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Effects of florfenicol on body weight, intestinal inflammatory response, intestinal fluid metabolism and microorganisms in broilers

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ABSTRACT

Importance: Although antibiotics can prevent and treat diseases, their overuse can be harmful. Despite this, the effects of orally administered florfenicol on intestinal health in broilers are unclear.
Objective: This study examined oral florfenicol's effects on intestinal function in broilers to guide its clinical use.
Methods: One hundred and twenty Arbor Acres broilers (8-d-old) were divided randomly into the control (N) and florfenicol groups (F). The broilers in group F were fed 100 mg/kg body weight florfenicol for seven days. On day 15, the broilers were euthanized and sampled to analyze fluid metabolism-related genes and proteins, jejunal morphological and microbiota.
Results: The results revealed a decrease in body weight and an increase in diarrhea rate in broilers in group F compared to group N. The villus length and villus length/crypt depth (V/C) of the jejunum were lower in group F than in group N, whereas the crypt depth was higher. The levels of tumor necrosis factor α, Toll-like receptor 4, lipopolysaccharide, nuclear factor kappa-B, interleukin (IL)-6 and IL-10 in group F were higher in group F than in group N, whereas the levels of cyclic adenosine monophosphate and aquaporins (AQP) 4 and AQP5 were lower. *Proteobacteria* were more abundant in group F than in group N, whereas *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were less abundant.
Conclusions and Relevance: Oral florfenicol might adversely affect the intestinal mucosa, intestinal mucosal immune system, intestinal microbiota balance, and water metabolism in broilers. This study provides a theoretical basis for the rational use of florfenicol.

Keywords: Florfenicol; broiler; inflammation; water metabolism; intestinal microbiota

INTRODUCTION

Antibiotics are natural or synthetic compounds that can destroy microbial pathogens or inhibit their growth and are used widely in poultry production [1]. Florfenicol is a broad-spectrum veterinary chloramphenicol antibiotic that inhibits many types of Gram-positive and Gram-negative bacteria and is characterized by its wide distribution in the body and easy absorption [2]. Florfenicol, a derivative of chloramphenicol, is safer and has a wider

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Conflict of Interest
The authors declare no conflicts of interest.

Data Availability Statement
The datasets used during the current study are available from the corresponding author upon reasonable request.

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antibacterial spectrum than chloramphenicol. Florfenicol has no potential risk of causing aplastic anemia [3] and has lower bacterial resistance than chloramphenicol, making it the best choice for the treatment and control of infectious diseases in poultry [4].

In China, the rate of florfenicol use in broiler chicken farms is 78%, ranking first among commonly used antibiotics [5]. The irrational application of antibiotics can also have a negative impact on animal health. Florfenicol has been reported to reduce chicken growth and the kidney index [6]. Low florfenicol doses significantly increased the body weight of Oreochromis niloticus [7] and rats [8]. Florfenicol elevated the hepatic inflammatory factor levels [9] and induced intestinal inflammatory responses in animals [8]. Recent studies have reported that the oral administration of 100 mg/kg·bw florfenicol in mice can have the following effects: damage the jejunal mucosa; regulate the expression of immune factors, such as interleukin (IL)-17, IL-22; reduce the abundance of beneficial bacteria in the jejunum mucosa; affect the intestinal barrier function of mice [10], which can lead to slower growth making animals more susceptible to infections.

In animals, florfenicol is absorbed mainly through the gastrointestinal tract. It can denature the vagus nerve and reduce the release of acetylcholine, resulting in slow gastrointestinal peristalsis and abnormal digestive and absorption function [11]. In early clinical studies, florfenicol was administered to chicks at 30 mg/kg for seven days, resulting in unsteady standing and drained loose stools [12]. Improper florfenicol use seriously affects animals, but most studies on intestinal microbes have been conducted on mice [10,13]. Therefore, understanding the effects of florfenicol on the intestinal structure, function, and immune response of broilers is important.

The effects of florfenicol on organisms such as swine [14], fish [15], cattle [16], and rabbits [17] have been studied. On the other hand, the digestive and immune systems of broilers differ from those of other animals. Hence, the effects of florfenicol on broilers are unclear. This study hypothesized that florfenicol disrupts the intestinal flora balance in broilers, damages the intestinal mucosa, disrupts intestinal water metabolism, stimulates the body to produce immunity passively, and indirectly affects broiler growth. Therefore, this study examined the effects of the continuous feeding of florfenicol to broilers on body weight, intestinal inflammatory response, intestinal water metabolism, and microbial balance.

METHODS

Animals

All experimental procedures were approved by the Animal Experimental Ethics Committee of Shanxi Agricultural University (approval number SXAU-EAW-2019Ab. XJ. 003020181). One hundred twenty-one-day-old Arbor Acres broilers were obtained from Shanxi Daxiang Agro-Livestock Group Co., Ltd. (China). They were provided access to feed and water *ad libitum* for seven days via nipple drinkers and divided randomly into two groups: control group (N), normal water was given daily, and a florfenicol group (F), in which 100 mg/kg body weight florfenicol (purity: 20%, Henan Baiyun Mugang Biotechnology Co., Ltd, China) was added to their drinking water. Each group consisted of 60 broilers, with five broilers per cage. These broilers were fed a diet (Feed code Q/140702, DXY010-2016) manufactured by Shanxi Daxiang Agro-Livestock Group Co., Ltd. (China). The primary ingredients of the experimental diets were corn, soybean meal, calcium hydrogen phosphate, rock flour, sodium chloride,

copper sulfate, ferrous sulfate, zinc sulfate, manganese sulfate, vitamins, L-lysine sulfate, DL-methionine, and phytase, which contained crude protein (min 21%), crude fiber (max 5%), crude ash (max 7%), Ca (0.6%–1.5%), P (min 0.45%), sodium chloride (0.6%–0.8%), methionine (min 0.37%), and moisture (max 14%). The broilers were maintained for 23 h under fluorescent light and 1 h in the dark throughout the study period (8 to 14 days of age). The room temperature was initially set at 33°C, gradually decreasing to reach 25°C at 15 days of age.

After fasting for 12 h at eight days of age, broilers in each group were administered a 3% D-xylose solution (Shanghai Yuanye Biotechnology Co., Ltd., China) at a dose of 20 mL/kg body weight. Blood was collected from a vein under the wing of the broilers at 10 days of age and centrifuged to obtain serum. The serum D-xylose content in the samples was measured using the resorcinol method.

Sample collection

The broilers were weighed, euthanized with nitrogen, and subjected to cervical dislocation. The spleen, kidneys, and lungs were removed immediately and weighed. The organ index was measured as the organ weight (mg) per body weight (g). A 3-cm long section of mid-jejunum was soaked in 4% paraformaldehyde, and another 3-cm long section of jejunal tissue was wrapped in tin foil and stored at −80°C for testing. The jejunum contents were placed in a sterile tube. The weight was recorded, and the contents were diluted with phosphate buffer to a 1:9 ratio (weight: volume), mixed, and tested for the microbial content.

The number of broilers with diarrhea was recorded and calculated using the following equation: Diarrhea Rate = Number of Broilers With Diarrhea/Total Number of Broiler Chickens in the Group ×100%.

Histological analysis

The jejunum was fixed with 4% paraformaldehyde for 48 h, cut into 5 μm sections, and stained with hematoxylin and eosin using standard protocols [18]. The villus length and crypt depth were measured using Image Pro Plus software (version 7.0).

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

The total RNA from the jejunum was extracted using TRIzol reagent (Thermo Scientific Inc., USA). cDNA was synthesized using the Takara Reverse Transcription Kit (Takara Biological Co. Ltd., China) according to the manufacturer's instructions. The RNA and cDNA concentration and purity were quantified using an ND-2000 UV-vis spectrophotometer (Thermo Scientific Inc., USA). Target gene expression was detected using SYBR Premix Ex Taq II. qRT-PCR was performed on mRNA using an ABI 7500 system (Applied Biosystems, USA). **Tables 1** and **2** list the qRT-PCR system and primer sequences, respectively. β-actin was

Table 1. Quantitative real-time polymerase chain reaction system

Regent	Amount
SYBR premix EX Taq II 2×	12.5 μL
Forward primer (10 μM)	1 μL
Reverse primer (10 μM)	1 μL
cDNA	2 μL
RNase free dH_2O	8.5 μL
Total	25 μL

The reaction conditions were as follows: pre-denaturation at 95°C for 30 sec, denaturation at 95°C for 5 sec, annealing temperature as shown in **Table 2**, 40 cycles; extension at 72°C for 30 sec.

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Table 2. Primers sequence used for quantitative real-time polymerase chain reaction

Gene	Gene ID	Primers sequence	Product size	Tm
β-actin	396526	F: ATGAAGCCCAGAGCAAAAGA R: GGGGTGTGTTGAAGGTCTCAAA	223 bp	55
TLR4	417241	F: AGTCTGAAATTGCTGAGCTCAAAT R: GCGACGTTAAGCCATGGAAG	190 bp	53.3
TNF-α	374125	F: TGTGTATGTGCAGCAACCCGTAGT R: GGCATTGCAATTTGGACAGAAGT	229 bp	58.5

TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor α.

used as an internal reference gene. The transcription level of the target genes was evaluated using the $2^{-\Delta\Delta Ct}$ method.

Protein expression analysis

The proteins in the jejunum of the broilers were extracted using RIPA Lysis Buffer containing phenylmethanesulfonyl fluoride (Applygen Technologies Inc., China). The samples were centrifuged at 10,000 ×g at 4°C for 10 min. The supernatant was extracted, placed into Eppendorf tubes, and stored at −80°C. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a polyvinylidene fluoride membrane, and blocked in 5% skim milk at room temperature (23°C ± 2°C) for 2 h. The samples were incubated with the primary antibodies against aquaporins 4 (AQP4, Cat No. 16473-1-AP, Proteintech Group, Inc., China) and AQP5 (Cat No. 20334-1-AP, Proteintech Group, Inc., China), which had been diluted with Tris-HCl and Tween (TBST) at a 1:700 ratio at 4°C overnight. The membrane was washed three times with TBST and incubated with horseradish peroxidase labeled secondary antibody (Cat No. PR30009, Proteintech Group, Inc., China) at 37°C for 1 h. The membrane was washed three times, and the protein bands were analyzed using Image J software (NIH, USA).

Lipopolysaccharide (LPS), cyclic adenosine monophosphate (cAMP), nuclear factor kappa-B (NF-κB), IL-6, and IL-10 in the jejunum of the broilers were detected using an ELISA kit (Enzyme-linked Biotechnology, China).

Microbial diversity analysis

Bacterial genomic DNA was isolated and extracted from the jejunal contents using a DNA Isolation Kit from MOBIO (China). The concentration and purity of the DNA were detected using an ND 2000 UV-vis spectrophotometer. The hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified with primers 338F(5'-ACTCCTACGGGGAGGCAGCAG-3') and 806R(5'-GACTACHVGGGTWTCTAAT-3'). The PCR amplification was performed as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 10 min, and ending at 4°C. Pyrosequencing of the PCR products was performed using an Illumina MiSeq platform (Majorbio Bio-Pharm Technology Co., Ltd., China). Operational taxonomic units (OTUs) were clustered at a 97% similarity cutoff using UPARSE (version 7.10). The taxonomy of the resulting OUT was analyzed using an RDP Classifier version 2.2.5 against the 16S rRNA database with a confidence threshold of 0.7. The Shannon, Chao, Simpson, Ace, species similarity, community composition, and Principal Component Analysis (PCA) were performed using a *t*-test and Anoism test.

Statistical analysis

The data were analyzed using GraphPad Prism software (version 9.0; GraphPad Software Inc., USA) with one-way ANOVA to compare the differences between the groups. The results are expressed as the mean ± SD.

The values of $p < 0.01$ and p values < 0.05 were considered significant.

RESULTS

D-xylose content, body weight, organ index, and diarrhea rate

The serum D-xylose content, body weight, and diarrhea rate of the chickens were significantly lower in group F than in group N, as shown in **Fig. 1** ($p = 0.039$, $p = 0.031$, $p < 0.001$, respectively). The spleen, kidney, and lung indices were similar in the two groups ($p = 0.17$; $p = 0.58$; $p = 0.85$).

Pathological changes in jejunum

Fig. 2 shows the pathological changes in the jejunum of broilers. The villi of the jejunum in group F were atrophic and fractured, and the mucosal layer structure was disordered compared to group N (**Fig. 2A**). The villus length and villus length/crypt depth (V/C) were significantly lower in group F than in group N ($p = 0.0074$ and $p = 0.044$, respectively), and the crypt depth was significantly higher ($p = 0.0079$; **Fig. 2B**).

Changes of inflammation-related factors and AQPs in jejunum

Fig. 3 presents the changes in gene and protein expression between groups N and F. The relative mRNA expression levels of Toll-like receptor 4 (TLR4) and tumor necrosis factor α (TNF-α) were significantly higher in group F than in group N ($p = 0.019$; $p = 0.023$; **Fig. 3A**). The cAMP levels in the jejunum of the broilers in group N were higher than in group F ($p = 0.0032$), whereas the LPS levels were significantly lower ($p = 0.041$; **Fig. 3B**). The levels of NF-κB, IL-6, and IL-10 were significantly higher in group F than in group N ($p < 0.001$,

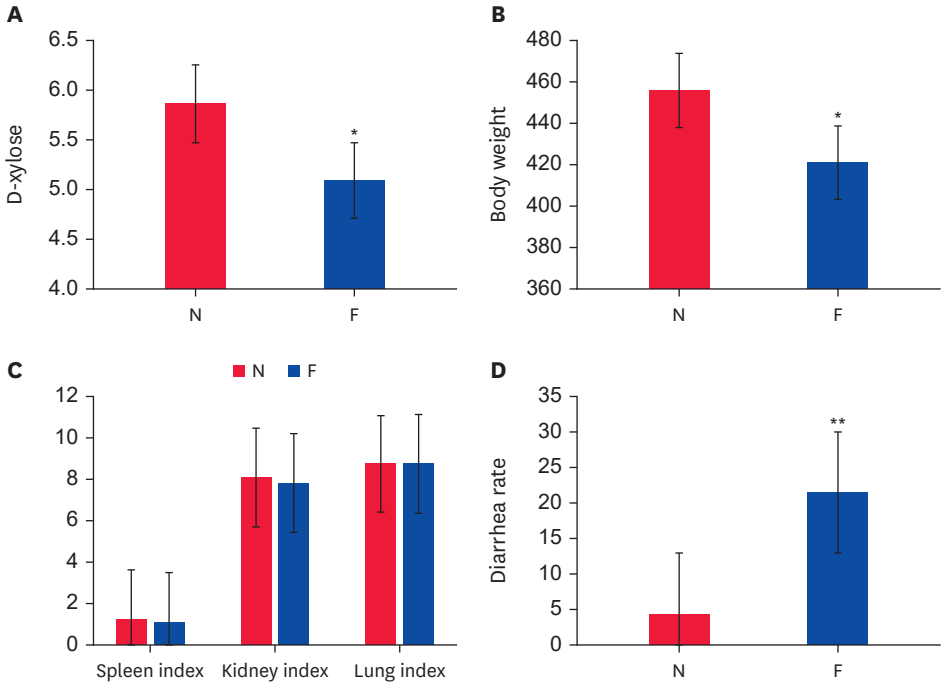

Fig. 1. Effects of florfenicol on the D-xylose content (A), body weight (B), organ index (C), and diarrhea rate (D). N, control group; F, florfenicol group.
*$p < 0.05$; **$p < 0.01$.

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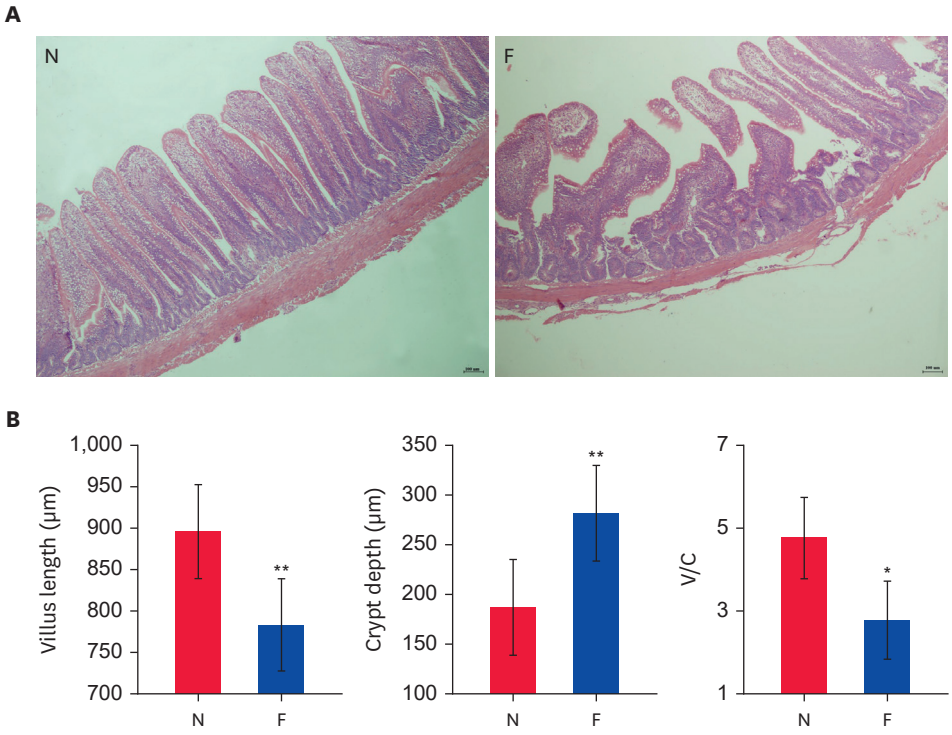

Fig. 2. Effects of florfenicol on the morphological structure of the jejunum in broilers. (A) Hematoxylin and eosin staining of jejunum tissue (Scale bar, 100 μm). (B) Changes in the jejunum villus length, crypt depth, and V/C. Scale bars: 100 μm.
N, control group; F, florfenicol group.
*$p < 0.05$; **$p < 0.01$.

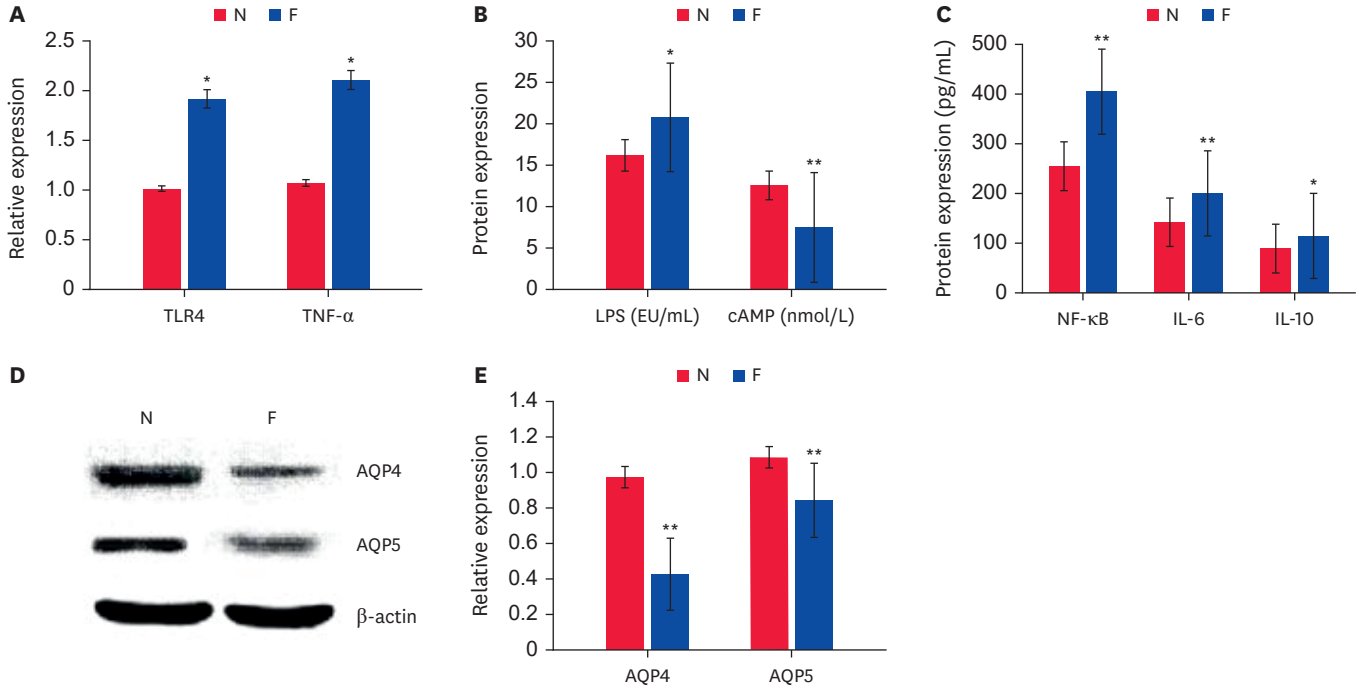

Fig. 3. Effects of florfenicol on gene and protein expression in jejunum of broilers. (A) Transcription of TLR4 and TNF-α. (B, C) Expression of cAMP, LPS, NF-κB, IL-6, and IL-10 measured by ELISA. (D) Expression of AQP4 and AQP5 measured by Western blot. (E) Relative expression of AQP4 and AQP5 normalized to β-actin.
N, control group; F, florfenicol group; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; cAMP, cyclic adenosine monophosphate; NF-κB, nuclear factor kappa-B; IL, interleukin; AQP, aquaporin.
*$p < 0.05$; **$p < 0.01$.

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$p = 0.0024$, $p = 0.048$, respectively; **Fig. 3C**). Compared to group N, the levels of AQP4 and AQP5 expression were significantly lower in group F than in group N ($p < 0.001$ and $p = 0.0033$, respectively; **Fig. 3D and E**).

Changes of microbial diversity in jejunum

The hypervariable V3-V4 region of the jejunal bacterial 16S rRNA gene was assessed; 175,818 tags were obtained from all samples, excluding low-quality sequences, with an average of 87909 tags in each group. The bacteria of the jejunal contents had a wide range on the X-axis, high species abundance, and a flat curve, suggesting that the species distribution of each sample was uniform and could be used for subsequent analysis (**Fig. 4A and B**).

The ACE, Chao, and Shannon indices were significantly lower in group F than in group N ($p = 0.048$; $p < 0.001$; $p = 0.0069$), whereas the Simpson index was significantly higher ($p = 0.039$; **Fig. 4C and D**). PCA analysis revealed significant differences in the gut microbiota composition between groups N and F (**Fig. 4E**).

Relative quantitative analysis of each sample was conducted using Quantity One software. *Proteobacteria*, *Firmicutes*, *Actinobacteria,* and *Bacteroidetes* were the dominant phyla at the phylum in the two groups. The *Proteobacteria* in the jejunum of broilers was significantly more abundant in group F than in group N ($p < 0.001$), whereas *Firmicutes*, *Actinobacteria,* and *Bacteroidetes* were significantly less abundant (*p*-values were all less than 0.001; **Fig. 4F**).

At the genus level, the main dominant genera were *Escherichia Shigella*, *Lactobacillus*, *Kitasatospora,* and *Burkholderia Paraburkholderia*. *Escherichia Shigella* was significantly more abundant in group F than in group N ($p < 0.001$). In contrast, *Lactobacillus*, *Bacillus,* and *Burkholderia Paraburkholderia* were significantly less abundant in group F than in group N ($p < 0.001$; **Fig. 4G**).

DISCUSSION

With the promotion of large-scale farming, antibiotics are used widely as feed additives to prevent and treat bacterial diseases. In actual production processes, however, the misuse of antibiotics by farmers has become common [19]. Research on the recommended doses or higher doses of florfenicol in chickens has focused on the immune response. A previous study reported that administering florfenicol at 100 mg/kg body weight may cause immunosuppression in chickens [20]. In contrast, the recommended dose (0.15 g/L, drinking water) of florfenicol can inhibit the growth performance of broilers without causing significant damage to their immune function [21]. The impact of the unreasonable use of florfenicol on the intestinal function was rarely reported. In this experiment, the broilers were administered florfenicol at a dose of 100 mg/kg body weight for seven days to explore the effect of florfenicol on intestinal function.

The small intestine is the primary site for the digestion and absorption of nutrients and water in animals, and the intestinal mucosa is an important barrier ensuring the animal's internal environment is not damaged [22]. The villus length and crypt depth can reflect the digestion and absorption abilities of the intestine, and the V/C ratio is an indicator commonly used to measure digestion and absorption [23]. In these experiments, florfenicol supplementation resulted in significant weight loss and an increased rate of diarrhea in broilers, which may be due to a disruption of the villus length and V/C, leading to absorption dysfunction in

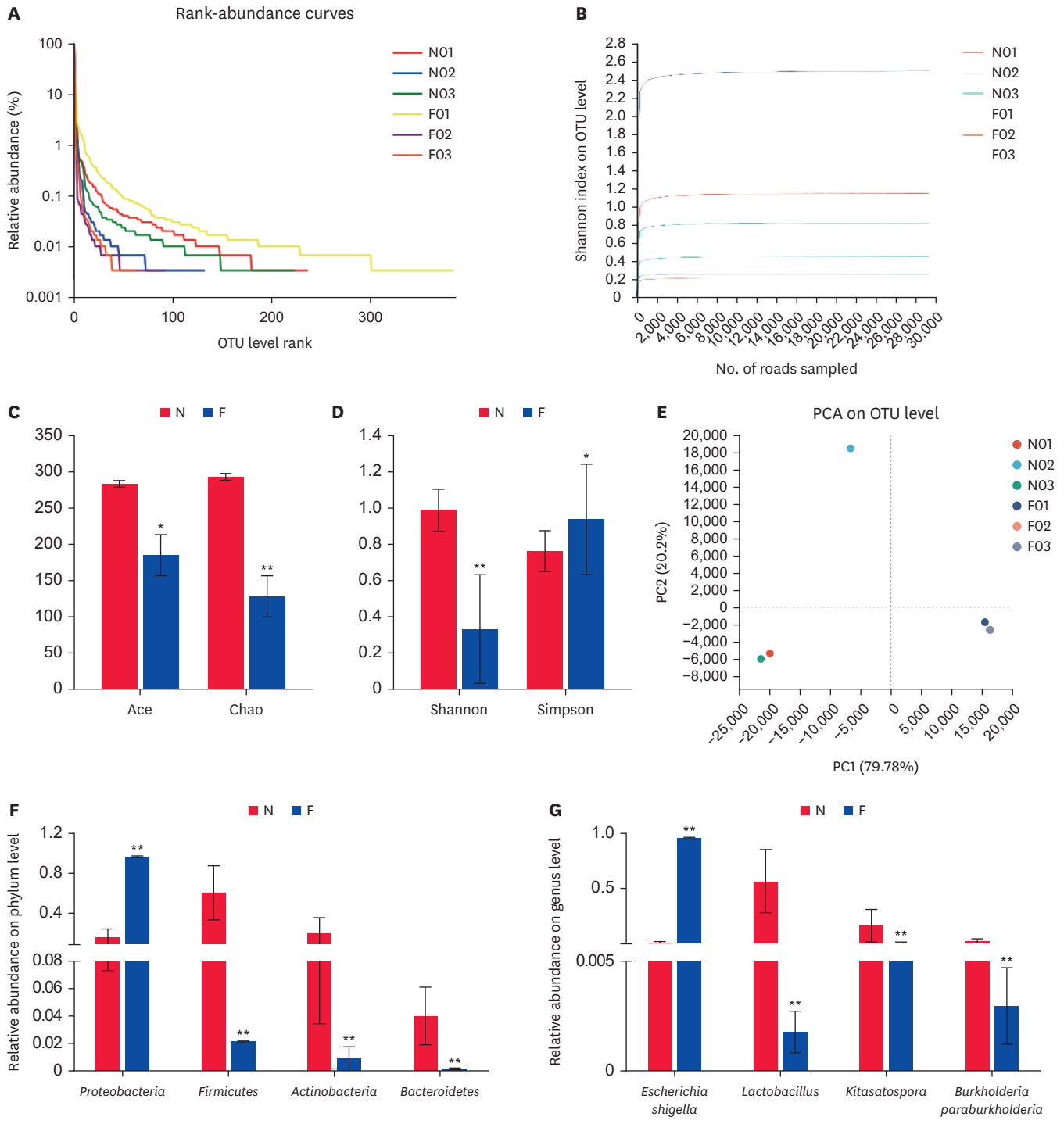

Fig. 4. Structure and diversity of the gut microbiota in groups N and F. (A, B) Rank-abundance curves and Shannon index on OTU level. (C, D) ACE, Chao, Shannon and Simpson indices in each group. (E) PCA on the OTU level. (F, G) Comparison of the bacterial OTUs at the phylum and genus level between the two groups. N, control group; F, florfenicol group; OUT, operational taxonomic unit.
$^{*}p < 0.05$; $^{**}p < 0.01$.

the intestinal epithelium, which causes diarrhea, reduces nutrient utilization in broilers, and retards growth. The serum D-xylose content is considered an indicator of intestinal absorption disorders. D-xylose is not metabolized by the liver but is absorbed through the

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small intestine and excreted through the kidneys [24]. The decrease in the serum D-xylose content of the broiler chickens in group F indirectly suggests that the intestinal absorption of nutrients and water is impaired.

The kidney is an important organ for water and fluid transport, and the spleen is an important immune organ in poultry. The immune system cannot function normally when the spleen is damaged [25]. Florfenicol is associated with a short period of anorexia, reduced water intake, and diarrhea and can affect the renal function of organisms [26]. Florfenicol has been reported to reduce the spleen index significantly in mice [27]. Severe diarrhea occurs in 70-d-old pigs when they are administered 120 mg/kg florfenicol orally for seven days and has a damaging effect on the kidneys [28]. In this study, the spleen, kidney, and lung indices of the broilers were similar in groups F and N. This may be due to the short-term addition of florfenicol, which mainly impairs the intestinal function and destroys the intestinal mucosal immune system, causing less damage to the spleen, kidneys, and lungs.

The long-term or high-dose application of antibiotics can cause an imbalance in the gut microbiota, damage the mucosal immune system, increase the number and functions of intestinal intraepithelial lymphocytes, and release large quantities of inflammatory factors [29]. The broilers in group F showed a higher LPS level than group N, possibly due to florfenicol disrupting the intestinal microbial balance, leading to the production of LPS by numerous harmful bacteria. LPS, a major bacterial toxin, can directly stimulate the proliferation of B cells to mediate humoral immunity, cause the overexpression of inflammatory factors, and disrupt the intestinal mucosal barrier, causing diarrhea [30].

LPS is also a ligand that binds to TLR4, phosphorylates p65 through the junction protein, and activates the NF-κB signal, causing TNF-α and IL-6 overexpression. The diversity of the bacterial community is lower when NF-κB expression is higher, and the damage to the intestinal mucosa is more severe, resulting in a significant decrease in intestinal immune function [31]. In the present study, the elevated jejunal TLR4 and NF-κB levels in group F may be because florfenicol stimulates the intestinal barrier and affects intestinal microbial homeostasis, leading to an inflammatory response in broilers, which is involved in the development of diarrhea.

TNF-α and IL-6 participate in the growth and differentiation of various tissues and cells and are the basic cytokines regulating the immune function in the inflammatory reaction of the body. TNF-α and IL-6 overexpression will damage cells and tissues [32], whereas TNF-α inflammatory processes characterized by increased inflammation can promote IL-6 and IL-10 secretion [33]. Hence, the overproduction of these pro-inflammatory cytokines, as well as certain anti-inflammatory cytokines like IL-10, can be detrimental. The serum IL-10 content was significantly higher when chickens were fed florfenicol at 100 mg/kg body weight for seven days than in the control group, suggesting that florfenicol may cause immunosuppression in chickens [20]. In this study, the TNF-α, IL-6, and IL-10 levels were significantly higher in the jejunum of the broilers in group F than in group N, which was consistent with previous studies [10, 20], possibly due to the host's attempt to counteract the exacerbated pro-inflammatory response by boosting the production of anti-inflammatory cytokines, such as IL-10.

AQPs, which are water carriers, are distributed throughout the body. AQPs perform a bidirectional transport function across cells by absorbing and secreting water from small

intestinal epithelial cells. The abnormal expression of AQPs can damage the tight junctions among intestinal cells, leading to increased intestinal permeability, blocked transmembrane transport, and impaired intestinal water transport, resulting in diarrhea [34]. Among these, AQP4 and AQP5 have been studied extensively in chickens [35]. AQP4 can repair intestinal damage and regulate intestinal mucosal barrier [36]. Another study showed that knocking out AQP4 in mice resulted in higher fecal water content and significantly lower colonic water permeability [37]. In this study, AQP4 and AQP5 expression in the intestines of broilers treated with florfenicol was significantly lower, and the diarrhea rate was higher than in group N, suggesting that decreased expression of aquaporins may lead to water metabolism disorders by disrupting the intercellular tight junctions and increasing intestinal permeability. In a mouse model of irritable bowel syndrome, the expression of colonic AQPs was upregulated by activation of the cAMP/PKA signaling pathway [38]. In contrast, the expression of AQPs was inhibited by the high NF-κB expression [39]. In this experiment, the addition of florfenicol resulted in a decrease in the levels of cAMP and AQP5 and an increase in diarrhea in the jejunum of broiler chickens. Moreover, florfenicol-induced diarrhea is associated with the cAMP/PKA and NF-κB signaling pathway.

Large microbial community resides in the intestine that adheres to the intestinal mucosa symbiotically and has functions such as protecting the host, participating in nutrient absorption, immune regulation, and biological barriers [40]. Climate, dietary structure, application of antibiotics, and harmful microorganisms can cause intestinal microbiota imbalances, among which antibiotics have a prominent impact [41]. The overuse of antibiotics can cause an imbalance in the intestinal microbiota and the proliferation of harmful bacteria, leading to antibiotic-related diseases and even death [42]. Studies have shown that after the intragastric administration of ceftriaxone sodium in mice, the number of dominant gut bacteria, such as *Bifidobacterium*, *Lactobacillus*, and *Escherichia coli*, was reduced significantly. In contrast, yeast and mold increased significantly [43]. In this study, no change was observed in the dominant gut microbiota types of broilers in the two groups, but there was a change in the abundance of the microbiota in group F. A significant increase in *Proteobacteria* and *Escherichia-Shigella* was observed in the group F broilers at the phylum and genus level compared to group N. The addition of florfenicol increased the number of harmful bacteria in the guts of the broilers. LPS, the main component of the cell wall of Gram-negative bacteria, showed a significant increase in content, suggesting that an increase in Gram-negative bacteria is the major cause of intestinal microbial imbalance.

Florfenicol has been used extensively to treat bacterial diseases, but the potential risks associated with its overuse are a concern. In this study, high-dose florfenicol adversely affected intestinal function and broiler growth but did not significantly impact the organ indices. Short-term and recommended therapeutic doses of florfenicol can induce renal injury and reduce the growth performance of young broilers, which differs from the results of the present study and may be due to the earlier use of florfenicol in broilers [6]. On the other hand, the antibiotic regimens for food animals, such as chickens, usually last for more than two weeks [44]. Studies on the long-term use of florfenicol have focused mainly on intestinal tissue and gut microbiota in aquatic animals [45]. Based on these findings, the effects of the long-term application of florfenicol on broilers remain to be studied. Owing to the lack of valid data and previous studies in this area, this gap will be explored further.

Antibiotics are frequently administered to entire flocks of broilers and layers through water or feed for preventive purposes, even without clinical indications [46]. Currently,

resistance to florfenicol is increasing because of global trade and misuse [47]. Antibiotics may generate economic benefits ranging from 1 to 10% when used as growth promoters in poultry production [48], but they may increase antimicrobial resistance (AMR) risk in society [44]. The extensive use of antibiotics in animals contributes to AMR among human enteric microbes [49]. The emergence of florfenicol-resistant bacteria may result in treatment failure, leading to a higher incidence and severity of infections. In addition, it may be a source of resistant bacteria/genes that may pose a risk to human health [50]. This study can help improve the use of florfenicol in the livestock sector and prevent the spread of AMR in animal and human populations.

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