Effects of compound feed additive on growth performance and intestinal microbiota of broilers

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ABSTRACT The purpose of this experiment was to determine the effectiveness of compound feed additive (**CFA**) to replace antibiotics for broiler production. A total of 350 one-day-old Arbor Acres broilers were randomly divided into 7 groups, 5 replications in each group and 10 broilers in each replication. Group A was the control; group B was supplemented with 75 mg/kg chlortetracycline; groups C, D, and E were supplemented with 0.03, 0.06, and 0.09% CFA including glucose oxidase, curcumin, and *Lactobacillus acidophilus*; group F was supplemented with 0.03% CFA plus 0.50% glucose; group G was supplemented with 0.50% glucose. The feeding period was divided into the early (1−21 d) and later stages (22−42 d). The results showed that average daily gain (**ADG**) and feed conversion rate (**F/G**) in group F in later stage were significantly better than those in the control and antibiotic groups; the diarrhea rates in the groups containing CFA in both stages was significantly lower than that in the control and antibiotic groups, indicating that CFA was better than antibiotics to improve growth and decrease diarrhea rate for broilers. Pathogenic *E. coli* challenge significantly increased diarrhea rates and decreased ADG for broilers; however, CFA addition could alleviate the above negative responses by increasing gut *Lactobacillus* abundance and decreasing *Shigella* abundance. It can be concluded that CFA can replace antibiotics to regulate intestinal microbiota, reduce diarrhea rate, and improve broiler growth.

Key words: antibiotics alternative, broilers, compound feed additive, growth performance, gut microbiota

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INTRODUCTION

The use of antibiotics for animal-production promotion can be traced to 1950s. Since the Food and Drug Administration of America (**FDA**) approved the use of antibiotics for livestock and poultry farming in 1950, the annual production of antibiotics in the world is nearly 210,000 tons, and the domestic use is nearly 180,000 tons (Zhang et al., 2018). The abuses of antibiotics in animal farming usually result in antibiotic residues in animal body and products as well as appearance of antibiotic-tolerance and antibiotic-resistance microbes, which causes serious consequences in production performance, disease prevention, and food safety. Therefore, it is more important to study the substitutes of antibiotics.

Glucose oxidase (**GOD**) is an aerobic dehydrogenase that can oxidize β-D-glucose to produce gluconic acid and hydrogen peroxide for inhibiting pathogenic bacteria proliferation and promoting animal growth (Wu et al., 2020). *Lactobacillus acidophilus* is one kind of beneficial microorganisms which can colonize the gastrointestinal tract of animals to maintain the normal gut microbiota. It can produce organic acids and active peptides to inhibit the proliferation of pathogenic bacteria (Cesare et al., 2020). *Lactobacillus acidophilus* is able to induce the immune system to secrete immune cells to enhance animal immunity for speeding up the clearance of pathogens and inflammation (Dev et al., 2020).

Curcumin is one kind of bioactive polyphenol extracted from traditional Chinese Medicine. It is a mixture of curcumin and demethoxycurcumin (Hernandez-Patlan et al., 2019). Some studies have shown that adding 200.00 mg/kg curcumin in broiler diet could improve growth performance, and adding 1.50% turmeric powder and 180.00 mg/kg zinc to diets could effectively alleviate the decline of production performance caused by *E. coli* for chickens (Galli et al., 2020).

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Even though the effectiveness of individual GOD, *Lactobacillus acidophilus* and curcumin has been studied, their compatibility has not been found. This research focused on studying their combination as feed additive to replace antibiotics for improving broiler health and production performance.

MATERIALS AND METHODS

Materials Preparation

Lactobacillus acidophilus, glucose oxidase and curcumin were provided by Henan Delin Biological Products Co., Ltd. Xinxiang, China. Chlortetracycline was provided by Fujian Pucheng Zhengda Biochemical Co., Ltd. Pucheng, China. Pathogenic *Escherichia coli* (***E. coli***) k88 was provided by Microbial Laboratory, Henan Agricultural University. The composition of compound feed additive (**CFA**) obtained previously in vitro in our laboratory for inhibiting pathogenic *E.coli* proliferation was: 17.0% glucose oxidase (1,700 U/g), 66.0% curcumin (99% purity), 17.0% *Lactobacillus acidophilus* (3.0×10^8 CFU/g).

Experimental Design and Broiler Management

A total of 350 one-day-old Arbor Acres broilers were randomly divided into 7 groups, 5 replications in each group, and 10 chickens (half male and half female) in each replication. Group A was the basal diet (control group); group B was supplemented with 75.00 mg/kg chlortetracycline (antibiotic group); groups C, D and E were supplemented with 0.03, 0.06, and 0.09% CFA; group F was supplemented with 0.03% CFA plus 0.50% glucose; group G was supplemented with 0.50% glucose. The soybean-corn-fish meal diets of broilers were prepared according to NRC standard (NRC, 1994), in which metabolizable energy, protein, calcium, and phosphorus concentrations were 12.42 MJ/Kg, 21.26, 1.03, 0.70% in the early feeding stage; 12.75 MJ/Kg, 19.07%, 1.01%, 0.63% in the later feeding stage, respectively. Diets and water were given ad libitum. All the broilers used in this experiment were managed according to the guidelines of Animal Care and Use Ethics Committee in Henan Agricultural University (No. 2021052).

The Experimental Design for the Broilers Challenged With E. coli *k88*

After 42-d feeding experiment, 1-week adaptive stage was conducted, in which the broilers in each group were fed with its corresponding diet. Thereafter, according to the growth performance and diarrhea rates of broilers during the previous 42-d feeding experiment, 12 broilers (half male and half female) with almost the same body weight in groups A, B, C, F, G were selected and fed their corresponding diets.

Six broilers in each group were challenged with 1.0 mL normal saline once by intraperitoneal injection for each broiler as the control, and 6 broilers in each group were challenged with 1.0 mL *E. coli* (2.3×10^8 CFU/mL, which was obtained by another *E. coli*-challenge pre-experiment) by intraperitoneal injection once in another house, named as groups A-1, B-1, C-1, F-1, G-1. Each broiler was raised in a single cage. The *E. coli*-challenge feeding period was 14 d. Diets and water were given ad libitum.

Diarrhea Rate and Growth Performance Measurements

The broilers in each replication were weighed on the 1st, 21st and 42nd day during normal feeding experiment, or on the 1st and 14th day during *E.coli*- challenge feeding experiment. Feed intake in each replication was recorded daily. Diarrhea rate (**DR**), average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion rate (**F/G**) were calculated. The diarrhea rate was calculated as the following: Diarrhea rate (%) = (The number of diarrhea broilers / (the total number of broilers × the total experimental days) × 100%. Diarrhea was defined as watery manure with irregular shape.

Microbiota Analysis in Rectum Contents of Broilers Challenged With E. coli

To investigate gut microbiota in rectum contents of broilers, the samples were collected from the rectum contents of 4 broilers in groups A, B, C, A-1, B-1, and C-1 during *E.coli*-challenge experiment, respectively. DNA was extracted from the samples using Soil DNA Kit (Omega Biotek, Norcross, GA). The final DNA concentration and purity were investigated by NanoDrop 2000 UV−vis spectrophotometer (Thermo Scientific, Wilmington, NC), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 region of 16S rRNA gene was amplified with 338F: 5′-ACTCCTACGGGAGGCAGCA-3′and 806R: 5′-TCGGACTACHVGGGTWTCTAAT-3′by PCR (GeneAmp 9700, ABI, Foster, CA). The PCR amplification program was set as follows: an initial denaturation at 98°C for 3 min; 30 cycles of 98°C for 30 s, annealing at 57°C for 30 s, elongation at 72°C for 30 s; and a final extension at 72°C for 10 min. PCR reactions were carried out in 20 μL reaction mixture containing 10 ng template DNA, 4 μL 5 × FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer and 0.4 μL FastPfu polymerase, and deionized water was used to adjust the reactive volume to 20 μL. The amplified PCR products were extracted by 1% agarose, purified by the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA), and further quantified using QuantiFluorTM-ST (Promega (Beijing) Biotech Co., Ltd. Beijing, China). The purified amplification products were pooled in equimolar

amounts and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA).

Statistical Analysis

Statistical analyses were performed using SPSS Statistics Software (version 20.0, IBM, New York, NY). Data were evaluated by one-way ANOVA, and the comparative analysis was conducted by using the method of Duncan's test. Statistical results were shown in mean ± SEM, and $P < 0.05$ was considered as statistical significance.

RESULTS AND DISCUSSION

Effect of CFA on Growth Performance of Broilers

Table 1 showed that ADG and F/G had no significant differences among all the groups in the early feeding stage ($P > 0.05$); however, ADG in group F was significantly higher than that in other groups ($P < 0.05$), and F/G in group F was significantly lower than that in other groups in the later feeding stage ($P < 0.05$). Diarrhea rates in groups C, D, E, and F were significantly lower than that in groups A, B, and G in both feeding stages ($P < 0.05$), indicating that CFA was better than antibiotics to decrease diarrhea rate for broilers. The previous reports showed that adding probiotics, herbs or its extract to broiler diets could increase growth performance, immune response and intestinal barrier function as well as regulate gut microbiota and reduce the incidence of diarrhea of broilers (Cross et al., 2007; Zhang et al., 2016), corresponding with this study. The reason why broiler growth performance in group F were the best among all the groups in later feeding stage may be that glucose as substrate of glucose oxidase can increase the function of glucose oxidase for inhibiting pathogenic bacterium proliferation.

Effect of CFA on Growth Performance and Microflora in Rectal Contents of Broilers Challenged With E. coli

E. coli challenge of broilers indicated that diarrhea rates were 11.90 and 36.90% for groups A and A-1 ($P < 0.05$), 19.05 and 46.43% for groups B and B-1 ($P < 0.05$), 4.76 and 19.05% for groups C and C-1 ($P < 0.05$), 2.38 and 14.29% for groups F and F-1 ($P < 0.05$), 10.71% and 33.33% for groups G and G-1 ($P < 0.05$). This study indicated that *E. coli* challenge significantly increased boiler diarrhea rates, compared with the broilers challenged with normal saline ($P < 0.05$). In addition, ADG was 103.33 g and 87.86 g for groups A and A-1 ($P < 0.05$), 90.00 g and 67.02 g for groups B and B-1 ($P < 0.05$), 97.50 g and 91.91 g for groups C and C-1 ($P > 0.05$), 97.38 g and 82.98 g for groups F and F-1 ($P > 0.05$), 90.71 g and 85.83 g for groups G and G-1 ($P > 0.05$). This study showed that *E. coli* challenge significantly decreased ADG and increased diarrhea rate, especially in groups A-1 and B-1 ($P < 0.05$). It was speculated that CFA was better than antibiotics to decrease diarrhea rates and alleviate the negative effect of *E. coli* on growth performance for broilers.

Figure 1 showed that the relative abundance of *Lactobacillus* in rectum of group C-1 was significantly higher than that of other groups ($P < 0.05$), corresponding with lower diarrhea rate and higher ADG in this group; the relative abundance of *Turicibacter* in group A was significantly higher than that in the other groups ($P < 0.05$); the relative abundance of *Bacteroides* in group A-1 was significantly higher than that in other groups ($P < 0.05$); the relative abundance of *Enterococcus* in group B-1 was significantly higher than that in other groups ($P < 0.05$); the abundance of *Shigella* in group A-1 and group B-1

Table 1. Effect of different levels of CFA on 1 to 42 d growth performance of broilers.

Groups	Initial weight, g	Final weight, g	ADFI, g/d	ADG, g/d	F/G	Diarrhea rate, %
			1–21 d			
A	42.70 ± 0.67	729.62 ± 28.48	41.05 ± 1.47^{ab}	32.71 ± 1.37	1.25 ± 0.06	7.24 ± 1.36^{a}
B	41.90 ± 1.08	739.16 ± 57.54	40.99 ± 0.73^{ab}	33.20 ± 2.77	1.24 ± 0.06	5.43 ± 1.26^{a}
C	42.90 ± 0.89	702.00 ± 28.63	40.45 ± 0.92^{ab}	31.38 ± 1.34	1.29 ± 0.02	1.71 ± 0.32^{b}
D	42.20 ± 0.45	735.44 ± 20.03	41.64 ± 0.93^{ab}	33.01 ± 0.98	1.26 ± 0.06	1.14 ± 0.36^{b}
E	42.80 ± 0.57	709.44 ± 31.76	39.01 ± 0.79^{b}	31.74 ± 1.50	1.23 ± 0.09	1.24 ± 0.44^{b}
F	42.80 ± 0.91	748.09 ± 51.73	40.85 ± 1.01^{ab}	33.58 ± 2.43	1.22 ± 0.09	1.05 ± 0.28^{b}
G	42.60 ± 1.08	734.60 ± 21.87	42.26 ± 0.67^{a}	32.95 ± 1.06	1.28 ± 0.01	6.38 ± 1.24^{a}
			22–42 d			
A	737.13 ± 10.49	2,317.50 ± 56.14^{bc}	129.24 ± 1.95^{ab}	75.26 ± 2.47^{bc}	1.72 ± 0.05^{a}	3.69 ± 0.55^{ab}
B	734.38 ± 10.56	2,296.00 ± 102.85^{bc}	129.78 ± 2.69^{ab}	74.37 ± 5.17^{bc}	1.75 ± 0.07^{a}	4.76 ± 0.82^{a}
C	729.75 ± 13.71	2,339.00 ± 93.64^{bc}	130.26 ± 2.23^{ab}	76.63 ± 4.04^{bc}	1.70 ± 0.04^{a}	0.83 ± 0.33^{c}
D	736.75 ± 9.07	2,274.00 ± 126.83^{bc}	125.18 ± 2.97^{b}	73.20 ± 6.06^{c}	1.71 ± 0.04^{a}	0.48 ± 0.25^{c}
E	728.00 ± 6.05	2,244.25 ± 73.81^{c}	124.42 ± 1.65^{b}	72.20 ± 3.33^{c}	1.72 ± 0.04^{a}	0.60 ± 0.47^{c}
F	729.00 ± 5.58	2,464.00 ± 52.49^{a}	134.15 ± 2.85^{a}	82.62 ± 2.49^{a}	1.62 ± 0.07^{b}	0.24 ± 0.16^{c}
G	729.13 ± 7.81	2,371.00 ± 84.29^{b}	133.32 ± 1.96^{a}	78.19 ± 4.02^{b}	1.71 ± 0.03^{a}	2.98 ± 0.61^{b}

$^{a\text{-}c}$The different superscript lowercase letters in the same column indicate significant difference ($P < 0.05$), while the same or without superscript lowercase letters in the same column indicate insignificant difference ($P > 0.05$). Statistical analysis is conducted within the early feeding stage or later feeding stage, respectively. A indicates the control group; B indicates antibiotics group; C, D, E, F and G indicate 0.03%, 0.06% and 0.09% CFA, 0.03% CFA + 0.50% glucose, 0.50% glucose, respectively.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; F/G, ADFI/ADG.

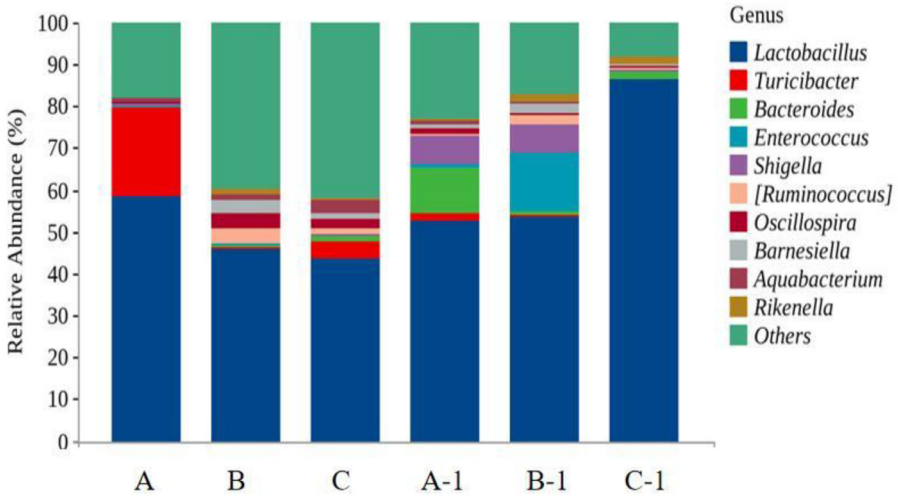

Figure 1. The rectum microbiota of broilers challenged with *E. coli* at genus level. Note: A: the control group challenged with normal saline; B: 75.00 mg/kg chlortetracycline group challenged with normal saline; C: 0.03% CFA challenged with normal saline; A-1, B-1, C-1: groups A, B, C challenged with *E. coli* instead of normal saline.

was significantly higher than that in other groups ($P < 0.05$); the abundance of *Pasteurella* in group B-1 was significantly higher than that in group C-1 ($P < 0.05$). It was speculated that CFA was better than antibiotics to decrease diarrhea rates through increasing beneficial bacterium abundance such as *Lactobacillus* and decreasing harmful bacterium abundance such as *Shigella*, corresponding with the previous report (Zhang et al., 2016). Therefore, the effect of CFA on improving broiler growth and reducing diarrhea rate may be achieved through effective regulation of intestinal microflora (Kabir, 2009).

In conclusion, the compound feed additive composed of glucose oxidase, *Lactobacillus acidophilus*, and curcumin can regulate the balance of intestinal microbiota, inhibit proliferation of pathogens to reduce diarrhea rate, and improve growth performance of broilers. It is possible to replace antibiotics in chicken farming for safe food production.

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DISCLOSURES

All the authors declare no conflicts of interest.

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