# Effects of Antimicrobial Peptide Sublancin on Growth Performance, Nutrient Utilization, and Cecal Microbiota in Broiler Chickens (Postprint)

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## Abstract

Two experiments were conducted to investigate the effects of the antimicrobial peptide Sublancin on growth performance, nutrient utilization, and cecal microbiota in broiler chickens. Experiment 1: A total of 432 one-day-old Arbor Acres (AA) broiler roosters were randomly allocated to 4 groups (6 replicates per group with 18 birds per replicate), including a control group (fed a basal diet without antibiotics), an antibiotic group (fed the basal diet + 20 mg/kg colistin sulfate), a low-dose antimicrobial peptide group (fed the basal diet + 150 mg/kg Sublancin), and a high-dose antimicrobial peptide group (fed the basal diet + 300 mg/kg Sublancin). The experiment lasted for 42 days. On days 1, 21, and 42 of the experiment, broilers were weighed and feed consumption was recorded to calculate average daily feed intake, average daily gain, and feed conversion ratio. On days 21 and 42, one bird per replicate was randomly selected for collection of cecal digesta to analyze cecal microbiota. Experiment 2: A total of 288 one-day-old Arbor Acres broiler roosters were randomly divided into 3 groups (8 replicates per group with 12 birds per replicate), including a control group (fed a basal diet without antibiotics), an antibiotic group (fed the basal diet + 80 mg/kg chlortetracycline), and an antimicrobial peptide group (fed the basal diet + 300 mg/kg Sublancin). The experiment lasted for 28 days. On days 1 and 21 of the experiment, broilers were weighed and feed consumption was recorded to calculate average daily feed intake, average daily gain, and feed conversion ratio. Excreta were collected from days 19 to 21 to determine apparent nutrient metabolizability and nitrogen retention. On day 22, each broiler was orally administered 1 mL of Escherichia coli K88 suspension (10^9 CFU/mL), and on day 28, one bird per replicate was selected for collection of cecal digesta to analyze cecal microbiota. The results showed that compared with the control group, dietary supplementation with 300 mg/kg antimicrobial peptide Sublancin or 20 mg/kg colistin sulfate significantly improved average

daily gain and feed conversion ratio of broilers during the starter phase (1–21 d), grower phase (22–42 d), and the overall period (1–42 d) ($P<0.05$), and significantly reduced the numbers of Escherichia coli and total aerobic bacteria in the cecum on days 21 and 42 ($P<0.05$). There was no significant difference in growth performance between the 300 mg/kg Sublancin group and the antibiotic group ($P>0.05$). Compared with the control group, dietary supplementation with 300 mg/kg antimicrobial peptide Sublancin or 80 mg/kg chlortetracycline significantly improved apparent crude protein metabolizability and nitrogen retention rate ($P<0.05$). The nutrient apparent metabolizability and nitrogen retention rate of broilers in the antimicrobial peptide group were not significantly different from those in the antibiotic group ($P>0.05$). The nitrogen retention amount in the antimicrobial peptide group was significantly higher than that in the control and antibiotic groups ($P<0.05$). Compared with the control group, dietary supplementation with 300 mg/kg antimicrobial peptide Sublancin or 80 mg/kg chlortetracycline significantly reduced the number of Escherichia coli in the cecum of broilers challenged with E. coli K88 ($P<0.05$), with no significant difference between the antimicrobial peptide group and the antibiotic group ($P>0.05$). It was concluded that the antimicrobial peptide Sublancin has potential value as an antibiotic alternative in broiler diets. Dietary supplementation with 300 mg/kg antimicrobial peptide Sublancin can improve growth performance of broiler chickens by enhancing nutrient utilization and reducing the number of harmful intestinal bacteria.

## Full Text

# Effects of Antimicrobial Peptide Sublancin on Growth Performance, Nutrient Utilization and Cecal Microbiota of Broilers

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### Abstract

Two experiments were conducted to investigate the effects of the antimicrobial peptide sublancin on growth performance, nutrient utilization, and cecal microbiota in broilers.

**Experiment 1:** A total of 432 one-day-old Arbor Acres male broilers were ran-

domly allocated to four groups (six replicates per group, 18 birds per replicate). The groups consisted of a control group fed a basal diet without antibiotics, an antibiotic group fed the basal diet plus 20 mg/kg colistin sulfate, a low-dose antimicrobial peptide group fed the basal diet plus 150 mg/kg sublancin, and a high-dose antimicrobial peptide group fed the basal diet plus 300 mg/kg sublancin. The experiment lasted 42 days. Body weight and feed intake were recorded on days 1, 21, and 42 to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR). On days 21 and 42, one bird per replicate was randomly selected for collection of cecal digesta to analyze cecal microbiota.

**Experiment 2:** A total of 288 one-day-old Arbor Acres male broilers were randomly divided into three groups (eight replicates per group, 12 birds per replicate). The groups included a control group fed a basal diet without antibiotics, an antibiotic group fed the basal diet plus 80 mg/kg chlortetracycline, and an antimicrobial peptide group fed the basal diet plus 300 mg/kg sublancin. The experiment lasted 28 days. Body weight and feed intake were recorded on days 1 and 21 to calculate ADFI, ADG, and FCR. Feces and urine were collected from days 19 to 21 to determine apparent nutrient metabolic rate and nitrogen retention. On day 22, each broiler was orally administered 1 mL of *Escherichia coli* K88 suspension (10  CFU/mL). On day 28, one bird per replicate was selected for collection of cecal digesta to analyze cecal microbiota.

The results showed that, compared with the control group, dietary supplementation with 300 mg/kg sublancin or 20 mg/kg colistin sulfate significantly increased ADG and improved FCR during the starter phase (1–21 d), finisher phase (22–42 d), and overall period (1–42 d) ($P < 0.05$), while significantly reducing populations of *E. coli* and total aerobic bacteria in the cecum on days 21 and 42 ($P < 0.05$). No significant differences in growth performance were observed between the 300 mg/kg sublancin group and the antibiotic group ($P > 0.05$). In Experiment 2, dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline significantly increased the apparent metabolic rate of crude protein and nitrogen retention rate compared with the control group ($P < 0.05$). The nutrient apparent metabolic rate and nitrogen retention rate did not differ significantly between the antimicrobial peptide group and the antibiotic group ($P > 0.05$). However, nitrogen retention in the antimicrobial peptide group was significantly higher than in both the control and antibiotic groups ($P < 0.05$). Additionally, dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline significantly reduced cecal *E. coli* populations in broilers challenged with *E. coli* K88 ($P < 0.05$), with no significant difference between the antimicrobial peptide and antibiotic groups ($P > 0.05$).

These findings indicate that antimicrobial peptide sublancin has potential value as an antibiotic alternative in broiler diets. Dietary supplementation with 300 mg/kg sublancin can improve broiler growth performance by enhancing nutrient utilization and reducing populations of harmful intestinal bacteria.

**Keywords:** antimicrobial peptide sublancin; broilers; growth performance; in-

testinal microbiota; antibiotic alternative

## Introduction

Antibiotics have been widely used in animal production for over 60 years [1]. In large-scale pig and poultry farms, antibiotics are primarily employed for disease prevention, treatment, and growth promotion. According to a 2012 World Health Organization (WHO) report, more than 50% of global antibiotic production is used in food-producing animals, with approximately 90% utilized for growth promotion and feed efficiency improvement, yielding significant economic benefits for livestock production. However, irrational use and abuse of antibiotics in practice, combined with long-term administration, have led to the development of bacterial resistance or insensitivity. This antibiotic resistance issue has caused increasingly more antibiotics to become less effective or even ineffective, posing a major threat to animal health, food safety, and public human health [2-3]. Consequently, identifying alternatives to conventional antibiotics for animal production has become an urgent priority.

Antimicrobial peptides (AMPs) are biologically active small polypeptides produced by living organisms and widely distributed across the biological world. They serve as important components of natural immunity and host defense against infection [4-5]. AMPs exhibit broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, fungi, viruses, and protozoa. Most AMPs exert their antibacterial activity by disrupting bacterial cell membrane integrity, while some can penetrate cell membranes and bind to intracellular targets to inhibit bacterial growth. Their unique mechanisms make resistance development difficult, rendering them a hot research topic as antibiotic alternatives [6-7].

Sublancin is a bacteriostatic substance isolated by Hansen' s research team at the University of Maryland from the fermentation broth of *Bacillus subtilis* 168. It is a cationic peptide composed of 37 amino acids with two disulfide bonds, exhibiting remarkable stability across pH 1.5–9.5 and high temperature environments [8-10]. Sublancin demonstrates activity against Gram-positive bacteria, including *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Clostridium perfringens*, but shows no significant inhibitory effect on Gram-negative bacteria [11-12]. Paik et al. [13] proposed that sublancin acts on specific molecules involved in bacterial cell membrane synthesis, creating pores in the membrane to exert its antibacterial activity. Kouwen et al. [14] found that sublancin' s bacteriostatic activity is associated with bacterial mechanosensitive ion channels, suggesting it may inhibit closure of these channels, causing rapid efflux of intracellular contents and bacterial lysis or death. Wang et al. [11] reported that sublancin may inhibit bacterial division by suppressing bacterial energy metabolism. Current research on sublancin has primarily focused on its structure, expression regulation, bactericidal activity, and mechanisms. How-

ever, the effects of sublancin as a feed additive on broiler growth performance and nutrient utilization remain incompletely understood. Therefore, this study employed both healthy broilers and *E. coli*-infected broilers as models to investigate the effects of sublancin on growth performance, apparent nutrient metabolic rate, nitrogen retention, and cecal microbiota, aiming to provide experimental evidence for sublancin as an antibiotic alternative in animal production.

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## 1. Materials and Methods

### 1.1 Preparation of Antimicrobial Peptide Sublancin

Sublancin was prepared by the National Feed Engineering Technology Research Center. A novel recombinant plasmid was constructed and transformed into *Bacillus subtilis* W800 to achieve high-level expression of sublancin. The peptide was then purified using an AKTA purification system to obtain a lyophilized powder with purity up to 99.6%. The amino acid sequence is GLGKAQCAALWLQCASGGTIGCGGGAVACQNYRQFCR, with a relative molecular mass of approximately 3,879.8 u. The activity units of the lyophilized powder were determined through antibacterial activity assays to establish appropriate supplementation levels.

### 1.2 Experimental Design and Diets

This study utilized one-day-old healthy Arbor Acres (AA) male broilers with an average initial body weight of (41.90 ± 3.21) g. The basal diet was a corn-soybean meal type formulated according to China' s "Feeding Standard of Broilers" (2004). The composition and nutrient levels are presented in Table 1 . All diets were provided in mash form.

**Experiment 1:** A total of 432 one-day-old AA male broilers were randomly divided into four groups with six replicates per group and 18 birds per replicate. The control group received the basal diet without antibiotics, the antibiotic group received the basal diet plus 20 mg/kg colistin sulfate, the low-dose antimicrobial peptide group received the basal diet plus 150 mg/kg sublancin, and the high-dose antimicrobial peptide group received the basal diet plus 300 mg/kg sublancin. Broilers had ad libitum access to feed and water throughout the 42-day experiment, which was divided into two phases: starter (1–21 d) and finisher (22–42 d).

**Experiment 2:** A total of 288 one-day-old AA male broilers were randomly allocated to three groups with eight replicates per group and 12 birds per replicate. The control group received the basal diet without antibiotics, the antibiotic group received the basal diet plus 80 mg/kg chlortetracycline, and the antimicrobial peptide group received the basal diet plus 300 mg/kg sublancin. The supplementation level of sublancin was based on results from Experiment 1. Broilers had ad libitum access to feed and water during the 28-day experiment.

Days 1–21 constituted the normal rearing phase, and on day 22, all broilers were challenged with *E. coli* K88 by oral administration of 1 mL bacterial suspension (10  CFU/mL).

### 1.3 Management Practices

Broilers were housed in three-tier cages (90 cm × 40 cm) with nipple drinkers, allowing ad libitum access to feed and water. Temperature and humidity were computer-controlled, and lighting was manually adjusted. Room temperature was maintained at 33 °C for the first three days, then reduced by 3 °C weekly until reaching a constant 24 °C. Lighting was provided 24 hours daily with adequate ventilation. All broilers were vaccinated against Newcastle disease on days 7 and 28, and against infectious bursal disease on days 14 and 21. The experiment was conducted at the Livestock and Poultry Experimental Base of the Feed Efficiency and Safety Supervision, Inspection and Testing Center (Beijing), Ministry of Agriculture, with all procedures performed in accordance with animal welfare protocols and requirements of China Agricultural University.

### 1.4 Analytical Methods

**1.4.1 Experiment 1  Growth Performance Measurement:** On days 1, 21, and 42, broilers were weighed and feed consumption recorded to calculate ADFI, ADG, and FCR.

**Cecal Microbiota Analysis:** On days 21 and 42, one bird per replicate was randomly selected, euthanized, and cecal digesta collected, wrapped in gauze, and snap-frozen at -80 °C for subsequent analysis. Body weights of selected birds are shown in Table 2 .

Cecal digesta were thawed at room temperature, homogenized, and approximately 0.5 g of sample was dissolved in 4.5 mL sterile physiological saline, thoroughly mixed, and serially diluted to 10  . For lactobacillus cultivation, dilutions of $10^{3}$ to 10  were selected and 0.1 mL of diluted solution was inoculated into anaerobic roll tubes. For *E. coli* cultivation, dilutions of $10^{2}$ to 10  were selected and 0.2 mL was plated onto culture medium. For total aerobic bacteria, dilutions of 10  to 10  were selected and 0.2 mL was plated. All media were incubated at 37 °C for 24 hours (lactobacillus and *E. coli*) or 48 hours (total aerobic bacteria) before colony counting. Each dilution was plated in duplicate, and plates or roll tubes with 30–300 colonies were used for enumeration.

*E. coli* was cultured using MacConkey agar medium with the following composition and pH: peptone, 20.0 g/L; lactose, 10.0 g/L; bile salts, 5.0 g/L; sodium chloride, 5.0 g/L; neutral red, 0.075 g/L; agar, 20.0 g/L; pH 7.4 ± 0.2. Lactobacillus was cultured using MRS agar medium, and total aerobic bacteria using plate count agar medium. All media were purchased from OXOID, UK.

**1.4.2 Experiment 2  Growth Performance Measurement:** On days 1 and 21, broilers were weighed and feed consumption recorded to calculate ADFI,

ADG, and FCR.

**Apparent Nutrient Metabolic Rate and Nitrogen Retention:** Feces and urine were collected from days 19–21, mixed thoroughly, weighed, dried at 60 °C for 48 hours, and ground to pass through a 40-mesh sieve for routine nutrient analysis. Dry matter and crude protein contents in feed and excreta samples were determined according to AOAC (2006) standard methods. Gross energy was measured using an oxygen bomb calorimeter (Parr 1281, USA) to calculate apparent metabolic rates of various nutrients. Feed intake from days 19–21 and excreta weight were recorded. Nitrogen content in feed and excreta was measured to calculate nitrogen retention amount and rate.

Nitrogen retention (g/d) = Nitrogen intake - Nitrogen excretion in feces and urine
Nitrogen retention rate (%) = 100 × Nitrogen retention amount / Nitrogen intake

**Cecal Microbiota Analysis:** On day 28, one bird per replicate was randomly selected, euthanized, and cecal digesta collected, wrapped in gauze, and snap-frozen at -80 °C for analysis. Body weights of selected birds are shown in Table 2. The cultivation and enumeration methods for lactobacillus and *E. coli* in cecal digesta were identical to those described in section 1.4.1.2.

### 1.5 Statistical Analysis

Data were analyzed using the ANOVA procedure of the General Linear Model (GLM) in SAS 9.2, with replicate as the experimental unit. When significant differences among groups were detected, means were compared using Student-Newman-Keuls test. Statistical significance was declared at $P < 0.05$.

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## 2. Results

### 2.1 Experiment 1

**2.1.1 Growth Performance** As shown in Table 3 , during the starter phase (1–21 d), dietary supplementation with 150 or 300 mg/kg sublancin or 20 mg/kg colistin sulfate significantly increased ADG and improved FCR compared with the control group ($P < 0.05$). No significant differences in ADG or FCR were observed between the antibiotic group and antimicrobial peptide groups ($P > 0.05$). During the finisher phase (22–42 d), dietary supplementation with 300 mg/kg sublancin significantly increased ADG compared with the control group ($P < 0.05$). Compared with the 150 mg/kg sublancin group and control group, supplementation with 300 mg/kg sublancin or 20 mg/kg colistin sulfate significantly improved FCR ($P < 0.05$). Throughout the entire experimental period (1–42 d), dietary supplementation with 300 mg/kg sublancin significantly increased ADG and improved FCR compared with the control group ($P < 0.05$).

No significant differences in growth performance were found between the antibiotic group and antimicrobial peptide groups ($P > 0.05$). Dietary supplementation with sublancin or colistin sulfate had no significant effect on ADFI at any stage ($P > 0.05$).

**2.1.2 Cecal Microbiota** As presented in Table 4 , dietary supplementation with 150 or 300 mg/kg sublancin or 20 mg/kg colistin sulfate significantly reduced populations of *E. coli* and total aerobic bacteria in the cecum on both days 21 and 42 compared with the control group ($P < 0.05$). No significant differences in these parameters were observed between the 150 and 300 mg/kg sublancin groups ($P > 0.05$). Compared with the 150 mg/kg sublancin group, dietary supplementation with 20 mg/kg colistin sulfate significantly decreased cecal *E. coli* and total aerobic bacteria populations on days 21 and 42 ($P < 0.05$). Dietary supplementation with sublancin or colistin sulfate had no significant effect on cecal lactobacillus populations on days 21 or 42 ($P > 0.05$).

### 2.2 Experiment 2

**2.2.1 Growth Performance** As shown in Table 5 , dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline significantly increased ADG and improved FCR compared with the control group ($P < 0.05$), but had no significant effect on ADFI ($P > 0.05$). No significant differences in growth performance were observed between the antimicrobial peptide group and antibiotic group ($P > 0.05$).

**2.2.2 Apparent Nutrient Metabolic Rate and Nitrogen Retention** As presented in Table 6 , dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline had no significant effect on apparent metabolic rates of dry matter or energy ($P > 0.05$), but significantly increased the apparent metabolic rate of crude protein ($P < 0.05$). No significant differences in nutrient apparent metabolic rates were found between the antimicrobial peptide group and antibiotic group ($P > 0.05$). Nitrogen retention in the antimicrobial peptide group was significantly higher than in both the control and antibiotic groups ($P < 0.05$), reaching 8.49 g/d. Compared with the control group, dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline significantly increased nitrogen retention rate ($P < 0.05$), though no significant difference was observed between the antimicrobial peptide and antibiotic groups ($P > 0.05$).

**2.2.3 Cecal Microbiota** As shown in Table 7 , dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline significantly reduced cecal *E. coli* populations in broilers challenged with *E. coli* K88 compared with the control group ($P < 0.05$). No significant difference in cecal *E. coli* populations was observed between the antimicrobial peptide group and antibiotic group ($P > 0.05$). Dietary supplementation with sublancin or chlortetracycline had no

significant effect on cecal lactobacillus populations in *E. coli* K88-challenged broilers ($P > 0.05$).

## 3. Discussion

The increasing severity of antibiotic resistance has made research into antibiotic alternatives increasingly important [15]. Antimicrobial peptides possess unique biological characteristics and antibacterial mechanisms that show potential to revolutionize clinical medicine, pharmacology, food processing, and agriculture as antibiotic replacements [16-18]. Bals et al. [19] demonstrated that antimicrobial peptides can promote animal growth and enhance disease resistance. Wen et al. [20] found that adding antimicrobial peptides to weaned piglet diets produced antidiarrheal effects comparable to antibiotics, with appropriate doses showing better growth-promoting effects than antibiotics. Chen et al. [21] reported that adding cecropin AD-yeast preparation to duck diets significantly enhanced serum metabolic hormone activity, increased insulin-like growth factor-1 (IGF-1) concentration, accelerated nutrient synthesis, significantly reduced blood urea nitrogen concentration, decreased nitrogen excretion, and produced no adverse effects on immune organs. Wang et al. [22] showed that adding antibacterial protein to feed significantly improved daily growth rate, relative weight gain, feed conversion ratio, survival rate, and disease resistance in *Litopenaeus vannamei*. These findings demonstrate the potential of antimicrobial peptides as novel feed additives to replace antibiotics.

Our laboratory' s previous research has demonstrated that sublancin possesses both in vitro and in vivo antibacterial activity. Oral administration of sublancin to mice was found to induce mixed Th1 and Th2 immune responses in ovalbumin (OVA)-immunized mice, enhancing both humoral and cellular immune responses [23]. However, few reports have examined the role of sublancin as an antibiotic replacement in broiler production and disease control. Building on our previous research, this study used broilers as experimental models to further investigate the effects of sublancin on growth performance and intestinal microbiota. Based on these results, we subsequently administered *E. coli* challenge to observe the effects of dietary sublancin supplementation on growth performance, nutrient utilization, and intestinal microbiota, aiming to provide a theoretical basis for sublancin as an antibiotic alternative in feed additives.

Colistin sulfate and chlortetracycline have long been used as growth promoters in livestock production [17,24] with good efficacy. However, due to bacterial resistance development induced by colistin sulfate, the Ministry of Agriculture issued a ban on its use. Consequently, researchers have sought new alternatives. In this study, colistin sulfate and chlortetracycline were selected as positive controls to investigate whether sublancin could serve as a novel additive to replace these antibiotics in livestock production. The results demonstrated that dietary sublancin supplementation improved broiler growth performance, con-

sistent with findings by Wang et al. [25] and Lu et al. [26] that antimicrobial peptide supplementation significantly improved production efficiency in broilers. Furthermore, Choi et al. [27] reported that dietary supplementation with synthetic antimicrobial peptide-p5 increased broiler ADG and FCR. This study also found that sublancin supplementation had no significant effect on ADFI, consistent with results from Bao et al. [28] and Ohh et al. [29]. The observation that sublancin and antibiotics (colistin sulfate and chlortetracycline) improved growth performance to a similar extent suggests that sublancin has potential as an antibiotic alternative in broiler diets, a finding consistent with Ohh et al. [29] and Choi et al. [27]. The growth-promoting mechanisms of antibiotics include inhibiting pathogens, reducing subclinical pathogen infection, and decreasing nutrient competition with gut microbiota through modulation of microbial composition [16-17,30]. In this study, the improved growth performance observed with sublancin supplementation may be attributed to enhanced nutrient absorption and utilization and reduced populations of harmful intestinal microorganisms.

In Experiment 1, dietary supplementation with 300 mg/kg sublancin significantly improved broiler growth performance. In Experiment 2, this same dose significantly increased nitrogen retention. Ohh et al. [29] and Choi et al. [27,31] similarly reported that antimicrobial peptide supplementation in broiler diets increased nitrogen retention and consequently improved growth performance. In this study, sublancin supplementation had no significant effect on apparent metabolic rates of dry matter or energy, consistent with findings from Ohh et al. [29] and Choi et al. [27,31]. Analysis of cecal microbiota revealed that dietary supplementation with 300 mg/kg sublancin or 20 mg/kg colistin sulfate significantly reduced cecal *E. coli* populations in healthy broilers. Under *E. coli* K88 challenge, dietary supplementation with 300 mg/kg sublancin or 80 mg/kg chlortetracycline significantly reduced cecal *E. coli* populations, similar to results reported by Wu et al. [24]. These findings indicate that sublancin may inhibit proliferation of harmful intestinal bacteria, thereby enhancing broiler resistance to infection.

Beyond direct antibacterial effects, antimicrobial peptides can indirectly modulate immune function by inhibiting microbial proliferation, including recruitment of immune cells, thereby regulating inflammatory responses and reducing infection severity. Our laboratory' s previous research demonstrated that sublancin reduced intestinal injury and decreased mortality in *S. aureus*-infected mice by downregulating intestinal nuclear factor- B (NF- B) and inducible nitric oxide synthase (iNOS) expression [11,30]. Additionally, sublancin reduced levels of pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- (TNF- ) in mouse ileum [30]. However, whether sublancin modulates host immune function and whether its effects on cecal microbiota are related to immune function require further investigation.

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## 4. Conclusion

Antimicrobial peptide sublancin demonstrates potential value as an antibiotic alternative in broiler diets. Dietary supplementation with 300 mg/kg sublancin can improve broiler growth performance by enhancing nutrient utilization and reducing populations of harmful intestinal bacteria.

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