# Effects of Plant Essential Oils on Growth Performance, Blood Parameters, and Immune Function in Weaned Piglets: Postprint

**Authors:** Zhou Xuanwu, Wang Yu, Chen Daiwen, Yu Bing, morning light, Li Yan, He Jun

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## Abstract

This experiment aimed to investigate the effects of dietary supplementation with plant essential oil (PEO) on growth performance, blood indices, and immune capacity of weaned piglets. Twenty-four weaned piglets (30 days of age) of similar body weight from "Duroc × Landrace × Yorkshire" crossbreed were selected and randomly allocated into 4 groups, with 6 replicates per group and 1 piglet per replicate. Each group was fed experimental diets supplemented with 50 mg/kg colistin sulfate (CS group) and 50 (Group I), 100 (Group II), and 200 mg/kg PEO (Group III) to the basal diet, respectively. The experiment consisted of a 6-day pre-trial period and a 14-day formal trial period. The results showed: 1) The average daily gain (ADG) of weaned piglets in Group III was significantly higher than that in the CS group ($P<0.05$), and showed an increasing trend compared with Groups I and II ($P=0.05$, $P=0.06$); the feed-to-gain ratio (F/G) of weaned piglets in Group III decreased compared with other groups, but the difference among groups was not significant ($P>0.05$). 2) The digestibility of dry matter (DM) in Group I was significantly lower than that in other groups ($P<0.05$), and the digestibility of crude protein (CP) in Group III showed an increasing trend compared with Group I ($P=0.07$). 3) The red blood cell count in Group III was significantly higher than that in the CS group ($P<0.05$); the mean hemoglobin concentration in Group II was significantly higher than that in the CS group ($P<0.05$); the serum glucose content in Group III was higher than that in other groups, but the difference was not significant ($P>0.05$); the serum albumin content in Group III was significantly higher than that in other groups ($P<0.05$). 4) The serum immunoglobulin G (IgG) content in Group III was significantly higher than that in the CS group and Group I ($P<0.05$), and the serum immunoglobulin A (IgA) content in Group III was significantly higher than that in Group I ($P<0.05$). In conclusion, dietary supplementation with 200 mg/kg PEO has the efficacy of improving blood effective component

content, enhancing immune capacity, strengthening post-weaning piglet health, and promoting growth; therefore, PEO has potential utilization value in weaned piglets.

## Full Text

## Introduction

With the advancement of intensive swine production, early and ultra-early weaning technologies have effectively improved sow utilization rates and generated significant economic benefits. However, piglets weaned too early often suffer from weaning stress during the first 1–2 weeks post-weaning, leading to early weaning syndrome. The discovery and application of antibiotics have effectively mitigated this prominent issue. Research has shown that supplementing diets of 7–35 day-old weaned piglets with various antibiotic combinations can protect piglet health, reduce diarrhea, and promote growth [1]. However, extensive antibiotic use has increasingly caused problems such as host microbiota disturbance, bacterial resistance, and drug residues. Consequently, identifying safer, more stable, and effective antibiotic alternatives has become a primary objective.

Plant essential oils (PEO) are volatile aromatic substances extracted from vegetation with complex compositions and broad biological activities, including antimicrobial, anti-inflammatory, antioxidant, anticancer, and insecticidal effects [2]. Essential oils are now widely used in food, medical, and agricultural fields. Gabriella et al. [3] found that essential oils can affect ruminal methanogen activity, reducing methane (CH ) and ammonia emissions without adversely affecting feed digestion or rumen fermentation. Huang et al. [4] reported that dietary oregano oil improved broiler growth performance, increased intestinal Lactobacillus and Bifidobacterium populations, and enhanced disease resistance. Diao et al. [5] demonstrated that adding a thymol mixture at certain doses enhanced digestive enzyme activity in piglets, improved digestive and antioxidant capacity, and improved growth performance. Fang et al. [6] noted that supplementing weaned piglet diets with 50 mg/kg cinnamon oil improved antioxidant capacity and nutritional status. Zhong et al. [7] found that adding 200 mg/kg thymol-cinnamaldehyde complex to sow diets enhanced immunity and reproductive performance. However, few studies have reported on thymol-cinnamaldehyde complex essential oils in weaned piglets. Therefore, this experiment investigated the effects of different dietary PEO levels on growth performance, blood indices, and immune capacity in "Duroc × Landrace × Yorkshire" weaned piglets to evaluate its efficacy in alleviating weaning stress and promoting growth, providing reference for modern swine production.

## Materials and Methods

### 1.1 Experimental Materials

Colistin sulfate (CS) contained 10% CS. PEO primarily consisted of 13.5% cinnamaldehyde and 4.5% thymol, with dextrin powder as the carrier. Both additives were provided by Chengdu Hualuo Biological Technology Co., Ltd. (China Animal Husbandry Co., Ltd.). Major instruments and reagents included a chemiluminescence analyzer, ELISA kits (Beijing Chenglin Biotechnology Co., Ltd.), and a microplate reader [Molecular Devices (Shanghai) Co., Ltd.].

### 1.2 Experimental Design and Diets

A single-factor design was employed using 24 "Duroc × Landrace × Yorkshire" weaned piglets (30 days of age) with similar body condition, randomly divided into 4 groups with 6 replicates per group (1 pig per replicate) and individually housed. The four groups received basal diets supplemented with 50 mg/kg colistin sulfate (CS group), 50 mg/kg PEO (Group I), 100 mg/kg PEO (Group II), and 200 mg/kg PEO (Group III). The preliminary period lasted 6 days, followed by a 14-day formal experimental period. The basal diet was formulated according to NRC (2012) nutrient requirements for weaned piglets. Diet composition and nutrient levels are shown in Table 1 .

### 1.3 Animal Management

The experiment was conducted at the Research Farm of Sichuan Agricultural University, Ya' an Campus. Pig houses were thoroughly cleaned and disinfected before the trial. Piglets had ad libitum access to feed and water, with feeding at 08:00, 12:00, 16:00, and 20:00 daily. House temperature was maintained at 24–26°C with relative humidity of 75–85%. Pens were cleaned daily, and no medications were administered during the experimental period.

### 1.4 Sample Collection and Analysis

**1.4.1 Growth Performance** Piglets were weighed at 08:00 on days 1 and 15 after fasting to record body weight and calculate weight gain (DG) and average daily gain (ADG). Daily feed intake was accurately recorded to calculate total feed intake (TFI) and average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was calculated based on ADFI and ADG.

**1.4.2 Nutrient Digestibility** From days 11–14, a digestibility trial was conducted using acid-insoluble ash (AIA) as an endogenous indicator. Determination of AIA, dry matter (DM), crude protein (CP), ether extract (EE), crude ash, and energy in feed and feces followed the methods of Diao et al. [5].

**1.4.3 Blood Indices** On day 15 at 07:00, 5 mL of fresh blood was collected from each piglet via anterior vena cava into EDTA tubes for routine blood analy-

sis at Ya' an People' s Hospital. An additional 10 mL was collected into regular anticoagulant tubes, left at room temperature for 0.5 h, centrifuged at 3,500 r/min for 10 min, and the supernatant stored at -20°C for serum biochemical analysis using a chemiluminescence analyzer. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations were determined using porcine ELISA kits.

### 1.5 Statistical Analysis

Data were processed using Excel 2010 and analyzed by one-way ANOVA using SPSS 21.0. Duncan' s multiple comparison test was applied. Results are expressed as means ± standard deviation. Differences were considered significant at $P < 0.05$ and a trend at $0.05 \quad P < 0.10$.

## Results

### 2.1 Effects of PEO on Growth Performance of Weaned Piglets

As shown in Table 2 , ADG in Group III was significantly higher than in the CS group ($P < 0.05$) and showed an increasing trend compared with Groups I and II ($P = 0.05$, $P = 0.06$). The F/G in Groups II and III was lower than in the CS and Group I, but differences were not significant ($P > 0.05$).

### 2.2 Effects of PEO on Nutrient Digestibility of Weaned Piglets

Table 3 shows that DM digestibility in Group I was significantly lower than in other groups ($P < 0.05$). CP digestibility in Group III was higher than in the CS and Group II ($P > 0.05$) and showed an increasing trend compared with Group I ($P = 0.07$). Additionally, EE, energy, and ash digestibility in Group III were higher than in other groups, but differences were not significant ($P > 0.05$).

### 2.3 Effects of PEO on Blood Routine Indices of Weaned Piglets

Table 4 indicates that white blood cell counts in Groups I, II, and III were higher than in the CS group, but differences were not significant ($P > 0.05$). Red blood cell counts in Groups I and III were significantly higher than in the CS group ($P < 0.05$). Mean corpuscular volume in Group II was significantly lower than in Group I and the CS group ($P < 0.05$). Mean corpuscular hemoglobin in Group I was significantly higher than in Group II ($P < 0.05$). Mean corpuscular hemoglobin concentration in Group II was significantly higher than in the CS group ($P < 0.05$).

### 2.5 Effects of PEO on Serum Biochemical Indices of Weaned Piglets

As presented in Table 5 , serum glucose content in Group III was higher than in other groups, but the difference was not significant ($P > 0.05$). Serum triglyceride content in Groups I and III was significantly lower than in the CS group (P

$< 0.05$), and Group II showed a decreasing trend compared with the CS group ($P = 0.05$). Serum total cholesterol content in the CS and Group III was significantly lower than in Group I ($P < 0.05$), and Group II showed a decreasing trend compared with Group I ($P = 0.71$). Additionally, serum albumin content in Group III was significantly higher than in all other groups ($P < 0.05$).

### 2.6 Effects of PEO on Serum Immunoglobulin Content of Weaned Piglets

Table 6 shows that serum IgG content in Group III was significantly higher than in the CS group and Group I ($P < 0.05$). Serum IgG in Group II also increased compared with the CS group and Group I, but not significantly ($P > 0.05$). Serum IgA content in Group III showed an increasing trend compared with the CS group ($P = 0.05$) and was significantly higher than in Group I ($P < 0.05$).

## Discussion

Post-weaning piglet health is crucial for sustainable swine industry development. Early and ultra-early weaning technologies have improved sow utilization and production efficiency, but prematurely weaned piglets with underdeveloped physiological systems are highly susceptible to weaning syndrome due to environmental changes and improper management. While antibiotics have alleviated this problem, their abuse has caused microbiota disturbance, bacterial resistance, and drug residues. Furthermore, Wang et al. [8] and Ruan [9] reported that antibiotic dosages in livestock production far exceed animal adaptation levels, with most unabsorbed portions excreted as parent compounds or metabolites, posing serious environmental threats. Numerous studies indicate that PEO has potential as an antibiotic alternative [4,10–11], as it is completely absorbed through the gastrointestinal wall, participates in metabolic regulation, and is excreted as safe products without harming the animal or environment [12–13].

Research also shows that PEO can stimulate animals to increase saliva, bile, and digestive enzyme secretion while enhancing enzyme activity [14–15], accelerate intestinal epithelial cell renewal, stimulate villus development, and increase small intestinal absorption area [16]. Huang et al. [17] reported that dietary supplementation with 0.1% PEO (containing 0.3% benzoic acid) significantly improved ADG and apparent digestibility of DM and energy in 1–14 day-old weaned piglets, though ADFI and F/G were not significantly affected. Our study found that adding 200 mg/kg PEO to weaned piglet diets significantly improved ADG compared with the CS group and showed increasing trends compared with Groups I and II, consistent with Zhang et al. [18]. Fang et al. [19] reported that 100 mg/kg PEO improved weaned piglet health and reduced F/G. Our results showed that 100 and 200 mg/kg PEO reduced F/G, while Group III significantly improved DM digestibility and increased CP and ash digestibility compared with the CS group, aligning with Li et al. [20] and Zeng et al. [21]. However, some studies found no significant effects of PEO on weaned piglet

growth performance [22–23], possibly due to differences in feeding environments, PEO composition, and supplementation levels.

Blood is an opaque red liquid circulating within the body, and its effective components serve as important indicators of metabolic function, tissue and cell permeability, and health status [24]. Studies show that premature weaning significantly alters blood composition, decreasing total protein, albumin, glucose, hemoglobin, and mean corpuscular volume while increasing mean corpuscular hemoglobin concentration [25]. Our results indicate that 200 mg/kg PEO supplementation increased hematocrit and hemoglobin content, similar to Rahimi et al. [26]. Additionally, Huang et al. [17] reported that 0.1% PEO significantly increased red blood cell (RBC) counts and lymphocyte counts in piglets. Our study also found that 50, 100, and 200 mg/kg PEO effectively increased RBC counts, though lymphocyte counts were not significantly affected. Groups II and III showed increased mean corpuscular hemoglobin concentration but decreased mean corpuscular volume compared with the CS and Group I. Reports indicate that PEO can affect blood composition and enhance oxygen, nutrient, and metabolite transport [12], suggesting that dietary PEO influences blood effective components and metabolic levels.

Fang et al. [6] found that 50 mg/kg cinnamon oil significantly increased serum total protein and albumin in weaned piglets. Diao et al. [5] reported that 100 mg/kg thymol with benzoic acid significantly increased serum albumin on days 14 and 42 while reducing serum urea and triglycerides. Our results showed that different PEO levels increased serum total protein compared with the CS group, with Group III significantly increasing serum albumin and decreasing serum triglycerides compared with the CS group, consistent with Rahimi et al. [26] and Liu [27]. Other studies showed that 0.1% PEO significantly increased serum glucose and cholesterol while decreasing serum urea nitrogen [24]. Our results indicated that 100 and 200 mg/kg PEO increased serum glucose and decreased total cholesterol, though serum urea was unaffected. Zhang et al. [18] found that PEO enhanced immunoglobulin secretion, increasing serum IgG and IgA but not IgM. Our results showed that Group III had significantly higher serum IgG than the CS and Group I, similar to Li et al. [20] but contrasting with Huang et al. [17], possibly due to different PEO levels and compositions. Group III also showed a trend for higher serum IgA than the CS group and significantly higher than Group I, consistent with Liu [27]. Thus, 200 mg/kg PEO (thymol + cinnamaldehyde) affects blood effective components, enhances metabolism, and improves immune capacity.

In conclusion, dietary supplementation with 200 mg/kg PEO improves blood routine indices, enhances immunity, alleviates weaning stress, and promotes growth performance, demonstrating potential value for weaned piglets.

## References

[1] Wang S F, Gong Y S. Effects of different antibiotic combinations on perfor-

mance and intestinal microflora of suckling piglets[J]. Acta Agriculturae Boreali-occidentalis Sinica, 2007, 16(5): 63-66.

[2] Brenes A, Roura E. Essential oils in poultry nutrition: main effects and modes of action[J]. Animal Feed Science and Technology, 2010, 158(1/2): 1-14.

[3] Cobellis G, Trabalza-Marinucci M, Marcotullio M C, et al. Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria in vitro[J]. Animal Feed Science and Technology, 2016, 215: 25-36.

[4] Huang G Q, Xie W, Wang B. Effects of oregano oil on broiler performance and intestinal microflora[J]. Chinese Journal of Veterinary Medicine, 2008, 44(11): 69-70.

[5] Diao H, Zheng P, Yu B, et al. Effects of benzoic acid on growth performance, serum biochemical indices, nutrient digestibility and jejunal digesta enzyme activities in weaned piglets[J]. Chinese Journal of Animal Nutrition, 2013, 25(4): 768-777.

[6] Fang Q H, Hou Y Q, Zhao D, et al. Effects of plant essential oils on growth performance and blood biochemical indices of weaned piglets[J]. Feed Industry, 2014, 35(17): 44-47.

[7] Zhong M, Wu D, Lin Y, et al. Phytogenic feed additive for sows: effects on sow feed intake, serum metabolite concentrations, IgG level, lysozyme activity and milk quality[J]. Journal of Agricultural Science and Technology, 2011, 1(6A): 802-810.

[8] Wang R, Wei Y S. Pollution characteristics and control of tetracycline antibiotics and heavy metals in livestock manure[J]. Journal of Agro-Environment Science, 2013, 32(9): 1705-1719.

[9] Ruan C X. Effects of combined tetracycline and copper pollution on soil nitrification and plant growth[D]. Master' s Thesis. Nanjing: Nanjing Forestry University, 2010.

[10] Hong J C, Steiner T, Aufy A, et al. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers[J]. Livestock Science, 2012, 144(3): 253-262.

[11] Zeng Z K, Zhang S, Wang H L, et al. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review[J]. Journal of Animal Science and Biotechnology, 2015, 6(1): 7.

[12] Worwood V A. The complete book of essential oils and aromatherapy: over 600 natural, non-toxic fragrant recipes create Health-Beauty-A Safe Home Environment[M]. California: New World Library, 1991.

[13] Michiels J, Missotten J, Dierick N, et al. In vitro degradation and in vivo passage kinetics of carvacrol, thymol, eugenol and trans-cinnamaldehyde along

the gastrointestinal tract of piglets[J]. Journal of the Science of Food and Agriculture, 2008, 88(13): 2371-2381.

[14] Jang I S, Ko Y H, Yang H Y, et al. Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens[J]. Asian Australasian Journal of Animal Sciences, 2004, 17(3): 394-400.

[15] Platel K, Srinivasan K. Stimulatory influence of select spices on bile secretion in rats[J]. Nutrition Research, 2000, 20(10): 1493-1503.

[16] Jiang W, Zhao X. Application of plant essential oils in pig production[J]. Modern Pig Farming, 2016(3): 72-74.

[17] Huang Y, Yoo J S, Kim H J, et al. Effects of dietary supplementation with blended essential oils on growth performance, nutrient digestibility, blood profiles and fecal characteristics in weanling pigs[J]. Asian-Australasian Journal of Animal Sciences, 2010, 23(5): 607-613.

[18] Zhang Q, Piao X S, Zhang H Y, et al. Effects of plant essential oil supplementation in low-energy diets on growth performance, antioxidant activity and immune function in piglets[C]//Proceedings of the 11th National Animal Nutrition Symposium of Chinese Association of Animal Science and Veterinary Medicine. Beijing: Chinese Association of Animal Science and Veterinary Medicine, 2012.

[19] Fang R J, Li M J, Zhou X B, et al. Effects of plant essential oil extracts on performance and blood biochemical indices of weaned piglets[J]. Feed Industry, 2010, 31(16): 9-12.

[20] Li P F, Piao X S, Ru Y J, et al. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health[J]. Asian-Australasian Journal of Animal Sciences, 2012, 25(11): 1617-1626.

[21] Zeng Z K, Xu X, Zhang Q, et al. Effects of essential oil supplementation of a low-energy diet on performance, intestinal morphology and microflora, immune properties and antioxidant activities in weaned pigs[J]. Animal Science Journal, 2015, 86(3): 279-285.

[22] Gois F D, Cairo P L G, De Souza Cantarelli V, et al. Effect of Brazilian red pepper (Schinus terebinthifolius Raddi) essential oil on performance, diarrhea and gut health of weanling pigs[J]. Livestock Science, 2016, 183: 24-27.

[23] Janczyk P, Pieper R, Urubschurov V, et al. Investigations on the effects of dietary essential oils and different husbandry conditions on the gut ecology in piglets after weaning[J]. International Journal of Microbiology, 2009, 2009: 730809.

[24] Yan F Y, Yin Y L, Kong X F, et al. Anti-weaning stress effects of Acanthopanax senticosus extract in piglets[J]. Scientia Agricultura Sinica, 2010, 43(21): 4490-4496.

[25] Zhou X M, Gao D Y, Wang Q L, et al. Effects of weaning stress on blood and biochemistry in piglets[J]. Chinese Journal of Veterinary Medicine, 1999, 25(9): 6-8.

[26] Rahimi S, Teymour Zadeh Z, Karimi Torshizi M A, et al. Effect of three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens[J]. Journal of Agricultural Science and Technology, 2011, 13(4): 527-539.

[27] Liu M. Effects of plant essential oils on performance, intestinal microorganisms and immune function in piglets[D]. Master' s Thesis. Zhengzhou: Henan Agricultural University, 2011.

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