Oat bran and wheat bran impact net energy by shaping microbial communities and fermentation products in pigs fed diets with or without xylanase

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Abstract

Background: Dietary fiber can be fermented in gut of pigs and the end products of fermentation were short-chain fatty acids (SCFA). The SCFA had positive effects on gut bacteria and host immune system. In addition, SCFA can provide a part of available energy for pigs. However, there were limited reports on the relationship between dietary fiber, gut bacteria, and energy metabolism. Therefore, this study investigated how dietary fiber and enzyme addition impacted energy metabolism by acting on the microbial community and SCFA.

Methods: Wheat bran (WB) was added to the corn-soybean meal-based diet at the levels of 12% and 27%, and oat bran (OB) at 15% and 36%. One of each diet was supplemented with or without 5000 U/kg feed of xylanase, so a total of 10 diets were allotted to 60 growing pigs (initial body weight: 27.2 ± 1.2 kg) using a randomized complete block design. The experiment was conducted in 10 consecutive periods using 6 similar open-circuit respiration chambers. Each pig was used for one 20-day period. During each period, six pigs were allowed 14 d to adapt to the diets in metabolic cages followed by 6 d (from d 15 to d 20) in respiration chambers to measure heat production (HP).

Results: Pigs fed 36% OB diets had greater (P < 0.05) nutrient digestibility and net energy (NE) values compared to those fed 27% WB diets. Apparent digestibility coefficients of dry matter (DM) and crude protein (CP) were lower (P < 0.05) in pigs fed 27% WB diets compared with those fed 12% WB diets. Enzyme addition improved (P < 0.05) the NE values (11.37 vs. 12.43 MJ/kg DM) in diets with 27% WB. Supplementation of xylanase did not affect NE values for basal diets, OB diets and 12%WB diets. Compared with diets with 36% OB, pigs fed 27% WB-based diets excreted more total SCFA, acetate and propionate (expressed as g/kg feed DM) in fecal samples of pigs (P < 0.05). Pigs in the WB diets had greater proportion of phylum Bacteroidetes while phylum Firmicutes were greater in pigs fed OB diets (P < 0.05). Pigs fed WB diets had greater (P < 0.05) abundance of Succinivibrio and Prevotella, which were associated with fiber degradation and SCFA production.

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Background
Dietary fiber (DF) means carbohydrate polymers with ten or more monomeric units which are not hydrolyzed by the endogenous enzymes in the small intestine of humans [1]. The microbial conversions of DF to monosaccharides in the gut involves a number of principal reactions mediated by the carbohydrate-active enzymes. These enzymes are from specific members of the gut microbiota and have the function to cleave the glycosidic bonds between sugar monomers or between carbohydrate and non-carbohydrate structures. Major end products from these fermentations are the short-chain fatty acids (SCFA) [2], mainly acetate, propionate, and butyrate. Recent studies show that SCFA from microbial fermentation generates 11% to 24% of the total digestible energy (DE) as wheat bran (WB) level increases from 10% to 30% when fed to pigs [3]. It suggests that DF has a final impact on energy metabolism by shaping microbes and regulating SCFA production [4]. However, there is little information on the relationship between DF, gut bacteria, SCFA, and energy metabolism [5].

Oat bran (OB) and WB are rich in DF that has negative effects on nutrient digestibility due to their anti-nutritive properties, but they may act as substances and energy sources to stimulate the intestinal microbiota [6]. Dietary fiber in OB is easier fermentable energy source for bacteria in the large intestine due to their lower proportion of lignified fiber types and higher proportion of soluble fiber types compared with fiber in WB. Specially, OB is rich in mixed linkages β-glucan which to a high degree is soluble and insoluble arabinoxylan [7]. By contrast, WB contained rich insoluble arabinoxylan. In the small intestine, β-glucan in OB is partly digested while arabinoxylan in OB or WB is hardly digested [7, 8]. Accordingly, arabinoxylan is considered as one of the main components for DF in OB and WB to resist fermentation by bacteria in gastrointestinal tract. By adding xylanase to feeds, xylose backbone of arabinoxylan can be cleaved, thereby improving accessibility and utilization of nutrients in WB or OB diets and providing some fermentable oligosaccharides degraded from indigestible polysaccharides to intestinal bacteria [9]. Substrates available to intestinal bacteria and physiology of lumen change along gastrointestinal tract, and consequently intestinal bacteria are altered. Therefore, DF and exogenous enzymes, independently or in combination interaction are important means of regulating intestinal bacteria and ultimately affecting energy metabolism [4–6].

Therefore, we hypothesize that the diets supplemented with different fiber sources, with or without xylanase, could exert different impacts on nutrient digestibility and NE values of diets, and these differences were related to the activity of certain bacteria. Specially, our objectives are to determine effects of dietary characteristics and xylanase addition on nutrient digestibility, NE values of diets, fecal SCFA concentrations, and fecal bacterial communities of growing pigs.

Methods
Experimental diets and enzyme
As shown in Table 1, WB diets contained 12% or 27% WB which was added at the expense of corn and soybean meal. Oat bran diets contained 15% or 36% OB which was added at the expense of corn and soybean meal. Enzyme was added to one half of each diet at the rate of 40 g/1000 kg diet to supply enzyme activity of 5000 U/kg complete feed (as-fed basis; Table 1). The enzyme was first mixed in the vitamin-mineral premix and then mixed with other ingredients. A total of 10 diets were fed to 60 growing pigs with a randomized complete block design. Diets were formulated on NE basis to meet estimated AA requirements for growing pigs [10]. Diets were formulated based on total dietary fiber (TDF) content in OB and WB. Specially, DF content (calculated values) between 36% OB diets and 27% WB diets or between 15% OB diets and 12% WB diets was similar. The xylanase used in the current study was endo-1, 4-β-xylanase in granular form. The xylanase was provided by Bestzyme Co. Ltd. (Jinan, China) with enzyme activity of 200,000 U/g.

Animals and experimental procedure
The animal procedures used in this experiment, including animal care and use, were approved by the Animal Care and Use Ethics Committee of China Agricultural University (Beijing, China). Sixty growing barrows (initial BW of 27.2 ± 1.2 kg) were allotted to 10 diets with 6 replicate pigs per diet in a randomized block design. The

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experiment was conducted in 10 consecutive periods using 6 similar open-circuit respiration chambers as described by Lyu et al. [11]. Each pig was used for one 20-d period. During each period, 6 pigs were allowed 14 d to adapt to diets in metabolic cages followed by 6 d in respiration chambers to measure heat production (HP). Pigs were fed their assigned diets at 2.3 MJ ME/(kg BW0.6·d) on basis of BW measured on d 0, 7, and 14 [12]. Pigs were fed equal-sized dried meals twice daily at 08:30 and 15:30 h in mash form and had free access to water via a low-pressure nipple drinker. Urine was collected in buckets containing 50 mL of 6 mol/L HCl. From d 15 to 19 of each period, total feces and urine were collected from the chambers once daily at 8:30 h and then stored at −20 °C. On d 19 (from 7:30 to 9:00 h) of each period, six freshly voided fecal samples from each dietary treatment were collected to measure SCFA concentrations and bacterial community. Fecal samples were acquired using a 5-mL centrifuge tube, put in liquid nitrogen and then were stored at −80 °C. Diet samples were collected in each collection period and then stored at −20 °C. At the end of experiment, diet samples were mixed and subsampled based on treatments to measure xylanase activity. From d 19 to 20 of each period, pigs were fasted.

The design of 6 open-circuit respiration chambers were reported by van Milgen et al. [13] and Lyu et al. [11]. Volume of each chamber was approximately 7.8 m³, and relative humidity in chamber was controlled at 70%. During the feeding period, the temperature was 22 °C, and it was increased to 24 °C on the fasted day. The measurement range of analyzers was 19.5% to 21% for O₂, 0 to 1% for CO₂, 0 to 0.1% for CH₄, and 0 to 0.1% for NH₃ with a sensitivity of 0.2% within the measurement range.

### Sample preparation and chemical analyses

Diets were collected during each feeding period to measure chemical composition. Fecal samples were oven-dried for 72 h at 65 °C and were ground through a 1-mm screen for further chemical analysis. Urine samples were pooled separately within pig.

Xylanase activity in diets was analyzed using spectrophotometric method as described by Lærke et al. [14]. The chemical analyses of ingredients and diets included dry matter (DM), crude protein, ether extract, ash, Ca, P, gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) in ingredients and diets. Non-starch.

### Table 1 Ingredients of experimental diets (as-fed basis)

| Item         | Basal diet | Test diets |
|--------------|------------|------------|
|              |            | 30% OB     | 27% WB | 15% OB | 12% WB |
| ingredients, % |            |            |        |        |        |
| Corn         | 71.60      | 45.12      | 51.74  | 60.57  | 62.77  |
| Soybean meal | 24.96      | 15.73      | 18.04  | 21.11  | 21.88  |
| Oat bran     | –          | 36.00      | –      | 15.00  | –      |
| Wheat bran   | –          | –          | 27.00  | –      | 12.00  |
| Dicalcium phosphate | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 |
| Limestone    | 0.90       | 0.90       | 0.90   | 0.90   | 0.90   |
| Salt         | 0.35       | 0.35       | 0.35   | 0.35   | 0.35   |
| Premix²      | 0.50       | 0.50       | 0.50   | 0.50   | 0.50   |
| Lys HCl      | 0.50       | 0.32       | 0.36   | 0.42   | 0.44   |
| Met          | 0.07       | 0.04       | 0.05   | 0.06   | 0.06   |
| Thr          | 0.16       | 0.10       | 0.12   | 0.14   | 0.14   |
| Val          | 0.06       | 0.04       | 0.04   | 0.05   | 0.05   |

1 OB Oat bran, WB Wheat bran. The xylanase was provided by Bestzyme Co. Ltd. (Jinan, China). Enzyme was added to one half of each diet at the rate of 40 g/1000 kg diet to supply enzyme activity of 5000 U/kg complete feed (as-fed basis).

2 Vitamin-mineral premix supplied the following per kg of complete diet: vitamin A as retinyl acetate, 5512 IU; vitamin D₃ as cholecalciferol, 2200 IU; vitamin E as DL-alpha-tocopheryl acetate, 30 IU; vitamin K₃ as menadione nicotinamide bisulfite, 2.2 mg; vitamin B₁₂, 27.6 μg; riboflavin, 4 mg; pantothenic acid as DL-calcium pantothenate, 14 mg; niacin, 30 mg; choline chloride, 400 mg; folacin, 0.7 mg; thiamin as thiamine mononitrate, 1.5 mg; pyridoxine as pyridoxine hydrochloride, 3 mg; biotin, 44 μg; Mn as MnO, 40 mg; Fe as FeSO₄·H₂O, 75 mg; Zn as ZnO, 75 mg; Cu as CuSO₄·5H₂O, 25 mg; I as KI, 0.3 mg; Se as Na₂SeO₃, 0.3 mg.
polysaccharides (NSP) and their constituent sugars in OB and WB were determined based on alditol acetates by gas-liquid chromatography (Agilent GC 6890, USA) with a flow of 20 mL/min and split 40:1. The column temperature was 220°C and the injector and detector temperature were 250°C.

Fecal SCFA concentrations were measured using the method reported by Liu et al. [19]. About 0.5 g of fecal sample was placed in a centrifuge tube with 8 mL of distilled water. The mixture was centrifuged at 13,000 r/min for 10 min to obtain the supernatant. One milliliter of supernatant was diluted 1:50 with water and filtered through a 0.22-mm nylon membrane filter. Filtered supernatant was analyzed using high-performance ion exchange chromatograph (ICS 3000 Dionex, USA).

Analysis for bacterial microbiota by 16S RNA sequences
Bacterial DNA was extracted from fecal samples using the DNA Kit (Omega Bio-tek, Norcross, GA, USA). NanoDrop 2000 UV-VIS spectrophotometer (Thermo Scientific, Wilmington, USA) was used to determine DNA concentration and purification. Quality of DNA was assessed by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′-ACTCCTACGG GAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGT TWCTTAAAT-3′) by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR products were extracted from a 2% agarose gel and then purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA).

Pooled and purified amplicons in equimolar and paired-end were sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols introduced by Majorbio BioPharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH. Operational taxonomic units (OTU) were clustered with 0.97 similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/). The taxonomy of each 16S rRNA gene sequence was analyzed by Ribosomal Database Project (RDP) Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 0.70.

Calculations
The apparent total tract digestibility (ATTD) of nutrients, total heat production (THP), retained energy (RE), and NE values in diets were calculated using the following equations [12, 20]: ATTD = [(Fi − Ff)/Fi] × 100%, where Fi was total intake of energy (kJ) or nutrients (g), and Ff was the total fecal output of energy (kJ) or nutrients (g); THP = 16.18 × O₂ + 5.02 × CO₂ + 2.17 × CH₄ – 5.99 × urinary nitrogen excretion, where THP was in kilojoule, O₂ was oxygen consumption in liters, CO₂ was carbon dioxide production in liters, and CH₄ was methane production in liters. Urinary nitrogen excretion was in grams. The data collection for O₂, CO₂, and CH₄ used the standard procedure as described by Lyu et al. [11]. In addition, to eliminate the effects of activity of pigs, FHP was calculated using the equation for THP with gas data obtained from 22:30 h (d 19) to 06:30 h (d 20). To calculate the FHP throughout the day, the 8-h FHP was extrapolated to a 24-h period; RE = (ME intake – THP), where RE was in kilojoule, and ME intake and THP were in kilojoule per day. The retained energy as protein (REp) was calculated based a recognized formula (nitrogen × 6.25 × 23.84, kJ/g), whereas RE as lipids (REL) was calculated as the difference between RE and REp [21]: NE = (RE + FHP)/DM intake, where NE was in kilojoule per kilogram DM; RE and FHP were in kilojoule per day and DM intake was in kilograms per day. The DE of diets was calculated by subtracting energy lost in the feces from GE. The ME of diets was calculated by subtracting energy lost in the urine and methane from DE. Energy lost as methane (CH₄E) was calculated assuming 1 L of CH₄ contained 39.54 kJ energy [20]. The DE, metabolizable energy (ME) and NE content of OB and WB in diets containing 36% OB and 27% WB were calculated using the difference method, respectively [22].

Statistical analysis
All data of the experiment were averaged per pig and were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The experimental unit was the individual pig. Diet, enzyme and their interactions were treated as the fixed effects and collection, period and chamber as random effects. The LSMEANS statement with Tukey’s adjustment was used to separate mean values. To eliminate the effect of ME intake, the THP and RE data were adjusted for each collection period by covariance analysis for ME intake of 2322 kJ/(kg BW⁰.⁶⁰·d) (mean value for the experiment). A mixture of two fecal samples was used to analyze bacterial communities. Bacterial diversity was analyzed using standardized OTU reads with the help of R software. Relative abundance of bacteria at phylum and family levels were showed as bar plots. Heatmap was used to analyze bacterial communities at genus level. The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was adopted to classify abundances of differential bacteria from phylum to genus. The logarithmic LDA value of 2.0 was used to be the criterion. The comparative analysis between OB-based diets (n = 12) and WB-based (n = 12) diets or between high-fiber diets (36% OB diets
Results

Chemical composition of ingredients and diets

Wheat bran contained more content of NDF, ADF, hemicellulose, and IDF but lower content of SDF than OB (Supplementary Table S1). For NSP composition, OB contained more soluble NSP (7.8% vs. 3.9%), especially glucose. By contrast, WB contained more insoluble NSP (28.7% vs. 15.6%), mainly arabinose and xylose. In addition, OB contained more content of CP (22.0% vs. 15.1%), starch (28.1% vs. 8.2%) and EE (7.6% vs. 3.2%) than WB. Dietary fiber content was similar between 36% OB diets and 27% WB diets or between 15% OB diets and 12% WB diets, which was consistent to the study design (Table 1 and Table 2).

Nutrient digestibility, nitrogen balance, and energy metabolism

There were interactions \((P < 0.05)\) between diet and enzyme supplementation on ATTD of DM, GE, CP and ADF (Table 3). In the enzyme-free treatments, 27% WB diets had lower \((P < 0.05)\) ATTD of DM, GE, CP, ADF and OM than OB diets and basal diets. Pigs fed 27% WB diets had lower \((P < 0.05)\) ATTD of DM, CP and ADF than those fed 12% WB diets. By contrast, there were no differences on the ATTD of nutrients between 36% OB diets and 15% OB diets. The nitrogen output from feces was greater \((P < 0.05)\) in 27% WB diets than that in basal diets and 15% OB diets. Total nitrogen retention was not affected by dietary characteristics. Supplementation of enzyme improved \((P < 0.05)\) ATTD of DM, CP and ADF in 27% WB diets, and no differences were observed for the enzyme effects in other dietary treatments.

No interactions between diet and enzyme on the energy retention were found (Supplementary Table S2). The THP, FHP, and RE data were not affected by dietary characteristics. Enzyme supplementation improved \((P < 0.05)\) ATTD of DM, CP and ADF in 27% WB diets, and no differences were observed for the enzyme effects in other dietary treatments.

No interactions between diet and enzyme on the energy retention were found (Supplementary Table S2). The THP, FHP, and RE data were not affected by dietary characteristics. Enzyme supplementation improved \((P < 0.05)\) ATTD of DM, CP and ADF in 27% WB diets, and no differences were observed for the enzyme effects in other dietary treatments.

The total SCFA were considered as the sum of lactate, formate, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate, but the lactate and formate were not listed because of their minor concentrations (Table 6). The concentrations of SCFA were expressed in mg/kg feed DM. There were interactions \((P < 0.05)\) between diet and enzyme supplementation on concentrations of

### Table 2 Chemical composition of experimental diets (DM basis)

| Item          | Basal diet | 36% OB | 27% WB | 15% OB | 12% WB |
|---------------|------------|--------|--------|--------|--------|
| GE, MJ/kg DM  | 18.21      | 18.37  | 19.00  | 19.10  | 18.62  |
| CP, % DM      | 19.5       | 19.7   | 21.1   | 21.6   | 18.5   |
| Ether extract, % DM | 3.6       | 3.5    | 5.9    | 5.6    | 3.8    |
| NDF, % DM     | 10.3       | 10.9   | 17.9   | 15.7   | 19.4   |
| ADF, % DM     | 3.9        | 4.1    | 5.3    | 5.1    | 6.1    |
| TDF, % DM     | 14.9       | 14.6   | 23.6   | 24.3   | 24.1   |
| SDF, % DM     | 2.9        | 2.7    | 7.8    | 7.7    | 2.8    |
| IDF, % DM     | 12.0       | 11.9   | 15.8   | 16.6   | 21.3   |
| Ash, % DM     | 4.8        | 4.9    | 5.5    | 6.0    | 5.6    |
| Enzyme activity, U/kg | –      | 5325   | –      | 5120   | –      |

1Data are the means of two replicates of analyzed values
2OB Oat bran; WB Wheat bran; GE Gross energy; DM Dry matter; CP Crude protein; NDF Neutral detergent fiber; ADF Acid detergent fiber; TDF Total dietary fiber; SDF Soluble dietary fiber; IDF Insoluble dietary fiber. The – and + symbol represents enzyme-free diets and enzyme addition diets, respectively.
| Item                      | Xylanase-free diets |          |          |          | Xylanase supplementation diets |          |          | SEM | P-value | Diet | Xylanase | D×X² |
|---------------------------|---------------------|----------|----------|----------|--------------------------------|----------|----------|-----|---------|------|----------|------|
|                           | Basal diet          | 36% OB   | 27% WB   | 15% OB   | 12% WB                         | Basal diet | 36% OB   | 27% WB | 15% OB | 12% WB | Diet Xylanase D×X² |
| Average BW                | 350                 | 35.5     | 350      | 35.6     | 365                            | 36.6      | 38.2     | 35.9 | 35.1    | 35.6 | 0.96     | 0.572 | 0.212 | 0.031 |
| DMI, kg/d                 | 1.21                | 1.23     | 1.28     | 1.25     | 1.28                           | 1.26      | 1.37     | 1.34 | 1.25    | 1.28 | 0.04     | 0.295 | 0.051 | 0.042 |
| ATTD, %                   | DM                  | 91.0a    | 86.8bc   | 80.7d    | 88.8abc                         | 85.2c     | 89.9bc   | 86.0bc | 85.3c   | 89.4bc | 87.2bc   | 1.00  | < 0.001 | 0.043 | 0.041 |
|                           | GE                  | 90.2a    | 86.0b    | 79.6c    | 87.9bc                          | 84.2bc    | 89.1bc   | 85.4b  | 81.6bc  | 88.4bc | 86.4b    | 1.08  | < 0.001 | 0.082 | 0.048 |
|                           | CP                  | 896a     | 86.9a    | 80.2c    | 88.2a                           | 84.8ab    | 88.5a    | 84.7ab | 85.7ab  | 89.0a  | 87.0a    | 1.44  | < 0.001 | 0.045 | 0.043 |
|                           | NDF                 | 63.5ab   | 70.0a    | 53.3b    | 63.4ab                          | 57.5ab    | 61.0bc   | 63.5ab | 58.9ab  | 65.2bc | 60.7ab   | 2.74  | 0.003    | 0.861 | 0.021 |
|                           | ADF                 | 65.2a    | 58.1bc   | 44.9c    | 61.4a                           | 56.2ab    | 63.8a    | 53.7ab | 53.5ab  | 62.1a  | 60.4a    | 3.29  | 0.022    | 0.025 | 0.036 |
|                           | OM                  | 92.1a    | 88.8bc   | 82.6c    | 90.3ab                          | 86.7bc    | 91.1a    | 88.1ab | 86.6bc  | 90.8ab | 88.5b    | 0.93  | < 0.001 | 0.122 | 0.064 |
|                           | UE/DE               | 180      | 1.97     | 247      | 1.85                           | 1.77      | 1.54     | 2.02  | 1.63    | 1.47   | 1.54     | 0.26  | 0.412    | 0.118 | 0.035 |
|                           | Ch4/E/DE            | 0.58     | 0.40     | 0.57     | 0.52                            | 0.45      | 0.51     | 0.31  | 0.34    | 0.55   | 0.51     | 0.08  | 0.142    | 0.243 | 0.385 |
| Nitrogen balance, g/d    | Intake              | 36.8b    | 40.4ab   | 40.1ab   | 39.0b                          | 39.3ab    | 38.3b    | 45.0b  | 41.7b   | 39.0b  | 39.1b    | 1.27  | 0.002    | 0.075 | 0.053 |
|                           | FN                  | 4.0c     | 5.4abc   | 7.7a     | 4.8bc                          | 6.0bc     | 4.7bc    | 7.1ab  | 7.2ab   | 4.5bc  | 5.2bc    | 0.58  | < 0.001 | 0.702 | 0.181 |
|                           | UN                  | 7.1      | 7.0      | 6.3      | 7.7                            | 6.5       | 6.2      | 7.6   | 7.0     | 7.7    | 8.7      | 0.80  | 0.544    | 0.298 | 0.395 |
|                           | RN                  | 25.6     | 28.0     | 26.1     | 26.5                            | 26.8      | 27.5     | 30.3  | 27.5    | 26.7   | 25.2     | 1.28  | 0.324    | 0.142 | 0.057 |

*abc* Values with different characters in superscripts were different (P < 0.05) in the same row

1. Treatment means are reported as least squares means and are based on 6 observations per treatment. OB Oat bran, WB Wheat bran; BW Body weight (mean values in collection period); DM Dry matter; DMI Dry matter intake; ATTD Apparent total tract digestibility; GE Gross energy; CP Crude protein; NDF Neutral dietary fiber; ADF Acid detergent fiber; OM Organic matter; UE Urinary energy; CH4E Methane energy; FN Nitrogen output from feces; UN Urinary nitrogen excretion; RN Total retained nitrogen; SEM Standard error of the mean

2. D×X is the interaction between diet and xylanase supplementation
Table 4: Effect of dietary characteristics and enzyme addition on energy utilization and energy values of experimental diets (DM basis)\textsuperscript{1}

| Item                   | Xylanase-free diets | Xylanase supplementation diets | SEM       | P-value       |
|------------------------|---------------------|--------------------------------|-----------|---------------|
|                        | Basal diet 36% OB   | 27% WB 15% OB 12% WB          | Basal diet 36% OB 27% WB 15% OB 12% WB | Diet Xylanase D×X |
| Energy utilization, %  | ME/DE               | NE/ME                          | SEM       | P-value       |
|                        | 97.6                | 97.6                           | 96.9      | 97.7          | 97.9          | 97.7          | 98.0          | 98.0          | 97.6          | 0.28          | 0.801         | 0.073         | 0.241         |
|                        | 82.8                | 82.7                           | 79.2      | 82.2          | 81.5          | 83.4          | 81.6          | 83.3          | 81.7          | 82.6          | 1.56          | 0.924         | 0.186         | 0.709         |
| Energy values, MJ/kg DM| DE                  | ME                             | NE        |                |               |               |               |               |               |               |               |               |               |
|                        | 16.40\textsuperscript{a} | 16.38\textsuperscript{a}     | 14.81\textsuperscript{c} | 16.43\textsuperscript{c} | 15.55\textsuperscript{abc} | 16.36\textsuperscript{a} | 16.32\textsuperscript{a} | 15.22\textsuperscript{c} | 16.40\textsuperscript{a} | 16.07\textsuperscript{ab} | 0.16          | < 0.001       | 0.130         | 0.232         |
|                        | 16.01\textsuperscript{a} | 15.99\textsuperscript{a}     | 14.35\textsuperscript{c} | 16.04\textsuperscript{a} | 15.20\textsuperscript{ab} | 16.02\textsuperscript{a} | 15.94\textsuperscript{ab} | 14.92\textsuperscript{c} | 16.07\textsuperscript{a} | 15.68\textsuperscript{abc} | 0.16          | < 0.001       | 0.049         | 0.034         |
|                        | 13.26\textsuperscript{a} | 13.23\textsuperscript{a}    | 11.37\textsuperscript{c} | 13.19\textsuperscript{a} | 12.38\textsuperscript{ab} | 13.36\textsuperscript{a} | 13.00\textsuperscript{a} | 12.43\textsuperscript{ab} | 13.13\textsuperscript{a} | 12.95\textsuperscript{ab} | 0.27          | < 0.001       | 0.043         | 0.041         |

\textsuperscript{a,b,c}Values with different characters in superscripts were different (P < 0.05) in the same row.

\textsuperscript{1}Treatment means are reported as least squares means and are based on 6 observations per treatment. OB Oat bran, WB Wheat bran; DE Digestible energy; ME Metabolizable energy; NE Net energy; SEM Standard error of the mean.

\textsuperscript{2}D×X is the interaction between diet and xylanase supplementation.
acetate, propionate, butyrate, and isovalerate. In the enzyme-free dietary groups, pigs fed WB-based diets excreted more \((P < 0.05)\) acetate and propionate and total SCFA in feces than those fed a basal diet. The acetate and propionate concentrations in feces were greater \((P < 0.05)\) in pigs fed 27% WB diets compared with those fed 36% OB diets and a basal diet. The acetate concentrations in feces were greater \((P < 0.05)\) in pigs fed 27% WB diets compared with those fed 12% WB diets. By contrast, the increase of fiber level in OB diets did not affect the acetate and propionate concentrations in fecal samples. Enzyme supplementation increased \((P < 0.05)\) acetate, propionate, isobutyrate and total SCFA excretion in pigs fed 12% WB diets.

The richness and biodiversity of bacterial communities

The indices of Chao and Shannon at the OTU level were used to elevate bacterial richness and diversity. The two indices were not affected by fiber source, fiber level or enzyme addition (Fig. 1). The bacterial composition was presented at the phylum, family, and genus levels, respectively (Fig. 2 and Supplementary Fig. S1). Firmicutes and Bacteroidetes, were the two main phyla of bacteria in the fecal samples of growing pigs (Supplementary Fig. S1A). The predominant families in Firmicutes consisted of Ruminococcaceae, Lachnospiraceae, Veillonellaceae, and Christensenellaceae, while Prevotellaceae, Bacteroidales_S24-7_group, and Erysipelotrichaceae were the predominant families in Bacteroidetes (Supplementary Fig. S1B).

Cladogram of LEfSe showed all differential bacteria from phylum to genus level (Fig. 3 and Fig. 4). The relative abundances of main kinds of bacteria were greater \((P < 0.05)\) in high-fiber diets than those in low-fiber diets (Fig. 3a), including Marvinbryantia, [Eubacterium]_hallii_group, Lachnospiraceae_FCS020_group and Lachnospiraceae_ND3007_group, which belonged to family Lachnospiraceae, and Bifidobacterium, which belonged to family Bifidobacteriaceae. The abundances of Senegalimassilia and norank_f__Peptococcaceae were decreased in high-fiber diet than those in low-fiber diets \((P < 0.05)\). The abundance of Anaerotruncus, Ruminococcaceae_UGC_014, and Solobacterium was greater \((P < 0.05)\) in pigs fed OB diets than pigs fed WB, which resulted in an increase \((P < 0.05)\) in the population of phylum Firmicutes (Fig. 3b). Pigs fed the WB diets had greater \((P < 0.05)\) proportion of phylum Bacteroidetes, family Prevotellaceae and order Bacteroidia compared to those fed the OB diets, which was due to increased relative abundances of unclassified_f__Prevotellaceae, Prevotella_1, and dgA_11_gut_group \((P < 0.05)\). The increased abundance of Succinivibrio \((P < 0.05)\) led to greater proportion of Spirochaetae \((P < 0.05)\) in pigs fed WB diets compared with those fed OB diets.

When pigs were fed diets with 36% OB (Fig. 4a), enzyme addition decreased \((P < 0.05)\) the relative abundances of unclassified_f__Lachnospiraceae, Lachnospiraceae_NK4A136_group, Lachnospiraceae_FCS020_group, Coprococcus_3, Marvinbryantia, Eubacterium_hallii_group, and Blautia belonging to family Lachnospiraceae. Meanwhile, xylanase addition decreased \((P < 0.05)\) the relative abundances of

### Table 5 Nutrient digestibility and energy values of the oat bran and wheat bran

| Item                | Ingredient | SEM | P-value |
|---------------------|------------|-----|---------|
| ATTD, %             |            |     |         |
| Gross energy        | 83.0\(^a\) | 81.5\(^b\) | 63.3\(^c\) | 71.8\(^d\) | 6.3 | 0.045 |
| Crude protein       | 83.1\(^a\) | 79.5\(^b\) | 52.4\(^c\) | 77.5\(^d\) | 6.8 | 0.034 |
| Neutral detergent fiber | 73.6\(^a\) | 65.3\(^b\) | 47.0\(^c\) | 62.8\(^d\) | 7.8 | 0.020 |
| Acid detergent fiber | 52.2\(^a\) | 43.8\(^b\) | 28.0\(^c\) | 52.8\(^d\) | 4.7 | 0.031 |
| Organic matter      | 83.7\(^a\) | 83.4\(^b\) | 58.0\(^c\) | 75.0\(^d\) | 5.0 | 0.044 |

**ATTD, %**: Apparent total tract digestibility (ATTD) of nutrient and energy values of oat bran and wheat bran were calculated using difference method in diets containing 36% oat bran or 27% wheat bran. **DE**: Digestible energy; **ME**: Metabolizable energy; **NE**: Net energy; **SEM**: Standard error of the mean

**Table 5**: Values with different characters in superscripts were different \((P < 0.05)\) in the same row

**Table 5**: Treatment means are reported as least squares means and are based on 6 observations per treatment

**Table 5**: The – and + symbol represents enzyme-free dietary treatment and enzyme addition dietary treatment, respectively.
| Item | Xylanase-free diets | Xylanase supplementation diets | SEM | P-value |
|------|---------------------|-------------------------------|-----|---------|
|      | Basal diet 36% OB | 27% WB | 15% OB | 12% WB | Basal diet 36% OB | 27% WB | 15% OB | 12% WB |         | Diet      | Xylanase | D×X²   |
| Acetate | 0.68<sup>a</sup> | 1.23<sup>abc</sup> | 2.22<sup>ab</sup> | 1.24<sup>abc</sup> | 1.59<sup>cd</sup> | 1.07<sup>abc</sup> | 1.66<sup>bcd</sup> | 1.73<sup>bc</sup> | 1.22<sup>abc</sup> | 2.50<sup>a</sup> | 0.123 | < 0.001 | 0.002 | < 0.001 |
| Propionate | 0.36<sup>a</sup> | 0.81<sup>cde</sup> | 1.28<sup>ab</sup> | 0.74<sup>cde</sup> | 0.99<sup>abcd</sup> | 0.62<sup>abc</sup> | 1.15<sup>bcd</sup> | 0.92<sup>bcd</sup> | 0.76<sup>cde</sup> | 1.54<sup>a</sup> | 0.096 | < 0.001 | 0.015 | 0.002 |
| Isovalerate | 0.04<sup>b</sup> | 0.09<sup>abc</sup> | 0.09<sup>abc</sup> | 0.09<sup>abc</sup> | 0.06<sup>bc</sup> | 0.13<sup>b</sup> | 0.14<sup>a</sup> | 0.07<sup>abc</sup> | 0.07<sup>abc</sup> | 0.06<sup>a</sup> | 0.016 | < 0.001 | 0.003 | 0.074 |
| Butyrate | 0.19<sup>b</sup> | 0.53<sup>b</sup> | 0.59<sup>b</sup> | 0.40<sup>bc</sup> | 0.45<sup>cd</sup> | 0.26<sup>c</sup> | 0.44<sup>bc</sup> | 0.35<sup>b</sup> | 0.39<sup>ab</sup> | 0.67<sup>a</sup> | 0.065 | < 0.001 | 0.521 | 0.018 |
| Isovalerate | 0.09<sup>c</sup> | 0.17<sup>abc</sup> | 0.24<sup>a</sup> | 0.18<sup>abc</sup> | 0.11<sup>bc</sup> | 0.11<sup>bc</sup> | 0.22<sup>ab</sup> | 0.23<sup>ab</sup> | 0.17<sup>abc</sup> | 0.26<sup>a</sup> | 0.025 | < 0.001 | 0.009 | 0.024 |
| Valerate | 0.09<sup>b</sup> | 0.20<sup>ab</sup> | 0.17<sup>bc</sup> | 0.23<sup>ab</sup> | 0.15<sup>bc</sup> | 0.15<sup>bc</sup> | 0.28<sup>a</sup> | 0.14<sup>ab</sup> | 0.18<sup>ab</sup> | 0.24<sup>ab</sup> | 0.037 | 0.023 | 0.184 | 0.221 |
| Total SCFA | 1.48<sup>c</sup> | 2.88<sup>cde</sup> | 4.65<sup>de</sup> | 2.90<sup>cde</sup> | 3.41<sup>bcd</sup> | 2.29<sup>de</sup> | 3.95<sup>bc</sup> | 3.58<sup>bcd</sup> | 2.83<sup>cde</sup> | 5.42<sup>a</sup> | 0.295 | < 0.001 | 0.012 | 0.004 |

<sup>a,b,c,d,e</sup>Values with different characters in superscripts were different (P < 0.05) in the same row.

<sup>1</sup>Treatment means are reported as least squares means and are based on 6 observations per treatment. OB Oat bran; WB Wheat bran; SCFA Short-chain fatty acid; SEM Standard error of the mean

<sup>2</sup>SCFA concentration (mg/g Feed DM) = SCFA concentration (mg/g feces DM) × feces DM (g)/feed DM (g)

<sup>3</sup>D×X is the interaction between diet and xylanase supplementation.
Ruminococcaceae_UCG_005, Ruminococcus_1, Faecalibacterium, and Candidatus_Saccharibacterium, and increased ($P < 0.05$) the abundances of Escherichia_Shigella and Olsenella. When pigs were fed the diet with 27% WB (Fig. 4b), enzyme addition increased ($P < 0.05$) the relative abundances of Christensenellaceae_R-7_group and Ruminococcaceae_UCG_002, thereby increasing ($P < 0.05$) the abundances of families Christensenellaceae and Ruminococcaceae. Enzyme addition decreased ($P < 0.05$) the relative abundances of genus Eubacterium_ruminantium_group and Lactobacillus and decreased ($P < 0.05$) the abundances of families Lachnospiraceae, Bacteroidales, and Lactobacillaceae when pigs were fed diet with 27% WB. When pigs were fed the diet containing 12% OB (Fig. 4c), enzyme addition improved ($P < 0.05$) the relative abundances of Blautia, Catenibacterium, and Prevotella_1. When pigs were fed diet with 12% WB (Fig. 4d), enzyme addition increased ($P < 0.05$) the relative abundances of Faecalibacterium, Ruminococcaceae_UCG_013, Ruminococcaceae_UCG_014, thereby increasing ($P < 0.05$) the abundances of family Ruminococcaceae. In addition, enzyme addition in diet with 12% WB decreased ($P < 0.05$) the abundance of Chlamydia.

**Discussion**

The high inclusion levels of DF in 36% OB diets and 27% WB diets decreases ATTD of DM and GE compared with basal diets. The results are consistent with the previous studies that report DF negatively affects nutrient digestibility due to their anti-nutritive effects [23–25]. The differences in chemical composition of OB and WB lead to different results on nutrient digestibility, SCFA concentrations and abundance of bacteria in feces, and energy values in diets. Wheat bran contains a higher proportion of insoluble lignified fiber and is therefore...
less degraded than DF in OB. As a result, pigs fed 36% OB diets have greater nutrient digestibility compared with those fed 27% WB diets. Meanwhile, 27% WB diets contain lower nutrient (DM and CP) digestibility than 12% WB diets. However, nutrient digestibility is not affected by inclusion levels of OB. The results confirm DF in WB has greater negative effects on nutrient digestibility than that in OB. In addition, OB contains more starch, CP, and EE content, which is the main reason why OB has greater NE values than WB. Wheat

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**Fig. 2** Effects of fiber level and type fecal on microbial community structure in growing pigs. Distribution of fecal bacteria on the phylum (a) and family (c) levels in pigs fed high or low-fiber diets. Distribution of fecal bacteria on the phylum (b) and family (d) levels in pigs fed OB or WB based diets. The Welch's t test was applied to identify the differences in the relative abundance of gut microbiota between two groups, and one asterisk means \( P < 0.05 \). Phyla and families with proportions less than 1% are not listed. HFG, diet containing 36% oat bran or 27% wheat bran (without enzyme); LFG, diets containing 15% oat bran or 12% wheat bran (without enzyme).
Bran has a typical NSP composition of gramineous grain by-products that mainly consists of insoluble arabinoxylan and cellulose [26], whereas OB is rich in mixed linkages β-glucan which to a high degree is soluble and insoluble arabinoxylan. In addition, only trace levels of cellulose are found in OB [7]. In the small intestine, β-glucan in OB is partly digested while arabinoxylan in OB or WB is hardly digested [7, 8]. In pigs, fiber in OB was readily fermented by bacteria along gastrointestinal tract [27]. By contrast, most of DF in WB enters hindgut to contribute to microbial fermentation and SCFA generation. Therefore, supplementation of diets with high levels WB rather than OB caused fermentation of fiber and undigested nutrients to shift from the small intestine to hindgut, thereby contributing to growth of fiber-degrading bacteria in hindgut of pigs [27, 28]. In the current study, the abundances of Firmicutes in pigs fed OB diets are greater while the abundances of Bacteroidetes in WB diets
are greater. Similarly, Liu et al. [19] showed that corn bran-based diets rich in IDF (mainly insoluble arabinoxylan) increased the population of Bacteroidetes while reducing the abundances of Firmicutes. Bacteroidetes has special polysaccharide utilization loci, which generates numerous carbohydrate enzymes to cleave the linkages that existed in complex polysaccharide structures [29]. Compared with OB diets, WB diets increase the abundance of Succinivibrio which is correlated positively with propionate and butyrate concentrations in feces [30]. The Prevotellaceae are a dominant family within Bacteroidetes phylum and produces numerous enzymes, including xylanases and β-glucanases, that are reported to be positively associated with arabinoxylan degradation in the gastrointestinal tract of animals [31, 32]. Prevotella has a strong ability to generate acetate [33]. The greater abundances of Prevotella in WB groups compared to OB groups is also reported by Zhang et al. [34] who observes that digestion of NSP and xylose in ileal digesta is correlated positively with abundance of Prevotella. Meanwhile, the concentrations of acetate, propionate and total SCFA are greater in pigs fed 27% WB diet than those fed 36% OB diet, which is consistent with the results about fecal bacteria. Our findings support the hypothesis that differences in chemical composition for OB diets and WB diets affect the abundance of the gut bacteria and concentrations of SCFA, and is ultimately reflected in energy metabolism.
Inconsistent results regarding the effects of NSP-degrading enzyme on nutrient digestibility based on cereal by-products have been reported widely [28, 35, 36]. In the current study, the addition of enzyme improves ATTD of nutrients and NE values of diets in pigs fed 27% WB diets. By contrast, the addition of enzyme to the basal diets or OB diets does not impact the ATTD of nutrients and NE values of diets. This observation suggests that the effect of enzymes on nutrient digestibility is related to type and concentration of substrates. Hemicellulose in WB consists predominantly of xylose and arabinose residues, and the concentration of arabinoxylan in WB is double than that in OB [37]. Consequently, diets containing 27% WB have the greatest arabinoxylan concentration among all diets. According to the theory of enzymatic kinetics, the higher the substrate concentration is, the faster the enzymatic reaction within a certain concentration range [38]. Yin et al. [24] reported that enzyme supplementation had a greater effect on ATTD of nutrients in pigs fed high NSP wheat bran-based diets compared with those fed low NSP wheat or wheat flour diets. Zeng et al. [28] also reported supplementation of carbohydrase increased ATTD of DM (74.5% vs. 78.0%) and CP (75.4% vs. 79.7%) in pigs fed wheat-wheat bran (10% wheat and 20% WB) diets, but not in wheat (10% wheat) diets. It confirms that effects of enzyme are highly related to type and concentration of the substrates. Additionally, another possible explanation is that OB contains significant quantities of insoluble arabinoxylans along with soluble β-glucans [26], so the use of xylanase does not eliminate the anti-nutritional effects of β-glucans. Soluble β-glucans increase viscosity of digesta, which, in turn, prevent arabinoxylans to contact with xylanase. The explanation is confirmed by a previous study that reports that xylanase is more efficient in degrading arabinoxylan in wheat than in rye. In addition, Yin et al. [39] reported that β-glucanase or an enzyme cocktail (β-glucanase, xylanase and protease) added to barley diets (higher proportion of β-glucans) improves ATTD of NDF (60.9%, 68.1%, 66.6% for control diets, β-glucanase addition diets and cocktail addition diets, respectively) but adding xylanase alone has no effect on the ATTD of NDF although values varied greatly (60.9% vs. 67.1%). It indicates that the effects of enzyme depend on whether the type of enzyme matches the dietary composition. Furthermore, the enzyme addition not only has an impact on digestion of specific NSP but also other nutrients by improving probability for nutrients to contact with endogenous enzymes [14]. In the current study, the enzyme addition improves the NE values only for 27% WB diets. Because DF provides a negligible net amount of DE to growing pigs [40], the explanation for this result is that the enzyme supplementation improves ATTD of CP and DM, resulting in more energy sources available to pigs. Exogenous enzymes influenced the intestinal microbiota by enhancing nutrient delivery to the host, providing fermentable oligosaccharides degraded from indigestible polysaccharides, and affecting the fermentation site and viscosity and flow rate of digesta [9, 14]. In general, addition of carbohydrase enzymes influenced more bacterial abundances in pigs fed high-fiber diets compared to pigs fed low-fiber diets. The results were consistent with those of digestibility, SCFA, and energy metabolism in high-fiber diets (mainly 27% WB diets) and low-fiber diets. Zhao et al. [41] reports that SCFA concentrations in feces are positively correlated with the IDF digestibility in diets. Enzyme addition increases SCFA concentrations in feces when pigs are fed 12% WB diets, which may be attributed to increased fiber digestibility (57.5% vs. 60.7% for NDF and 56.2% vs. 60.4% for ADF). However, there is an inconsistent result for effects of enzyme on 27% WB diets. This maybe because enzyme addition hydrolyzed cell wall components, promoting the digestion of nutrients in the foregut, resulting in a decrease in the substrate available for hindgut fermentation. Enzyme supplementation decreases abundance of Lachnospiraceae, Bacteroidales, and Lactobacillaceae for 27% WB-fed pigs, which seems to support this explanation. However, comprehensive explanation is not available because SCFA concentrations in feces do not represent the SCFA production in intestinal tract.

Conclusions
In conclusion, diets supplied by high level of OB or WB promote the growth of fiber-degrading bacteria. The differences in chemical composition between WB and OB lead to differences in nutrient digestibility and bacterial communities, which were ultimately reflected in energy metabolism. Enzyme supplementation improves nutrient digestibility as well as NE values for 27% WB but not for other diets, which indicates that effects of enzyme are determined by type and level of NSP in diets. However, the SCFA production and absorption is not measured in this study, leading to an inability to comprehensively explain the correlation between fatty acids and energy metabolism. Therefore, the effect of dietary structure on digestive properties in different intestinal segments deserves further study.

Supplementary information
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Additional files 1: Table S1. Chemical composition of fibrous ingredients (%), as-fed basis. Table S2. Effect of dietary characteristics and xylanase addition on heat production and energy retention of growing pigs’.
Abbreviations
AA: Amino acid; ADF: Acid detergent fiber; ATTD: Apparent total tract digestibility; BSNE: The basal diet without xylanase; BW: Body weight; CH₄: Energy lost as methane; CP: Crude protein; DE: Digestible energy; DFD: Dietary fiber; DM: Dry matter; EE: Ether extract; FG: Wheat bran diets and oat bran diets without xylanase; FHP: Fasting heat production; GE: Gross energy; HFG: Diet containing 36% oat bran and diet containing 27% wheat bran (without enzyme); HP: Heat production; DIF: Soluble dietary fiber; LDA: Linear discriminant analysis effect size; LFSHE: Linear discriminant analysis effect size; LFG: Diets containing 15% oat bran or 12% wheat bran (without enzyme); ME: Metabolizable energy; NDF: Neutral detergent fiber; NE: Net energy; NSP: Non-starch polysaccharide; OB: Oat bran; OBNE: Oat bran diets without xylanase; OLT: Operational taxonomic units; OM: Organic matter; RDP: Ribosomal database project; RET: Retained energy; RE: Retained energy as lipids; RE: Retained energy as protein; SCFA: Short-chain fatty acids; SDF: Soluble dietary fiber; TDF: Total dietary fiber; THP: Total heat production; UE: Gross energy of urine; WB: Wheat bran; WBNE: Wheat bran diet without xylanase

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Authors’ contributions
ZQL, SZ, and CHL designed the experiment. ZYW and CHL gave advice on the experiment design. ZQL analyzed the study data and prepared the manuscript. The manuscript was edited by LW, CHL, JJW. All the authors have read and approved the final manuscript.

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Ethics approval and consent to participate
The experimental protocols used in this experiment, including animal care and use, were reviewed and approved by the Animal Care and Use Ethics Committee of China Agricultural University (Beijing, China).

Consent for publication
Not applicable.

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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