Effects of a Compound Preparation of Plant Essential Oils and Sodium Butyrate on Growth Performance, Serum Antioxidant Indices, Fecal Microbiota, and Ammonia Emission in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of a compound preparation of plant essential oil and sodium butyrate on growth performance, serum antioxidant indices, fecal microbiota, and ammonia emission in weaned piglets. Three hundred healthy 28-day-old Duroc × Landrace × Yorkshire piglets with a body weight of (11.20$±$0.29) kg were randomly allocated into three groups, with four replicates per group and 25 piglets per replicate. The control group received a basal diet, the plant essential oil group (EO group) received the basal diet supplemented with 1000 mg/kg plant essential oil preparation, and the compound group of plant essential oil and sodium butyrate (ES group) received the basal diet supplemented with 1000 mg/kg compound preparation of plant essential oil and sodium butyrate. The experimental period lasted 28 days. The results showed that: 1) Compared with the control group, the final body weight of the EO and ES groups increased by 2.64% (P>0.05) and 3.40% (P<0.05), respectively, and the average daily gain increased by 4.74% (P<0.05) and 6.48% (P<0.05), respectively. The average daily feed intake of both the EO and ES groups was significantly higher than that of the control group (P<0.05), while the feed conversion ratio was lower than that of the control group (P>0.05). 2) On day 14, the serum total antioxidant capacity (T-AOC) of the EO and ES groups was significantly higher than that of the control group (P<0.05), and the serum malondialdehyde (MDA) content was significantly lower than that of the control group (P<0.05). On day 28, the serum T-AOC of the EO group was significantly higher than that of the control group (P<0.05). 3) The number of Escherichia coli in the feces of the EO and ES groups on days 7, 14, and 21 was significantly lower than that of the control group (P<0.05), and the number of Escherichia coli in the feces of the ES group on days 7 and 14 was significantly

lower than that of the EO group (P<0.05). The number of Lactobacillus in the feces of the EO and ES groups on days 7 and 14 was significantly higher than that of the control group (P<0.05), the number of Lactobacillus in the feces of the ES group on day 7 was significantly higher than that of the EO group (P<0.05), and the number of Lactobacillus in the feces of the ES group on day 21 was significantly higher than that of the control group (P<0.05). 4) The ammonia nitrogen content and urease activity in the feces of the EO and ES groups on days 14 and 28 were significantly lower than those of the control group (P<0.05). On day 14, the ammonia nitrogen content in the feces of the ES group was significantly lower than that of the EO group (P<0.05); on day 28, the ammonia nitrogen content and urease activity in the feces of the ES group were significantly lower than those of the EO group (P<0.05). These results indicate that both the plant essential oil preparation and the compound preparation of plant essential oil and sodium butyrate have beneficial effects on the growth performance of piglets,

Full Text

Effects of Plant Essential Oil and Sodium Butyrate Compound Preparation on Growth Performance, Serum Antioxidant Indices, Fecal Flora, and Ammonia Loss in Weaned Piglets

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Abstract

This experiment was conducted to investigate the effects of plant essential oil and sodium butyrate compound preparation on growth performance, serum antioxidant indices, fecal flora, and ammonia loss in weaned piglets. A total of 300 healthy 28-day-old "Duroc × Landrace × Large White" piglets with an average body weight of (11.20$±$0.29) kg were randomly allocated to three groups with four replicates per group and 25 piglets per replicate. The control group was fed a basal diet, the plant essential oil group (EO group) received the basal diet supplemented with 1,000 mg/kg plant essential oil preparation, and the plant essential oil and sodium butyrate compound group (ES group) received the basal diet supplemented with 1,000 mg/kg of a compound preparation containing both plant essential oil and sodium butyrate. The experimental period lasted 28 days.

The results demonstrated several significant effects. First, compared with the control group, the final body weight of piglets in the EO and ES groups increased

by 2.64% (P>0.05) and 3.40% (P<0.05), respectively, while their average daily gain increased by 4.74% (P<0.05) and 6.48% (P<0.05), respectively. Both the EO and ES groups exhibited significantly higher average daily feed intake than the control group (P<0.05), and their feed-to-gain ratio was lower than that of the control group (P>0.05). Second, on day 14, the serum total antioxidant capacity (T-AOC) in both EO and ES groups was significantly higher than in the control group (P<0.05), whereas serum malondialdehyde (MDA) content was significantly lower (P<0.05). On day 28, the serum T-AOC in the EO group remained significantly higher than in the control group (P<0.05). Third, the fecal Escherichia coli counts in both EO and ES groups were significantly lower than in the control group on days 7, 14, and 21 (P<0.05), with the ES group showing significantly lower counts than the EO group on days 7 and 14 (P<0.05). The fecal Lactobacillus counts in both treatment groups were significantly higher than in the control group on days 7 and 14 (P<0.05), with the ES group exhibiting significantly higher counts than the EO group on day 7 (P<0.05) and significantly higher counts than the control group on day 21 (P<0.05). Fourth, on both days 14 and 28, the fecal ammoniacal nitrogen content and urease activity in EO and ES groups were significantly lower than in the control group (P<0.05). On day 14, the ES group showed significantly lower fecal ammoniacal nitrogen content than the EO group (P<0.05), and on day 28, the ES group demonstrated significantly lower fecal ammoniacal nitrogen content and urease activity than the EO group (P<0.05). These findings indicate that both plant essential oil preparation and the compound preparation of plant essential oil with sodium butyrate exert positive effects on growth performance, serum antioxidant indices, fecal flora, and ammonia loss in piglets, with the compound preparation showing superior efficacy.

Keywords: plant essential oil; sodium butyrate; piglets; growth performance; microbial flora

Introduction

Plant essential oils and sodium butyrate represent safe and environmentally friendly antibiotic alternatives that have attracted widespread attention from animal scientists worldwide. Plant essential oils demonstrate significant effects in regulating animal intestinal flora, exerting antimicrobial activity, enhancing immunity, providing antioxidant benefits, and improving growth performance. Sodium butyrate, whose main active component is butyric acid, serves as a primary energy source for gastrointestinal epithelial cells, thereby improving intestinal health and promoting animal growth. Previous research by Wen et al. found that dietary supplementation with 1,000 mg/kg sodium butyrate effectively influenced intestinal flora populations and small intestinal mucosal morphology in weaned piglets, significantly enhancing growth performance. Our laboratory' s preliminary research demonstrated that plant essential oils and sodium butyrate can synergistically inhibit the growth of Escherichia coli and

Salmonella in vitro. However, few studies have investigated the practical application of combined plant essential oil and sodium butyrate supplementation in production settings. Therefore, this experiment was designed to evaluate the effects of a plant essential oil and sodium butyrate compound preparation on growth performance, serum antioxidant indices, fecal flora, and ammonia loss in weaned piglets, providing a theoretical reference for its practical application in swine production.

Materials and Methods

1.1 Experimental Design A total of 300 healthy 28-day-old "Duroc × Landrace × Large White" piglets with an average body weight of (11.20$±$0.29) kg were randomly allocated to three groups with four replicates per group and 25 piglets per replicate. The control group received a basal diet, the plant essential oil group (EO group) received the basal diet supplemented with 1,000 mg/kg plant essential oil preparation, and the plant essential oil and sodium butyrate compound group (ES group) received the basal diet supplemented with 1,000 mg/kg of a compound preparation containing both plant essential oil and sodium butyrate. The experimental period lasted 28 days. The basal diet was formulated according to the nutrient requirements for swine established by NRC (1998) and contained antibiotics (chlortetracycline). The composition and nutrient levels of the basal diet are presented in Table 1 . The plant essential oil preparation contained 15% cinnamaldehyde and 5% thymol, while the compound preparation contained 40% sodium butyrate, 15% cinnamaldehyde, and 5% thymol. Both preparations were microencapsulated using solid dispersion technology and were purchased from Zhejiang Wanfang Biotechnology Co., Ltd.

1.2 Management Practices The feeding trial was conducted at Zhengxin Animal Husbandry Co., Ltd. in Anji County, Zhejiang Province. Piglets were weaned at 28 days of age and transferred to disinfected nursery pens. Following a 5-day transition period, the formal experimental period began at approximately 33 days of age and lasted 28 days. Piglets had ad libitum access to feed and water throughout the trial, and were vaccinated and dewormed according to the farm' s standard procedures.

1.3 Measurements 1.3.1 Growth Performance

Piglets were weighed at 08:00 after overnight fasting at the beginning and end of the experiment to calculate average daily gain. Daily feed consumption was recorded to calculate average daily feed intake and feed-to-gain ratio.

1.3.2 Serum Antioxidant Indices

On days 14 and 28 of the experiment, eight piglets were randomly selected from each group and 5 mL of blood was collected from the anterior vena cava into procoagulant tubes after overnight fasting. After standing for 20 minutes, serum was separated by centrifugation at 3,500 rpm for 5 minutes. Serum antioxidant

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

1.3.3 Fecal Microbial Flora

On the mornings of days 7, 14, 21, and 28, approximately 50 g of uncontaminated fecal samples were collected from each replicate. Plate counting methods were used to enumerate Lactobacillus and Escherichia coli populations in the feces.

1.3.4 Fecal Ammonia Loss Indices

On days 14 and 28, approximately 50 g of uncontaminated fecal samples were collected from each replicate and fixed with 10% hydrochloric acid. Fecal ammoniacal nitrogen content and urease activity were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4 Statistical Analysis Data were analyzed using one-way ANOVA with SPSS 16.0 statistical software. Results are expressed as means ± standard error, with $P<0.05$ considered statistically significant.

Results

2.1 Effects on Growth Performance As shown in Table 2 , compared with the control group, the final body weight of piglets in the EO and ES groups increased by 2.64% ($P>0.05$) and 3.40% ($P<0.05$), respectively, while their average daily gain increased by 4.74% ($P<0.05$) and 6.48% ($P<0.05$), respectively. Both the EO and ES groups exhibited significantly higher average daily feed intake than the control group ($P<0.05$), and their feed-to-gain ratio was lower than that of the control group ($P>0.05$). These results indicate that dietary supplementation with either plant essential oil preparation or the compound preparation improved piglet growth performance, with the compound preparation demonstrating superior efficacy.

2.2 Effects on Serum Antioxidant Indices Table 3 presents the effects on serum antioxidant indices. On day 14, both EO and ES groups showed significantly higher serum total antioxidant capacity than the control group ($P<0.05$) and significantly lower serum malondialdehyde content ($P<0.05$). On day 28, the EO group maintained significantly higher serum total antioxidant capacity compared to the control group ($P<0.05$), while no significant differences were observed in total superoxide dismutase activity or malondialdehyde content between groups ($P>0.05$). These findings suggest that dietary supplementation with plant essential oil preparation or the compound preparation enhanced the antioxidant capacity of weaned piglets.

2.3 Effects on Fecal Escherichia coli and Lactobacillus Counts Table 4 summarizes the effects on fecal microbial populations. Compared with the control group, both EO and ES groups showed significantly reduced fecal Escherichia coli counts on days 7, 14, and 21 ($P<0.05$), with the ES group also showing significantly lower counts on day 28 ($P<0.05$). The ES group exhibited significantly lower Escherichia coli counts than the EO group on days 7 and 14 ($P<0.05$). Regarding Lactobacillus populations, both treatment groups showed significantly higher counts than the control group on days 7 and 14 ($P<0.05$). The ES group demonstrated significantly higher Lactobacillus counts than the EO group on day 7 ($P<0.05$) and significantly higher counts than the control group on day 21 ($P<0.05$). These results indicate that both preparations increased fecal Lactobacillus populations while reducing Escherichia coli populations, with the compound preparation showing greater effectiveness.

2.4 Effects on Fecal Ammonia Loss Indices As presented in Table 5 , on day 14, the EO and ES groups showed 16.9% ($P<0.05$) and 25.1% ($P<0.05$) reductions in fecal ammoniacal nitrogen content, respectively, and 17.5% ($P<0.05$) and 23.0% ($P<0.05$) reductions in urease activity, respectively, compared with the control group. The ES group exhibited significantly lower fecal ammoniacal nitrogen content than the EO group on day 14 ($P<0.05$). On day 28, the EO and ES groups showed 10.8% ($P<0.05$) and 21.2% ($P<0.05$) reductions in fecal ammoniacal nitrogen content, and 10.6% ($P<0.05$) and 25.4% ($P<0.05$) reductions in urease activity, respectively. The ES group demonstrated significantly lower fecal ammoniacal nitrogen content and urease activity than the EO group on day 28 ($P<0.05$). These findings indicate that both preparations significantly reduced fecal ammoniacal nitrogen content and urease activity, with the compound preparation showing superior effects.

Discussion

3.1 Effects on Growth Performance Zhou et al. investigated the effects of plant essential oils on growth performance, blood indices, and immune function in weaned piglets, finding that dietary supplementation with 200 mg/kg plant essential oil enhanced immune capacity, improved post-weaning health, and promoted growth. Zhe et al. reported that microencapsulated sodium butyrate added during late gestation and lactation increased sow feed intake during lactation, reduced body weight loss, shortened the weaning-to-estrus interval, and improved offspring birth and weaning weights. The plant essential oil preparation and compound preparation used in this study were produced through microencapsulation technology, providing intestinal slow-release functionality. Our results demonstrate that both preparations improved piglet growth performance, with the compound preparation showing superior effects to the plant essential oil preparation alone, indicating a positive interactive effect between plant essential oils and sodium butyrate in improving piglet growth performance.

3.2 Effects on Serum Antioxidant Indices The active components of plant essential oils primarily include esters, phenols, aromatic hydrocarbons, aldehydes, and alcohols, which possess free radical-scavenging and antioxidant properties. Duan et al. found that birch leaf essential oil has potential developmental value in antibacterial and antioxidant products. Yue et al. reported that dietary sodium butyrate supplementation significantly improved growth performance in gestating and lactating sows and their newborn piglets, significantly increased serum superoxide dismutase activity, and significantly reduced serum malondialdehyde content. Ju et al. demonstrated that both sodium butyrate and coated sodium butyrate improved nutrient metabolism and maintained antioxidant and anti-inflammatory functions in broilers under lipopolysaccharide stress. In our study, on day 14, both EO and ES groups showed significantly higher serum total antioxidant capacity and total superoxide dismutase activity, along with significantly lower serum malondialdehyde content, compared with the control group. These results indicate that both plant essential oil preparation and the compound preparation effectively improved the antioxidant capacity of weaned piglets.

3.3 Effects on Intestinal Microflora Piglet diarrhea is a major concern in intensive swine production, with many researchers considering intestinal flora imbalance as an important and direct contributing factor. Fecal microbial analysis can reflect changes in the digestive tract microbial community. Li et al. found that dietary supplementation with a plant essential oil compound containing cinnamaldehyde and thymol significantly reduced fecal Escherichia coli counts, increased Lactobacillus populations, and decreased diarrhea incidence during the first week post-weaning, demonstrating beneficial effects on intestinal flora. Sodium butyrate has also been widely reported to improve intestinal microflora. Lu et al. found that dietary supplementation with 1,000 mg/kg sodium butyrate significantly improved growth performance and reduced intestinal Clostridium and Escherichia coli populations in weaned piglets. Cerisuelo et al. reported that the combination of plant essential oils and sodium butyrate effectively controlled Salmonella proliferation, and our previous research demonstrated that this combination effectively inhibited Escherichia coli and Clostridium perfringens growth in vitro. Jerzsele et al. found that a combination of ginger oil, carvacrol, and sodium butyrate showed therapeutic and preventive potential against necrotic enteritis. Our study confirms that dietary supplementation with both preparations affected fecal Escherichia coli and Lactobacillus populations, with the compound preparation showing superior effects. While the exact mechanism underlying the enhanced antimicrobial effect of the combined treatment remains unclear, a widely accepted hypothesis suggests that plant essential oils and sodium butyrate exhibit synergistic effects on cell membranes, accelerating membrane structure disruption and functional loss.

3.4 Effects on Fecal Ammonia Loss Indices In intensive animal production, decomposition of animal excreta by microorganisms and enzymes produces

harmful gases such as ammonia (NH_3) and hydrogen sulfide (H_2S), which pose health risks to animals and humans, reduce animal welfare, and cause environmental pollution. Urease is a key enzyme that accelerates fecal decomposition, and inhibiting its activity represents an effective approach for reducing ammonia volatilization. Cinnamaldehyde has been identified as an effective urease inhibitor. Wang found that dietary supplementation with 120 mg/kg cinnamaldehyde significantly reduced fecal urease activity, decreased ammoniacal nitrogen content in fecal-urine mixtures, and improved total nitrogen retention, thereby delaying urea decomposition and reducing ammonia concentration in pig houses. Zhou et al. reported that 250 mg/kg microencapsulated cinnamaldehyde significantly reduced ammonia concentration in pig houses and improved average daily gain in weaned piglets; at 350 mg/kg, it significantly improved dietary protein digestibility, total nitrogen retention, and serum total protein content while significantly reducing urease activity produced by fecal microorganisms and slowing urea decomposition in fecal-urine mixtures. Our findings are consistent with these reports, showing that both EO and ES groups exhibited significantly reduced fecal urease activity and ammoniacal nitrogen content on days 14 and 28, with the compound preparation demonstrating stronger urease inhibition than plant essential oil alone. This enhanced inhibitory effect on urease activity is significant for reducing ammonia emissions from piglets and improving housing environments.

Conclusion

Both plant essential oil preparation and plant essential oil combined with sodium butyrate compound preparation exert positive effects on piglet growth performance, serum antioxidant indices, fecal flora, and ammonia loss, with the compound preparation demonstrating superior efficacy.

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