Effects of Acidifiers and Oregano Oil Replacing Colistin Sulfate on Growth Performance, Diarrhea Rate, and Cecal Microbial Count in Weaned Piglets: Postprint

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Abstract

This experiment aimed to investigate the effects of acidifiers and oregano oil as replacements for colistin sulfate on growth performance, diarrhea rate, and cecal microorganism counts in weaned piglets. The experiment adopted a single-factor design, selecting 120 26-day-old weaned piglets with similar parity and body weight, which were randomly divided into 3 groups with 5 replicates per group and 8 piglets per replicate. All groups received diets containing 75 mg/kg chlortetracycline + 10 mg/kg enramycin; additionally, the three groups were supplemented with 0.10% acidifier + 0.10% colistin sulfate, 0.15% acidifier, and 0.15% acidifier + 0.03% oregano oil, respectively. The experimental period lasted 14 days. The results showed: 1) There were no significant differences in average daily gain, feed-to-gain ratio, and diarrhea rate among the groups ($P>0.05$). Compared with the other two groups, piglets in the 0.15% acidifier group showed a tendency for increased average daily feed intake ($P=0.066$). 2) The cecal Salmonella count in the 0.10% acidifier + 0.10% colistin sulfate group was significantly lower than that in the 0.15% acidifier group ($P<0.05$); the cecal Bifidobacterium and Lactobacillus counts in the 0.15% acidifier + 0.03% oregano oil group were significantly higher than those in the 0.15% acidifier group ($P<0.05$), but there was no significant difference between the 0.15% acidifier + 0.03% oregano oil group and the 0.10% acidifier + 0.10% colistin sulfate group ($P>0.05$). It can be concluded that supplementation of 0.15% acidifier or 0.15% acidifier + 0.03% oregano oil in diets containing 75 mg/kg chlortetracycline + 10 mg/kg enramycin had similar effects on growth performance in weaned piglets as supplementation with 0.10% colistin sulfate; dietary supplementation with 0.15% acidifier + 0.03% oregano oil also helped increase the number of beneficial cecal bacteria, with effects comparable to those of 0.10%

colistin sulfate supplementation. Therefore, dietary supplementation with acidifiers and oregano oil can be used as a replacement for colistin sulfate in weaned piglet diets.

Full Text

Effects of Acidifier and Oregano Oil as Replacements for Colistin Sulfate on Growth Performance, Diarrhea Rate, and Caecal Microbial Populations in Weaned Piglets

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Abstract

This experiment investigated the effects of dietary acidifier and oregano oil as replacements for colistin sulfate on growth performance, diarrhea rate, and caecal microbial populations in weaned piglets. Using a single-factor design, 120 piglets weaned at 26 days of age with similar parity and body weight were randomly allocated to three groups, each consisting of five replicates of eight piglets. All groups received basal diets containing 75 mg/kg chlortetracycline and 10 mg/kg enramycin, with the following additional treatments: Group 1: 0.10% acidifier + 0.10% colistin sulfate; Group 2: 0.15% acidifier; and Group 3: 0.15% acidifier + 0.03% oregano oil. The 14-day feeding trial yielded several key findings. First, no significant differences were observed among groups in average daily gain (ADG), feed-to-gain ratio (F/G), or diarrhea rate ($P > 0.05$). However, the 0.15% acidifier group showed a trend toward increased average daily feed intake (ADFI) compared to the other two groups ($P = 0.066$). Second, the 0.10% acidifier + 0.10% colistin sulfate group exhibited significantly lower caecal *Salmonella* counts than the 0.15% acidifier group ($P < 0.05$). The 0.15% acidifier + 0.03% oregano oil group demonstrated significantly higher populations of *Bifidobacterium* and *Lactobacillus* in the caecum compared to the 0.15% acidifier group ($P < 0.05$), though these values did not differ significantly from the colistin sulfate group ($P > 0.05$). These results indicate that supplementation with either 0.15% acidifier or 0.15% acidifier + 0.03% oregano oil produces growth performance effects comparable to 0.10% colistin sulfate in weaned piglets. Moreover, the combined acidifier and oregano oil treatment enhances beneficial caecal bacteria populations similarly to colistin sulfate. Therefore, dietary acidifier and oregano oil can effectively replace colistin sulfate in weaned piglet diets.

Keywords: acidifier; oregano oil; colistin sulfate; weaned piglets; growth per-

formance; caecal microorganisms

Introduction

Early weaning technology has become a cornerstone of modern swine production, enabling improved annual sow productivity and enhanced economic efficiency [1]. However, the abrupt changes in environment and diet at weaning, coupled with immature immune and digestive systems, insufficient gastric acid secretion, and imbalanced intestinal microflora, frequently trigger "early weaning syndrome" in piglets [2-4]. This syndrome represents a major obstacle to swine industry development, causing substantial economic losses [5]. While dietary antibiotics have been widely used in livestock production to enhance disease resistance and growth performance [6-7], their prolonged and indiscriminate use has led to serious concerns regarding drug residues in animal products and environmental contamination [8-9]. In the current context of "antibiotic-free feed," identifying suitable antibiotic alternatives has become an urgent priority.

Research on antibiotic alternatives has primarily focused on acidifiers, probiotics, and plant extracts, with both acidifiers and plant extracts demonstrating efficacy in improving piglet growth performance and immune function. Henry et al. [10] reported that dietary acidifiers enhance piglet growth performance while reducing diarrhea rates. Similarly, oregano oil has shown promise in preventing and treating intestinal infections, reducing diarrhea, and improving growth performance [11]. Despite the variety of available acidifiers and plant extracts, standardized usage protocols remain lacking, and few studies have investigated the synergistic effects of combined acidifier and plant extract supplementation on piglet growth and intestinal microflora. In practical production settings, swine farms commonly use acidifiers in combination with colistin sulfate for disease prevention. With colistin sulfate now banned in many jurisdictions, alternative strategies are urgently needed. This study was designed to evaluate the effects of replacing colistin sulfate with either 0.15% acidifier alone or 0.15% acidifier + 0.03% oregano oil on growth performance, diarrhea rate, and caecal microbial populations in weaned piglets, thereby providing a theoretical basis for the rational application of these additives.

Materials and Methods

1.1 Experimental Materials

Colistin sulfate (10% purity) was purchased commercially, while the acidifier (containing 28.0% formic acid and 14.0% propionic acid) and oregano oil were provided by Anpario (Shanghai) Biological Technology Co., Ltd., UK.

1.2 Experimental Animals and Design

One hundred twenty piglets weaned at 26 days of age, with similar parity and body weight, were randomly assigned to three dietary treatment groups. Each group comprised five replicates with eight piglets per replicate. All groups received basal diets supplemented with 75 mg/kg chlortetracycline and 10 mg/kg enramycin. The three treatment groups received additional supplements as follows: (1) 0.10% acidifier + 0.10% colistin sulfate, (2) 0.15% acidifier, and (3) 0.15% acidifier + 0.03% oregano oil. The experimental period lasted 14 days.

1.3 Experimental Diets and Management

Piglets were fed corn-soybean meal-based diets formulated according to NRC (2012) and Chinese Feeding Standards for Swine (NY/T 65-2004). The composition and nutrient levels of experimental diets are presented in Table 1 . Animals were group-housed with ad libitum access to feed and water. Sanitation, disinfection, and immunization protocols followed standard farm procedures.

1.4 Sample Collection and Processing

On day 14 of the experiment, one piglet was randomly selected from each replicate and euthanized. Approximately 6 cm of the mid-caecum was immediately excised, double-ligated at both ends, wrapped in aluminum foil, and then sealed with plastic wrap and cotton thread. Samples were labeled and stored for subsequent analysis.

1.5 Measurement Indices

1.5.1 Growth Performance Piglet numbers and feed intake were recorded daily throughout the trial, with any culled or deceased animals weighed and documented. Individual body weights were obtained on days 1 and 14 after overnight fasting, and feed consumption was recorded by group. Average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated accordingly.

1.5.2 Diarrhea Rate Diarrhea incidence was monitored and recorded twice daily before morning and afternoon feeding. Diarrhea rate was calculated as: (Total diarrhea episodes / (Total piglets × experimental days)) × 100%.

1.5.3 Caecal Microbial Quantification Approximately 0.5 g of caecal content was aseptically transferred to a penicillin bottle containing 5 mL sterile saline, vortexed for 3-5 minutes, and serially diluted (10^{1} to $10^{}$). Aliquots were plated onto selective media in triplicate across five dilution gradients and incubated at 37°C for 24 hours before colony counting. The culture media used for different bacterial groups are listed in Table 2 .

1.6 Data Processing and Statistical Analysis

Data were processed using Excel 2013 and analyzed via one-way ANOVA with SPSS 17.0 software. Duncan's multiple range test was used for post-hoc comparisons. Results are expressed as means ± standard error. Statistical significance was declared at $P < 0.05$, with trends noted at 0.05 P 0.10.

Results

2.1 Effects on Growth Performance and Diarrhea Rate

As shown in Table 3 , no significant differences were observed among groups in ADG, ADFI, or F/G ($P > 0.05$). However, the 0.15% acidifier group exhibited a trend toward higher ADFI compared to the 0.10% acidifier + 0.10% colistin sulfate group ($P = 0.066$), representing a 10.94% increase. The 0.15% acidifier + 0.03% oregano oil group also showed a 3.56% increase in ADFI compared to the colistin sulfate group, though this difference was not statistically significant. Diarrhea rates did not differ significantly among groups ($P > 0.05$), but the 0.15% acidifier and 0.15% acidifier + 0.03% oregano oil groups showed 22.04% and 20.10% lower diarrhea rates, respectively, compared to the colistin sulfate group.

2.2 Effects on Caecal Microbial Populations

Table 4 presents the caecal microbial data. The 0.10% acidifier + 0.10% colistin sulfate group had significantly lower *Salmonella* counts than the 0.15% acidifier group ($P < 0.05$). The 0.15% acidifier + 0.03% oregano oil group exhibited significantly higher *Bifidobacterium* and *Lactobacillus* populations compared to the 0.15% acidifier group ($P < 0.05$), though these values were not significantly different from the colistin sulfate group ($P > 0.05$). No significant differences were detected among groups in *Escherichia coli* or total aerobic bacteria counts ($P > 0.05$).

Discussion

3.1 Impact on Growth Performance and Diarrhea Rate

Early weaning syndrome causes significant economic losses for swine producers. While antibiotics effectively improve growth performance and disease prevention, concerns about drug residues and bacterial resistance have intensified [12-13]. Research indicates that acidifiers and plant extracts can match antibiotics' growth-promoting effects and serve as viable alternatives [14-15]. Wang et al. [16] found that bamboo vinegar as an antibiotic substitute produced comparable final body weight, ADFI, ADG, and F/G in weaned piglets. Acidifiers appear most effective during the first two weeks post-weaning, when gastric acid

secretion is insufficient and normalizes only after 4-5 weeks [17]. Hahn et al. [18] similarly reported that dietary lactic acid supplementation improved ADG and ADFI during weeks 1-3 post-weaning while significantly reducing pH in the stomach, duodenum, jejunum, and ileum. Zhang et al. [19] demonstrated that organic acids alone or combined with cactus extract improved F/G, reduced diarrhea rates, and enhanced overall growth performance, confirming the synergistic potential of acidifier-plant extract combinations. Oregano oil, extracted from *Origanum* plants, exhibits strong antimicrobial and growth-promoting properties without drug residues, making it an environmentally friendly feed additive. Huang et al. [20] reported that oregano oil reduced F/G and diarrhea rates compared to chlortetracycline.

In the current study, the 0.15% acidifier and 0.15% acidifier + 0.03% oregano oil groups reduced F/G by 2.02% and 3.53%, respectively, compared to the colistin sulfate group, while decreasing diarrhea rates by 22.04% and 20.10%. These findings suggest that acidifiers alone or combined with oregano oil can effectively reduce F/G and diarrhea incidence. Giannenas [21] also reported positive effects of formic acid on piglet performance. Lu [22] found that 0.4% acidifier supplementation significantly increased ADG and crude protein digestibility while reducing F/G. Since F/G directly impacts farm profitability, the superior performance of the 0.15% acidifier + 0.03% oregano oil group is particularly noteworthy. Overall, when added to diets containing 75 mg/kg chlortetracycline + 10 mg/kg enramycin, both the 0.15% acidifier and 0.15% acidifier + 0.03% oregano oil treatments produced growth performance comparable to, or even better than, the colistin sulfate treatment, indicating their potential as effective replacements.

3.2 Impact on Caecal Microbial Populations

The intestinal microbiota plays a crucial role in animal health and growth. Early weaning disrupts microbial balance, and various stressors can trigger dysbiosis, leading to diarrhea [23-24]. The caecum harbors the largest and most diverse bacterial community due to its size and retention time. *Lactobacillus* and *Bifidobacterium* are beneficial bacteria, *Salmonella* is pathogenic, and *E. coli* is a facultative pathogen that can cause diarrhea during stress or immunosuppression [25]. While antibiotics inhibit specific microbes and improve diarrhea, they also affect gut pH. Acidifiers help restore microbial balance, potentially by reducing harmful bacteria [26]. Blomberg et al. [27] found that formic and lactic acids significantly reduced intestinal *E. coli* in piglets. Combined slow-release acidifier-antibiotic treatments have also proven more effective than either additive alone in reducing *E. coli* and increasing *Lactobacillus* populations [28].

Oregano oil's active components possess strong surface activity and lipid solubility, enabling rapid penetration of pathogenic cell membranes, causing content leakage and disrupting mitochondrial respiration, ultimately leading to bacterial death. It exhibits potent bactericidal effects against Gram-negative bacteria such as *E. coli* and *Salmonella* while significantly increasing beneficial bacteria

like *Bifidobacterium* and *Lactobacillus* [29-30], thereby reducing diarrhea and mortality rates [31].

Our results show that the colistin sulfate group significantly reduced caecal *Salmonella* compared to the 0.15% acidifier group, while the combined acidifier-oregano oil treatment significantly increased beneficial *Lactobacillus* and *Bifidobacterium* populations. This synergistic effect likely stems from oregano oil' s potent antimicrobial activity combined with the gut-modulating effects of acidifiers [15]. Overall, when added to diets containing 75 mg/kg chlortetracycline + 10 mg/kg enramycin, the 0.15% acidifier + 0.03% oregano oil treatment produced caecal microbial effects comparable to, or slightly better than, the colistin sulfate treatment, confirming that this combination can effectively replace colistin sulfate in piglet diets.

Conclusion

Based on diets supplemented with 75 mg/kg chlortetracycline + 10 mg/kg enramycin, the following conclusions can be drawn:

1. Replacement of 0.10% colistin sulfate with either 0.15% acidifier or 0.15% acidifier + 0.03% oregano oil improved production performance and reduced diarrhea rates in weaned piglets.
2. The 0.15% acidifier + 0.03% oregano oil combination increased beneficial caecal bacteria while reducing pathogenic bacteria, with effects similar to 0.10% colistin sulfate. Therefore, acidifier combined with oregano oil can serve as an effective replacement for colistin sulfate in weaned piglet diets.

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