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Non Ruminant Nutrition

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Growth performance, visceral organ weights, and gut health of weaned pigs fed diets with different dietary fiber solubility and lipid sources

Jinsu Hong,[†] Saymore Petros Ndou,[†] Seidu Adams,[‡] Joy Scaria,[‡] and
Tofuko Awori Woyengo[†,‖,1]

[†]Department of Animal Science, South Dakota State University, Brookings, SD 57007, USA, [‡]Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD 57007, USA, [‖]Department of Animal Science, Aarhus University, Blichers Allé 20, DK-8830, Tjele, Denmark

[1]Corresponding author: woyengo@anis.au.dk

Abstract

The objective of this study was to determine the interactive effects of dietary fiber solubility and lipid source on growth performance, visceral organ weights, gut histology, and gut microbiota composition of weaned pigs. A total of 280 nursery pigs [initial body weight (BW) = 6.84 kg] weaned at 21 d were housed in 40 pens (7 pigs/pen). The pigs were fed four diets (10 pens/diet) in a randomized complete block design in two phases: Phase 1 from 0 to 2 wk and Phase 2 from 2 to 5 wk. The diets were corn-soybean meal-based with either sugar beet pulp (SBP) or soybean hulls (SBH) as a fiber source and either soybean oil (SBO) or choice white grease (CWG) as a lipid source in a 2 × 2 factorial arrangement. The BW and feed intake were determined by phase, whereas visceral organ weights, intestinal histology, and gut microbial composition were determined at the end of the trial. Dietary fiber solubility and lipid source did not interact ($P > 0.05$) on average daily feed intake and average daily gain across all phases. However, the gain to feed ratio (G:F) for CWG-containing diets was lower ($P < 0.05$) than that for SBO-containing diets for Phase 1. Also, G:F for SBP-containing diets was lower ($P < 0.05$) than that for SBH-containing diets for Phase 1 and for the entire study period. Pigs fed SBP-containing diets had greater ($P < 0.05$) stomach weight, and tended to have greater ($P < 0.10$) small and large intestine weights relative to BW than those fed SBH-containing diets. Duodenal villous height to crypt depth ratio for CWG-based diets tended to be greater ($P = 0.09$) than that for SBO-based diets. Fiber solubility and lipid source interacted ($P < 0.05$) on relative abundance of *Bacteroides* in the colon such that the relative abundance of the *Bacteroides* for CWG was greater ($P < 0.05$) than that for the SBO in SBP-based diet, but not in SBH-based diet. Relative abundance of *Butyricicoccus* in the colon for SBH-based diet was greater ($P < 0.05$) than that for SBP-based diet. In conclusion, inclusion of SBH instead of SBP in corn-soybean meal-based diets for weaned pigs can result in increased feed efficiency and relative abundance of *Butyricicoccus* in the colon, which is associated with improved gut health. Also, inclusion of SBO instead of CWG in the diets for weaned pigs can result in improved feed efficiency during Phase 1 feeding; however, the pigs may recover from the low feed efficiency induced by dietary inclusion of CWG instead of SBO after Phase 1 feeding.

Key words: fiber solubility, growth performance, gut health, lipid source, weaned pig

Abbreviations

ADF	acid detergent fiber
ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CD	crypt depth
CP	crude protein
DM	dry matter
EE	ether extract
G:F	gain to feed ratio
GIT	gastrointestinal tract
IDF	insoluble dietary fiber
NDF	neutral detergent fiber
OTU	operational taxonomic unit
PCoA	principal coordinates analysis
PUFA	polyunsaturated fatty acids
SDF	soluble dietary fiber
SFA	saturated fatty acids
TDF	total dietary fiber
VFA	volatile fatty acid
VH	villous height
VH:CD	villous height to crypt depth.

Introduction

Moderate amounts of fibrous feedstuffs can be added in diets for weaned pigs to improve growth performance and gut health. Insoluble dietary fiber (IDF) can increase performance of weaned pigs by stimulating feed intake (Gerritsen et al., 2012) and by reducing gut infections through increasing the rate of passage of digesta in gastrointestinal tract (GIT) that result in reduced attachment of pathogens to GIT mucosa (Molist et al., 2014). Soluble dietary fiber (SDF), which is more fermentable than IDF (Jaworski and Stein, 2017), can improve performance by generating volatile fatty acid (VFA) during its fermentation in the GIT; VFAs are a source of energy for intestinal epithelial cells, and hence, they promote intestinal mucosal growth and integrity (Wang et al., 2004; Tao et al., 2019). Conversely, SDF may reduce small intestinal nutrient digestibility if it increases digesta viscosity (Owusu-Asiedu et al., 2006).

Fibrous feedstuffs have relatively low energy value (Hansen et al., 2006), and hence, lipids are often added in high-fiber diets to improve the dietary energy level. Dietary lipids reduce fiber fermentation in the rumen (Maia et al., 2007). Thus, dietary lipids that escape digestion in the small intestine can reduce the dietary fiber fermentation in the hindgut of pigs (Yan et al., 2013). Unsaturated fatty acids are more digestible than saturated fatty acids (Powles et al., 1994), implying that replacement of dietary unsaturated fatty acids with saturated fatty acids may result in reduced fiber fermentation in hindgut. Since “viscous” SDF reduces small intestinal nutrient digestibility, it can negatively interact with dietary fatty acids as it can increase the flow of the fatty acids to the hindgut, leading to reduced hindgut fermentation of organic matter. Thus, the effects of adding a combination of fibrous feedstuffs and fat in diets for weaned pigs on growth performance and hindgut fermentation can vary depending on fiber type and fat type.

Some of the insoluble and soluble fiber-rich feedstuffs that can be added in weaned pig diets include soybean hulls (SBH) and sugar beet pulp (SBP), respectively. The unsaturated fatty acids-rich feedstuffs that can be added in the swine diets include corn oil and soybean oil (SBO), whereas the saturated fatty acids-rich feedstuffs that can be added in the swine diets include beef tallow and choice white grease (CWG). Ndou et al. (2019) determined the effects of dietary inclusion of cellulose (IDF) or pectin (SDF) each with either corn oil or beef tallow on nutrient digestibility of pigs. In their (Ndou et al., 2019) study, they observed an interaction between fiber source and fat source such that the addition of beef tallow to pectin-containing diet, but not to cellulose-containing diet, reduced ileal digestibility of total fatty acids. However, information is lacking on the effects of dietary inclusion of SBH or SBP each with either SBO or CWG on growth performance, nutrient digestibility, and indicators of gut health such as intestinal morphology and gut microbial composition of weaned pigs. Soluble fiber in SBP, unlike that in pectin, has limited effects on digesta viscosity (Flis et al., 2017). Furthermore, effects of purified fibers (such as cellulose and pectin) on growth performance, nutrient digestibility, and the indicators of gut health of pigs may differ from the effects of fibers in the matrix of fibrous feedstuffs such as SBH and SBH. Thus, there was a need to fill this gap in knowledge.

Because of the limited effects of SDF in SBP on digesta viscosity (Flis et al., 2017), both SBH and SBP may have limited effects on fatty acid digestibility in the small intestine, and hence fatty acids flow to the hindgut. Because of the lower content of unsaturated fatty acids in CWG than in SBO, fatty acids in CWG can be less digestible than fatty acids in SBO, and hence, CWG may more negatively affect fiber fermentation in the hindgut of pigs than SBO. It was hypothesized that replacement of SBO with CWG in SBH-containing diets has limited effects on growth performance and gut microbial composition of weaned pigs, whereas replacement of SBO with CWG in SBP-containing diets negatively affects growth performance and gut microbial composition of weaned pigs. This is because SDF in SBP can improve growth performance and gut mucosal growth and integrity of weaned pigs mainly via its fermentation in the hindgut, and CWG can negatively affect fiber fermentation in the hindgut. The objective of this study was to determine the interactive effects of dietary fiber solubility and lipid source on growth performance, visceral organ weights, gut histomorphology, and gut microbial composition of weaned pigs fed SBP- or SBH-containing diets with either SBO or CWG supplementation.

Materials and Methods

The experimental animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (#18-088E).

Animals and housing

A total of 280 pigs [initial body weight (BW) of 6.84 ± 0.98 kg; Large White-Landrace female × Large White-Hampshire male; Pig Improvement Company] weaned at 21 d of age were obtained from Swine Education and Research Facility, South Dakota State University (Brookings, SD, USA). The pigs were fed an antibiotic-free commercial starter diet during the first 10 d post-weaning. Pigs were then individually weighed and housed in 40 pens (7 pigs/pen). Pens (1.8 × 2.4 m) had fully slatted-concrete floors, metal spindle walls (1.0 m high), and solid polyvinyl chloride gates. Each pen was equipped with a cup drinker, a double-spaced dry feeder, and a heat lamp. Room temperature was maintained at 28 ± 1 °C during the first week. Thereafter, the room temperature was maintained at 24 ± 2 °C throughout the experiment.

Experimental diets

Four experimental diets fed included a corn-soybean meal-based diet with SBP or SBH as fibrous feedstuff and SBO or CWG as a lipid source in a 2 × 2 factorial arrangement (Table 1). The experimental diets were formulated to contain similar total dietary fiber, crude fat, Ca, standardized total tract digestible P, and standardized ileal digestible Lys, Met, and Thr contents. The diets were fed as mash and were formulated to meet or exceed NRC (2012) nutrient recommendations for nursery pigs. The four experimental diets were fed in 2 phases: Phase 1 from days 0 to 14 and Phase 2 from days 14 to 35 of the trial. The SBP and SBH were included at 10% in Phase 1 diets and at 12% in Phase 2 diets. Lipid sources were added in diets at 4.5%.

Experimental design and procedure

The four diets were allotted to the 40 pens (10 pens per diet) in a randomized complete block design with sex as block. Pigs had an ad libitum access to diets and fresh water during the entire period. Individual pig BW and feed intake per pen were measured by phase to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

At the end of the Phase 2, one pig from each pen with BW that was closest to the pen average BW was selected and then euthanized by captive bolt penetration. Visceral organs including heart, liver, kidneys, and spleen were isolated and collected from the euthanized pigs, blot dried, and weighed. The stomach, small intestine, cecum, and large intestine were also collected from the eviscerated pig carcasses, digesta emptied, blot dried, and weighed. Digesta samples were collected from the proximal colon and immediately stored in a −80 °C freezer for further analysis of microbial composition.

Table 1. Ingredient and analyzed compositions of the experimental diets[1]

	Phase 1				Phase 2			
	SBP		SBH		SBP		SBH	
Item	SBO	CWG	SBO	CWG	SBO	CWG	SBO	CWG
Ingredient, %								
Corn	42.31	42.28	43.28	43.27	52.53	52.49	51.57	51.56
Soybean meal	30.00	30.00	28.50	28.50	28.00	28.00	28.50	28.50
Whey powder	10.00	10.00	10.00	10.00	0.00	0.00	0.00	0.00
SBP	10.00	10.00	0.00	0.00	12.00	12.00	0.00	0.00
SBH	0.00	0.00	10.00	10.00	0.00	0.00	12.00	12.00
SBO	4.50	0.00	4.50	0.00	4.50	0.00	4.50	0.00
CWG	0.00	4.50	0.00	4.50	0.00	4.50	0.00	4.50
Limestone	0.75	0.73	1.02	1.02	0.64	0.64	0.95	0.96
Monocalcium phosphate	1.02	1.05	1.06	1.06	0.96	1.00	0.98	0.98
Salt	0.59	0.60	0.65	0.66	0.57	0.57	0.63	0.63
L-Lysine·HCl	0.43	0.44	0.50	0.50	0.42	0.42	0.43	0.43
L-Threonine	0.13	0.13	0.16	0.16	0.14	0.14	0.14	0.14
DL-Methionine	0.07	0.07	0.13	0.13	0.04	0.04	0.10	0.10
L-Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Calculated composition, as fed								
NE, kcal/kg	2,561	2,531	2,486	2,457	2,555	2,525	2,453	2,424
SID AA[4], %								
Lys	1.35	1.35	1.35	1.35	1.23	1.23	1.23	1.23
Met	0.39	0.39	0.39	0.39	0.36	0.36	0.36	0.36
Thr	0.79	0.79	0.79	0.79	0.73	0.73	0.73	0.73
Trp	0.27	0.27	0.22	0.22	0.25	0.25	0.20	0.20
Ca, %	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.70
STTD[5] P, %	0.40	0.40	0.40	0.40	0.33	0.33	0.33	0.33
Total dietary fiber, %	24.31	24.31	24.35	24.35	22.19	22.18	21.86	21.85
Analyzed composition, % as fed								
Dry matter	88.5	88.3	88.1	88.0	87.6	88.0	87.8	88.4
Crude protein	18.8	19.3	19.6	18.9	17.0	17.8	18.4	17.9
Crude ash	6.55	6.59	5.59	6.05	5.38	5.42	5.55	5.21
Ether extract	4.90	4.59	4.52	4.37	3.79	5.30	4.19	4.82
Total dietary fiber	14.12	14.20	16.21	15.52	16.13	15.36	17.52	17.51
Soluble dietary fiber	0.98	1.21	0.40	0.36	1.48	1.47	0.57	0.50
Insoluble dietary fiber	13.14	12.99	15.81	15.16	14.65	13.89	16.95	17.01
Neutral detergent fiber	14.74	14.81	16.81	16.72	15.49	15.59	17.01	17.32
Acid detergent fiber	6.19	6.24	7.67	7.41	6.48	6.56	10.24	10.29

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.

[2] Provided the following per kilogram of diet: 11,011 IU vitamin A, 1,652 IU vitamin D3, 55 IU vitamin E, 0.04 mg vitamin B_{12}, 4.4 mg menadione, 9.9 mg riboflavin, 61 mg pantothenic acid, 55 mg niacin, 1.1 mg folic acid, 3.3 mg pyridoxine, 3.3 mg thiamine, and 0.2 mg biotin.

[3] Provided the following per kilogram of diet: 165 mg Zn as $ZnSO_4$, 23 mg Fe as $FeSO_4$, 17 mg Cu as $CuSO_4$, 44 mg Mn as $MnSO_4$, and 0.30 mg Se as Na_2SeO_3.

[4] SID AA = standardized ileal digestible amino acid.

[5] STTD = standardized total tract digestible.

For gut histomorphology, the 5 cm of segments of duodenum (at 70 cm below the pylorus), ileum (at 70 cm cranial to ileal–cecal junction), and jejunum (at the middle of the rest of small intestine) were cleaved off from the small intestine. The segments were gently flushed with saline and placed in a 50-mL conical tube filled with 10% formalin, and stored for later analysis.

Sample preparation and analyses

The fibrous feedstuffs (SBP and SBH) and experimental diets were ground to pass through a 0.75-mm screen using a centrifugal mill (model Zm200; Retsh GmbH, Haan, Germany). The samples were analyzed for dry matter (DM) by oven drying at 135 °C for 2 h (method 930.15), crude protein (CP) by a combustion procedure (method 990.03), ether extract (EE; method 2003.06), and crude ash (method 942.05) as per AOAC (2007). The samples were analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF) as described by Van Soest et al. (1991) on an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY), and for insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) by using a Megazyme Total Dietary Fiber kit (Megazyme International Ireland Ltd, Wicklow, Ireland) according to AOAC-991.43 and AACC-33-07.01 methods (AOAC, 2012; McCleary et al., 2012). The total dietary fiber (TDF) was calculated as sum of IDF and SDF. The fat sources were analyzed for fatty acid profile (method 996.06) and peroxidation (method 965.33) as per AOAC (2007).

Intestinal tissues for histology analysis were sent to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University for staining with hematoxylin and eosin. The villous height (VH; from the top of the villi to the villous-crypt junction) and crypt depth (CD; from the villous-crypt junction to the base) were measured at 4× magnification using a microscope (Micromaster, Fisher Scientific, Waltham, MA, USA) equipped with a 0.55× wifi camera eyepiece (MC500-W 3rd Gen., Meiji Techno Co. LTD., Saitama, Japan) and Micro-Capture software (Meiji Techno Co. LTD., Saitama, Japan) in 20 well-oriented villi and crypt columns. The villous height-to-crypt depth (VH:CD) ratio was calculated by dividing VH by CD.

For analysis of gut microbial composition, the extracted DNA samples from colonic digesta samples were analyzed for sequencing and bioinformatics. The total microbial DNA was extracted using QIAamp PowerFecal Pro DNA kit (QIAGEN, MD, USA) following the manufacturer's instructions. The quality of the DNA was determined using NanoDrop one (Thermo Fisher Scientific, DE, USA) and quantified using Qubit Fluorometer 3.0 (Invitrogen, CA, USA). The extracted DNA was stored for further analysis. The extracted DNA samples were used for the sequencing of the hypervariable V3-V4 regions of the bacterial 16S rRNA using Illumina MiSeq platform. The library preparation for metagenomic sequencing was performed using 0.3 ng of DNA with a Nextera XT library preparation kit (Illumina, San Diego, CA, USA) and sequenced on the MiSeq Platform. The variations in bacterial communities within the colonic digesta of weaned pigs were analyzed using 16S rRNA microbial community analysis package in Quantitative Insights into Microbial Ecology framework (QIIME, Version 2.0). Briefly, 32 samples were quality filtered, demultiplexed, and denoised using dada2. The outputs were transferred to R for analysis using phyloseq. The Shannon diversity, Simpson diversity, Choa 1 diversity, and ACE diversity indices were used to estimate the α-diversity index and the Bray NMDS dissimilarity index was used to calculate the β-diversity index. The taxonomy was assigned to ASVs using dada2 package to implement the naive Bayesian classifier method against GreenGenes (http://greengenes.lbl.gov). The operational taxonomic units (OTUs) were clustered with 97% similarity cut off using USEARCH and Chimeric sequences, subsequently filtered out to obtain OTUs for species classification. The sequences have been deposited into the NCBI database, accession number PRJNA723299.

Statistical analysis

Data were subjected to ANOVA using the MIXED procedure (SAS Inst. Inc., Cary, NC) in a randomized complete block design with pen as the experimental unit. Phase was the repeated term in models involving time. Initial BW was used as a covariate for growth performance data. Main effects of fiber solubility and fat source and their interactions were determined. Treatment means were separated by the probability of difference when interactions between fiber solubility and fat source were significant. To test the hypotheses, $P < 0.05$ was considered significant. If pertinent, tendency ($0.05 \le P < 0.10$) was also reported.

Results

As expected, the SBP contained more SDF and less IDF than SBH (Table 2). The SBO contained less saturated fatty acids (SFA) and more polyunsaturated fatty acids (PUFA) than CWG. The peroxide value for CWG was greater than that for SBO.

There were no interactions ($P > 0.05$) between dietary fiber solubility and lipid source on ADG, ADFI, and G:F (Table 3). However, the main effects of dietary fiber solubility were observed for Phase 1 and for entire study period whereby pigs fed SBH-containing diets had greater ($P < 0.05$) G:F than those that consumed SBP-containing diets. Also, the G:F for pigs fed CWG-containing diets was lower ($P < 0.05$) than that for pigs fed SBO-containing diet for Phase 1. However, G:F for pigs fed CWG-containing diets tended to be greater ($P = 0.06$) than that for pigs fed SBO-containing diets for Phase 2. The overall G:F for pigs fed the SBP-containing diets was greater ($P < 0.05$) than that of pigs fed SBH-containing diets, whereas the overall G:F for pigs fed the SBO-containing diets did not differ from that of pigs fed CWG-containing diets.

There were no ($P > 0{\cdot}10$) interactions between dietary fiber solubility and lipid source on visceral organ weights (Table 4) and gut histomorphology (Table 5). Pigs fed SBP-containing diets had greater ($P < 0.05$) relative weight of stomach, and tended to have greater relative weight of the small intestine ($P = 0.079$) and large intestine ($P = 0.057$) than pigs fed SBH-containing diets. Pigs fed CWG-containing diets tended to have greater ($P = 0.09$) VH:CD in duodenum than pigs fed SBO-containing diets.

Table 2. Fiber and lipid composition of feedstuffs[1], as-fed basis

Item	SBP	SBH	SBO	CWG
Total dietary fiber, %	51.7	66.5	–	–
Soluble dietary fiber, %	14.7	6.00	–	–
Insoluble dietary fiber, %	37.0	60.5	–	–
IDF:SDF ratio	2.52	10.08	–	–
Saturated fatty acids (SFA), %	–	–	15.24	35.53
Polyunsaturated fatty acids (PUFA), %	–	–	80.75	62.42
PUFA:SFA ratio	–	–	5.30	1.76
Peroxide value, meq of active O_2/kg lipid	–	–	10.75	21.98

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.

Table 3. Growth performance of nursery pigs fed diets with different fiber solubility and lipid sources[1]

	SBP		SBH			P-value		
Item[2]	SBO	CWG	SBO	CWG	SEM	Fiber	Lipid	F × L[3]
Body weight, kg								
Initial	6.82	6.83	6.85	6.86	0.228	0.920	0.977	0.991
Phase 1	11.13	10.89	11.57	11.32	0.411	0.460	0.671	0.997
Phase 2	23.38	23.68	24.02	23.83	0.699	0.697	0.957	0.807
Average daily gain, g								
Phase 1	308	290	337	318	14.1	0.161	0.365	0.986
Phase 2	583	609	593	596	15.9	0.924	0.524	0.616
Overall	447	450	464	455	8.8	0.200	0.785	0.495
Average daily feed intake, g								
Phase 1	487	506	501	504	17.1	0.795	0.651	0.754
Phase 2	1,004	1,012	1,018	991	26.4	0.923	0.802	0.634
Overall	747	760	759	745	11.3	0.903	0.999	0.251
Gain to feed ratio								
Phase 1	0.630	0.568	0.671	0.629	0.015	0.003	0.003	0.530
Phase 2	0.581	0.604	0.581	0.603	0.012	0.972	0.058	0.945
Overall	0.605	0.586	0.626	0.616	0.011	0.023	0.182	0.670

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.
[2] Experimental diets were fed in 2 phases; Phase 1 from days 0 to 14 and Phase 2 from days 14 to 35.
[3] F × L, fiber by lipid interaction.

Table 4. Relative visceral organ weights of nursery pigs fed diets with different fiber solubility and lipid sources[1]

	SBP		SBH			P-value		
Item, g/ kg body weight	SBO	CWG	SBO	CWG	SEM	Fiber	Lipid	F × L[2]
Heart	5.85	5.75	5.56	5.45	0.108	0.070	0.511	0.984
Liver	27.10	26.37	24.62	26.64	0.609	0.211	0.463	0.121
Spleen	2.10	2.14	1.99	2.20	0.085	0.857	0.335	0.476
Stomach	9.03	9.37	8.22	8.72	0.185	0.010	0.126	0.779
Small intestine	47.92	52.51	44.82	47.30	1.613	0.079	0.133	0.649
Cecum	2.89	3.00	3.00	2.91	0.115	0.959	0.935	0.572
Large intestine	16.96	17.26	15.30	15.85	0.546	0.057	0.582	0.873

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.
[2] F × L, fiber by lipid interaction.

Fiber solubility and lipid source did not affect the alpha diversity of colonic microbiota of pigs measured using Simpson's index, Shannon index, Chao 1 index, and ACE index (Figure 1). A separation of the samples was shown by a principal coordinates analysis (PCoA) for dietary treatments with PCoA1 and PCoA2, respectively, accounting for 29% and 20% of the total variability among communities (Figure 2). The effects of diets on colonic microbiota at phylum and genus levels are presented in Tables 6 and 7, respectively. At the phylum level, pigs fed SBH-containing diets had greater ($P < 0.05$) relative abundancy of Proteobacteria than pigs fed SBO-containing diets. At the genus level, there were interactions ($P < 0.05$) between dietary fiber solubility and lipid source on relative abundance of *Lachnospira, Peptococcus* and *Bacteroides* in the colon. For *Lachnospira*, its relative abundance for the CWG was greater ($P < 0.05$) than that for the SBO in the SBH-based diet, but not in the SBP-based diet. With regard to *Peptococcus,* its relative abundance for the CWG was lower ($P < 0.05$) than for the SBO in SBH-based diet, but not in the SBP-based diet. Concerning the *Bacteroides*, its relative abundance for the CWG was greater ($P < 0.05$) than for the SBO in the SBP-based diet, but not in the SBH-based diet. Dietary fiber solubility and lipid source did not interact on relative abundance of other microorganisms at genus level. Pigs fed SBH-containing diets had greater ($P < 0.05$) relative abundance of *Butyricicoccus* and *Campylobacter* and lower ($P < 0.05$) relative abundance of *Prevotella.1* than pigs fed SBP-containing diets. Pigs fed CWG-containing diets had lower ($P < 0.05$) relative abundance of *Coprococcus* and greater ($P < 0.05$) relative abundance of *Anaerovibrio* than pigs fed SBO-containing diets.

Discussion

As expected, SBP had a greater content of SDF and lower content of IDF than SBH. Cellulose is the major non-starch polysaccharide in both SBP and SBH, although its content in SBH is greater than that in SBP. For instance, cellulose constituted 50% (Bach Knudsen, 2014) and 35.5% (Zhou et al., 2018) of total non-starch polysaccharide in SBH and SBP, respectively. Pectin and arabinans are the major non-cellulosic polysaccharides in SBP, whereas pectin, xylans, and galactomannans are the major non-cellulosic polysaccharides in SBH. For instance, uronic acid (which is the major component of pectin) and arabinose (which is the major component of arabinans), respectively, constituted 26% and 24% of total non-starch polysaccharide in

Table 5. Gut histomorphology of nursery pigs fed diets with different fiber solubility and lipid sources[1]

	SBP		SBH			P-value		
Item[2]	SBO	CWG	SBO	CWG	SEM	Fiber	Lipid	F × L[3]
Duodenum								
VH, um	526.4	567.9	541.5	528.1	29.42	0.661	0.621	0.350
CD, um	357.2	311.3	338.0	333.6	22.91	0.962	0.292	0.384
VH:CD	1.53	1.84	1.61	1.63	0.098	0.489	0.093	0.140
Jejunum								
VH, um	487.2	440.2	472.9	476.9	20.39	0.601	0.311	0.218
CD, um	250.5	262.9	254.3	269.4	17.08	0.759	0.432	0.943
VH:CD	2.01	1.71	1.86	1.82	0.116	0.882	0.157	0.262
Ileum								
VH, um	412.2	397.1	413.8	402.9	22.62	0.864	0.577	0.917
CD, um	231.4	250.8	229.3	218.2	13.19	0.222	0.759	0.282
VH:CD	1.79	1.61	1.83	1.89	0.113	0.170	0.609	0.293

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.

[2] VH, villous height and CD, crypt depth.

[3] F × L, fiber by lipid interaction.

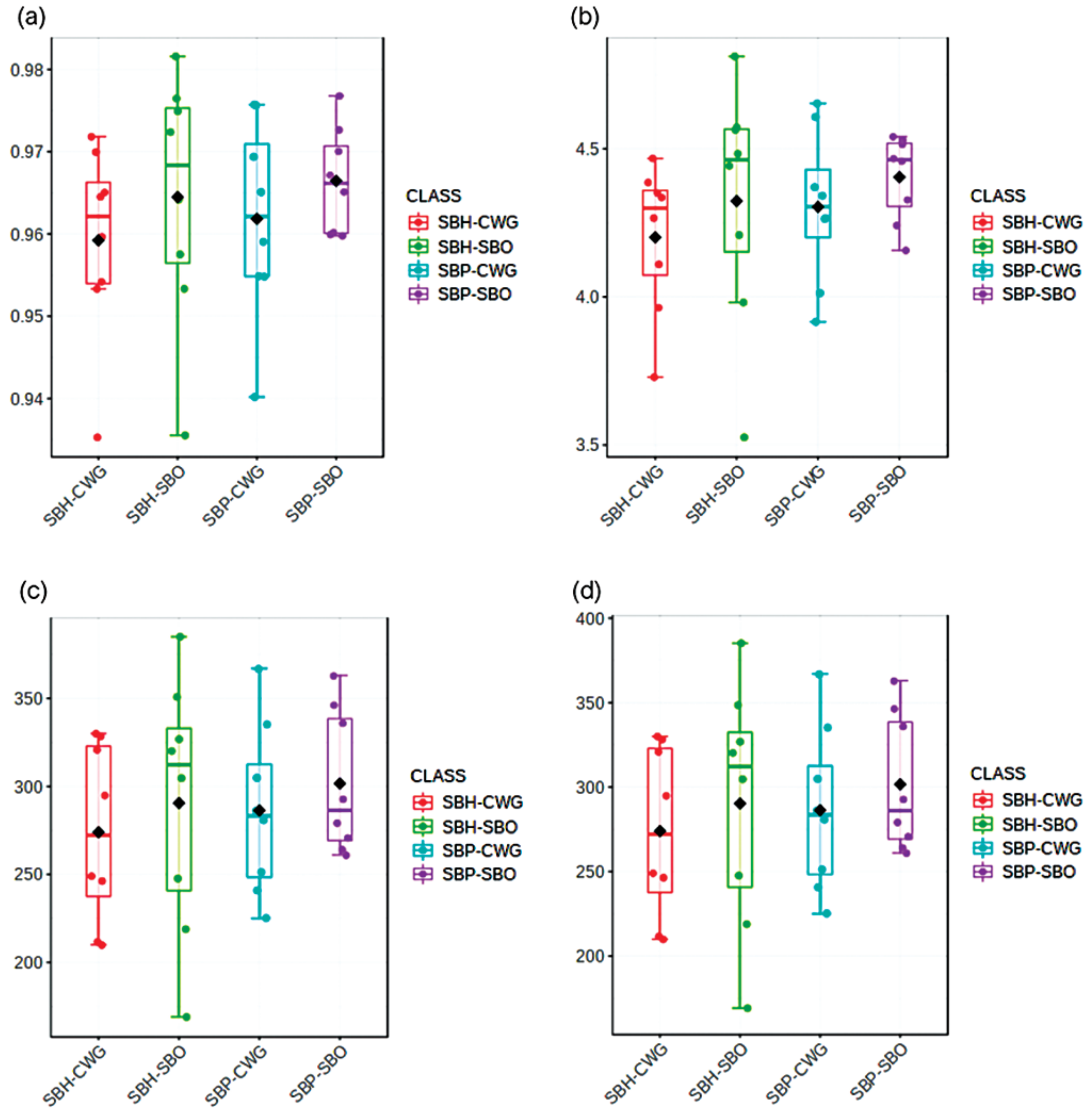

Figure 1. Box plot analysis of alpha diversity of colonic microbiota using Simpson, Shannon, Chao1, and ACE indices in pigs fed the experimental diets; (a) Simpson index (P-value: 0.641; ANOVA), (b) Shannon index (P-value: 0.554; ANOVA), (c) Chao1 index (P-value: 0.786; ANOVA), and (d) ACE index (P-value: 0.786; ANOVA).

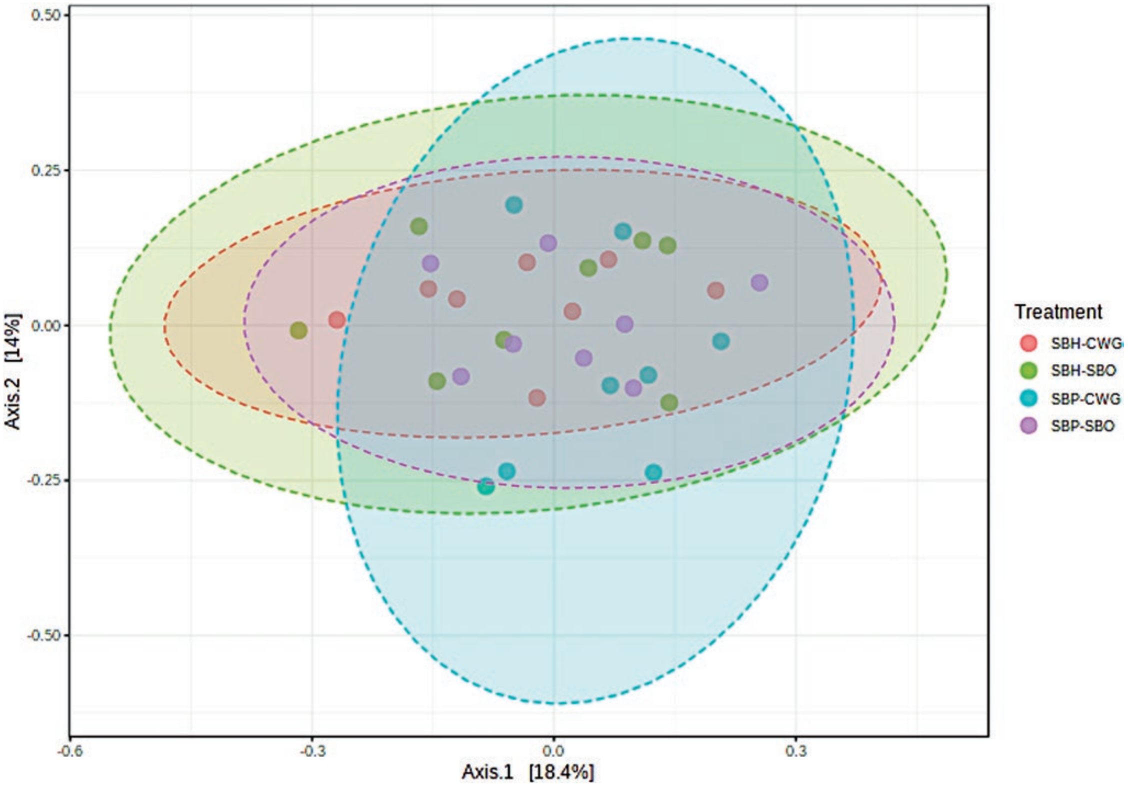

Figure 2. Principal coordinates analysis (PCoA) plots of Bray–Curtis computed distances in colonic microbiota between pigs fed the experimental diets (P-value: <0.223; PERMANOVA).

Table 6. Relative abundance of colonic microbiota at Phylum level in nursery pigs fed diets with different fiber solubility and lipid sources[1]

	SBP		SBH			P-value		
Relative abundance, %	SBO	CWG	SBO	CWG	SEM	Fiber	Lipid	F × L[2]
Firmicutes	61.10	59.49	60.75	61.74	2.660	0.723	0.907	0.628
Bacteroidetes	23.64	25.45	20.74	20.58	2.338	0.107	0.727	0.677
Proteobacteria	3.13	3.30	6.46	5.76	1.246	0.027	0.833	0.730
Actinobacteria	2.50	2.09	2.05	2.75	0.352	0.759	0.688	0.126
Tenericutes	1.71	2.42	2.34	1.64	0.476	0.876	0.986	0.151
Spirochaetes	0.57	1.73	2.08	1.10	0.569	0.448	0.875	0.069
TM7	2.46	1.68	1.63	1.20	0.497	0.194	0.235	0.732
Cyanobacteria	2.03	1.56	1.41	1.92	0.484	0.788	0.964	0.321
Euryarchaeota	1.64	0.53	0.98	1.26	0.449	0.935	0.358	0.132
Chlamydiae	0.92	1.20	0.72	0.80	0.395	0.453	0.650	0.789
Deferribacteres	0.30	0.55	0.73	0.45	0.359	0.647	0.965	0.472
WPS2	0.00	0.00	0.11	0.80	0.405	0.271	0.398	0.398

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.
[2] F × L, fiber by lipid interaction.

SBP (Zhou et al., 2018), whereas uronic acid, xylose (which is the major component of xylans), and mannose (which is the major component of galactomannans), respectively, constituted 17%, 12%, and 8% of total non-starch polysaccharide in SBH (Bach Knudsen, 2014). Pectin is the most soluble non-starch polysaccharide in both SBP and SBH (Bach Knudsen, 2014; Zhou et al., 2018). Thus, the greater content of pectin in SBP than in SBH may partly explain why SBP had greater content of SDF than SBH. Also, as expected, SBO had a higher content of polyunsaturated fatty acids than CWG. Peroxide value is the measure of degree of fat rancidity (DeRouchey et al., 2004). Kerr et al. (2020) observed that growth performance of pigs fed diet containing peroxidized SBO (with peroxide value of 3.05 mg/kg diet) did not differ from that of pigs fed the control diet (with peroxide value of 0.27 mg/kg diet). The calculated peroxide values of the SBO diets and CWG diets fed in the current study were 0.48 and 0.99 mg/kg diet, respectively (data not presented). These peroxide values for diets fed in the current study were within the range of values that were reported by Kerr et al. (2020), implying that the performance of pigs in

Table 7. Relative abundance of colonic microbiota at Genus level in nursery pigs fed diets with different fiber solubility and lipid sources[1]

	SBP		SBH			P-value		
Relative abundance, %	SBO	CWG	SBO	CWG	SEM	Fiber	Lipid	F × L[2]
Lactobacillus	11.02	11.86	9.49	10.17	1.027	0.128	0.463	0.940
Streptococcus	10.70	8.70	10.78	11.05	0.974	0.222	0.384	0.255
Prevotella.1	8.13	9.12	6.94	6.33	0.961	0.047	0.841	0.412
Blautia	5.71	5.67	6.72	5.84	0.436	0.189	0.296	0.348
Eubacterium	1.44	1.59	1.74	1.50	0.205	0.595	0.816	0.357
Faecalibacterium	4.92	5.64	5.27	5.46	0.509	0.875	0.375	0.611
Roseburia	5.25	4.64	4.61	4.69	0.415	0.478	0.535	0.409
Coprococcus	5.06	4.31	4.46	3.86	0.273	0.064	0.020	0.773
Dialister	4.09	4.15	4.54	4.84	0.471	0.235	0.701	0.801
Lachnospira	4.37^{ab}	4.04^{ab}	3.49^{b}	4.85^{a}	0.402	0.934	0.215	0.045
Prevotella.2	3.73	4.12	3.32	3.75	0.436	0.382	0.359	0.962
Dorea	3.79	3.47	3.59	3.11	0.223	0.207	0.084	0.721
Ruminococcus.1	3.45	3.25	3.69	3.06	0.315	0.931	0.194	0.501
Oscillospira	3.07	2.91	3.24	3.20	0.435	0.597	0.819	0.889
Megasphaera	2.51	2.96	2.58	2.46	0.388	0.593	0.674	0.468
Ruminococcus.2	2.31	2.08	2.67	2.70	0.292	0.103	0.735	0.655
Butyricicoccus	1.95	2.05	2.85	2.87	0.296	0.007	0.850	0.878
Acidaminococcus	1.33	1.47	1.56	1.56	0.284	0.576	0.797	0.801
Bulleidia	1.23	1.28	1.18	0.85	0.145	0.109	0.356	0.193
Campylobacter	0.80	0.36	1.02	1.77	0.357	0.031	0.680	0.107
p75a5	1.20	0.73	0.95	0.92	0.128	0.796	0.060	0.104
Anaerovibrio	0.64	1.60	0.19	1.06	0.366	0.186	0.018	0.892
Desulfovibrio	0.24^{b}	0.62^{ab}	0.95^{a}	0.59^{ab}	0.215	0.127	0.988	0.097
Treponema	0.18	0.73	0.85	0.46	0.247	0.417	0.745	0.066
Turicibacter	0.24	0.56	0.20	0.68	0.250	0.869	0.123	0.762
Bifidobacterium	0.48	0.43	0.26	0.26	0.138	0.165	0.858	0.833
Vestibaculum	0.26	0.24	0.34	0.55	0.262	0.465	0.718	0.682
Flexispira	0.37	0.28	0.37	0.35	0.174	0.849	0.758	0.845
Peptococcus	0.04^{b}	0.15^{b}	0.64^{a}	0.20^{b}	0.105	0.005	0.127	0.015
Escherichia	0.24	0.24	0.25	0.19	0.130	0.879	0.854	0.815
Bacteroides	0.00^{b}	0.42^{a}	0.00^{b}	0.00^{b}	0.102	0.048	0.048	0.048
Methanobrevibacter	0.00	0.00	0.08	0.20	0.077	0.081	0.419	0.419

[1] SBP, sugar beet pulp; SBH, soybean hulls; SBO, soybean oil; and CWG, choice white grease.

[2] F × L = fiber by lipid interaction.

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

the current study may not have been affected by the dietary peroxide values.

The IDF has an ability to increase feed intake and digestive capacity of GIT by reducing intestinal stasis and increasing activity of digestive enzyme (Molist et al., 2014). Inclusion of SBP in diets for weaned pigs did not affect activity of digestive enzymes in the small intestine (Lizardo et al., 1997). Thus, the improvement in feed efficiency of weaned pigs due to replacement of SBP with SBH in diets was potentially due to improved digestion and absorption, although the digestive capacity was not measured in the current study. Unsaturated fatty acids compared with saturated fatty acids have a greater ability to increase cholecystokinin section (Beardshall et al., 1989) and hence pancreatic digestive enzyme secretion, and to increase activity of pancreatic lipase in the small intestine (Van Kuiken and Behnke, 1994). Thus, the improvement in feed efficiency of weaned pigs during the first 2 wk post-weaning due to replacement of CWG with SBO in diets could be attributed to improved nutrient digestibility by the replacement. The digestive capacity and feed intake of weaned pigs are low, especially during the first 2 wk after weaning (Molist et al., 2014). Thus, the lack of the significant effect of fiber solubility on feed efficiency during Phase 2 feeding could be attributed to the fact that digestive capacity and feed intake of weaned pigs had improved after 2 wk post-weaning. Also, the improvement in feed efficiency due to replacement of CWG with SBO in diets for weaned pigs was expected to be more pronounced during Phase 1 of feeding than during Phase 2 of feeding. However, it is not clear why replacement of CWG with SBO in diets for weaned pigs tended to reduce the feed efficiency for Phase 2.

The SDF swells and retains more water than IDF (Knudsen and Hansen, 1991; Wenk, 2001). The swelling of fiber in the GIT can result in stretching of the GIT walls and hence increase in weight of the GIT (Jiménez-Moreno et al., 2010; Rezaei et al., 2018). Previous studies showed that the SBP had a greater water holding capacity than SBH (Giger-Reverdin, 2000; Kim et al., 2015). Thus, the increase in relative weight of the stomach and small intestine of pigs due to replacement of SBH with SBP in diets could be attributed to the greater content of SDF in the SBP than in SBH. Lizardo et al. (1997) similarly reported increased stomach weight of weaned pigs due to inclusion of SBP in wheat-soybean meal-based diets for weaned pigs. The SDF is more fermentable in GIT of pigs than insoluble fiber (Jha and Berrocoso, 2016). Volatile fatty acids, which are some of the end products of carbohydrate (including fiber) and protein fermentation in GIT, stimulate proliferation of cells in GIT (Pell et al., 1995). Pectin, which constitutes the bulk of SDF in SBP, is fermented in lower part of the small intestine and in the hindgut (Drochner et al., 2004). Thus, the greater fermentability of fiber in the SBP than in SBH could partly explain the increase in relative weight of the small intestine, and explain the increase in relative weight of the large intestine of pigs due to replacement of SBH with SBP in diets.

There were no effects of fiber solubility and fat source on small intestinal histomorphology. Villous height in small intestine of weaned pigs is positively associated with luminal availability of nutrients, especially energy-yielding nutrients (Pluske et al., 1996). As previously mentioned, dietary insoluble fiber and unsaturated fatty acids can increase nutrient digestibility in the small intestine. Thus, we had hypothesized that replacement of SBP with SBH would result in increased villous height because of the higher content of insoluble fiber in latter than in the former. Also, we had hypothesized that replacement of CWG with SBO would result in increased villous height because of the higher content of unsaturated fatty acids in SBO than in CWG. Thus, the reason for the lack of effect of fiber solubility and fat source on small intestinal histomorphology is not clear. However, it should be noted that the negative effects of SDF on intestinal histomorphology of pigs is dependent on the extent to which the SDF increases digesta viscosity (Molist et al., 2014), which may explain the lack of effect of SBP on the small intestinal histomorphology. Also, Zhou et al. (2017) reported a linear increase in ileal digestibility of amino acids due to a linear increase in dietary level of canola oil, which is rich in unsaturated fatty acids, from 0 to 6%, implying that the effect of unsaturated fatty acids on luminal nutrient availability and hence intestinal histomorphology is dependent on dietary level of unsaturated fatty acids. Finally, relatively high amount of IDF in SBH may have caused abrasive damage to villi, leading to reduced villous height.

Microorganisms under the Bacteroidetes phylum produce several fiber-degrading enzymes that enable them to ferment fiber (Flint et al., 2012). Since SDF is more fermentable than IDF, the increase in relative abundance of *Prevotella* and *Bacteroides* genera that are under Bacteroidetes phylum and hence numerical increase in overall relative abundance of Bacteroidetes phylum in colonic digesta of the pigs due to replacement of SBH with SBP in diets is attributed to the higher content of SDF in the SBP than in SBH. The abundance of *Prevotella* genus (that is under Bacteroidetes phylum) in the GIT of pigs was positively correlated with uronic acid (that is a component of pectin) intake (Ivarsson et al., 2014), which may explain why the relative abundance of this genus was particularly greater in pigs fed SBP-containing diet than in those fed SBH-containing diets. Other studies have also reported increased abundance of Bacteroidetes in GIT of pigs (Ndou et al., 2018) or humans (Scott et al., 2014) due to consumption of soluble fiber.

The abundance of Proteobacteria phylum in GIT is partly affected by GIT pH; it decreases with a decrease in pH (Isaacson and Kim, 2012; Xu et al., 2020). Indeed, the abundance of proteobacteria decreased with an increase in fermentable fiber intake and increased with an increase in protein intake (Liu et al., 2017). Although not measured in the current study, the pH in the hindgut of pigs fed SBP-containing diets was expected to lower than that in pigs fed SBH-containing diets due to the higher fermentability of fiber in SBP than in SBH. Thus, the decrease in the relative abundance of *Campylobacter* genus that is under the Proteobacteria phylum and hence the decrease in relative abundance of Proteobacteria phylum in colon due to replacement of SBH with SBP in diets for pigs may have been due to lower pH in the hindgut of pig fed SBP-containing diets than in pigs fed SBH-containing diets.

Inclusion of wheat bran-derived arabinoxylan oligosaccharides in diets for mice resulted in increased abundance of *Butyricicoccus* genera in GIT (Suriano et al., 2017). Also, Nielsen et al. (2014) reported that arabinoxylans were a better substrate for butyric acid production by microorganisms in pigs than resistant starch. Ivarsson et al. (2014) observed a positive correlation between xylose intake and butyric acid production in GIT of pigs, implying that it is a xylose component of arabinoxylans that promote the growth of butyric acid-producing microorganisms in the GIT. The SBH has a higher content of xylose than SBP because, as previously mentioned, xylans are one of the major non-starch polysaccharides in SBH. For instance, the xylose content (on DM basis) in SBH was 8.8%, whereas that in SBP was 3.1% (Miron et al., 2001). Thus, in the current study, the greater in relative abundance of *Butyricicoccus* in colon of pigs fed SBH-containing diets than that in the colon of pigs fed SBP-containing diets could be attributed to the greater content of xylose in SBH than in SBP. Because the increase in relative abundance of butyric acid-producing microorganisms such as *Butyricicoccus* that is associated with improved gut health because butyric acid improves gut barrier function and has anti-inflammatory affects in the gut (Eeckhaut et al., 2016; Boesmans et al., 2018; He et al., 2018), the replacement of SBP with SBH in weaned pig diets can result in improved gut health.

The abundance of *Peptococcus* in feces of mice was reduced by inclusion of resistant starch (a highly fermentable dietary fiber) in high-fat diets (Zhang et al., 2020), whereas consumption of a low-fat diet resulted in increased abundance of *Peptococcus* in feces of men (Cuevas-Sierra et al., 2021). Cao et al. (2003) reported that inclusion of cellulose in diets for chickens at 10% increased the count of *Peptococcaceae* in the cecal digesta. Results from these studies of Cao et al. (2003), Zhang et al. (2020) and Cuevas-Sierra et al. (2021) indicate that the abundance of *Peptococcus* in hindgut is negatively correlated with the availability of fermentable fiber in the hindgut and positively correlated with availability of fat and IDF in the hindgut. Thus, the increase in relative abundance of *Peptococcus* in colon of pigs due to replacement of SBP with SBH in SBO-containing diets could have been due to the greater IDF in SBH than in SBP. However, it is not clear why the relative abundance of *Peptococcus* in colon of pigs was unaffected by the replacement of SBP with SBH in CWG-containing diets.

As previously mentioned, SBH has a greater content of cellulose than SBP. Fermentation of cellulose results in production of VFA with high molar ratio of acetic acid (Sunvold et al., 1995). Fermentation of cellulose by cellulolytic microorganisms (such as *Ruminococcus*) into acetic acid results in generation of H_2, which is then utilized by methane-producing microorganisms such as *Methanobrevibacter* to synthesize methane (Pavlostathis et al., 1990). Thus, the increase in relative abundance of *Methanobrevibacter* in colon of pigs due to replacement of SBP with SBH could be attributed to the greater content of cellulose in the SBH than in the SBP. Zhang et al. (2018) similarly reported that replacement of wheat bran with SBH in diets for pigs increased abundance of *Methanobrevibacter* in feces. In the current study, the relative abundance of *Ruminococcus* in colon was increased numerically by the replacement of SBP with SBH.

The small intestinal digestibility of unsaturated fatty acids is greater than that of saturated fatty acids (Powles et al., 1994). Thus, the small intestinal digestibility of fatty acids in SBO is expected to be generally greater than that of fatty acids in CWG. Fat inhibits microbial fermentation of carbohydrates (Palmquist, 1994), implying that fat that escape small intestinal fermentation can inhibit fiber fermentation in the hindgut of pigs. The abundance of Bacteroidetes phylum compared with that of

Firmicutes phylum is more negatively affected by ingestion of fat (Hildebrandt et al., 2009). Thus, it had been hypothesized that replacement of CWG with SBO in the SBP-containing diet would result in increased relative abundance of *Bacteroides* in colon of the pigs. This is because fiber in SBP (compared to that in SBH) is expected to be more fermentable and hence to be more affected by fat-induced reduction in microbial fermentation. It is not clear why the relative abundance of *Bacteroides* was increased by the replacement of SBO with CWG in SBP-containing diet.

Inclusion of corn oil (at the expense of olive oil or milk fat) in diets for mice resulted in increased abundance of *Coprococcus* in GIT (Abulizi et al., 2019). In the current study, pigs fed SBO-containing diets had greater relative abundance of *Coprococcus* in colon than pigs fed CWG-containing diets. Corn oil, like SBO, has a higher content of polyunsaturated fatty acids than olive oil, milk fat, or CWG (Rodrigues and Gioielli, 2003; Liu et al., 2018; Okazaki and Katayama, 2021). Thus, it appears that consumption of fat that is rich in polyunsaturated fatty acids can result in increased relative abundance of *Coprococcus* in GIT; however, the mechanisms by which this is achieved are not clear. The increase in relative abundance of *Anaerovibrio* due to replacement of SBO with CWG in diets for pigs could have been due to greater availability of fat in the hindgut of pigs fed the CWG-containing diets. This is because pigs have lower small intestinal digestibility of fat in CWG than in SBO (Powles et al., 1994). *Anaerovibrio* produce enzymes that hydrolyze triglycerides to liberate glycerol, which is then used as a source of energy by the same microorganisms (Liu et al., 2017; Yang et al., 2020), and hence, their relative abundance in hindgut is likely to increase with an increase in availability of fat in the hindgut. Generally, fat source compared with fiber solubility had limited effects on fecal microbial composition. A previous study with mice (Morrison et al., 2020) also demonstrated lower effect of fat than of fiber on gut microbial composition.

Fiber solubility and fat source did not interact on most of the response criteria measured in the current study. This is contrary to results from a previous study (Ndou et al. 2019) in which fiber source (cellulose vs. pectin) and fat source (corn oil vs. beef tallow) interacted on apparent ileal digestibility of fatty acids in growing pigs. In this study of Ndou et al. (2019), dietary replacement of cellulose with pectin reduced the apparent ileal digestibility of total fatty acids for both corn oil- and beef tallow-containing diets, but the magnitude of reduction in the digestibility for beef tallow-containing diet was greater than that for corn oil-containing diet. They (Ndou et al., 2019) attributed this interaction between fiber source and fat source on apparent ileal digestibility of total fatty acids to the fact that pectin increases digesta viscosity, and that the digestibility of fat in beef tallow is lower than that in corn oil due to the greater content of saturated fatty acids in the former than in the latter. As previously mentioned, SDF in SBP has limited effect on digesta viscosity (Flis et al., 2017). Also, the beef tallow has higher content of saturated fatty acids than CWG (Liu et al., 2018). Finally, fats were included in diets at 4.5% in the current study and at 6% in the study of Ndou et al. (2019). Thus, the difference between the current study and that of Ndou et al. (2019) with regard to interactions between fiber source and fat source could be attributed to differences in SDF source, saturated fatty acid source, and level of inclusion of fat in diets among the studies.

In conclusion, inclusion of SBH instead of SBP in corn-soybean meal-based diets for weaned pigs can result in improved feed efficiency. Also, inclusion of SBH instead of SBP in corn-soybean meal-based diets for weaned pigs can result in improved gut microbial composition of weaned pigs through increased relative abundance of *Butyricicoccus* genus in colon. Inclusion of SBO instead of CWG in corn-soybean meal-based diets for weaned pigs can result in improved feed efficiency during Phase 1 feeding; however, the pigs may recover from the low feed efficiency induced by dietary inclusion of CWG instead of SBO after Phase 1 feeding. Dietary lipid sources have limited effects on colonic microbial composition. Also, fiber sources (SBH and SBP) and lipid sources (SBO and CWG) fed in the current study may not interact on growth performance, digestive organ weights, small intestinal histomorphology, and composition of most of bacterial genera in colon of weaned pigs when the fiber sources and lipid sources are included in diets at 10% to 12%, and at 4.5%, respectively.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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