Effects of Fermented Grape Seed Meal on Digestive Physiology, Immune Organ Indices, and Antioxidant Indices in 5-12-Week-Old Wulong Geese (Postprint)

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Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of different dietary levels of fermented grape seed meal on digestive physiology, immune organ indices, and serum antioxidant indicators in 5-12 week-old Wulong geese, in order to determine its efficacy and appropriate supplementation level. A total of 288 five-week-old Wulong geese were randomly divided into 6 groups, with 6 replicates per group and 8 geese per replicate (half male and half female). The control group (Group I) was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 2% (Group II), 4% (Group III), 6% (Group IV), 8% (Group V), and 10% (Group VI) fermented grape seed meal, respectively. The experimental period lasted 8 weeks. The results showed that: 1) Compared with the control group, Group V exhibited extremely significant increases in the activities of amylase, trypsin, and pancreatic lipase in the duodenum and jejunum, as well as amylase and trypsin in the pancreas ($P<0.01$); Group IV showed an extremely significant increase in pancreatic lipase activity ($P<0.01$); and Group V showed a significant increase in pepsin activity ($P<0.05$). 2) Compared with the control group, Group IV showed a significant reduction in intestinal Escherichia coli count ($P<0.05$), while Group V showed a significant increase in intestinal Lactobacillus count ($P<0.05$); there was no significant difference between Groups IV and V ($P>0.05$). 3) Compared with the control group, Group IV showed significant increases in intestinal villus height and muscular layer thickness ($P<0.05$), while Group V showed an extremely significant decrease in crypt depth ($P<0.01$). 4) There were no significant differences in immune organ indices among all groups ($P>0.05$). Compared with the control group, Group V showed an extremely significant increase in serum total antioxidant capacity ($P<0.01$); Group IV showed an extremely significant decrease in serum malondialdehyde content (P<0.01), and Group V showed extremely significant increases in serum total superoxide dismutase and glutathione peroxidase activities (P<0.01). These results indicate that fermented grape seed meal can significantly enhance digestive enzyme activities, optimize intestinal tissue structure, and improve antioxidant capacity in 5-12 week-old Wulong geese, without affecting immune organ indices. It is recommended that the appropriate supplementation level of fermented grape seed meal in the diet is 6%-8%.

Full Text

Preamble

Effects of Fermented Grape Seed Meal on Digestive Physiology, Immune Organ Indices and Serum Antioxidant Indices of Wulong Geese Aged 5–12 Weeks

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Abstract: This experiment investigated the effects of dietary fermented grape seed meal at different inclusion levels on digestive physiology, immune organ indices, and serum antioxidant indices in 5–12-week-old Wulong geese to determine its efficacy and optimal supplementation level. A total of 288 five-week-old Wulong geese were randomly allocated into 6 groups with 6 replicates per group and 8 geese per replicate (half male and half female). The control group (Group I) received a basal diet, while treatment groups received the basal diet supplemented with 2% (Group II), 4% (Group III), 6% (Group IV), 8% (Group V), or 10% (Group VI) fermented grape seed meal. The experiment lasted 8 weeks. The results showed: (1) Compared with the control group, Group V exhibited extremely significant increases in amylase, trypsin, and lipase activities in the duodenum and jejunum, as well as amylase and trypsin activities in the pancreas (P<0.01). Group IV showed an extremely significant increase in pancreatic lipase activity (P<0.01), and Group V demonstrated a significant increase in pepsin activity (P<0.05). (2) Group IV had significantly reduced *Escherichia coli* counts in the intestine compared to the control (P<0.05), while Group V showed significantly increased *Lactobacillus* counts (P<0.05), with no significant difference between Groups IV and V (P>0.05). (3) Group IV exhibited significantly increased intestinal villus height and muscle layer thickness (P<0.05), while Group V showed an extremely significant decrease in crypt depth (P<0.01). (4) No significant differences were observed in immune organ indices among all groups (P>0.05). Compared with the control, Group V showed an extremely significant increase in serum total antioxidant capacity (P<0.01); Group IV demonstrated an extremely significant decrease in serum malondialdehyde content (P<0.01), while Group V exhibited extremely significant increases in serum total superoxide dismutase and glutathione peroxidase activities (P<0.01). In conclusion, fermented grape seed meal can significantly enhance digestive enzyme activity, optimize intestinal tissue structure, and improve antioxidant capacity in 5–12-week-old Wulong geese without affecting immune organ indices. The recommended optimal dietary inclusion level is 6%–8%.

Keywords: fermented grape seed meal; geese; digestive physiology; immune organ indices; antioxidant indices

Grape seed meal is a byproduct derived from grape seeds after oil extraction in wineries. Microbial fermentation can mitigate its antinutritional factors and excessive lignin content. As an important green feed additive, probiotics can improve the intestinal environment, regulate microecological balance, induce intestinal immune responses, replace antibiotics, and enhance antioxidant capacity. Applying fermented grape seed meal in feed holds significant economic and social value for reducing waste resources and developing novel feed materials. Aerobic probiotics such as *Bacillus* spp. consume oxygen in the gastrointestinal tract, inhibiting harmful bacteria and reducing intestinal diseases. Previous studies have shown that probiotics can decrease *E. coli* populations and increase *Lactobacillus* counts in the intestine [1-5]. Probiotics also promote digestive enzyme secretion and enhance substance metabolism, particularly *Bacillus subtilis* and *Bacillus licheniformis*, which exhibit strong protease, lipase, and amylase activities [6-7]. Wang [8] reported that fermented soybean meal significantly increased protease activity in the duodenum, jejunum, and ileum of piglets and improved the *Lactobacillus* to *E. coli* ratio in the colon. Qiu [9] found that fermented cottonseed meal increased villus height and decreased crypt depth in broiler chickens, effectively optimizing intestinal structure and absorption capacity while significantly improving immune organ indices. Grape seed meal contains abundant polyphenols, primarily oligomeric proanthocyanidins (OPC), which possess exceptional antioxidant capacity—50 times that of vitamin E and 20 times that of vitamin C—scavenging excess free radicals and earning international recognition for their antioxidant effects [10]. Bian et al. [11] demonstrated that grape seed proanthocyanidins enhance antioxidant enzyme system activity, thereby strengthening antioxidant capacity and reducing lipid peroxidation. Zhang [12] showed that grape seed oil increased glutathione peroxidase (GSH-Px) and superoxide dismutase activities in mouse liver and kidney tissues. Zhu [13] reported that beneficial bacteria in microbially fermented feed regulated microecological balance and enhanced disease resistance in meat ducks. While previous research has investigated the antioxidant functions of grape seed meal in humans and animals and the effects of fermented feed on animal digestive physiology, studies on the impacts of fermented grape seed meal on digestive enzyme activity, intestinal microbiota, digestive organ structure, and antioxidant function in geese remain unexplored.

This experiment used 5–12-week-old Wulong geese to investigate the effects of different dietary levels of fermented grape seed meal on digestive physiology, intestinal microbiota, immune organ indices, and antioxidant indices, aiming to determine its utilization efficacy and optimal inclusion level, enrich the nutritional database of grape seed meal, and provide theoretical guidance for feed resource development and goose production.

1.1 Experimental Materials

Grape seed meal (crude fiber [CF] 50.81%, crude protein [CP] 11.52%, calcium 0.55%, phosphorus 0.22%) was purchased commercially. Fermented grape seed meal was prepared in our laboratory through composite fermentation of grape seed meal (plus auxiliary materials) with *Penicillium oxalicum* (F67), *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Lactobacillus*. The product contained CF 35.99%, CP 8.35%, calcium 0.59%, total phosphorus 0.49%, and small peptides (<1,000 u) 1.78%, and was proven safe and non-toxic through toxicological testing. Experimental geese were provided by the Laiyang Tiansen Huoyan Goose Breeding Center, a demonstration base of the National Waterfowl Industry Technology System.

1.2 Experimental Design

A total of 288 healthy five-week-old Wulong geese with uniform body weight were randomly allocated into 6 groups using a random numbering method, with 6 replicates per group and 8 geese per replicate (half male and half female). Group I served as the control group receiving the basal diet, while treatment groups received the basal diet supplemented with 2% (Group II), 4% (Group III), 6% (Group IV), 8% (Group V), or 10% (Group VI) fermented grape seed meal. The experimental period lasted 8 weeks.

1.3 Experimental Diets

The basal diet was formulated according to the NRC *Nutrient Requirements of Poultry* (1994). All diets were designed to maintain consistent levels of energy, CP, calcium, and phosphorus. The composition and nutrient levels of experimental diets are shown in Table 1 .

Table 1 Composition and Nutrient Levels of Experimental Diets (Air-Dry Basis), %

Note: The multivitamin and trace elements provided per kilogram of diet: nicotinic acid 65 mg, pantothenate 15 mg, folic acid 0.5 mg, VD_3 200 IU, VA 1,500 mg, VB_1 2.2 mg, VB_2 5.0 mg, VB_6 2 mg, VE 12.5 mg, VK_3 1.5 mg, biotin 0.2 mg, choline 1,000 mg, Fe 85 mg, Zn 80 mg, Mn 80 mg, I 0.42 mg, Se 0.3 mg, Co 2.5 mg, Cu 6 mg. Nutrient levels were calculated values.

1.4 Management Practices

Before the experiment, the goose house was thoroughly disinfected. Geese were raised indoors throughout the trial on thick litter in partitioned pens with ad libitum access to water and feed, following the principle of frequent feeding in small amounts. The health status of the flocks was monitored regularly.

1.5 Sample Collection

At the beginning of the 12th week, 2 geese per replicate (half male and half female) were randomly selected, totaling 72 geese. At the end of the feeding trial, 10 mL of blood was collected from the wing vein and centrifuged at 3,000 r/min for 15 min to obtain serum samples. After slaughter and exsanguination, the abdominal cavity was immediately opened, intestinal segments were ligated at their boundaries, and cecal contents were collected in sterile centrifuge tubes and stored at -40 °C. The entire digestive tract was removed to isolate the glandular stomach, duodenum, pancreas, and jejunum, which were snap-frozen in liquid nitrogen and transferred to a -20 °C freezer. The thymus, spleen, and bursa of Fabricius were collected, blotted with filter paper to remove excess moisture and blood, trimmed of extraneous tissue and fat, and weighed.

Preparation of glandular stomach supernatant: Glandular stomach contents were thawed at room temperature, diluted 10-fold (w/v) with 4 °C deionized water, homogenized at low temperature, and centrifuged at 3,000 r/min for 15 min. The supernatant was aliquoted and stored at -20 °C.

Preparation of pancreatic supernatant: After thawing, pancreatic samples were taken from the head, body, and tail regions, diluted 10-fold (w/v) with 4 °C deionized water, ground at low temperature, and centrifuged at 3,000 r/min for 20 min at 4 °C. The supernatant was aliquoted and stored at -20 °C.

Preparation of duodenal and jejunal supernatants: The small intestine was longitudinally opened before complete thawing. Samples were diluted 10-fold (w/v) with 4 °C deionized water, homogenized for 45 s at 1,200 r/min in an ice bath, and centrifuged at 3,000 r/min for 15 min at 4 °C. The supernatant was aliquoted and stored at -20 °C.

1.6 Digestive Enzyme Activity Assays

Activities of pepsin (glandular stomach), trypsin (pancreas, duodenum, jejunum), amylase (pancreas, duodenum, jejunum), and pancreatic lipase (pancreas, duodenum, jejunum) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.7 Intestinal Microbiota Analysis

One gram of thawed cecal content was transferred to a sterile tube containing 9 mL of sterile diluent, mixed thoroughly with a vortex shaker to prepare a 10^{-1}

dilution. One milliliter of this dilution was transferred to another tube containing 9 mL of sterile diluent, vortexed for 1-2 min to prepare a 10^{-2} dilution, and serial dilutions up to 10^{-5} were prepared. Three drops of each dilution were inoculated onto appropriate media in duplicate: eosin-methylene blue (EMB) agar for *E. coli*, Bifidobacterium-selective (BBL) agar for bifidobacteria, and de Man, Rogosa, and Sharpe (MRS) agar for lactobacilli. *E. coli* was cultured aerobically at 37 °C for 24 h, while lactobacilli and bifidobacteria were cultured anaerobically at 35 °C for 48 h. Plates with 30-300 colonies were selected for counting. Bacterial counts were calculated using the formula: bacteria per gram of intestinal content = colony count × number of drops × dilution factor, and expressed as $\log_{10}$(CFU/g).

1.8 Antioxidant Indices Assays

Serum total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) activity, GSH-Px activity, and malondialdehyde (MDA) content were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.9 Statistical Analysis

Data were analyzed using SPSS 17.0 software. Significance testing was performed using LSD multiple comparison. Results are expressed as "mean ± standard deviation." P<0.05 and P<0.01 were considered significant and extremely significant, respectively.

2.1 Effects of Dietary Fermented Grape Seed Meal Levels on Digestive Enzyme Activity in Geese

As shown in Table 2 , Group V exhibited the highest activities of amylase, trypsin, and lipase in the duodenum, which were extremely significantly higher than the control group (P<0.01). No significant difference in trypsin activity was observed between Groups IV and V (P>0.05). Group V also showed the highest activities of amylase, trypsin, and lipase in the jejunum, extremely significantly higher than the control (P<0.01), with no significant differences in amylase and lipase activities between Groups IV and V (P>0.05). In the pancreas, Group V had the highest amylase and trypsin activities, extremely significantly higher than the control (P<0.01), while Group IV showed the highest lipase activity, extremely significantly higher than the control (P<0.01). No significant differences in pancreatic amylase and lipase activities were found between Groups IV and V (P>0.05). Group V demonstrated the highest pepsin activity, significantly higher than the control (P<0.05).

These results indicate that fermented grape seed meal supplementation can significantly or extremely significantly enhance digestive enzyme activities in the duodenum, pancreas, and jejunum, as well as increase pepsin activity, with optimal effects observed at inclusion levels of 6%-8%.

Table 2 Effects of Dietary Fermented Grape Seed Meal Levels on Digestive Enzyme Activity in Geese, U/g

Note: In the same column, values with the same lowercase letter or no letter superscript indicate no significant difference (P>0.05), adjacent lowercase letters indicate significant difference (P<0.05), and alternate lowercase letters indicate extremely significant difference (P<0.01). The same applies to Table 3 and Table 6.

2.2 Effects of Dietary Fermented Grape Seed Meal Levels on Intestinal Microflora Balance in Geese

As shown in Table 3 , Group IV had the lowest *E. coli* count, significantly lower than the control (P<0.05), with no significant difference between Groups IV and V (P>0.05). Group V had the highest *Lactobacillus* count, significantly higher than the control (P<0.05), with no significant difference between Groups IV and V (P>0.05). Group V also had the highest bifidobacteria count, though no significant differences were observed among all groups (P>0.05).

These results demonstrate that fermented grape seed meal can significantly reduce *E. coli* populations and increase *Lactobacillus* and bifidobacteria counts in the cecum, with optimal beneficial microflora ratios achieved at 6%-8% inclusion levels.

Table 3 Effects of Dietary Fermented Grape Seed Meal Levels on Cecal Microflora Balance in Geese, $\log_{10}$(CFU/g)

2.3 Effects of Dietary Fermented Grape Seed Meal Levels on Jejunal Histomorphology in Geese

As shown in Table 4 , Group IV exhibited the greatest villus height, significantly higher than the control (P<0.05) but not significantly different from Groups III and V (P>0.05). Group IV also showed the thickest muscle layer, significantly thicker than the control (P<0.05) but not significantly different from Group III (P>0.05). Group V had the shallowest crypt depth, extremely significantly lower than the control (P<0.01).

These results indicate that fermented grape seed meal can optimize intestinal structure, with the most rapid tissue development observed at 6%-8% inclusion levels.

Table 4 Effects of Dietary Fermented Grape Seed Meal Levels on Jejunal Histomorphology in Geese, m

Note: In the same row, values with the same lowercase letter or no letter superscript indicate no significant difference (P>0.05), adjacent lowercase letters indicate significant difference (P<0.05), and alternate lowercase letters indicate extremely significant difference (P<0.01). The same applies to Table 5.

2.5 Effects of Dietary Fermented Grape Seed Meal Levels on Immune Organ Indices in Geese

As shown in Table 5 , Group IV had the highest thymus index, though no significant differences were observed among groups (P>0.05). Groups IV and V showed the highest spleen index, with no significant differences among groups (P>0.05). Group IV also had the highest bursa of Fabricius index, with no significant differences among groups (P>0.05).

These results suggest that fermented grape seed meal may influence immune organ development, with the highest indices observed at 6%-8% inclusion levels, though differences were not statistically significant.

Table 5 Effects of Dietary Fermented Grape Seed Meal Levels on Immune Organ Indices in Geese, g/kg

2.6 Effects of Dietary Fermented Grape Seed Meal Levels on Serum Antioxidant Indices in Geese

As shown in Table 7 , Group V exhibited the highest serum T-AOC, extremely significantly higher than the control (P<0.01). Group IV had the lowest serum MDA content, extremely significantly lower than the control (P<0.01), with no significant differences among Groups III, IV, V, and VI (P>0.05). Group V showed the highest serum T-SOD and GSH-Px activities, extremely significantly higher than the control (P<0.01), with no significant difference between Groups IV and V (P>0.05).

These results demonstrate that fermented grape seed meal can enhance antioxidant capacity in geese, with optimal antioxidant indices achieved at 6%-8% inclusion levels.

Table 7 Effects of Dietary Fermented Grape Seed Meal Levels on Serum Antioxidant Indices in Geese

3.1 Effects of Dietary Fermented Grape Seed Meal Levels on Digestive Enzyme Activity in Geese

Xiong [14] reported that *Bacillus natto* preparations significantly increased amylase and trypsin activities in the duodenum and jejunum of broiler chickens at 21 and 42 days of age. Liu [15] found that fermented soybean meal significantly enhanced trypsin and lipase activities in the duodenum and jejunum of Arbor Acres broilers. Hu et al. [16] demonstrated that fermented rapeseed meal significantly increased protease, amylase, and lipase activities in the duodenum, jejunum, ileum, and cecum of broiler chickens at 21 and 42 days of age. Yang et al. [17] showed that supplementing 4% plant peptides in lactating sows' diets significantly increased pepsin activity in the pancreas and lipase and amylase activities in the duodenum of weaned piglets. Guo et al. [18] reported that dietary *Lactobacillus* supplementation increased amylase activity in the jejunum and pancreas of broilers and significantly enhanced trypsin activity in the jejunum and ileum and chymotrypsin activity in the duodenum. Tang et al. [19] confirmed that *Bacillus* spores could germinate and persist in the intestinal tract of Arbor Acres broilers for over 72 hours, improving digestive enzyme activity and microbial community structure. The present study indicates that 6%-8% fermented grape seed meal supplementation significantly enhances digestive enzyme activities in the duodenum, pancreas, and jejunum, as well as pepsin activity, consistent with previous research findings.

3.2 Effects of Dietary Fermented Grape Seed Meal Levels on Intestinal Microflora Balance in Geese

Xiong [14] reported that *Bacillus natto* preparations significantly reduced *E. coli* and *Salmonella* counts while increasing *Lactobacillus* populations in the ileum and cecum of broilers at 21 and 42 days of age. Guerra et al. [20] also reported that lactic acid bacteria effectively inhibit intestinal *E. coli* growth and reproduction. Juven et al. [21] and Reid et al. [22] found that bacteriocins produced by lactobacilli inhibit *Salmonella*, *E. coli*, and *Shigella*. Huang [23] demonstrated that oral administration of *Lactobacillus delbrueckii* increased villus height and crypt depth in piglets. Ma et al. [24] showed that dietary *Lactobacillus* powder alone significantly inhibited *E. coli* proliferation and promoted the growth of lactobacilli and bifidobacteria, while also stimulating villus growth and increasing intestinal wall thickness in the cecum and ileum. Hu et al. [25] reported that composite probiotic preparations and their adjuvants reduced cecal *E. coli* counts in broilers under certain conditions. The present study demonstrates that 6%-8% fermented grape seed meal supplementation significantly reduces cecal *E. coli* counts, increases *Lactobacillus* populations, and enhances bifidobacteria counts, consistent with these previous findings.

3.3 Effects of Dietary Fermented Grape Seed Meal Levels on Immune Organ Indices and Serum Antioxidant Indices in Geese

Grape seed meal is rich in vitamin E and proanthocyanidins, both possessing antioxidant functions, and the microbial strains used for fermentation can also produce antioxidant substances. Liu [15] reported that fermented soybean meal promoted immune organ development and significantly increased thymus, spleen, and bursa of Fabricius indices in Arbor Acres broilers. Hu et al. [16] found that fermented rapeseed meal significantly increased spleen and thymus indices in broilers at 21 and 42 days of age. Hu et al. [25] demonstrated that composite probiotic preparations significantly increased serum T-AOC in broilers. Zhang et al. [26] reported that appropriate yeast culture supplementation significantly reduced serum MDA content, increased serum GSH-Px activity, and significantly enhanced serum T-SOD activity and total antioxidant capacity in cashmere goats. Li et al. [10] showed that proanthocyanidins reduced serum MDA content in a dose-dependent manner. Microbial fermentation produces small peptides that participate in immune regulation and enhance immune function [27]. Zhu [28] reported that plant peptide supplementation during lactation stimulated intestinal development in weaned piglets, increasing villus height and decreasing crypt depth. Chen [29] demonstrated that active feed yeast powder enhanced immune function in weaned piglets. The present study indicates that 6%-8% fermented grape seed meal supplementation significantly improves antioxidant capacity in geese, consistent with previous research, though it did not significantly affect immune organ indices.

Conclusions:

1. Appropriate dietary supplementation with fermented grape seed meal significantly enhances digestive enzyme activities in the duodenum, pancreas, and jejunum, as well as pepsin activity.

2. Appropriate dietary supplementation with fermented grape seed meal significantly reduces cecal *E. coli* counts, increases *Lactobacillus* and bifidobacteria populations, improves villus height and muscle layer thickness, decreases crypt depth, and optimizes intestinal histomorphology.

3. Appropriate dietary supplementation with fermented grape seed meal can increase thymus, spleen, and bursa of Fabricius indices in geese, though not significantly, while significantly enhancing serum T-AOC, T-SOD, and GSH-Px activities and reducing serum MDA content.

4. The recommended optimal dietary inclusion level of fermented grape seed meal for geese is 6%-8%.

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