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Effects of *Saccharomyces boulardii* cell wall polysaccharide on growth performance, immunity, antioxidant ability and ileal mucosal morphology of Jinhua weaned piglets in Northern Xinjiang region

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Background: The high rates of diarrhea, mortality, and feed conversion ratio (F/G) among weaned piglets have significantly hindered the development of pig feeding practices in Jinhua, located in the North Xinjiang region. *Saccharomyces boulardii* cell wall polysaccharide (SBWP) is a probiotic that has the potential to enhance gastrointestinal immunity and health, while also reducing systemic inflammation, diarrhea rates, and mortality. This study investigates the effects of SBWP on the performance, immune capacity, antioxidant capacity, and mucosal morphology of weaned piglets from Jinhua, North Xinjiang region, through a 28-day feeding trial.

Methods: A total of 64 male weaned Jinhua piglets, aged 28 days and with a mean body weight of 4.23 ± 0.53 kg, were utilized in this study. The piglets were randomly assigned to four experimental groups, each consisting of four pens with four piglets per pen, based on varying percentages of SBWP as a feed supplement. The supplementation concentrations for the four groups were 0% (control group), 0.2%, 0.5%, and 1.0% of SBWP, respectively.

Results: Compared to the CON group, the 0.5% and 1.0% SBWP groups demonstrated a increase in average daily feed intake (ADFI) and average daily gain (ADG), alongside a decrease in the feed-to-gain ratio (F/G). Additionally, the average fecal score and diarrhea frequency in the 0.5% and 1.0% SBWP groups were reduced, with no mortality recorded in these groups, which represented a 37.50% reduction compared to the CON group. In terms of ileal mucosal immunity, the 0.5% and 1.0% SBWP supplementation groups demonstrated reduced concentrations of pro-inflammatory cytokines, including interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), as well as diamine oxidase (DAO). Conversely, both groups exhibited significantly elevated concentrations of the anti-inflammatory cytokine interleukin-10 (IL-10). Furthermore, the addition of 0.5% and 1.0% SBWP significantly increased secretory immunoglobulin A (SIgA). In serum immunity, both SBWP groups had

reduced concentrations of TNF- α , IL-1 β , IL-6 and DAO. Regarding the antioxidant capacity of the ileal mucosa, the content of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) in the 0.5% and 1.0% SBWP groups were decreased, while catalase (CAT), glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) were increased, and superoxide dismutase (SOD) activity was enhanced. Lastly, the addition of 0.5% and 1.0% SBWP to the basal diet resulted in a promotion in villus height and the ratio of villus height to crypt depth (VCR), thereby improving the morphology of the ileal mucosa. In conclusion, the growth performance of weaned piglets from Jinhua in northern Xinjiang improved by 0.5–1.0% when supplemented with SBWP in the basal diet. This enhancement is attributed to the increased antioxidant capacity, improved immune function, enhanced mucosal morphology, and reduced concentrations of inflammatory factors in both the ileal mucosa and serum.

KEYWORDS

feed additive, immunomodulation, growth evaluation, gut morphometry, swine, yeast wall

1 Introduction

The Jinhua Pig is a popular breed known for its thin skin, tender flesh, and unique flavor, particularly favored in southern China (Zhang et al., 2021). This breed originates from Jinhua in Zhejiang Province, located on the east coast of China, characterized by hot and humid weather (Gong et al., 2019). In recent years, a large-scale intensive livestock farm for Jinhua Pigs has been established in the Changji region, situated in the northern part of Xinjiang, which experiences cold, dry conditions and significant temperature fluctuations between day and night (Chen et al., 2022). Unfortunately, high rates of diarrhea and mortality among weaned piglets—exceeding 60% for diarrhea and 30% for mortality—are common in Jinhua pig farms, resulting in substantial economic losses. The primary cause of these elevated rates has been identified as stress related to weather adaptation, leading to heightened mucosal inflammatory responses and decreased nutritional intake. This cascade ultimately results in diminished intestinal mucosal immunity, oxidative injury, and increased pyroptosis of intestinal cells, thereby reducing intestinal

permeability, facilitating bacterial translocation, and increasing the incidence of mortality and diarrhea (Billoo et al., 2006). Although antibiotics are a cost-effective and rapid solution, their misuse poses significant risks, including nutritional metabolism disorders, developmental delays, and drug residues in piglets (Mengjian et al., 2020). Consequently, there is an urgent need to identify natural, green additives that could alleviate the diarrhea and mortality rates among weaned Jinhua piglets in the Xinjiang region (Dai et al., 2024). *S. boulardii* wall polysaccharides (SBWP) as a safe and effective immune activator have been shown to contain the abilities of anti-inflammatory, antioxidant, and immunomodulatory properties, and promote growing that may be able to mitigate the effects of Jinhua weaned piglet stressors (Rascón-Chu et al., 2021; Shao et al., 2021). The SBWP has a complex structure with main components of β -glucan (40%–60%), mannan (30%–40%), and chitin (2%–10%), which cannot be digested in the gastrointestinal tract, but can reduce inflammatory reaction and improve intestinal mucosal immuno-barrier by the pathogen-associated or damage-associated molecular patterns with a direct or mediate way (Mengjian et al., 2020; Liu et al., 2021). Besides, SBWP was able to improve antioxidant enzyme function and mitigate reactive oxygen species (ROS)-induced oxidation damage (Czerucka et al., 2007). Moreover, SBWP has a wide range of application prospects by wide range of sources, stable chemical properties, and non-drug resistance (Billoo et al., 2006). However, limited information exists on the impact of SBWP on Jinhua weaned piglets feeding in the North of Xinjiang region. Therefore, it is necessary to evaluate the effects of SBWP on performance, intestine, serum immunity capacity, and antioxidant capacity to minimize stress effects in Jinhua-weaned piglets in north the Xinjiang region.

Abbreviations: SBWP, *S. boulardii* Cell Wall Polysaccharide; BW, Body weight; IBW, Average initial body weight; FBW, Average final body weight; ADI, average daily feed intake; ADG, average daily weight gain; F/G, gain to feed ratio; IL-1 β , Interleukin-1 β ; IL-6, interleukin-6; IL-10, interleukin-10; TNF- α , tumor necrosis factor- α ; DAO, diamine oxidase; SigA, secretory Immunoglobulin A; PBS, phosphate buffered saline; MDA, malondialdehyde; H₂O₂, hydrogen peroxide, CAT, catalase; GSH-Px, glutathione peroxidase; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; VCR, The ratio of villus height to crypt depth; ROS, reactive oxygen species.

2 Materials and methods

The experiment was conducted in a regimental field farm, in Changji, China (43° 91 ' 22.01 " S, 87° 09 ' 93.84 " W). The experiment site is characterized by an average elevation of approximately 1200 m and an average annual temperature of 8.1°C.

2.1 Test materials

S. bouhardii was obtained from the College of Animal Science at Xinjiang Agricultural University. The SBWP was produced through a series of biotechnological processes including microbial fermentation, solvent extraction, and chromatographic purification, conducted under previously established protocols (Liu et al., 2021). Optimized ultrasound-assisted extraction parameters included 52.63% NaOH supplementation, 143.15 W ultrasonic power, and 86.20 min processing duration, achieving a maximum extraction yield of 37.54%. Primary SBWP components BLC-1 and BLC-2 exhibited molecular weights of 164.68 kDa and 13.21 kDa, constituting 24.57% and 66.08% of total composition, respectively. Monosaccharide analysis revealed BLC-1 contained 47.68% glucose and 39.18% mannose, while BLC-2 comprised 76.59% glucose and 6.86% mannose. Compositional analysis using Chinese National Standards (GB/T 6434-94, GB/T 6432-94, GB/T 6435-86) demonstrated SBWP contained 84% polysaccharides, 4% crude protein, 3% crude fat, 2% ash, and 7% moisture. Gentamicin sulfate (8 million IU/g potency) was procured from Hebei Jiupeng Pharmaceutical Co., Ltd. as a soluble powder for comparative antibiotic evaluation. The composition of the SBWP included polysaccharides ($\geq 84\%$), crude protein ($\leq 4\%$), crude fat ($\leq 3\%$), crude ash ($\leq 2\%$), and moisture ($\leq 7\%$).

2.2 Animal, diets, and experiment design

A total of 64 male Jinhua weaned piglets (4.23 ± 0.53 kg, 28 days old) were allocated to treatments based on initial body weight in a randomized complete block design, comprising four dietary treatments with four replicates ($n = 4$ animals/replicate). The treatments were arranged as a single factorial, consisting of: 1) a basal diet without supplement, 2) a basal diet supplemented with 0.2% SBWP, 3) a basal diet supplemented with 0.5% SBWP, and 4) a basal diet supplemented with 1.0% SBWP for the duration of the 28-day trial. The basal diet was formulated following the Chinese Feeding Standard (2020) for weaned piglets weighing between 5 and 8 kg and achieving a daily gain of 450 g/day, as detailed in Table 1 (Wu et al., 2024). The SBWP at varying concentrations was mixed with the basal diet in a single preparation to create a customized feed.

All weaned piglets were housed in pens measuring 2.25 x 2.04 m, which were equipped with slatted floors, one cup nipple drinker, and one feed trough. The ambient temperature was maintained between 18-23°C, and the piglets were weaned at 28 days of age.

They received 14 hours of artificial light per day and were provided with food and water *ad libitum* throughout the feeding trial, with feeding occurring four times daily at 08:00, 13:00, 18:00, and 23:00. Vaccination programs were implemented under farm management protocols.

The experimental immunization protocol was strictly implemented in accordance with the standardized vaccination program established by the production facility, with specific procedures as follows: Day 0: Pseudorabies Vaccine (Bartha-K61 strain, Intranasal instillation, 1 head dose); Day 3: Iron Dextran Supplementation (neck region, 200 mg iron/piglet); Day 7: Porcine Circovirus Type 2 Vaccine (Inactivated, Intramuscular injection, 2 mL/piglet), Day 14, Classical Swine Fever Vaccine (C-strain, Intramuscular injection); Day 21, Porcine Reproductive and Respiratory Syndrome Modified Live Vaccine (Intramuscular injection, 2 mL/piglet); Day 28 (Weaning): Mycoplasma hyopneumoniae Bacterin (Intramuscular injection, 2 mL/piglet).

Without adding antibiotics to the ingredients, the nutrient concentrations were calculated. The per kilogram of premix supply: Lysine, 2.3 g; Methionine, 1.2 g; Threonine, 0.5 g; NaCl, 0.30 g; Zn, 100 mg; Mn, 80 mg; Fe, 120 mg; Cu, 4 mg; Se, 0.25 mg; I, 0.30 mg; Co, 0.08 mg; vitamin A, 9000 IU; vitamin B1, 2.8 mg; vitamin B2, 2.2 mg; vitamin B6, 6.6 mg; vitamin B12, 0.02 mg; vitamin D, 2500 IU; vitamin E, 80 IU; vitamin K, 3.0 mg; nicotinic acid, 25 mg; D-pantothenic acid, 30 mg; folic acid, 2.0 mg; biotin, 0.2 mg; thiamine, 1.0 mg; choline, 800 mg.

2.3 Sample collection

Weaned piglets were fasted 24 hours before the collection of blood samples after the study. On the morning of the final day, blood samples (5 mL each) were obtained from each piglet via jugular venipuncture and placed into Vacutainer tubes (10 mL, Jiangsu

TABLE 1 Ingredient and mean chemical composition of basic diet (air-dry basis) %.

Ingredients		Nutrition level	
Corn	28	Dry matter	91.23
Expanded corn	30	Crude protein	18.21
Expanded soy	8	Digestible energy DE (Kcal/kg)	3694
Fermented soybean	14	Crude fat	3.5
Milk powder	1	Crude fiber	4.1
Whey powder	5	Lysine	1.25
Soybean oil	1	Methionine+Cysteine	0.73
Molasses	2.5	Calcium	0.87
Premix	5	Total Phosphorus	0.65
Dicalcium phosphate	0.7		
CaCO ₃	0.5		
NaCl	0.3		

Rongye Technology Co., Ltd.). The serum was then collected after coagulated at 4°C for 6 hours and separated by centrifugation at 2795 xg for 10 minutes at 4°C (Refrigerated centrifuge, CH-100 R, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.). The serum was subsequently stored at -80°C until cytokine analysis measurements were conducted (Zhang et al., 2021).

Two piglets from each pen were anesthetized using an intramuscular injection of 4% Nembutal solution at a dosage of 4 mg/kg body weight (BW). Following a 35-minute post-injection period, those two experimental piglets in each pen were humanely euthanized by exsanguination via severing of the neck veins. Immediately following euthanasia, a standardized 5-cm segment of the distal ileum was surgically excised and subsequently subjected to three sequential washes with sterile physiological saline (0.9% NaCl) to remove luminal contents while preserving mucosal integrity. The ileal segments were placed in aseptic tubes and immediately stored in a liquid nitrogen flask for subsequent analysis of immune and antioxidant activity, as well as histological measurements (Shanely et al., 2016). For intestinal morphological analysis, ileal tissue segments were rinsed with ice-cold 10% phosphate-buffered saline (PBS) for 3 times and promptly immersed in 10% neutral buffered formalin (NBF) solution (pH7.4) immediately following surgical resection. The tissue specimens were completely submerged in the fixative solution at a standardized tissue-to-fixative ratio of 1:10 (w/v) to ensure optimal penetration and preservation of mucosal architecture. The fixation process was maintained for precisely 24 hours under controlled ambient temperature conditions (22 ± 2°C) to prevent tissue autolysis while maintaining structural integrity.

2.4 Detection indicators and methods

2.4.1 The effect of SBWP on growth performance

The body weight of all weaned piglets was recorded at both the beginning and end of the trial, following a 24-hour fasting period. Additionally, feeders were weighed daily throughout the entire feeding trial to calculate the average daily feed intake (ADFI), average daily weight gain (ADG), and the gain-to-feed ratio (F/G) using the appropriate formulas:

$$\text{ADFI} = [\text{average daily feed intake}/\text{trail days}] \times 100\% ;$$

$$\text{ADG} = [\text{average daily weight gain}/\text{trail days}] \times 100\% ;$$

$$\text{F/G} = [\text{ADG}/\text{ADFI}] \times 100\% .$$

2.4.2 The effect of SBWP on diarrhea frequency and fecal score

Feces and mortality were continuously monitored and recorded by the same individual once daily at 19:30 throughout the study. A hierarchical system of average fecal scores was established to indicate the severity, extent, and presence of diarrhea, as detailed in Table 2. A diarrheal day was noted if the fecal score exceeded 3. A

piglet was classified as diarrheic if it had a fecal score greater than 3 for two consecutive days. After the study, the average fecal score and the average frequency of diarrhea were calculated using the following formulae (Huang et al., 2023).

Average fecal score (%)

$$= [\text{sum of fecal scores}/\text{the total number of piglets}] \times 100\% ;$$

Diarrhea frequency (%)

$$= [\text{the number of diarrhea piglets} \\ \times \text{diarrhea days}]/(\text{the total number of piglets} \\ \times \text{experimental days}) \times 100\% ;$$

2.4.3 Determination of immune parameters of serum and ileum mucosa

Serum samples and ileal segments were thawed at room temperature (26°C). A total of 1.0 g of mucosa was scraped using a scalpel, accurately weighed, and mixed with a 5.0 mL 0.9% sodium chloride solution in sterile mortars for 20 minutes. The supernatant was separated by centrifugation at 6987.5 xg for 10 minutes at 4°C, after which the tissue fragments were removed, and the supernatant was collected for immune analysis (Horn et al., 2017). Concentrations of Secretory Immunoglobulin A (SIgA), Immunoglobulin G (IgG), Immunoglobulin M (IgM), Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor Necrosis Factor-α (TNF-α), and Diamine Oxidase (DAO) were determined using an ELISA kit (Nanjing Jiancheng Bioengineering Institute Co., Ltd.) (Jiang et al., 2023).

2.4.4 Determination of antioxidant indexes of ileum mucosa

Ileal segments were thawed at room temperature (26°C). A 0.1 g piece of mucosa was scraped from the inner surface of the intestinal segment using a scalpel and placed in a 2 mL centrifuge tube, where it was thoroughly mixed with 0.9 mL of a 0.9% sodium chloride solution. The supernatant was collected following refrigerated centrifugation at 6987.5 xg for 10 minutes at 4°C, minutes. The activities of CAT, SOD, and GSH-Px, as well as the total antioxidant

TABLE 2 A hierarchical system of average fecal score.

Score	Trait
1	hard firm feces with a definite shape and dark brown color, without a fetid odor
2	slightly soft feces with a definite shape and dark brown color, without a fetid odor
3	partially formed feces with a loose shape and dark yellow color, with a light fetid odor
4	semiliquid feces with a coagulum and faint yellow, white color, with a distinct fetid odor
5	watery, mucous-like feces with blood or mucus and faint yellow, white, and grey color, with a pungent fetid odor

capacity (T-AOC), hydrogen peroxide (H₂O₂) content, and malondialdehyde (MDA) content, were measured according to the protocols outlined in the instructions of the commercial kits (Nanjing Jiancheng Bioengineering Institute Co., Ltd.) (Mahdavi et al., 2010).

2.4.5 Morphology measurements

Following the standardized fixation period, the tissue samples were carefully retrieved from the 10% NBF solution using sterile forceps. Excess fixative was gently removed by blotting on absorbent paper without applying mechanical pressure that could compromise tissue integrity. Eight slides per piglet were used to obtain the villi height and depth means. Ileal sections were cut to a thickness of 5 µm and stained with eosin and hematoxylin. Villus height and crypt depth were measured, and the ratio of villus height to crypt depth was calculated using a fully automated digital section scanning and application system (EasyScan 1, MOTIC CHINA GROUP CO., LTD.). Means of at least four villi per segment were analyzed for differences. Villus length is defined as the distance from the villus tip to the valley between the villi, while crypt depth is defined as the distance from the crypt opening to the base (Alqhtani et al., 2024).

2.5 Statistical analysis

The original data were initially calculated using Microsoft Excel (version 2022 for Windows, Microsoft Inc., Chicago, IL, USA). Statistical values and significant differences between means were analyzed and expressed as mean ± standard deviation using one-way analysis of variance (ANOVA) followed by Duncan's test, implemented in SPSS software (version 23.0 for Windows, SPSS Inc., New York, NY, USA). Specify the significance level ($P < 0.05$) and ($P < 0.01$) for the mean comparison using Duncan's test.

3 Result

3.1 The effect of SBWP on growth performance

As shown in Table 3, there was no significant statistical difference in the average initial body weight (IBW) between the groups, indicating that both the experimental design and the experimental group were appropriate. The average final body

weight (FBW), average daily gain (ADG), and average daily feed intake (ADFI) of group III were increased by 38.45%, 38.46%, and 14.86% ($P < 0.01$), respectively, compared to group I. The feed-to-gain ratio (F/G) of groups III and IV was significantly decreased by 18.4% ($P < 0.01$) and 9.33% ($P < 0.05$), respectively, than that of group I. However, there was no significant difference in F/G between groups III and IV ($P > 0.05$).

3.2 The effect of SBWP on diarrhea incidence

As shown in Table 4, the average diarrhea frequency in group III was significantly decreased by 45.22% ($P < 0.01$) compared to group I and 23.17% ($P < 0.05$) compared to group II. However, there was no significant difference when compared to group IV ($P > 0.05$). Similarly, the average fecal score of group III was extremely significantly decreased by 58.14% ($P < 0.01$) compared to group I and 25.86% ($P < 0.01$) compared to group II, while again showing no significant difference with group IV ($P > 0.05$). Additionally, there were 6 and 1 cases of mortality in groups I and II, respectively, during the first-week post-weaning; notably, there were no recorded mortalities in groups III and IV throughout the entire study, regardless of treatment.

3.3 The effect of SBWP on serum immune parameters

As shown in Table 5, the concentrations of IL-1β, IL-6, and DAO in group IV were significantly reduced by 45.16%, 10.07%, and 43.21%, respectively, compared to group I ($P < 0.05$). Additionally, the level of TNF-α in group III was the lowest, showing a significant decrease of 40.73% compared to group I ($P > 0.01$). No significant statistical differences were observed in the concentrations of serum IgM, IgG, and IL-10 across all experimental groups ($P > 0.05$).

3.4 The effect of SBWP on the ileum immune parameters

As shown in Table 6, the concentrations of SIgA and IL-10 in group IV were significantly increased of 4.78% and 5.88%,

TABLE 3 Main effects of SBWP on growth performance of Jinhua weaned piglets (n=16).

	Group I	Group II	Group III	Group IV	P
IBW (kg)	4.37 ± 0.55	4.25 ± 0.48	4.03 ± 0.55	4.27 ± 0.55	0.329
FBW (kg)	7.36 ± 0.58D	8.31 ± 0.81C	10.19 ± 0.86A	9.40 ± 0.59B	0.001
ADG (kg)	0.26 ± 0.02D	0.30 ± 0.03C	0.36 ± 0.03A	0.34 ± 0.02B	0.009
ADFI (kg)	0.63 ± 0.02D	0.67 ± 0.004C	0.74 ± 0.013A	0.70 ± 0.006B	0.004
F/G	2.50 ± 0.11Aa	2.25 ± 0.09Bb	2.04 ± 0.14Bc	2.08 ± 0.06Bc	0.000

IBW, average initial body weight; FBW, average final body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, ratio of ADFI to ADG. Mean comparison using Duncan's test and the comparisons are related to rows; $p < 0.05$, significant difference; $p < 0.01$, very significant difference. Lowercase letters represent significant differences ($p < 0.05$), and uppercase letters represent extremely significant differences ($p < 0.01$).

TABLE 4 Effects of SBWP on diarrhea and mortal of Jinhua weaned piglets (n=16).

	Group I	Group II	Group III	Group IV	P
Average diarrhea frequency (head/day)	1.15 ± 0.13Aa	0.82 ± 0.07Bb	0.63 ± 0.04Bc	0.67 ± 0.09Bc	0.000
Average fecal score (/day)	3.14 ± 0.25A	2.32 ± 0.39B	1.72 ± 0.93C	1.84 ± 0.11C	0.000
Mortality rate	37.50%	6.25%	0%	0%	

Mean comparison using Duncan's test and the comparisons are related to rows; $p < 0.05$, significant difference; $p < 0.01$, very significant difference. Lowercase letters represent significant differences ($p < 0.05$), and uppercase letters represent extremely significant differences ($p < 0.01$).

respectively ($P < 0.05$) when compared to group I. In contrast, the concentrations of IL-1 β , IL-6, TNF- α , and DAO in group IV were significantly decreased of 17.16%, 11.76%, 36.67%, and 20.00% ($P < 0.01$), respectively, in comparison to group I.

3.5 The effect of SBWP on ileum antioxidant indexes

As shown in Table 7, the concentrations of CAT, GSH-Px, T-AOC, and T-SOD in the diet with 0.5% SBWP increased by 25.19% ($P < 0.01$), 18.80% ($P < 0.01$), 31.02% ($P < 0.01$), and 14.87% ($P < 0.01$), respectively. Additionally, the concentrations of MDA and H₂O₂ in group III were significantly decreased by 25.42% and 8.97% ($P < 0.01$), respectively, when compared to group I.

3.6 The effect of SBWP on ileal morphology

As shown in Table 8, the ileal villus height of group IV was significantly improved by 15.70% ($P < 0.01$) compared to group I, although it was not significantly different from group III ($P > 0.05$). However, statistical analysis revealed no significant differences in crypt depth and villus width measurements among the experimental groups ($P > 0.05$). The ratio of villus height to crypt depth (VCR) of Group III was significantly improved by 27.17% ($P < 0.01$) compared to group I, while no significant difference was observed when compared to Group IV ($P > 0.05$).

4 Discussion

4.1 The effect of SBWP on growth performance and diarrhea of Jinhua weaned piglets

The high incidence of diarrhea, mortality, and subsequent adverse effects is a significant concern for the Jinhua pig farm in the Xinjiang region. A clear link has been established between environmental stress and weaning stress. The climate of the Xinjiang region, characterized by a substantial temperature variation between day and night and extremely dry and cold conditions, contrasts sharply with that of Zhejiang province, which experiences high temperatures and humidity. Furthermore, Jinhua piglets experience weaning stress due to inadequate management and an unsuitable climate, leading to increased digestive tract inflammation, elevated rates of diarrhea and mortality, and a reduced feed conversion ratio (Zhang et al., 2021). The present study demonstrates that the addition of 0.5% SBWP improved growth performance and feed utilization in Jinhua weaned piglets in north of Xinjiang region. A scientific evidence indicated that *S. boulardii* demonstrates three principal physiological characteristics: (1) exceptional environmental stress tolerance, (2) robust antioxidant capacity, and (3) broad-spectrum antimicrobial activity (Fu et al., 2022). Furthermore, this probiotic yeast strain synthesizes a diverse array of bioactive metabolites exhibiting multifunctional therapeutic properties, including but not limited to antioxidant, antimicrobial, antineoplastic, and anti-inflammatory activities. Recent literature indicates that *S.*

TABLE 5 Effects of SBWP on Serum Immune Parameters of Jinhua Weaned Piglets (n=8).

	Group I	Group II	Group III	Group IV	P
IL-1 β (pg/ml)	358.63 ± 23.46a	271.88 ± 20.46b	223.08 ± 11.26c	196.66 ± 15.94d	0.012
IgM (mg/ml)	20.35 ± 30.2	10.53 ± 1.40	10.75 ± 0.93	10.63 ± 1.21	0.741
IL-6 (pg/ml)	709.27 ± 77.93a	693.25 ± 39.92ab	656.43 ± 31.80ab	637.84 ± 50.20b	0.044
IgG (mg/ml)	11.14 ± 1.83	11.54 ± 1.39	11.79 ± 2.17	11.50 ± 1.44	0.903
IL-10 (pg/ml)	76.21 ± 5.6	79.53 ± 1.42	75.72 ± 17.41	75.11 ± 24.67	0.940
TNF- α (pg/ml)	239.97 ± 7.87A	190.12 ± 6.41B	142.22 ± 4.60D	165.36 ± 5.33C	0.000
DAO (μ g/ml)	0.81 ± 0.31a	0.78 ± 0.15a	0.46 ± 0.10b	0.43 ± 0.38b	0.018

IL-1 β , Interleukin-1 β ; IgM, Immunoglobulin M; IL-6, Interleukin-6; IgG, Immunoglobulin G; IL-10, Interleukin-10; TNF- α , Tumor Necrosis Factor- α ; DAO, Diamine Oxidase. Mean comparison using Duncan's test and the comparisons are related to rows; $p < 0.05$, significant difference; $p < 0.01$, very significant difference. Lowercase letters represent significant differences ($p < 0.05$), and uppercase letters represent extremely significant differences ($p < 0.01$).

TABLE 6 Effects of SBWP on ileum immune parameters of Jinhua weaned piglets (n=8).

	Group I	Group II	Group III	Group IV	P
SigA ($\mu\text{g}/\text{mg}$)	5.24 \pm 0.13b	5.27 \pm 0.21b	5.49 \pm 0.24a	5.45 \pm 0.21ab	0.039
IL-1 β (pg/g)	690.00 \pm 60.57A	631.74 \pm 19.07B	571.63 \pm 19.17C	557.93 \pm 17.19C	0.000
IL-6 (pg/g)	1309.71 \pm 55.01A	1167.32 \pm 141.31B	1156.14 \pm 33.54B	1174.82 \pm 97.76B	0.007
IL-10 (pg/g)	526.29 \pm 15.07b	530.15 \pm 52.19ab	556.48 \pm 7.86a	557.25 \pm 9.19a	0.045
TNF- α (pg/g)	798.83 \pm 32.74A	601.31 \pm 8.55B	505.89 \pm 16.56D	549.67 \pm 30.96C	0.000
DAO ($\mu\text{g}/\text{mg}$)	0.15 \pm 0.002A	0.14 \pm 0.01B	0.12 \pm 0.0046D	0.13 \pm 0.0043C	0.000

SigA, Secretory Immunoglobulin A; IL - 1 β , Interleukin - 1 β ; IgM, Immunoglobulin M; IL - 6, Interleukin - 6; IL - 10, Interleukin - 10; TNF - α , Tumor Necrosis Factor - α ; DAO, Diamine Oxidase.

Mean comparison using Duncan's test and the comparisons are related to rows; p <0.05, significant difference; p <0.01, very significant difference.

Lowercase letters represent significant differences (p < 0.05), and uppercase letters represent extremely significant differences (p < 0.01).

boulardii and its polysaccharide composition can mitigate *Escherichia coli* and diarrhea associated with weaning stress (Olga et al., 2024). AJL Mercado reported that *S. boulardii*, when used as a dietary supplement, significantly improved average daily gain (ADG) and reduced feed-to-gain (F/G) ratio and the incidence of diarrhea in early-weaned piglets (Mercado et al., 2022). M. Manafi found that probiotic *Bacillus* species and *S. boulardii* could enhance performance in broiler chickens (Manafi et al., 2018). Additionally, Wenxiu Zhang reported that diets supplemented with *S. boulardii* mafic-1701 improved the feed conversion ratio in weaned piglets (Zhang et al., 2020). The results of the study on the effects of SBWP on growth performance and diarrhea in weaned piglets in Jinhua were consistent with findings from previous research employing a similar experimental design. This study demonstrated that the inclusion of 0.5%-1.0% SBWP as a dietary supplement not only significantly improved Average Daily Feed (ADF) and Average Daily Gain (ADG) but also reduced the incidence of Feed-to-Gain (F/G) ratio, diarrhea, and mortality among the weaned piglets in Jinhua. Specifically, there were six deaths in group I and one death in group II, whereas no deaths occurred in groups III and IV throughout the trial. The primary mechanisms underlying these improvements may involve the reduction of inflammatory stress and enteral bacterial translocation, which contribute to enhanced intestinal health and growth performance in the weaned piglets of Jinhua. This study suggests that the incorporation of 0.5%-1.0% SBWP could lead to improved growth performance in weaned piglets in the Xinjiang region, warranting further validation in a large-scale production trial.

4.2 The effect of SBWP on the immune capacity of serum and ileum

SBWP, a type of polysaccharide, possesses a complex stereoscopic structure with a molecular weight ranging from 100 to 500 kDa. It primarily consists of β -glucan, mannan, and chitin, which contribute to its biological activity (Bzducha-Wróbel and Kieliszek, 2013; Rascón-Chu et al., 2021). The β -glucan and mannan found in SBWP are recognized as common pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) such as TLR, NLR, Dectin-1, and C-type lectin receptors, which are dependent on receptor gene expression (Czerucka et al., 2007). Furthermore, SBWP modulates the expression of immune factor genes by stimulating transmembrane receptor-related immune signaling pathways, including the TLR-MyD88-NF- κ B, complement receptor CR3-SYK, CR3-PI3K-MAPK, and TNFR-MAPK signaling pathways. This modulation enhances intestinal mucosal immune barrier function and reduces inflammatory cytokine concentrations (Pothoulakis, 2010). FBD Laguna demonstrated that the inflammatory response was diminished, and the physical barrier of the intestinal mucosa was improved by administering *Saccharomyces cerevisiae boulardii* CNCM I-1079 following vaccination (De Laguna et al., 2022). Mairead K. Heavy reported that pharmacodynamic parameters, such as colonic cytokine expression profiles and histological inflammation scores, were significantly improved and restored to healthy concentrations in studies utilizing *S. boulardii* as an extracellular matrix probiotic, which enhanced intestinal

TABLE 7 Effects of SBWP on Ileum Antioxidant of Jinhua Weaned Piglets (n=8).

	Group I	Group II	Group III	Group IV	P
MDA (nmol/mg prot)	0.59 \pm 0.08Aa	0.51 \pm 0.02Bb	0.44 \pm 0.04Bc	0.46 \pm 0.02Bbc	0.000
H ₂ O ₂ ($\mu\text{mol}/\text{g}$)	14.15 \pm 0.84Aa	13.51 \pm 0.23Bb	12.88 \pm 0.12Bc	13.10 \pm 0.23Bbc	0.000
CAT (U/mg prot)	18.14 \pm 1.81Cc	20.08 \pm 1.87BCb	22.71 \pm 1.03Aa	21.02 \pm 0.43ABb	0.000
GSH-Px (U/g prot)	35.51	37.86 \pm 1.83BCb	41.90 \pm 1.91Aa	40.65 \pm 0.89ABa	0.000
T-SOD (U/mg prot)	224.07 \pm 23.73b	230.81 \pm 12.45ab	257.40 \pm 42.14a	254.48 \pm 13.95a	0.037
T-AOC (mmol/mg prot)	48.00 \pm 1.00C	53.01 \pm 2.64B	62.89 \pm 3.01A	61.08 \pm 2.33A	0.000

MDA, Malondialdehyde; H₂O₂, Hydrogen Peroxide; CAT, Catalase; GSH - Px, Glutathione Peroxidase; T-SOD, Superoxide Dismutase; T-AOC, Total Antioxidant Capacity.

Mean comparison using Duncan's test and the comparisons are related to rows; p <0.05, significant difference; p <0.01, very significant difference.

Lowercase letters represent significant differences (p < 0.05), and uppercase letters represent extremely significant differences (p < 0.01).

TABLE 8 Effects of SBWP on the development of the ileal mucosal of Jinhua weaned piglets (n=8).

	Group I	Group II	Group III	Group IV	P
Villus height/ μm	281.29 \pm 11.73C	295.53 \pm 8.47B	323.15 \pm 8.34A	325.15 \pm 11.48A	0.000
Crypt depth/ μm	163.69 \pm 13.16	153.91 \pm 19.46	148.96 \pm 18.62	149.38 \pm 16.30	0.346
Villus width/ μm	137.40 \pm 16.04	138.16 \pm 16.92	140.30 \pm 15.79	135.34 \pm 20.72	0.937
VCR	1.73 \pm 0.12Bc	1.95 \pm 0.25ABbc	2.20 \pm 0.26Aa	2.14 \pm 0.31Aab	0.001

VCR, ratio of villus height to crypt depth.

Mean comparison using Duncan's test and the comparisons are related to rows; $p < 0.05$, significant difference; $p < 0.01$, very significant difference.

Lowercase letters represent significant differences ($p < 0.05$), and uppercase letters represent extremely significant differences ($p < 0.01$).

residence time and recovery in murine colitis (Heavey et al., 2024). In our study, consistent with previous research, the addition of 0.5%-1.0% SBWP to the basal diet led to a decrease in inflammatory factors (IL-1 β , IL-6, TNF- α , DAO) in serum and ileal mucosa, alongside an increase in anti-inflammatory factors (SIgA and IL-10) in the ileal mucosa. Therefore, this study suggests that 0.5-1.0% SBWP may serve as a potential dietary supplement to enhance the innate immune system and mitigate inflammation in Jinhua-weaned piglets exposed to the unadapted climate of the Xinjiang region.

4.3 The effect of SBWP on the antioxidant capacity of ileum

Environmental maladaptation and weaning stress can induce oxidative damage, while oxidative stress arises when the delicate balance between antioxidant and oxidative systems within cells is disrupted, further compromising piglet health and performance (Jiang et al., 2011; Sajjad et al., 2023). MDA and H₂O₂, products of lipid peroxidation and components of the oxygen reduction pathway, are known to be highly toxic to host cells and serve as biomarkers of oxidative damage (Kheradmand et al., 2010). Endogenous antioxidant enzymes such as SOD, CAT, and GSH-Px play a crucial role in scavenging harmful reactive oxygen species, and increased activity of these antioxidant enzymes has been employed to assess non-specific immune responses that are beneficial in inhibiting free radical formation and mitigating lipid superoxide damage in cells (Suthama et al., 2023). Additionally, T-AOC is a quantitative measure representing the total contribution of all antioxidant substances, while changes in serum diamine oxidase (DAO) concentrations serve as a sensitive marker of intestinal damage (Den et al., 2017). Yeast polysaccharides may enhance antioxidant capacity by elevating the concentrations of antioxidant enzymes and reducing intracellular ROS concentrations. Although the regulatory mechanism of SBWP remains unclear and warrants further investigation at the molecular level, three potential mechanisms have been proposed: 1. SBWP may enhance the capacity of endogenous antioxidant enzymes such as SOD, CAT, and GSH-Px by acting as a primary messenger that induces gene expression regulation (Pothoulakis, 2010). 2. SBWP may directly interact with free oxygen radicals, thereby reducing cellular damage from oxidative reactions (Rascón-Chu et al., 2021). 3. The interaction of free oxygen radicals may be

inhibited by the combination of oxidase and the three-dimensional structures of SBWP, leading to a reduction in oxidative damage within the intestinal mucosa (Czerucka et al., 2007). In the present study, diets supplemented with 0.5% and 1.0% SBWP demonstrated significantly elevated concentrations of SOD, CAT, GSH-Px, and T-AOC compared to the control group, while concentrations of MDA and H₂O₂ were also significantly increased. Furthermore, the addition of 0.5% and 1.0% SBWP significantly decreased the DAO content in both serum and ileum mucus. This finding indicates that the damage to the intestinal mucosal mechanical barrier and its high permeability were alleviated, leading to an improvement in the integrity of the intestinal mucosa.

4.4 The effect of SBWP on mucosa morphology of ileum

Reports on the effects of dietary supplementation with yeast polysaccharides on intestinal mucosal morphology are limited, and the precise mechanism of action of SBWP on ileal morphology remains incompletely understood. However, it is likely associated with antimicrobial, immunomodulatory, or antioxidant properties. Yeast wall polysaccharides may play a crucial role in regulating epithelial cell integrity, mucosal immunity, and immune responses to exogenous antigens by increasing the number of intraepithelial lymphocytes and macrophages in the gut (Zhu et al., 2012). Furthermore, yeast polysaccharides may enhance intestinal morphology by modulating epithelial cell apoptosis and pyroptosis through various signaling pathways, including MAPK-MLCK and TNF-R1/TRADD/CASP3 (Chen et al., 2006). K. Kumari observed that the height of intestinal villi and the depth of crypts were increased in groups supplemented with the chicken yeast *S. boulardii* (Kumari and Susmita, 2014). Additionally, Mairead K. Heavey's research on the probiotic *S. boulardii* in the extracellular matrix indicated enhanced intestinal residence time and recovery in murine colitis, with pharmacodynamic parameters, including colon length and villus height, showing significant improvement (Heavey et al., 2024). Zhang et al. demonstrated that dietary supplementation with yeast cell wall significantly greater villus height and villus height-to-crypt depth (VH: CD) ratio measurements compared to both control groups and those supplemented with yeast extract (Zhang et al., 2005). The results of our study align with those previously reported, demonstrating that a

diet containing 0.5-1.0% SBWP effectively reversed the adverse effects of an unadapted climate on ileal villus height. Notably, our findings indicated a significant improvement in the morphological structure of the intestinal mucosa, with the integrity of the villi enhanced by 15.70%. The addition of 1.0% SBWP further improved villus height and the villus-to-crypt ratio, thereby enhancing the efficiency of nutrient absorption and promoting the health of the intestinal mucosa compared to a diet devoid of SBWP. In contrast, the ileal morphology of the control group exhibited disordered and twisted villi, with blurred and loosely arranged connective tissue sheath fibers, a reduced number of connective tissue cells, and abrupt effaced villus tips. In the groups supplemented with 0.5% and 1.0% SBWP, the ileal villi appeared clean and intact, with improvements in length, density, and distribution, devoid of twisting and disruption. Consequently, the findings of the present study indicate that dietary supplementation with 0.5%-1.0% SBWP exerts beneficial effects on growth performance, mitigates the occurrence of diarrhea, and reduces mortality rates in Jinhua piglets. These improvements are primarily attributed to the enhancement of immune function, augmentation of antioxidant capacity, optimization of mucosal morphology, and attenuation of inflammatory responses.

5 Conclusion

In conclusion, the results indicated that dietary supplementation with SBWP at 0.5% and 1% can enhance growth performance, prevent diarrhea, and reduce the mortality of Jinhua-weaned piglets under the specific conditions of northern Xinjiang region.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by College of Veterinary Medicine, Xinjiang Agriculture University. The studies were

conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

WZh: Formal Analysis, Data curation, Writing – original draft. WR: Data curation, Writing – original draft. XM: Data curation, Writing – original draft. WZe: Formal Analysis, Writing – original draft. ML: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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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