Effects of Tea Seed Polysaccharide and Macleaya cordata Alkaloids on Intestinal Microbiota of Yellow-Feathered Broiler Chickens and Antibacterial Activity of Sanguinarine from Macleaya cordata: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with tea seed polysaccharides and Macleaya cordata alkaloids on the intestinal microbiota of yellow-feathered broilers and the antibacterial activity of sanguinarine from Macleaya cordata. A total of 750 healthy 1-day-old yellow-feathered broilers were selected and randomly divided into 5 groups with 6 replicates per group and 25 chickens per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with antibiotics (10 mg/kg colistin sulfate at 1–28 days of age and 5 mg/kg flavomycin at 29–56 days of age; antibiotic group), tea seed polysaccharides (0.04%; polysaccharide group), Macleaya cordata alkaloids (10 mg/kg at 1–28 days of age and 20 mg/kg at 29–56 days of age; Macleaya group), or tea seed polysaccharides (0.04%) + Macleaya cordata alkaloids (10 mg/kg at 1–28 days of age and 20 mg/kg at 29–56 days of age; polysaccharide + Macleaya group). The experimental period lasted 56 days. The agar diffusion disc method and tube liquid double dilution method were used to determine the antibacterial effects and minimum inhibitory concentration (MIC) of sanguinarine against Staphylococcus aureus, Escherichia coli, Salmonella, and Pasteurella. The results showed that the jejunal villus height/crypt depth (V/C) ratio in the polysaccharide, Macleaya, and polysaccharide + Macleaya groups was significantly higher than that in the control group ($P<0.05$). Compared with the control and antibiotic groups, the number of Lactobacillus in the jejunal contents of yellow-feathered broilers in the polysaccharide, Macleaya, and polysaccharide + Macleaya groups was extremely significantly increased ($P<0.01$). Sanguinarine showed moderate sensitivity to Staphylococcus aureus and Pasteurella, low sensitivity to Escherichia coli, and high sensitivity to Salmonella. The MICs of sanguinarine against Staphylococcus aureus, Escherichia coli, Pasteurella, and Salmonella were 25.00, 25.00, 12.50, and 1.56 g/mL, respectively. In conclusion, dietary supplementation with tea seed polysaccharides and Macleaya cordata alkaloids can both replace antibiotics, effectively improve the jejunal morphology of yellow-feathered broilers, and increase the number of Lactobacillus in the jejunum, with combined use showing comparable effects to individual supplementation; sanguinarine exhibits strong antibacterial activity against Salmonella, which is superior to that of sodium penicillin.

Full Text

Preamble

Effects of Tea Seed Polysaccharide and Macleaya cordata Alkaloids on Intestinal Flora of Yellow-Feathered Broilers and Antimicrobial Activity of Macleaya cordata Sanguinarine

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Abstract: This experiment was conducted to investigate the effects of dietary tea seed polysaccharide and Macleaya cordata alkaloids on intestinal flora of yellow-feathered broilers and the antimicrobial activity of Macleaya cordata sanguinarine. A total of 750 one-day-old healthy yellow-feathered broilers were randomly allocated to 5 groups with 6 replicates per group and 25 broilers per replicate. The control group was fed a basal diet, while experimental groups received the basal diet supplemented with either antibiotic (10 mg/kg colistin at 1-28 days of age and 5 mg/kg flavomycin at 29–56 days of age; antibiotic group), tea seed polysaccharide (0.04%; polysaccharide group), Macleaya cordata alkaloids (10 mg/kg at 1-28 days of age and 20 mg/kg at 29–56 days of age; Macleaya cordata group), or a combination of tea seed polysaccharide (0.04%) and Macleaya cordata alkaloids (10 mg/kg at 1–28 days of age and 20 mg/kg at 29–56 days of age; polysaccharide+Macleaya cordata group). The experiment lasted 56 days. The antibacterial effects and minimum inhibitory concentration (MIC) of Macleaya cordata sanguinarine against Staphylococcus aureus, Escherichia coli, Salmonella, and Pasteurella multocida were determined using the agar diffusion paper method and double dilution method in test tube liquid, respectively. The results showed that the villus height to crypt depth ratio (V/C) in the jejunum of yellow-feathered broilers in the polysaccharide group, Macleaya cordata group, and polysaccharide+Macleaya cordata group was significantly higher than that in the control group ($P<0.05$). Compared with the control and antibiotic groups, the number of Lactobacillus in jejunal contents was significantly increased in the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups ($P<0.01$). Macleaya cordata sanguinarine showed moderate sensitivity to Staphylococcus aureus and Pasteurella multocida, low sensitivity to Escherichia coli, and high sensitivity to Salmonella. The MIC values of Macleaya cordata sanguinarine against Staphylococcus aureus, Escherichia coli, Pasteurella multocida, and Salmonella were 25.00, 25.00, 12.50, and 1.56 g/mL, respectively. In conclusion, dietary supplementation with tea seed polysaccharide and Macleaya cordata alkaloids can effectively improve jejunal morphological structure and increase Lactobacillus counts in the jejunum of yellow-feathered broilers, with effects equivalent to antibiotics. The combined use of both additives produces effects comparable to individual supplementation. Macleaya cordata sanguinarine exhibits strong antibacterial activity against Salmonella, superior to that of penicillin sodium.

Keywords: tea seed polysaccharide; Macleaya cordata alkaloids; intestinal morphological structure; antimicrobial activity; minimum inhibitory concentration

In modern intensive livestock production, antibiotics are widely used and even abused to promote rapid animal growth and prevent diseases. However, antibiotic usage has led to serious problems including antibiotic residues and bacterial resistance, posing significant threats to human living environments and health. In response to this crisis, many countries and regions have banned or restricted antibiotic use in feed. Consequently, developing safe, green, and efficient feed additives has become an inevitable trend in the livestock industry.

Plant extracts are among the most commonly studied and promising antibiotic alternatives, characterized by being green, pollution-free, low-toxicity, and easily degradable. Macleaya cordata alkaloids possess strong antibacterial and anti-inflammatory properties [1], while polysaccharides can promote free radical scavenging and significantly influence specific immunity, non-specific immunity, cellular immunity, and humoral immunity in animals [2]. Previous studies have shown that Macleaya cordata sanguinarine can inhibit the proliferation of plant pathogenic bacteria, molds, and viruses [3]. Yu [4] reported that Macleaya cordata injection exhibited good antibacterial effects against avian Pasteurella, Escherichia coli, and Salmonella pullorum, and was effective in treating avian cholera and piglet white scours. Sun et al. [5] found that dietary Astragalus polysaccharide supplementation reduced Escherichia coli and Salmonella counts while increasing Lactobacillus and Bifidobacterium numbers in the ceca of 49-day-old broilers. However, research on the effects of tea seed polysaccharide and Macleaya cordata alkaloids on intestinal health in livestock and poultry, particularly broilers, remains limited. Therefore, this study aimed to investigate the effects of tea seed polysaccharide and Macleaya cordata alkaloids on intestinal health in yellow-feathered broilers and to evaluate the antibacterial efficacy and MIC of Macleaya cordata sanguinarine, providing a basis for their application in livestock production.

1.1 Experimental Materials

Macleaya cordata alkaloids (total alkaloid content 70%, containing 60% sanguinarine and 20% chelerythrine) was provided by the Hunan Engineering Research Center for Traditional Chinese Medicine Extraction. Tea seed polysaccharide (purity 43.4%) was provided by the Oil and Fat Teaching and Research Section of the College of Food Science and Technology, Hunan Agricultural University. Macleaya cordata sanguinarine (purity approximately 40%) was prepared as a 9.6 mg/mL solution in 20% methanol and provided by the Hunan Engineering Research Center for Traditional Chinese Medicine Extraction. Antibiotics used were colistin for the early period and flavomycin for the later period. Penicillin sodium (purity 95%) was prepared as a 4.8 mg/mL solution in 20% methanol.

Test strains including Staphylococcus aureus (BNCC186335), Escherichia coli (BNCC336953), Salmonella paratyphi (BNCC103169), and Pasteurella multocida (BNCC126487) were provided by the College of Veterinary Medicine, Hunan Agricultural University.

1.2.1 Feeding Trial

A total of 750 one-day-old yellow-feathered broilers from the same batch with identical genetic background and normal development were randomly divided into 5 groups with 6 replicates per group and 25 broilers per replicate. Initial body weight showed no significant differences among groups (P>0.05). A single-factor randomized design was employed. The control group received a basal diet, while experimental groups received the basal diet supplemented with antibiotic (10 mg/kg colistin at 1–28 days of age and 5 mg/kg flavomycin at 29–56 days of age; antibiotic group), tea seed polysaccharide (0.04%; polysaccharide group), Macleaya cordata alkaloids (10 mg/kg at 1–28 days of age and 20 mg/kg at 29–56 days of age; Macleaya cordata group), or a combination of tea seed polysaccharide (0.04%) and Macleaya cordata alkaloids (10 mg/kg at 1–28 days of age and 20 mg/kg at 29–56 days of age; polysaccharide+Macleaya cordata group). Antibiotic supplementation complied with current Chinese laws and regulations. Broilers were fed mash diets throughout the 56-day experimental period.

1.2.2 Antimicrobial Test

The four activated bacterial strains were inoculated onto slant culture media (containing peptone 10 g, beef extract 10 g, sodium chloride 5 g, agar 15–20 g, and water 1,000 mL; pH adjusted to 7.2–7.4 before sterilization at 121 °C for 30 min) and incubated at 37 °C for 24 h. A loopful of bacterial cells was transferred to physiological saline containing glass beads, shaken thoroughly, and counted using a hemocytometer to adjust the concentration to 10 -10 cells/mL.

1.3 Experimental Diets

The basal diets were formulated according to NRC (1994) and the Chinese Feeding Standard of Chickens (NY/T 33—2004) using corn, soybean meal, and other ingredients. The composition and nutrient levels are presented in Table 1 .

1.4 Management

The experiment was conducted in an open-type chicken house with high-low bed floor rearing. Lighting schedule was 14 h light:10 h dark (14 L:10 D). Temperature was maintained at 30-33 °C for days 1-7, 27-29 °C for days 8-14, 24-26 °C for days 15-21, 22-23 °C for days 22-28, and 20-21 °C for days 29-56 using boiler heating, with natural ventilation. Relative humidity was maintained at 55%-65%. Broilers had free access to feed and water and were vaccinated according to routine procedures. Chicken house hygiene was cleaned regularly.

1.5 Sample Collection

At 56 days of age, one broiler 接近 each replicate' s average body weight was selected from each replicate, slaughtered, and 1 g of jejunal content was collected for microbial analysis. Two 2-cm segments of jejunal tissue were fixed in 10% formalin solution for morphological analysis.

1.6.1 Jejunal Morphology

Fixed specimens were processed through dehydration, clearing, paraffin infiltration, embedding, trimming, sectioning, and routine hematoxylin-eosin (HE) staining to prepare paraffin sections. Sections were observed at 100× magnification using a light microscope (Motic AE67), with multiple non-consecutive fields examined and typical fields photographed. Villus height and crypt depth were measured using DT2000 universal image analysis software version 2.0, and the villus height to crypt depth ratio (V/C) was calculated.

1.6.2 Enumeration of Escherichia coli and Lactobacillus in Jejunal Contents

Approximately 0.5 g of jejunal content was weighed aseptically into a sterile test tube, and 4.5 mL of sterile diluent (physiological saline) was added. The mixture was shaken on a magnetic oscillator for 3-5 min to prepare a 10^{1} dilution. After centrifugation, 0.5 mL of supernatant was transferred to another tube containing 4.5 mL of sterile diluent for 10^{2} dilution, followed by serial dilutions to 10^{3}-10 . Aliquots (0.1 mL) from 10^{3}, 10 , and 10 dilutions were plated onto respective media using a spreader. Escherichia coli was cultured on eosin methylene blue medium (Beijing Luqiao Technology Co., Ltd.) at 37 °C under aerobic conditions for 24 h before counting. Lactobacillus was cultured on Lactobacillus medium (Beijing Luqiao Technology Co., Ltd.) at 37 °C under anaerobic conditions for 48 h before counting.

1.6.3 Antimicrobial Activity

Qualitative antibacterial test: The agar diffusion paper method was used. After plates were prepared, sterile cotton swabs dipped in bacterial suspension were used to cover the entire plate surface, which was then inverted and dried for 20 min. Filter paper discs (7 mm diameter, dry-heat sterilized) were soaked in test solutions, excess liquid removed, and placed sequentially on bacterial plates. Each plate contained one disc of each test substance and one control disc with 20% methanol solvent. Plates were incubated at 37 °C for 18-24 h, and the inhibition zone diameter (D) was measured. The criteria for antibacterial effect were: D 8 mm = not sensitive; 8 < D 13 mm = low sensitivity; 13 < D 18 mm = moderate sensitivity; D > 18 mm = high sensitivity. The test was repeated four times.

MIC determination: The double dilution method in test tube liquid was employed. Drug solutions were serially diluted twofold up to tube 24, with bacterial suspension added to each tube to achieve a concentration of 10 CFU/mL. Negative controls were prepared for tubes 1-23, and a positive control for tube 24. Tubes were incubated at 37 °C for 24 h, and results were judged by visual comparison with control tubes. The lowest drug concentration showing complete bacterial growth inhibition was recorded as the MIC. The procedure was repeated three times, and the average value was calculated.

1.7 Data Processing and Statistical Analysis

Experimental data were initially processed using Excel 2007 software and then analyzed using the one-way ANOVA procedure in SPSS 16.0 software. Significant differences among groups were further analyzed using Duncan' s multiple comparison test. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$. Results are expressed as "mean ± standard deviation."

2.1 Jejunal Morphological Structure

As shown in Table 2 , jejunal villus height and crypt depth in yellow-feathered broilers showed no significant differences among the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups compared with the control and antibiotic groups ($P>0.05$). However, the jejunal V/C ratio in the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups was significantly higher than that in the control group ($P<0.05$), with no significant differences observed compared with the antibiotic group ($P>0.05$). No significant differences in V/C ratio were detected among the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups ($P>0.05$).

2.2 Enumeration of Escherichia coli and Lactobacillus in Jejunal Contents

Table 3 shows that Escherichia coli counts in jejunal contents of yellow-feathered broilers showed no significant differences among the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups compared with the control and antibiotic groups ($P>0.05$), although values were numerically lower than the control. In contrast, Lactobacillus counts in jejunal contents were significantly increased in the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups compared with the control and antibiotic groups ($P<0.01$), with no significant differences among these three treatment groups ($P>0.05$).

2.3 Antibacterial Effects of Macleaya cordata Sanguinarine

Table 4 presents the inhibition zone diameters of Macleaya cordata sanguinarine against the four test strains as 14, 13, 22, and 18 mm. These results indicate that Macleaya cordata sanguinarine exhibited moderate sensitivity to Staphylococcus aureus and Pasteurella multocida, low sensitivity to Escherichia coli, and high sensitivity to Salmonella. Compared with penicillin sodium, Macleaya cordata sanguinarine showed enhanced antibacterial effects against three of the test strains, particularly Salmonella, except for a weaker effect against Pasteurella multocida.

2.4 MIC of Macleaya cordata Sanguinarine

Table 5 shows that the MIC values of Macleaya cordata sanguinarine were 25.00 g/mL for both Staphylococcus aureus and Escherichia coli, 12.50 g/mL for Pasteurella multocida, and 1.56 g/mL for Salmonella, demonstrating strong antibacterial activity against Salmonella. Compared with penicillin sodium, Macleaya cordata sanguinarine showed higher MIC values against Staphylococcus aureus and Pasteurella multocida.

3.1 Effects of Tea Seed Polysaccharide and Macleaya cordata Alkaloids on Jejunal Morphology in Yellow-Feathered Broilers

Small intestinal villus height and crypt depth are closely related to digestive and absorptive functions. More developed small intestines lead to better nutrient digestion and absorption [6]. Increased villus height and density, along with more mature epithelial cells, enlarge the intestinal absorption area and enhance absorptive capacity [7]. Shallower crypts increase the maturation rate and secretory function of intestinal epithelial cells [8]. The V/C ratio comprehensively reflects small intestinal function status; an increased ratio indicates improved intestinal mucosal structure and enhanced digestive and absorptive capacity. Dai [9] found that dietary supplementation with 0.5 and 1.0 mg/kg sanguinarine could prevent and treat piglet diarrhea. Sanguinarine in Macleaya cordata alkaloids possesses antibacterial and anti-inflammatory effects and can directly act on and repair damaged intestinal villi, thereby regulating intestinal health [10]. The current results showed no significant differences in jejunal villus height and crypt depth among groups, while the V/C ratio in the polysaccharide, Macleaya cordata, and polysaccharide+Macleaya cordata groups was significantly higher than that in the control group, with no significant differences compared with the antibiotic group. These findings indicate that dietary supplementation with tea seed polysaccharide and Macleaya cordata alkaloids improved jejunal mucosal structure comparably to antibiotics, effectively promoting nutrient digestion and absorption to enhance animal production efficiency. The combined use of both additives produced effects equivalent to individual supplementation.

3.2 Effects of Tea Seed Polysaccharide and Macleaya cordata Alkaloids on Escherichia coli and Lactobacillus Counts in Jejunal Contents

Microorganisms, animal bodies, and the environment exist in an interdependent relationship. Under microecological balance, normal intestinal microbial flora can improve intestinal structure and function while enhancing intestinal immune function and disease resistance [11]. Intestinal microbial flora provides signals for the development of important lymphocyte subpopulations and maintains intestinal T and B cell development, and can also influence systemic immune responses by affecting the ratio of T helper 1 (Th1) to T helper 2 (Th2) effector cells [12]. Exogenous additives can alter intestinal microbial composition. Lee et al. [13] demonstrated that dietary supplementation with 20 mg/kg sanguinarine significantly increased Lactobacillus counts in cecal contents of broilers. The current results showed that dietary tea seed polysaccharide and Macleaya cordata alkaloids had no significant effect on Escherichia coli counts in jejunal contents of yellow-feathered broilers but significantly increased Lactobacillus counts. Yuan et al. [14] reported that tea seed polysaccharide inhibited Escherichia coli in broiler ceca with effects comparable to antibiotics. The present results are consistent with this finding, showing that dietary tea seed polysaccharide reduced Escherichia coli counts in jejunal contents without significant differences, producing effects equivalent to antibiotics. The results also demonstrated that the combined use of tea seed polysaccharide and Macleaya cordata alkaloids had consistent effects on Escherichia coli and Lactobacillus compared with individual supplementation. This suggests that Macleaya cordata alkaloids and tea seed polysaccharide may improve intestinal flora balance by promoting the proliferation of beneficial bacteria and thereby indirectly inhibiting harmful bacteria, with no antagonistic or additive effects observed when used in combination.

3.3 Antimicrobial Activity of Macleaya cordata Sanguinarine

Macleaya cordata was originally used as a botanical pesticide. Its active ingredients are natural substances that degrade easily, cause minimal environmental pollution, and rarely induce pest resistance. Zhao et al. [15] found that Macleaya cordata alkaloids exhibited strong antibacterial effects against Staphylococcus aureus, Micrococcus tetragenus, and Bacillus subtilis, but no effect against Escherichia coli. Sanguinarine showed strong antibacterial activity against Staphylococcus aureus, Micrococcus tetragenus, Bacillus subtilis, and Bacillus cereus, but weak effects against Escherichia coli. Yu et al. [16] reported that total Macleaya cordata alkaloids had good antifungal effects against Mucor, while sanguinarine hydrochloride showed strong activity against Rhizopus, Aspergillus flavus, Aspergillus niger, Aspergillus oryzae, Mucor, and Trichoderma, but weak effects against Penicillium and yeast. The current study found low sensitivity of Macleaya cordata sanguinarine to Escherichia coli, consistent with the in vivo results. Macleaya cordata sanguinarine showed moderate sensitivity to Staphylococcus aureus and Pasteurella multocida and high sensitivity to Salmonella. Wang et al. [17] observed good antibacterial effects of Macleaya cordata alkaloids against Staphylococcus aureus, similar to the present results. However, Li et al. [18] reported that with increasing concentrations of Macleaya cordata alkaloids (total alkaloid content 64%, containing 45% sanguinarine and 19% chelerythrine), the inhibitory effects against Staphylococcus aureus, Salmonella, Escherichia coli, and Bacillus subtilis were enhanced, with the order of efficacy being Staphylococcus aureus > Bacillus subtilis > Escherichia coli > Salmonella. This differs from the current findings, possibly due to variations in active ingredient content. The present results demonstrated that the MIC values of Macleaya cordata sanguinarine were 25 g/mL for both Staphylococcus aureus and Escherichia coli, 12.5 g/mL for Pasteurella multocida, and 1.56 g/mL for Salmonella. These results indicate weaker antibacterial effects against Staphylococcus aureus and Pasteurella multocida but strong activity against Salmonella, with antimicrobial activity superior to penicillin sodium.

4 Conclusion

1. Dietary supplementation with tea seed polysaccharide and Macleaya cordata alkaloids can improve jejunal morphological structure in yellow-feathered broilers, with effects comparable to antibiotics.
2. Dietary supplementation with tea seed polysaccharide and Macleaya cordata alkaloids significantly increases Lactobacillus counts in jejunal contents. The combined use produces effects equivalent to individual supplementation, and both are superior to antibiotics, indicating that both additives can replace antibiotics.
3. Macleaya cordata sanguinarine exhibits strong antibacterial activity against Salmonella, superior to penicillin sodium, with an MIC of 1.56 g/mL.

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