

Effects of Combined *Bacillus subtilis* and Fructooligosaccharides on Broiler Chicken Growth Performance and Its Mechanism: Postprint

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Abstract

This experiment aimed to investigate the effects and mechanism of combined *Bacillus subtilis* 048 (BS048) and fructooligosaccharides (FOS) on the growth performance of Snow Mountain grass chickens. A total of 980 one-day-old male Snow Mountain grass chickens with similar body weight were selected and randomly divided into 2 groups, with 7 replicates per group and 70 chickens per replicate. The control group was fed a basal diet, while the experimental group was fed the basal diet supplemented with 0.1% (W/W) BS048 and 0.08% (W/W) FOS. The experimental period lasted for 80 days. The results showed that compared with the control group, the average daily gain (ADG), average daily feed intake (ADFI), jejunum digestive enzyme activity, and cecal volatile fatty acid (VFA) content of Snow Mountain grass chickens in the experimental group were significantly or extremely significantly increased ($P < 0.05$ or $P < 0.01$), while small intestinal chyme viscosity was extremely significantly decreased ($P < 0.01$); however, feed conversion ratio and small intestinal chyme pH showed no significant changes ($P > 0.05$). It can be concluded that the combined use of BS048 and FOS can not only reduce small intestinal chyme viscosity and increase jejunum digestive enzyme activity to enhance intestinal digestive function, but also promote cecal VFA production to maintain and improve intestinal health status, thereby improving the growth performance of Snow Mountain grass chickens.

Full Text

Effects of Combined Use of *Bacillus subtilis* and Fructooligosaccharide on Growth Performance of Broilers and Its Mechanism

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Abstract: This study investigated the effects of combined supplementation of *Bacillus subtilis* 048 (BS048) and fructooligosaccharide (FOS) on growth performance in Xueshan chickens and explored the underlying mechanisms. A total of 980 one-day-old male Xueshan chickens with similar body weight were randomly allocated into two groups, each consisting of seven replicates of 70 birds. The control group received a basal diet, while the experimental group received the basal diet supplemented with 0.1% (W/W) BS048 and 0.08% (W/W) FOS. The 80-day feeding trial demonstrated that compared with the control group, the experimental group exhibited significant or highly significant improvements in average daily gain (ADG), average daily feed intake (ADFI), jejunal digestive enzyme activities, and cecal volatile fatty acid (VFA) concentrations ($P < 0.05$ or $P < 0.01$). Additionally, intestinal chyme viscosity decreased extremely significantly ($P < 0.01$), whereas feed-to-gain ratio and intestinal chyme pH remained unchanged ($P > 0.05$). These findings indicate that the combined use of BS048 and FOS enhances intestinal digestive function by reducing chyme viscosity and increasing digestive enzyme activities, while promoting VFA production in the cecum to maintain and improve gut health, thereby improving growth performance in Xueshan chickens.

Keywords: *Bacillus subtilis*; fructooligosaccharide; Xueshan chickens; digestive function; growth performance

Introduction

The disadvantages of antibiotic use in animal production have become increasingly prominent in recent years, drawing greater attention to the safety, efficacy, and environmental impact of feed additives. Developing non-toxic, residue-free additives that promote animal growth while improving animal product quality has emerged as a critical research priority. *Bacillus* species are Gram-positive bacteria that are aerobic or facultatively anaerobic and produce highly resistant endospores under certain conditions, offering advantages such as strong stress resistance, high temperature and pressure tolerance, and easy storage. These bacteria enhance growth performance, improve feed utilization, boost immunity, provide antimicrobial benefits, and promote gastrointestinal microecological balance. Moreover, they maintain high viability during feed pelleting, storage, and passage through the acidic gastric environment, making them excellent and safe probiotics widely used in livestock and poultry production.

Fructooligosaccharide (FOS), also known as oligofructose or oligofructan, refers to oligosaccharides formed by linking n D-fructose units ($n < 8$) to sucrose molecules via β -1,2-glycosidic bonds. FOS possesses physicochemical properties including low caloric value, stability, safety, high viscosity, strong

hygroscopicity, and resistance to gastrointestinal digestion. Previous research has demonstrated that FOS significantly increases daily weight gain and feed conversion ratio while improving intestinal microflora structure and enhancing immunity in poultry, swine, ruminants, and aquatic animals. Most previous studies have investigated *Bacillus* or FOS individually regarding their effects on growth performance, digestive function, and intestinal microflora. However, limited research has examined their combined effects on yellow-feathered broilers.

Xueshan chickens represent a high-quality yellow-feathered broiler breed developed by Lihua Livestock Company of Jiangsu Province through selective cross-breeding of indigenous Chinese breeds including Tibetan chickens and Chahua chickens. This breed offers advantages such as superior meat quality, strong disease resistance, simple management, and high economic returns. This study aimed to elucidate the effects and mechanisms of combined BS048 and FOS supplementation on growth performance in Xueshan chickens, providing scientific evidence for developing novel *Bacillus* preparations and their application in high-quality yellow-feathered broiler production.

Materials and Methods

Experimental Animals and Diets The experiment utilized 980 one-day-old male Xueshan chickens (Jiangsu Lihua Livestock Company), BS048 preparation (Shandong Baolailaisheng Bioengineering Co., Ltd., containing 5×10^8 CFU/g viable bacteria), FOS (Korean Samyang Genex Company), total protein, amylase, lipase, and trypsin assay kits (Nanjing Jiancheng Bioengineering Institute), and other conventional reagents (analytical grade, Sangon Biotech Shanghai Co., Ltd.). Equipment included a Leici PHS-3C pH meter (Shanghai INESA Scientific Instrument Co., Ltd.), UV-2100 UV-Vis spectrophotometer (Unico Shanghai Instruments Co., Ltd.), and 7890B gas chromatograph (Agilent Technologies, USA).

Experimental Design and Management Nine hundred and eighty one-day-old male Xueshan chickens (61.5 ± 0.5 g) were randomly divided into two groups with seven replicates each (70 birds per replicate). The control group received a basal diet, while the experimental group received the basal diet supplemented with 0.1% (W/W) BS048 and 0.08% (W/W) FOS. Four basal diets (C101 for days 1-20, C102 for days 21-40, C103 for days 41-60, and C104 for days 61-80) were formulated according to the *Feeding Standard of Chickens* (NY/T 33-2004). Diet composition and nutrient levels are presented in Table 1.

The feeding trial was conducted at the experimental farm of Jiangsu Lihua Livestock Company for 80 days. Birds were raised on floor pens with *ad libitum* access to feed and water, 24-hour lighting, and standard vaccination proto-

cols. Daily health status, mortality, and feed consumption were recorded. Body weight was measured at days 40 and 80 after 12-hour feed deprivation (with free access to water). Average body weight (ABW), ADG, ADFI, and feed-to-gain ratio (F/G) were calculated.

Sample Collection and Analysis At days 40 and 80, 28 healthy birds with similar body weight were selected from each group (four per replicate) for slaughter after blood collection from the jugular vein. The abdominal cavity was opened, and intestinal segments (duodenum, jejunum, ileum, and cecum) were rapidly isolated. All chyme was gently expelled and collected into sample bags. pH values of duodenal, jejunal, and ileal chyme were measured using a pH meter. All chyme samples were briefly immersed in liquid nitrogen and then stored at -80°C .

Chyme viscosity: Duodenal, jejunal, and ileal contents were mixed uniformly. Approximately 1.00 ± 0.01 g of chyme was mixed with 6 mL distilled water, vortexed, and centrifuged at $3,000 \times g$ (5 mm) and incubated at 40°C for 5 minutes before measuring relative viscosity. Relative viscosity was calculated as: (time for chyme supernatant to flow through capillary)/(time for equal volume of distilled water to flow through capillary).

Digestive enzyme activities: Approximately 0.2 g of jejunal chyme was homogenized with physiological saline (1:9 W/V) for 3 minutes on ice and centrifuged at $1,500 \times g$ for 10 minutes. Supernatant was used to determine total protein content and activities of amylase, lipase, and trypsin using assay kits according to manufacturer instructions.

Volatile fatty acid (VFA) content: Cecal chyme samples were thawed, and approximately 2 g was mixed with ultrapure water (1:2 W/V), vortexed, and centrifuged at $10,000 \times g$ for 15 minutes at 4°C . One and a half milliliters of supernatant was mixed with 0.2 mL of 8.2% (W/V) metaphosphoric acid, incubated on ice for 40 minutes, and centrifuged at $10,000 \times g$ for 10 minutes at 4°C . Supernatant was analyzed for acetic, propionic, and butyric acid contents using gas chromatography.

Statistical Analysis Data were analyzed using SPSS 22.0 software. Independent samples t-tests were used for two-group comparisons. Significance was declared at $P < 0.05$, extreme significance at $P < 0.01$, and trends were noted at $0.05 < P < 0.10$. Results are expressed as mean \pm standard deviation.

Results

Growth Performance

As shown in Table 2, ADG and ADFI in the experimental group were significantly or highly significantly greater than those in the control group across all

growth phases. During the first half of the trial (days 1-40), ADG and ADFI increased by 6.047% ($P<0.05$) and 7.159% ($P<0.05$), respectively. During the second half (days 41-80), ADG and ADFI increased by 8.166% ($P<0.01$) and 7.048% ($P<0.05$), respectively. Over the entire trial period (days 1-80), ADG and ADFI increased by 7.359% ($P<0.01$) and 7.077% ($P<0.01$), respectively. No significant differences in F/G were observed between groups during any phase ($P>0.05$).

Intestinal Chyme Viscosity and pH

Table 3 shows that intestinal chyme viscosity in the experimental group decreased by 3.474% ($P<0.01$) and 5.180% ($P<0.01$) at days 40 and 80, respectively, compared with the control group. However, no significant differences were observed in pH values of duodenal, jejunal, or ileal chyme between groups ($P>0.05$).

Jejunal Digestive Enzyme Activities

As presented in Table 4, the experimental group exhibited significant increases in jejunal enzyme activities at both time points. Amylase activity increased by 44.594% ($P<0.01$) and 36.609% ($P<0.01$) at days 40 and 80, respectively. Lipase activity increased by 73.388% ($P<0.01$) and 73.278% ($P<0.05$) at days 40 and 80, respectively. Trypsin activity increased by 38.985% ($P<0.01$) and 91.795% ($P<0.01$) at days 40 and 80, respectively.

Cecal VFA Content

Table 5 demonstrates that the experimental group had significantly higher cecal VFA concentrations. Acetic acid content increased by 51.348% ($P<0.01$) and 41.704% ($P<0.01$) at days 40 and 80, respectively. Propionic acid content increased by 45.160% ($P<0.05$) and 52.676% ($P<0.05$) at days 40 and 80, respectively. Butyric acid content increased by 85.258% ($P<0.01$) and 100.178% ($P<0.01$) at days 40 and 80, respectively.

Discussion

Growth performance, comprising ADG, ADFI, and F/G, represents a critical determinant of broiler production economics. This study found that dietary supplementation with BS048 and FOS significantly improved ADG and ADFI in Xueshan chickens across different growth stages, consistent with findings by Yi et al. Recent studies have demonstrated that both *Bacillus subtilis* preparations and FOS significantly promote broiler growth performance. Our results further indicate that the synbiotic combination of BS048 and FOS markedly improves growth performance in Xueshan chickens, with synergistic effects superior to either component alone.

Animal growth and development depend primarily on energy and nutrients derived from dietary digestion and absorption in the intestine, establishing a close relationship between growth performance and intestinal digestive function. Previous research has shown that both *Bacillus subtilis* and FOS enhance intestinal digestive function. This study investigated the mechanisms underlying the growth-promoting effects of combined BS048 and FOS supplementation on intestinal digestive function in Xueshan chickens. Dietary BS048 and FOS significantly reduced intestinal chyme viscosity and increased jejunal digestive enzyme activities. Reduced chyme viscosity diminishes anti-nutritional effects, increasing contact opportunities between nutrients and endogenous digestive enzymes or intestinal mucosa, accelerating nutrient dissolution, and thereby promoting digestion and absorption. Concurrently, substantially increased digestive enzyme activities enhance chemical digestion of dietary macronutrients (starch, fat, protein) for intestinal absorption. These combined effects significantly increase the capacity and rate of dietary digestion and absorption, accelerating chyme passage and enabling greater daily feed intake, which aligns with our observed increases in ADFI.

VFAs are short-chain fatty acids (carbon atoms <5) including acetic, propionic, and butyric acids, produced by anaerobic microorganisms (e.g., *Bifidobacterium*, *Lactobacillus*) in the rumen of ruminants or hindgut of monogastric animals through carbohydrate fermentation. This study found that dietary BS048 and FOS significantly increased cecal VFA concentrations in Xueshan chickens. As an aerobic bacterium, *Bacillus* consumes substantial free oxygen in the intestine, reducing oxygen tension and inhibiting aerobic pathogens such as *E. coli* and *Salmonella* while optimizing conditions for anaerobic beneficial bacteria. FOS, resistant to hydrolysis by endogenous α -amylase, sucrase, and maltase, passes through the stomach and small intestine to the hindgut, where it is fermented by *Bifidobacterium* and lactic acid bacteria to produce VFAs, while being unavailable to harmful bacteria like *Clostridium* and *E. coli*. VFAs subsequently lower intestinal pH, inhibiting pathogen proliferation. Thus, combined BS048 and FOS optimizes intestinal microflora structure and maintains gut health, which is essential for optimal growth performance in Xueshan chickens.

In conclusion, combined supplementation of BS048 and FOS enhances growth performance in Xueshan chickens by reducing intestinal chyme viscosity, increasing jejunal digestive enzyme activities, and promoting cecal VFA production, thereby improving both digestive function and intestinal health.

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