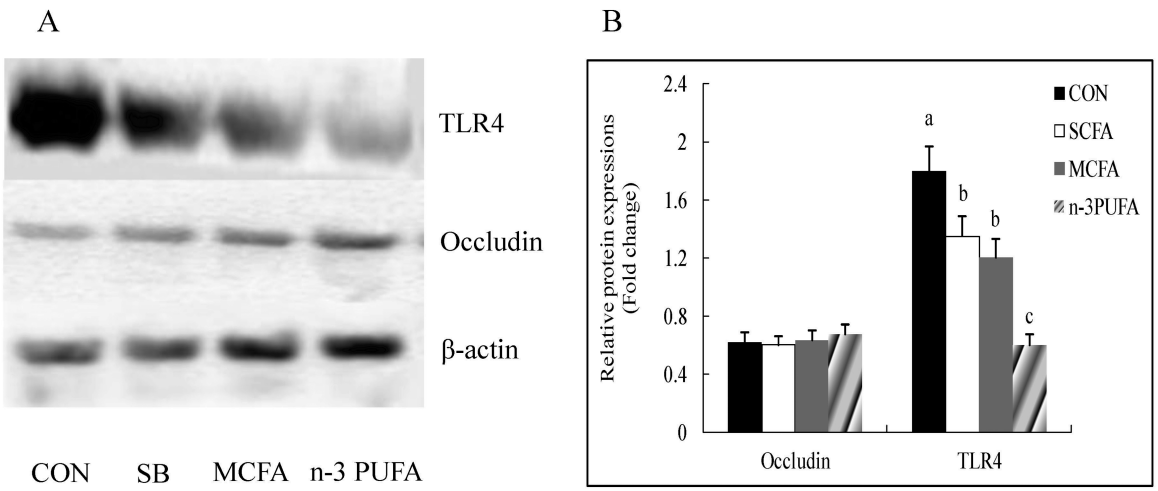

Figure 2. The effects of supplementing sow diet with SB, MCFA, or n-3 PUFA on the relative protein expressions in the jejunum mucosa of suckling piglets at 22 d of age. (A and B) The western blotting analysis. $n = 6$ for A and B. All data are mean ± SD; [a–c]values with different letter superscripts within the same index mean significant difference ($P < 0.05$). CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3 PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids; TLR4, toll-like receptor 4.

Table 7. Effects of supplementing sow diet with SB, MCFA, or n-3 PUFA on the microbial alpha diversity and microbial composition at the levels of phylum and genus in the caecum of suckling piglets at 22 d of age

	Treatments[1]					
	CON	SB	MCFA	n-3PUFA	SEM	P-value
Alpha diversity						
Simpson index	0.89	0.94	0.94	0.88	0.04	0.470
Chao1 index	587^{b}	755^{a}	883^{a}	815^{a}	34.3	<0.001
ACE index	603^{b}	784^{a}	902^{a}	815^{a}	32.2	<0.001
Shannon index	5.06	6.01	6.35	5.82	0.32	0.080
At phylum level						
Fiemicutes	65.0^{a}	42.2^{b}	44.3^{b}	45.4^{b}	1.57	<0.001
Bacteriodetes	14.2^{c}	31.5^{a}	32.3^{a}	33.9^{b}	2.17	<0.001
Proteobacteria	16.8^{b}	19.9^{ab}	18.6^{b}	21.8^{a}	2.94	0.098
Actinobacteria	7.55^{a}	0.63^{b}	1.03^{b}	0.35^{b}	0.32	<0.001
Spirochaetes	0.03^{c}	2.15^{a}	2.98^{a}	0.68^{b}	0.41	<0.001
Tenericutes	0^{c}	0.03^{c}	1.33^{a}	0.48^{b}	0.10	<0.001
Verrucomicrobia	0^{b}	0.05^{ab}	0.13^{ab}	0.23^{a}	0.05	0.030
Deferribacteres	0^{b}	0.18^{a}	0.05^{b}	0.03^{b}	0.03	<0.001
Synergistetes	0.13^{a}	0.05^{b}	0.05^{b}	0.03^{b}	0.03	0.042

[1]CON, basal diet; SB, basal diet + 1 g/kg coated sodium butyrate; MCFA, basal diet + 7.75 g/kg coated medium-chain fatty acids; n-3PUFA, basal diet + coated 68.2 g/kg n-3 polyunsaturated fatty acids. Data are presented as mean ± SEM (n = 6).
[a–c]Values in the same row with different letter superscripts mean significant difference ($P < 0.05$).

respectively) was higher than that of sows in the control and n-3 PUFA groups (22.1 and 22.4 Mcal/d, respectively). The findings of these studies indicate that the feed intake of sows will increase if fatty acids are added to the diet at an appropriate ratio; otherwise, feed intake will decrease, although energy intake will not. Second is the fatty acid source. Dietary fat sources (palm oil, fish oil, and soybean oil) did not affect the feed intake of lactating sows (Jin et al., 2017). Eastwood et al. (2014) reported that plant-based n-6:n-3 PUFA ratios of 9:1 to 1:1 did not change the feed intake of lactating sows, whereas changes were observed with the use of plant- and fish-based n-3 PUFAs. Third is the product form of fatty acids. Sodium butyrate did not improve the feed intake of lactating sows (Biagi et al., 2007; Wang et al., 2014). The smell of SB may explain why its growth-promoting effects were negated; therefore, there may be a difference in the palatability and intake of sow diets containing coated or noncoated fatty acids (Wang et al., 2014). These explain the changes in feed intake by sows fed diets with SB, MCFA, or n-3 PUFA. In present study, the weights and back fat thickness of sows at farrowing and weaning were not measured. To our knowledge, supplementing diets with different fat or fatty acid sources does not affect the weights and back fat thickness of sows at farrowing and weaning (Jang et al., 2017; Yin et al., 2017; Lan and Kim, 2018).

Decreasing the weaning-to-estrus interval of sows is one approach used to enhance their reproductive performance. A relationship has been shown to exist between nutritional status and weaning-to-estrus interval. Sows that lose excessive amounts of body weight during lactation will have extended weaning-to-estrus intervals and experience increased duration of an estrus (Einarsson and Rojkittikhun, 1993). In this study, supplementing diets with SB, MCFA, and n-3 PUFA during late pregnancy and lactation shortened the weaning-to-estrus interval of sows. Rosero et al. (2016) also reported that the addition of α-linolenic acid to sow diets caused a rapid return to estrus. The shortened estrus interval in sows fed diets with SB, MCFA, or n-3 PUFA may be due to the fatty acids providing sufficient energy to sows, preventing deterioration of physical condition, which is beneficial for their postpartum recovery (Rosero et al., 2016). In addition, fatty acids have antibacterial and anti-inflammatory effects, can enhance immunity, and can prevent the occurrence of sow reproductive diseases (Gessner et al., 2015). Importantly, MCFA was the most effective dietary treatment to induce a rapid return to estrus.

Colostrum Composition

In the present study, the addition of SB, MCFA, or n-3 PUFA to sow diets increased fat content in colostrum. Previous studies have shown that the composition of colostrum and milk from sows was significantly influenced by the source of dietary fat or fatty acids. Azain (1993) found that supplementing diet with 10% MCT increased the contents of fat and MCT in the milk of sows. The addition of fish oil to sow diet during late pregnancy and lactation increased the contents of fat and n-3 PUFA in colostrum and milk, and decreased the content of saturated fatty acids in colostrum and milk, and monounsaturated fatty acids in colostrum (Shen et al., 2015). Jin et al. (2017) reported that the addition of fish oil to sow diets elevated milk fat content, and that the consumption of fish oil possibly benefits piglets via increasing n-3 PUFA availability. Yao et al. (2013) reported that the content of C18:n-3 and total n-3 PUFA in colostrum and milk were decreased by an increase in the n6:n3 PUFA ratio in lactating sow diets. Increasing the n-3 PUFA content in sow diets resulted in a higher content of fatty acids in milk and piglet blood (Eastwood et al., 2014). Peng et al. (2010) reported that supplementation in sow diets with conjugated linoleic acid during the late gestation and lactation stages increased the concentrations of conjugated linoleic acid isomers in the umbilical cord plasma, colostrum, milk, and plasma of neonatal and weanling piglets. Inconsistent reports exist in the literature. Jang et al. (2017) reported that dietary SB supplementation did not affect the milk composition, including the content of fat, protein, lactose, total solids, and solids (nonfat). In the present study, the SB, MCFA, and n-3 PUFA content in colostrum, milk, and piglet blood were not measured. Previous studies suggest that supplementing specific fatty acids will increase the transfer of these fatty acids to suckling piglets via colostrum and milk (Azain 1993; Yao et al., 2013; Eastwood et al., 2014; Shen et al., 2015; Jin et al., 2017).

In the present study, dietary SB, MCFA, or n-3 PUFA supplementation increased the protein content in colostrum. Fat sources (palm oil, fish oil, or soybean oil) did not change the protein content in colostrum and milk of sows (Jin et al., 2017). The protein content in sow milk was not changed by SB supplementation (Jang et al., 2017). To our knowledge, data on the mechanisms mediating the effects of fat or fatty acid sources on the protein content in sow colostrum are limited. We speculated that the increased protein content in sow colostrum might have resulted from the special functions of SB, MCFA, or n-3 PUFA in regulating metabolism, gut permeability and microbiota balance, and immunity (Hontecillas et al., 2002; Calder, 2008; Liu, 2016; Chiu et al., 2017).

In addition, feeding sows with SB, MCFA, or n-3 PUFA increased the content of IgG, IgM, and IgA in colostrum. Fatty acids modulate immunoglobulins in sow milk. Supplementing diets with 0.1% coated SBs during late pregnancy and lactation increased the content of IgG and IgA in colostrum (Jang et al., 2017). The content of IgG and IgA in sow milk was increased by dietary supplementation with fish oil (Jin et al., 2017). The

findings of previous studies are inconsistent; for example, Eastwood et al. (2014) reported that increasing the ratio of n-3 PUFA in sow diets had no impact on the colostrum and piglet serum IgA and IgG concentrations. The mechanisms underlying the increased levels mammary immunoglobulins in sows with dietary fatty acid supplementation require further study.

The increased immunoglobulin, protein, and fat content in the colostrum further explains the improved survival and reduced incidence of diarrhea in suckling piglets from sows fed diets containing SB, MCFA, and n-3 PUFA. Notably, dietary n-3 PUFA supplementation was the most effective dietary treatment for increasing the content of fat and protein in colostrum; supplementing diets with SB was the most effective dietary treatment for increasing the content of IgG, IgM, and IgA in the colostrum.

Growth Performance of Suckling Piglets

In this study, supplementing sow diet with SB increased the survival rate of piglets at weaning. Previous studies have also reported the positive effects of fatty acids on the growth performance of suckling piglets. Supplementing sow diets with fatty acids during late pregnancy and lactation increased the average daily body weight gain of piglets at weaning (Lu et al., 2012; Tanghe et al., 2014; Balasubramanian et al., 2016). Gatlin et al. (2002) reported that supplementing sow diets with 10% MCTs, 8% fish or coconut oil increased the average daily body weight gain of suckling piglets (Lauridsen and Danielsen, 2004). Feed intake and milk composition in sows were the main factors limiting the growth performance of their nursing offspring (Jin et al., 2017). Therefore, the increased survival rate of piglets might be attributed to the changes in feed intake and milk composition in response to dietary SB supplementation.

In the present study, the addition of SB, MCFA, or n-3 PUFA to sow diets decreased the incidence of diarrhea in suckling piglets. Previous studies have shown that fatty acids provide energy and also have important antibacterial and anti-inflammatory functions (Gatlin et al., 2002; Farmer et al., 2010; McAfee et al., 2016). Previous studies have also shown that supplementing sow diets with fatty acids during late pregnancy and lactation decreases the incidence of diarrhea in suckling piglets (Lu et al., 2012; Tanghe et al., 2014; Balasubramanian et al., 2016). The decreased incidence of diarrhea in suckling piglets is likely due to changes in the composition of breast milk, and is consistent with the survival results reported for piglets at weaning. Of note, dietary SB supplementation was the most effective dietary treatment for decreasing the diarrhea rate of suckling piglets.

Nutrient Metabolism of Suckling Piglets

In this study, the addition of SB to sow diets increased the plasma total protein content in suckling piglets, which indicates that protein synthesis in the liver is adequate (Wang et al., 2009). Meanwhile, the addition of MCFA increased the plasma glucose content in suckling piglets. This supports the findings of Newcomb et al. (1991) who reported that addition of MCT to sow diets during late pregnancy increased the plasma glucose content in neonatal piglets, indicating that the energy supply to tissues and organs is adequate. In addition, dietary SB, MCFA, or n-3 PUFA treatment increased the plasma urinary nitrogen content in suckling piglets, which indicates increased amino acid metabolism. Increased plasma urinary nitrogen has traditionally indicated an increase in nitrogen metabolism.

In the present study, the addition of SB to sow diets increased the plasma triglycerides content in suckling piglets, and dietary supplementation of SB, MCFA, or n-3 PUFA decreased the plasma cholesterol content in suckling piglets while increasing the content of plasma free fatty acids, high-density lipoprotein, and the activity of plasma alkaline phosphatase in suckling piglets. Previous studies have reported similar results. Yu et al. (2017) reported that addition of SB to sow diets inhibited lipid synthesis and promoted lipolysis in neonatal piglets, while the addition of MCT to weaned piglet diets decreased plasma cholesterol and triglyceride content (Li et al., 2015). Decreased plasma cholesterol and triglyceride levels indicate fat metabolism, while blood free fatty acids reflect the strength of lipolysis metabolism in the animal body. High-density lipoproteins mediate reverse cholesterol transport from peripheral cells to the liver for re-metabolizing. Combined with the results observed for milk fat, it can be inferred that the addition of SB, MCFA, or n-3 PUFA to sow diets increased the milk fat intake of piglets, resulting in increased fat metabolism and an increased concentration of plasma free fatty acids.

Intestinal Mucosal Morphology of Suckling Piglets

In this study, the addition of SB, MCFA, or n-3 PUFA to sow diets had no impact on the villus height, but decreased the jejunum crypt depth of suckling piglets. Chwen et al. (2013) reported that addition of MCT to sow diet increased the daily body weight gain and jejunum villus height of suckling piglets. Liu et al. (2012) reported that supplementing sow diets with fish oil increased the jejunum villus height of weaned piglets and decreased the jejunum villus height/crypt depth ratio. In general, increased intestinal villus height and decreased crypt depth are beneficial for nutrient absorption (Nabuurs et al., 1993). The mechanisms by which fatty acids improve intestinal morphology have been partly elucidated. Fatty acids can promote the development of the intestinal tract by supplying energy to small intestinal cells; in addition, fatty acids may also reduce the damage induced by harmful intestinal bacteria on the intestinal mucosa through sterilization or bacteriostasis, thereby improving the morphological structure and function of the intestines (Liu, 2016).

Immune Status of Suckling Piglets

In this study, dietary supplementation with n-3 PUFA decreased plasma albumin content, dietary supplementation with SB or n-3 PUFA increased the plasma globulin content, and dietary supplementation with SB, MCFA, or n-3 PUFA decreased the albumin/globulin ratio in the blood of suckling piglets. Plasma globulin, which is secreted by B cells following transformation into plasma cells, increases with increasing in antibody levels. The decreased albumin/globulin ratio in the blood, which reflects immune status, indicates that globulin synthesis increased and immunity improved, and the increased total protein content in the blood indicates that there is adequate protein synthesis in the liver (Wang et al., 2009).

In the present study, the plasma IgG content of piglets in the SB, MCFA, and n-3 PUFA groups, the plasma IgA content of piglets in the SB group, and the plasma IgM content of piglets in the SB and MCFA groups were higher than in piglets in the control group. Many studies have shown that dietary supplementation with fatty acids enhances blood immunoglobulin levels. Fang et al. (2014) reported that the addition of SB to weaned piglet diets enhanced the plasma IgG content, while dietary supplementation with SCFA and MCFA increased the plasma IgG content of weaned piglets (Kuang et al., 2015). Rooke and Bland (2002) reported that the plasma IgG content of piglets was positively correlated with the IgG content in sow milk. The results of the present study indicated that dietary SB, MCFA, or n-3 PUFA supplementation could, to

some extent, regulate and enhance the immunity of suckling piglets. The increased plasma immunoglobulins in suckling piglets might be attributed to changes in the immunoglobulin content of sow colostrum in response to dietary SB, MCFA, or n-3 PUFA supplementation. Of note, similar to the results observed for immunoglobulins in the colostrum, SB supplementation was the most effective dietary treatment to enhance the immunoglobulin level in blood.

In this study, supplementing sow diets with SB, MCFA, or n-3 PUFA increased the mRNA expression of claudin and ZO-1 in the jejunum mucosa of suckling pigs. Tight junction, which are an important component of the intestinal mucosal barrier, are composed of transmembrane proteins (occludin and claudin) and a cytoplasmic protein (ZO), and play an important role in regulating the permeability of the intestinal mucosal barrier and preventing bacteria or endotoxins from entering the body. Sodium butyrate increased the mRNA expressions of occludin and ZO-1 in the porcine intestinal epithelial cells (IPEC-J2), and enhanced the protein expression of occludin in the jejunum mucosa of piglets (Huang et al., 2015). Dietary SB supplementation maintains the integrity of the intestinal barrier by inducing the expression of defensins in IPEC-J2 (Zeng et al., 2013). Yan et al. (2017) also reported that SB improved intestinal barrier function in IPEC-J2 cells through the selective upregulation of tight junction proteins and activation of the Akt signaling pathway.

The addition of SB, MCFA, and n-3 PUFA decreased the mRNA expression of TLR4 and its downstream effector MγD88 in the jejunum mucosa. TLR4 signals through MγD88-dependent and MγD88-independent pathways (Takeda et al., 2003). The MγD88-dependent pathway involves a series of signal transduction steps, which activate the nuclear transcription factor, NF-κB. Activated NF-κB rapidly transmits signals to the nucleus, enhancing the transcriptional expression of TNF-α and IL-1β, and simultaneously increases the secretion of pro-inflammatory cytokines such as IL-6 and IL-8, which further enhance the initial inflammatory signal and induces an inflammatory reaction (Pandey and Agrawal, 2006). In addition, compared with the control group, the mRNA expression of NF-κB p65 in the jejunum mucosa of piglets decreased in the n-3PUFA groups, the mRNA expression of anti-inflammatory IL-10 in the jejunum mucosa of piglets fed diets with SB, MCFA, and n-3 PUFA increased, and the mRNA expression of IL-1β and TNF-α in the jejunum mucosa of piglets in the MCFA and n-3PUFA groups decreased. Kuang et al. (2015) reported that the addition of SB and MCFA to the diets of weaned piglets decreased the mRNA expression IL-1β and TNF-α in the jejunum mucosa. The results of the present study indicate that dietary supplementation with fatty acids reduced inflammation by inhibiting TLR4 signaling, consequently improved intestinal health.

In general, supplementing sow diets with SB was better at regulating the immune function of suckling piglets than MCFA or n-3 PUFA.

Intestinal Microbiota of Suckling Piglets

Intestinal microorganisms participate in nutrient metabolism, and play an important role in intestinal morphology, immunity, digestion, and regulation of host gene expression (Bauer et al., 2006). An understanding of how fatty acid treatments affect intestinal bacterial communities may help to elucidate the association between their changes and improvements in growth performance and the intestinal health of suckling piglets. In this study, dietary supplementation with SB, MCFA, or n-3 PUFA increased the Chao1 and ACE indexes in the caecum content of suckling piglets. Alpha-diversity analysis includes Shannon, Simpson, Chao1, and ACE indexes, and other indicators. ACE and Chao1 indexes are used to evaluate the abundance of the microbial community, while the Shannon and Simpson indexes are commonly used to measure the diversity of the microbial community. Xu et al. (2016) reported that oral administration of SB increased the richness estimators (ACE and Chao) of the stomach, decreased the richness estimator (ACE) of the ileum, and increased the richness estimator (Chao) and the diversity of microbiota in the colon of neonatal piglets. Dietary SB decreased the ileal microbial diversity but increased that in the colon of weaned piglets (Huang et al., 2015). This discrepancy in response to fatty acid supplementation may be associated with the type and dosage of fatty acids or the different segments of gastrointestinal tract examined (Xu et al., 2016; Han et al., 2018).

In the present study, the dominant microflora in the piglet colon at the phylum level were *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. *Firmicutes* app. play an important role in carbohydrate metabolism (Walker et al., 2011), with *Ruminococcus*, *Dorea*, *Sarcina*, *Megasphaera*, *Anaerovibrio*, *Coprococcus*, and *Blautia* spp. belonging to *Firmicutes*. *Ruminococcus* has a multifunctional fibrous body with a complex structure, which degrades insoluble cellulose, such as xyloglucan and xylan, and as well as cellulose, and releases soluble oligosaccharides (Ravachol et al., 2016). *Flavobacterium* and *Prevotella* belong to *Bacteroidetes* and are mainly involved in the degradation of various soluble polysaccharides such as oligosaccharides, starch, and pectin (Larsbrink et al., 2014). *Proteobacteria* are mostly involved in protein fermentation (Cowieson and Bedford, 2009) and include *Succinivibrio* and *Desulfovibrio*. In this study, focusing on the phylum level, the addition of SB, MCFA, or n-3 PUFA to sow diets reduced the relative abundance of *Firmicutes*, *Actinobacteria*, and *Synergistetes* in the caecum content, whereas it increased the relative abundance of *Bacteroidetes* and *Spirochaetes*. The decreased relative abundance of *Firmicutes* in the caecum content of suckling piglets indicated reduced levels of carbohydrates and proteins entering the caecum. The increased relative abundance of *Bacteroidetes* in the caecum content of suckling piglets may help the host to obtain more energy from complex polysaccharides, which are resistant to the action of digestive enzymes. *Synergistetes* can induce diarrhea and vomiting in animals (Magalhaes et al., 2007). Therefore, the decreased relative abundance of *Synergistetes* in the caecum content of suckling piglets favors improvement of the intestinal microbiota and reduces the incidence of diarrhea in suckling piglets. This is consistent with the difference in the incidence of diarrhea in suckling piglets.

In conclusion, during late pregnancy and lactation, providing a sow diet containing MCFA was the most effective dietary treatment to shorten the weaning-to-estrus interval; dietary n-3 PUFA supplementation was the most effective treatment to increase the fat and protein content in the colostrum; and providing a sow diet with SB was most effective at promoting intestinal health and decreasing the preweaning mortality of suckling piglets. Dietary SB, MCFA, or n-3 PUFA supplementation favored improvements in the intestinal microbiota.

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