Effects of dietary β-1,3-glucan addition on the growth performance, mRNA expression in jejunal barrier, and cecal microflora of broilers challenged with *Clostridium perfringens*

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ABSTRACT This experiment aimed to explore the interaction of *β*-1,3-glucan and *Clostridium perfringens* on the growth performance, intestinal health and cecal microflora of broilers. A total of 384 one-day-old Arbor Acre broilers were sorted into 4 treatments with 6 replications. There were 2 factors in this trial: dietary *β*-1,3-glucan addition including 0 and 250 mg/kg, intestinal enteritis challenged with *Clostridium perfringens* attack or not. Results showed that *Clostridium perfringens* infection disrupted the integrity of the intestinal mucosa by reducing the jejunal *Occludin* and *Claudin-1* mRNA expression of broiler chickens at 21 d of age ($P < 0.05$). Meanwhile, when considering *Clostridium perfringens* as the main effect, it also decreased the mRNA expression of the glucose transporter recombinant sodium/glucose cotransporter 1 (***SGLT1***) at d 21 and the fatty acid transporter liver fatty acid-binding protein (***L-FABP***) at d 42 ($P < 0.05$) as well as affect cecum microbial diversity, especially in relative abundance of *Firmicutes* and *Bacteroidetes*. In addition, *Clostridium perfringens* infection reduced body weight, daily weight gain, and feed-gain ratio (**FCR**) in broilers at d 42 ($P < 0.05$). The dietary *β*-1,3-glucan could alleviate intestinal mucosal damage caused by the *Clostridium perfringens* to some extent. When considering *β*-1,3-glucan as the main effect, it increased the *SGLT1* at 42 d of age ($P < 0.05$), and stabilized gut microbiota disorder caused by *Clostridium perfringens*. More over dietary *β*-1,3-glucan addition increased body weight at 42-day-old ($P < 0.05$), and improved daily weight gain and FCR during 1 to 42 d ($P < 0.05$). In conclusion, dietary *β*-1,3-glucan could improve growth performance and intestinal health in broilers infected with *Clostridium perfringens*.

Key words: broilers, *β*-1,3-glucan, clostridium perfringens, growth performance, intestinal health

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INTRODUCTION

Necrotizing enteritis (**NE**) is a common intestinal toxic disease in the poultry industry especially under the condition of the antibiotic-free rearing program (Cervantes, 2015). NE is mainly induced by the pathogenic strain of *Clostridium perfringens*. Chronic intestinal mucosal damage induced by subclinical NE not only causes big difficulty in the production management of broilers (Smith, 2019; Williams, 2005) but also results in enormous economic loss (Paiva and McElroy, 2014). On the other hand, the NE has adverse effects on daily weight gain, and feed conversion efficiency in chickens due to weakening digestion and absorption (Van Immerseel et al., 2009; Cervantes, 2015; Emami and Dalloul, 2021). The diet was reported to changes in intestinal microbial ecology and fermentation end products, which in turn affects nutrition, physiology and immune function of the host (Makki et al., 2018). Previous studies have elaborated the roles of *β*-1,3-glucan such as modulating immune function (Vetvicka and Yvin, 2004) and suppressing visceral fat accumulation (Cao et al., 2016), which might be mediated by improving the intestinal environment (Cui et al., 2021). However, the physiological effects of *β*-glucan varied with their sources, which depend on molecular weight and three-dimensional structure or the ratio of *β*-1,3-glucan to *β*-1,6-glucan. The *β*-1,3-glucan of *Euglena gracilis* origin has well-proportioned molecular weight, structure and size (1 to 3 mm) (Barsanti et al., 2001), and could be utilized by many beneficial microorganisms as the carbon source. Zhang et al. (2020) reported that *β*-glucan could be a

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probiotic for broilers growth. Dietary β-glucan addition was found to change the microbiota of the gastrointestinal tract, promote beneficial microorganisms and reduce the number of pathogenic bacteria (Lee et al., 2020). Therefore, the current study was carried out to explore the interaction function between β-1,3-glucan and *Clostridium perfringens* on the growth performance, intestinal health, and cecal microflora of broilers.

MATERIALS AND METHODS

All the birds and experimental protocols in this study were approved by the Institution Animal Care and Use Committee of the Northwest A&F University (NWAFAC1008).

Animals and Experimental Design

Using a 2 × 2 factorial completely randomized trial design, 384 one-day-old healthy Arbor Acre broilers (half male and half female) were randomly divided into 4 treatments: 1) control group: basal diet + no attack, name it with Ctrl, 2) NE group: basal diet + *Clostridium perfringens* attack, name it with C. P, 3) β-1,3-glucan group: basal diet + 250 mg/kg β-1,3-glucan, name it with 250, and 4) β-1,3-glucan + NE group: 250 mg/kg β-1,3-glucan + *Clostridium perfringens* attack, name it with C.P250. There are 6 replicates for each group with 16 chickens per replicate. The β-1,3-glucan is derived from algae (*Euglena gracilis*) with 54.9% to 55.9% content and provided by Kemin (China) Technologies Co., Ltd. (Zhuhai, China). The nutrient levels of the basal diet were listed in Table 1. The protocol of *Clostridium perfringens* infection was based on the published method from Guo et al. (2021). The growth performance of each group was recorded on 21 and 42 d. All birds were given with feed and water ad libitum. All housing and handling were approved by Farm Management Procedures of Arbor Acres.

Sample Collection

On the 21st day and 42nd day, 1 chick from each repetition of each group was randomly selected, and the weight of each chick was recorded. After anesthesia was administered, the animals were sacrificed by cutting the carotid artery. We took 1 middle segments of the jejunum of approximately 1 cm long, and rinsed them with PBS to remove intestinal content. It was stored at −80° C until detection of mRNA contents. Contents of the cecum were obtained and stored at −80°C until the assessment of the abundance of the microbiota.

RNA Isolation of Jejunal Mucous Membrane

Total RNA from jejunal mucous samples were extracted using the TRIzol reagent (Invitrogen, Shanghai, China). Specifically, DNaseI was used during the RNA isolation process to avoid contamination with genomic DNA. The quantity and purity of total RNA were analyzed by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Shanghai, China), and the integrity of RNA was assessed with gel electrophoresis. Only RNA samples that had an OD260/280 > 1.8, OD260/230 > 2.0, and good integrity were used for further qRT-PCR.

Microbial DNA Extraction

DNA extraction for cecal contents was following the manufacturer's instructions of QIAamp DNA Stool Mini Kit (Qiagen, Germany). DNA Samples were measured on a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Shanghai, China) to assess DNA quantity and then stored at −80°C until sequencing analysis.

Quantitative Real-Time PCR Analysis

Approximately 1 μg of total RNA was used for cDNA synthesis using the PrimeScript RT reagent Kit

Table 1. Ingredient composition and calculated nutrient levels of the basal diets.

Ingredients	Content(%)		Ingredients	Content (%)	
	d 1−21	d 22−42		d 1−21	d 22−42
Corn	57.10	63.51	Threonine	0.13	0.21
Soybean meal (46% CP)	22.60	16.30	Choline chloride (50%)	0.08	0.10
DDGS	5.00	5.00	Vitamin premix[1]	0.05	0.05
Corn gluten meal (60% CP)	4.00	4.00	Mineral premix[2]	0.15	0.15
Cottonseed meal (45% CP)	3.00	4.00	Total	100.00	100.00
Wheat flour	2.50	—	Calculated nutrient level		
$CaHPO_4$	1.83	1.24	ME (Kcal/kg)	2900	3000
Soybean oil	1.20	2.30	Crude protein (%)	21.00	19.00
Limestone	0.90	1.36	Calcium (%)	0.90	0.90
L-Lysine sulphate (70%)	0.84	1.10	Total phosphorus (%)	0.70	0.70
Bentonite	0.08	0.08	None-phytate phosphorus (%)	0.45	0.35
NaCl	0.30	0.30	Lysine (%)	1.30	1.30
DL-Methionine (99%)	0.23	0.30	Methionine (%)	0.56	0.60
			Methionine + cysteine (%)	0.73	0.73

Abbreviations: CP, crude protein; DDGS, distillers dried grains with soluble; ME, metabolizable energy.

[1]The vitamin premix provides the following per kg of diets: 1−21 day of age VA 11.2 KIU, VD_3 3.36 KIU, VE 20 mg, VK_3, 4.0 mg, VB_1 2.2 mg, VB_2 7.28 mg, VB_6 4.8 mg. 22−42 day of age, V A 8.4 KIU, VD_3 2.52 KIU, VE 15.75 mg, VK_3 3.0 mg, VB_1 1.64 mg, VB_2 5.46 mg, VB_6 3.6 mg.

[2]The trace element premix provides the following per kg of diets: Cu 10 mg, Zn 75 mg, Fe 80 mg, Mn 80 mg, Se 0.3 mg, I 0.4 mg.

Table 2. Primer sequences for Real-time PCR assay.

Gene	GenBank accession numbers	Forward/Reverse(5'→3')
β-actin	NM_205518.1	F: AATCAAGATCATTGCCCCACCT R: TGGGTGTTGGTAACAGTCCG
PepT1	NM_204365	F: GCATTGTTTCTAGCTTGCGGT R: TCCTCCTGAGAACGGACTGT
SGLT1	NM_001293240.1	F: GATGTGCGGATACCTGAAGC R: AGGGATGCCAACATGACTGA
L-LABP	NM_204192	F: CAGGAGAGAAGGCCAAGTGTA R: TGGTGTCTCCGTTGAGTTCG
Claudin-1	NM_001013611.2	F: GCAGATCCAGTGCAAGGTGTA R: CACTTCATGCCCGTCACAG
ZO-1	XM_015278981.2	F: CTTCAGGTGTTTCTCTTCCTCCTC R: CTGTGGTTTCATGGCTGGATC
Occludin	NM_205128.1	F: ACGGCAGCACCTACCTCAA R: GGGCGAAGAAGCAGATGAG
MUC2	NM_001318434.1	F: TTCATGATGCCTGCTCTTGTG R: CCTGAGCCTTGGTACATTCTTG

Abbreviations: *β*-actin, the internal reference gene beta actin, *PepT1*, oligopeptide transporter 1, *SGLT1*, Recombinant Sodium/Glucose Cotransporter 1, *L-FABP*: Liver fatty acid-binding protein. *Claudin-1, ZO-1*, and *Occludin*, tight junctions protein1, *MUC2*: mucoprotein2. Same as below.

with gDNA eraser (TaKaRa, Dalian, China). qRT-PCR was performed using SYBR Green PCR Master Mix (TaKaRa). A 20 μL PCR mixture was quickly prepared and conducted in the iCycler iQ5 multicolor (Bio-Rad Laboratories, Shanghai, China) based on the parameters as follows: 95°C for 10 min; 40 cycles of 95° C for 10 s; 60°C for 30 s; 72°C for 30 s; and 72°C for 5 min. Primers were designed using Primer-BLAST and listed in Table 2. All samples were examined in triplicate. All data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) Bio-Rad Laboratories (Shanghai) Co., Ltd.

The 16S rRNA Gene Amplification and Data Processing

16S rRNA gene amplicons were used to determine the diversity and structural comparisons of the bacterial species in cecal samples using Illumina MiSeq sequencing (LC Bio Tech Co., Ltd, Zhejiang, China). The V3 + V4 hypervariable region of the 16S rRNA gene were amplified from microbial genomic DNA using forward primer 338 F (5' − ACTCCTACGGGAGGCAGCAG - 3') and reverse primer 806 R (5' - GGACTACNNGGGTATCTAAT - 3').

Paired−end reads were assigned to samples based on their unique barcode, and samples were truncated by cutting off the barcode and primer sequences. Paired−end reads were merged using FLASH (Magoč and Salzberg, 2011). Quality filtering on raw tags was performed using specific filtering conditions to obtain high−quality clean tags with FastQC. Verseach (v2.3.4) was used to filter chimeric sequences and to assign samples with ≥97% sequence similarity as the same operational taxonomic units (**OTUs**) (Rognes, et al., 2016). OTU abundance data were normalized for further processing using QIIME 2.34 (Bolyen, et al., 2019). The taxon abundance for each sample was determined according to phylum, class, order, family, and genus. The Alpha diversity was identified by studying the Observed species, Chao1, ACE, Shannon, and Simpson indices. Meanwhile, LEfSe analysis was performed to estimate the effect size of species that contributed to the differences between the samples. The threshold of the LDA score was set at a default value of 3.0 and the P value was less than 0.05. Correlations between variables were tested by Pearson correlation test and RDA analyses were performed by using R packages.

Statistical Analysis

The SPSS 21.0 software was used for all statistical analyses in this study. Univariate analysis of the data on the growth performance, relative expression of gene mRNA and alpha diversity of cecal microflora was used to analyze the main effect and interactive effect. All the analysis results were considered to be statistically significant at $P < 0.05$.

RESULTS

Growth Performance

The effects of *β*-1,3-glucan (**G**) and *Clostridium perfringens* (**C.P**) on broiler performance are shown in Table 3. The main effects analysis showed that dietary *β*-1,3-glucan addition significantly increased the body weight at 42 d of age, and the *β*-1,3-glucan treatment increased average daily weight gain (**ADG**) at 22 to 42 d of age as well as 1 to 42 d, while decreased feed-gain ratio (**FCR**) during 1 to 42 d ($P < 0.05$). There was also no interaction effect on BW, ADG, and FCR of production performance between these 2 treatment factors ($P > 0.05$). So, it can be seen that *β*-1,3-glucan can

Table 3. The effect of β-1,3-glucan supplementation on growth performance on broilers when exposed to *Clostridium perfringens*.

Items		Non-challenged		Challenged		SEM	*P* values		
		Ctrl	250	C.P	C.P250		250	C.P	Interaction
BW/g	d 1	46.34	45.73	46.75	46.87	0.224	0.592	0.101	0.434
	d 21	837	860	824	847	6.191	0.078	0.309	0.996
	d 42	3005	3121	2935	3042	16.605	0.003	0.038	0.892
ADFI/g	d 1−21	50.87	51.92	50.65	50.74	0.574	0.621	0.549	0.677
	d 22−42	179.89	178.20	175.34	178.43	1.401	0.807	0.451	0.405
	d 1−42	115.01	115.06	113.00	114.58	0.690	0.560	0.378	0.586
ADG/g	d 1−21	37.83	38.78	37.01	38.10	0.320	0.128	0.259	0.913
	d 22−42	105.43	107.67	100.55	104.55	0.699	0.038	0.010	0.538
	d 1−42	71.43	73.22	68.78	71.32	0.397	0.013	0.010	0.641
FCR	d 1−21	1.345	1.340	1.368	1.332	0.010	0.301	0.693	0.432
	d 22−42	1.706	1.655	1.744	1.707	0.006	0.003	0.003	0.592
	d 1−42	1.609	1.571	1.643	1.606	0.005	0.001	0.001	0.964

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion rate.
Ctrl: control group, 250: β-1,3-glucan group, C.P: NE group, C.P250: β-1,3-glucan + NE group.

Table 4. Relative expression of mRNA of tight junction genes in the jejunal mucosa of broiler at 21 and 42 d of age.

Items		Non-Challenged		Challenged		SEM	*P* values		
		Ctrl	250	C.P	C.P250		250	C.P	Interaction
21 d	*ZO-1*	1.032	0.952	0.892	0.718	0.244	0.230	0.084	0.651
	Occludin	1.008	0.743	0.654	0.368	0.242	<0.001	<0.001	0.811
	Claudin-1	0.910	0.858	0.642	0.606	0.231	0.645	0.015	0.931
	Muc2	0.892[b]	1.884[a]	1.244[ab]	1.113[b]	0.578	0.066	0.348	0.020
42 d	*ZO-1*	0.873	0.712	0.827	1.020	0.266	0.888	0.271	0.143
	Occludin	1.023	0.825	0.926	1.218	0.277	0.691	0.225	0.054
	Claudin-1	1.015[b]	1.090[b]	0.833[b]	1.915[a]	0.586	0.006	0.094	0.013
	Muc2	1.005	0.922	1.112	1.052	0.328	0.656	0.464	0.944

Ctrl: control group, 250: β-1,3-glucan group, C.P: NE group, C.P250: β-1,3-glucan + NE group.
[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

promote production performance, and *Clostridium perfringens* inhibit the growth of broilers.

The mRNA Expression of Mucosal Barrier and Nutrient Transport in the Jejunum

The intestinal mucosa acts as a selectively permeable barrier and develops important functions such as absorbing nutrients and defending against pathogens and toxins. We detected the mRNA expression of tight junctions and nutrient transport. As exhibited in Table 4. *Clostridium perfringens* significantly downregulated the mRNA expression of *Occludin* and *Claudin-1* at 21 d, and β-1,3-glucan treatment showed the same result on the mRNA expression of *Occludin*. But at 42 d of age, dietary β-1,3-glucan addition increased the mRNA expression of *Claudin-1*, while *Clostridium perfringens* had no effect, but an interaction was existed between dietary β-1,3-glucan and *Clostridium perfringens* challenge. The highest expression of *Claudin-1* in the combined group might repair mucosal damage caused by *Clostridium perfringens*.

β-glucans can increase the digesta retention time in the gastrointestinal tract (**GIT**) as non-starch polysaccharides, which affected the digestibility of other nutrients such as protein and starch in the intestine. Therefore, we examined the relative mRNA abundance of nutrient transports in the jejunum as shown in Table 5. C.P significantly decreased the mRNA expression of *SGLT1* at 21 d of age. At 42 d of age, β-1,3-glucans significantly increased the expression of *SGLT1*, there was a significant reduction in the

Table 5. Relative expression of mRNA of nutrient transport genes in the jejunal mucosa of broiler chickens at 21 and 42 d of age.

Items		Non-challenged		Challenged		SEM	*P* values		
		Ctrl	250	C.P	C.P250		250	C.P	Interaction
21 d	*Pept1*	0.587	0.634	0.454	0.618	0.395	0.599	0.708	0.769
	SGLT1	1.005	1.054	0.808	0.660	0.247	0.605	0.007	0.310
	L-FABP	0.845	0.588	0.863	0.758	0.287	0.214	0.511	0.595
42 d	*Pept1*	1.135	4.690	4.728	3.990	2.862	0.293	0.281	0.118
	SGLT1	1.008	1.406	1.042	2.148	0.755	0.017	0.189	0.229
	L-FABP	1.000[a]	0.576[b]	0.463[b]	0.662[b]	0.269	0.232	0.024	0.003

Ctrl: control group, 250: β-1,3-glucan group, C.P: NE group, C.P250: β-1,3-glucan + NE group.
[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

Table 6. The α diversity of cecal microflora of broilers at 21 d and 42 d.

Items		Non-challenged		Challenged		SEM	*P* values		
		Ctrl	250	C.P	C.P250		250	C.P	Interaction
21 d	Chao1	1,608.03	1,979.90	2,317.63	2,162.70	489.98	0.551	0.021	0.156
	ACE	1,731.38	2,113.29	2,467.26	2,348.81	526.82	0.503	0.021	0.210
	Simpson	0.83	0.94	0.96	0.94	0.132	0.436	0.244	0.274
	Shannon	6.37	7.71	8.21	7.67	1.537	0.513	0.149	0.132
42 d	Chao1	2,095.56	1,980.31	2,197.07	2,104.44	300.65	0.440	0.402	0.932
	ACE	2,180.94	2,073.18	2,285.88	2,194.84	325.66	0.497	0.440	0.954
	Simpson	0.98	0.98	0.95	0.98	0.028	0.446	0.227	0.182
	Shannon	8.20	7.87	7.84	7.99	0.510	0.691	0.598	0.274

Ctrl: control group, 250: β-1,3-glucan group, C.P: NE group.

expression of *L-FABP* when exposed with C.P, and the interaction effect was found between C.P and β-1,3-glucans.

The Cecal Microbiota

Increased viscosity caused by β-1,3-glucans in turn could affect the growth of bacteria in the upper GIT. To get the diversity and abundance of microflora in cecal content, we performed 16s rDNA gene sequencing. The α diversity of cecal microflora was showed in Table 6. The Chao1 and Simpson index were increased when the birds were challenged with *Clostridium perfringens* at 21 d of age.

The microflora composition at phylum level at 21-d were showed in Figure 1A, the addition of β-1,3-glucan without C.P infection was able to reduce the relative abundance of Firmicutes; while it increased the relative abundance of Firmicutes under the conditions of C.P infection. For Bacteroidetes, C.P infection increased the abundance of *Bacteroidetes* when β-1,3-glucan was not added ($< 0.01\% \rightarrow 1.85\%$), and decreased the abundance of *Bacteroidetes* when β-1,3-glucan was added ($0.62\% \rightarrow < 0.01\%$). The results of the relative abundance of the main microflora phylum levels for each cecum sample at 42 d of age are shown in Figure 1B. *Clostridium perfringens* attack increased the relative abundance of Firmicutes and decreased the relative abundance of *Bacteroidetes* compared to the

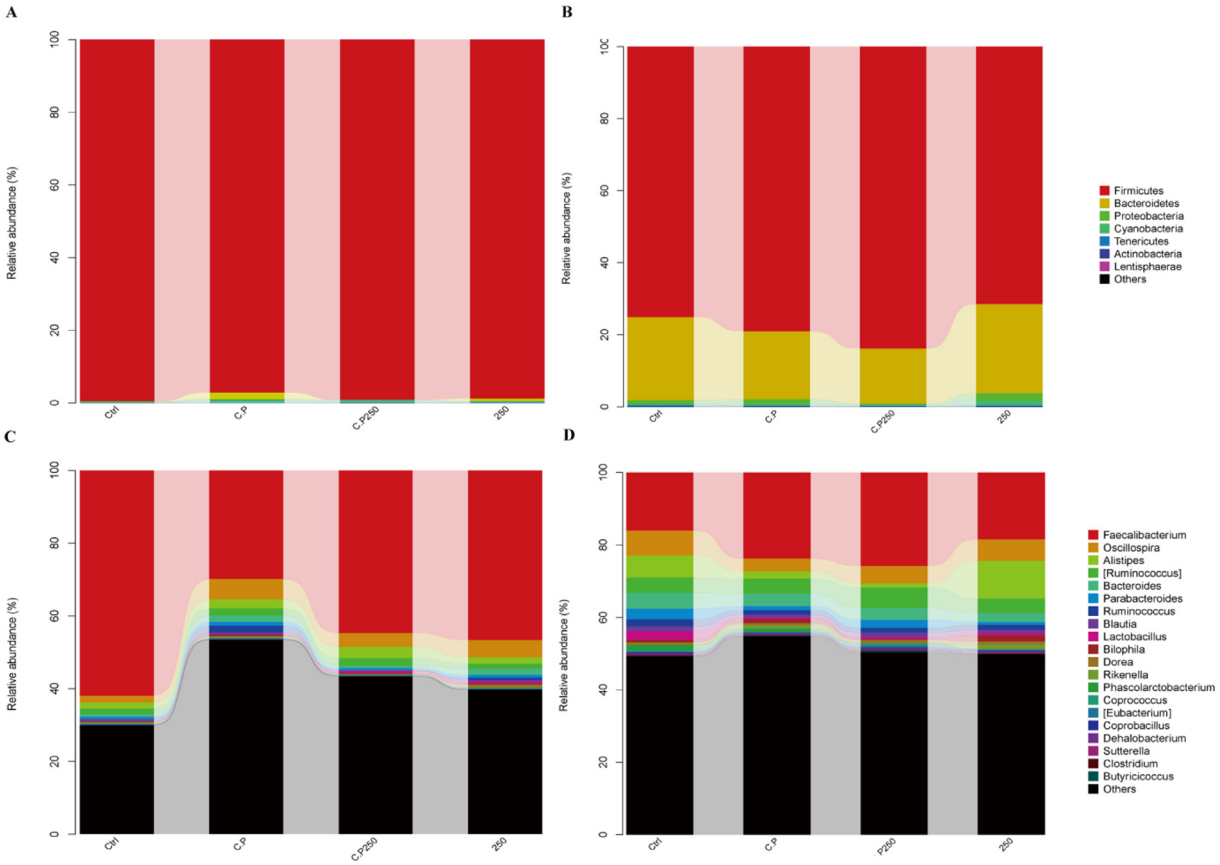

Figure 1. Cecum microflora's composition broilers at 21 days and 42 days. Relative abundance of microorganisms at phylum level (A) 21 days, n = 6, (B) 42 days, n = 6. Relative abundance of microorganisms at genus level (C) 21 days, n = 6 (D) 42 days, n = 6.

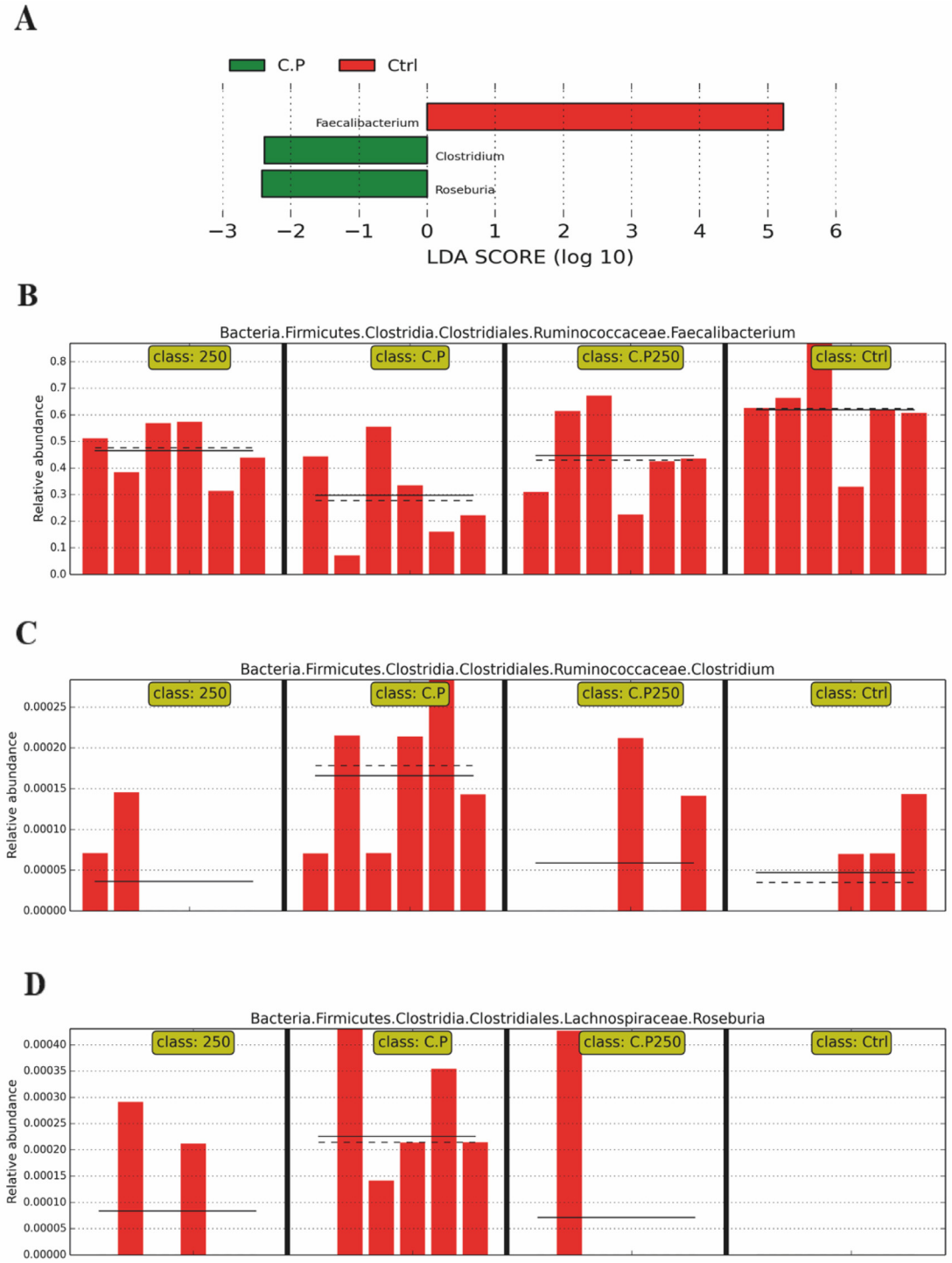

Figure 2. LEfSe analysis identified the taxonomic biomarkers of cecal microorganisms at 21 d. (A) Total taxonomic biomarkers, LDA score > 2.0, the relative abundance of biomarker in each repeat of each group; (B) *Faecalibacterium*, (C) *Clostridium*, (D) *Roseburia*.

control group, *Clostridium perfringens* attack had a tendency to reduce the relative abundance of *Cyanobacteria*. The relative abundance of the main microflora genus levels of each cecum at 21 d of age was shown in Figure 1C. Addition of β-1,3-glucan decreased the relative abundance of *Faecalibacterium* and *Unclassified_Ruminococcaceae* when birds were not given C.P infection while increased these bacteria when giving infection. *Clostridium perfringens* attack increased the relative abundance of *Faecalibacterium* and decreased the relative abundance of *Unclassified_Ruminococcaceae* (Figure 1D). NE reduced the relative abundance of *Oscillospira* and *Alistipes* at 42 d of age. β-1,3-glucan increase the relative abundance of *Alistipes* at 42-d.

LEfSe analysis identified a total of three cecum microbial taxonomic biomarkers were identified (set LDA score > 2.0, Figure 2A) at d 21, whereas, at d 42 there was nothing. The highest relative abundance (P = 0.042) of *Faecalibacterium* (genus

Faecalibacterium) was found in the control group (Figure 2B), and the highest relative abundance of *Clostridium* (genus *Clostridium*, Figure 2C; $P = 0.049$) and *Roseburia* (genus *Roseburia*, Figure 2D; $P = 0.028$) was found in the NE group.

DISCUSSION

The growth performance of livestock and poultry is the most direct and effective indicator to evaluate their economic value. In this study, NE significantly reduced body weight, average daily gain, and feed conversion efficiency in broilers, which is consistent with the results of many studies (Jozefiak et al., 2012; Li et al., 2018). Most of the β-1,3-glucan has been studied for its effect on the immune function and growth performance of livestock (Karunaratne et al., 2020), and less reports focused on its effect on intestinal health of livestock under the infection model. Shao et al. (2016) investigated the effect of yeast β-1,3-glucan on intestinal health combining with *Salmonella* typhimurium infection in broiler chickens, and found that β-1,3-glucan significantly improved the Salmonella-induced reduction in production performance, which is consistent with the results of the present study.

Tight junction proteins are an important component of the intestinal epithelial mechanical barrier that keeps pathogenic bacteria in the intestinal and inhibits the passage of macromolecules from the intestinal lumen to the intestinal epithelium (Ballard et al., 1995). The tight junctional structure between intestinal epithelial cells consists of several proteins, *JAM, Occludin, ZOs*, and *Claudins*. In the current study, *Clostridium perfringens* significantly reduced the expression of *Occludin* and *Claudin-1* tight junction proteins at 42 d of age, disrupting the integrity of the intestinal barrier function, which was consistent with the findings from others (Li et al., 2018; Wu et al., 2019). In this study, *C. perfringens* attack increased the expression of MUC2 in the small intestine, which is consistent with Li's findings (Li et al., 2018), which might be related to *C. perfringens* upregulating the number of mucin-2 secreting goblet cells. *Clostridium perfringens* is capable of encoding dozens of hydrolytic enzymes that have been reported to degrade mucin in the mucus layer, which was used as a substrate for its own growth and reproduction (Ficko-Blean and Boraston, 2006). β-1,3-glucan can inhibit *Clostridium perfringens*-induced secretion of MUC2.

Nutrient transport carriers in the intestine are located on the brush border membrane of the small intestine and play an important role in the absorption of nutrients (Chin et al., 2017; Santos et al., 2019). Currently, many studies have reported the effect of β-1,3-glucan or *Clostridium perfringens* attack on the expression of nutrient transport carriers in the small intestine of broiler chickens (Broom, 2017; Dierick et al., 2019; Vecchio et al., 2021). In this study, *Clostridium perfringens* tapping reduced *SGLT1* expression at 21-d and *L-FABP* expression at 42-d, thereby reducing absorption of glucose and fatty acids in the small intestine, which may also be a potential cause of reduced growth performance. Guo et al. (2014) and Hosseini et al. (2017) noted that infection with *Clostridium perfringens* significantly reduced the expression of *SGLT1* and *PepT1* mRNA in the jejunum of broiler chickens, who reported that *SGLT1, PepT1*, and *L-FABP* expression were significantly downregulated in duodenum of broiler chickens with necrotizing enterocolitis.

The gastrointestinal tract of poultry is a structurally complex microecosystem in which the hindgut hosts a large number of microorganisms and play an important role in nutrient absorption (Wen et al., 2019), normal development of intestinal morphology and digestive function (Gong et al., 2020), establishment of immune function (Mesa et al., 2020), and resistance to disease in poultry (Shang et al., 2018b). *Clostridium perfringens* in this study was able to increase the relative abundance of the Firmicutes phylum and decrease the relative abundance of the *anaphyla*. The biological taxonomic status of *Clostridium perfringens* belongs to the Firmicutes phylum and its massive colonization in the intestine is responsible for the increased abundance of the Firmicutes phylum. The phylum *Clostridium perfringens* breaks down macromolecular organic matter such as sugars and proteins in the intestine to provide energy to the host (Wexler and H., 2007), and the lower abundance may reduce productive performance. The *Clostridium perfringens* is relatively abundant in the infected *Clostridium perfringens* group. The phylum Metaplasma contains many pathogenic bacteria such as *Salmonella, E. coli*, and *H. henselae*, and they all colonize the poultry intestine (Mora et al., 2010), suggesting that these conditionally pathogenic bacteria may coexist with NE.

The β-configured glucosidic linkages that define these polysaccharides render them inaccessible to the limited repertoire of digestive enzymes encoded by the chicken genome. As a result, the various β-glucans become fodder for the broiler gut microbiota in the lower gastrointestinal tract, where they influence community composition and metabolic output, including fermentation to short chain fatty acids (**SCFAs**). The ability of bacterial species in the GIT to utilize β-1,3-glucans was defined by Abigail Salyers in the late 1970s. A survey of 154 strains from 22 species of human colonic bacteria, including *Bifidobacterium, Peptostreptococcus, Lactobacillus, Ruminococcus, Coprococcus, Eubacterium*, and *Fusobacterium* species, revealed no laminarin fermenters, except for a single *Peptostreptococcus* productus strain (Salyers et al., 1977). More recently, the ability of Lactobacillus and Bifidobacterium species to utilize laminarin for growth has been demonstrated (Seong et al., 2019). Notably, growth of bacteroides species on laminarin was found to induce specifically β-1,3-glucanase activity, portending the discovery of β-1,3-glucan-specific polysaccharide utilization loci in select Bacteroides and other Bacteroidetes (Déjean et al., 2020). β-1,3-glucan can regulate digestive tract microorganisms and promote intestinal health with prebiotic

potential (Shang et al., 2018). At the genus level, β-1,3-glucan did not have a significant effect on the top 10 dominant genera in relative abundance. *Faecalibacterium* (genus Faecalibacterium) had the highest relative abundance in the control group, suggesting that *Clostridium perfringens* attack reduced this potentially beneficial bacterium. It can produce short-chain fatty acids, mainly butyric acid, which promotes the normal development of the host immune system (Miquel et al., 2013). It has been shown that the relative abundance of *Faecalibacterium* is much lower in the gut of patients with Crohn's disease (Willing et al., 2011).

LEfSe analysis showed a higher abundance of *Faecalibacterium* in the cecum of normal broiler chickens, implying that *Faecalibacterium* spp. has potential as a biomarker to detect the presence of enteritis in the intestine of livestock. Many studies have shown that *Roseburia*, a genus with high butyric acid production, plays an important role in the control of intestinal inflammatory processes. It has been shown that *Roseburia* is closely related to the production of intestinal anti-inflammatory regulatory T cells, which play an important role in the repair of host epithelial cells and in the regulation of inflammatory processes (Willing et al., 2011). In this experiment, the relative abundance of *Clostridium* was higher in the attack group compared to the control group at 21 d of age, and its relative abundance decreased with the addition of β-1,3-glucan, indicating that β-1,3-glucan could inhibit the colonization of *Clostridium perfringens* in the intestine to some extent and relieve its damage to the intestine. *Lactobacillus paracasei DG* reduces the abundance of *Clostridium* in feces and regulates fecal butyrate to promote gastrointestinal health (Ferrario et al., 2014). Turunen et al. (2011) showed that long-term dietary intervention with β-glucan in patients with enteritis significantly reduced the abundance of *C. perfringens* in the intestine, which is consistent with the results in the present study. Therefore, β-1,3-glucan mainly repairs enteritis damage by reducing the number of enteritis pathogenic bacteria *Clostridium* and increasing probiotics such as *Faecalibacterium*.

In summary, the NE can weaken the absorption of nutrients in the small intestine of broiler chickens, destabilize the integrity of the intestinal mucosal barrier and the structure of the cecum microbiota. Dietary 250 mg/kg β-1,3-glucan addition benefit for maintaining the intestinal health to a certain extent through alleviating the intestinal mucosal damage caused by C.P infection.

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DISCLOSURES

We declare that there are no financial or personal relationships between our study and other organizations or person that can influence our work. And there is no professional or other personal interest of any nature or kind in any product, service or company that could influence the position we presented in, or the review of, the manuscript entitled.

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