



## Original Research Article

## Diets enriched with finely ground wheat bran alter digesta passage rate and composition of the gut microbiome in sows

Zijie Wang<sup>a</sup>, Wenhui Wang<sup>a</sup>, Song Xu<sup>a</sup>, Jian Ding<sup>b</sup>, Xiangfang Zeng<sup>a</sup>, Hu Liu<sup>a,\*</sup>, Fenglai Wang<sup>a</sup><sup>a</sup> State Key Lab of Animal Nutrition, College of Animal Science & Technology, China Agricultural University, Beijing 100193, China<sup>b</sup> National Animal Husbandry Service, Ministry of Agriculture and Rural Affairs of the People's Republic of China, Beijing 100000, China

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## ABSTRACT

We investigated the effects of finely ground wheat bran on the nutrient digestibility, digesta passage rate, and gut microbiota structure in sows. A 3 × 3 Latin square design with 3 test periods and 3 experimental diets was used. Six non-pregnant sows (parity: 5 to 7) were randomly assigned to 3 experimental diets with 2 replicates per treatment in each period. Each period lasted 19 d (12 d for adaptation and 7 d for experiment). The experimental diets included (a) a basal corn and soybean meal diet (CON), (b) a basal diet with 20% coarse wheat bran (CWB; particle size: 605 μm), and (c) a basal diet with 20% fine wheat bran (FWB; particle size: 438 μm). The results demonstrated that the apparent total tract digestibility of neutral detergent fiber, acid detergent fiber and energy were reduced ( $P < 0.05$ ) in the FWB and CWB groups compared with those in the CON group. Viscosity of digesta increased ( $P < 0.001$ ) in FWB-fed sows. The passage rate of digesta from the mouth to the ileum decreased ( $P < 0.001$ ) in FWB-fed sows. Peptide YY (PYY) concentration increased ( $P = 0.01$ ) in FWB-fed sows after 30 min of feeding. In the FWB group, the relative abundance of Lactobacillaceae at the family level increased ( $P < 0.05$ ) in the ileal digesta. At the class level, the relative abundance of Clostridia in feces decreased ( $P < 0.05$ ) in FWB-fed sows. FWB enhanced the concentration of butyrate in feces compared with CON and CWB ( $P = 0.04$ ). These results suggest that dietary supplementation with finely ground wheat bran reduces the passage rate of digesta, increases the abundance of beneficial microorganisms, and elevates the concentration of short-chain fatty acids and PYY in sows. These findings indicate that the addition of finely-ground wheat bran to the diets of sows is more effective than using coarse wheat bran for improving their satiety and intestinal microbial composition.

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## 1. Introduction

Dietary fiber (DF) refers to plant polysaccharides and lignin that cannot be hydrolyzed by endogenous enzymes in the mammalian intestine (Knudsen, 1997). The DF that is not digested by

endogenous enzymes in the small intestine enters the large intestine, facilitating microbial growth and production of short-chain fatty acids (SCFA) (Serena et al., 2008). Gut microbiota regulate the intestinal tract physiology of a host. An important function of microbiota is to degrade DF into SCFA (e.g., acetate, propionate, and butyrate). These SCFA affect the gut microbial ecology, physiology and overall health of the host (Ríos-Covián et al., 2016).

Gastric emptying rate, passage rate of digesta and total transit time are affected by the type of DF (Serena et al., 2008) and physicochemical properties of DF can influence the passage kinetics of digesta (Schop et al., 2020). Further, different DF types possess varying physicochemical properties. Soluble dietary fiber (SDF) can delay gastric emptying due to its high water-holding capacity (WHC) and high viscosity. Compared with SDF, insoluble dietary fiber (IDF) can increase fecal excretion due to

\* Corresponding author.

E-mail address: [liuhu0674@cau.edu.cn](mailto:liuhu0674@cau.edu.cn) (H. Liu).

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reduced microbial degradation of fiber within the large intestine (Serena, 2005). Therefore, the passage rate of digesta, nutrient digestibility, microbial community structure and physiological metabolic processes are influenced by DF characteristics (Knudsen et al., 1993; Owusu-Asiedu et al., 2006). The passage rate of digesta in the gastrointestinal tract impacts transit time and total time of digestion by the endogenous digestive juice and microbes, and influences the degree of digestion (Owusu-Asiedu et al., 2006).

Wheat bran primarily contains IDF (Messia et al., 2016) which is not easily fermented by large intestine microorganisms, reducing the nutritional value of wheat bran. Further, its physiological effects in the host are limited (Lin et al., 2021). If the wheat bran can be reasonably processed, for example through fine grinding, the value of wheat bran can be used to improve the digestive physiology of sows and utilization of feed. Many *in vitro* studies have demonstrated that fine grinding can improve the physicochemical properties of wheat bran by decreasing particle size, increasing porosity and increasing specific surface area (Silva and Bloom, 2012; Santala et al., 2014). Feed processing of fine grinding not only increases the content of SDF, WHC and fermentability of wheat bran, but also improves lipid metabolism and inflammatory response by modulating the gut microbiota structure in pregnant sows (Wang et al., 2022). We hypothesized that finely ground wheat bran will reduce the passage rate of digesta and increase digestion and decomposition of large intestinal microorganisms. Therefore, the current study aimed to investigate the effects of finely ground wheat bran on the 1) ileal and fecal nutrient digestibility and microbial population composition; 2) digesta passage rate through the small intestine and total tract; 3) plasma concentrations of hormones; and 4) SCFA concentrations in the ileum and feces.

## 2. Materials and methods

### 2.1. Ethical approval

All experimental protocols in the current study were approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China). Protocols were based on the National Research Council's Guide for the Care and Use of Laboratory Animals (AW42022202-1-1).

### 2.2. Wheat bran samples

Wheat bran was purchased from Hebei Flour Mill (Hebei, China), and a shredding machine equipped with a 1.0 mm screen (Jiangyin Hongda powder equipment limited company, Jiangsu, China) was used to grind wheat bran into a fine sample.

### 2.3. Experimental diets

Three experimental diets were used in this experiment. The experimental diets included a basal corn and soybean meal diet (CON; basal diet), a basal diet with 20% coarse wheat bran (CWB; particle size, 605  $\mu\text{m}$ ) and a basal diet with 20% fine wheat bran (FWB; particle size, 438  $\mu\text{m}$ ). The particle size of wheat bran was determined using the fourteen-sieve method of the ANSI/ASAE S319.4-2008 standard (Stark and Chewning, 2011). All experimental diets met or exceeded the nutrient requirements of pregnant sows, based on the nutrient requirements of swine (NRC, 2012). The composition and nutrient content of the experimental diets are shown in Table 1.

**Table 1**

Ingredient composition and nutrient concentrations of experimental diets (% as-fed basis).

Item	CON <sup>1</sup>	CWB <sup>2</sup>	FWB <sup>3</sup>
Corn	77.30	59.35	59.35
Soybean meal	17.55	15.50	15.50
Coarse wheat bran <sup>4</sup>		20.00	
Fine wheat bran <sup>5</sup>			20.00
Soybean oil	2.00	2.00	2.00
Dicalcium Phosphate	1.30	1.30	1.30
Limestone	1.05	1.05	1.05
Salt	0.30	0.30	0.30
Vitamin and mineral premix <sup>6</sup>	0.50	0.50	0.50
Calculated composition			
ME <sup>7</sup> , kcal/kg	3.285	3.159	3.159
SID lysine	0.58	0.57	0.57
Calcium	0.78	0.80	0.80
Total phosphorus	0.70	0.72	0.72
Analyzed composition			
GE <sup>8</sup> , kcal/kg	3,729	3,662	3,662
Crude protein	12.68	15.31	15.30
Neutral detergent fiber	15.70	18.30	18.28
Total dietary fiber	10.74	15.19	15.21
Soluble dietary fiber	1.49	2.20	2.90
Insoluble dietary fiber	9.25	12.99	12.31

<sup>1</sup> CON = a corn-soybean meal diet.

<sup>2</sup> CWB = diet contained coarse wheat bran with particle size of 605  $\mu\text{m}$ .

<sup>3</sup> FWB = diet contained fine wheat bran with particle size of 438  $\mu\text{m}$ .

<sup>4</sup> Coarse wheat bran, with particle size of 605  $\mu\text{m}$ . Analyzed coarse wheat bran (as fed-basis): dry matter, 87.37%; ash, 5.02%; crude protein, 16.93%; neutral detergent fiber, 51.65%; acid detergent fiber, 13.23%; total dietary fiber, 43.77%; soluble dietary fiber, 4.01%; insoluble dietary fiber, 39.76%.

<sup>5</sup> Fine wheat bran, with particle size of 438  $\mu\text{m}$ . Analyzed fine wheat bran (as fed-basis): dry matter, 86.98%; ash, 4.55%; crude protein, 16.79%; neutral detergent fiber, 49.11%; acid detergent fiber, 13.01%; total dietary fiber, 43.92%; soluble dietary fiber, 5.98%; insoluble dietary fiber, 37.94%.

<sup>6</sup> The vitamin and mineral premix provided the following per kilogram of the diet: 12,000 IU vitamin A; 3,000 IU vitamin D<sub>3</sub>; 15 IU vitamin E; 1.8 mg vitamin K<sub>2</sub>; 1.0 mg thiamine; 3.0 mg riboflavin; 1.5 mg pyridoxine; 0.015 mg vitamin B<sub>12</sub>; 15 mg pantothenic acid; 30 mg nicotinic acid; 0.2 mg biotin; 1.5 mg folic acid; 100 mg Zn; 85 mg Fe; 12 mg Mn; 20 mg Cu; 1.2 mg I; 0.4 mg Se.

<sup>7</sup> ME = metabolizable energy.

<sup>8</sup> GE = gross energy.

### 2.4. Experimental design

This experiment was conducted at the Feng Ning Swine Research Unit of China Agricultural University (Chengdejiuyun Agricultural and Livestock Co., Ltd., Hebei, China). A 3 × 3 Latin square design with 3 test periods and 3 experimental diets was used.

### 2.5. Animals and experimental procedures

Six Yorkshire × Landrace non-pregnant sows (average initial BW = 250 kg; parity = 5 to 7) were fed a standard diet 1 wk before the experiment began. Sows were housed in a metabolism stall (1.80 m × 0.65 m) equipped with a feeder, nipple drinker and slatted floor. The average ambient temperature was maintained at 22 °C. After a week-long adaptation period, a T-cannula was installed in the distal ileum of each sow. Installation of the cannula and postoperative care of the sows was performed according to a previous study (Stein et al., 1998). During the 10-d recovery period, the sows were allowed *ad libitum* access to water and a standard diet. After the recovery period, the sows were assigned randomly to 1 of the 3 experimental diets, and each diet was fed to 2 sows during each of the 3 periods. Each experimental period included 12 d of acclimatization to their assigned test diet, followed by 2 d of ileal digesta collection (d 13 and d 14), 4 d of fecal sampling (from d 15 to 18), and 1 d of blood collection (d 19). The

feeding levels were equal among diets and set at 152 kcal/kg BW<sup>0.75</sup> per day based on the BW recorded on d 0 and 19 of each period. These feeding levels were equal to 1.5 times the metabolizable energy maintenance requirements of pregnant sows (NRC, 2012). Daily feed rations were offered in 2 equal meals at 08:00 and 17:00.

## 2.6. Sample collection

Feed samples were collected during each phase of the experiment and stored at  $-20^{\circ}\text{C}$  until analysis. On d 13, the digesta samples were collected every 15 min for 4 h by fastening a plastic bag on the cannula. Digesta samples were stored at  $-80^{\circ}\text{C}$  to determine microbial composition and SCFA concentrations. On d 14, 0.2% Cr<sub>2</sub>O<sub>3</sub> was added to the morning meal (08:00). Digesta samples were collected every 30 min (from 08:00 to 17:00), weighed and then stored at  $-20^{\circ}\text{C}$  for further analysis. Cr<sub>2</sub>O<sub>3</sub> was detected in the subsample of each digesta sample. The rest of the digesta samples were thawed, homogenized, subsampled and dried for further analysis. On d 15, fresh feces were collected and stored at  $-80^{\circ}\text{C}$  to analyze microbial communities and SCFA concentrations. On d 16 and d 17, the morning meal was supplemented with 1% ferric oxide, and we recorded the time ferric oxide first appeared in the feces. On d 18, 0.2% Cr<sub>2</sub>O<sub>3</sub> was added to the morning meal (08:00), and feces were collected during excretion at any time until 17:00. The collected feces were stored immediately at  $-20^{\circ}\text{C}$  to prevent microbial fermentation. At the end of each fecal collection period, all collected feces were thawed, homogenized, sub-sampled and dried for further analysis. Blood samples were collected from the anterior vena cava in tubes without anticoagulant 30 min before feeding, and 30, 60 and 90 min after feeding on d 19. All blood samples were centrifuged for 15 min at  $3,000 \times g$  at  $4^{\circ}\text{C}$ . Plasma samples were collected and stored at  $-20^{\circ}\text{C}$  for analysis of glucose, insulin, glucagon-like peptide-1 (GLP-1), ghrelin and peptide YY (PYY).

## 2.7. Chemical analysis

The viscosities of the diets and digesta were analyzed using a digital viscometer (Bangxi Instrument Technology Co., Ltd, Shanghai, China). Briefly, ileal digesta (30 g) were weighed into 50-mL tubes. The samples were centrifuged at  $4,000 \times g$  for 30 min at  $4^{\circ}\text{C}$ , 5 mL supernatant was added into the sample container, and CEP-40 rotator was selected to determine the viscosity. At  $37^{\circ}\text{C}$  and 60 rpm, the value was read after stabilizing for 30 s. The WHC of the diets and digesta was measured using a centrifugation approach (Owusu-Asiedu et al., 2006). Briefly, the diet (2 g) and ileal digesta (7 g) were weighed into 50-mL tubes. Then, 30 mL 0.9% NaCl containing 0.2% NaN<sub>3</sub> was added and the samples were placed in a shaking water bath at  $39^{\circ}\text{C}$  for overnight culture. The samples were centrifuged at  $4,000 \times g$  for 20 min at  $25^{\circ}\text{C}$ , the supernatant was removed, dried at  $103^{\circ}\text{C}$  for 20 h and weighed. The results were expressed as gram water retained per gram dry residue.

Diets, digesta and fecal samples were analyzed for dry matter (DM) (Method 934.01; AOAC, 2007), crude protein (CP) (Method 976.05; AOAC, 2007), ether extract (EE) (Thiex et al., 2003), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991). Wheat bran and diet samples were analyzed for total dietary fiber, SDF and IDF using the methods described by Prosky et al. (1998). Cr<sub>2</sub>O<sub>3</sub> content in digesta and fecal samples was detected using an atomic absorption spectrophotometer (Fan and Sauer, 2002). The gross energy of the diets, digesta and fecal samples was determined using an isoperibol calorimeter (Parr 6300 Calorimeter, Moline, IL, USA).

## 2.8. Analyses of glucose, insulin, GLP-1, ghrelin, and PYY levels

The plasma glucose concentrations at different time points were analyzed using an automatic biochemical analyzer (Thermo Fisher Scientific, Waltham, MA, US). Plasma concentrations of GLP-1 (KDEIA00105D, the minimum test dose: 0.125 mol/L), ghrelin (KDEIA00112D, the minimum test dose: 15 pg/mL) and PYY (KDEIA00093D, the minimum test dose: 5 pg/mL) were detected using an enzyme-linked immunosorbent assay (ELISA) kit (Beijing Kangjia Hongyuan Biotechnology Co., Ltd., Beijing, China). Plasma insulin concentration was quantified via radioimmunoassay using a GC-2010 immune counter (Anhui Zhongke Zhongjia Scientific Instrument Co., Ltd., Hefei, China).

## 2.9. Microbiota composition of digesta and feces

The microbiota of digesta and feces was assayed using 16 S rRNA high-throughput sequencing. Microbial genomic DNA was recovered from the digesta and fecal samples using a DNA extraction kit (Omega Bio-tek, Norcross, GA, US). Amplification of the V3–V4 hypervariable region of bacterial 16 S rRNA was achieved using primers F338 (5'-ACTCCTACGGGAGGCAGCAG-3') and R806 (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR reaction conditions were as follows: 27 cycles of pre-denaturation at  $95^{\circ}\text{C}$  for 3 min, denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $55^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 30 s. Amplification products were sequenced on an Illumina MiSeq platform (Majorbio Pharm Technology, Shanghai, China). Data were analyzed using the online platform Majorbio Cloud Platform (www.majorbio.com).

## 2.10. SCFA analyses

SCFA concentrations in digesta and feces were determined using the gas chromatography method (Shang et al., 2019). Briefly, 0.5 g of feces or 1.0 g of digesta were weighed into a tube (10 mL). Then, a 2-ethylbutyric acid (200  $\mu\text{L}$  of 1.0 mg/mL) solution and a mixed hydrochloric acid-formic acid solution (50:50, vol:vol) (5 mL) were added to the centrifuge tube and mixed well. The tube was vibrated ultrasonically for 5 min and then centrifuged at  $1,000 \times g$  at  $4^{\circ}\text{C}$  for 10 min. The supernatant was transferred into a centrifuge tube (1.5 mL) and centrifuged at  $14,000 \times g$  at  $4^{\circ}\text{C}$  for 10 min. The supernatant was filtered through a membrane and placed in a gas chromatograph for further analysis.

## 2.11. Calculations

Apparent total tract digestibility (ATTD) and apparent ileal digestibility (AID) values were calculated using the indirect marker method (Fan and Sauer, 2002). The equations used for digestibility calculation were the following:

$$\text{AID} = [1 - (\text{Cr}_{\text{diet}}/\text{Cr}_{\text{ileal digesta}}) \times (\text{N}_{\text{ileal digesta}}/\text{N}_{\text{diet}})] \times 100\%$$

$$\text{ATTD} = [1 - (\text{Cr}_{\text{diet}}/\text{Cr}_{\text{feces}}) \times (\text{N}_{\text{feces}}/\text{N}_{\text{diet}})] \times 100\%$$

where Cr represents the content of Cr<sub>2</sub>O<sub>3</sub> in diet, digesta and feces, and N represents the contents of nutrient in diet, digesta and feces.

The Cr<sub>2</sub>O<sub>3</sub> concentrations at each time point were calculated according to the linear relationship of first-order kinetics (Owusu-Asiedu et al., 2006):  $Y = a + bX$ , where Y is the Cr<sub>2</sub>O<sub>3</sub> concentration (g of Cr<sub>2</sub>O<sub>3</sub>/g of DM), and X is the time (h). The slope (b) of the line is the Cr<sub>2</sub>O<sub>3</sub> excretion rate, which describes the rate of digesta passage. The mean retention time was determined from the concentration of Cr<sub>2</sub>O<sub>3</sub> using the formula (Faichney, 1975): mean retention

**Table 2**  
Effects of finely ground wheat bran on feed and digesta characteristics of sows<sup>1</sup>.

Item	CON <sup>2</sup>	CWB <sup>3</sup>	FWB <sup>4</sup>	SEM <sup>5</sup>	P-value
<b>Feed</b>					
Water holding capacity, g/g	1.18 <sup>c</sup>	1.45 <sup>b</sup>	2.45 <sup>a</sup>	0.02	<0.001
Viscosity, mPa·s	1.13 <sup>b</sup>	1.38 <sup>b</sup>	1.66 <sup>a</sup>	0.10	0.01
<b>Ileal digesta</b>					
Quantity collected, g/kg of feed DM	953.60 <sup>b</sup>	1,065.00 <sup>b</sup>	1,860.60 <sup>a</sup>	170.40	0.02
Water holding capacity, g/g	2.47 <sup>b</sup>	2.98 <sup>b</sup>	3.68 <sup>a</sup>	0.24	0.02
Viscosity, mPa·s	1.38 <sup>b</sup>	1.48 <sup>b</sup>	2.15 <sup>a</sup>	0.08	<0.001
Passage rate, % of Cr <sub>2</sub> O <sub>3</sub> /h	1.38 <sup>a</sup>	1.32 <sup>a</sup>	1.05 <sup>b</sup>	0.03	<0.001
Mean retention time, h	4.43 <sup>b</sup>	4.40 <sup>b</sup>	4.88 <sup>a</sup>	0.05	<0.01
Total tract retention time, h	33.83 <sup>b</sup>	28.33 <sup>c</sup>	36.83 <sup>a</sup>	0.52	<0.001

a, b, c Different superscripts represents a significant difference,  $P < 0.05$ .

<sup>1</sup> Data are shown as mean  $\pm$  SEM ( $n = 6$ ).

<sup>2</sup> CON = a corn soybean meal diet.

<sup>3</sup> CWB = diet contained coarse wheat bran with particle size of 605  $\mu\text{m}$ .

<sup>4</sup> FWB = diet contained fine wheat bran with particle size of 438  $\mu\text{m}$ .

<sup>5</sup> SEM = standard error of the mean.

time =  $\sum C_i t_i / \sum C_i$ , where  $i$  stands for 1, 2, 3 ...,  $C_i$  is the concentration of Cr<sub>2</sub>O<sub>3</sub> at time  $t_i$ .

### 2.12. Statistical analyses

Data were analyzed using the general linear model (GLM) procedures in SAS (version 9.2; SAS Inst. Inc., Cary, NC, USA) with each sow as the experimental unit. Diets were treated as the fixed effect, while period and parity were considered as the random effect. The least squares procedure was employed to calculate mean values, and Tukey's test was used to calculate the differences among dietary treatments. Data are presented as mean  $\pm$  standard error of the mean (SEM). Figures were created using GraphPad Prism (version 5; GraphPad Software Inc., San Diego, CA, USA). The composition and diversity of the microbiota community expressed as standardized operational taxonomic unit (OTU) readings were analyzed using R software (version 3.3.1; R Software Inc., Auckland, New Zealand). The relative abundance of microbial species at different levels was analyzed using the Kruskal Wallis H test. Differences were considered to be statistically significant when  $P < 0.05$ , and tendencies were declared at  $0.05 \leq P < 0.10$ .

## 3. Results

### 3.1. Diets and digesta characteristics

Diets containing wheat bran (CWB and FWB) exhibited higher WHC than the CON group ( $P < 0.001$ ; Table 2). The FWB diet had a higher WHC ( $P < 0.001$ ), compared with the CWB diet. The FWB diet had a higher viscosity than the CON and CWB diets ( $P = 0.01$ ).

The quantity of digesta collected at the end of the ileum was greater in the FWB group than in the CON and CWB groups ( $P = 0.02$ ; Table 2). Similarly, digesta viscosity in FWB-fed sows was greater ( $P < 0.001$ ) than that in CON or CWB-fed sows. Finely ground wheat bran supplementation increased the WHC of digesta in the ileum ( $P = 0.02$ ) compared with the CWB. Supplementation of finely ground wheat bran to the diet decreased the digesta passage rate in the small intestine ( $P < 0.001$ ), corresponding with increased digesta retention time ( $P < 0.01$ ) compared to CON and CWB. There were no significant differences in digesta characteristics between the CON and CWB-fed sows. Dietary supplementation with finely ground wheat bran increased the total tract retention time in sows ( $P < 0.001$ ) compared with CON and CWB-fed sows. However, dietary incorporation of coarse wheat bran reduced the

total tract retention time in sows ( $P < 0.001$ ) compared with CON and FWB-fed sows.

### 3.2. DM feed intake and nutrient digestibility

CON-fed sows had lower levels of total DM intake and daily DM intake than CWB or FWB-fed sows ( $P < 0.001$ ; Table 3), however, there were no significant differences between the CWB and FWB groups. Dietary treatment had no effect on the AID of DM, CP, NDF and ADF ( $P > 0.05$ ; Table 3). AID of EE ( $P = 0.03$ ) and energy ( $P < 0.001$ ) was lower in the CWB- and FWB-fed sows than in the CON-fed sows. Finely ground wheat bran consumption resulted in a lower AID of EE and energy than CWB ( $P < 0.05$ ). Dietary treatment had no effect on the ATTD of DM, CP and EE ( $P > 0.05$ ). Dietary wheat bran (CWB and FWB) reduced the ATTD of energy compared with the CON diet ( $P < 0.05$ ). Control sows demonstrated the greatest ATTD of NDF ( $P = 0.02$ ) and ADF ( $P < 0.01$ ) compared to CWB or FWB sows, but there was no difference between CWB and FWB sows.

**Table 3**

Effects of finely ground wheat bran on DM feed intake and nutrient digestibility of sows<sup>1</sup>.

Item	CON <sup>2</sup>	CWB <sup>3</sup>	FWB <sup>4</sup>	SEM <sup>5</sup>	P-value
<b>DM feed intake, kg</b>					
Total DM feed intake	43.66 <sup>b</sup>	46.68 <sup>a</sup>	46.70 <sup>a</sup>	0.05	<0.001
Daily DM feed intake	2.30 <sup>b</sup>	2.46 <sup>a</sup>	2.46 <sup>a</sup>	0.003	<0.001
<b>Apparent ileal digestibility, %</b>					
DM	78.34	77.80	78.18	0.58	0.80
Energy	78.43 <sup>a</sup>	68.28 <sup>b</sup>	63.14 <sup>c</sup>	0.30	<0.001
CP	66.61	63.56	63.56	1.32	0.30
EE	74.34 <sup>a</sup>	69.17 <sup>b</sup>	64.76 <sup>c</sup>	1.77	0.03
NDF	41.69	46.50	50.05	4.85	0.50
ADF	62.05	56.48	66.04	2.90	0.11
<b>Apparent total tract digestibility, %</b>					
DM	85.33	85.61	85.38	0.74	0.96
Energy	89.21 <sup>a</sup>	84.95 <sup>b</sup>	84.51 <sup>b</sup>	0.78	<0.05
CP	86.61	86.75	86.32	0.90	0.94
EE	69.02	68.71	65.42	1.90	0.37
NDF	75.88 <sup>a</sup>	58.16 <sup>b</sup>	63.03 <sup>b</sup>	3.72	0.02
ADF	84.39 <sup>a</sup>	71.25 <sup>b</sup>	74.31 <sup>b</sup>	2.42	<0.01

ADF = acid detergent fiber; CF = crude fiber; CP = crude protein; DM = dry matter; EE = ether extract; NDF = neutral detergent fiber.

a, b, c Different superscripts represents a significant difference,  $P < 0.05$ .

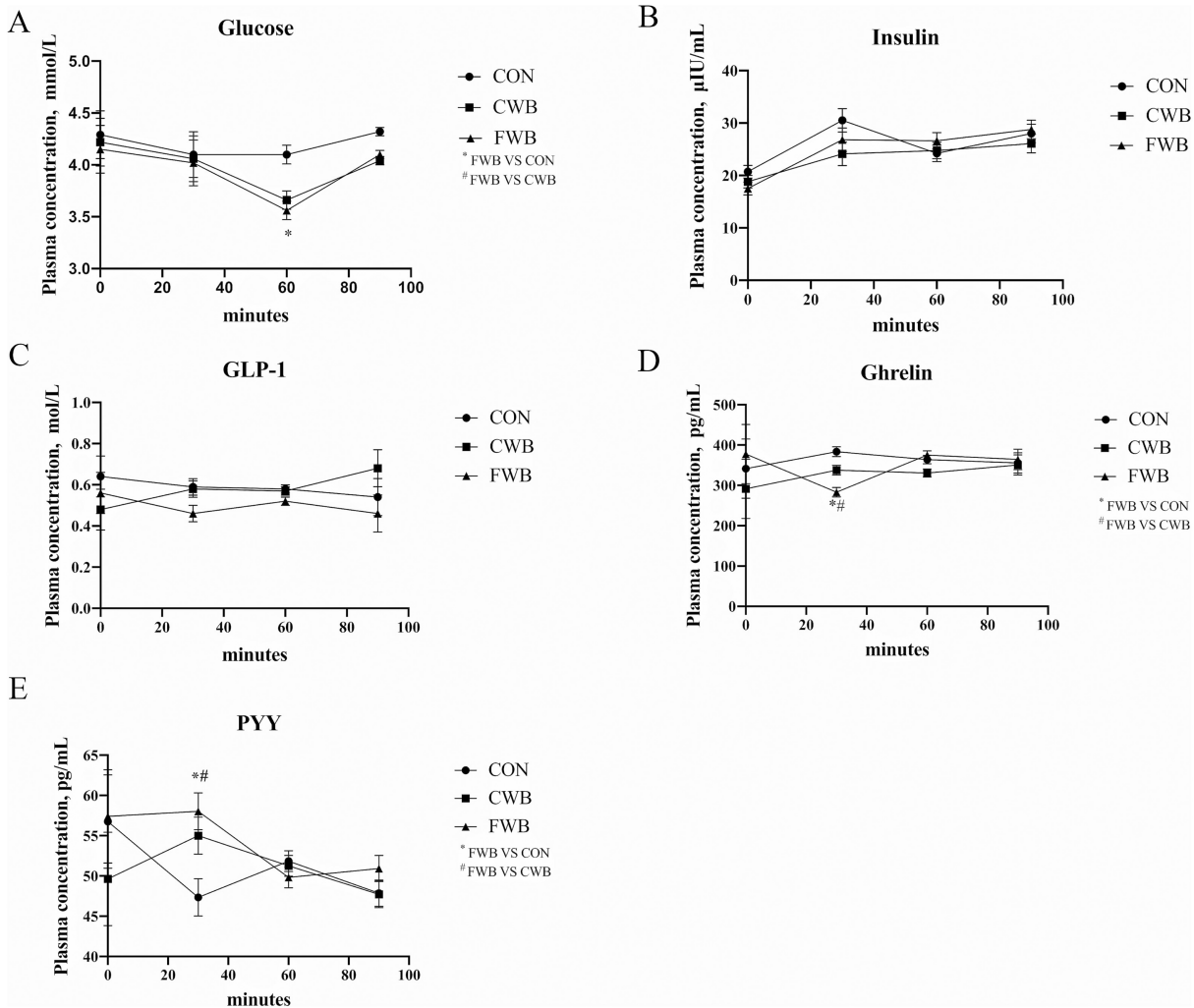
<sup>1</sup> Data are shown as mean  $\pm$  SEM ( $n = 6$ ).

<sup>2</sup> CON = a corn soybean meal diet.

<sup>3</sup> CWB = diet contained coarse wheat bran with particle size of 605  $\mu\text{m}$ .

<sup>4</sup> FWB = diet contained fine wheat bran with particle size of 438  $\mu\text{m}$ .

<sup>5</sup> SEM = standard error of the mean.



**Fig. 1.** Effects of finely ground wheat bran on plasma glucose, insulin, GLP-1, ghrelin and PYY at different times in sows. (A) Plasma glucose concentrations at different time points. (B) Plasma insulin concentrations at different time points. (C) Plasma GLP-1 concentrations at different time points. (D) Plasma ghrelin concentrations at different time points. (E) Plasma PYY concentrations at different times points. The x-axis represents minutes after a meal. \* Denotes a significant difference ( $P < 0.01$ ) from the CON group; # denotes a significant difference ( $P < 0.01$ ) from the CWB group;  $n = 6$ . CON = a corn soybean meal diet; CWB = diet contained coarse wheat bran with particle size of 605  $\mu\text{m}$ ; FWB = diet contained fine wheat bran with particle size of 438  $\mu\text{m}$ ; GLP-1 = glucagon-like peptide-1; PYY = peptide tyrosine.

### 3.3. Plasma glucose and hormones

The concentration of glucose in the plasma was not affected by dietary treatment before feeding, 30 min after feeding, or 90 min after feeding (Fig. 1A). However, 60 min after feeding, the concentration of plasma glucose was higher in CON sows than in CWB and FWB-fed sows ( $P < 0.01$ ; Fig. 1A). The plasma insulin concentration pattern was similar to that of the plasma glucose; however, the difference was not significant (Fig. 1B). Dietary treatment exhibited no effect on GLP-1 concentration (Fig. 1C). In FWB-fed sows, the concentration of ghrelin decreased ( $P < 0.01$ ; Fig. 1D) 30 min after feeding compared to CON- and CWB-sows; however, the concentration of PYY increased ( $P = 0.01$ ; Fig. 1E).

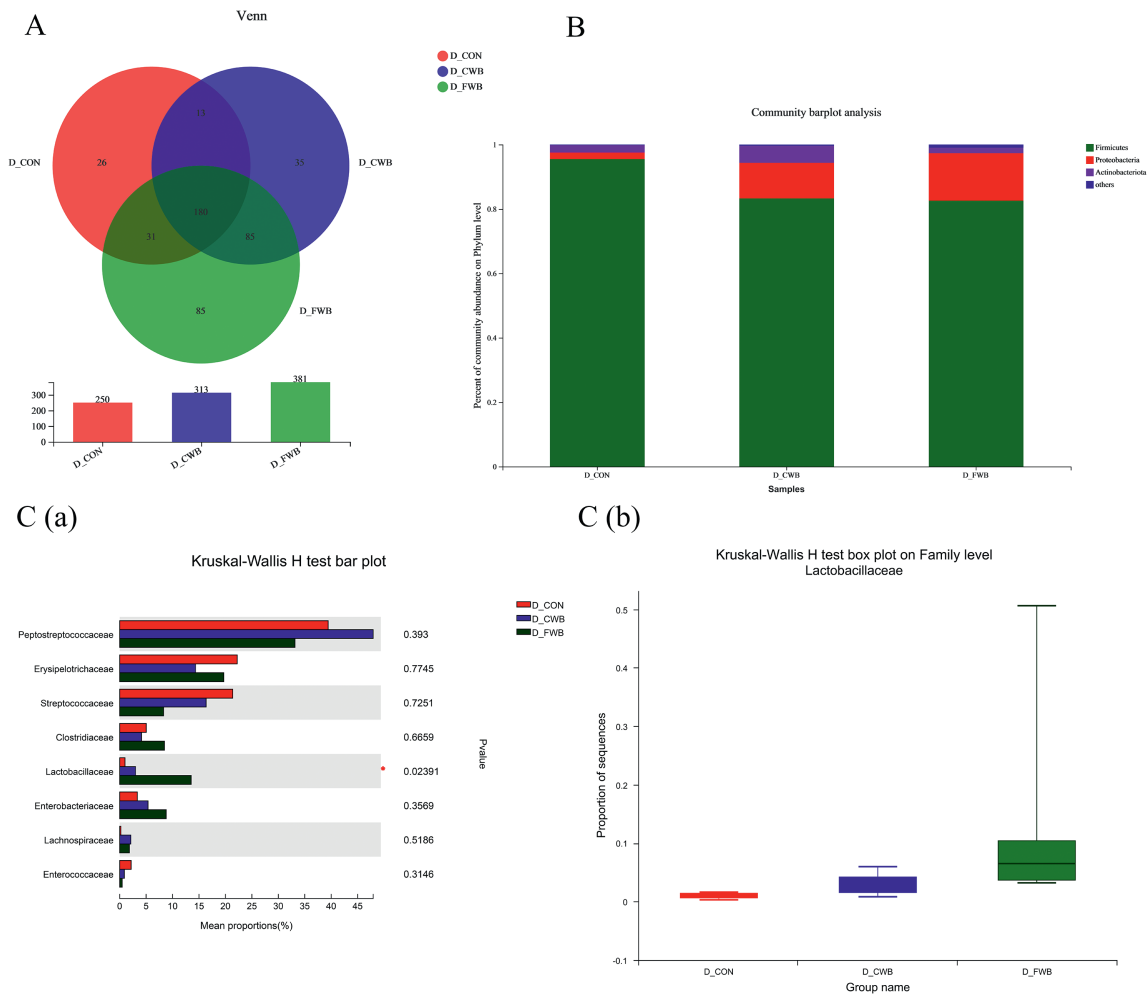
### 3.4. Ileal bacterial populations

Overall, 250, 313 and 381 OTUs were recorded in the CON, CWB and FWB groups, respectively, of which 180 OTUs were shared among the 3 groups (Fig. 2A). At the phylum level, Firmicutes and Proteobacteria were the most abundant, followed by

Actinobacteriota (Fig. 2B). At the family level, the relative abundance of Lactobacillaceae in the FWB group was higher than that in CON- or CWB-fed sows ( $P < 0.05$ ; Fig. 2C).

### 3.5. Fecal microbiota composition

Overall, 827, 837 and 872 OTUs were identified in the CON, CWB and FWB groups, respectively, of which 619 OTUs were shared among the 3 groups (Fig. 3A). At the phylum level, Firmicutes and Bacteroidetes were the dominant bacteria, followed by Spirochaetes (Fig. 3B). At the class level, the relative abundance of Clostridia was lower in the FWB group than in the CON group ( $P < 0.05$ ), but not in the CWB group (Fig. 3C). At the order level, the relative abundance of Lachnospirales decreased in the CWB- and FWB-fed sows ( $P < 0.05$ ; Fig. 3D). At the genus level, the relative abundance of *norank\_f\_p-2534-18B5\_gut\_group* increased in CWB- and FWB-fed sows ( $P < 0.05$ ) compared to CON-fed sows. However, compared with FWB-fed sows, the relative abundance of *norank\_f\_p-2534-18B5\_gut\_group* reduced in CWB-fed sows ( $P < 0.05$ ; Fig. 3E).



**Fig. 2.** Effects of finely ground wheat bran on ileal digesta microbiota composition of sows. (A) OTU Venn of 3 dietary treatments. (B) Percent of community abundance at the phylum level. The results were analyzed by Student's *t*-test and presented as mean values of different bacteria,  $n = 6$ . (C-a) The difference of microbiota at the family level; the results analyzed by Kruskal–Wallis H test,  $n = 6$ ; the y-axis represents the microbiota name at a certain classification level; the x-axis represents the average relative abundance in different groups of species; and the columns with different colors represent different groups. The rightmost is the *P*-value,  $*P \leq 0.05$ . (C-b) The relative abundance of Lactobacillaceae in different groups, the x-axis represents different samples, columns of different colors represent different groups, and the y-axis represents the abundance proportion of a microbiota in different samples. OTU = operational taxonomic unit.

### 3.6. SCFA concentration in ileal digesta and feces

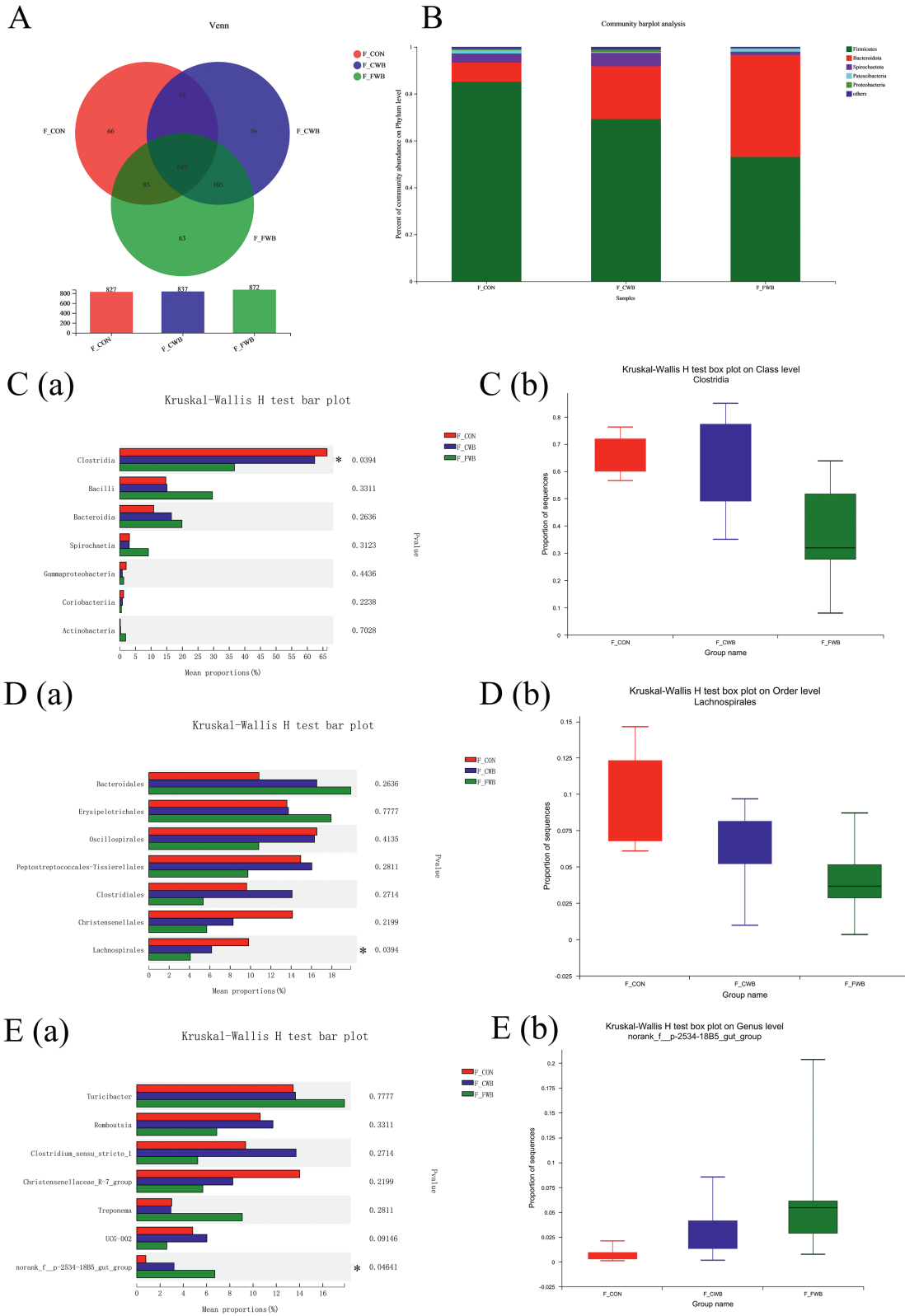
Dietary treatment had no effect on the acetate, propionate and butyrate contents in the ileal digesta (Fig. 4A). Acetate and propionate concentrations in feces were not affected by the dietary treatment (Fig. 4B). The concentration of butyrate in feces was higher in FWB-fed sows than in CON or CWB sows ( $P = 0.04$ ); however, there was no difference between CON and CWB sows.

## 4. Discussion

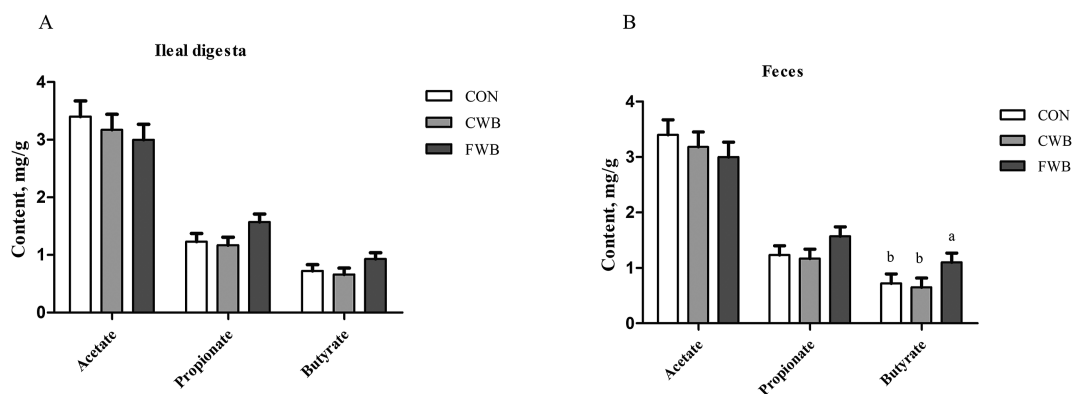
The structure and physicochemical characteristics of DF, such as WHC and viscosity, influence digestion and metabolism of feed-stuffs in the digestive tract (Owusu-Asiedu et al., 2006). In the current study, a simple T-cannula was used to investigate the effects of finely ground wheat bran on the digestive physiology, gut hormones, and gut microbiota in sows.

Finely ground wheat bran increased digesta viscosity, slowed digesta passage rate and increased total tract retention time compared to corn soybean meal and CWB diets. Hydration of DF will enhance viscosity, but this depends upon the chemical

structure of DF and other cell wall compounds connected to DF (Wenk, 2001). In general, the viscosity of SDF is greater than IDF (Brockman, 2014). A small quantity of SDF, such as guar gum or  $\beta$ -glucan, has very high viscosity when hydrated in the digestive tract (Dikeman et al., 2006). However, wheat bran contains high IDF, which has low viscosity even when hydrated in the digestive tract (Gallaher et al., 2000). In our previous study, finely ground wheat bran exhibited higher SDF and  $\beta$ -glucan content than coarse wheat bran (Wang et al., 2022). In the current study, dietary supplementation with finely ground wheat bran increased the viscosity of the feed. This demonstrated that enhanced SDF concentrations increased the feed viscosity, resulting in enhanced digesta viscosity. Sugar beet pulp is rich in SDF and can reduce the passage rate of digesta in the small intestine due to higher digesta viscosity (Knudsen and Hansen, 1991). Finely ground wheat bran may act in a manner similar to sugar beet pulp in decreasing the passage rate of digesta. Cereal bran is rich in IDF and can increase digesta passage rate throughout the entire gastrointestinal tract owing to the decrease in digesta viscosity (Jenkins et al., 2000). Conversely, the increased viscosity of finely ground wheat bran led to the increase in total tract retention time. Thus, dietary supplementation with



**Fig. 3.** Effects of finely ground wheat bran on fecal microbiota composition of sows. (A) OTU Venn of 3 dietary treatments. (B) Percent of community abundance at the phylum level, the results were analyzed by Student's t-test and presented as mean values of different bacteria,  $n = 6$ . (C-a) The difference of microbiota at the class level. (C-b) The relative abundance of Clostridia in different groups. (D-a) The difference of microbiota at the order level. (D-b) The relative abundance of Lachnospirales in different groups. (E-a) The difference of microbiota at the genus level. (E-b) The relative abundance of *norank\_f\_p-2534-18B5\_gut\_group* in different groups. The results of (C-a), (D-a), and (E-a) analyzed by Kruskal–Wallis H test,  $n = 6$ ; the y-axis represents the microbiota name at a certain classification level; the x-axis represents the average relative abundance in different groups of species; and the columns with different colors represent different groups. The rightmost is the  $P$ -value,  $*P \leq 0.05$ . (C-b), (D-b) and (E-b) The x-axis represents different samples; columns of different colors represent different groups; and the y-axis represents the abundance proportion of a microbiota in different samples. OTU = operational taxonomic unit.



**Fig. 4.** Effects of finely ground wheat bran on ileal digesta and fecal short-chain fatty acids of sows. (A) Acetate, propionate, and butyrate concentrations in ileal digesta. (B) Acetate, propionate and butyrate concentrations in feces. Values are presented as mean  $\pm$  SEM ( $n = 6$ ). <sup>a, b</sup> Means without common letters differ at  $P < 0.05$ . CON = a corn soymeal diet; CWB = diet contained coarse wheat bran with particle size of 605  $\mu\text{m}$ ; FWB = diet contained fine wheat bran with particle size of 438  $\mu\text{m}$ ; SEM = standard error of the mean.

finely ground wheat bran may have important effects on the apparent digestibility of nutrients in the small intestine and degradation of nutrients in the large intestine. Physicochemical properties of DF affect digesta quantity in terminal ileum. The increased quantity of digesta in the distal ileum may be due to the increase in WHC and viscosity caused by SDF (Smulikowska et al., 2002). In the present study, finely ground wheat bran increased the quantity of digesta in the distal ileum. The diet containing finely ground wheat bran increased WHC and viscosity, suggesting that fine wheat bran plays a crucial role in increasing digesta quantity.

Dietary supplementation with coarse wheat bran and fine wheat bran decreased the AID and ATTD of energy in the current study. Dietary fiber itself has a low energy concentration, which reduces the energy density of diets and daily energy intake of animals (Slavin, 2005), thereby reducing the AID and ATTD of energy compared to the control diet. The inclusion of 20% coarse wheat bran and 20% fine wheat bran in the diet decreased the ATTD of NDF and ADF. Coarse wheat bran increased the total tract passage rate. An increase in total tract passage rate reduces the effective time for digestion and absorption of nutrients (Hooda et al., 2001) in the gastrointestinal tract, resulting in reduced digestibility. Hydration of DF increases the viscosity of digesta, hinders contact between digesta and digestive enzymes, and therefore decreases the digestibility of nutrients (Murray et al., 1999). In the current study, increased digesta viscosity caused by finely ground wheat bran likely hindered the contact between digesta and digestive enzymes, which reduced the digestibility of NDF, ADF and energy. The interaction between digesta viscosity and passage rate of digesta is suggested to influence the digestion characteristics of diets.

Dietary fiber can modify some gut hormones such as ghrelin, GLP-1 and PYY, which regulate satiety and energy intake (Sánchez et al., 2012). Ghrelin is mainly produced in the gastric mucosa and regulates satiety (Tarini and Wolever, 2010). Fermentable fiber polysaccharides are rapidly and widely fermented in the proximal colon and may regulate ghrelin levels (Tarini and Wolever, 2010). GLP-1 and PYY are hormones secreted by hindgut L cells that signal satiety after food intake and participate in short-term regulation of food intake (Silva and Bloom, 2012). Fermentable DF increased the secretion of PYY and GLP-1 in a rodent model (Chaudhri et al., 2006). A diet containing 3% barley  $\beta$ -glucan reduced the concentration of ghrelin and increased the concentration of PYY in the plasma, which resulted in increased satiety after eating (Vitaglione et al., 2009). In the current study, dietary supplementation with fine wheat bran decreased plasma ghrelin concentration and enhanced plasma PYY concentration 30 min after feeding, indicating that fine wheat bran elicited enhanced satiety after feeding

compared with coarse wheat bran. These changes in gut hormone levels may be attributed to the following aspects: 1) Increasing the fiber content of a diet increases the amount of digesta in the intestinal tract, influencing the secretion of gut hormones; 2) finely ground wheat bran reduces the passage rate of digesta and increases the retention time of digesta in the gastrointestinal tract, indirectly promoting the sense of satiety and reducing the secretion of ghrelin; and 3) the concentration of butyrate is involved in increasing the secretion of PYY and decreasing the secretion of ghrelin in the intestine (Klosterbuer et al., 2012). Finely ground wheat bran consumption increased fecal butyrate content in the current study, which may be the primary reason for the changes in ghrelin and PYY concentrations.

In the present study, fine wheat bran increased the relative abundance of Lactobacillaceae in the ileal digesta of the sows. The relative abundance of Clostridia in the feces of the sows declined when they consumed FWB. Bacteria prefer carbohydrates with specific chemical structures as fermentation substrates (Owusu-Asiedu et al., 2006). Thus, feed components which differ in the types of DF and speed of digestion (Serena et al., 2008) could facilitate the growth of specific microbiota. A study on humans suggested that wheat bran extract, a food-grade soluble fiber preparation, had prebiotic activity and could increase the abundance of *Lactobacillus* and *Bifidobacterium* (François et al., 2012). The stimulation of *Bifidobacterium* species after the intake of FWB demonstrates that the high SDF of finely-ground wheat bran has prebiotic effects that coarse wheat bran with a higher IDF content does not possess. Clostridia are generally regarded as pathogenic bacteria (Yang et al., 2019), and decreased relative abundance of Clostridia can attenuate inflammation of the gut in pigs (Shang et al., 2019). Dietary supplementation with finely-ground wheat bran reduced the relative abundance of Clostridia, suggesting that finely-ground wheat bran could prevent intestinal inflammation to some extent.

The enhanced relative abundance of Lactobacillaceae caused by finely-ground wheat bran may be due to the combined effects of increased digesta viscosity and decreased nutrient digestibility. Increased digesta viscosity elicited by SDF supplementation leads to changes in the physiology and ecosystem of the gut (Bedford and Classen, 1992), thereby hindering the contact between digesta and digestive enzymes and reducing the effective absorption of nutrients. Decreased energy digestibility increases the undigested energy and amount of digesta in the distal ileum, which serves as a substrate for the microbiome, thus providing a stable environment for microbes to proliferate. Alternatively, a slower digesta passage rate with low oxygen tension ensures a relatively stable

environment for colonization and proliferation of microbiota in the small intestine (De Boever et al., 2000). The decreased relative abundance of Clostridia in feces caused by finely-ground wheat bran may be due to the elevated content of SDF and  $\beta$ -glucan, which are not preferred substrates for fermentation by Clostridia (Owusu-Asiedu et al., 2006).

SCFA are the final products of the anaerobic fermentation of DF by the gut microbiome. These end products have multiple beneficial effects on mammalian energy metabolism (Besten et al., 2013). Butyrate can provide energy to colonic epithelial cells, promote the proliferation of probiotics, reduce inflammation and maintain intestinal function, all of which have a positive impact on gut health (Cornick et al., 2015; Makki et al., 2018). In the present study, dietary supplementation with finely-ground wheat bran improved butyrate concentration and finely-ground wheat bran positively affected gut health. The degree and speed of DF degradation by gut microorganisms is related to water solubility, chemical structure, particle size and other factors of the fiber (Anguita et al., 2006). Structural characteristics, such as type, quantity and bonding mode of monosaccharides in polysaccharide molecules, largely determine the fermentation of fibers in the large intestine (Henningsson et al., 2001). The smallest size fraction of corn bran is the most extensively fermented fraction and produces the highest levels of butyrate (Thakkar et al., 2020). The increased concentration of butyrate in FWB-fed sows may be due to the decrease in particle size of wheat bran, increase in specific surface area, and enzymes secreted by microorganisms which degrade the fiber content of the wheat bran more adequately. In addition, finely-ground wheat bran prolonged the total tract retention time, which enables microorganisms to fully interact with the fiber. Finally, the quantity of digesta in the terminal ileum increased in FWB-fed sows, suggesting that it provides a sufficient amount of substrate for the microorganisms residing in the large intestine.

## 5. Conclusions

Dietary supplementation with finely-ground wheat bran decreased digesta passage rate, increased the abundance of beneficial microorganisms and enhanced the concentrations of SCFA and PYY in sows. Therefore, dietary supplementation with fine wheat bran exhibited a better effect on improving satiety and intestinal microbial composition of sows.

## Author contributions

**Zijie Wang** conducted the animal experiment and prepared the first manuscript draft. **Wenhui Wang** and **Song Xu** assisted in collection of samples. **Xiangfang Zeng** and **Jian Ding** assisted in preparing the manuscript. **Hu Liu** assisted in preparing the manuscript and data curation. **Fenglai Wang** contributed to designing the studies and providing financial support. All authors have read and approved the final manuscript.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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