Effects of Compound Plant Extracts as an Alternative to In-Feed Antibiotics on Growth Performance and Serum Parameters in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of dietary supplementation with compound plant extract (containing pomegranate peel, Artemisia argyi, Schisandra sphenanthera, and Evodia rutaecarpa) as a replacement for feed antibiotics on growth performance and serum indices of weaned piglets. Experiment 1: 160 weaned piglets (approximately 8 kg, 30 days of age) were randomly allocated to 4 dietary treatments: a control group receiving basal diet supplemented with chlortetracycline (107 mg/kg) + flavomycin (40 mg/kg), and three treatment groups receiving basal diet supplemented with 0.05%, 0.10%, or 0.20% compound plant extract, respectively. Each treatment comprised 4 replicates of 10 piglets each, and the experimental period was 28 d. The results of Experiment 1 demonstrated that compared with the control group, compound plant extract supplementation did not significantly affect ADG, ADFI, or F/G ($P>0.05$); however, the 0.10% level exhibited improved ADG, which was 2.44%higher than that of the control. Experiment 2: 120 weaned piglets (approximately 11 kg, 40 days of age) were randomly divided into 3 groups: an antibiotic-free group receiving basal diet only, an antibiotic group receiving basal diet supplemented with chlortetracycline (67 mg/kg) + flavomycin (20 mg/kg), and a plant extract group receiving basal diet supplemented with 0.10% compound plant extract. The trial lasted 35 d. The results of Experiment 2 revealed that compared with the antibiotic-free group, 0.10% compound plant extract significantly increased ADG by 21.39%($P<0.05$), significantly decreased serum ALB, CHOL, LDL-C, and NO contents ($P<0.05$), significantly enhanced GSH-Px activity ($P<0.05$), and significantly reduced CER activity ($P<0.05$). Compared with the antibiotic group, 0.10% compound plant extract significantly elevated ADG by 15.07%($P<0.05$) and significantly decreased serum UN, LDL-C, and

NO contents (P<0.05). In conclusion, dietary supplementation with 0.10% compound plant extract can effectively replace feed antibiotics (chlortetracycline + flavomycin), enhance growth performance of weaned piglets, and exhibits anti-stress and antioxidant functions.

Full Text

Effects of Composite Plant Extracts Replacing Feed Antibiotics on Growth Performance and Serum Indexes of Weaned Piglets

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Abstract

This study was conducted to investigate the effects of composite plant extracts (CPE) (from pomegranate peel, argy wormwood leaf, Kadsura japonica L., and Fructus evodiae rutaecarpae) replacing feed antibiotics on growth performance and serum indexes of weaned piglets. In experiment 1, a total of 160 weaned piglets weighing approximately 8 kg at 30 days of age were randomly allocated into 4 groups with 4 replicates per group and 10 piglets per replicate. The control group received a basal diet supplemented with aureomycin (107 mg/kg) and flavomycin (40 mg/kg), while the other three groups received the basal diet supplemented with CPE at 0.05%, 0.10%, and 0.20% for 28 days. In experiment 2, a total of 120 weaned piglets weighing approximately 11 kg at 40 days of age were randomly allocated into 3 groups with 4 replicates per group and 10 piglets per replicate. The antibiotic-free group received the basal diet, while the other two groups received the basal diet supplemented with aureomycin (67 mg/kg) + flavomycin (20 mg/kg) (antibiotics group) and CPE at 0.10% for 35 days, respectively. The results of experiment 1 showed that compared with the control group, the CPE groups showed no significant changes in average daily gain (ADG), average daily feed intake (ADFI), and feed/gain ratio (F/G) (P>0.05), though the 0.10% CPE group demonstrated better ADG, which was 2.44% higher than the control group. The results of experiment 2 showed that compared with the antibiotic-free group, the 0.10% CPE group significantly increased ADG by 21.39% (P<0.05), significantly decreased serum albumin (ALB), cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), and nitric oxide (NO) contents (P<0.05), significantly increased serum glutathione peroxidase (GSH-Px) activity (P<0.05), and significantly decreased ceruloplasmin (CER) activity (P<0.05). Compared with the antibiotics group, the 0.10% CPE group significantly increased ADG by 15.07% (P<0.05) and significantly decreased serum urea nitrogen (UN), LDL-C, and NO contents (P<0.05). In conclusion, supplementation with 0.10% CPE can replace feed antibiotics (aure-

omycin + flavomycin), improve growth performance, and exert anti-stress and antioxidant functions in weaned piglets.

Keywords: composite plant extracts; weaned piglets; antibiotics; growth performance; serum indexes

Introduction

The long-term use and abuse of antibiotics have led to drug residues and antibiotic resistance issues that directly threaten human survival and health. Statistics indicate that China' s total annual antibiotic usage is 150,000–200,000 tons, accounting for approximately 50% of global consumption, with livestock production using 52% of China' s total antibiotics [1]. Another report shows that the average annual antibiotic dosage per kilogram of body weight is 172 mg/kg for pigs, 148 mg/kg for chickens, and 45 mg/kg for cattle [2]. Evidently, pig production uses more antibiotics than other livestock sectors, and as the world' s largest pig producer, China uses the majority of its antibiotics in pig farming. Weaned piglets are particularly vulnerable due to low immunity and high disease susceptibility, receiving the highest antibiotic doses. Therefore, solving antibiotic-free feeding of weaned piglets is crucial for the sustainable development of the pig industry and human health.

Plant extracts, rich in various bioactive components and characterized by being natural, green, and free from drug resistance and residues, represent one of the most promising antibiotic alternatives. The composite plant extract feed additive developed by Zhejiang Academy of Agricultural Sciences is formulated from pomegranate peel, Kadsura japonica L., Fructus evodiae rutaecarpae, and argy wormwood leaf, which has demonstrated significant growth-promoting, immunity-enhancing, and anti-diarrheal effects in mice [3]. This study further investigated the effects of this composite plant extract replacing feed antibiotics on growth performance and blood indexes of weaned piglets to provide a scientific basis for its application in pig production.

1.1 Experimental Materials

Pomegranate peel, argy wormwood leaf, Kadsura japonica L., and Fructus evodiae rutaecarpae (all purchased from Zhejiang Chinese Medical University Pharmacy) were ground and sieved through a 200-mesh screen. The materials were mixed at a ratio of 1:3:9:9 (pomegranate peel:argy wormwood leaf:Kadsura japonica L.:Fructus evodiae rutaecarpae), then extracted at 80 °C for 6 hours with a solid-to-water ratio of 1:20 in a closed system, followed by concentration and spray-drying into powder form.

1.2 Experimental Design and Diets

Experiment 1: A total of 160 healthy weaned "Landrace × Large White" piglets weighing approximately 8 kg at 30 days of age were randomly allocated into 4 groups with 4 replicates per group and 10 piglets per replicate. The control group received a basal diet supplemented with aureomycin (107 mg/kg) + flavomycin (40 mg/kg), while the other three groups received the basal diet supplemented with 0.05%, 0.10%, and 0.20% CPE, respectively. Piglets had ad libitum access to feed and water during the 28-day experimental period. The basal diet was formulated according to the NRC (2012) nutrient requirements for 11–25 kg growing pigs. The composition and nutrient levels are shown in Table 1 .

Experiment 2: A total of 120 healthy weaned "Landrace × Large White"piglets weighing approximately 11 kg at 40 days of age were randomly allocated into 3 groups with 4 replicates per group and 10 piglets per replicate. The antibiotic-free group received the basal diet, the antibiotics group received the basal diet supplemented with aureomycin (67 mg/kg) + flavomycin (20 mg/kg), and the CPE group received the basal diet supplemented with 0.10% CPE. Piglets had ad libitum access to feed and water during the 35-day experimental period. The basal diet was the same as in experiment 1.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis), %

Items	Content
Ingredients	
Corn	
Soybean meal	
Puffing soybean	
Wheat bran	
Fish meal	
Fermented soybean meal	
Plasma protein meal	
$CaHPO_4$	
Limestone	
NaCl	
Premix[1]	
Total	
Nutrient levels[2]	
Crude protein (CP)	
Digestible energy (DE, MJ/kg)	
Lysine (Lys)	
Threonine (Thr)	
Tryptophan (Trp)	
Methionine (Met)	
Calcium (Ca)	
Total phosphorus (Total P)	

[1]The premix provided the following per kilogram of diet: VA 3,950 IU, VD_3 595 IU, VE 23 IU, VB_2 5.50 mg, VB_{12} 0.03 mg, biotin 0.15 mg, nicotinic acid 30

mg, folacin 1 mg, choline chloride 600 mg, VK_3 1 mg, pantothenic acid 15 mg, thiamin 1.5 mg, VB_6 8 mg, Cu ($CuSO_4 \cdot 5H_2O$) 200 mg, Fe ($FeSO_4 \cdot 7H_2O$) 110 mg, Zn ($ZnSO_4 \cdot 7H_2O$) 120 mg, Mn ($MnSO_4 \cdot H_2O$) 40 mg, Se ($NaSe_2O_3$) 0.3 mg, I (IO_3) 1 mg.
[2]Nutrient levels were calculated values.

1.3 Feeding Management

The experiments were conducted at the Haining Science and Technology Ranch Experimental Base of Zhejiang Academy of Agricultural Sciences. All piglets were housed in enclosed pig pens with automatic feeders, good ventilation, and temperature maintained at 20–25 °C. Piglets were fed twice daily (09:00 and 16:00) and had ad libitum access to water via nipple drinkers. Manure was cleaned twice daily to maintain pen hygiene, and pens were disinfected twice weekly.

1.4 Sample Collection and Index Determination

In both experiments, all piglets were fast-weighed on day 1 and at the end of the experiment, and feed intake per pen was recorded to calculate ADG, ADFI, and F/G. In experiment 2, at the end of the experiment, 6 piglets were randomly selected from each group, and blood was collected from the anterior vena cava using 5 mL disposable syringes into centrifuge tubes. Serum was separated to determine total protein (TP), albumin (ALB), urea nitrogen (UN), glucose (GLU), cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA), and nitric oxide (NO) contents, as well as total antioxidant capacity (T-AOC) and activities of ceruloplasmin (CER), alkaline phosphatase (AKP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). All indexes were measured using kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer's instructions.

1.5 Statistical Analysis

All data were initially processed using Excel 2010 and analyzed using SPSS 17.0 statistical software with ANOVA. All results are expressed as "mean ± standard deviation," with $P<0.05$ indicating significant difference.

2.1 Effects of CPE Replacing Feed Antibiotics on Growth Performance of Weaned Piglets

The results of experiment 1 (Table 2) showed that compared with the control group, complete replacement of feed antibiotics with 0.05%, 0.10%, and 0.20% CPE had no significant effects on ADG, ADFI, or F/G ($P>0.05$). However, the 0.10% CPE group showed a trend toward improved ADG, which was 2.44% higher than the control group. The mortality and culling rates for the control, 0.05% CPE, 0.10% CPE, and 0.20% CPE groups were 12.25%, 6.25%, 3.13%, and 6.25%, respectively (not statistically analyzed, not listed in table).

During experiment 2, piglets remained healthy with no mortality or culling. The results (Table 3) showed that compared with the antibiotic-free group, the antibiotics group showed no significant changes in ADG, ADFI, or F/G ($P>0.05$), while the CPE group significantly increased ADG by 21.39% ($P<0.05$) with no significant changes in ADFI or F/G ($P>0.05$). Compared with the antibiotics group, the CPE group significantly increased ADG by 15.07% ($P<0.05$) with no significant changes in ADFI or F/G ($P>0.05$).

2.2.1 Serum Biochemical Indexes

As shown in Table 4 , there were no significant differences in TP and GLU contents among groups ($P>0.05$). Compared with the antibiotic-free group, both the antibiotics and CPE groups significantly increased serum ALB content ($P<0.05$) and significantly decreased serum CHOL content ($P<0.05$). Compared with the antibiotics group, the CPE group significantly decreased serum UN content ($P<0.05$). The CPE group also significantly decreased serum LDL-C content compared with both the antibiotic-free and antibiotics groups ($P<0.05$).

2.2.2 Serum Antioxidant Indexes

As shown in Table 5 , there were no significant differences in serum SOD and CAT activities, T-AOC, or MDA content among groups ($P>0.05$). Compared with the antibiotic-free group, both the antibiotics and CPE groups significantly increased GSH-Px activity ($P<0.05$), with no significant difference between the antibiotics and CPE groups ($P>0.05$).

2.2.3 Serum Anti-Stress Indexes

As shown in Table 6 , there were no significant differences in serum AKP activity among groups ($P>0.05$). Compared with the antibiotic-free group, both the antibiotics and CPE groups significantly decreased serum CER activity and NO content ($P<0.05$). Compared with the antibiotics group, the CPE group showed no significant difference in serum CER activity ($P>0.05$) but significantly decreased serum NO content ($P<0.05$).

Discussion

In this study, experiment 1 showed high mortality and culling rates, while experiment 2 had no piglet deaths or health-related culling. This discrepancy can be attributed to two factors: First, the initial body weight of piglets in experiment 1 was 2–3 kg lower than in experiment 2, and experiment 1 piglets were newly weaned compared to two weeks post-weaning in experiment 2, making them more susceptible with weaker resistance and under weaning stress. Second, to ensure objective results, no medication was administered to sick piglets throughout the experimental period, leading to higher mortality and culling in experiment 1.

Plant extracts possess unique modes of action and are termed "resistance reversal agents" for antibiotics [4]. Their biological functions primarily include growth promotion, immunomodulation, antioxidant activity, anti-inflammatory, antibacterial, antiviral, and antiparasitic effects [5-11]. The application effects of plant extracts in piglets have shown initial promise. For example, extracts from Macleaya cordata, Ginkgo biloba, licorice, Origanum vulgare, Acanthopanax senticosus, and Forsythia suspensa have been found to alleviate weaning stress, improve health, and promote growth in piglets [12-16]. However, research on composite plant extracts, particularly regarding their application in replacing feed antibiotics for piglets, remains scarce.

This study found that adding 0.10% CPE to weaned piglet diets could completely replace the "aureomycin + flavomycin" antibiotic combination, achieving comparable results even when the antibiotic doses exceeded the limits specified in Ministry of Agriculture Announcement No. 168 (Feed Drug Additive Usage Standards) by 60%, while reducing mortality and culling rates. Experiment 2 results showed that compared with the antibiotic-free group, 0.10% CPE improved F/G by up to 16.27%, though the difference was not significant, possibly due to large variations in ADFI and ADG among replicates. To date, no studies have reported on the composite extraction of these four plants (pomegranate peel, argy wormwood leaf, Kadsura japonica L., and Fructus evodiae rutaecarpae) or the application of individual extracts from these plants as antibiotic replacements in piglets, with only sporadic reports on additional Schisandra extract supplementation [17]. However, mouse trials demonstrated that the composite extract of these four plants was superior to individual plant extracts [3], suggesting a synergistic effect from the combination. Due to the diversity of plant species and variations in extraction methods and reagents, the active components and contents of plant extracts differ significantly. Nevertheless, plant extracts are generally rich in polysaccharides, polyphenols, flavonoids, and other active components that can improve intestinal microecology, enhance immune function, and exert antioxidant effects to prevent disease and promote growth [18]. This study also found that low-dose antibiotics failed to significantly promote growth. Existing research indicates that sub-inhibitory antibiotic concentrations may promote bacterial proliferation and worsen infections, providing no significant disease prevention [19], which may be a major factor contributing to current antibiotic overuse.

ALB is the most abundant protein in serum, accounting for 40–60% of TP, with important physiological functions closely related to animal health. In this study, both CPE and antibiotics increased serum ALB content in weaned piglets, indicating improved health status. Serum UN content indirectly reflects protein metabolism and amino acid balance, decreasing when amino acid deposition increases [20]. The CPE reduced serum UN and CHOL contents, specifically LDL-C, suggesting enhanced protein and lipid synthesis. Similar to our results, Fu et al. [21] found that polyphenols from pomegranate peel improved serum and liver protein deposition in mice.

Research on the antioxidant effects of plant extracts is extensive, particularly regarding organic solvent-extracted components such as essential oils, sterols, and flavonoids, which enhance antioxidant function by increasing antioxidant enzyme secretion and activity while reducing lipid oxidation products like MDA to prevent peroxidation damage [22-24]. The CPE used in this study contained water-extracted active components that increased serum GSH-Px activity, likely due to the strong antioxidant properties of polyphenols and polysaccharides [21,25-26], though specific active components require further identification and verification.

CER is an acute-phase protein that increases during stress and catalyzes NO synthesis [27]. This study found that CPE supplementation decreased both serum CER activity and NO content in weaned piglets, indicating significant anti-stress effects. This may be related to the specific functions of CPE in regulating body temperature, adjusting central nervous system function, providing anti-convulsant effects, adaptogenic activity, and hormone-like effects [28].

In conclusion, 0.10% composite plant extract (containing pomegranate peel, argy wormwood leaf, Kadsura japonica L., and Fructus evodiae rutaecarpae) can replace the feed antibiotic combination of aureomycin (67 mg/kg) + flavomycin (20 mg/kg) by exerting anti-stress and antioxidant functions to improve the health and growth performance of weaned piglets.

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