Effects of Lactobacillus plantarum, Tartary Buckwheat Flavonoids, and Their Combination on Growth Performance, Nutrient Digestibility, and Serum Indices in Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with Lactobacillus plantarum and tartary buckwheat flavonoids and their combination on growth performance, nutrient digestibility, and serum indices of weaned piglets. Fifty weaned piglets aged (35±3) days with an average body weight of (7.85±0.67) kg were selected and randomly divided into 5 groups, with 10 replicates per group and 1 piglet per replicate. The negative control group (NC group) was fed a basal diet, while the other groups were fed the basal diet supplemented with 40 mg/kg colistin sulfate (positive control group, PC group), 1×10^9 CFU/kg Lactobacillus plantarum (LP group), 40 mg/kg tartary buckwheat flavonoids (BF group), and 1×10^9 CFU/kg Lactobacillus plantarum + 40 mg/kg tartary buckwheat flavonoids (LB group). The experimental period lasted 28 days. The results showed: 1) The average daily gain of piglets in the BF group during days 1-28 was significantly higher than that in the NC and PC groups ($P<0.05$), increasing by 52.74% and 34.14%, respectively. 2) The nutrient digestibility of piglets in the NC group was significantly lower than that in the other groups ($P<0.05$); the digestibility of gross energy, dry matter, organic matter, and phosphorus in the LB group was significantly higher than that in the other groups ($P<0.05$). 3) The serum contents of total protein, albumin, urea nitrogen, glucose, free fatty acids, immunoglobulin G, immunoglobulin A, and immunoglobulin M in the BF group were significantly higher than those in the NC group ($P<0.05$). The serum total antioxidant capacity, superoxide dismutase, glutathione peroxidase, and catalase activities in the LP group were significantly higher than those in the NC and PC groups ($P<0.05$), while serum malondialdehyde content was significantly lower than that in the NC and PC groups ($P<0.05$). It can be concluded that tartary buckwheat flavonoids im-

proved the growth performance and immune function of weaned piglets, while Lactobacillus plantarum improved the antioxidant capacity of piglets, and their combined application also improved dietary nutrient digestibility.

Full Text

Effects of *Lactobacillus plantarum*, Buckwheat Flavone and Their Combination on Growth Performance, Nutrient Digestibility and Serum Indices of Weaned Piglets

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with *Lactobacillus plantarum*, buckwheat flavone, and their combination on growth performance, nutrient digestibility, and serum indices of weaned piglets. Fifty piglets at (35±3) days of age with an average body weight of (7.85±0.67) kg were randomly allocated to five groups, each consisting of 10 replicates with one piglet per replicate. The negative control group (NC) received a basal diet, while other groups received the basal diet supplemented with 40 mg/kg colistin sulfate (positive control, PC), 1×10 CFU/kg *Lactobacillus plantarum* (LP), 40 mg/kg buckwheat flavone (BF), or 1×10 CFU/kg *Lactobacillus plantarum* plus 40 mg/kg buckwheat flavone (LB). The experimental period lasted 28 days. The results showed: (1) The average daily gain (ADG) of piglets during days 1–28 in the BF group was significantly higher than that in the NC and PC groups ($P<0.05$), representing increases of 52.74% and 34.14%, respectively. (2) Nutrient digestibility in the NC group was significantly lower than in all other groups ($P<0.05$), while the LB group exhibited significantly higher digestibility of gross energy, dry matter, organic matter, and phosphorus compared to the remaining groups ($P<0.05$). (3) Serum total protein, albumin, urea nitrogen, glucose, free fatty acids, immunoglobulin G, immunoglobulin A, and immunoglobulin M concentrations in the BF group were significantly higher than those in the NC group ($P<0.05$). Serum total antioxidant capacity, superoxide dismutase, glutathione peroxidase, and catalase activities in the LP group were significantly higher than those in the NC and PC groups ($P<0.05$), whereas serum malondialdehyde concentration was significantly lower ($P<0.05$). These findings indicate that buckwheat flavone improved growth performance and immune function, *Lactobacillus plantarum* enhanced antioxidant capacity, and their combination improved nutrient digestibility in weaned piglets.

Keywords: weaned piglets; *Lactobacillus plantarum*; buckwheat flavone; growth performance; nutrient digestibility; serum indices

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Weaned piglets often experience growth depression, poor feed efficiency, diarrhea, and even mortality due to physiological, environmental, and nutritional stress factors. While sub-therapeutic antibiotics in feed can prevent disease and promote growth, concerns about bacterial resistance, drug residues, and threats to human health have prompted worldwide restrictions on antibiotic use in animal feed, making the search for alternatives critically important. Currently studied antibiotic substitutes include probiotics (oligosaccharides, beneficial bacteria), plant extracts, antimicrobial peptides, and bacteriophages. Probiotics, as safe, non-polluting, residue-free, and non-resistant biological feed additives, have been widely applied in livestock production. Our previous research demonstrated that *Lactobacillus plantarum* GF103 exhibits excellent probiotic characteristics in vitro and that dietary supplementation with this strain improves growth performance and maintains intestinal health by promoting balanced gut microbiota in weaned piglets. Plant extracts are bioactive substances derived from plants with one or more biological functions, with commonly used compounds including essential oils, polysaccharides, and flavonoids. Buckwheat flavone, present in various buckwheat tissues at concentrations of 0.51%–6.28%, possesses antioxidant, anti-cancer, antibacterial, and immune-enhancing properties. While research on flavonoid plant extracts in livestock has focused primarily on compounds like soy isoflavones, the effects of buckwheat flavone on antioxidant capacity and immune function in weaned piglets remain unexplored, and no studies have reported on the combined application of *Lactobacillus plantarum* and buckwheat flavone. Therefore, this experiment aimed to provide a theoretical basis for the use of these additives by investigating their individual and combined effects on growth performance, nutrient digestibility, and serum indices in weaned piglets.

1.1 Experimental Materials

Lactobacillus plantarum GF103 was prepared by the Laboratory of Animal Science at the Feed Research Institute of the Chinese Academy of Agricultural Sciences, with a viable count 2×10 CFU/g. Buckwheat flavone was provided by Nanjing Jingzhu Biotechnology Co., Ltd., with a flavone content 70%.

1.2 Experimental Design

A single-factor completely randomized design was employed. Fifty "Landrace × Large White" crossbred weaned piglets at (35±3) days of age with an average body weight of (7.85±0.67) kg were randomly divided into five groups, each containing 10 replicates with one piglet per replicate. The groups were: negative control (NC) fed the basal diet without antibiotics, *Lactobacillus plantarum*, or buckwheat flavone; positive control (PC) fed the basal diet plus 40 mg/kg

colistin sulfate; *Lactobacillus plantarum* group (LP) fed the basal diet plus 1×10 CFU/kg *Lactobacillus plantarum*; buckwheat flavone group (BF) fed the basal diet plus 40 mg/kg buckwheat flavone; and combination group (LB) fed the basal diet plus both 1×10 CFU/kg *Lactobacillus plantarum* and 40 mg/kg buckwheat flavone. All supplementation levels refer to active ingredient content. The 28-day experiment was divided into two phases: days 1–14 and days 15–28.

1.3 Experimental Diets and Management

A corn-soybean meal basal diet was formulated according to NRC (1998) nutrient requirements for piglets, with experimental additives incorporated as per the design. The composition and nutrient levels of the basal diet are presented in Table 1 . The premix provided per kilogram of diet: VA 8,000 IU, VD 3,000 IU, VE 20 IU, VK 2 mg, VB 4 mg, VB 3.6 mg, VB 40 mg, VB 4 mg, VB 0.02 mg, biotin 0.15 mg, folic acid 1.0 mg, D-pantothenic acid 11 mg, nicotinic acid 10 mg, antioxidant 100 mg, Cu 10 mg, Fe 80 mg, Mn 80 mg, Zn 75 mg, I 0.40 mg, Se 0.30 mg. Digestible energy was a calculated value, while other nutrients were measured values.

The trial was conducted at Yixiong Pig Farm in Changping District, Beijing. Piglets were housed on slatted floors, fed wet-mixed powder ad libitum, and provided free access to water. The house was well-ventilated with temperature maintained at 20–25°C. Manure was removed daily, and disinfection was performed twice weekly. Routine immunization and deworming procedures were followed throughout the experiment.

1.4.1 Growth Performance

Growth performance parameters included average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G). Body weight (BW) was recorded individually after overnight fasting on days 1, 15, and 29 for ADG calculation. Feed addition was recorded by group, and residual feed was weighed on days 15 and 29 for ADFI calculation. The F/G was calculated as ADFI/ADG using the following formulas:

ADG (g/d) = Total weight gain per piglet / Number of feeding days
ADFI (g/d) = Total feed intake per pen / (Number of piglets per pen × Number of feeding days)
F/G = ADFI / ADG

1.4.2 Nutrient Digestibility

Titanium dioxide (TiO) was used as an external marker for digestibility determination over a 10-day period. TiO was added to the diet at 0.1% from day 19, with a 4-day adaptation period followed by 6 consecutive days of feed and fecal sampling. Approximately 50 g of feed was collected daily from each group' s feeder and pooled by group after sampling. Fresh feces (approximately 50 g per piglet) were collected daily by group, with marked pigs not resampled on the

same day. A minimum of six piglets per group were sampled within 1–2 hours, and 10% HCl was added at 10% of fecal weight for nitrogen fixation. Daily fecal samples were stored frozen. After collection, feces were dried at 65°C for 48 hours, equilibrated at room temperature for 24 hours, and ground through a 40-mesh sieve.

Gross energy (GE) and contents of dry matter (DM), crude protein (CP), ether extract (EE), ash, calcium (Ca), and phosphorus (P) in feed and fecal samples were analyzed according to *Feed Analysis and Feed Quality Detection Technology* (3rd edition). Titanium content was determined using the method of Deng et al. [10]. Nutrient digestibility was calculated as:

Nutrient digestibility (%) = [1 - (Ti in feed / Ti in feces) × (Nutrient in feces / Nutrient in feed)] × 100

1.4.3 Serum Indices

On day 29 at 08:00, 10 mL of blood was collected from the anterior vena cava of six randomly selected piglets per group, centrifuged at 3,000 rpm for 20 minutes, and serum was stored at -20°C for analysis. Serum concentrations of total protein (TP), albumin (ALB), globulin (GLB), urea nitrogen (UN), glucose (GLU), free fatty acids (NEFA), insulin-like growth factor I (IGF-I), and growth hormone (GH) were measured. Immunological indices included immunoglobulin G (IgG), IgM, and IgA. Antioxidant indices comprised total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and malondialdehyde (MDA).

TP, ALB, GLB, UN, GLU, IgG, IgA, and IgM were measured using a KHB-1280 automatic biochemical analyzer. IGF-I and GH were determined by radioimmunoassay using a GC-1200 automatic gamma counter. NEFA, T-AOC, SOD, GSH-Px, CAT, and MDA were measured colorimetrically using an L-3180 semi-automatic biochemical analyzer.

1.5 Statistical Analysis

Data were processed using Excel 2007 and analyzed by one-way ANOVA using SPSS Version 17.0. Duncan' s multiple range test was applied when significant differences were detected. Differences were considered significant at $P<0.05$ and trending at $0.05 P<0.10$.

2.1 Effects on Growth Performance

As shown in Table 2 , initial body weight did not differ among groups ($P>0.05$), meeting experimental requirements. Final body weight in the BF group was significantly higher than in the NC and LP groups ($P<0.05$), while not differing from the LB and PC groups ($P>0.05$). During days 1–14 and 15–28, ADG did not differ significantly among groups ($P>0.05$), though BF group ADG was 65.88% and 46.42% higher than NC ($0.05 P<0.10$), and 12.37% and 49.94%

higher than PC (0.05 P<0.10), respectively. Over the entire period (days 1-28), BF group ADG was significantly higher than NC, PC, and LP groups (P<0.05), while not differing from LB (P>0.05). Although F/G did not differ significantly among groups throughout the trial (P>0.05), BF group F/G of 1.63 showed a decreasing trend compared to NC and PC (0.05 P<0.10).

2.2 Effects on Nutrient Digestibility

Table 3 shows that digestibility of GE, DM, OM, CP, EE, Ca, and P in the PC, LP, BF, and LB groups was significantly higher than in NC (P<0.05). The LB group exhibited significantly higher digestibility of GE, DM, OM, and P compared to all other groups (P<0.05).

2.3 Effects on Serum Biochemical Indices

Table 4 reveals no significant differences in serum GLB concentration among groups (P>0.05), though BF showed an increasing trend versus NC (0.05 P<0.10). Serum ALB/GLB ratio and IGF-I concentration did not differ among groups (P>0.05). Serum TP in BF was significantly higher than in PC, LP, and NC (P<0.05), while not differing from LB (P>0.05). Serum ALB in BF was significantly higher than all other groups (P<0.05). Serum UN in BF was significantly higher than in LB, LP, and NC (P<0.05), while not differing from PC (P>0.05). Serum GLU in BF and LB was significantly higher than in LP and NC (P<0.05), while not differing from PC (P>0.05). Serum NEFA in BF was significantly higher than in LP and NC (P<0.05), while not differing from LB and PC (P>0.05). Serum GH in LP was significantly higher than all other groups (P<0.05), with no differences among BF, NC, LB, and PC (P>0.05).

2.4 Effects on Serum Immune Indices

As presented in Table 5 , serum IgG in BF was significantly higher than in LP and NC (P<0.05), while not differing from LB and PC (P>0.05). Serum IgA in BF was significantly higher than all other groups (P<0.05), with no differences among LB, NC, PC, and LP (P>0.05). Serum IgM in BF was significantly higher than in PC, LP, and NC (P<0.05), while not differing from LB (P>0.05); LB IgM was significantly higher than NC (P<0.05), while not differing from PC and LP (P>0.05).

2.5 Effects on Serum Antioxidant Indices

Table 6 demonstrates that serum T-AOC in LP was significantly higher than in PC, BF, and NC (P<0.05), while not differing from LB (P>0.05). Serum SOD activity in both LP and LB was significantly higher than in PC, BF, and NC (P<0.05). Serum GSH-Px activity in LP was significantly higher than all other groups (P<0.05), while LB was significantly higher than PC, BF, and NC (P<0.05). Serum CAT activity in LP was significantly higher than all

other groups (P<0.05), with LB and PC significantly higher than BF and NC (P<0.05). Serum MDA concentration in LP and LB was significantly lower than in PC, BF, and NC (P<0.05).

3.1 Impact on Growth Performance

Weaning stress and gastrointestinal microbiota imbalance are primary causes of reduced appetite, diarrhea, and growth retardation in piglets. Minimizing stress and improving microbial balance are crucial for enhancing growth performance. Colistin sulfate, with potent anti-Gram-negative activity, has been considered a last-resort treatment but is now banned in animal feed due to food safety and public health concerns. This experiment used colistin sulfate as a positive control to identify suitable growth-promoting alternatives. *Lactobacillus plantarum* is a homofermentative lactic acid bacteria that survives gastric passage and colonizes the intestine beneficially. Flavonoids are important plant secondary metabolites that can modulate immune function and growth axes, thereby affecting immunity, growth, and reproduction. In this study, BF supplementation significantly improved overall ADG and F/G compared to NC, indicating that buckwheat flavone can replace antibiotic growth promotion. Similar results have been reported for bamboo leaf flavonoids and ampelopsis grossedentata flavonoids in weaned piglets. However, *Lactobacillus plantarum* alone or combined with buckwheat flavone did not differ significantly from NC, consistent with some previous studies showing no significant effects on ADG, ADFI, or F/G, though others have reported improvements. The PC group showed 13.9% higher ADG than NC without statistical significance, possibly due to high within-group variation.

3.2 Impact on Nutrient Digestibility

Weaning substantially alters piglet gut microbiota, increasing *E. coli* and decreasing *Lactobacillus*. Dietary probiotics can modulate microbiota structure, improve digestibility, and promote growth. Previous findings on *Lactobacillus* and flavonoids have been inconsistent. Some studies demonstrated improved apparent nutrient digestibility with *Lactobacillus* or soy isoflavone supplementation, while others found no significant changes in GE, DM, OM, CP, EE, NDF, Ca, or P digestibility. In this study, PC, LP, BF, and LB groups all showed significantly higher digestibility of GE, DM, OM, CP, EE, Ca, and P than NC, suggesting both additives enhance nutrient digestibility. *Lactobacillus* may improve digestibility by increasing villus length, deepening gastric gland foveolae, lowering intestinal pH, and promoting digestive enzyme secretion, thereby enhancing enzyme activities. The combination of *Lactobacillus* and buckwheat flavone synergistically improved GE, DM, OM, and P digestibility beyond individual supplementation, though the underlying mechanism requires further investigation.

3.3 Impact on Serum Biochemical Indices

Serum TP concentration reflects hepatic protein synthesis capacity. Albumin maintains colloidal osmotic pressure and transports metabolites, while globulin concentration indicates immune status. The albumin-to-globulin ratio reflects splenic immune function; a decreased ratio suggests enhanced specific immune response. In this study, buckwheat flavone supplementation significantly increased serum TP and ALB compared to NC, with GLB showing an increasing trend, indicating improved protein digestion and synthesis consistent with growth performance findings. Similar results have been reported for ampelopsis grossedentata flavonoids.

Serum UN, the end product of protein catabolism, reflects amino acid metabolism and utilization. Previous studies on probiotics and flavonoids have shown inconsistent effects on serum UN, with some reporting no influence and others demonstrating reduction. In this study, *Lactobacillus* had no effect, while buckwheat flavone increased serum UN, possibly related to altered protein intake from improved feed consumption and digestibility, or changes in protein turnover and hepatic amino acid metabolism. The specific role and mechanism of buckwheat flavone warrant further investigation.

Glucose is the primary energy substrate for cellular function, while NEFA is an important energy source from neutral fat catabolism. The parallel changes in serum GLU and NEFA in this study suggest buckwheat flavone enhanced intestinal glucose absorption and energy metabolism, which within normal ranges can improve immune function.

Growth hormone stimulates bone and cartilage growth and regulates protein, lipid, and carbohydrate metabolism, while IGF-I enhances intestinal mucosal mass and villus height to increase nutrient absorption. Except for significantly increased GH in the LP group, no differences were observed among groups, consistent with some reports but contrasting with others showing flavonoid-induced increases in GH and IGF-I. These discrepancies may relate to animal growth stage, flavonoid composition and dosage, or the pulsatile nature of GH secretion. IGF-I may better reflect growth status due to its positive correlation with body weight and gain.

3.4 Impact on Serum Immune Indices

Immunoglobulins are primary mediators of humoral immunity and important indicators of immune function. IgG comprises 75% of total immunoglobulins and plays a major role in specific immunity. IgA is the primary antibody for mucosal immunity and serves as the initial barrier against infection. IgM is the first antibody produced in humoral immune response, with bactericidal and opsonic activities 500–1,000 times greater than IgG, playing a crucial role in early defense. In this study, *Lactobacillus* supplementation did not affect serum IgG, IgM, or IgA, consistent with some previous reports. However, buckwheat flavone significantly increased all three immunoglobulins compared to NC, indicating

enhanced immune function. Research has shown that buckwheat flavonoids can increase spleen index, phagocytic activity, and serum hemolysin levels, thereby improving specific and non-specific immunity. The significantly higher serum IgA in the BF group suggests well-developed mucosal immunity and intestinal health. Collectively, buckwheat flavone may promote growth not through the growth axis but by enhancing immunity, particularly intestinal health, to improve nutrient absorption.

3.5 Impact on Serum Antioxidant Indices

Free radicals generated during metabolism accelerate aging, reduce immune function, and induce disease. Antioxidant capacity is closely linked to health status through enzymatic (SOD, GSH-Px, CAT) and non-enzymatic (vitamins C and E, glutathione) systems. T-AOC reflects overall antioxidant capacity and free radical scavenging ability. SOD removes superoxide anions, GSH-Px captures free radicals to reduce lipid peroxidation, and CAT converts hydrogen peroxide to water. MDA, a lipid peroxidation product formed from free radical attack on polyunsaturated fatty acids, indicates the degree of lipid peroxidation and cellular oxidative damage.

In this study, buckwheat flavone did not significantly affect serum T-AOC compared to NC. This may be because most antioxidant studies on buckwheat flavone are in vitro, whereas this in vivo trial measured partial antioxidant indicators after complex biological processing. The lack of effect could also relate to insufficient dosage or interactions with dietary antioxidants. In contrast, *Lactobacillus* significantly increased T-AOC, SOD, GSH-Px, and CAT activities while decreasing MDA, indicating enhanced free radical scavenging, reduced oxidative damage, and improved health status.

4 Conclusion

Under the conditions of this experiment, dietary buckwheat flavone supplementation improved ADG and immune function in weaned piglets. Dietary *Lactobacillus plantarum* alone or combined with buckwheat flavone enhanced antioxidant capacity. Both additives individually and in combination improved nutrient digestibility in weaned piglets.

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Note: Figure translations are in progress. See original paper for figures.