

Effects of Star Anise, Its Essential Oil and Residue on Growth Performance, Intestinal Development, and Nutrient Digestibility in Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of star anise, essential oil, and residue on growth performance, intestinal development, and nutrient digestibility in broiler chickens. A single-factor completely randomized design was adopted, with 384 1-day-old Arbor Acres broiler chicks randomly divided into 4 groups, with 12 replicates per group and 8 birds per replicate. Broilers in the control group were fed a corn-soybean meal basal diet, while those in the treatment groups were fed the basal diet supplemented with 5,000 mg/kg star anise powder (star anise group), 200 mg/kg star anise essential oil (essential oil group), and 5,000 mg/kg star anise residue (residue group), respectively. The trans-anethole content in the diets of the star anise and essential oil groups was 0.204 g/kg, while that in the control and residue groups was below 0.005 g/kg. The experimental period lasted 21 days. The results showed: 1) The average daily gain and average daily feed intake of broilers in the star anise and essential oil groups were significantly higher than those in the control group ($P < 0.05$), with no significant difference between the residue and control groups ($P > 0.05$). Compared with the control and residue groups, dietary supplementation with star anise and star anise essential oil significantly increased the 21-day body weight of broilers ($P < 0.05$). 2) Compared with the control and residue groups, the relative length of the jejunum in broilers from the star anise and essential oil groups was significantly increased ($P < 0.05$), while the relative weight of the small intestine in the essential oil group was significantly increased ($P < 0.05$); furthermore, the relative weight of the jejunum in broilers from the star anise and essential oil groups was significantly higher than that in the control group ($P < 0.05$). 3) Compared with the control and residue groups, dietary supplementation with star anise and star anise essential oil significantly increased the

villus height in the duodenum and jejunum ($P < 0.05$). 4) The apparent and true digestibility of crude protein and the apparent and true metabolizable energy in the essential oil group were significantly higher than those in all other groups ($P < 0.05$). It can be concluded that dietary supplementation with 5 g/kg star anise or 0.2 g/kg star anise essential oil can improve growth performance, intestinal development, and nutrient digestion and utilization in broiler chickens, whereas supplementation with 5 g/kg star anise residue had no such effect.

Full Text

Preamble

Effects of Star Anise and Its Essential Oil and Leavings on Growth Performance, Intestinal Development and Nutrient Digestibility of Broilers

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Abstract: This experiment was conducted to investigate the effects of star anise, its essential oil, and leavings on growth performance, intestinal development, and nutrient digestibility in broilers. A single-factor completely randomized design was employed, allocating 384 one-day-old Arbor Acres broilers into 4 groups with 12 replicates of 8 birds each. The control group received a corn-soybean meal basal diet, while experimental groups were fed the basal diet supplemented with 5,000 mg/kg star anise powder (star anise group), 200 mg/kg star anise essential oil (essential oil group), and 5,000 mg/kg star anise leavings (leavings group), respectively. The trans-anethole content in diets for the star anise and essential oil groups was 0.204 g/kg, whereas that in the control and leavings groups was below 0.005 g/kg. The experimental period lasted 21 days. The results showed: 1) Average daily gain and average daily feed intake in the star anise and essential oil groups were significantly higher than in the control group ($P < 0.05$), with no significant differences between the leavings and control groups ($P > 0.05$). Dietary supplementation with star anise and its essential oil significantly increased 21-day body weight compared to the control and leavings groups ($P < 0.05$). 2) The relative length of jejunum in the star anise and essential oil groups was significantly higher than in the control and leavings groups ($P < 0.05$), while the relative weight of small intestine in the essential oil group was significantly higher ($P < 0.05$). Additionally, the relative weight of jejunum in the star anise and essential oil groups was significantly higher than in the control group ($P < 0.05$). 3) Dietary supplementation with star anise and its essential oil significantly increased villous height in the duodenum and jejunum ($P < 0.05$). 4) The apparent and true digestibility of crude protein and apparent and true metabolic energy in the essential oil group were significantly higher than in all other groups ($P < 0.05$). In conclusion, dietary

supplementation with 5 g/kg star anise or 0.2 g/kg star anise essential oil can improve growth performance, intestinal development, and nutrient digestibility and utilization in broilers, whereas 5 g/kg star anise leavings showed no such effects.

Keywords: star anise; star anise essential oil; star anise leavings; broilers; growth performance; intestinal development; nutrient digestibility

Star anise (*Illicium verum* Hook. f.) is a plant of the Magnoliaceae family and an important “medicinal and edible” economic tree species in southern China, possessing appetite-stimulating, antibacterial, and analgesic properties. Its main active components include trans-anethole, estragole, and anisaldehyde [1-4]. Star anise essential oil is an extract obtained from star anise through steam distillation, primarily containing trans-anethole. Due to its natural properties, multifunctionality, non-toxic side effects, and lack of drug resistance, it has been widely used in medicine, food, and other industries. Star anise leavings are the residual material after essential oil extraction, comprising the main portion of the processed material. However, research on the effects of these leavings is currently lacking. Furthermore, natural plants and their extracts have broad application value as feed additives and are considered potential alternatives to natural plant antibiotics and medicinal growth promoters [1,3], making them an increasingly important research focus. Previous studies have reported on the application of star anise and its extracts in livestock and poultry, primarily for stimulating appetite, inhibiting bacteria, and improving antioxidant performance [4-7]. However, few studies have investigated their effects on intestinal development and nutrient digestibility in broilers, and comparative studies on the effects of star anise, its essential oil, and leavings are scarce. This study investigated the effects of dietary supplementation with star anise, star anise essential oil, and star anise leavings (at equivalent trans-anethole content for star anise and its essential oil) on growth performance, intestinal development, and nutrient digestibility in broilers, aiming to provide a theoretical basis for the comparative application of these products in broiler production.

1.1 Experimental Materials

Broilers were purchased from Ningyang Xiling Family Farm in Tai’an City, Shandong Province. Star anise was purchased from Wuma Market, Taishan District, Tai’an City, Shandong Province, dried at 65°C, and ground to pass through a 60-mesh sieve. Star anise essential oil and leavings were extracted by the Animal Nutrition and Metabolism Laboratory of Shandong Agricultural University. The essential oil was extracted by steam distillation: 150 g (accurate to 0.1 g) of ground, dried star anise was placed in a flat-bottom flask with 1,500 mL water and distilled continuously for 3 hours until no obvious oil traces remained in the distillate. A small amount of sodium chloride was added to the distillate, and after layering, the upper light yellow oily liquid was collected in a brown essential oil bottle and stored at 4°C for later use. The star anise leavings were the residual material after steam distillation for essential oil extraction,

dried at 65°C.

The active component contents of star anise, its essential oil, and leavings are shown in Table 1. Active components were analyzed using a Shimadzu GC-2014 gas chromatograph equipped with a DB-23 capillary column (30 m×0.25 mm×0.25 μm) and a flame ionization detector (FID) at 260°C. The injector temperature was maintained at 210°C, with high-purity nitrogen as carrier gas at a total flow rate of 28 mL/min and a split ratio of 20:1. High-purity hydrogen served as fuel gas, with an injection volume of 1 μL. The initial column temperature was 80°C, increased to 245°C at 5°C/min, with a total analysis time of 35 minutes. The contents of trans-anethole, estragole, and anisaldehyde were expressed as relative contents in crude fat and calculated using the external standard method.

1.2 Experimental Design

Three hundred eighty-four healthy one-day-old Arbor Acres (AA) broilers were randomly allocated to 4 treatments with 12 replicates of 8 birds each (equal sex ratio), with similar initial body weight among replicates ($P>0.05$). The experiment employed a single-factor completely randomized design. The control group was fed a corn-soybean meal basal diet formulated according to NRC (1994) and Chinese Feeding Standard of Chickens (NY/T 33-2004), with composition and nutrient levels shown in Table 2. Experimental groups were fed the basal diet supplemented with 5 g/kg star anise powder (star anise group), 0.2 g/kg star anise essential oil (essential oil group), and 5 g/kg star anise leavings (leavings group), respectively. The trans-anethole content in diets for the star anise and essential oil groups was 0.204 g/kg, while that in the control and leavings groups was below 0.005 g/kg. The experimental period was 21 days. Broilers were housed in cages with free access to water and feed, and were vaccinated according to routine immunization programs.

1.3 Sample Collection and Preparation

On day 21 of the experiment, one healthy broiler with body weight close to the average was selected from each replicate, fasted for 12 hours, and euthanized by cervical venous exsanguination. The intestinal tract was isolated, digesta removed, and the length and weight of each segment measured. Approximately 3 cm sections from the middle of duodenum, jejunum, and ileum were gently rinsed with physiological saline, placed in fixative solution, and preserved for intestinal morphological analysis.

On day 16, four healthy broilers with similar body weight ($P>0.05$) were selected from each replicate for metabolic trials. The total collection method was used, with replaceable stainless steel collection trays used to continuously collect excreta from each replicate for 72 hours, during which feed intake and excreta output were accurately recorded. Eighteen healthy broilers with similar body weight ($P>0.05$) were selected from spare birds for endogenous excreta

collection: they were fasted for 24 hours, then continued fasting for 48 hours while collecting excreta for 48 hours under the same feeding conditions. After the trial, the 72-hour excreta from each replicate were thoroughly mixed. A portion of excreta (10% of total) was mixed with 10 mL of 10% sulfuric acid for nitrogen fixation to determine crude protein (CP) content, while the remaining excreta was dried and ground for determination of dry matter (DM) and gross energy (GE).

1.4 Measurement Indicators

1.4.1 Growth Performance

Body weight and feed intake were measured weekly to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

1.4.2 Intestinal Development Indices

The ratio of each intestinal segment length to live body weight represented the relative length, while the ratio of each segment weight (with digesta removed using consistent technique) to live body weight represented the relative weight.

1.4.3 Intestinal Morphology Indices

Fixed duodenal, jejunal, and ileal samples (24-hour fixation) were processed through rinsing, gradient alcohol dehydration, xylene clearing, and paraffin embedding. Sections were cut at 5 μ m thickness, stained with routine hematoxylin-eosin, and observed under a microscope. Villous height and crypt depth were measured using DigiLab-C professional software, with five fields per slice averaged as the final result. The villus-to-crypt ratio (V/C) was calculated. Small intestinal villous height was defined as the vertical distance from the intestinal gland opening to the villus tip, while crypt depth was the vertical distance from the crypt opening to its base.

1.4.4 Nutrient Digestibility

CP content in diets and excreta was determined by the Kjeldahl method (GB/T 6432-1994), and GE was measured using a WHR-15 oxygen bomb calorimeter (Changxing Higher Education Instrument Equipment Development Co., Ltd., Changsha, Hunan). Digestibility of CP and GE and metabolic energy were calculated according to reference [8] using the following formulas:

Apparent digestibility (AD) of a nutrient = $100 \times (\text{nutrient intake} - \text{nutrient excretion}) / \text{nutrient intake}$;

True digestibility (TD) of a nutrient = $100 \times (\text{nutrient intake} - \text{nutrient excretion} + \text{nutrient amount from digestive tract origin}) / \text{nutrient intake}$;

Apparent metabolic energy (AME) = $(\text{GE intake} - \text{GE excretion}) / \text{feed intake}$;

True metabolic energy (TME) = (GE intake - GE excretion + endogenous GE) / feed intake.

1.5 Statistical Analysis

Experimental data were analyzed using one-way ANOVA and Duncan's multiple comparison tests with SAS 9.2 General Linear Model procedure, with significance level set at $P < 0.05$.

2.1 Effects of Star Anise and Its Essential Oil and Leavings on Growth Performance of Broilers

As shown in Table 3, ADG and ADFI in the star anise and essential oil groups were significantly higher than in the control group ($P < 0.05$), while no significant differences were observed between the leavings and control groups ($P > 0.05$). Dietary supplementation with star anise and its essential oil significantly increased 21-day body weight compared to the control and leavings groups ($P < 0.05$). The F/G ratio in the leavings group was significantly higher than in the control group ($P < 0.05$), with no significant differences among other groups ($P > 0.05$).

2.2 Effects of Star Anise and Its Essential Oil and Leavings on Intestinal Development Indices of Broilers

As shown in Table 4, the relative length of jejunum in the star anise and essential oil groups was significantly higher than in the control and leavings groups ($P < 0.05$), and the relative weight of jejunum was significantly higher than in the control group ($P < 0.05$). The relative weight of duodenum in the star anise and essential oil groups was significantly higher than in the leavings group ($P < 0.05$), though no significant differences were found between the control and experimental groups ($P > 0.05$). The relative weight of small intestine in the essential oil group was significantly higher than in the control and leavings groups ($P < 0.05$). No significant differences were observed among groups for relative length of duodenum, or relative length and weight of ileum, or relative length of small intestine ($P > 0.05$).

2.3 Effects of Star Anise and Its Essential Oil and Leavings on Intestinal Morphology Indices of Broilers

As shown in Table 5, villous height in duodenum and jejunum and V/C ratio in jejunum in the star anise and essential oil groups were significantly higher than in the control and leavings groups ($P < 0.05$), with star anise showing the most pronounced effect on jejunal villous height. The V/C ratio in duodenum was significantly higher in the star anise group compared to the control and leavings groups ($P < 0.05$), with no significant differences among the other three groups ($P > 0.05$). Ileal villous height in the essential oil group was significantly higher than in the leavings group ($P < 0.05$). No significant differences were observed

among groups for crypt depth in duodenum, jejunum, and ileum, or for V/C ratio in ileum ($P>0.05$).

2.4 Effects of Star Anise and Its Essential Oil and Leavings on Nutrient Digestibility of Broilers

As shown in Table 6, apparent and true CP digestibility in the essential oil group were significantly higher than in the control, leavings, and star anise groups ($P<0.05$). AME and TME in the essential oil group were significantly higher than in the control and leavings groups ($P<0.05$), but not significantly different from the star anise group ($P>0.05$). Apparent GE digestibility in the essential oil group was significantly higher than in the leavings group ($P<0.05$), with no significant differences among other groups ($P>0.05$).

3.1 Effects of Star Anise and Its Essential Oil and Leavings on Growth Performance of Broilers

As an aromatic Chinese herbal medicine, star anise possesses appetite-stimulating, gastric juice secretion-promoting, and intestinal motility-exciting effects [4,9], thereby inducing feed intake and improving animal growth performance. Currently, few reports exist on the application of star anise and its essential oil in broiler production. Osman et al. [7] reported that mixed supplementation of star anise, clove, and oregano extracts significantly improved ADG and F/G in broilers. Soltan et al. [10] found that dietary supplementation with 0.50 and 0.75 g/kg star anise increased ADG in broilers. Ghalib et al. [11] demonstrated that combined supplementation of star anise and rosemary improved ADFI in broilers. The present results showed that dietary supplementation with star anise and its essential oil significantly increased ADG and 21-day body weight, while star anise leavings significantly increased F/G. These findings align with Osman et al. [7] and Soltan et al. [10], possibly due to the characteristic anise aroma [12] that induces feed intake in broilers. Increased feed intake consequently improves ADG. In contrast, star anise leavings lack this characteristic aroma as the active components have been extracted, and the remaining components may hinder digestion and absorption, resulting in increased F/G and reduced growth performance.

3.2 Effects of Star Anise and Its Essential Oil and Leavings on Intestinal Development of Broilers

Nutrient digestion and absorption in broilers primarily occurs in the small intestine; therefore, intestinal development directly affects nutrient utilization and consequently growth performance [13-14]. The small intestine is the main site for nutrient digestion, absorption, and utilization, and healthy intestinal morphology is crucial for optimal digestive function and animal development [14]. Villous height and crypt depth are key indicators of small intestinal digestive and absorptive function. Villous height affects absorption area—greater height

provides larger surface area for nutrient absorption. Crypt depth influences digestive juice secretion—shallower crypts indicate stronger secretory capacity. The V/C ratio comprehensively reflects small intestinal functional status; an increased ratio indicates enhanced digestive and absorptive capacity and improved intestinal mucosal structure [15]. Reisinger et al. [16] reported that dietary supplementation with a blend of star anise, oregano, and citrus essential oils significantly increased ileal crypt depth and jejunal villous height in broilers. Hong et al. [17] found that dietary star anise and oregano significantly increased duodenal villous height. The present results indicated that while star anise and its essential oil and leavings did not significantly affect ileal development, star anise and its essential oil improved jejunal development to varying degrees, with star anise significantly increasing small intestinal relative weight. However, star anise leavings adversely affected duodenal relative weight. Additionally, star anise and its essential oil significantly increased duodenal and jejunal villous height and jejunal V/C ratio, with star anise also significantly increasing duodenal V/C ratio, while star anise leavings significantly decreased ileal villous height. These morphological results are consistent with the effects on intestinal relative weight. The intestinal development-promoting effects of star anise and its essential oil may be closely related to their ability to improve intestinal microflora structure [4-7]. The differential effects on various intestinal segments may be attributed to structural differences along the small intestine. The negative effects of star anise leavings on intestinal development may result from extraction of active components, leaving residual components that cannot be utilized, which further compromises growth performance.

3.3 Effects of Star Anise and Its Essential Oil and Leavings on Nutrient Digestibility of Broilers

Aromatic plants stimulate appetite and digestion by increasing bile flow through stimulation of digestive enzyme production in the liver, pancreas, and intestinal mucosa, thereby promoting nutrient digestion and absorption in the small intestine [7,16]. As an aromatic Chinese herbal medicine, star anise stimulates appetite, gastric juice secretion, and intestinal motility [4,9], thereby improving nutrient digestibility. The present study found that dietary supplementation with star anise essential oil significantly improved apparent and true CP digestibility and AME and TME in broilers, while star anise showed less pronounced effects. Amad et al. [18] reported that dietary supplementation with 150, 750, and 1,500 mg/kg mixed star anise and thyme essential oils significantly improved apparent digestibility of CP and ether extract in broilers, with effects increasing linearly with dosage. Cho et al. [19] demonstrated that dietary star anise and thyme essential oil significantly improved DM and GE digestibility in broilers. These findings are similar to our results. The improved nutrient digestibility associated with star anise is closely related to its appetite-stimulating, gastric juice secretion-promoting, and intestinal motility-exciting effects [4,9]. The less pronounced effect of star anise compared to its essential oil may be attributed to the higher purity and more characteristic flavor of the

essential oil, which more effectively stimulates feed intake and improves nutrient digestibility. The minimal effect of star anise leavings is likely related to the extraction of active components. Furthermore, improved nutrient digestibility is closely associated with enhanced intestinal development.

Conclusion

Dietary supplementation with star anise and its essential oil can improve growth performance in broilers to varying degrees, whereas star anise leavings may compromise growth performance.

Dietary supplementation with star anise and its essential oil can improve intestinal development and morphology, thereby enhancing small intestinal function, while star anise leavings show no such effects.

Dietary supplementation with star anise and its essential oil can improve the digestion and utilization of CP and GE in broilers, while star anise leavings show no such effects.

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