Effects of Dietary Supplementation with Different Plant Essential Oils on Growth Performance, Intestinal Development, Immune Organ Indices, and Slaughter Performance of Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of different plant essential oil products supplemented in the basal diet on growth performance, intestinal development, immune organ indices, and slaughter performance of broiler chickens. A total of 2,304 one-day-old Ross 308 commercial male broiler chicks were selected and randomly allocated into 6 groups with 12 replicates per group and 32 birds per replicate. Group 1 was the control group fed the basal diet; Group 2 was the negative control group with antibiotics (20 mg/kg virginiamycin) added to the basal diet; Groups 3-6 each had one plant essential oil product added to the basal diet, namely 100 mg/kg plant essential oil 1 (containing 5% carvacrol, EO1 group), 300 mg/kg plant essential oil 2 (containing 5% carvacrol, EO2 group), 300 mg/kg plant essential oil 3 (containing 8% mixture of carvacrol and thymol, EO3 group), and 300 mg/kg plant essential oil 4 (containing 2% mixture of carvacrol and thymol, EO4 group). The experimental period was 39 days. The results showed: 1) Compared with the control group, the feed conversion ratio of broilers in the negative control group and EO2 group during 22–31 days of age was significantly reduced ($P<0.05$), decreasing by 2.58% and 3.51%, respectively; compared with the control group, the average daily gain of broilers in the negative control group during 1–38 days of age increased and the feed conversion ratio decreased, but the differences were not significant ($P>0.05$); supplementation of different plant essential oils in the diet did not significantly affect the average daily gain, average daily feed intake, and feed conversion ratio of broilers during 1–38 days of age ($P>0.05$). 2) Compared with the control group, dietary supplementation of different plant essential oils tended to increase the duodenal villus height of broilers at 39 days

of age (P=0.0753), and the villus height to crypt depth ratio was also increased (P>0.05). 3) There were no significant differences in spleen index and bursa of Fabricius index of broilers among all groups at 22 and 39 days of age (P>0.05). There were no significant differences in carcass yield, breast muscle percentage, leg muscle percentage, wing weight percentage, and abdominal fat percentage of broilers among all groups (P>0.05). In conclusion, under the conditions of this experiment, dietary supplementation of different plant essential oils did not significantly improve the growth performance of broiler chickens, but could promote duodenal villus development.

Full Text

Effects of Dietary Different Plant Essential Oils on Growth Performance, Intestinal Development, Immune Organ Indexes and Slaughter Performance of Broilers

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Abstract

This experiment investigated the effects of different plant essential oil products supplemented in basal diets on growth performance, intestinal development, immune organ indexes, and slaughter performance of broiler chickens. A total of 2,304 one-day-old Ross 308 male broiler chicks were randomly allocated into 6 groups with 12 replicates per group and 32 birds per replicate. Group 1 served as the control group fed a basal diet; group 2 was the negative control group fed the basal diet supplemented with antibiotics (20 mg/kg virginiamycin); and groups 3–6 were fed the basal diet supplemented with four different plant essential oil products, respectively: 100 mg/kg plant essential oil 1 (containing 5% carvacrol, EO1 group), 300 mg/kg plant essential oil 2 (containing 5% carvacrol, EO2 group), 300 mg/kg plant essential oil 3 (containing 8% mixture of carvacrol and thymol, EO3 group), and 300 mg/kg plant essential oil 4 (containing 2% mixture of carvacrol and thymol, EO4 group). The experimental period lasted 39 days. The results showed: (1) Compared with the control group, the feed-to-gain ratio (F/G) of broilers during 22–31 days of age in the negative control group and EO2 group was significantly reduced (P<0.05) by 2.58% and 3.51%, respectively. The negative control group exhibited increased average daily gain (ADG) and decreased F/G during 1–38 days of age compared with the control group, though these differences were not significant (P>0.05). Dietary supplementation with different plant essential oils did not significantly affect ADG, average daily feed intake (ADFI), or F/G of broilers during 1–38

days of age (P>0.05). (2) Compared with the control group, dietary plant essential oils tended to increase duodenal villus height at 39 days of age (P=0.0753), and the villus height-to-crypt depth ratio was also increased (P>0.05). (3) No significant differences were observed among groups in spleen index or bursa of Fabricius index at 22 and 39 days of age (P>0.05). Similarly, no significant differences were found among groups in carcass yield, breast muscle yield, thigh muscle yield, wing yield, or abdominal fat yield (P>0.05). In conclusion, under the conditions of this experiment, dietary supplementation with different plant essential oils did not significantly improve growth performance of broilers but promoted duodenal villus development.

Keywords: plant essential oil; growth performance; slaughter performance; intestinal development; immune organ; broilers

Introduction

For decades, antibiotics have played a positive role in reducing livestock mortality and promoting growth. However, their negative impacts, including drug resistance, residues, and food safety concerns, have prompted many countries to legislate their prohibition. Since the European Union banned antibiotics as feed additives in poultry, plant essential oils (EO) have attracted considerable attention as a novel feed additive and become a research hotspot. Plant essential oils are aromatic, volatile oil liquids extracted from plants through distillation. Naturally extracted plant essential oils primarily contain compounds such as thymol, carvacrol, and cinnamaldehyde, which possess extensive pharmacological properties including antimicrobial, antioxidant, and anti-inflammatory effects [1]. Previous studies have demonstrated that plant essential oils can positively affect broiler growth performance [2], enhance antioxidant capacity [3], and improve carcass quality [4]. However, their application in production practice still faces many challenges due to poor product stability, insufficient scale of animal trials, and unclear mechanism elucidation [5]. This study evaluated different types of plant essential oil products available on the market to investigate their effects on growth performance, intestinal development, immune organ indexes, and slaughter performance of broilers, aiming to provide a basis for the application of plant essential oil products in broiler production.

1.1 Experimental Materials

Four plant essential oil products were selected for evaluation. Products 1 and 2 both contained 5% carvacrol as the active ingredient but were sourced from different manufacturers. Products 3 and 4 both contained mixtures of carvacrol and thymol, with effective concentrations of 8% and 2%, respectively, and were also from different manufacturers.

1.2 Experimental Animals and Grouping

A total of 2,304 one-day-old Ross 308 male broiler chicks were randomly divided into 6 groups with 12 replicates per group and 32 birds per replicate. Group 1 was the control group (CON) fed a basal diet; group 2 was the negative control group (NC) fed the basal diet supplemented with antibiotics (20 mg/kg virginiamycin); and groups 3–6 were fed the basal diet supplemented with four different plant essential oil products at the manufacturers'recommended dosages: 100 mg/kg plant essential oil 1 (containing 5% carvacrol, EO1 group), 300 mg/kg plant essential oil 2 (containing 5% carvacrol, EO2 group), 300 mg/kg plant essential oil 3 (containing 8% mixture of carvacrol and thymol, EO3 group), and 300 mg/kg plant essential oil 4 (containing 2% mixture of carvacrol and thymol, EO4 group). The basal diet was formulated according to the breeding company' s recommended standards and NRC (1994) standards, with composition and nutrient levels shown in Table 1 . The experimental diets were fed in four phases: crumbles during 1–7 days of age and pellets during the other phases. The experimental period lasted 39 days.

1.4 Feeding Management

Broilers were raised in small pens with ad libitum access to feed and water. Daily observation of flock health and mental status was conducted. Whole-house brooding was employed with temperature controlled by a warm-air furnace water heating system. The initial house temperature was 32 °C at chick placement, then reduced by 2 °C weekly until reaching 26 °C. Routine immunization programs were implemented. Temperature, humidity, lighting, and sanitary indicators in the chicken house complied with hygienic requirements for broiler raising (GB/T 14925–1994).

1.5.1 Growth Performance

At 7, 21, 31, and 38 days of age, all birds in each pen were weighed after feed withdrawal and feed consumption was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G), body weight (BW), and survival rate.

1.5.2 Intestinal Development and Morphology

At 22 and 39 days of age, 12 birds per group (one per replicate) were slaughtered. The lengths of duodenum, jejunum, and ileum were measured, and each intestinal segment was weighed after digesta removal. Relative intestinal weight and weight per unit length were calculated as follows:
Relative intestinal weight (%) = intestinal weight (g) / body weight (g)
Weight per unit length (g/cm) = intestinal weight (g) / intestinal length (cm).

A 1-cm segment from the middle of the duodenum was fixed overnight in 4% paraformaldehyde, embedded in paraffin, sectioned, stained with hematoxylin-

eosin (HE), and mounted. Villus height (VH) and crypt depth (CD) were measured under a light microscope, and the VH/CD ratio was calculated.

The spleen and bursa of Fabricius were weighed to calculate immune organ indexes as follows:
Immune organ index (g/kg) = immune organ weight (g) / body weight (kg).

1.5.3 Slaughter Performance

At 39 days of age, 12 birds per group (one per replicate) were randomly selected, weighed, slaughtered, and dissected to determine carcass yield, thigh muscle yield, breast muscle yield, wing yield, and abdominal fat yield.

1.6 Data Processing

Experimental data were analyzed using the GLM model in SPSS 17.0 statistical software, with each replicate as the experimental unit. If significant differences among groups were detected, Duncan's multiple comparison test was performed. $P<0.05$ was considered significant, and $0.05 \leq P<0.10$ was considered a tendency. Results are expressed as "mean ± standard deviation."

2.1 Effects of Plant Essential Oils on Growth Performance of Broilers

As shown in Table 2 , no significant differences were observed among groups in ADG, ADFI, F/G during 1–7 days of age, or body weight at 7 days of age ($P>0.05$). Compared with the control and negative control groups, the EO3 and EO4 groups showed significantly increased F/G during 8–21 days of age ($P<0.05$). During 22–31 days of age, the negative control group and EO2 group had significantly lower F/G compared with the control group, decreasing by 2.58% and 3.51%, respectively ($P<0.05$). No significant differences were found among groups in ADG, ADFI, F/G during 32–38 days of age, or body weight at 38 days of age ($P>0.05$). Over the entire period (1–38 days of age), the negative control group showed increased ADG and decreased F/G compared with the control group, but these differences were not significant ($P>0.05$). Dietary plant essential oils did not significantly affect ADG, ADFI, or F/G of broilers ($P>0.05$), but all groups except EO3 showed some improvement in growth performance. The EO2 group demonstrated the best effects, with 38-day body weight increased by 24.4 g ($P>0.05$), F/G decreased by 0.56% ($P>0.05$), and survival rate increased by 1.6% ($P>0.05$) compared with the control group.

2.2 Effects of Plant Essential Oils on Intestinal Development and Duodenal Villus Morphology of Broilers

As shown in Table 3 , the negative control group had significantly lower weight per unit length of duodenum and jejunum at 22 days of age compared with the control group ($P<0.05$). In contrast, the EO1, EO2, EO3, and EO4 groups all

had significantly higher weight per unit length of jejunum at 22 days of age compared with the negative control group (P<0.05). No significant differences were observed among groups in weight per unit length or relative weight of intestinal segments at 39 days of age (P>0.05).

The control group had significantly greater crypt depth in duodenum at 22 days of age than the negative control, EO2, EO3, and EO4 groups (P<0.05). Dietary plant essential oils tended to increase duodenal villus height at 39 days of age compared with the control group (P=0.0753). The VH/CD ratio at 22 and 39 days of age was lower in the control group than in other groups, though the differences were not significant (P>0.05).

2.3 Effects of Plant Essential Oils on Immune Organ Indexes of Broilers

As shown in Table 4 , dietary plant essential oils had no significant effects on spleen index or bursa of Fabricius index of broilers at 22 and 39 days of age (P>0.05).

2.4 Effects of Plant Essential Oils on Slaughter Performance of Broilers

As shown in Table 5 , dietary plant essential oils had no significant effects on carcass yield, breast muscle yield, thigh muscle yield, wing yield, or abdominal fat yield of broilers (P>0.05).

3.1 Effects of Plant Essential Oils on Growth Performance of Broilers

Studies have shown that plant essential oils promote broiler growth. Li et al. [6] reported that adding 100 and 200 mg/kg plant essential oils (containing 85% carvacrol and thymol) to diets of 1-day-old Arbor Acres (AA) broilers significantly increased ADFI and ADG and decreased F/G by 42 days of age, though the F/G reduction was not significant. Zhu et al. [7] found that dietary thymol essential oil increased ADG and decreased F/G of Chinese partridge chickens during 22–42 days of age. Weber et al. [8] demonstrated that dietary supplementation with 300 mg/kg mixture of thymol, eugenol, piperine, and benzoic acid significantly improved growth performance of broilers under different rearing conditions. In this experiment, F/G tended to increase when no antibiotics were added, and although supplementation with 100 and 300 mg/kg plant essential oils did not significantly improve growth performance, they were superior to the control group in terms of F/G and survival rate. This indicates that the effects of plant essential oils in practical application are not consistent, which may be related to the dosage and active ingredient content of the essential oils used in this experiment, as well as differences in essential oil types and production processes.

3.2 Effects of Plant Essential Oils on Intestinal Development and Duodenal Villus Morphology of Broilers

The small intestine is the primary site for nutrient digestion and absorption in poultry. Changes in small intestine length and weight can alter nutrient absorption and consequently affect animal growth performance [9]. Few studies have reported on the effects of plant essential oils on intestinal development. Bravo et al. [10] reported that plant essential oils (active ingredients: carvacrol and cinnamaldehyde) increased intestinal villus height, reduced energy requirements for intestinal maintenance, and effectively protected intestinal mucosa and promoted intestinal development, consistent with Jamroz et al. [11] who found that plant essential oils increased the thickness of muscular stomach and jejunal mucosa, better promoting gastrointestinal development. However, this experiment showed that supplementation with plant essential oils (active ingredients: carvacrol and thymol) had no significant effect on intestinal development of broilers at 22 and 39 days of age. Compared with the control group, the negative control (antibiotic) group had significantly lower weight per unit length of duodenum and jejunum at 22 days of age, which may be related to the mechanism of antibiotic action. Sunde et al. [12] summarized previous research suggesting that antibiotic use reduces intestinal wall thickness, facilitating nutrient absorption. In this experiment, the weight per unit length of intestinal segments in plant essential oil groups was similar to that of the control group, indicating that the plant essential oils used in this experiment were not ideal for promoting intestinal development, possibly due to their active ingredients, as the combination of carvacrol and cinnamaldehyde may be more beneficial for intestinal development.

Morphological parameters such as small intestinal villus height and crypt depth are widely recognized as important indicators of small intestinal absorptive function and mechanical barrier function. Reduced villus height and deepened crypts may impair nutrient absorption and result in poorer growth performance. Khattak et al. [2] found that dietary supplementation with 300 g/t plant essential oils increased villus height and surface area, indicating improved nutrient absorption. However, other studies found that a mixture of oregano, cinnamon, and pepper plant extracts had no significant effects on duodenal villus height, crypt depth, or villus surface area of broilers. These inconsistent results may be related to plant essential oil types. This study showed that dietary plant essential oils significantly reduced crypt depth at 22 days of age and increased the VH/CD ratio at 22 and 39 days of age, similar to the findings of Mao et al. [13] in broilers, suggesting that plant essential oils have some promoting effect on villus development.

3.3 Effects of Plant Essential Oils on Immune Organ Indexes of Broilers

The thymus, bursa of Fabricius, and spleen are the primary immune organs in poultry, and immune organ indexes are important indicators for evaluating broiler immune function [14]. Studies have shown that thymol essential oil not only improved growth performance but also increased thymus index while decreasing spleen index in Chinese partridge chickens [15]. Under the conditions of this experiment, neither plant essential oils nor antibiotics had significant effects on bursa of Fabricius index or spleen index of broilers, suggesting that the effects of plant essential oils on immune organ development require further investigation.

In conclusion, under the conditions of this experiment, dietary supplementation with plant essential oils did not significantly improve growth performance of broilers but promoted duodenal villus development.

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