Effects of Plant Polyphenols on Antioxidant Capacity, Intestinal Morphology, and Meat Quality of Yellow-Feathered Broilers (Postprint)

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Abstract

This experiment aimed to investigate the effects of plant polyphenols on antioxidant capacity, intestinal morphology, and meat quality in yellow-feathered broiler chickens. A total of 288 one-day-old male yellow-feathered broiler chickens were selected and randomly divided into 4 groups, with 6 replicates per group and 12 birds per replicate. The groups were fed a basal diet (control group), basal diet + 100 g/t antibiotic (antibiotic group), basal diet + 500 mg/kg plant polyphenols (BP500 group), and basal diet + 1,000 mg/kg plant polyphenols (BP1000 group), respectively. The experimental period lasted 56 days. The results showed: 1) At 21 days of age, the serum catalase (CAT) activity in broilers of the BP500 and BP1000 groups was significantly higher than that in the antibiotic group ($P<0.05$); at 56 days of age, the serum CAT activity in broilers of the BP1000 group was significantly higher than that in the other three groups ($P<0.05$). 2) At 21 days of age, the ileal villus height/crypt depth ratio (V/C) in broilers of the BP500 and BP1000 groups was significantly lower than that in the antibiotic group ($P<0.05$); at 56 days of age, the ileal V/C in broilers of the BP500 group was significantly higher than that in the control and antibiotic groups ($P<0.05$). 3) At 56 days of age, the breast meat redness (a*) value in the BP1000 group was significantly higher than that in the other three groups ($P<0.05$); the breast muscle water loss rate in the BP500 and BP1000 groups was significantly lower than that in the other two groups ($P<0.05$); the 24 h drip loss of breast muscle in the BP500 and BP1000 groups was significantly lower than that in the other two groups ($P<0.05$); the 48 h drip loss of breast muscle in the BP1000 group was significantly lower than that in the control and antibiotic groups ($P<0.05$). It can be concluded that dietary supplementation with 500 and 1,000 mg/kg of plant polyphenols can both improve intestinal morphology, enhance systemic antioxidant capacity, and improve meat quality

in yellow-feathered broiler chickens. The recommended supplementation level of plant polyphenols is 1,000 mg/kg.

Full Text

Effects of Botanical Polyphenol on Antioxidant Capacity, Intestinal Morphology and Meat Quality of Yellow Broilers

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Abstract

This experiment was conducted to investigate the effects of botanical polyphenol on antioxidant capacity, intestinal morphology and meat quality of yellow broilers. A total of 288 one-day-old male yellow broilers were randomly allocated to 4 groups with 6 replicates per group and 12 chickens per replicate. Broilers in the four groups were fed a basal diet (control group), the basal diet + 100 g/t colistin sulfate (antibiotic group), the basal diet + 500 mg/kg botanical polyphenol (BP500 group) and the basal diet + 1,000 mg/kg botanical polyphenol (BP1000 group), respectively. The experiment lasted for 56 days. The results showed as follows: 1) On day 21, the serum catalase (CAT) activity in the BP500 and BP1000 groups was significantly higher than that in the antibiotic group ($P<0.05$); on day 56, the serum CAT activity in the BP1000 group was significantly higher than that in the other three groups ($P<0.05$). 2) On day 21, the ileal villus height/crypt depth (V/C) ratio in the BP500 and BP1000 groups was significantly higher than that in the antibiotic group ($P<0.05$); on day 56, the ileal V/C ratio in the BP500 group was significantly higher than that in the control and antibiotic groups ($P<0.05$). 3) On day 56, the breast muscle redness value (a*) in the BP1000 group was significantly higher than that in the other three groups ($P<0.05$); the water loss rate of breast muscle in the BP500 and BP1000 groups was significantly lower than that in the other two groups ($P<0.05$); the 24 h drip loss of breast muscle in the BP500 and BP1000 groups was significantly lower than that in the control and antibiotic groups ($P<0.05$); the 48 h drip loss of breast muscle in the BP1000 group was significantly lower than that in the control and antibiotic groups ($P<0.05$). It is concluded that dietary supplementation with 500 and 1,000 mg/kg botanical polyphenol can improve intestinal morphology, enhance antioxidant capacity and improve meat quality of yellow broilers. The recommended addition level of botanical polyphenol is 1,000 mg/kg.

Key words: botanical polyphenol; yellow broilers; antioxidant capacity; intestinal morphology; meat quality

Introduction

Since antibiotics were widely applied in animal production, they have played an important role in preventing and treating livestock diseases and promoting growth, greatly improving the economic benefits of farming. However, with the rapid development of animal husbandry, the long-term low-dose prophylactic use or excessive and indiscriminate use of antibiotics has become common, causing increasingly prominent problems such as toxin residues and accumulation in livestock, development of drug-resistant pathogens, and decreased animal immunity, which seriously threaten human health. In recent years, the emergence of super-resistant bacteria has sounded the alarm for humans, making it urgent to develop green, environmentally friendly, and non-toxic antibiotic alternatives without residue.

Botanical polyphenols are a class of natural plant extracts widely present in plants, featuring polyphenolic structures and serving as natural antioxidants that can scavenge harmful free radicals and activate the antioxidant defense system [1-2]. Previous studies have reported that dietary supplementation with botanical polyphenols not only improves the health and product quality of livestock and poultry but also avoids harmful residues and toxic side effects [3-5]. Therefore, botanical polyphenols, with their wide sources and low cost, can serve as pure natural, pollution-free, residue-free, and non-resistant antibiotic alternatives with broad market prospects and application potential, representing an inevitable trend in future feed industry development and holding significant importance for the stable, sustainable, and healthy development of animal husbandry. In poultry, botanical polyphenols as feed additives can also promote growth performance, immune function, and antioxidant capacity, and improve meat quality and intestinal morphology [6-9]. However, only a few botanical polyphenol components have been confirmed effective in livestock and poultry, and more applications need to be explored. Therefore, this laboratory developed a polymeric botanical polyphenol. By adding different levels of this polymeric botanical polyphenol to the diet, this study investigated its effects on the antioxidant capacity, intestinal morphology and meat quality of yellow broilers, providing a theoretical basis for the application of botanical polyphenols in poultry diets.

Materials and Methods

1.1 Test Materials

The botanical polyphenol was prepared in our laboratory, with main components being monomeric polyphenols (flavonoids) and complex polyphenols (proanthocyanidins). The measured total phenol content was 11.59 mg/g, and the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity was 1.97 times that of Trolox.

1.2 Experimental Design

A total of 288 healthy one-day-old male yellow broilers (fast-growing yellow broilers, bred by Guangdong Wens Group) were randomly divided into 4 groups with 6 replicates per group and 12 chickens per replicate, with similar body weight among replicates ($P>0.05$). The control group was fed a basal diet formulated according to the NRC (1994) and "Feeding Standard of Yellow Chickens" (NY/T 33-2004) for yellow broilers. The composition and nutrient levels of the basal diet are shown in Table 1 . The antibiotic group was fed the basal diet + 100 g/t colistin sulfate, while the experimental groups were fed the basal diet + 500 mg/kg botanical polyphenol (BP500 group) and the basal diet + 1,000 mg/kg botanical polyphenol (BP1000 group), respectively. The experimental period lasted 56 days, divided into two stages: 1-21 days and 22-56 days.

1.3 Management

Broilers were raised in three-tier cages with boiler temperature control (33°C on day 1, 30-32°C in week 1, 27-29°C in week 2, 24-26°C in week 3, and 20-21°C thereafter), artificial lighting, natural ventilation, and free access to water and feed with ad libitum intake and measurement. Normal immunization procedures were followed. Daily observations and records were made on flock behavior and health status, as well as temperature and humidity in the chicken house.

1.4 Sample Collection and Measurements

1.4.1 Serum Antioxidant Indices On days 21 and 56, one chicken was randomly selected from each replicate for blood collection from the wing vein. After standing at room temperature for 2 hours, blood samples were centrifuged at 3,000 r/min for 10 minutes to separate serum, which was stored at -20°C for subsequent analysis. Serum catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, total antioxidant capacity (T-AOC) and malondialdehyde (MDA) content were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.2 Antibody Levels Newcastle disease virus (NDV) vaccine was first administered at 14 days of age via drinking water, and second vaccination was completed at 28 days of age in the same manner. Blood was collected from the wing vein on day 14 after the first vaccination and on days 5, 9, and 13 after the second vaccination. After centrifugation at low temperature, serum was separated and stored at -20°C. Anti-NDV antibody levels were determined by enzyme-linked immunosorbent assay (ELISA).

1.4.3 Intestinal Morphology Indices On days 21 and 56 after slaughter, approximately 1 cm segments from the terminal jejunum and middle ileum were collected and fixed in 10% formalin. After 24 hours of fixation, the intestinal segments were processed through water flushing, gradient alcohol dehydration, xylene clearing, and paraffin embedding. Sections were cut at 6 m thickness, stained with routine hematoxylin-eosin (HE), and finally sealed with neutral resin. Intestinal morphology changes were observed under a biological microscope. Three intact and straight villi were selected to measure villous height and crypt depth, and the villus height/crypt depth ratio (V/C) was calculated.

1.4.4 Meat Quality Indices 1.4.4.1 Water Loss Rate

On day 56 after slaughter, the right breast muscle was collected for water loss rate determination using the compression method. A 1 cm × 1 cm × 1 cm cube was cut perpendicular to the muscle fiber direction and weighed (W). The meat cube was placed between two layers of medical gauze, with 18 layers of filter paper (medium-speed qualitative analysis filter paper) on each side, sandwiched between iron plates, and placed on the platform of a modified steel expansion-compression instrument. Pressure was applied to 35 kg and maintained for 5 minutes. After pressure removal, the meat sample was weighed again (W). Water loss rate (%) = [(W - W) / W] × 100.

1.4.4.2 Drip Loss

On day 56 after slaughter, the right breast muscle was collected and trimmed into 2 cm × 2 cm × 1 cm pieces and weighed (W). The meat sample was hooked with a thin wire at one end so that muscle fibers hung vertically downward. The other end of the wire was passed through the bottom of a plastic cup, suspending the meat sample inside the cup. The plastic cup was placed in a fresh-keeping bag, which was sealed and stored in a 4°C refrigerator. After 24 hours, the meat sample was removed and weighed (W) after gently wiping surface moisture with clean filter paper. The weighed meat sample was re-hung and returned to the refrigerator. After another 24 hours, the meat sample was removed and weighed (W) after wiping surface juice with filter paper. 24 h drip loss (%) = [(W - W) / W] × 100; 48 h drip loss (%) = [(W - W) / W] × 100.

1.4.4.3 Meat Color

On day 56 after slaughter, the left breast muscle was collected. After 30-45 minutes, a CR-400 colorimeter (Konica Minolta, Japan) was used to measure lightness (L*), redness (a*) and yellowness (b*) values on the muscle surface near the bone side. Three measurements were taken and the average was used as the final result.

1.4.4.4 pH Value

On day 56 after slaughter, the left breast muscle was collected. After 45 minutes, the electrode of a Testo-205 portable pH meter (Testo, Germany) was completely embedded in the meat sample to ensure full contact with muscle tissue fluid. The pH value was recorded after the reading stabilized. The muscle was then stored in a 4°C refrigerator and measured again after 24 hours. Each meat sample was measured three times and the average was used as the final result.

1.4.4.5 Muscle MDA Content

On day 56 after slaughter, approximately 10 g of the right breast muscle was collected, wrapped in tin foil, snap-frozen in liquid nitrogen, and then transferred to a -20°C refrigerator for storage. Muscle MDA content was measured using an assay kit purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Experimental data were expressed as mean ± standard deviation. One-way ANOVA was performed on all data using SPSS 19.0 software, and Duncan' s multiple comparison was conducted for significant differences. $P<0.05$ was considered statistically significant.

Results

2.1 Effects of Botanical Polyphenol on Serum Antioxidant Indices of Yellow Broilers

The effects of botanical polyphenol on serum antioxidant indices of yellow broilers at 21 and 56 days of age are shown in Table 2 . On day 21, serum CAT activity in the BP500 and BP1000 groups was significantly higher than that in the antibiotic group ($P<0.05$). On day 56, serum CAT activity in the BP1000 group was significantly higher than that in the other three groups ($P<0.05$).

2.2 Effects of Botanical Polyphenol on Serum Antibody Levels of Yellow Broilers

The effects of botanical polyphenol on antibody levels of broilers are shown in Table 3 . On day 14 after the first vaccination (28 days of age), the serum anti-NDV antibody level in the control group was significantly higher than that in the antibiotic and BP1000 groups ($P<0.05$), while the antibody level in the BP500 group was slightly higher than that in the antibiotic and BP1000 groups ($P>0.05$). On day 9 after the second vaccination (37 days of age), the anti-NDV antibody level in the BP500 group was significantly higher than that in the other groups ($P<0.05$). On days 5 (33 days of age) and 13 (51 days of age) after the second vaccination, no significant differences in anti-NDV antibody levels were observed among groups (P>0.05).

2.3 Effects of Botanical Polyphenol on Intestinal Morphology of Yellow Broilers

The effects of botanical polyphenol on intestinal morphology of broilers at 21 and 56 days of age are shown in Figure 1 [Figure 1: see original paper], Figure 2 [Figure 2: see original paper] and Table 4 . As shown in Figures 1 and 2, villi in the jejunum and ileum of all groups were generally finger-shaped and columnar. The control and antibiotic groups showed poorly developed villi in both intestinal segments, mostly irregularly arranged, thick and short with considerable shedding. In contrast, the BP500 and BP1000 groups exhibited longer villi in both segments, arranged neatly and tightly with clear boundaries between villi. Overall, the BP500 group showed better intestinal villus development.

As shown in Table 4, on day 21, the jejunal crypt depth was greatest in the control group, significantly greater than that in the antibiotic group (P<0.05), while the antibiotic group had the shallowest crypt depth and the BP500 and BP1000 groups showed no significant differences from other groups (P>0.05). The ileal villus height was shortest in the antibiotic group, significantly lower than that in the control group (P<0.05), while the control group had the highest ileal villus height and no significant differences were observed between the BP500 and BP1000 groups (P>0.05). The ileal crypt depth in the antibiotic and BP500 groups was significantly lower than that in the control and BP1000 groups (P<0.05). The ileal V/C ratio in the antibiotic group was significantly higher than that in the other three groups (P<0.05). On day 56, the ileal villus height was shortest in the BP500 group, significantly lower than that in the antibiotic and BP1000 groups (P<0.05), while the antibiotic group had the highest ileal villus height and no significant differences were observed between the control and BP1000 groups (P>0.05). The ileal crypt depth in the BP500 group was significantly lower than that in the other three groups (P<0.05). The ileal V/C ratio in the BP500 group was significantly higher than that in the control and antibiotic groups (P<0.05).

2.4 Effects of Botanical Polyphenol on Meat Quality of Yellow Broilers

The effects of botanical polyphenol on meat quality of yellow broilers at 56 days of age are shown in Table 5 . The breast muscle redness value (a*) in the BP1000 group was significantly higher than that in the other groups (P<0.05). The water loss rate of breast muscle in the BP500 and BP1000 groups was significantly lower than that in the control and antibiotic groups (P<0.05). The 24 h drip loss of breast muscle in the BP500 and BP1000 groups was significantly lower than that in the control and antibiotic groups (P<0.05), and the 48 h drip loss of breast muscle in the BP1000 group was significantly lower than that in the control and antibiotic groups (P<0.05).

Discussion

3.1 Effects of Botanical Polyphenol on Serum Antioxidant Indices of Yellow Broilers

The results of this study indicate that dietary supplementation with 500 and 1,000 mg/kg botanical polyphenol enhanced the antioxidant capacity of broilers. Shu Gang [10] reported that bamboo leaf flavonoids significantly increased serum SOD and CAT activities and T-AOC while decreasing serum MDA content in broilers. Li Hong et al. [11] found that dietary supplementation with 0.3% chestnut involucre polyphenol significantly improved antioxidant capacity in broilers. The results of this experiment are basically consistent with these reports on the antioxidant effects of botanical polyphenols (tea polyphenols). The antioxidant activity of botanical polyphenols is mainly attributed to their structure containing multiple phenolic hydroxyl groups that are easily oxidized, which can directly combine with free radicals and oxidants to scavenge free radicals and inhibit lipid peroxidation, or indirectly scavenge free radicals by promoting the body' s antioxidant system [12-13].

3.2 Effects of Botanical Polyphenol on Antibody Levels of Yellow Broilers

Antibody levels reflect humoral immune capacity to a certain extent. The results of this study showed that on day 14 after the first vaccination, the anti-NDV antibody level in the BP1000 group was significantly higher than that in the control group; on day 9 after the second vaccination, the anti-NDV antibody level in the BP500 group was significantly higher than that in the other groups, indicating that botanical polyphenol had some influence on the humoral immune response of yellow broilers. These findings on the promoting effect of botanical polyphenols on humoral immunity are similar to reports by Li Zhen et al. [14], Ma Meihu [15] and Qiu Guangheng et al. [16]. Most researchers believe that the specific mechanism by which botanical polyphenols regulate immune function is through their antioxidant properties protecting biological membranes from free radical damage, thereby maintaining membrane fluidity and keeping the body in a dynamic redox balance state.

3.3 Effects of Botanical Polyphenol on Intestinal Morphology of Yellow Broilers

Small intestinal villi are the direct sites for absorption function, and villus height is significantly correlated with cell number. Increased villus height can enlarge the surface area for nutrient absorption in the small intestine, so villus length is directly related to animal growth and development. Only mature villus epithelial cells have nutrient absorption function; therefore, short villi mean fewer mature cells and lower nutrient absorption capacity [17-19]. Small intestinal crypts are tubular glands formed by epithelial invagination at the villus base into the lamina propria. Crypt depth mainly reflects the generation rate of epithelial cells. Epithelial cells continuously move from the crypt base toward the villus tip, forming new villus cells with absorptive capacity to replace normal cell shedding. If this process slows down, the cell generation rate at the base decreases, resulting in deeper crypts [20-21]. The V/C ratio comprehensively reflects small intestinal villus absorptive function. A decreased V/C ratio indicates reduced digestive and absorptive function, often accompanied by diarrhea; an increased V/C ratio indicates enhanced digestive and absorptive function and decreased diarrhea rate [22-23]. In this experiment, the ileal villus height of 21-day-old broilers in the BP1000 group was significantly higher than that in the antibiotic group; dietary supplementation with 500 and 1,000 mg/kg botanical polyphenol increased the ileal V/C ratio of 56-day-old broilers. This indicates that botanical polyphenol can improve intestinal morphology of yellow broilers to a certain extent, consistent with the findings of Sun Danfeng [9], Li Denghui [24] and Yuan Zehong [25].

3.4 Effects of Botanical Polyphenol on Meat Quality of Yellow Broilers

The meat color a* value indicates the content and state of myoglobin in muscle. Muscle water-holding capacity, the ability of muscle tissue to retain water, is an important indicator for evaluating poultry meat quality and is currently assessed using water loss rate, drip loss, cooking loss or cooked meat rate. Lipid peroxidation is one of the main causes of meat quality deterioration, and MDA is one of the most important products of lipid peroxidation in muscle. Jiang Shouqun [7] reported that dietary supplementation with 40 mg/kg soybean isoflavone in Lingnan yellow broilers slightly increased the breast muscle a* value at 24 h post-slaughter, improved breast muscle water-holding capacity, and decreased breast muscle MDA content. Song Yang et al. [26] reported that dietary supplementation with Paulownia flower flavonoid extract could improve muscle water-holding capacity in Arbor Acres broilers. In this experiment, the meat color a* value in the BP1000 group was significantly higher than that in the other three groups, indicating that botanical polyphenol can inhibit myoglobin oxidation to metmyoglobin to a certain extent, maintaining meat color stability. The water loss rate and drip loss of breast muscle in the BP500 and BP1000 groups were significantly reduced, indicating that botanical polyphenol can enhance muscle water-binding capacity. Dietary supplementation with botanical polyphenol showed a trend toward decreasing MDA content in breast muscle, suggesting that botanical polyphenol can inhibit lipid peroxidation to some extent and maintain chicken freshness. Overall, the results of this experiment are basically consistent with domestic reports on botanical polyphenols improving chicken meat quality.

Conclusion

Dietary supplementation with 500 and 1,000 mg/kg botanical polyphenol significantly increased serum antioxidant enzyme CAT activity in yellow broilers, thereby enhancing their antioxidant capacity, with 1,000 mg/kg showing better effects. Both 500 and 1,000 mg/kg botanical polyphenol supplementation improved humoral immune response to NDV to some extent. Dietary supplementation with 500 and 1,000 mg/kg botanical polyphenol improved intestinal morphology in broilers, with 1,000 mg/kg showing better effects. Both supplementation levels improved meat quality of yellow broilers, with 1,000 mg/kg showing better effects. Taking all factors into consideration, the recommended dietary supplementation level of botanical polyphenol is 1,000 mg/kg.

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