



Original Research Article

Time-course effects of different fiber-rich ingredients on energy values, microbiota composition and SCFA profile in growing pigs



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ABSTRACT

This study was to investigate time-course effects of different types of dietary fiber on the energy values, fecal microbiota and short-chain fatty acid (SCFA) concentration in growing pigs. A total of 24 barrows (initial body weight, 19.8 ± 0.5 kg) were assigned to 4 dietary treatments based on body weight (BW) in a completely randomized design, including a basal diet (CON) and 3 fiber-rich diets replacing corn, soybean meal and soybean oil in the CON diet with 20% sugar beet pulp (SBP), defatted rice bran (DFRB) or soybean hull (SBH), respectively. Fresh feces were sampled on d 7, 14 and 21, followed by 5 d total feces and urine collections. The results showed that there were no differences in DE and ME between any of the fiber ingredients on d 7, 14 or 21. However, fiber inclusion decreased the DE and ME of the diet ($P < 0.05$) regardless of the time effect. Principal coordinate analysis (PCoA) revealed distinctly different microbial communities on the DFRB diet and SBH diet across different times ($P < 0.05$) and the fecal microbiota of the 4 diet groups demonstrated notably distinct clusters at each time point ($P < 0.05$). With adaptation time increased from 7 to 21 d, cellulose-degrading bacteria and SCFA-producing bacteria (e.g., *Ruminococcaceae_UCG-014*, *Rikenellaceae_RC9_gut_group* and *Bifidobacterium*) increased in the fiber inclusion diets, and pathogenic genera (e.g., *Streptococcus* and *Selenomonas*) were increased in the basal diet ($P < 0.05$). Furthermore, the gut microbiota of growing pigs adapted more easily and quickly to the SBP diet compared to the DFRB diet, as reflected by the concentration of propionate, butyrate, isovalerate and total SCFA which increased with time for growing pigs fed the DFRB diet ($P < 0.05$). Collectively, our results indicated at least 7 d adaptation was required to evaluate the energy values of fiber-rich ingredients, as the hindgut microbiota of growing pigs may need more time to adapt to a high fiber diet, especially for insoluble dietary fiber.

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1. Introduction

Feed represents a major cost in swine production, in which the ingredients supplying energy account for the highest part of the total feed cost (Noblet et al., 2022). Previous studies have investigated decreased energy levels by adding fiber-rich ingredients to swine diets to reduce the costs of pig production (Zijlstra and Beltranena, 2013; Li et al., 2021). High-fiber ingredients, such as soybean hulls (SBH), defatted rice bran (DFRB) and sugar beet pulp (SBP), are enriched with dietary fibers, which play important roles in the maintenance of gut health by regulating gut microbiota and metabolite compositions and improving gastrointestinal barrier function (Gill et al., 2021). However, the pig intestinal tract needs

time to adapt to diets with different sources of fiber to achieve a new intestinal homeostasis (Castillo et al., 2007; Molist et al., 2009).

Previous studies have showed that an adaptation period of 5 to 7 d is generally used to evaluate energy values and nutrient digestibility of cereals (Lyu et al., 2019; Rodriguez et al., 2020) and protein ingredients (Ma et al., 2018; Zhong and Adeola, 2019). However, digestion of dietary fiber is predominantly reliant on fermentation occurring in the hindgut of pigs by resident microbiota, which may require a longer adaptation period to fiber-rich diets compared to a corn-soybean meal basal diet (Zhao et al., 2018b; Zhang et al., 2019). Dietary fibers derived from different fiber-rich ingredients may have different physicochemical properties, especially solubility (Flis et al., 2017), which influence fermentation time. Soluble dietary fiber (SDF) enriched ingredients could increase digesta viscosity, resulting in the reduction of digesta flow rate, thus increasing fermentation time in the hindgut (Navarro et al., 2018a). Conversely, insoluble dietary fiber (IDF) enriched ingredients could increase the rate of digesta passage and shorten fermentation time for digesta in the hindgut (Zhao et al., 2018b). Additionally, intestinal microbiota and the gastrointestinal tract itself may need more time to adapt to fiber-rich ingredients, as the intestinal microbiota composition is also directly affected by fiber-rich ingredients (Heinritz et al., 2016; Zhao et al., 2018a). Researchers have reported that the relative abundance of fiber degrading bacteria, such as *Prevotella*, decreased during short-term pea fiber diet feeding, but increased as the feeding time was prolonged (Luo et al., 2018, 2019), indicating that the adaptation period may be related to the microbial composition.

To date, an adaptation duration of 14 to 21 d is recommended to determine the energy values of fiber-rich ingredients (Van Der Peet-Schwering et al., 2002; Lyu et al., 2018; Zhao et al., 2018b). However, little is known in pigs about whether differing adaptation times to fiber-rich ingredients of varying solubility are needed for energy evaluation. Additionally, time-course alterations in gut microbiota composition and metabolite profiles in pigs after fiber intake also remains unclear. Therefore, the objective of this study was to investigate the time-course effects of different types of dietary fiber on energy values, fecal microbiota and short-chain fatty acid (SCFA) production in growing pigs.

2. Materials and methods

2.1. Animal ethics statement

The experimental protocols were reviewed and approved by the Animal Welfare and Ethical Committee of the Institute of Animal Science, Chinese Academy of Agriculture Sciences, Beijing, P. R. China (Ethics Approval Code: IAS2019-32).

2.2. Animals, diets and experimental design

Twenty-four barrows (initial body weight = 19.8 ± 0.5 kg) were individually housed in metabolism crates and divided into 4 groups with 6 pigs per group in a completely randomized design. Four diets consisting of a corn-soybean basal diet (CON), a SBP diet, a SBH diet, and a DFRB diet were formulated (Table 1). In the 3 fiber-rich diets, corn, soybean meal and soybean oil in the CON diet were replaced by 20% fiber ingredients in such a way that the ratios of corn, soybean meal and soybean oil were equal in the CON diet. All barrows were acclimated to the crates for 5 d and fed the same diet before the start of the experiment, and then each group of pigs was randomized to 1 of the 4 diets. Vitamins and minerals were included in all diets to meet or exceed the requirements of growing

pigs according to the NRC (2012). Pigs were provided with ad libitum access to water and fed a daily amount of diet equivalent to 4% BW at the beginning of each experimental period, with 2 equal meals offered at 08:00 and 16:00, respectively.

2.3. Sample collection

Fresh pig feces in each group were sampled (2 tubes per pig) from the rectum in the morning at d 7, 14 and 21 after the experiment initiation. Fresh feces were loaded into 5 mL centrifuge tubes, snap-frozen in liquid nitrogen, and then stored at -80°C to analyze the SCFA concentration and microbiota composition. Then total feces and urine were collected for 5 consecutive days from d 8 to 12, d 15 to 19, and d 22 to 26 by a time-to-time method, respectively. Urine buckets with a preservative of 50 mL of 3 mol/L HCl were placed under the metabolism crates for urine collection. Urine in buckets was emptied every morning and a 20% subsample was sampled and stored at -20°C . Feces were collected twice daily and stored at -20°C . At the end of the experiment, urine and fecal samples were thawed and mixed for each pig in each collection period. Feces were dried at 65°C for 72 h followed by cooling and weighing, and stored at -20°C for chemical analysis.

2.4. Chemical analysis and calculation

The feeds, ingredients of diets and oven-dried feces were ground to pass through a 0.5-mm screen and analyzed in duplicate for dry matter (DM, method 934.01), crude protein (CP, method 990.03), extract ether (EE, method 954.02), ash (method 942.05), neutral (NDF) and acid detergent fiber (ADF; method 973.18), IDF (method 991.43) and total dietary fiber (TDF, method 991.43) according to AOAC (2012). The content of SDF (%) was calculated as the difference between TDF (%) and IDF (%). The gross energy (GE) concentration in feeds, ingredients of diets, feces and urine samples was analyzed using an automatic adiabatic bomb calorimeter (model 6400, Parr Instruments, Moline, IL, USA). Fecal SCFA concentration was analyzed according to a previous study (Wu et al., 2016) with a gas chromatography (GC) system (Agilent HP 6890 Series, Santa Clara, CA, USA).

2.5. Bacterial DNA extraction, amplification and 16S rRNA gene sequencing

Fecal microbial DNA was isolated with a DNA Kit (Omega, Biotek, Norcross, GA, USA) according to the provided protocol. The V3–V4 hypervariable regions of the 16S rRNA gene were amplified using polymerase chain reaction (PCR) with gene-specific primers 338F (5'-ACTCCTRCGGGAGGCAGCAG-3') and 806R (5'-GGAC-TACCVGGGTATCTAAT-3'). The PCR product was further purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The purified amplicon sequencing was carried out with an Illumina sequencing platform using Miseq PE300 (Wu et al., 2020). Raw data were processed on the platform of Majorbio I-Sanger Cloud Platform (www.i-sanger.com). UPARSE (version 7.1, <http://drive5.com/uparse/>) was used for denoising, chimera detection, and operational taxonomic unit (OTU) clustering (97% similarity cutoff), and RDP classifier (<http://rdp.cme.msu.edu/>) was used for taxonomic assignment.

2.6. Statistical analysis and calculation

The direct method was used to calculate the DE, ME and apparent total tract digestibility (ATTD) of energy and nutrient

Table 1
Ingredient composition and analysis of experimental diets (as-fed basis, %).

Item	Basal diet	Defatted rice bran diet	Soybean hull diet	Sugar beet pulp diet
Ingredients				
Corn	69.78	55.21	55.21	55.21
Soybean meal	24.00	18.99	18.99	18.99
Soybean oil	2.00	1.58	1.58	1.58
Test ingredient	0.00	20.00	20.00	20.00
Dicalcium phosphate	1.15	1.15	1.15	1.15
Limestone	0.70	0.70	0.70	0.70
L-Lysine-HCl	0.30	0.30	0.30	0.30
DL-Methionine	0.05	0.05	0.05	0.05
L-Threonine	0.10	0.10	0.10	0.10
L-Tryptophan	0.02	0.02	0.02	0.02
Choline chloride	0.10	0.10	0.10	0.10
Premix ¹	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30
Celite	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
Analyzed compositions				
Dry matter	90.42	90.38	90.56	91.42
Crude protein	16.84	17.03	15.35	15.26
Ether extract	5.68	5.22	5.10	4.97
Neutral detergent fiber	12.86	15.42	18.76	17.90
Acid detergent fiber	3.99	4.29	11.28	7.32
Ash	5.32	6.70	6.00	6.45
Total dietary fiber	16.50	19.74	27.60	26.31
Insoluble dietary fiber	15.53	18.96	23.80	20.63
Soluble dietary fiber	0.97	0.78	3.81	5.67
SDF:TDF ratio	5.88	3.96	13.79	21.57
Gross energy, MJ/kg	16.96	16.60	16.54	16.66

SDF = soluble dietary fiber; TDF = total dietary fiber.

¹ Provided the following quantities per kilogram of diet: vitamin A, 9,140 IU; vitamin D₃, 4,405 IU; vitamin E, 11 IU; menadione sodium bisulfite, 7.30 mg; riboflavin, 9.15 mg; D-pantothenic acid, 18.33 mg; niacin, 73.50 mg; choline chloride, 1285 mg; vitamin B₁₂, 200 µg; biotin, 900 µg; thiamine mononitrate, 3.67 mg; folic acid, 1,650 µg; pyridoxine hydrochloride, 5.50 mg; I (as potassium iodide), 1.85 mg; Mn (as manganese sulfate), 110.10 mg; Cu (as copper sulfate), 7.40 mg; Fe (as ferrous sulfate), 73.50 mg; Zn (as zinc sulfate), 73.50 mg; Se (as sodium selenite), 500 µg.

composition of the experimental diets, and the indirect method was used to calculate DE and ME contents in ingredients as described by Liu et al. (2021).

Data of DE, ME and ATTD of GE and nutrient composition as well as SCFA contents were evaluated using the BOXPLOT procedure to remove outliers (Gao et al., 2020). Data were analyzed using the PROC GLM of SAS (Version 9.4, SAS Institute, Cary, NC, USA) with Duncan's multiple comparison test. Linear and quadratic effects of the adaptation time were determined using the GLM procedure.

The difference of α -diversity indices (Sobs, ACE, Shannon and Chao 1) among diet treatments and different time points were conducted using Wilcoxon ran-sum test. The principal coordinate analysis (PCoA) based on the unweighted UniFrac distance and ANOSIM test was performed using the Majorbio I-Sanger Cloud Platform (www.i-sanger.com). The significant difference among the 4 diet treatments and each time point was tested by a Kruskal–Wallis test followed by a Welch's test with $P < 0.05$ indicating statistical significance and $0.05 < P < 0.1$ indicating a significant trend.

3. Results

3.1. Chemical composition in test ingredients and diets

The contents of GE and nutrient composition of the diets and the test ingredients are presented in Tables 1 and 2. The content of SDF, which is more easily fermented in the colon, and the ratio of SDF to TDF were lowest in DFRB (0.19% and 0.56%, respectively), intermediate in SBH (4.33% and 5.47%, respectively), and highest in SBP (13.66% and 23.35%, respectively). Accordingly, the DFRB diet had the lowest SDF content and SDF/TDF (0.78% and 3.96%, respectively), and the SBP diet had the highest SDF content and SDF/TDF (5.67% and 21.57%, respectively).

3.2. Energy values, ATTD of GE, and nutrient composition of experimental diets

The DE and ME (DM basis) of all experimental diets, except for ME of SBH diet, were not affected by the increase in adaptation time from 7 to 21 d (Table 3). Whereas the adaptation duration had quadratic effects on the DE and ME of the basal diet, the DFRB diet and the SBH diet ($P < 0.05$). The fiber-rich diets had lower DE (15.27, 14.93 and 15.00 MJ/kg DM for the SBP diet, DFRB diet and SBH diet, respectively) and ME (15.02, 14.70 and 14.74 MJ/kg DM for the SBP diet, DFRB diet and SBH diet, respectively) than those of the basal diet (16.29, 16.08 MJ/kg DM for DE and ME, respectively; $P < 0.01$). Additionally, the DE and ME of the SBP diet (15.27 and 15.02 MJ/kg DM, respectively) were greater than the DFRB diet (14.93 and 14.70 MJ/kg DM, respectively) and the SBH diet (15.00 and 14.74 MJ/kg DM, respectively; $P < 0.05$).

The adaptation time did not affect the ATTD of GE and most nutrients of each diet, except for ATTD of CP for the CON and SBP diets ($P < 0.05$, Table 4), which linearly increased with the adaptation time from 7 to 21 d ($P < 0.05$). Besides, the fiber-rich diets had lower ATTD of GE, DM and CP than the values of the basal diet ($P < 0.05$), whereas the ATTD of the ADF for the SBP diet was greater than the values of the basal diet and DFRB diet ($P < 0.05$), the ATTD of the NDF for the SBP diet was greater than the values of all other diets ($P < 0.05$).

3.3. Energy values of the test fiber-rich ingredients

The DE and ME values of each fiber-rich ingredient were not affected by the adaptation times, except for DE of the SBP diet, which tended to increase with the adaptation time from 7 to 21 d ($P = 0.07$). There were no linear or quadratic effects on the energy values of each fiber-rich ingredient with increasing adaptation duration (Table 5).

Table 2
Analyzed composition of the ingredients (as-fed basis, %).

Item	Corn	Soybean meal	Defatted rice bran	Soybean hull	Sugar beet pulp
Nutrient compositions					
Dry matter	90.47	91.40	91.06	92.51	93.39
Crude protein	8.42	47.53	16.31	9.48	9.58
Ether extract	4.81	2.74	2.46	2.24	1.48
Neutral detergent fiber	8.21	17.49	24.36	62.64	41.31
Acid detergent fiber	1.92	7.19	10.08	44.82	23.76
Ash	6.20	6.25	10.54	4.69	10.30
Total dietary fiber	9.67	16.06	34.06	79.02	58.50
Insoluble dietary fiber	9.53	15.76	33.86	74.69	44.84
Soluble dietary fiber	0.14	0.30	0.19	4.33	13.66
SDF:TDF ratio	1.48	1.86	0.56	5.47	23.35
Gross energy, MJ/kg	17.00	18.07	16.27	16.13	15.33

SDF = soluble dietary fiber; TDF = total dietary fiber.

SBH had higher determined DE content than DFRB at d 7 ($P < 0.05$), whereas, there was no difference observed at d 14 and 21. No significant difference in ME among fiber-rich ingredients was observed at d 7 and 14, but significantly lower ME content in DFRB was found at d 21 compared with SBP ($P < 0.05$).

3.4. Variation in α -diversity and β -diversity

Good's coverage (>0.995) and rarefaction curves indicated that the sequencing results could reflect the microbial diversity and bacterial communities information in the samples (Fig. S1). The results of α -diversity showed that Sobs, Shannon and Chao 1 indices in pig feces of each group were not changed with the prolongation of time from 7 to 21 d ($P > 0.05$; Fig. 1A, B, and 1D). However, Simpson index of the SBP diet group increased first and then decreased ($P < 0.05$; Fig. 1C). Compared with the basal diet group, the SBH diet group decreased Shannon index at d 14, and the SBP diet group decreased Simpson index at d 21 ($P < 0.05$; Fig. 1B and C). Among the different fiber inclusion diets, the SBP diet group had a higher Shannon index compared with the SBH diet group, but a lower Simpson index compared with the SBH diet and DFRB diet groups at d 7 and 21 ($P < 0.05$; Fig. 1B and C).

Beta-diversity (PCoA) revealed that fecal microbial community structure of the CON diet group and SBP diet group displayed no clear difference with different adaptation time ($P = 0.313$, $P = 0.116$, respectively; Fig. 2A and D), but distinct microbial communities in the DFRB diet group and SBH diet group were observed ($P < 0.05$,

Fig. 2B and C). On d 7 ($R = 0.39$, $P < 0.001$, Fig. 3E), d 14 ($R = 0.52$, $P < 0.001$, Fig. 3F), and d 21 of the trial ($R = 0.53$, $P < 0.001$, Fig. 3G), the composition of microbiota among the 4 diet groups demonstrated notable separation.

3.5. Comparison of the microbial composition

At the phylum level, Firmicutes and Bacteroidetes were the predominant phyla, comprising over 90% of total phyla with Spirochaetes and Proteobacteria in the feces (Fig. S2A). The abundance of Firmicutes for the basal diet group and Actinobacteria for the SBH diet group was decreased ($P < 0.05$, Figs. S3A and S3D), and the abundance of Tenericutes for the basal diet group and Spirochaetes and Tenericutes for the DFRB diet group was increased ($P < 0.05$, Figs. S3A and S3C) with time from 7 to 21 d. Furthermore, fiber inclusion diets fed to pigs decreased the relative abundance of Firmicutes, reached a significant level at d 14 ($P < 0.05$ Fig. S2D), and increased Bacteroidetes in the fecal microbiota ($P > 0.05$, Fig. S2A–C, and S2E). The abundance of Proteobacteria decreased after dietary fiber intake and reached a significant level at 21 d ($P = 0.025$, Figs. S2A, S2B, and S2D).

At the genus level, the top 10 genera with highest relative abundance in feces were *Prevotellaceae_NK3B31_group*, *Lactobacillus*, *Treponema_2*, *unclassified_f_Lachnospiraceae*, *norank_f_Muribaculaceae*, *Rikenellaceae_RC9_gut_group*, *Megasphaera*, *Christensenellaceae_R-7_group*, *Ruminococcaceae_UCG-005*, and *Clostridium_sensu_stricto_1*, which comprised approximately 50% of total genera

Table 3
Energy values of experimental diets fed to growing pigs.

Item	Adaptation time			Mean	SEM	P-value		
	D7	D14	D21			ANOVA	Linear	Quadratic
DE, MJ/kg DM								
Basal diet	16.36 ^a	16.09 ^a	16.42 ^a	16.29 ^a	0.11	0.099	0.717	0.037
SBP diet	15.25 ^{bc}	15.17 ^b	15.4 ^b	15.27 ^b	0.11	0.360	0.366	0.268
DFRB diet	15.01 ^c	14.76 ^b	15.02 ^c	14.93 ^c	0.09	0.129	0.920	0.048
SBH diet	15.34 ^b	15.34 ^b	15.06 ^c	15.00 ^c	0.19	0.072	0.323	0.033
SEM	0.10	0.18	0.09	0.08				
P-value	<0.01	<0.01	<0.01	<0.01				
ME, MJ/kg DM								
Basal diet	16.18 ^a	15.85 ^a	16.22 ^a	16.08 ^a	0.12	0.085	0.805	0.030
SBP diet	15.07 ^b	14.92 ^b	15.09 ^b	15.02 ^b	0.12	0.561	0.905	0.291
DFRB diet	14.81 ^b	14.54 ^{bc}	14.78 ^c	14.70 ^c	0.09	0.113	0.797	0.041
SBH diet	15.11 ^{bA}	14.37 ^{cB}	14.81 ^{cAB}	14.74 ^c	0.17	0.030	0.250	0.014
SEM	0.11	0.17	0.08	0.08				
P-value	<0.01	<0.01	<0.01	<0.01				

DE = digestible energy; ME = metabolizable energy; Basal diet = corn-soybean diet; SBP diet = sugar beet pulp diet; DFRB diet = defatted rice bran diet; SBH diet = soybean hull diet.

^{A, B} Within a row, means without a common superscript differ at $P < 0.05$.

^{a, b, c} Within a column, means without a common superscript differ at $P < 0.05$.

Table 4
Apparent total tract digestibility (ATTD) of GE and nutrients for experimental diets fed to growing pigs.

Item	Adaptation time			Mean	SEM	P-value		
	D 7	D 14	D 21			ANOVA	Linear	Quadratic
ATTD of gross energy, %								
Basal diet	87.22 ^a	85.75 ^a	87.54 ^a	86.84 ^a	0.58	0.096	0.701	0.035
SBP diet	83.73 ^b	83.28 ^a	84.53 ^b	83.85 ^b	0.61	0.364	0.366	0.273
DFRB diet	81.73 ^c	80.39 ^b	81.81 ^c	81.29 ^c	0.51	0.131	0.920	0.049
SBH diet	83.98 ^b	80.23 ^b	82.44 ^c	82.11 ^c	1.01	0.072	0.322	0.033
SEM	0.54	0.98	0.46	0.44				
P-value	<0.01	<0.01	<0.01	<0.01				
ATTD of dry matter, %								
Basal diet	87.68 ^a	86.37 ^a	87.48 ^a	87.18 ^a	0.51	0.179	0.788	0.071
SBP diet	83.81 ^b	84.14 ^a	84.71 ^b	84.22 ^b	0.60	0.577	0.309	0.867
DFRB diet	81.18 ^c	80.29 ^b	80.9 ^d	80.76 ^d	0.42	0.371	0.664	0.180
SBH diet	84.47 ^b	81.13 ^b	83.00 ^c	82.77 ^c	1.01	0.114	0.340	0.057
SEM	0.52	0.92	0.46	0.41				
P-value	<0.01	<0.01	<0.01	<0.01				
ATTD of crude protein, %								
Basal diet	79.02 ^{a,AB}	78.03 ^{a,B}	82.68 ^{a,A}	80.15 ^a	1.19	0.050	0.048	0.116
SBP diet	70.03 ^{b,B}	69.80 ^{b,B}	74.82 ^{b,A}	71.66 ^b	1.30	0.029	0.021	0.144
DFRB diet	69.62 ^b	68.41 ^b	71.96 ^c	69.90 ^b	1.36	0.245	0.288	0.190
SBH diet	75.22 ^a	68.72 ^b	72.13 ^c	71.83 ^b	1.64	0.055	0.224	0.029
SEM	1.41	1.80	0.83	0.91				
P-value	<0.01	0.010	<0.01	<0.01				
ATTD of ether extract, %								
Basal diet	71.98 ^a	66.90	74.40 ^a	71.09 ^a	2.39	0.110	0.485	0.048
SBP diet	66.81 ^{ab}	69.29	67.94 ^b	68.01 ^{ab}	1.46	0.501	0.591	0.301
DFRB diet	65.02 ^b	65.36	66.10 ^b	66.47 ^b	2.72	0.791	0.639	0.627
SBH diet	73.56 ^a	69.92	68.53 ^b	70.16 ^{ab}	1.73	0.378	0.173	0.868
SEM	2.09	1.99	1.75	1.24				
P-value	0.043	0.361	0.018	0.045				
ATTD of neutral detergent fiber, %								
Basal diet	71.27 ^b	69.07 ^{ab}	70.75 ^b	70.36 ^b	1.47	0.555	0.808	0.297
SBP diet	75.80 ^a	76.15 ^a	76.86 ^a	76.25 ^a	0.90	0.626	0.347	0.990
DFRB diet	59.11 ^c	61.51 ^b	60.25 ^c	60.29 ^c	0.97	0.317	0.490	0.195
SBH diet	69.09 ^b	62.41 ^b	70.12 ^b	67.10 ^b	3.46	0.270	0.843	0.117
SEM	1.15	2.93	1.44	1.19				
P-value	<0.01	<0.01	<0.01	<0.01				
ATTD of acid detergent fiber, %								
Basal diet	60.88 ^b	57.03 ^b	63.33 ^b	60.41 ^b	2.47	0.224	0.494	0.114
SBP diet	70.30 ^a	71.08 ^a	73.57 ^a	72.19 ^a	1.25	0.381	0.372	0.311
DFRB diet	27.10 ^c	31.03 ^c	33.65 ^c	30.66 ^c	2.15	0.147	0.056	0.960
SBH diet	67.98 ^{ab}	60.01 ^{ab}	72.81 ^a	66.87 ^a	4.98	0.222	0.525	0.116
SEM	2.51	4.18	2.09	1.86				
P-value	<0.01	<0.01	<0.01	<0.01				

SBP diet = sugar beet pulp diet; DFRB diet = defatted rice bran diet; SBH diet = soybean hull diet.

^{A, B} Within a row, means without a common superscript differ at $P < 0.05$.

^{a, b, c} Within a column, means without a common superscript differ at $P < 0.05$.

Table 5
Energy values of fiber-rich ingredients fed to growing pigs (DM-basis).

Item	Adaptation time			Mean	SEM	P-value		
	D7	D14	D21			ANOVA	Linear	Quadratic
DE, MJ/kg DM								
SBP	11.23 ^{ab}	11.89	11.71	11.61	0.54	0.068	0.536	0.540
DFRB	10.56 ^b	10.41	10.40	10.45	0.47	0.970	0.818	0.910
SBH	12.29 ^a	9.98	10.68	10.91	0.91	0.260	0.250	0.210
SEM	0.41	0.98	0.41	0.39				
P-value	0.049	0.379	0.091	0.133				
ME, MJ/kg DM								
SBP	11.02	11.55	11.36 ^a	11.31	0.59	0.810	0.691	0.623
DFRB	10.31	9.98	9.72 ^b	9.99	0.44	0.670	0.380	0.960
SBH	11.90	9.38	10.14 ^{ab}	10.39	0.83	0.150	0.170	0.130
SEM	0.45	0.91	0.41	0.38				
P-value	0.111	0.252	0.032	0.058				

DE = digestible energy; ME = metabolizable energy; SBP = sugar beet pulp; DFRB = defatted rice bran; SBH = soybean hull.

^{a, b, c} Within a column, means without a common superscript differ at $P < 0.05$.

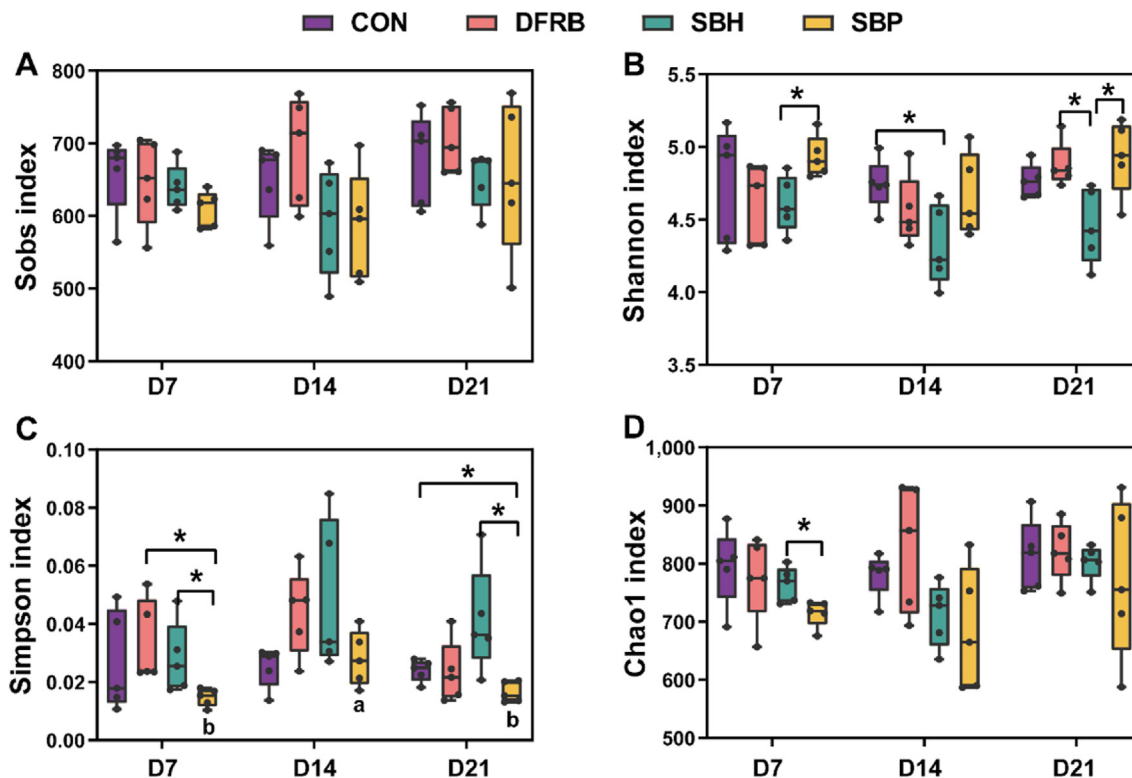


Fig. 1. Alpha-diversity of fecal microbiota. The box plot of Sobs (A), Shannon (B), Simpson (C), and Chao 1 (D) indexes (OTU level) in feces after dietary fiber intake with extension of adaptation time. Data are expressed as min to max showing all points ($n = 5$ pigs/group). * Indicates significant difference ($P < 0.05$). ^{a, b} Mean values with different letters are statistically significant ($P < 0.05$). CON = basal diet; SBP = sugar beet pulp; DFRB = defatted rice bran; SBH = soybean hull.

(Fig. 3A). The relative abundance of certain genera increased (e.g., *Prevotellaceae_NK3B31_group*, *unclassified_f_Prevotellaceae*, and *norank_f_Bacteroidales_RF16_group*), whereas others decreased (e.g., *Christensenellaceae_R-7_group*, *Parabacteroides*) during the 3 wk adaptation to dietary fiber (Fig. 3A and B). Although some genera did not change consistently at the 7-d adaptation point, the relative abundance of those genera all showed increment (e.g., *Alloprevotella*, *Sphaerochaeta*) or decrement (e.g., *Megasphaera*, *Ruminococcaceae_NK4A214_group*, *Ruminococcaceae_UCG-002*) at d 14 and 21 with dietary inclusion. Furthermore, 4 genera were identified which had significant differences in all three adaptation points (*Prevotellaceae_NK3B31_group*, *Lactobacillus*, *Selenomonas*, *Treponema_2*, Fig. 3C).

3.6. Alterations of specific microbiota

The abundance of *Selenomonas* was increased with the extension of adaptation time for the CON diet ($P < 0.05$, Fig. 4A). *Ruminococcus_1* and *unclassified_o_Bacteroidetes* were decreased at d 14 ($P < 0.05$), but increased to initial levels at d 21 for the SBH diet. For pigs fed the SBP diet, *Rikenellaceae_RC9_gut_group* increased significantly at d 21, whereas *Marvinbryantia* and *Christensenellaceae_R-7_group* decreased with time. In the DFRB diet group, *Lactobacillus* and *Bifidobacterium* increased with time, and reached significant levels at d 14. Additionally, *Rikenellaceae_RC9_gut_group*, *Ruminococcaceae_UCG-014*, and *norank_f_norank_o_Mollicutes_RF39* increased from 7 to 21 d ($P < 0.05$), whereas *Streptococcus*, *Megasphaera*, *Mitsuokella*, *Blautia* and *Coprococcus_3* decreased from d 7 to 21.

A total of 11, 20 and 7 different genera were identified in the feces of the pigs fed different diets after 7, 14 and 21 d of adaptation, respectively ($P < 0.05$, Figs. S4A–C). The alterations in relative abundance by dietary fiber intake attracted more attention.

Compared with pigs fed the basal diet after 7 d, the abundance of *Prevotellaceae_NK3B31_group*, *Megasphaera* and *Selenomonas* were more than 2-fold greater for pigs fed the DFRB diet (Fig. 5A). The SBH diet increased the abundances of *Treponema_2*, *Ruminococcus_1*, *Lachnospiraceae_NK4A136_group* and *Selenomonas*, and decreased the abundance of *Anaerovibrio* and *Mitsuokella* more than 2-fold compared with the basal diet (Fig. 5A). Additionally, the SBP diet improved *Prevotellaceae_NK3B31_group* abundance, but decreased the abundance of most genera, such as *Megasphaera*, *Anaerovibrio* and *Mitsuokella*, compared to the basal diet (Fig. 5A). Dietary fiber intake increased 6 differential genera and decreased 7 differential genera compared with the basal diet after 14 d adaptation (Fig. 5B). Furthermore, different types of fiber had different effects on microbiota; for example, the abundance of *Ruminococcus_1* and *Selenomonas* was decreased for pigs fed the SBH diet, whereas it was increased for pigs fed the SBP diet and/or the DFRB diet. After 21 d adaptation time (Fig. 5C), *Prevotellaceae_NK3B31_group* and *Sphaerochaeta* were found to be 2-fold greater for pigs fed the SBP diet and DFRB diet compared with pigs fed the basal diet. Lastly, the abundance of *Selenomonas* decreased more than 2-fold in all fiber-rich diet groups compared with the basal diet group.

3.7. Concentration of SCFA in feces

There was no difference in the concentrations of acetate, isobutyrate, isovalerate, valerate and total SCFA for all dietary treatment groups from 7 to 21 d (Fig. 6A and 6D–G). However, the adaptation time affected the concentration of propionate for the SBP diet group ($P < 0.05$; Fig. 6B) and the concentration of butyrate increased with the extension of adaptation time for the DFRB diet group ($P < 0.05$; Fig. 6C). Further, the concentrations of propionate, isovalerate and total SCFA linearly increased with adaptation time

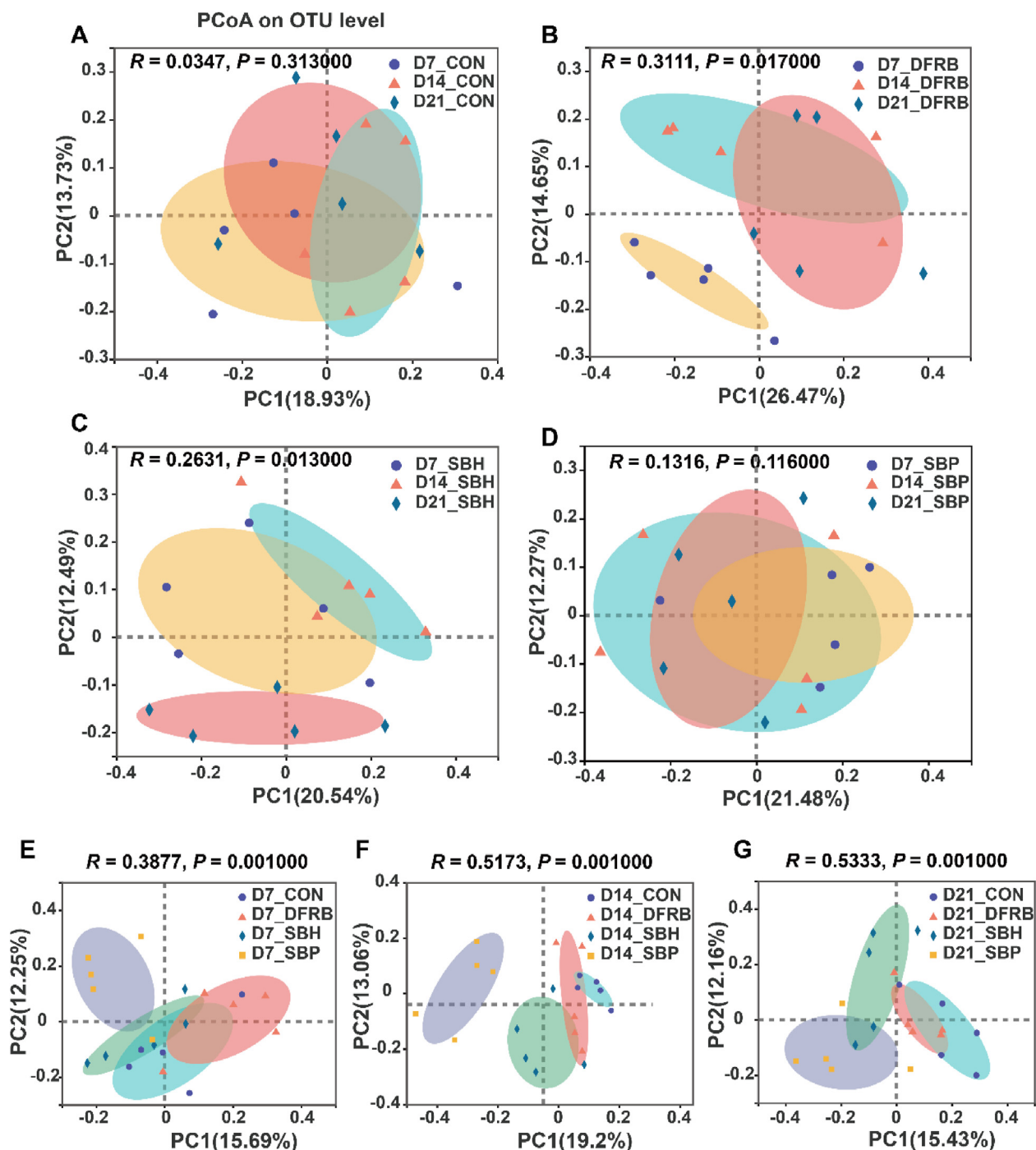


Fig. 2. Beta-diversity of fecal microbiota. PCoA (OTU level) of community membership based on the Unweighted Unifrac and ANOSIM test in CON (A), DFRB (B), SBH (C), and SBP group (D) at different time points, the 4 different diet treatments on d 7 (E), d 14 (F), and on d 21 (G) ($n = 5$ pigs/group). CON = basal diet; SBP = sugar beet pulp; DFRB diet = defatted rice bran; SBH diet = soybean hull.

from 7 to 21 d for pigs fed the DFRB diet ($P < 0.05$; Fig. 6B, F, and 6G). Among dietary treatments, the SBH diet group had the highest acetate concentration, whereas the basal diet group had the highest concentration of isobutyrate and isovalerate ($P < 0.05$). The DFRB diet group had the lowest acetate production, and the SBP diet group had the lowest valerate production ($P < 0.05$). Pigs fed the SBH diet and CON diet had similar propionate and total SCFA concentrations, which were higher than in pigs fed the DFRB and SBP diets ($P < 0.05$).

4. Discussion

In the present study, the analyzed values of dietary fiber contents and the ratio of SDF to TDF (SDF/TDF) of the 3 fiber-rich ingredients were within the range of values reported previously (NRC, 2012; Jaworski and Stein, 2017; Lyu et al., 2018). The 3 fiber-rich diets had lower digestible and metabolizable energy, and ATTD of most nutrients than the corn-soybean meal basal diet, as the fiber-rich ingredients had higher contents of dietary fiber, which

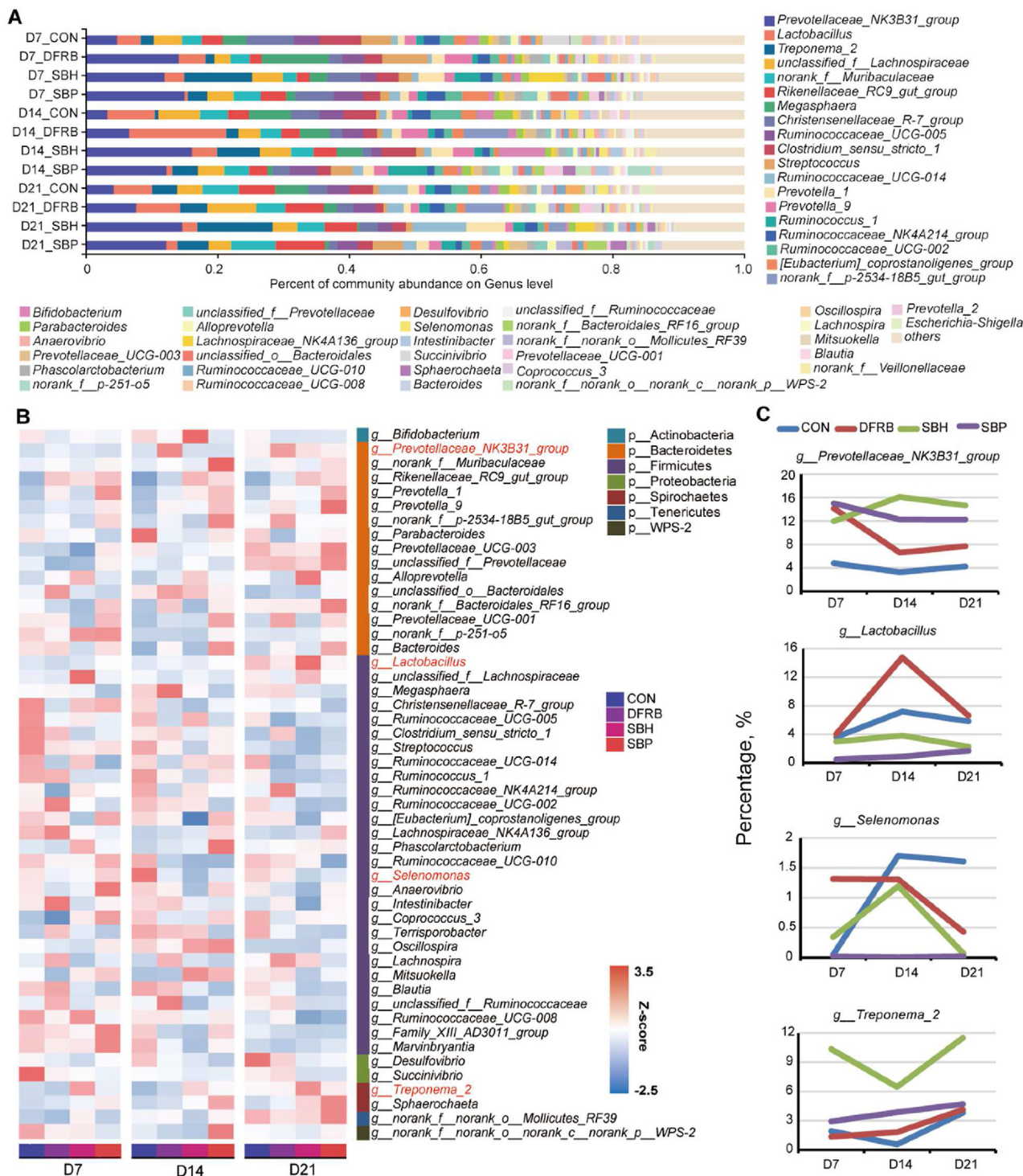


Fig. 3. Community composition analysis on genus level. The relative abundance (A) or trends change (B) of the bacterial genus (top 50) in feces; the specifically altered genera in feces (C). CON = basal diet; SBP = sugar beet pulp; DFRB = defatted rice bran; SBH = soybean hull.

had negative effects on energy values and nutrient digestibility (Chen et al., 2013; Zhao et al., 2018b; Zhong and Adeola, 2019). Thus, the fiber content in diets and ingredients can be used as an important independent variable to predict energy digestibility and it has been reported that NDF, rather than other dietary fiber types, as an independent variable can increase the accuracy of prediction equations for DE in feed ingredients or diets for growing pigs (Choi et al., 2020). The diet containing SDF-rich ingredients (the SBP diet)

had greater values of NDF and ADF digestibility the basal diet and DFRB diet, which was consistent with previous research (Yan et al., 2017; Lyu et al., 2018; Liu et al., 2021). These may be attributed to SDF being easily and rapidly fermented by microbiota in the hindgut to produce SCFA (Gao et al., 2015; Zhao et al., 2019, 2021), and may also increase digesta fermentation time in the hindgut by increasing the viscosity of digesta (Freire et al., 2000; Chen et al., 2017; Navarro et al., 2018b). In addition, fiber composition of

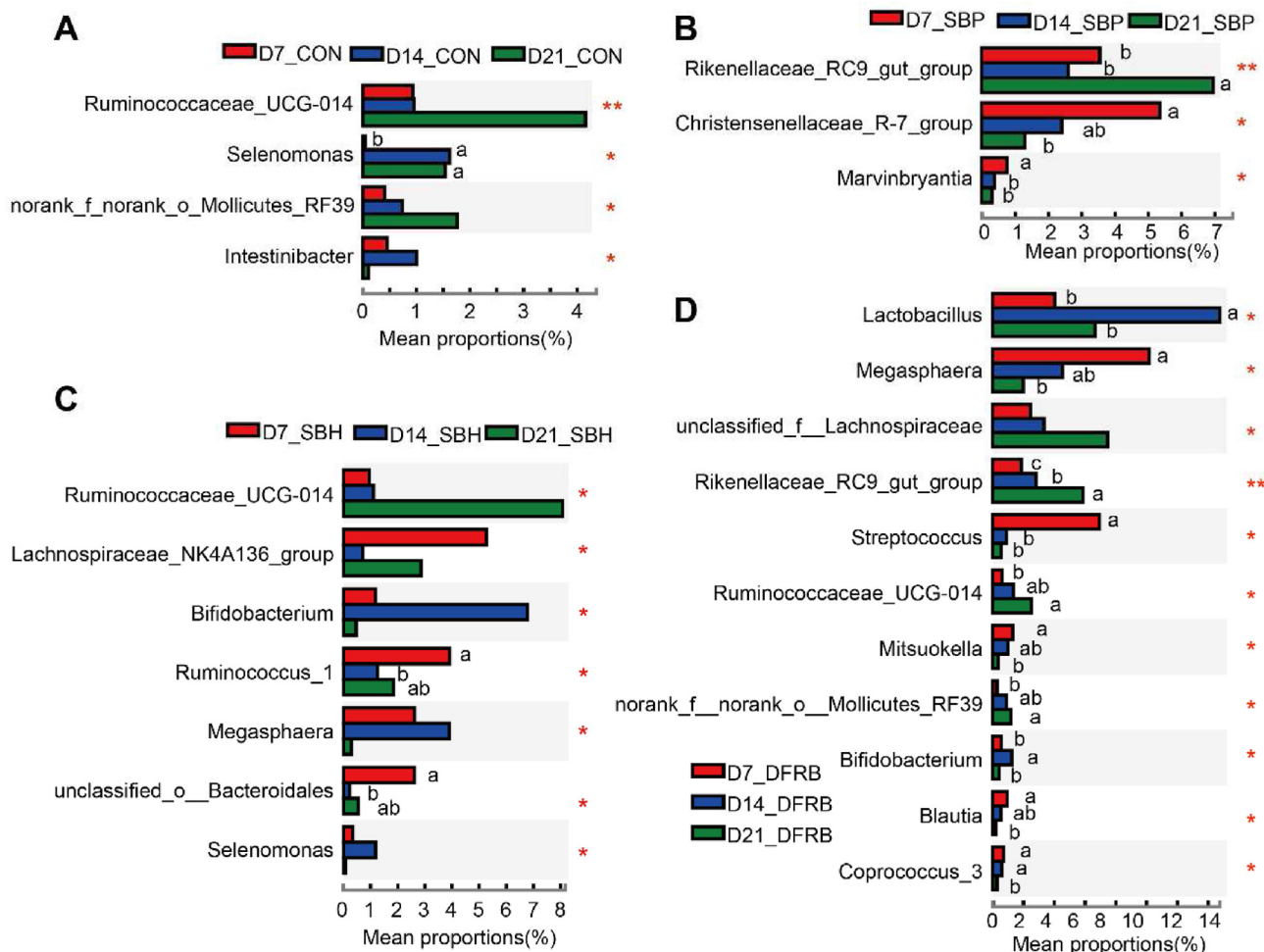


Fig. 4. Differentially abundant genera (top 50) in feces after different adaptation time within CON (A), SBP (B), SBH (C), and DFRB group (D) analyzed using Kruskal–Wallis H test with Welch’s Post-hoc test. * $P < 0.05$, ** $P < 0.01$, respectively. Different letters (a, b, and c) indicate $P < 0.05$ in pairwise comparisons. CON = basal diet; SBP = sugar beet pulp; DFRB = defatted rice bran; SBH diet = soybean hull.

non-starch polysaccharides may influence digestibility and different types of ingredients may also cause the gut microbiota composition to differ; thus, SDF-rich fibers could improve the abundance of cellulolytic bacteria (Zhao et al., 2019), such as *Ruminococcus_1*, which may also contribute to the higher ATTD of ADF and NDF in pigs fed SBH or SBP diets in our study.

Previous researchers suggested that a 5–7 d adaptation period was generally required for pigs to adapt to the test diets (Adeola, 2001; Zhong and Adeola, 2019; Rodriguez et al., 2020). For fiber-rich diets, however, pigs may need a longer adaptation duration owing to the different physicochemical properties (especially solubility) of the dietary fiber (Bakker, 1996; Zhang et al., 2019). Interestingly, a study found that adaptation time to achieve a constant fecal marker concentration is longer with a low-fiber diet compared with a high-fiber diet, and the ATTD of energy and nutrients achieved stability after 4 d for pigs fed the high-fiber diet. This was shorter than 5 d that is required for pigs fed the low-fiber diet (Choi and Kim, 2019), which may be due to the increased passage rate of digesta for the high-fiber diet, and a greater volume of digesta present in the intestinal tract. The study provided a minimum adaptation period for constant marker concentrations in feces from pigs fed diets with varying fiber concentrations. The differences between the aforementioned study and our study may be that the above experiment only had

an 8 d total fecal collection period and determined the ATTD of energy and nutrients on each sampling day, which may not have been enough to measure whether the pigs could adapt to the high-fiber diet quickly. In our study, the ATTD of energy and nutrients were determined using the mixed feces for each pig during the 5-d collection periods, and found that there was no difference in the energy values and the ATTD of most nutrients across different duration times for the basal diets and the fiber-rich diets, which was also in line with previous findings (Fan et al., 2017; Lyu et al., 2018; Zhang et al., 2019). Nevertheless, a lower energy values and energy digestibility of pigs fed the DFRB diet and the SBH diet was observed over the 14-d adaptation compared with the 7 or 21 d adaptation. Previous studies also reported that the lowest DE and digestibility of nutrients were found in pigs fed wheat bran diets (Zhao et al., 2018b) or fed a palm kernel meal diet (Huang et al., 2018) for 14 d compared with 7, 21, or 28 d adaptation. The reason for this result may be due to the increased excretion of endogenous ash and N prior to the terminal ileum of pigs caused by the high dietary fiber (Wilfart et al., 2007). Moreover, the largest number of differential genera was identified at d 14, which may suggest that adaptive changes in microbiota reach a peak, leading to lower fermentative capabilities and nutrient digestibility. It has been reported that at least 14 to 21 d might be needed for pigs to adapt to high-fiber diets with wheat

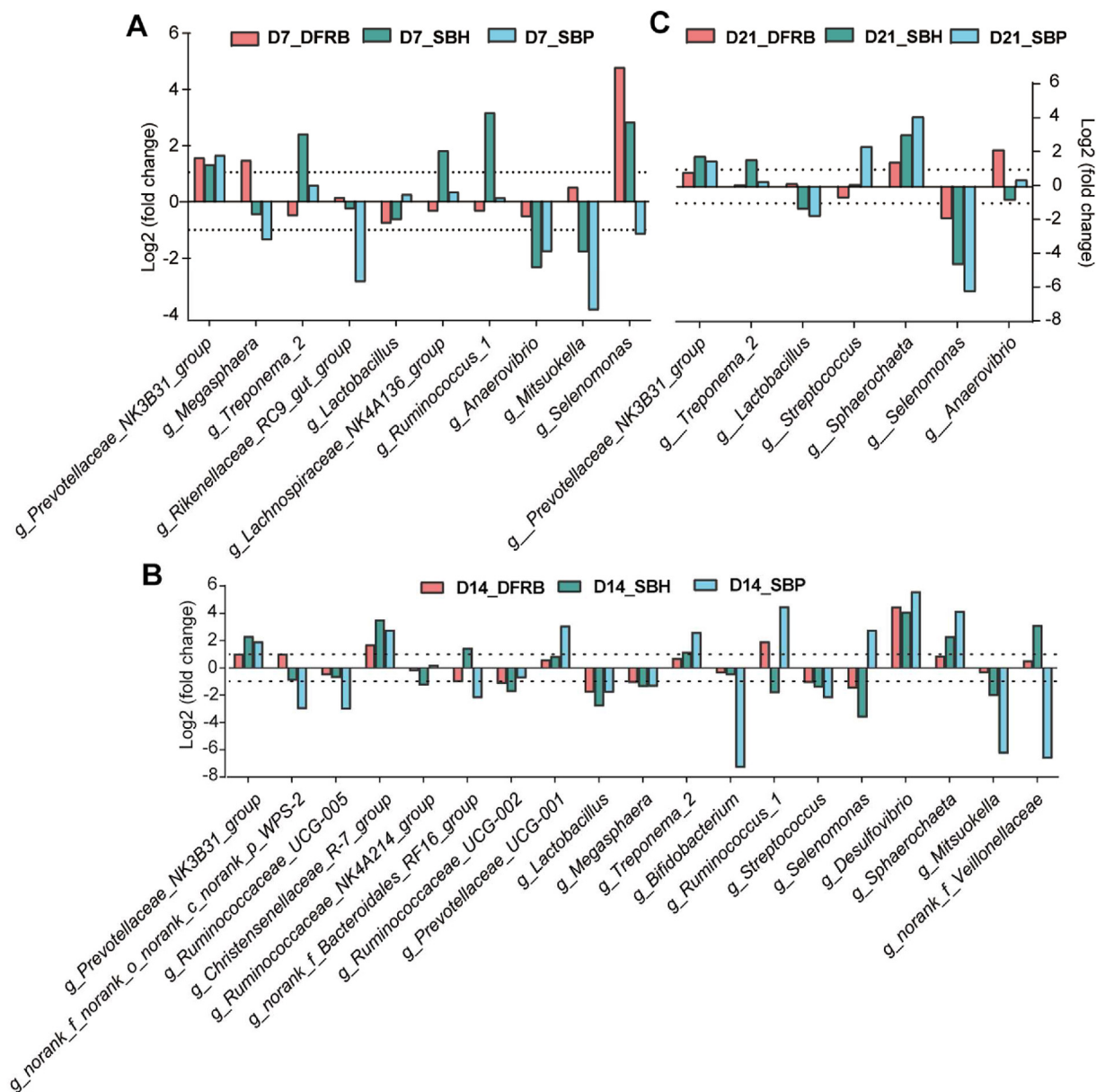


Fig. 5. Fold change of differentially abundant genera from feces in higher fiber diet groups vs. Con group on d 7 (A), d 14 (B), and d 21 (C). Positive fold changes indicate that a genus is enriched in the high-fiber diet groups (DFRB, SBH, or SBP), while negative fold changes suggest that a genus is enriched in the Con group. SBP = sugar beet pulp; DFRB = defatted rice bran; SBH diet = soybean hull.

bran (Zhao et al., 2018b). Another study showed that the adaptation time of growing pigs to diets supplemented with raw potato starch was 5 wk, reflected by the ATTD of nutrients and SCFA content in feces (Martinez-Puig et al., 2003). Besides, addition of SBP to pig diets was more easily adapted by hindgut microbiota, compared to dietary addition of wheat bran (WB; Roca-Canudas et al., 2007; Molist et al., 2009). The results were consistent with the present study that the gut microbiota in growing pigs more easily and quickly adapted to the SBP diet (rich in SDF) compared to the DFRB diet (rich in IDF), as reflected in the PCoA analysis where there was no considerable distinction between the composition of the microbial community at different times with the SBP diet. The lowest number of differential genera for pigs fed the SBP diet across the 3 adaptation times may also suggest that the gut microbiota from pigs receiving the SBP diet adapted more easily. Therefore, it is necessary to select an appropriate

adaptation time for nutritional evaluation of fiber-rich ingredients according to the growth stage of animals and physicochemical characteristics of the dietary fibers.

Dietary fiber is one of the major factors affecting the diversity and community of bacteria in the gut (Bach Knudsen et al., 2012) by affecting the digestion site and gut environment (Högberg and Lindberg, 2004). Alpha-diversity indices were independent of the adaption time (Le Sciellour et al., 2018), which was consistent with our results. In addition, this is also consistent with a previous report that piglets fed SBP but not WB decreased α -diversity (Chao 1 or Shannon indices) compared to those fed a CON diet (Shang et al., 2021), indicating that high SDF content fiber may not increase the microbiota diversity because it is easy to ferment. In our results, growing pigs fed high SDF content fiber (i.e., SBP, SBH) had a lower α -diversity (Shannon index) compared to pigs fed IDF-enriched fiber (i.e., DFRB). However, the Sobs index and Chao 1 index were

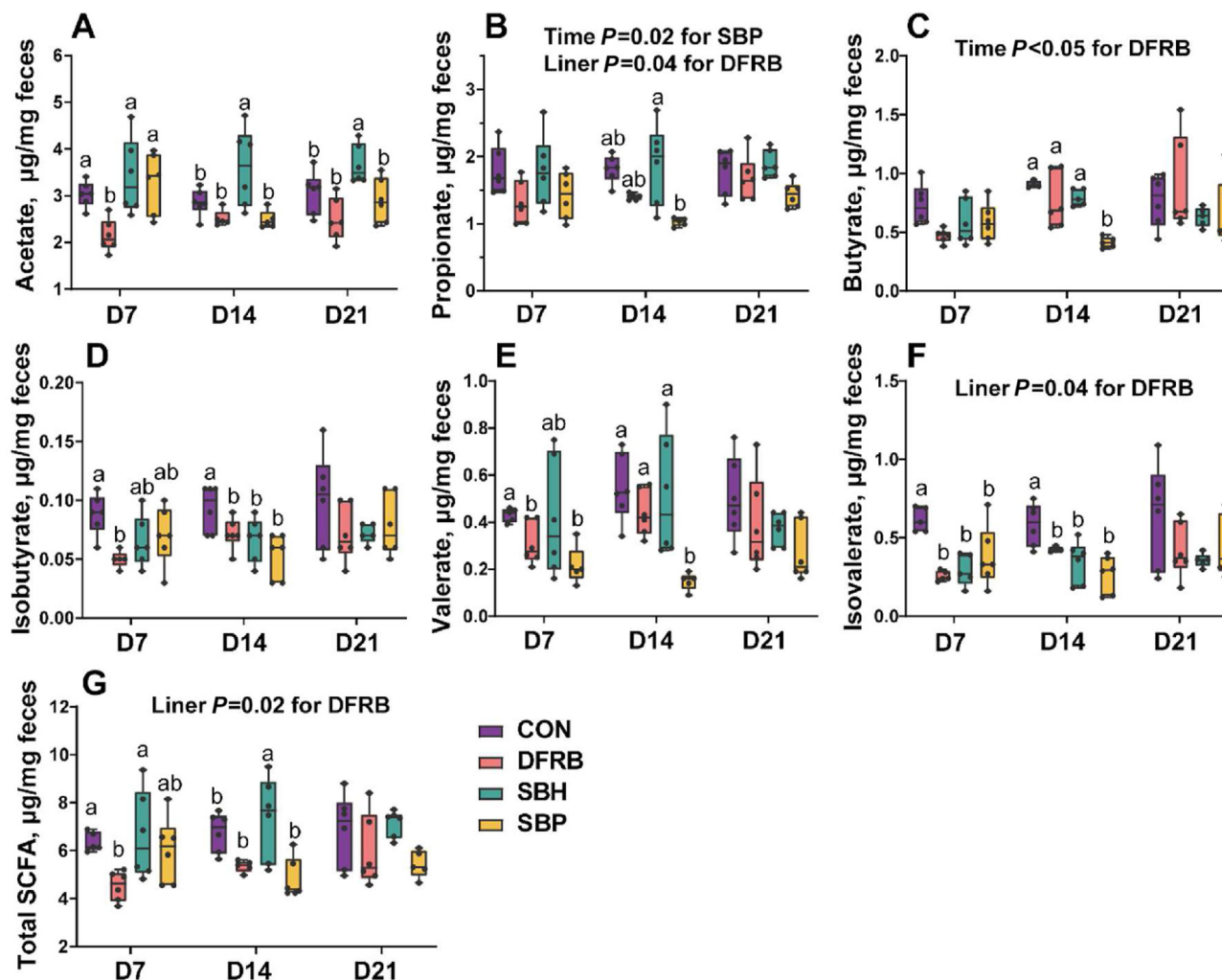


Fig. 6. Concentration of short-chain fatty acids (SCFA, µg/mg) in feces (wet basis). Concentration of acetate (A), propionate (B), butyrate (C), isobutyrate (D), valerate (E), isovalerate (F), and total SCFA (G). Different letters (a, b, c, and d) indicate significant differences ($P < 0.05$). CON = basal diet group; SBP = sugar beet pulp diet group; DFRB diet = defatted rice bran diet group; SBH diet = soybean hull diet group.

similar among the different diet treatments, which was consistent with a previous study (Pu et al., 2020). In the current study, the dominant bacteria in the pig hindgut were Firmicutes and Bacteroidetes, which accounted for about 85% of the gut microbiota in agreement with previous studies (Holman et al., 2017; Liu et al., 2018). Proteobacteria and Spirochaetes were also present in fecal microbiota but in smaller amounts, as previously shown (Looft et al., 2014; Le Scieillour et al., 2018). *Ruminococcus_1* and *Lachnospiraceae*, belonging to Firmicutes, can degrade fibers and produce SCFA, such as butyrate (Kim et al., 2018; Xie et al., 2019). The phylum of Bacteroidetes also contained bacteria (e.g., *Prevotellaceae_NK3B31_group*, *Rikenellaceae_RC9_gut_group*) that are reported to be capable of utilizing fiber (Qiu et al., 2019; Wang et al., 2020). Therefore, Firmicutes and Bacteroidetes abundance are important indicators for the ability of gut microbiota to degrade fiber and produce SCFA (Zhao et al., 2019). Our results showed that dietary fiber inclusion numerically decreased the relative abundance of Firmicutes and increased Bacteroidetes in the feces of pigs, which is consistent with a previous study (Ferrario et al., 2017). However, another study reported that an increased Firmicutes to Bacteroidetes ratio in human gut microbiota was observed with a

high-resistant starch diet (Maier et al., 2017). The different effects of dietary fiber on microbiota composition could be attributed to the different fermentation properties of dietary fiber and intestinal environment of the host. In addition, we observed an increased abundance of *Ruminococcus_1* for pigs fed the SBH diet or SBP diet, which is in agreement with findings reported by Zhao et al. (2019). *Ruminococcus_1* was reported to be able to ferment complex polysaccharides and produce SCFA (Xie et al., 2019; Su et al., 2020), which may explain the higher ATTD of ADF and SCFA production in pigs fed the SBH or SBP diet in our study. The relative abundance of *Lactobacillus* was also affected by different types of fiber supplementation in the present study, and the pigs fed the DFRB diet had the highest abundance of *Lactobacillus*. Genera *Lactobacillus* are widely known probiotics because of their multiple health promotion effects, such as suppression of intestinal inflammation and improvement of intestinal barrier function, maintenance of microbial homeostasis, and prevention of diseases (Wang et al., 2018; Zhang et al., 2018). However, as similarly shown in a previous study (Zhao et al., 2019), the high *Streptococcus* abundance, which is regarded as pathogenic bacteria, in pigs fed the SBP diet was also observed in the present study. *Streptococcus* abundance in pigs fed

the DFRB diet was low, which may be due to a higher SDF/TDF content in the SBP than the DFRB diet. From these studies, it may be inferred that dietary fibers might differently modulate the metabolism due to their physicochemical properties that shape the gut microbiota composition in the host.

The previous study reported that *Selenomonas* specifically increased in the basal diet (Lv et al., 2019) and was related to obesity (Li et al., 2017). In this study, the relative abundance of *Selenomonas* was increased from d 7 to 21 for pigs fed the corn-soybean meal basal diet, which may be due to the fact that the basal diet had a high energy level. Dietary fiber can improve animal health by regulating gut microbiota. Our results showed that fiber inclusion in the diet increased the relative abundance of probiotics (e.g., *Lactobacillus* and *Bifidobacterium*), cellulose-degrading bacteria and SCFA-producing bacteria (e.g., *Ruminococcaceae_UCG-014*, *Rikenellaceae_RC9_gut_group*, and *Bifidobacterium*), and decreased the relative abundance of pathogenic bacteria (e.g., *Streptococcus*, *Selenomonas*). It has been reported that *Rikenellaceae_RC9_gut_group* is related to fiber degradation in the hindgut (Qiu et al., 2019), and was enriched in the high SDF ratio group (Tao et al., 2019), which supported our findings that pigs fed the SBP diet showed an incremental growth in relative abundance of *Rikenellaceae_RC9_gut_group* as adaptation time increased from 7 to 21 d. With time, pigs fed the DFRB diet increased abundance of *Ruminococcaceae_UCG-014*, which is capable of degrading and utilizing cellulose and hemicellulose components to produce butyrate and has a potential role in maintaining intestinal health (Dai et al., 2018). These findings were consistent with the results of the increased butyrate and SCFA production for the DFRB diet along with time from 7 to 28 d.

Increasingly differential genera were observed for pigs fed fiber-rich diets at d 14, which may indicate that there were still large modulations in microorganisms after 14 d adaptation time. Differential genera decreased at 21 d, which indicated the new gut homeostasis might reshape after 21 d adaptation to fiber-rich diets. The results were closely related to the quadratic effect of determined DE and ME. However, the adaptation time may vary across different fiber ingredients. Sappok et al., (2015) reported that adaptation of microbiota of pigs fed fiber-rich diets, especially for slow-fermenting substrates, takes longer than 19 d. This suggests that high IDF-content fiber ingredients may need more time for microbial adaptation. In the present study, growing pigs fed the DFRB diet had the most differential genera and the total SCFA, butyrate, isobutyrate and isovalerate content in feces linearly increase with time, indicating that the microbiota of pigs fed the BFRB diet may need a longer adaptation period, which is in agreement with the previous study that the adaptation time of pigs fed a diet with added sugar beet pulp was significantly shorter than that of pigs fed a diet with added wheat bran (Castillo et al., 2007; Molist et al., 2009), indicating that the gut microbiota of pigs may more easily adapt to fermentable SDF.

5. Conclusion

In conclusion, dietary fiber inclusion decreased energy values of diets regardless of the adaptation time. No significant differences among adaptation times were observed for the DE or ME of all ingredients, indicating at least 7 d adaptation is required for pigs to evaluate the energy values of DFRB, SBP and SBH. However, gut microbiota had different adaptation times for different types of dietary fiber. With time increased from 7 to 21 d, cellulose-degrading bacteria and SCFA-producing bacteria (e.g., *Ruminococcaceae_UCG-014*, *Rikenellaceae_RC9_gut_group*, and *Bifidobacterium*) were increased in fiber inclusion diets. Nevertheless, the

precise mechanisms underlying the potential beneficial effects of dietary fiber need to be further explored.

Author Contributions

Qingtao Gao: Conceptualization, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing; **Zhengqun Liu:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing; **Kai Li:** Investigation; **Guosong Bai:** Investigation; **Lei Liu:** Investigation, Methodology; **Ruqing Zhong:** Conceptualization, Data curation, Writing – review & editing; **Liang Chen:** Conceptualization, Data curation, Funding acquisition; **Hongfu Zhang:** Supervision.

Declaration of competing interest

No conflict of interest exists in the submission, and all authors have approved the manuscript.

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Appendix Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2022.10.003>.

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