Postprint: Experimental Study on Replacing Chlortetracycline and Zinc Bacitracin with Cinnamaldehyde in Nursery Pig Diets

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Abstract

This study investigated the effects of cinnamaldehyde as a replacement for the combined use of chlortetracycline and zinc bacitracin in nursery pig diets. A total of 180 healthy 42-day-old Duroc × Landrace × Large White crossbred nursery pigs were selected and allocated into control group, experimental group A, and experimental group B based on similar body weight and equal sex ratio, with 60 pigs per group, 4 replicates per group, and 15 pigs per replicate. Pigs in the control group were fed the conventional nursery diet containing chlortetracycline, zinc bacitracin, and colistin; while chlortetracycline and zinc bacitracin were removed from the diet and supplemented with 400 and 600 mg/kg cinnamaldehyde preparation for experimental groups A and B, respectively. The experimental period lasted 21 days. The results revealed that replacing the combined chlortetracycline and zinc bacitracin with cinnamaldehyde preparation significantly reduced the diarrhea rate and diarrhea index in nursery pigs ($P<0.05$). The average daily gain of pigs in groups A and B was higher than that of the control group, with group B achieving statistical significance ($P<0.05$). Serum total protein content in group B was significantly higher than that in the control group ($P<0.05$); serum alanine aminotransferase activity in experimental groups was lower than that in the control group, with group B reaching significance ($P<0.05$); serum aspartate aminotransferase activity in group B was significantly higher than that in both the control group and group A ($P<0.05$). Serum superoxide dismutase, catalase, glutathione peroxidase activities and total antioxidant capacity in groups A and B were significantly ($P<0.05$) or extremely significantly ($P<0.01$) higher than those in the control group; serum malondialdehyde content in groups A and B was extremely significantly lower than that in the control group ($P<0.01$). Levels of classical swine fever antibodies, porcine reproductive and respiratory syndrome antibodies, and porcine circovirus antibodies in groups A and B were all significantly higher than those

in the control group (P<0.05); pseudorabies antibody levels also exhibited an upward trend, with group B attaining significance (P<0.05). No significant difference was observed in serum foot-and-mouth disease antibody levels among the three groups (P>0.05). These results suggest that cinnamaldehyde can replace the combined use of chlortetracycline and zinc bacitracin in nursery pig diets, with superior efficacy compared to the antibiotic combination. Cinnamaldehyde can effectively prevent and control diarrhea occurrence, enhance antioxidant capacity, promote growth, and improve feed conversion efficiency in nursery pigs. The appropriate supplementation level of cinnamaldehyde preparation in nursery pig diets is 600 mg/kg.

Full Text

Substitution of Cinnamyl Aldehyde for Aureomycin and Bacitracin Zinc in Nursery Pig Diet: An Experimental Study

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Abstract: This study investigated the effects of substituting cinnamyl aldehyde for the combined use of aureomycin and bacitracin zinc in nursery pig diets. A total of 180 healthy 42-day-old three-way crossbred (Duroc × Landrace × Yorkshire) nursery pigs were selected and divided into a control group, experimental group A, and experimental group B according to similar body weight and equal sex ratio. Each group comprised 60 pigs with 4 replicates of 15 pigs each. The control group was fed the conventional nursery diet used on the farm, which contained aureomycin, bacitracin zinc, and colistin. Experimental groups A and B were fed the same basal diet with aureomycin and bacitracin zinc removed and replaced with 400 mg/kg and 600 mg/kg of cinnamyl aldehyde preparation, respectively. The trial lasted for 21 days. The results demonstrated that substituting cinnamyl aldehyde preparation for the combined antibiotics significantly reduced the diarrhea rate and diarrhea index of nursery pigs ($P < 0.05$). Average daily gain in experimental groups A and B was higher than that in the control group, with group B reaching statistical significance ($P < 0.05$). Serum total protein content in group B was significantly higher than in the control group ($P < 0.05$). Serum glutamic-pyruvic transaminase activity in the experimental groups was lower than in the control group, with group B showing a significant difference ($P < 0.05$). Serum glutamic-oxaloacetic transaminase activity in group B was significantly higher than in both the control group and group A ($P < 0.05$). The activities of

superoxide dismutase, catalase, glutathione peroxidase, and total antioxidant capacity in serum of groups A and B were significantly ($P < 0.05$) or extremely significantly ($P < 0.01$) higher than those in the control group, while serum malondialdehyde content was extremely significantly lower ($P < 0.01$). The levels of CSFV-Ab, PRRSV-Ab, and PCV-Ab in groups A and B were all significantly higher than in the control group ($P < 0.05$), and PRV-Ab level also showed an upward trend, with group B reaching significance ($P < 0.05$). No significant differences were observed in serum FMDV-Ab levels among the three groups ($P > 0.05$). These findings indicate that cinnamyl aldehyde can effectively replace the combined use of aureomycin and bacitracin zinc in nursery pig diets, with even better efficacy than the antibiotic combination. Cinnamyl aldehyde effectively prevents and controls diarrhea, enhances antioxidant capacity, promotes growth, and improves feed conversion efficiency in nursery pigs. The optimal supplementation level of cinnamyl aldehyde preparation in nursery pig diets is 600 mg/kg.

Keywords: cinnamyl aldehyde; nursery pigs; application effects
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Cinnamyl aldehyde is a novel plant extract [1] that occurs naturally in cinnamon oil and serves as the main functional component of traditional Chinese medicinal herbs such as cinnamon. It finds extensive applications in spices, pharmaceuticals, daily chemical products, feed, papermaking, and food processing [2]. Research has demonstrated that cinnamyl aldehyde possesses antimicrobial and antiviral properties, enhances gastrointestinal motility, improves feed utilization efficiency, and promotes animal growth [3]. Tiihonen et al. [4] reported that dietary supplementation with cinnamyl aldehyde promoted the proliferation of beneficial gut microbiota in broilers. Jamroz et al. [5] confirmed that feed additives containing cinnamyl aldehyde stimulated the growth of lactobacilli in the posterior intestine while inhibiting *Escherichia coli*. Wang et al. [6] revealed that cinnamyl aldehyde induces oxidative stress in *E. coli*, causing excessive accumulation of intracellular reactive oxygen species and enhanced superoxide dismutase activity, which leads to oxidative damage of cell membrane lipids, structural disruption, and ultimately bacterial death. Zhang and Shen [7] reported that cinnamon enhances reticuloendothelial system function and improves macrophage phagocytic capacity in mice. However, experimental studies on cinnamyl aldehyde application in pigs remain scarce, necessitating research on its health-promoting and growth-enhancing effects in swine. Furthermore, current production practices involve excessive antibiotic supplementation in pig diets, particularly for nursery pigs, which may create serious safety concerns [8]. Therefore, this study aimed to evaluate the efficacy of cinnamyl aldehyde as an antibiotic substitute in nursery pig diets to provide technical solutions for safe pig production.

1.1 Experimental Material

The cinnamyl aldehyde preparation contained 10% cinnamyl aldehyde and was provided by Guangzhou Xinnong Feed Science and Technology Co., Ltd.

1.2 Experimental Design

A total of 180 healthy 42-day-old three-way crossbred (Duroc × Landrace × Yorkshire) nursery pigs were selected and allocated to the control group, experimental group A, and experimental group B based on similar body weight and equal sex ratio. Each group consisted of 60 pigs with 4 replicates of 15 pigs each. The control group received the conventional nursery diet used on the farm, which contained multiple antibiotics (Table 1). Experimental groups A and B were fed the basal diet with aureomycin and bacitracin zinc removed and supplemented with 400 mg/kg and 600 mg/kg of cinnamyl aldehyde preparation, respectively.

1.3 Experimental Diets

The composition and nutrient levels of the conventional nursery diet (control group diet) are presented in Table 1.

Table 1 Composition and nutrient levels of nursery pig diet on pig farm (air-dry basis), %

Item	Content
Ingredients	
Corn	
Emulsified oil meal	
Whey meal	
Honey	
Barley bud	
Soybean meal	
Extruded soybean	
Imported fish meal	
Rice protein meal	
Premix[1]	
Total	
Nutrient levels[2]	
DE/(MJ/kg)	
CP	
Lys	
Met	
Ca	

[1] One kilogram of premix contained: Lys 75.0 g, Met 37.5 g, vitamin premix

35.0 g, CaHPO 375.0 g, limestone 218.8 g, NaCl 70.0 g, FeSO ·H O 11.0 g, ferrous fumarate 7.5 g, CuSO ·5H O 20.0 g, MnSO ·H O 1.25 g, ZnSO ·H O 8.0 g, zinc oxide 75.0 g, iodine premix (1%) 0.75 g, selenium premix (1%) 0.75 g, piglet multi-enzymes 5.0 g, phytase 2.75 g, acidifier 25.0 g, flavor agent 7.5 g, edulcorant 10.0 g, chlortetracycline premix 37.5 g, zinc bacitracin premix 5.0 g, and colistin premix 2.5 g.
[2] Calculated values.

1.4 Feeding Management

The feeding trial was conducted at Sulvyuan Breeding Pig Farm in He County, Anhui Province, with a duration of 21 days. Experimental pigs were housed on nursery beds, with 15 pigs per bed constituting one replicate. Each nursery bed was equipped with heating plates of identical structure and size. Pigs had ad libitum access to feed and water, and were managed according to conventional farm practices.

1.5 Measurements

1.5.1 Health Status Daily observations recorded morbidity in each group to calculate diarrhea rate and diarrhea index using the following formulas:
Diarrhea rate (%) = 100 × (number of pigs with diarrhea × diarrhea days) / (total pigs × trial days)
Diarrhea index = sum of diarrhea scores / number of pigs

The scoring standard for diarrhea severity is shown in Table 2.

Table 2 The standard for evaluation of diarrhea degree in pigs

Shape of feces	Diarrhea scores	Diarrhea degree
Formed or granular	0	Normal
Soft feces, formable	1	Mild
Thick, unformed, no fecal-water separation	2	Moderate
Liquid, unformed, with fecal-water separation	3	Severe

1.5.2 Growth Performance Body weight was measured at the beginning and end of the trial using electronic scales (by difference method) to calculate average daily gain. Feed consumption was recorded for each replicate to determine average daily feed intake and feed-to-gain ratio.

1.5.3 Nutrient Digestibility During the third week of the trial, approximately 1.2 kg of fresh feces was collected from each replicate, sun-dried, labeled, and sealed to prevent mold and moisture absorption. Approximately 250 g of diet samples from the control group were collected and preserved. Dry matter, crude protein, crude fat, crude fiber, crude ash, calcium, phosphorus, and acid-insoluble ash contents were determined in both diet and fecal samples.

Apparent digestibility of these nutrients was calculated using the endogenous indicator method [9].

1.5.4 Serum Biochemical Parameters At the end of the trial, 8 pigs per group (2 per replicate) were selected for fasting jugular vein blood collection (10 mL). Serum was prepared to measure glucose (Glu), total protein (TP), urea nitrogen (UN), immunoglobulin G (IgG), malondialdehyde (MDA) content, and the activities of glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC). All parameters were determined using kits and recommended methods from Nanjing Jiancheng Technology Co., Ltd.

1.5.5 Disease Antibodies At the end of the trial, 8 pigs per group (2 per replicate) were selected to determine serum levels of classical swine fever virus antibody (CSFV-Ab), porcine reproductive and respiratory syndrome virus antibody (PRRSV-Ab), pseudorabies virus antibody (PRV-Ab), foot-and-mouth disease virus antibody (FMDV-Ab), and porcine circovirus antibody (PCV-Ab). These antibody parameters were measured by the Preventive Medicine Laboratory of Anhui Agricultural University.

1.6 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 17.0 software, with Duncan' s multiple comparison test used for post-hoc analysis. $P < 0.05$ indicated significant difference, and $P < 0.01$ indicated extremely significant difference.

2.1 Health Status

As shown in Table 3 , diarrhea rates in experimental groups A and B were reduced by 27.43% and 54.86% respectively compared with the control group, with significant differences among all groups ($P < 0.05$). Diarrhea indices in groups A and B decreased by 60% ($P < 0.05$) and 66% ($P < 0.05$) respectively compared with the control group, though no significant difference was observed between the two experimental groups ($P > 0.05$).

Table 3 Diarrhea rate and diarrhea index of pigs

Item	Control group	Experimental group A	Experimental group B
Diarrhea rate/%	1.75 ± 0.21^{a}	1.27 ± 0.19	0.79 ± 0.13
Diarrhea index	0.50 ± 0.11^{a}	0.20 ± 0.07	0.17 ± 0.05

In the same row, values with no letter or the same small letter superscripts indicate no significant difference ($P > 0.05$), different small letters indicate significant difference ($P < 0.05$), and different capital letters indicate extremely significant difference ($P < 0.01$). The same applies below.

2.2 Growth Performance and Nutrient Digestibility

Table 4 shows that average daily gain in experimental groups A and B was higher than in the control group, with group B reaching statistical significance ($P < 0.05$). Feed-to-gain ratio in groups A and B was lower than in the control group, though not significantly ($P > 0.05$). Although some differences in nutrient digestibility were observed among the three groups, none reached statistical significance ($P > 0.05$).

Table 4 Growth performance of pigs

Item	Control group	Experimental group A	Experimental group B
Initial weight/kg	13.07 ± 0.28	13.05 ± 0.25	13.10 ± 0.72
Final weight/kg	22.79 ± 0.83[a]	23.35 ± 0.89	23.62 ± 0.62
ADG/g	462.86 ± 42.39[a]	490.48 ± 38.95	500.95 ± 47.51
ADFI/kg	0.79 ± 0.03	0.76 ± 0.04	0.82 ± 0.04
F/G	1.71 ± 0.10	1.69 ± 0.15	1.64 ± 0.12
EE digestibility	0.89 ± 0.001	0.90 ± 0.001	0.89 ± 0.008
CP digestibility	0.59 ± 0.006	0.59 ± 0.003	0.60 ± 0.007
CF digestibility	0.39 ± 0.001	0.38 ± 0.001	0.38 ± 0.018
Ash digestibility	0.83 ± 0.001	0.80 ± 0.004	0.81 ± 0.002
Ca digestibility	0.53 ± 0.002	0.51 ± 0.032	0.53 ± 0.006
P digestibility	0.83 ± 0.04	0.82 ± 0.04	0.82 ± 0.04

2.3 Serum Biochemical Parameters

Table 5 reveals no significant differences in serum Glu, UN, or IgG contents among the three groups ($P > 0.05$). Serum TP content in group B was significantly higher than in the control group ($P < 0.05$), while group A also showed higher TP content than the control group, though not significantly ($P > 0.05$). Serum GPT activity in the experimental groups was lower than in the control group, with group B showing a significant reduction ($P < 0.05$). Serum GOT activity in group B was significantly higher than in both the control group and group A ($P < 0.05$). The activities of SOD, CAT, GSH-Px, and T-AOC in groups A and B were significantly ($P < 0.05$) or extremely significantly ($P < 0.01$) higher than in the control group, while serum MDA content was extremely significantly lower ($P < 0.01$).

Table 5 Serum biochemical parameters of pigs

Item	Control group	Experimental group A	Experimental group B
Glu/(mmol/L)	5.86 ± 0.20	5.96 ± 0.10	6.18 ± 0.58
TP/(g/L)	59.18 ± 0.60[a]	59.72 ± 1.51	61.47 ± 1.68
UN/(mmol/L)	5.45 ± 0.05	5.75 ± 0.22	5.62 ± 0.05
GOT/(U/L)	52.02 ± 1.30[a]	52.42 ± 1.51[a]	58.24 ± 0.47

Item	Control group	Experimental group A	Experimental group B
GPT/(U/L)	65.01 ± 0.67[a]	63.56 ± 0.71	60.58 ± 0.59
SOD/(U/L)	115.39 ± 2.33[a]	118.07 ± 4.13	120.34 ± 2.12
CAT/(U/L)	1.22 ± 0.05	1.42 ± 0.03	1.65 ± 0.10
GSH-Px/(U/L)	278.98 ± 2.62	297.41 ± 7.42	292.17 ± 3.33
T-AOC/(U/L)	4.30 ± 0.14[a]	4.68 ± 0.17	4.79 ± 0.24
MDA/(mmol/mL)	5.06 ± 0.24	4.20 ± 0.14	4.15 ± 0.27
IgG/(g/L)	1.41 ± 0.03	1.49 ± 0.07	1.45 ± 0.10

2.4 Disease Antibodies

As shown in Table 6 , the levels of CSFV-Ab, PRRSV-Ab, and PCV-Ab in the experimental groups were all significantly higher than in the control group ($P < 0.05$). PRV-Ab level also demonstrated an upward trend, with group B reaching statistical significance ($P < 0.05$). No significant differences were observed in serum FMDV-Ab levels among the three groups ($P > 0.05$).

Table 6 Serum antibody content of pigs (OD value)

Item	Control group	Experimental group A	Experimental group B
CSFV-Ab	0.41 ± 0.09[a]	0.56 ± 0.05	0.54 ± 0.02
PRRSV-Ab	0.57 ± 0.02[a]	0.66 ± 0.02	0.64 ± 0.03
PCV-Ab	1.84 ± 0.09[a]	2.10 ± 0.14	2.22 ± 0.11
FMDV-Ab	0.08 ± 0.02	0.09 ± 0.07	0.09 ± 0.06
PRV-Ab	0.38 ± 0.03[a]	0.43 ± 0.02	0.45 ± 0.02

3.1 Effects on Health Status of Nursery Pigs

The substitution of cinnamyl aldehyde for aureomycin and bacitracin zinc in nursery pig diets significantly reduced diarrhea rate and diarrhea index, indicating that cinnamyl aldehyde not only effectively replaces the combined antibiotics but also provides superior control of piglet diarrhea. Based on these health indicators, the optimal supplementation level of cinnamyl aldehyde preparation in nursery pig diets is 600 mg/kg.

Previous research supports these findings. Zhang et al. [10] reported that cinnamyl aldehyde inhibits various pathogens including *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella typhi* and *paratyphi A*, *Aerobacter aerogenes*, *Proteus vulgaris*, *Diplococcus pneumoniae*, *Bacillus anthracis*, *E. coli*, *Salmonella*, and *Vibrio cholerae*. Wang et al. [6] demonstrated that cinnamyl aldehyde kills *E. coli* through oxidative damage and inhibits *Pseudomonas aeruginosa* proliferation. Castillo et al. [11] found that cinnamyl aldehyde possesses intracellular antioxidant functions that protect intestinal villi from free radical and toxin damage, thereby increasing villus height. Tiihonen et al. [4] observed that dietary cinnamyl aldehyde increased butyric acid proportion in

intestinal contents, which contributes to small intestinal mucosal repair and proliferation. Manzanilla et al. [12] reported that butyrate significantly reduced crypt depth and increased villus height in piglet small intestine. Additionally, Tiihonen et al. [4] and Liu et al. [13] demonstrated that cinnamyl aldehyde promotes proliferation of beneficial gut microbiota and improves intestinal structure and immune function in broilers. Wang et al. [14] found cinnamyl aldehyde to have stronger antibacterial effects against *S. aureus* and *E. coli* than oregano oil, citral, and carvacrol. Li et al. [15] reported that cinnamon ether extract strongly inhibits various foodborne pathogens, making it a highly effective natural antimicrobial agent. Collectively, these findings suggest that cinnamyl aldehyde prevents and controls piglet diarrhea by inhibiting harmful microorganisms, promoting beneficial bacteria, and protecting and repairing intestinal mucosal epithelium.

3.2 Effects on Serum Biochemical Parameters

The elevated serum TP content in the cinnamyl aldehyde-treated groups, reaching significance in group B, likely reflects improved health status and enhanced protein synthesis (anabolic) efficiency due to smoother metabolic processes. The significantly or extremely significantly higher activities of SOD, CAT, GSH-Px, and T-AOC, coupled with extremely significantly lower MDA content in groups A and B, can be attributed to the antioxidant properties of cinnamyl aldehyde. As an antioxidant, cinnamyl aldehyde protects other oxidizable substances, thereby enhancing the antioxidant enzyme system and reducing oxidative products. Castillo et al. [11] confirmed the antioxidant activity of cinnamyl aldehyde. Jiao [16] reported that dietary cinnamyl aldehyde promoted growth and reduced stress in growing cattle. Mu et al. [17] demonstrated that cinnamyl aldehyde inhibits polyphenol oxidase. Li et al. [3] showed that cinnamyl aldehyde composite preservatives effectively inhibited bacterial growth and lipid oxidation in shrimp, significantly extending shelf life. Li et al. [18] found that cinnamyl aldehyde compound preservatives provided excellent antiseptic effects on spicy snack foods, with efficacy 5–20 times greater than calcium propionate and potassium sorbate.

As an antioxidant, cinnamyl aldehyde also protects cell membrane structure and function in tissues such as the liver. This protective effect may explain the lower serum GPT activity (an enzyme primarily located in hepatocytes) in the experimental groups, with group B showing a significant reduction. Intact hepatocyte membrane structure limits the leakage of intracellular enzymes such as GPT, resulting in lower serum levels [19]. The significantly higher serum GOT activity in group B compared to both the control and group A requires further investigation to elucidate its underlying mechanism.

Although not statistically significant, the consistently higher serum Glu content in groups A and B may reflect improved gastrointestinal health compared to the antibiotic combination, promoting enhanced glucose absorption by small intestinal mucosal cells. Additionally, cinnamyl aldehyde treatment significantly

increased levels of CSFV-Ab, PRRSV-Ab, PCV-Ab, and PRV-Ab (in group B), suggesting that cinnamyl aldehyde stimulates antibody synthesis against these pathogens. A similar stimulatory effect was observed on IgG synthesis. However, no apparent effect on FMDV-Ab synthesis was detected, though these preliminary findings warrant further investigation into the mechanisms of cinnamyl aldehyde' s immunomodulatory effects.

3.3 Effects on Growth Performance and Feed Utilization

The higher average daily gain in groups A and B, with significant improvement in group B, and the lower feed-to-gain ratio in both experimental groups demonstrate that cinnamyl aldehyde promotes growth and improves feed conversion efficiency more effectively than the combined antibiotics. These improvements likely result from enhanced health status, increased hepatocyte membrane stability and function, and strengthened antioxidant capacity, which collectively facilitate more efficient conversion of dietary nutrients into body tissue. Previous reports indicate that cinnamyl aldehyde can increase nitrogen retention in animals by over 7% [3] and promote growth in beef cattle [16]. The current study found no significant effects on nutrient digestibility, suggesting that improved feed conversion efficiency primarily results from enhanced post-absorptive utilization of nutrients for tissue synthesis rather than improved digestion. Based on growth performance and feed utilization indicators, the optimal supplementation level of cinnamyl aldehyde preparation in nursery pig diets is 600 mg/kg.

Conclusions

1. Cinnamyl aldehyde can effectively replace the combined use of aureomycin and bacitracin zinc in nursery pig diets, with superior efficacy compared to the antibiotic combination.
2. Cinnamyl aldehyde effectively prevents and controls diarrhea, improves nutritional and metabolic status, enhances antioxidant capacity, promotes growth, and increases feed conversion efficiency in nursery pigs.
3. The optimal supplementation level of cinnamyl aldehyde preparation in nursery pig diets is 600 mg/kg.

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