Effects of Probiotic-Fermented Apple Pomace on Growth Performance, Serum Biochemical Indices, and Fecal Microbiota in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of probiotics fermented apple pomace on growth performance, serum biochemical indices, and fecal microbiota in early-weaned piglets. A total of 120 weaned piglets with an average body weight of (5.87$±$0.10) kg were randomly assigned to five groups (with 3 replicates per group and 8 piglets per replicate): the negative control group was fed a basal diet (without antibiotics), the positive control group was fed the basal diet supplemented with 0.1% mixed antibiotics, and the experimental groups were fed experimental diets containing 4%, 6%, and 8% probiotics fermented apple pomace added to the basal diet. The experimental period lasted 35 days. The results showed that, compared with the negative control group, dietary supplementation with antibiotics and 6% probiotics fermented apple pomace both significantly increased average daily feed intake and average daily gain in weaned piglets ($P<0.05$), and significantly decreased feed-to-gain ratio, fecal Escherichia coli count, and diarrhea rate ($P<0.05$). Supplementation with antibiotics and 6% probiotics fermented apple pomace could significantly decrease serum urea nitrogen and total cholesterol contents ($P<0.05$), and significantly increase growth hormone, insulin, triiodothyronine, and thyroxine contents ($P<0.05$). With the increase of probiotics fermented apple pomace supplementation level, growth performance, diarrhea rate, fecal Escherichia coli count, as well as serum urea nitrogen, total cholesterol, and hormone indices exhibited a quadratic trend ($P<0.05$), with the best effect observed at the 6% supplementation level. Compared with the positive control group, supplementation with probiotics fermented apple pomace could extremely significantly increase total fecal bacterial count ($P<0.01$), while having no significant effects on other indices ($P>0.05$). Therefore, supplementation with 6% probiotics fermented apple pomace can improve growth performance, regulate intestinal microecological balance, reduce fecal Escherichia coli count and diarrhea rate, increase serum endocrine hormone contents, and decrease urea nitrogen and cholesterol contents in weaned piglets.

Full Text

Probiotic Fermented Apple Pomace Affects Growth Performance, Serum Biochemical Indicators and Fecal Microbial Flora of Weaned Piglets

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Abstract

This experiment was conducted to investigate the effects of probiotic fermented apple pomace on growth performance, serum biochemical indicators, and fecal microbial flora of early-weaned piglets. A total of 120 weaned piglets with an average body weight of (5.87$\pm$0.10) kg were randomly allocated to 5 groups (3 replicates per group, 8 piglets per replicate): a negative control group fed a basal diet without antibiotics, a positive control group fed the basal diet supplemented with 0.1% mixed antibiotics, and three treatment groups fed the basal diet supplemented with 4%, 6%, or 8% probiotic fermented apple pomace. The experimental period lasted 35 days. The results showed that, compared with the negative control group, dietary supplementation with antibiotics and 6% probiotic fermented apple pomace significantly increased average daily feed intake and average daily gain ($P<0.05$), and significantly decreased feed-to-gain ratio, fecal *E. coli* count, and diarrhea rate ($P<0.05$). Antibiotic and 6% probiotic fermented apple pomace supplementation also significantly reduced serum urea nitrogen and total cholesterol contents ($P<0.05$), while significantly increasing growth hormone, insulin, triiodothyronine, and thyroxine levels ($P<0.05$). With increasing probiotic fermented apple pomace supplementation, growth performance, diarrhea rate, fecal *E. coli* count, and serum urea nitrogen, total cholesterol, and hormone indices exhibited quadratic trends ($P<0.05$), with the 6% supplementation level showing the best results. Compared with the positive control group, probiotic fermented apple pomace supplementation extremely significantly increased total fecal bacterial count ($P<0.01$) but had no significant effects on other indices ($P>0.05$). These findings indicate that dietary supplementation with 6% probiotic fermented apple pomace can improve growth performance, regulate intestinal microecological balance, reduce fecal

E. coli count and diarrhea rate, increase serum endocrine hormone levels, and decrease urea nitrogen and cholesterol contents in weaned piglets.

Keywords: probiotic fermented apple pomace; weaned piglet; growth performance; serum biochemical indicators; fecal microbial flora

In 2014, China' s apple cultivation area and production reached 2.3553 million hectares and 39.15 million tons, respectively, with 25% of the harvest used for producing concentrated apple juice, fruit vinegar, and other by-products. The annual output of apple pomace from concentrated juice processing alone has reached 1.2 million tons. Research has shown that apple pomace is rich in nutrients, vitamins, and malic acid, which can be directly absorbed and utilized by microorganisms, making it suitable as livestock feed. However, its relatively low protein content limits the utilization of other components when used as animal feed. Therefore, specific processes are needed to increase the protein content of apple pomace through microbial fermentation, transforming it into nutrient-rich microbial protein feed—an important approach to solving the utilization problem of apple pomace. Studies have also indicated that during the fermentation process for producing protein feed from apple pomace, not only is the protein content increased, but numerous bioactive components such as enzymes, active peptides, and free amino acids are produced, which play important roles in animal nutrition. Some research has found that fermented products and their metabolic byproducts can improve piglet growth performance and intestinal health, and may replace antibiotics in feed. Therefore, this experiment was designed to investigate the effects of fermented apple pomace on growth performance, serum biochemical indicators, and fecal microbial flora of weaned piglets, providing a reference basis for its application in livestock production.

1.1 Experimental Animals and Design

This experiment was conducted at the Xinhai Pig Breeding Farm of Shaanxi Xinhai Animal Husbandry Co., Ltd. in Xingping City, Shaanxi Province. The total experimental period was 38 days, including a 3-day pre-trial period and a 35-day formal experimental period. During the pre-trial period, pigs were fed a basal diet without antibiotics or probiotic fermented apple pomace, with composition and nutrient levels shown in Table 1 . A total of 120 (21$±$1)-day-old weaned "Duroc × Landrace × Large White" crossbred piglets with an initial body weight of (5.87$±$0.10) kg were selected and randomly divided into 5 groups according to a single-factor randomized block design, with 3 replicates per group and 8 piglets per replicate. The experimental groups were as follows: negative control (NC) group fed the basal diet; positive control (PC) group fed the basal diet supplemented with 0.1% mixed antibiotics (colistin sulfate, bacitracin zinc, and chlortetracycline); and experimental groups I, II, and III fed the basal diet supplemented with 4%, 6%, and 8% probiotic fermented apple pomace, respectively.

1.2.1 Fermentation Raw Materials

The solid-state fermentation medium consisted of apple pomace:oil residue:urea at a ratio of 17:2:1, with a substrate moisture content of 60% after sterilization. The microbial inoculum mainly included *Aspergillus niger*, *Saccharomyces cerevisiae* (Angel Yeast), *Lactobacillus*, and *Bacillus*.

1.2.2 Fermentation Process

The *A. niger* slant culture was inoculated into tissue culture bottles containing wheat bran medium and cultured at 30 °C for 5 days. Then, 2% of this culture was inoculated into plastic trays containing wheat bran medium and cultured at 30 °C for 10 days. This culture was then inoculated together with Angel Yeast into the solid-state fermentation medium for solid-state fermentation. After drying, *Lactobacillus* and *Bacillus* were added at a certain proportion and mixed to produce the final product.

1.2.3 Composition of Fermented Apple Pomace

After adding oil residue supplements to air-dried apple pomace and fermenting with the mixed microbial inoculum, the protein content of the feed was significantly increased. Additionally, *Lactobacillus* and *Bacillus* that improve animal intestinal flora were added, transforming the pomace into a bioactive protein feed that provides both protein and probiotic benefits. The nutritional composition was as follows: contents of Angel Yeast, *Lactobacillus*, and *Bacillus* were 1.4\$×10$^{\{4\},\,5.5\times10}$\{7\}, *and*8.7×10^\{5\}\$ CFU/g, respectively; crude protein, gross energy, polypeptide, and free amino acid contents were 29.29%, 17.47 MJ/kg, 4.04%, and 0.07%, respectively; and activities of protease, cellulase, and pectinase were 114.06, [value missing], and [value missing] units, respectively.

1.3 Experimental Diets and Management

Experimental diets were formulated according to NRC (2012) standards, with diet composition and nutrient levels shown in Table 1. Before the experiment, pig houses were thoroughly disinfected, and all experimental piglets were uniformly dewormed and vaccinated. During the experimental period, pigs were fed at 07:30, 14:30, and 21:30 daily, with free access to feed and water. Feed supply and residual amounts were recorded daily to calculate average daily feed intake.

1.4.1 Fecal Sample Collection

On days 7, 14, 21, 28, and 35 of the experimental period, fresh fecal samples were collected from 3 randomly selected piglets per replicate before daily feeding. Samples were mixed, and 10 g was placed in a 10 mL sterilized centrifuge tube with glycerol and stored at -80 °C for subsequent fecal microbial flora analysis.

1.4.2 Blood Sample Collection and Serum Preparation

On days 21 and 35 of the experiment, one pig per replicate per group was randomly selected, and 10 mL of blood was collected from the anterior vena cava. The blood was placed at an angle for 30 minutes, then centrifuged at 3,000 rpm for 15 minutes to collect serum, which was stored at -20 °C for serum biochemical indicator analysis.

1.5.1 Growth Performance

Pigs were weighed on an empty stomach at the start of the experiment, on day 21, and on day 35. Average daily gain was calculated based on initial and final weights. Daily feed intake was recorded for each group to calculate average daily feed intake and feed-to-gain ratio. Fecal consistency was observed daily at 16:30, and the number of diarrheic pigs was recorded to calculate diarrhea rate.

Diarrhea rate (%) = [Total diarrhea incidents / (Total number of pigs × Experimental days)] × 100.

1.5.2 Serum Biochemical Indicators

Serum biochemical indicators including glucose (GLU), urea nitrogen (UN), total protein (TP), albumin (ALB), triglycerides (TG), and total cholesterol (TC) were measured using commercial kits. Serum hormone indicators including growth hormone (GH), insulin (INS), triiodothyronine (T3), and thyroxine (T4) were measured using enzyme-linked immunosorbent assay (ELISA) kits. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.5.3 Fecal Microbial Flora

Fecal samples were analyzed for total bacterial count and *E. coli* using plate dilution methods.

Total bacterial count: Depending on the sample batch, three consecutive dilutions (10^{-4} to 10^{-6}) were selected. One milliliter of each diluted sample was pipetted into sterile petri dishes, with two dishes per dilution. Simultaneously, 1 mL of blank diluent was added to two sterile petri dishes as controls. Fifteen to twenty milliliters of plate count agar medium cooled to 46 °C (maintained in a 46$±1°Cwaterbath)waspouredintoeachdish,whichwasthenrotatedtoensureuniformmixing.Aftertheagarsol
h. Colony counts were expressed as CFU. The average of two plates per dilution was multiplied by the corresponding dilution factor to obtain the total bacterial count per gram of sample.

***E. coli* count:** Three consecutive dilutions (10^{-4} to 10^{-6}) were selected, and 1 mL of each dilution was inoculated into three tubes of lauryl sulfate tryptose (LST) broth and incubated at 37$±1°Cfor24±2h.Tubeswithgasproductionintheinvertedvialweresubjectedtoc
gas−producingtubeswereincubatedforanadditional24h(48±2htotal)beforeconfirmatorytesting.Non−
gas−producingtubeswererecordedascoliform−negative.Usinganinoculatingloop,oneloopfulofculturefrome

producingLSTtubewastransferredtobrilliantgreenlactosebile(*BGLB*)*brothandincubatedat*36±1°*Cfor*48±$2 h. Gas-producing tubes were recorded as coliform-positive. Based on the number of confirmed positive LST tubes, the most probable number (MPN) table was used to report the MPN value of coliforms per gram of sample.

1.6 Data Processing

Data were analyzed using SPSS 17.0 software via one-way ANOVA, with Duncan's multiple range test for post-hoc comparisons. t-tests were used to compare differences between the PC group and the 4%, 6%, and 8% probiotic fermented apple pomace groups. Linear and quadratic effects of increasing probiotic fermented apple pomace supplementation on various measured indices were examined. Significance was declared at P<0.05, and extreme significance at P<0.01.

2.1 Effects of Probiotic Fermented Apple Pomace on Growth Performance of Weaned Piglets

As shown in Table 2 , no significant differences were observed in initial weight among groups (P>0.05). Final weight was significantly higher in all groups except the NC and III groups (P<0.05). Average daily gain in group II was extremely significantly higher by 4.90%, 2.44%, and 4.84% compared with the NC, I, and III groups, respectively (P<0.01), while the PC group was significantly higher than the NC and III groups (P<0.05). Compared with the NC group, average daily feed intake in group II was extremely significantly increased by 2.45% (P<0.01), and was also significantly increased in the PC and I groups (P<0.05). Feed-to-gain ratio in the PC and II groups was extremely significantly lower than in the NC and III groups (P<0.01). Compared with the PC group, probiotic fermented apple pomace supplementation tended to increase feed-to-gain ratio (P=0.077). With increasing probiotic fermented apple pomace supplementation, average daily gain, average daily feed intake, and feed-to-gain ratio showed quadratic trends (P<0.05), with the 6% supplementation level showing the best results.

2.2 Effects of Probiotic Fermented Apple Pomace on Diarrhea Rate of Weaned Piglets

As shown in Table 3 , during days 1-21, diarrhea rates in the PC, I, and II groups were significantly reduced by 17.98%, 13.47%, and 15.73% compared with group III (P<0.05), while the PC group was significantly reduced by 15.11% compared with the NC group (P<0.05). Groups I and II showed reductions of 10.45% and 14.29% compared with the NC group, respectively, though these differences were not significant (P>0.05). During days 22-35, diarrhea rates in the NC and III groups were significantly higher than in the PC and II groups (P<0.05), while group I was significantly lower than the NC group (P<0.05). Overall, diarrhea rate decreased initially and then increased with increasing probiotic fermented apple pomace supplementation, showing a quadratic trend (P<0.05), with the 6% supplementation group having the lowest diarrhea rate.

2.3 Effects of Probiotic Fermented Apple Pomace on Serum Biochemical Indicators of Weaned Piglets

As shown in Table 4 , no significant differences were observed among groups in serum TP, ALB, GLU, or TG contents (P>0.05), though TP and ALB contents in group II and the PC group were notably higher than in the NC group. For serum UN content, the PC and II groups showed reductions of 34.67% and 38.21% at day 21 (P<0.05) and 15.94% and 16.52% at day 35 (P<0.05) compared with the NC group. For serum TC content, the PC, I, and II groups all showed significant decreasing trends compared with the NC group (P<0.05). With increasing probiotic fermented apple pomace supplementation, serum UN and TC contents showed quadratic trends (P<0.05), and serum TC content at day 21 showed a linear decreasing trend (P=0.011). No significant changes were observed in any serum biochemical indicators when comparing probiotic fermented apple pomace groups with the PC group (P>0.05). These results indicate that dietary supplementation with 6% probiotic fermented apple pomace can increase serum TP and ALB contents and significantly reduce UN and TC contents.

2.4 Effects of Probiotic Fermented Apple Pomace on Serum Hormone Indicators of Weaned Piglets

As shown in Table 5 , serum GH, INS, T3, and T4 contents in the PC and II groups were significantly higher than in the NC and III groups (P<0.05), with extremely significant increases in GH content (P<0.01). With increasing probiotic fermented apple pomace supplementation, serum INS, T3, and T4 contents at day 21 showed quadratic trends (P<0.05), while GH content showed a quadratic trend (P=0.059). At day 35, serum GH, INS, and T3 contents showed quadratic trends (P<0.05). These results demonstrate that dietary supplementation with 6% probiotic fermented apple pomace had the most pronounced effects on serum GH, INS, T3, and T4 contents in weaned piglets.

2.5 Effects of Probiotic Fermented Apple Pomace on Fecal Microorganisms of Weaned Piglets

As shown in Table 6 , throughout the feeding period, total bacterial counts in the PC group were significantly lower than in the NC and probiotic fermented apple pomace groups (P<0.05), while probiotic fermented apple pomace groups showed increasing trends compared with the NC group, though not significantly (P>0.05). For *E. coli*, the PC and II groups showed extremely significant reductions on day 7 (P<0.01) and significant reductions on days 14 and 21 (P<0.05) compared with the NC and III groups. On days 28 and 35, the PC, I, and II groups all showed significant reductions compared with the NC group (P<0.05), while group III also showed a decrease, though not significant (P>0.05). With increasing probiotic fermented apple pomace supplementation, *E. coli* count showed a significant quadratic trend (P<0.05). Total bacterial counts were extremely significantly lower in the PC group than in probiotic fermented apple pomace groups (P<0.01). These results indicate that dietary supplementation with probiotic fermented apple pomace can significantly increase total bacterial count and reduce *E. coli* count in weaned piglets, with the 6% supplementation level showing the best effect.

3.1 Effects of Probiotic Fermented Apple Pomace on Growth Performance of Weaned Piglets

Weaned piglets often experience a series of "early weaning stress syndrome" symptoms including decreased feed intake, reduced immunity, growth stagnation, and diarrhea, which severely affect survival rates and economic efficiency in pig production. The probiotic fermented apple pomace used in this experiment was produced through solid-state fermentation of fresh pomace using a mixed microbial preparation consisting of yeast, lactic acid bacteria, and bacillus. In addition to containing large numbers of live microorganisms, probiotic fermented apple pomace contains proteinases, amylases, cellulases, active peptides, and free amino acids produced through microbial metabolism, which can improve piglet digestive capacity and feed utilization, thereby enhancing growth performance. Furthermore, aerobic bacteria such as bacillus and yeast create an anaerobic environment favorable for intestinal lactic acid bacteria proliferation, which produces large amounts of lactic acid and inhibits pathogenic *E. coli* overgrowth. This reduces intestinal pH, improves intestinal microecological balance, enhances digestive capacity, improves animal immunity, and reduces morbidity and diarrhea rates, while also improving diet palatability and increasing feed intake.

The results of this experiment indicate that higher supplementation levels of probiotic fermented apple pomace do not necessarily lead to better growth performance, suggesting an optimal dosage exists. Based on comprehensive evaluation of average daily gain, average daily feed intake, feed-to-gain ratio, and diarrhea rate, the 6% probiotic fermented apple pomace supplementation and the PC group showed the best results, significantly increasing average daily gain and feed intake while reducing feed-to-gain ratio and diarrhea rate, thereby improving growth performance. Wu et al. reported that feeding quails with 3% fermented apple pomace feed improved egg quality and production performance. García et al. found that dietary probiotic supplementation significantly improved growth performance and feed conversion ratio while reducing diarrhea incidence in weaned piglets. These findings are consistent with the results of the present study.

3.2 Effects of Probiotic Fermented Apple Pomace on Serum Biochemical Indicators of Weaned Piglets

Serum biochemical indicators comprehensively reflect the metabolic status of the organism. For instance, serum TP and UN contents accurately reflect protein metabolism and amino acid balance in the diet. In this experiment, no significant changes were observed in serum TP and ALB contents among groups, but

6% probiotic fermented apple pomace supplementation significantly reduced serum UN content, indicating that probiotic fermented apple pomace can increase nitrogen retention and promote protein synthesis and growth, likely due to its high content of active peptides and free amino acids. Zeng et al. reported that fermenting residue feed with probiotics significantly increased small peptides, oligopeptides, and free amino acid contents, thereby improving dietary protein utilization and animal growth performance.

Blood lipid and lipoprotein contents reflect metabolic regulation under steady-state conditions, particularly the fundamental regulation of fatty acid cycling between adipose tissue and the liver. Serum TG and TC contents reflect fat metabolism in animals. In this experiment, no significant changes were observed in serum TG content among groups, but serum TC content was significantly reduced at 6% probiotic fermented apple pomace supplementation compared with the NC group, indicating that appropriate supplementation can improve fat utilization to provide energy for protein synthesis, promote growth, and thereby improve piglet growth performance.

3.3 Effects of Probiotic Fermented Apple Pomace on Serum Hormone Levels of Weaned Piglets

Hormones along the "hypothalamus-pituitary-target organ" axis, including GH, INS, and thyroid hormones, play crucial regulatory roles in animal growth. GH is central to the growth axis, with primary physiological functions of promoting protein deposition and bone growth, specifically by facilitating amino acid uptake into cells and enhancing DNA and RNA synthesis to promote protein synthesis, resulting in positive nitrogen balance. For piglets, GH is the primary physiological factor for increasing protein deposition. INS is the main hormone regulating glucose metabolism, promoting glucose uptake and utilization by tissue cells, accelerating glycogen synthesis, and facilitating amino acid uptake while inhibiting protein catabolism and gluconeogenesis, thereby promoting cell growth. INS also synergizes with GH to promote growth and development. Thyroid hormones are important for protein synthesis and promote organ and tissue differentiation by regulating carbohydrate and fat metabolism. T3 is the primary active form of thyroid hormone in animals, promoting protein synthesis and affecting growth and development mainly by regulating GH gene expression and synthesis in the pituitary and modulating INS levels.

The results of this experiment demonstrate that dietary supplementation with 6% probiotic fermented apple pomace significantly increased endogenous hormones related to growth (GH, INS, and thyroid hormones) in weaned piglets, thereby promoting growth. Wang et al. found that dietary antibiotic combinations significantly increased endogenous hormone (GH, INS, and T3) levels. Lu et al. reported that dietary supplementation with 10% and 20% fermented soybean meal significantly increased serum GH levels, promoted growth, and reduced feed-to-gain ratio in piglets. These findings are consistent with the present study.

3.4 Effects of Probiotic Fermented Apple Pomace on Fecal Microorganisms of Weaned Piglets

One of the main manifestations of weaning stress is the imbalance of intestinal microbial flora, leading to changes in dominant bacterial populations and overgrowth of pathogenic or opportunistic bacteria such as *E. coli* and *Salmonella*, which disrupts intestinal microecological balance, releases endotoxins, or produces other toxic effects, causing digestive dysfunction and reduced growth performance. Studies have shown that applying single or mixed probiotics, primarily lactic acid bacteria, during the weaning period can intervene and correct intestinal microecological imbalances, thereby ensuring optimal growth performance. Dong et al. reported that feeding probiotics to weaned piglets significantly reduced fecal *E. coli* counts and improved growth performance and immunity. The present study demonstrates that dietary supplementation with 6% probiotic fermented apple pomace significantly reduced fecal *E. coli* counts, showing good antibacterial effects against pathogens and producing results similar to antibiotics, thereby reducing diarrhea rates and improving growth performance.

In conclusion, dietary supplementation with probiotic fermented apple pomace can significantly increase average daily gain and feed intake while reducing feed-to-gain ratio in weaned piglets. It can also significantly reduce fecal *E. coli* counts and diarrhea rates. The optimal supplementation level of probiotic fermented apple pomace in weaned piglet diets is 6%.

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