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# Effects of Partial Replacement of Wheat Bran with Poplar Wood Composite Fiber on Growth Performance, Nutrient Apparent Digestibility, Immune Function, and Gut Microbiota in Growing Pigs

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Keywords: dietary fiber; poplar wood composite fiber; wheat bran replacement; growth performance; nitrogen metabolism; gut microbiota; growing pigs



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Article

# Effects of Partial Replacement of Wheat Bran with Poplar Wood Composite Fiber on Growth Performance, Nutrient Apparent Digestibility, Immune Function, and Gut Microbiota in Growing Pigs

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## Simple Summary

This study investigated the effects of replacing wheat bran with poplar wood composite fiber (PWCF) in pig diets. The research involved 140 growing pigs fed either a control diet or an experimental diet with 2% wheat bran replaced by PWCF for 60 days. The results showed that PWCF did not negatively affect pig growth performance, nutrient digestibility, or immune function. Instead, it helped regulate nitrogen metabolism by lowering blood urea nitrogen (BUN) levels and serum total free amino acids (TFAAs), and enhanced antioxidant capacity by increasing catalase activity. Notably, PWCF supplementation led to significant increases in the relative abundance of certain gut bacteria known to degrade dietary fiber. Overall, PWCF can be a suitable alternative fiber source in pig diets, offering benefits such as nitrogen metabolism regulation and gut microbiota modulation, without compromising growth or health. This supports the use of PWCF in diversifying fiber ingredients in pig feed.

## Abstract

The objective of this study was to evaluate the effects of partially replacing wheat bran with poplar wood composite fiber (PWCF) on growth performance, immune status, apparent total tract digestibility (ATTD), and gut microbial composition in growing pigs. A total of 140 healthy crossbred (Duroc × Landrace × Yorkshire) growing pigs with an initial body weight of  $47.25 \pm 0.49$  kg were randomly assigned to two dietary treatments, with five replicates per treatment and fourteen pigs per replicate. The control (CT) group was fed a corn–soybean meal–based diet, whereas the experimental group received the same diet in which 2% wheat bran was replaced by PWCF. The experiment lasted for 60 days. Compared with the CT group, replacing wheat bran with PWCF did not affect body weight, average daily feed intake, feed conversion ratio, or average daily gain on days 30 or 60 ( $P > 0.05$ ). In addition, no negative effects were observed on ATTD of nutrients and serum immunoglobulin A (IgA), IgG, and IgM levels at either time point, indicating that PWCF can serve as a suitable partial substitute for wheat bran in growing pig diets. However, it could regulate nitrogen metabolism by reducing blood urea nitrogen (BUN) concentration and the BUN/creatinine ratio, as well as decreasing total free amino acids in serum ( $P < 0.05$ ). In addition, the antioxidant capacity can be improved by increasing catalase activity. Gut microbiota analysis showed that the replacement significantly increased the relative abundances of *Treponema*, Lachnospiraceae\_XPB1014\_group, Prevotellaceae\_UCG-001, Prevotellaceae\_UCG-003, Prevotellaceae\_UCG-004, and norank\_f\_Oscillospiraceae ( $P < 0.05$ ). These changes suggest that PWCF modulates gut microbiota and enriches fiber-degrading bacterial populations. Overall,

substituting wheat bran with PWCF did not impair growth performance, immunity, or digestibility, while altering microbial community composition. These findings support the potential application of PWCF as an alternative fiber source, contributing to greater diversity in feed formulation.

**Keywords:** dietary fiber; poplar wood composite fiber; wheat bran replacement; growth performance; nitrogen metabolism; gut microbiota; growing pigs

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## 1. Introduction

Dietary fiber (DF) refers to a group of food components that are resistant to hydrolysis by endogenous digestive enzymes and are therefore poorly digested and absorbed in the small intestine. It is widely present in plant-derived foods such as cereals, fruits, and vegetables [1]. In recent years, increasing evidence has shown that DF can influence growth performance [2], immune function [3], apparent total tract digestibility (ATTD) [4], and gut microbial composition in growing pigs [5]. Previous studies have demonstrated that moderate inclusion of DF in diets does not impair average daily gain or overall growth performance in growing pigs [6,7]. However, supplementation with a high level of soybean hulls (20%) has been reported to reduce the gain-to-feed ratio [8]. Previous studies have also shown that DF supplementation can improve ileal nutrient digestibility [7] while reducing the ATTD of crude protein (CP) and energy [9]. In addition, DF has been reported to promote intestinal development and support gut health [10,11]. Similar findings indicate that DF inclusion may decrease meal frequency while increasing average meal size, and reduce the apparent fecal digestibility of dry matter (DM), organic matter, crude ash, nitrogen, and gross energy (GE). However, it may enhance the digestibility of crude fiber (CF) and neutral detergent fiber (NDF) [12]. Collectively, these results suggest that the effects of DF in pig diets are complex and may depend on the inclusion level and fiber source. Therefore, further studies are required to determine the optimal DF supplementation level for growing pigs.

Wheat bran is a widely used feed ingredient due to its broad availability and low cost. It is rich in DF, crude protein, and minerals, and can stimulate intestinal motility, help maintain microbial balance, improve diet palatability, and enhance satiety in livestock and poultry [13,14]. However, the application of wheat bran in animal diets also has notable limitations. During storage, wheat bran is susceptible to contamination by pathogenic microorganisms and the production of various mycotoxins, such as deoxynivalenol and zearalenone. These toxins can impair immune function and exert chronic negative effects on growth performance, as well as reproductive health [15]. Wheat bran has been reported to harbor relatively high microbial loads. The microorganisms commonly detected in wheat bran include bacterial genera such as *Pseudomonas aeruginosa*, *Micrococcus*, and *Lactobacillus*, as well as fungi including *Aspergillus*, *Penicillium*, and *Streptomyces*, together with their associated mycotoxins. This microbial contamination increases the difficulty of wheat bran storage and poses potential risks to feed safety [16]. Poplar wood powder contains a high level of DF, exceeding 87% [17]. However, limited information is available regarding its potential as an alternative fiber source to replace wheat bran in pig diets.

Therefore, in the present study, a poplar wood-based composite fiber was developed using poplar powder as the main ingredient, and its feasibility as a partial substitute for wheat bran was evaluated. The present study aimed to evaluate the effects of dietary supplementation with poplar wood composite fiber (PWCF) on growth performance, immune status, ATTD, and gut microbial composition in growing pigs. By examining these parameters, this study sought to determine whether PWCF could partially replace wheat bran in growing pig diets. Such substitution may contribute to reducing feed costs and improving feed efficiency, thereby providing a theoretical basis for the practical application of alternative DF sources in swine nutrition.

## 2. Materials and Methods

The experiment was conducted at the Tianpeng Experimental Farm located in Langfang, China. All animal procedures in this study were approved by the Animal Care and Use Committee of the Feed Research Institute, Chinese Academy of Agricultural Sciences (IFR-CAAS20220725).

### 2.1. Experimental Design and Animal Management

A total of 140 healthy growing pigs (Duroc × Landrace × Yorkshire) with similar initial body weight ( $47.25 \pm 0.49$  kg) were randomly assigned to two dietary treatment groups. Each treatment included five replicates, with fourteen pigs per replicate. The control group (CT) was fed the basal diet, whereas the fiber treatment group (FF) received the same diet in which 2% wheat bran was replaced with 2% PWCF (Hebei Weierli Animal Pharmaceutical Group Co., Ltd., Hebei, China). The basal diet was formulated as a pelleted feed according to the nutrient requirements for growing–finishing pigs recommended by the NRC (2012), and its nutrient composition is presented in Table 1. The nutrient composition of the PWCF used is shown in Table 2. The experimental period lasted for 60 days.

**Table 1.** Ingredient composition and calculated nutrient levels of the basal diet (as-fed basis, %).

Items	Treatment groups	
	CT	FF
Corn	63.87	63.87
Soybean meal, 46% CP	19.00	19.00
Rice bran meal	8.00	8.00
Wheat bran	4.00	2.00
Soybean oil	1.90	1.90
Dicalcium phosphate	0.18	0.18
Limestone	1.50	1.50
L-Lysine	0.70	0.70
DL-Methionine	0.05	0.05
L-Threonine	0.14	0.14
L-Tryptophan	0.01	0.01
Salt	0.40	0.40
Phytase	0.02	0.02
Vitamin premix <sup>1</sup>	0.03	0.03
Mineral premix <sup>1</sup>	0.20	0.20
Poplar wood composite fiber <sup>2</sup>	0.00	2.00
Total	100.00	100.00
Nutrient composition		
Crude protein <sup>3</sup>	15.45	15.41
Net energy, kcal/kg <sup>4</sup>	2475	2475
Calcium <sup>3</sup>	0.67	0.68
Total phosphorus <sup>3</sup>	0.51	0.51
Lysine <sup>4</sup>	1.00	1.00
Methionine <sup>4</sup>	0.28	0.28
Threonine <sup>4</sup>	0.60	0.60
Tryptophan <sup>4</sup>	0.16	0.16

<sup>1</sup>Premix supplied per kilogram of diets: vitamin A, 7245 IU; vitamin D<sub>3</sub>, 1470 IU; vitamin E, 40 IU; vitamin K<sub>3</sub>, 1.79 mg; vitamin B<sub>1</sub>, 2.14 mg; vitamin B<sub>2</sub>, 4.37 mg; vitamin B<sub>6</sub>, 2.68 mg; vitamin B<sub>12</sub>, 0.024 mg; niacin, 25 mg; calcium pantothenate, 15 mg; folic acid, 4.15 mg; 2%biotin, 0.42 mg; iron (FeSO<sub>4</sub>·H<sub>2</sub>O), 150 mg; manganese (MnSO<sub>4</sub>·H<sub>2</sub>O), 100 mg; copper (CuSO<sub>4</sub>·5H<sub>2</sub>O), 23 mg; selenium (Na<sub>2</sub>SeO<sub>3</sub>), 0.5 mg; cobalt (CoCl<sub>2</sub>), 1 mg; iodine (Ca(IO<sub>3</sub>)<sub>2</sub>), 23 mg; Zinc 79 mg. <sup>2</sup>Poplar wood composite fiber is composed of 50% fiber sourced from white poplar.

<sup>3</sup>Values were analyzed. <sup>4</sup>Values were calculated.

**Table 2.** Nutrient composition and fiber physical characteristics of the poplar wood composite fiber (PWCF) (air dry basis, %).

Items	Composition
Nutrient composition	
Moisture	5.42
Crude protein	3.55
Crude fiber	44.25
Neutral detergent fiber (NDF)	70.51
Acid detergent fiber (ADF)	59.55
Ether extract (EE)	2.65
Nitrogen-free extract	14.00
Ash	30.13
Calcium	1.50
Phosphorus	0.03
Fiber physical characteristics	
Water-holding capacity (g/mL)	6.25
Swelling capacity (g/mL)	6.17

### 2.2. Growth Performance

Body weight (BW) was recorded at the beginning of the experiment (day 0) and on days 30 and 60. Feed intake was measured simultaneously. Average daily gain (ADG), average daily feed intake (ADFI), and the feed-to-gain ratio (F: G) were calculated to evaluate growth performance.

### 2.3. Determination of Serum Biochemical, Immunoglobulins, and Antioxidant-Related Indices

At the end of the experiment, one pig from each replicate was selected for blood sampling, blood samples were collected from the anterior vena cava of the experimental pigs using anticoagulant blood collection tubes, and the samples were centrifuged at  $3000 \times g$  and  $4^\circ\text{C}$  for 15 min; the supernatant was collected for the determination of serum biochemical, immunoglobulins, and antioxidant-related indices. Serum levels of glucose (GLU), triglyceride (TG), total protein (TP), and blood urea nitrogen (BUN) were measured using commercial assay kits from Chengdu Makalu Biotechnology Co., Ltd., China, on an automatic biochemical analyzer (Erba XL-200, Germany), with the specific procedures carried out following the corresponding kit instructions.

Blood ammonia and creatinine were determined using a commercial assay kit (Beijing Boxbio Science & Technology Co., Ltd.). Serum amino acids were analyzed using a high-performance liquid chromatography system (LC-20AT, Japan). Immunoglobulins in serum, including immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM), were detected with assay kits purchased from Shanghai Enzyme-Linked Biotechnology Co., Ltd., and the specific operations were performed in accordance with the kit instructions. Serum catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturers' instructions.

### 2.4. Nutrient Composition and Fiber Physical Characteristics Determination, and Apparent Total Tract Digestibility Calculation

At the end of the experiment, approximately 200 g of fresh fecal samples were collected from three randomly selected pigs per pen and thoroughly mixed to form a composite sample. The samples were dried at  $65^\circ\text{C}$  for 48 h to obtain air-dried material for subsequent analysis. Dry matter (DM), CP, EE, Ca, and ash content in both feed and fecal samples were analyzed using AOAC official methods 930.15, 990.03, 920.39, 967.30, and 942.05, respectively. Gross energy (GE) was determined via adiabatic bomb calorimetry (Parr 6300, Parr Instrument Company, Moline, IL, USA). The contents of NDF and acid detergent fiber (ADF) were quantified according to the protocols described by Van

Soest [18]. Furthermore, the water-holding capacity and swelling capacity of poplar wood composite fiber were determined according to the methods described by previous studies [19,20]. Acid-insoluble ash (AIA) was used as an indigestible marker, and the apparent digestibility of dietary nutrients was evaluated according to the procedures of the Association of Official Analytical Chemists (AOAC, 942.05) [21].

The ATTD was calculated using the following formula:

$$\text{ATTD} = [1 - (A_{\text{feed}} * A_{\text{feces}}) / (N_{\text{feed}} * N_{\text{feces}})] \times 100,$$

where:  $A_{\text{feed}}$  = Content of AIA in feed (%);  $A_{\text{feces}}$  = Content of AIA in feces (%);  $N_{\text{feed}}$  = Content of a certain nutrient in feed (%);  $N_{\text{feces}}$  = Content of a certain nutrient in feces (%).

### 2.5. Fecal Microbiota Analysis

On the final day of the experiment, fresh fecal samples were collected via rectal massage and then frozen and stored at -80 °C for subsequent analysis. Following the procedures described in a previous study [22], total microbial DNA was extracted from fecal samples using a commercial kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR products were purified with a DNA purification kit (Axegen Biosciences, USA). Purified amplicons were sequenced on a high-throughput sequencing platform. Sequence data were processed using the DADA2 pipeline to denoise reads and generate amplicon sequence variants (ASVs) under default parameters. Taxonomic assignment of all ASVs was performed in QIIME 2 using a naïve Bayes classifier against the SILVA 138 reference database. Alpha diversity indices were calculated using Mothur (version 1.30), whereas beta diversity was analyzed using the vegan package (version 3.3.1). Principal coordinate analysis (PCoA) based on Bray–Curtis distances was conducted to visualize differences in microbial community composition, and analysis of similarities (ANOSIM) was applied to assess the statistical significance of group separation. Differences in microbial taxa between treatments were further evaluated using the Wilcoxon rank-sum test. All bioinformatic analyses were performed using the Majorbio Cloud Platform (<https://cloud.majorbio.com/>) (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

### 2.6. Statistical Analysis

All experimental data were analyzed using SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, USA). Differences between treatments were evaluated using the Student's t-test. Results are presented as mean ± standard error of the mean (SEM). Statistical significance was declared at  $P < 0.05$ .

## 3. Results

### 3.1. Growth Performance and Apparent Total Tract Nutrients Digestibility

Dietary supplementation with PWCF had no significant effects on BW or F: G in growing pigs ( $P > 0.05$ ) (Table 3). In addition, during the period from days 31 to 60, pigs in the FF group exhibited slightly higher ADG and ADFI compared with the CT group, although these differences were not statistically significant ( $P > 0.05$ ). As shown in Table 4, no significant differences were observed between the FF group and CT group in the apparent digestibility of DM, CP, calcium, P, EE, or GE ( $P > 0.05$ ).

**Table 3.** Growth performance of growing pigs fed diets in which wheat bran was partially replaced by poplar wood composite fiber.

Items	Treatment groups		SEM	P-value
	CT	FF		
Body weight, kg				
0d	47.4	47.7	0.81	0.511
30d	71.9	71.5	1.18	0.777
60d	99.3	99.2	1.85	0.965
Average daily gain, g/d				
1~30d	817	791	29.3	0.584
31~60d	911	923	31.6	0.746
1~60d	864	861	27.7	0.875
Average daily feed intake, g/d				
1~30d	2151	2104	62.9	0.632
31~60d	2656	2695	65.3	0.819
1~60d	2403	2400	58.7	0.969
Average daily feed intake: Average daily gain (F: G)				
1~30d	2.63	2.67	0.05	0.586
31~60d	2.92	2.93	0.05	0.870
1~60d	2.78	2.80	0.04	0.667

CT = basal diet; FF = CON + 2% poplar wood composite fiber (replacing 2% wheat bran).

**Table 4.** Effects of partial replacement of wheat bran with poplar wood composite fiber on apparent total tract digestibility in growing pigs.

Items	Treatment groups		SEM	P-value
	CT	FF		
Dry matter, %	79.2	78.8	1.16	0.825
Crude protein, %	75.3	77.2	1.71	0.464
Calcium, %	34.8	36.4	4.49	0.810
Phosphorus, %	35.8	39.0	4.06	0.590
Ether extract, %	72.9	75.0	2.20	0.512
Gross energy, %	80.2	80.6	1.18	0.807

CT = basal diet; FF = CON + 2% poplar wood composite fiber (replacing 2% wheat bran).

### 3.2. Serum Biochemical Parameters

There were no significant differences observed in serum GLU, TC, TP, creatinine, or blood ammonia concentrations between the FF group and the CT group on either day 30 or day 60 ( $P > 0.05$ ) (Table 5). However, on day 60, serum BUN levels tended to be lower in the FF group than in the CT group ( $P = 0.084$ ). In addition, the BUN/creatinine ratio was not significantly affected on day 30 ( $P > 0.05$ ), whereas it was significantly lower in the FF group than in the control group on day 60 ( $P < 0.05$ ).

**Table 5.** Effects of partial replacement of wheat bran with poplar wood composite fiber on serum biochemical parameters in growing pigs.

Items	Treatment groups		SEM	P-value
	CT	FF		
Glucose, mmol/L				
30 d	5.47	4.97	0.272	0.210
60 d	4.44	4.56	0.187	0.656
Triglyceride, mmol/L				
30 d	0.22	0.27	0.030	0.115

60 d	0.22	0.29	0.025	0.194
	Total protein, mg/mL			
30 d	53.2	52.2	1.97	0.715
60 d	56.6	58.5	1.84	0.498
	Blood urea nitrogen, mmol/L			
30 d	0.57	0.68	0.053	0.197
60 d	0.72	0.58	0.057	0.084
	Creatinine, $\mu$ mol/mL			
30 d	0.22	0.22	0.009	0.576
60 d	0.23	0.21	0.012	0.490
	Blood ammonia, $\mu$ mol/mL			
30 d	0.33	0.31	0.013	0.233
60 d	0.34	0.30	0.024	0.194
	Blood urea nitrogen/ Creatinine			
30 d	2.71	3.10	0.310	0.388
60 d	3.22	2.64	0.190	0.048

CT = basal diet; FF = CON + 2% poplar wood composite fiber (replacing 2% wheat bran).

### 3.3. Serum Free Amino Acid Profiles

As shown in Table 6, on day 30, no significant differences were observed in the serum concentrations of aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, isoleucine, lysine, or leucine between the FF group and the CT group ( $P > 0.05$ ). However, the concentrations of phenylalanine and total free amino acids (TFAA) tended to be lower in the FF group than in the CT group ( $P = 0.082$  and  $P = 0.059$ , respectively). On day 60, no significant differences were observed in the concentrations of aspartic acid, glutamic acid, serine, histidine, glycine, arginine, tyrosine, valine, methionine, isoleucine, phenylalanine, lysine, or leucine between the two groups ( $P > 0.05$ ). However, the FF group showed significantly lower concentrations of threonine and TFAA than the CT group ( $P < 0.05$ ), and alanine tended to be decreased ( $P = 0.065$ ).

**Table 6.** Effects of partial replacement of wheat bran with poplar wood composite fiber on serum free amino acids in growing pigs.

Items	Treatment groups		SEM	P-value
	CT	FF		
30 d (mg/L)				
Aspartic acid	8.49	8.04	0.692	0.660
Glutamic acid	40.23	37.24	2.314	0.383
Serine	24.75	22.01	1.778	0.324
Histidine	54.08	52.08	2.305	0.562
Glycine	94.50	80.56	6.504	0.160
Threonine	25.59	27.14	3.771	0.779
Arginine	43.34	36.45	3.819	0.219
Alanine	61.89	54.66	3.532	0.165
Tyrosine	34.41	29.49	2.793	0.234
Valine	41.64	40.93	1.660	0.776
Methionine	17.95	18.42	1.582	0.834
Isoleucine	21.58	21.72	1.226	0.937
Phenylalanine	25.41	30.44	1.924	0.082
Lysine	40.93	39.51	2.705	0.715
Leucine	32.09	30.37	1.761	0.505
Total free amino acid	572.35	526.09	13.221	0.059
60 d (mg/L)				
Aspartic acid	8.32	6.43	0.772	0.102

Glutamic acid	34.42	26.46	2.968	0.074
Serine	17.40	15.39	1.470	0.361
Histidine	12.22	10.50	1.308	0.383
Glycine	78.54	68.74	4.615	0.154
Threonine	47.64	20.59	4.432	<0.001
Arginine	36.38	31.77	5.268	0.545
Alanine	39.68	30.33	3.252	0.065
Tyrosine	21.90	20.67	2.742	0.757
Valine	34.56	32.68	1.889	0.497
Methionine	9.33	11.86	1.513	0.252
Isoleucine	15.33	14.47	1.359	0.692
Phenylalanine	11.64	11.62	1.123	0.991
Lysine	36.30	29.31	4.487	0.329
Leucine	28.82	25.33	1.852	0.228
Total free amino acid	433.97	357.65	15.190	0.010

CT = basal diet; FF = CON + 2% poplar wood composite fiber (replacing 2% wheat bran).

### 3.4. Serum Immunoglobulin Levels and Antioxidant-Related Indices

As shown in Table 7, no significant differences were observed in the serum concentrations of IgG, IgA, or IgM between the FF group and CT group, both on day 14 and day 28 ( $P > 0.05$ ). As for the antioxidant-related indices, as shown in Table 8, on day 14, compared with the control group, the FF group significantly increased the serum CAT concentration in growing pigs ( $P < 0.05$ ), but had no significant effects on MDA or SOD levels ( $P > 0.05$ ). However, on day 28, no significant differences were observed in serum MDA, CAT, or SOD levels between the FF group and the control group.

**Table 7.** Effects of partial replacement of wheat bran with poplar wood composite fiber on immune parameters in growing pigs.

Items	Treatment groups		SEM	P-value
	CT	FF		
	Immunoglobulin G, mg/mL			
30 d	34.9	35.0	0.546	0.853
60 d	34.4	34.0	0.864	0.644
	Immunoglobulin A, $\mu$ g/mL			
30 d	181	182	3.99	0.818
60 d	184	182	2.92	0.571
	Immunoglobulin M, mg/mL			
30 d	3.67	3.77	0.071	0.356
60 d	3.60	3.45	0.088	0.282

CT = basal diet; FF = CON + 2% poplar wood composite fiber (replacing 2% wheat bran).

**Table 8.** Effects of partial replacement of wheat bran with poplar wood composite fiber on serum biochemical parameters in growing pigs.

Items	Treatment groups		SEM	P-value
	CT	FF		
	Malondialdehyde, nmol/mL			
30 d	4.18	3.89	0.157	0.120
60 d	3.84	3.73	0.148	0.642
	Catalase, U/mL			
30 d	9.80	10.87	0.224	0.013
60 d	10.34	10.61	0.420	0.615
	Superoxide dismutase, U/mL			



**Figure 1.** Effects of partial replacement of wheat bran with poplar wood composite fiber on fecal microbial composition in growing pigs. (A) ACE index; (B) Chao1 index; (C) Shannon index; (D) Simpson index; (E) PCoA based on Bray–Curtis distances at the genus levels; (F) Microbial community composition at the genus level; (G) Differential taxa identified by the Wilcoxon rank-sum test. CT = basal diet; FF = CON + 2% poplar wood composite fiber (replacing 2% wheat bran).

## 4. Discussion

In traditional nutrition research, DF has long been regarded as an anti-nutritional factor because it cannot be degraded by endogenous digestive enzymes and may reduce nutrient digestibility. Therefore, diets formulated for monogastric animals have typically contained relatively low levels of DF [23]. Although monogastric animals cannot directly utilize DF, gut microorganisms possess the capacity to ferment fiber substrates, producing beneficial microbial metabolites that may exert probiotic effects on the host [24]. At present, the application of DF in swine nutrition remains challenging, as multiple factors must be considered, including the wide variety of fiber sources, the complexity of fiber composition, and differences among pig breeds [25]. In this study, wheat bran was partially replaced with a defined proportion of DF to evaluate the effects of DF supplementation on growth performance, immune status, nutrient digestibility, and gut microbiota in growing pigs.

### 4.1. Partial Replacement of Wheat Bran with PWCF Did Not Affect Growth Performance in Growing Pigs

DF levels in pig diets are often restricted because of their potential anti-nutritional properties, which may reduce the digestibility of protein and energy [26]. In the present study, partial replacement of wheat bran with PWCF did not adversely affect average daily gain or feed conversion ratio in growing pigs. These findings suggest that PWCF can be incorporated at a low inclusion level without compromising growth performance. Consistent with our results, Wang et al. reported that pigs fed diets containing 5%, 10%, or 15% alfalfa meal exhibited improved feed utilization efficiency, whereas ADFI and ADG were not significantly affected [27]. Together, these results indicate that moderate supplementation with certain fiber sources may be well tolerated in growing pigs and may not impair productive performance. Consistent with the present findings, previous studies have shown that moderate DF inclusion does not necessarily impair growth performance in growing pigs. However, other research has reported that high-fiber diets may reduce ADG and increase the F: G. For instance, when DF levels increased from 5% to 7%, ADG was significantly decreased in growing pigs [28,29]. These results indicate that excessive DF inclusion can exert negative effects on growth performance. Similarly, pigs fed high-fiber diets have been reported to exhibit poorer growth outcomes, including reduced carcass weight and dressing percentage, as well as a higher carcass fat iodine value [30]. Such adverse effects may be attributed to the fact that DF can increase digesta viscosity and limit interactions between nutrients and digestive enzymes in the small intestine, thereby reducing nutrient digestion and absorption [31].

### 4.2. Partial Replacement of Wheat Bran with PWCF Had No Effects on the Apparent Total Tract Digestibility of Nutrients in Growing Pigs

Apparent total tract digestibility is an important indicator for evaluating feed efficiency, improving swine production performance, and reducing environmental burden [32]. Previous studies have shown that increasing DF levels to 6.86% significantly decreased the digestibility of CP, EE, CF, and ADF in growing pigs [33]. Similarly, in corn-based diets, increasing DF content resulted in a linear reduction in the ATTD of CP, DM, ash, and organic matter [34]. This decline may be attributed to the increased proportion of plant cell wall components associated with DF sources, which are generally resistant to digestion [35]. Other studies have also reported that the digestibility of DM, GE, and NDF decreases as DF levels increase in growing pig diets [36]. In addition, DF fractions have been negatively correlated with DE and metabolizable energy concentrations, indicating that high-fiber diets may reduce energy utilization in pigs [37]. However, in the present

study, supplementation with PWCF did not exert any negative effects on ATTD, suggesting that this fiber source can effectively substitute wheat bran at the tested inclusion level.

#### *4.3. Effects of Partial Replacement of Wheat Bran with PWCF on Immune Function and Serum Biochemical Parameters in Growing Pigs*

The content of immunoglobulins in serum can directly reflect the immune capacity of the organism. In the present study, supplementation of PWCF in the diet to partially replace wheat bran exerted no adverse effects on the immune capacity of growing pigs. However, several studies have shown that high-fiber diets reduce the contents of IgM and IgG in the plasma of growing pigs, impair their immune capacity, and induce certain intestinal damage [25]. Relevant studies have demonstrated that TP is closely associated with protein absorption and utilization [38]. Meanwhile, GLU, TG, LDL, HDL, TC, and other lipid metabolism indices are closely associated with fat deposition and metabolism in pigs [39]. However, DF supplementation reduces the contents of GLU and TG in the blood of growing pigs, which may be associated with the increased viscosity and oil-holding capacity of high-DF diets, thereby slowing down the diffusion of glucose and free fatty acids across the intestinal epithelium [40,41]. In the present experiment, supplementation of PWCF in the diet did not reduce the contents of GLU and TG, which indicates that dietary supplementation of this fiber exerts no adverse effects on lipid absorption in growing pigs. Meanwhile, serum CAT concentration was significantly increased in growing pigs on day 30. Although CAT does not directly participate in nitrogen metabolism, its role in scavenging H<sub>2</sub>O<sub>2</sub>, protecting nitrogen-metabolizing enzymes, and maintaining redox homeostasis may indirectly contribute to the normal progression of nitrogen metabolism [42].

#### *4.4. Effects of Partial Replacement of Wheat Bran with PWCF on Nitrogen Metabolism in Growing Pigs*

BUN is negatively correlated with dietary efficiency and lean tissue deposition [43]. Therefore, plasma BUN concentration serves as an indicator of dietary protein supply and utilization [44]. Supplementation of PWCF in the present study reduced the levels of BUN in serum, and this observation is contrary to the findings of Malmlof and Lenis et al., who found that feeding pigs high-fiber diets decreased the postprandial mean concentrations of urea in portal and arterial blood [45,46]. However, the results of the present study are also in contrast to those of Van Der Meulen, who demonstrated that when corn starch was completely replaced with raw potato starch in growing pigs, the postprandial mean concentrations of urea in portal and arterial blood were increased compared with the low-fiber control group [47]. Changes in blood urea concentration depend on the dietary protein level and the fermentability of DF [48]. Therefore, based on the concept of ideal protein ratio, the use of DF combined with a reduced dietary protein level may lower the BUN concentration, thereby decreasing urea excretion via urine. In summary, compared with other DF, PWCF can effectively replace wheat bran and reduce the content of BUN in the serum of growing pigs.

The BUN/creatinine ratio is considered a sensitive indicator for evaluating protein catabolism, and its variation depends on the balance between BUN production and creatinine excretion [49]. In the present study, no significant difference in the BUN/creatinine ratio was observed between the two groups on day 30, whereas the ratio was significantly lower in the FF group on day 60. This change was primarily driven by the declining trend in BUN, while creatinine concentration remained stable throughout the experimental period, suggesting that the reduced ratio was mainly attributable to a decrease in protein catabolism [50]. Mechanistically, long-term FF treatment may suppress muscle protein degradation or amino acid deamination, thereby reducing ammonia production and subsequently decreasing hepatic urea synthesis, which ultimately leads to a lower BUN concentration.

As a precursor for urea synthesis, blood ammonia can help explain the mechanism underlying the decrease in BUN [51]. In the present study, blood ammonia concentration did not differ significantly between the two groups and did not decrease in parallel with BUN. This may suggest that reduced ammonia generation was not the sole direct cause of the decline in BUN. At the same

time, the stable blood ammonia concentration indicates that FF treatment did not impair hepatic ammonia detoxification capacity, thereby maintaining a safe and stable nitrogen metabolic state.

The plasma amino acid profile directly reflects amino acid metabolic homeostasis [52]. On day 30, FF treatment only tended to reduce total amino acid concentration, indicating that the animals may have maintained metabolic balance through compensatory regulation. However, on day 60, TFAA concentration was significantly reduced, with threonine showing a particularly marked decline. The decrease in total amino acids may be associated with altered amino acid transport, accelerated amino acid catabolism, or reduced intestinal absorption, which may, in turn, feed back to suppress urea synthesis and thereby contribute to the reduction in BUN. In addition, the decrease in essential amino acids may limit protein synthesis [53]. As an essential amino acid, the marked reduction in threonine may be related to reduced endogenous release due to lower protein breakdown, as well as possible interference with intestinal absorption. Long-term threonine deficiency may further impair intestinal mucosal barrier integrity, immune function, and energy metabolism [54].

In summary, FF treatment may reduce BUN concentration and the BUN/creatinine ratio by suppressing protein catabolism and amino acid deamination, thereby decreasing ammonia production and hepatic urea synthesis. Meanwhile, FF treatment reshaped amino acid homeostasis, leading to significant decreases in TFAAs and threonine, whereas stable blood ammonia concentration ensured the safety of nitrogen metabolism.

#### 4.5. Effects of Partial Replacement of Wheat Bran with PWCF on Gut Microbiota in Growing Pigs

The gut microbial community is composed of various bacterial species in specific proportions, among which interspecific interactions constrain each other's functions and enable mutual dependence to establish an ecological balance [55]. The gut microbiota plays a pivotal role in the interactions between diet and host physiology, and represents one of the most important determinants of intestinal health [56]. DF acts as a substrate during fermentation and facilitates the proliferation of selective microbiota, thereby leading to alterations in the composition of the gut microbiota [23]. It has been well established that DF exerts a positive effect on maintaining the diversity of the gut microbial community and intestinal health in pigs [57]. PWCF is rich in hemicellulose dominated by xylan, which has a main chain composed of xylose residues, simple side chains, and a low degree of branching, making it an excellent carbon source for intestinal fiber-degrading bacteria [58].

Compared with the CT group, the FF group exhibited a higher relative abundance of *Treponema*, Lachnospiraceae, and Prevotellaceae. *Treponema* is a typical fiber-degrading spirochete whose genome is enriched in coding genes for xylanase, endoglucanase, and other hydrolases, enabling it to efficiently hydrolyze the xylan backbone. The high xylan content in poplar wood provides a specific substrate for this genus, allowing it to dominate the fiber-degrading ecological niche [59]. Although wheat bran also contains arabinoxylan, it has numerous and highly branched side chains, making it more readily utilized by rapidly fermenting bacteria (e.g., *Lactobacillus*, Ruminococcaceae), and thus exhibits lower selectivity for such fiber-degrading bacteria than the high xylan in poplar wood [60]. Meanwhile, PWCF contains a certain proportion of lignin yet does not reach a high degree of lignification; it is mainly distributed in the intercellular layers and cell corners, forming a "fiber-lignin" composite structure [61]. Prevotellaceae is sensitive to fluctuations in ammonia concentration and pH [62]. Moderate lignin retards protein degradation and reduces ammonia accumulation, thus favoring its growth more; whereas wheat bran, with a high protein content and rapid fermentation, easily leads to elevated ammonia concentrations and inhibits Prevotellaceae [63].

On the other hand, PWCF is rich in hemicellulose dominated by xylan, which has a main chain composed of xylose residues, simple side chains, and a low degree of branching, making it an excellent carbon source for intestinal fiber-degrading bacteria [64]. Relevant studies have demonstrated that *Treponema*, Lachnospiraceae, and Prevotellaceae are common xylan-degrading

genera and families with abundant xylanases, indicating that supplementation with PWCF can effectively increase the relative abundance of DF-degrading genera [59,65,66]. Wang et al. also reported similar findings that the proportions of Prevotellaceae, Ruminococcaceae, and Lachnospiraceae are positively correlated with DF intake. These bacterial families are associated with the fermentation of plant-derived non-starch polysaccharides into short-chain fatty acids (SCFAs). *Treponema* has the capacity to degrade DF, and it may enhance the metabolism of succinic acid and lactic acid during the degradation process, thereby acidifying the intestinal tract and inhibiting pathogenic bacteria to promote nutrient absorption and improve intestinal health [67].

Likewise, several studies have demonstrated that DF supplementation in the diet promotes the growth of beneficial bacteria (e.g., Lachnospiraceae and Prevotellaceae) and inhibits the growth of pathogenic bacteria, thereby exerting a certain anti-inflammatory effect. This finding is consistent with the results of the present study: the proportion of Lachnospira in the FF group was significantly higher than that in the CT group, demonstrating that DF supplementation can effectively reduce the incidence of intestinal inflammation and decrease the risk of intestinal microbial dysbiosis [68,69].

The present study only analyzed the changes in the relative abundance of *Treponema*, Lachnospiraceae, and Prevotellaceae, but did not determine their core metabolites, especially SCFAs such as acetic acid, propionic acid, and butyric acid, making it impossible to directly verify whether the alterations in microbial abundance are actually translated into differences in metabolic functions. These results indicated that PWCF can alter the composition of the gut microbiota and increase the relative abundance of bacterial genera capable of degrading PWCF, whereas whether it exerts beneficial effects on intestinal barrier function requires further investigation.

## 5. Conclusion

The present study demonstrated that long-term partial replacement of wheat bran with PWCF in growing pig diets did not impair growth performance, immune status, apparent total tract digestibility of nutrients, or serum biochemical parameters. However, it could regulate nitrogen metabolism by reducing BUN concentration and the BUN/creatinine ratio, as well as decreasing TFAAs in serum. This dietary intervention also reshapes the gut microbial community and increases the relative abundance of dietary fiber-degrading bacterial taxa. These findings suggest that PWCF can serve as an effective alternative fiber source to partially substitute wheat bran, providing a theoretical basis for diversifying fiber ingredients in swine feed formulation.

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## Abbreviations

The following abbreviations are used in this manuscript:

ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AIA	Acid-insoluble ash
AOAC	Association of Official Analytical Chemists
ASV	Amplicon sequence variants
ATTD	Apparent total tract digestibility
BUN	Blood urea nitrogen
BW	Body weight
CAT	Catalase
CF	Crude fiber
CP	Crude protein
CT	Control group
DM	Dry matter
FF	Fiber treatment group
F: G	Feed-to-gain ratio
GE	Gross energy
GLU	Glucose
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MDA	Malondialdehyde
NDF	Neutral detergent fiber
PCoA	Principal coordinate analysis
PWCF	Poplar wood composite fiber
SCFAs	Short-chain fatty acids
SOD	Superoxide dismutase
TFAA	Total free amino acid
TG	Triglyceride
TP	Total protein

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