Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.2 / 001-012

http://dx.doi.org/10.1590/1806-9061-2022-1721

Original Article

Ganoderma Lucidum Extract Modulates Cecal Microbiota Community in Broilers under Dextran Sulfate Sodium Challenge During the Starter Phase

■Author(s)

Chuang KB[I] https://orcid.org/0000-0003-4094-5781
Yu YH[I] https://orcid.org/0000-0001-9629-8478

[I] Department of Biotechnology and Animal Science, National Ilan University, Yilan, 26047, Taiwan.

■Mail Address

Corresponding author e-mail address
Yu-Hsiang Yu
No. 1, Sec. 1, Shennong Rd., Yilan City, Yilan County, Zip code: 26047, Taiwan.
Phone: +886-3-931-7716
Email: yuyh@niu.edu.tw

■Keywords

Broiler, dextran sulfate sodium, *Ganoderma lucidum*, inflammation, microbiota.

Submitted: 09/September/2022
Approved: 02/January/2023

ABSTRACT

In this study, we investigated the effects of *Ganoderma lucidum* extract (GLE) supplementation on the cecal microbiota of broilers challenged with dextran sulfate sodium (DSS) during the starter phase. A total of 32 one-day-old, unsexed broiler chicks were randomly divided into four dietary treatments with eight birds per treatment and reared individually for 14 days (n = 8). The diet treatments were: non-DSS challenge, DSS challenge only, DSS challenge plus 0.5 mL/L GLE, and DSS challenge plus 1 mL/L GLE. The results showed that DSS challenge plus 0.5 mL GLE alleviated inflammatory gene expression in the duodenum of broilers ($p \leq 0.01$). The alpha diversity of bacterial species in the cecal digesta increased in the group treated with DSS plus 1 mL/L GLE compared with the DSS challenge-only group ($p \leq 0.01$). Principal component analysis and principal coordinate analysis indicated distinct clusters between groups treated with DSS-only and DSS plus GLE (0.5 and 1 mL/L). The abundance of the genera *Ruminiclostridium 9*, *Enterococcus*, and *Sellimonas* increased in the group treated with DSS plus GLE (0.5 and 1 mL/L) compared with the other groups ($p \leq 0.01$). Comparative microbial function analysis demonstrated that the immune system was promoted in the group treated with DSS plus GLE (0.5 and 1 mL/L) compared to the DSS challenge-only group ($p \leq 0.001$). These results demonstrated that GLE supplementation can modulate the cecal microbial community of broilers under DSS challenge during the starter phase.

INTRODUCTION

In poultry production, sub-therapeutic doses of antibiotics can be used to promote growth and protect health by inhibiting intestinal pathogen growth and modulating the immune status (Lee *et al*., 2012). The mechanism of antibiotic action may involve the prevention of gastrointestinal infections and microbiota modification in the intestines of broilers. However, in 2006, the European Union banned the use of antibiotic growth promoters in animal feed. During the starter phase, broiler chicks are much more susceptible to infection by pathogenic bacteria owing to immature immunity and gut microbiota (Wickramasuriya *et al*., 2022). Therefore, there is an urgent need to develop alternatives to antibiotics to reduce immunological stress and modulate the gut microbiota to provide optimal nutrient utilization for growth in broilers during the starter phase.

Probiotics, prebiotics, and medicinal plants have gained interest as potential alternatives to antibiotics for poultry (Aljumaah *et al*., 2020; Chen & Yu, 2020; Alkhulaifi *et al*., 2022). *Ganoderma lucidum* has been recognized as a medicinal mushroom and exhibits multiple pharmacological functions, including immunomodulatory, antitumor,

antimicrobial, and antioxidant properties (Cör *et al*., 2018). The primary bioactive compounds in *G. lucidum* are triterpenoids and polysaccharides (Yang *et al*., 2007; Wu & Wang, 2009). Polysaccharides purified from *G. lucidum* can induce immune cell proliferation and cytokine production (Mao *et al*., 1999; Chen *et al*., 2004). In broilers, dietary supplementation with *G. lucidum* extract (GLE) improves growth performance and modulates the gut microbiota (Ogbe *et al*., 2008; Ogbe *et al*., 2009; Sofyan *et al*., 2012; Liu *et al*., 2016; Chen & Yu, 2020). GLE supplementation promotes immunity in broilers (AL-Zuhariy & Hassan, 2017; Chen & Yu, 2020). Dietary supplementation with GLE can modulate gut morphology and microbial composition of broilers under lipopolysaccharide challenge (Chuang & Yu, 2022).

Dextran sulfate sodium (DSS), a synthetic sulfated polysaccharide, is commonly used to mimic chronic gut inflammation and microbial dysbiosis in rodents (Laroui *et al*., 2012; Håkansson *et al*., 2015; Shen *et al*., 2021). It has been proposed that the toxic effect of DSS induces intestinal inflammation and is likely the result of damage to the intestinal epithelial cells allowing the dissemination of proinflammatory intestinal contents into underlying tissue (Yan *et al*., 2009). Administration of DSS to the drinking water of broilers causes intestinal bleeding, diarrhea, and body weight loss (Menconi *et al*., 2015; Kuttappan *et al*., 2016; Chen & Yu, 2022). The addition of DSS to the drinking water of broilers disrupts the intestinal structure, promotes an inflammatory response, and induces necrotic enteritis (Menconi *et al*., 2015; Chen & Yu, 2022).

The intestinal microbiota plays a critical role in the immune response and nutrient utilization (Diaz Carrasco *et al*., 2019). Disturbance of the gut microbiota attenuates nutrient metabolism and the immune system, leading to impaired growth performance in broilers (Diaz Carrasco *et al*., 2019). Our previous study demonstrated that GLE supplementation boosted immunity and modulated gut microbial composition in broilers (Chen & Yu, 2020). Dietary supplementation with GLE modulates gut morphology and cecal microbiota of broilers under lipopolysaccharide challenge (Chuang & Yu, 2022). However, little is known about whether GLE supplementation can reverse the DSS-induced disturbance of gut microbial composition in broilers.

Therefore, this study was designed to examine the effects of GLE supplementation on the cecal microbiota community in broilers under DSS challenge during the starter phase. The results provide a theoretical basis for the use of GLE for the alleviation of inflammation-induced microbial dysbiosis in the poultry industry.

MATERIALS AND METHODS

The animal protocol was approved by the National Ilan University Institutional Animal Care and Use Committee (IACUC, protocol number 109-9).

G. lucidum extract preparation

GLE is a commercially available poultry feed additive (Life Rainbow Biotech, Yilan, Taiwan). GLE from powdered fruiting bodies was prepared using a hot water extraction method, and the polysaccharide concentration in GLE was verified using phenol-sulfuric acid. The polysaccharide quantity in GLE was 40 mg/kg (Chen & Yu, 2020).

Animal experiment

Thirty-two one-day-old healthy unsexed Arbor Acres broiler chicks (43.6 ± 1.18 g) were obtained from a commercial hatchery and randomly assigned to four treatments, with eight birds per treatment. The broilers were reared individually in stainless-steel cages. The experimental diets were: non-DSS challenge (C), DSS challenge only (D), DSS challenge plus 0.5 mL/L GLE (LD), and DSS challenge plus 1 mL/L GLE (HD). GLE was supplied to the chickens' drinking water from days 1 to 14, and DSS (molecular weight = 40,000; Bioman, New Taipei City, Taiwan) was supplied to the chickens' drinking water from days 3 to 14. All the treatment groups had free access to feed and water. Diets were formulated to meet the nutrient requirements recommended by the National Research Council (1994) (Table 1). No antibiotics or coccidiostats were included in the diet. During the experimental period, the house temperature and lighting schedule were provided according to Arbor Acres broiler management guidelines. Birds were immunized against Newcastle disease (ND) and infectious bursal disease (IBD) at 4 days of age. Body weight and feed intake of the birds were measured daily. Growth performance (daily gain, daily feed intake, and feed conversion ratio) was evaluated in two phases (days 1–7 and days 8–14). Mortality was recorded daily throughout the experiment.

Gut morphology analysis

At 14 days of age, six birds per group were randomly chosen (n = 6) and sacrificed by carbon dioxide inhalation to collect the small intestine and cecum, as previously described (Chen & Yu, 2020).

Table 1 – Composition of basal diets.

Item	Day 1 to 14
Ingredient, g kg^{-1}, as fed basis	
Corn, yellow	554.2
Soybean meal	355.2
Fish meal	39.9
Vegetable oil	35.2
Limestone	15.2
Salt	3.0
Monocalcium phosphate	9.2
Mineral premix[1]	2.0
Vitamin premix[2]	2.0
DL-methionine	2.0
L-lysine	1.0
Choline chloride	0.5
Calculated value, g kg^{-1}	
Dry matter	88.9
Crude protein	221.6
Analyzed calcium	10.2
Analyzed total phosphorus	6.9
Lysine	11.2
Methionine+Cystine	8.5
ME, kcal/kg	3081.1

[1]Supplied per kg of diet:32 mg of Mn ($MnSO_4 \cdot H_2O$), 16 mg of Fe ($FeSO_4 \cdot 7H_2O$), 24 mg of Zn (ZnO), 2 mg of Cu ($CuSO_4 \cdot 5H_2O$), 800 µg of I (KI), 200 µg of Co ($CoSO_4$), and 60 µg of Se.

[2]Supplied per kg of diet:1.8 mg of all-trans-retinyl acetate, 0.02 mg of cholecalciferol, 8.3 mg of alpha-tocopheryl acetate, 2.2 mg of menadione, 2 mg of pyridoxine HCl, 8 mg of cyanocobalamin, 10 mg of nicotine amid, 0.3 mg of folic acid, 20 mg of D-biotin, and 160 mg of choline chloride.

Small intestinal segment samples were embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin. Ten well-oriented villi from each sample were selected to measure the gut morphology using an Olympus CX43 microscope (Olympus Corporation, Tokyo, Japan). The cecal tissue was washed in phosphate-buffered saline, and the length was measured.

Quantitative reverse transcription polymerase chain reaction

At 14 days of age, three broilers per group were randomly chosen (n = 3) and sacrificed by carbon dioxide inhalation at the end of the experiment. Total RNA was isolated from the small intestine (duodenum, jejunum, and ileum) samples of broilers separately using the REzol reagent extraction method (Protech Technology Enterprise, Taipei City, Taiwan), according to the manufacturer's protocol. cDNA was synthesized from total RNA using an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The expression of inflammatory and internal control genes was determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The sequences of the forward and reverse oligonucleotide primers were as follows: cyclooxygenase 2 (*cox2*):5'-AAC ACA ATA GAG TCT GTG ACG TCT T-3' and 5'-TAT TGA ATT CAG CTG CGA TTC GG-3'; inducible nitric oxide synthase (*inos*):5'-AGG CCA AAC ATC CTG GAG GTC-3' and 5'-TCA TAG AGA CGC TGC TGC CAG-3'; interleukin 6 (*il-6*):5'-AGG ACG AGA TGT GCA AGA AGT TC-3' and 5'-TTG GGC AGG TTG AGG TTG TT-3'; *18S* rRNA:5'-ATA ACG AAC GAG ACT CTG GCA-3' and 5'-CGG ACA TCT AAG GGC ATC ACA-3'. The reaction was performed using Miniopticon Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and iQ SYBR Green Supermix kit (Bio-Rad, Hercules, CA). *18S* rRNA was used as an internal control. Gene expression levels were normalized to *18S* rRNA expression to obtain the relative expression levels using the $2^{-\Delta\Delta Ct}$ method.

16S ribosomal RNA gene sequencing

Cecal digesta from broilers were freshly collected and four replicates per group (n = 4) were used for 16S ribosomal RNA (rRNA) gene sequencing. Total genomic DNA was extracted using a ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA, USA). DNA concentration and quantity were measured using a spectrophotometer (NanoDrop, Wilmington, DE, USA). The distinct V3-V4 regions of the 16S rRNA genes were amplified using specific primers (5'-GTGCCAGCMGCCGCGGTAA-3' and 5'-GGACTACHVGGGTWTCTAAT-3'). The PCR products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Germantown, MD, USA) and used for library construction. 16S rRNA gene sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) and 300 bp paired-end reads were generated.

Sequence filtering and taxonomic assignments

Raw reads were pre-processed to remove adapters and low-quality reads using QIIME 2 software (version 2017.4). After quality control, chimera reads were removed, and overlapped paired-end clean reads were merged using the UCHIME software (version 4.2). Bacterial operational taxonomic units (OTUs) were assigned at 97% sequence similarity using Mothur software (version 1.39.5). Bacterial OTU taxa were classified using the Genomes Online Database (Gold. jgi. doe. gov). Alpha diversity (bacterial species richness and evenness) and beta diversity were analyzed using QIIME 2 software. Beta diversity was analyzed using unweighted and weighted UniFrac metrics

and distances were visualized by principal coordinate analysis (PCoA). Principal component analysis (PCA) ordinations were used to visualize the clustering of samples based on their genus-level compositional profiles. PICRUSt software (version 1.1.4) was used to predict the functional enrichment of the microbial communities in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Statistical analysis

Data were analyzed using one-way ANOVA followed by multiple comparisons using Tukey's honest significant difference test to detect statistically significant differences between groups. A significance was set at $p \leq 0.05$. PCA and PCoA based on UniFrac distance and permutational multivariate analysis of variance (PERMANOVA) were performed to compare microbiota composition.

RESULTS

Effect of *G. lucidum* extract on growth performance and inflammation-associated gene expression of broilers in response to dextran sulfate sodium challenge

The effects of dietary GLE supplementation on the growth performance of broilers under DSS challenge are shown in Table 2. No significant differences in growth performance (body weight, daily gain, daily feed intake, and feed conversion ratio) were observed between the groups during the experimental period. The survival rate of broilers fed only a basal diet, DSS challenge-only, DSS challenge plus 0.5 mL/L GLE, or DSS challenge plus 1 mL/L GLE were 100%, 75%, 87.5%, and 100%, respectively (Table 2). The effects of dietary GLE supplementation on the gut morphology of broilers under DSS challenge are shown in Table 3. Villus length was reduced in the duodenum of broilers challenged with DSS plus 1 mL/L GLE compared with the C and LD groups ($p \leq 0.05$). No significant differences in jejunal and ileal morphology were found among the groups (Table 3). DSS challenges (D, LD, and HD) decreased the cecum length of broilers compared with the C group ($p \leq 0.001$). The effect of dietary GLE supplementation on inflammation-associated gene expression in the small intestine of DSS-challenged broilers is shown in Table 4. DSS challenge-only increased the *cox2* mRNA expression in the duodenum of broilers ($p \leq 0.01$); whereas the *cox2* mRNA expression was reduced in broilers challenged with DSS plus 0.5 mL/L GLE ($p \leq 0.01$). The *inos* mRNA expression was increased in the duodenum of broilers challenged with DSS ($p \leq 0.01$), whereas GLE supplementation (0.5 and 1 mL/L) decreased the *inos* mRNA expression. DSS challenge only increased *il-6* mRNA expression in the duodenum of broilers compared to the C group ($p \leq 0.05$). The *cox2* mRNA expression was induced in the jejunum of broilers challenged with DSS (with or without GLE) ($p \leq 0.01$). DSS challenge only increased *il-6* mRNA expression

Table 2 – Effect of *Ganoderma lucidum* extract on the growth performance of broilers under dextran sulfate sodium challenge.

	C[1]	D	LD	HD	SEM	*p* value
Body weight (g/bird)						
1 d	43.5[2]	43.8	43.6	43.5	0.22	0.714
7 d	126.6	125.3	126.2	130.4	3.23	0.217
14 d	382.3	331.4	318.9	316.4	10.84	0.236
Daily gain (g/d/bird)						
1-7 d	11.9	11.3	11.8	12.4	0.47	0.225
8-14 d	36.5	29.4	27.5	26.6	1.27	0.114
1-14 d	24.2	20.5	19.7	19.5	0.78	0.232
Daily feed intake (g/d/bird)						
1-7 d	13.9	13.7	12.8	13.7	0.52	0.873
8-14 d	45.6	44.9	44.4	36.5	1.99	0.572
1-14 d	29.8	29.3	28.6	25.1	1.18	0.697
Feed conversion ratio						
1-7 d	1.2	1.2	1.1	1.1	0.05	0.620
8-14 d	1.2	1.7	1.6	1.5	0.09	0.684
1-14 d	1.2	1.5	1.4	1.3	0.07	0.723
Survival rate						
1-14 d (%)	100.0	75.0	87.5	100.0	5.24	0.278

[1]C = Basal diet; D = Basal diet plus dextran sulfate sodium challenge; LD = Basal diet plus 0.5 mL/L GLE and DSS challenge; HD = Basal diet plus 1.0 mL/L GLE and DSS challenge.

[2]Data are mean values of 8 replicates (n = 8).

Table 3 – Effect of *Ganoderma lucidum* extract on the gut morphology of broilers under dextran sulfate sodium challenge.

		C[1]	D	LD	HD	SEM	*p* value
Duodenum	Villus length (μm)	1200.4[2,a]	1122.1[ab]	1193.2[a]	1039.6[b]	21.77	0.019
	Crypt depth (μm)	132.3	126.6	125.2	101.0	4.93	0.106
	Villus length: Crypt depth	9.1	9.2	9.9	10.4	0.34	0.572
Jejunum	Villus length (μm)	584.5	613.0	659.9	573.2	18.91	0.391
	Crypt depth (μm)	120.9	105.2	103.8	93.9	6.23	0.518
	Villus length: Crypt depth	5.2	6.6	6.3	6.2	0.32	0.448
Ileum	Villus length (μm)	415.3	475.0	504.9	460.8	17.23	0.334
	Crypt depth (μm)	113.8	88.2	118.0	96.7	7.10	0.420
	Villus length: Crypt depth	3.9	6.2	4.4	5.1	0.35	0.086
Cecum length (cm)		9.2[a]	5.7[b]	5.8[b]	5.6[b]	0.34	≤ 0.001

[1]C = Basal diet; D = Basal diet plus dextran sulfate sodium challenge; LD = Basal diet plus 0.5 mL/L GLE and DSS challenge; HD = Basal diet plus 1.0 mL/L GLE and DSS challenge.

[2]Data are mean values of 6 replicates (n = 6).

[a-b]Means of a row with no common superscript are significantly different ($p \leq 0.05$).

in the jejunum of broilers compared to the C group ($p \leq 0.05$). The *il-6* mRNA expression was increased in the ileum of broilers challenged with DSS plus 0.5 mL/L GLE compared with the C and D groups ($p \leq 0.01$).

Table 4 – Effect of *Ganoderma lucidum* extract on the intestinal inflammatory gene expression of broilers under dextran sulfate sodium challenge.

	C[1]	D	LD	HD	SEM	*p* value
Duodenum						
cox2	0.81[2,c]	9.9[a]	2.2[bc]	6.9[ab]	1.18	0.007
inos	0.8[b]	5.2[a]	0.7[b]	2.2[b]	0.58	0.006
il-6	0.7[b]	57.2[a]	36.5[ab]	49.8[ab]	8.50	0.047
Jejunum						
cox2	1.2[b]	24.2[a]	17.2[a]	16.7[a]	2.97	0.005
inos	0.8	5.0	3.2	3.3	0.76	0.133
il-6	1.2[b]	788.7[a]	379.1[ab]	466.1[ab]	102.13	0.020
Ileum						
cox2	0.8	52.8	35.6	29.9	8.53	0.255
inos	1.2	8.1	12.6	2.6	2.18	0.159
il-6	1.0[c]	577.3[bc]	1610.4[a]	969.2[ab]	195.21	0.004

[1]C = Basal diet; D = Basal diet plus dextran sulfate sodium challenge; LD = Basal diet plus 0.5 mL/L GLE and DSS challenge; HD = Basal diet plus 1.0 mL/L GLE and DSS challenge.

[2]Data are mean values of 3 replicates (n = 3).

[a-c]Means of a row with no common superscript are significantly different ($p \leq 0.05$).

Effect of *G. lucidum* extract on cecal bacterial community composition of broilers in response to dextran sulfate sodium challenge

The average high-quality reads from the cecal digesta of C, D, LD, or HD were 23402, 23344, 21979, and 24187, respectively. DSS challenge only decreased bacterial species richness (Chao1 and Fisher alpha estimator) in the cecal digesta compared to the C group ($p \leq 0.01$) (Table 5). The bacterial species richness increased in the cecal digesta of broilers challenged with DSS plus 1 mL/L GLE compared with the D group

Table 5 – Microbial diversity in the cecal contents of broilers under dextran sulfate sodium challenge.

	C[1]	D	LD	HD	SEM	*p* value
Chao1	48.8[2,a]	37.5[c]	41.3[bc]	46.0[ab]	1.23	0.003
Fisher alpha	5.1[a]	3.8[c]	4.3[bc]	4.8[ab]	0.14	0.002
Shannon	2.6[bc]	2.4[c]	2.7[b]	3.1[a]	0.07	0.001
Enspie	3.4[b]	3.5[b]	4.4[a]	4.8[a]	0.19	0.004

[1]C = Basal diet; D = Basal diet plus dextran sulfate sodium challenge; LD = Basal diet plus 0.5 mL/L GLE and DSS challenge; HD = Basal diet plus 1.0 mL/L GLE and DSS challenge.

[2]Data are mean values of 4 replicates (n = 4).

[a-c]Means of a row with no common superscript are significantly different ($p \leq 0.05$).

($p \leq 0.01$). GLE supplementation (0.5 and 1 mL/L) plus DSS challenge increased bacterial species evenness (Shannon and Enspie estimator) in the cecal digesta of broilers compared with the D group ($p \leq 0.01$). PCA was conducted to examine the functional distinction of the microbiota and revealed significant discrimination among the groups (Fig. 1A). The PCoA of qualitative traits (unweighted UniFrac distances) and quantitative traits (weighted UniFrac distances) indicated that the microbiota of cecal samples was separated among the groups (Fig. 1B and 1C).

Effects of *G. lucidum* extract on cecal bacterial taxonomic distribution in broilers in response to dextran sulfate sodium challenge

The results of bacterial taxonomy in the cecal digesta of broilers challenged with DSS are shown in Table 6. The abundance of the phylum Firmicutes decreased and the phylum Proteobacteria increased in the D group compared with the other groups ($p \leq 0.001$). DSS challenge plus GLE supplementation (0.5 and 1 mL/L) promoted the abundance of the phylum Firmicutes and reduced the abundance of the phylum Proteobacteria in the cecal digesta of broilers compared to the D

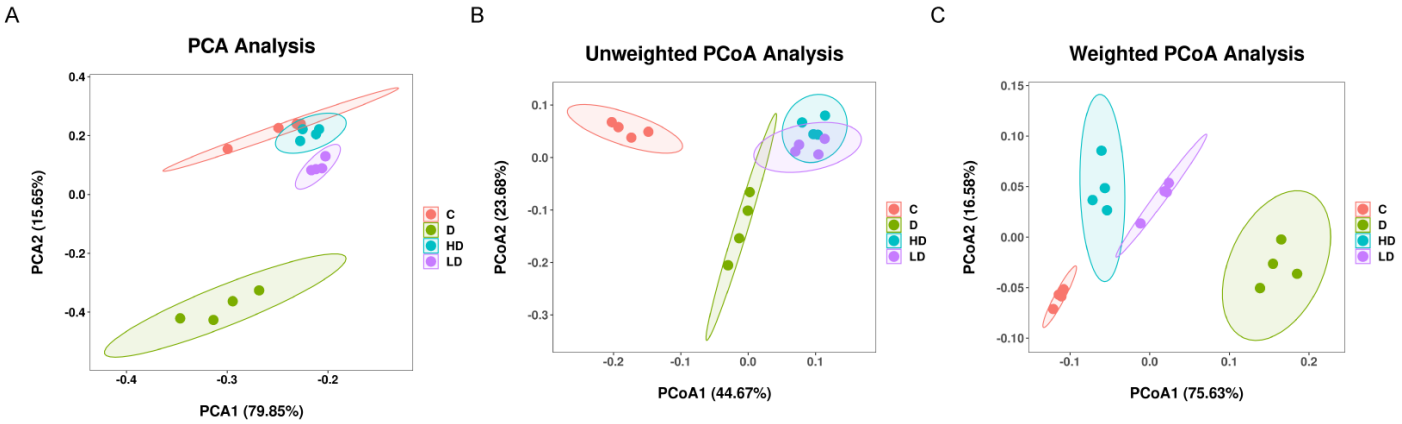

Figure 1 – Advanced analysis of the bacterial communities in the cecal digesta of broilers under dextran sulfate sodium challenge. (A) Principal component analysis of the cecal digesta of broilers in non-DSS challenge group (C), DSS challenge only group (D), DSS challenge plus 0.5 mL/L GLE group (LD), and DSS challenge plus 1 mL/L GLE group (HD) (n = 4). Principal coordinate analysis of quantitative traits (unweighted UniFrac distances) (B) and qualitative traits (weighted UniFrac distances) (C) of the cecal bacterial communities from C, D, LD, and HD (n = 4).

Table 6 – Bacterial taxonomy within the cecal contents of broilers under dextran sulfate sodium challenge.

	Relative abundance (%)					
	C[1]	D	LD	HD	SEM	*p* value
Phylum						
Firmicutes	93.6[2,a]	61.9[d]	82.1[c]	91.1[b]	3.22	≤ 0.001
Proteobacteria	6.3[d]	38.1[a]	17.9[b]	8.7[c]	3.24	≤ 0.001
Genus						
Lachnospiraceae_unclassified	47.3[a]	37.4[b]	39.1[b]	37.3[b]	1.27	≤ 0.001
Ruminococcus torques group	27.2[a]	8.8[d]	17.3[c]	23.3[b]	1.81	≤ 0.001
Escherichia-Shigella	6.1[c]	36.6[a]	14.9[b]	8.1[c]	3.12	≤ 0.001
Ruminiclostridium 9	3.6[c]	2.9[c]	12.7[a]	5.7[b]	1.01	≤ 0.001
Enterococcus	0.5[d]	2.6[c]	4.8[b]	8.3[a]	0.76	≤ 0.001
Sellimonas	1.8[c]	1.6[c]	2.8[b]	4.0[a]	0.25	≤ 0.001
Erysipelatoclostridium	0.3[b]	4.2[a]	0.1[b]	0.4[b]	0.52	0.006
Enterobacteriaceae_unclassified	0.2[d]	1.4[b]	3.0[a]	0.5[c]	0.28	≤ 0.001
Butyricicoccus	2.0[a]	0.5[b]	0.8[b]	0.7[b]	0.16	≤ 0.001
Flavonifractor	0.4[c]	1.4[a]	0.9[b]	1.2[ab]	0.10	≤ 0.001
Negativibacillus	1.6[a]	0.1[b]	0.1[b]	1.6[a]	0.20	≤ 0.001
Oscillibacter	1.0[a]	0.4[b]	0.3[b]	1.1[a]	0.10	≤ 0.001

[1]C = Basal diet; D = Basal diet plus dextran sulfate sodium challenge; LD = Basal diet plus 0.5 mL/L GLE and DSS challenge; HD = Basal diet plus 1.0 mL/L GLE and DSS challenge.

[2]Data are mean values of 4 replicates (n = 4).

[a-d]Means of rows with no common superscript are significantly different ($p \leq 0.05$).

group ($p \leq 0.001$). At the genus level, DSS challenges (D, LD, and HD groups) decreased the abundance of the genera *Lachnospiraceae_unclassified* and *Butyricicoccus* compared to the C group ($p \leq 0.001$). The abundance of the genera *Escherichia-Shigella* and *Erysipelatoclostridium* was higher, and the abundance of the genus *Ruminococcus torques group* was lower in the D group than in the other groups ($p \leq 0.01$). DSS challenge plus GLE supplementation (0.5 and 1 mL/L) increased the abundance of the genera *Ruminococcus torques group*, *Ruminiclostridium 9*, *Enterococcus*, and *Sellimonas* in the cecal digesta of broilers compared with the D group ($p \leq 0.001$). The abundance of the genus *Enterobacteriaceae_unclassified* in the LD group was higher than that in the other groups ($p \leq 0.001$). DSS challenge only increased the abundance of the genus *Flavonifractor* compared to the LD group ($p \leq 0.001$). The abundances of the genera *Negativibacillus* and *Oscillibacter* were lower in the D and LD groups than in the other groups ($p \leq 0.001$). An overview of the heat map of the 35 most abundant genera in the cecal digesta is shown in Fig. 2A. The results of the heat map showed that similar bacterial community clusters, such as *Flavonifractor*, *Romboutsia*, *Clostridium sensu stricto 1*, *Blautia*, *Pseudoflavonifractor*, *Enterococcus*, and *Peptostreptococcaceae_unclassified*, were observed in the D, LD, and HD groups. Some bacterial community clusters were specifically decreased in group D, such as the genera *Ruminococcus torques group*, *Eisenbergiella*, *Weissella*, and *Ruminococcaceae UCG-*

013. Some bacterial community clusters, such as the genera *Intestinimonas*, *Sellimonas*, and *Pediococcus*, were specifically increased in the LD and HD groups. DSS challenge only resulted in unique bacterial community clusters compared to other groups, such as *Erysipelatoclostridium* and *Escherichia-Shigella*. The functional prediction of the cecal microbiota is presented in Fig. 2B. The results demonstrated that some microbial functions were specifically decreased in group D, such as membrane transport, biosynthesis of other secondary metabolites, immune system, transcription, lipid metabolism, and endocrine system. DSS challenge plus GLE supplementation (0.5 and 1 mL/L) increased cell motility, metabolism of terpenoids and polyketides, replication and repair, and transport and catabolism functions compared with

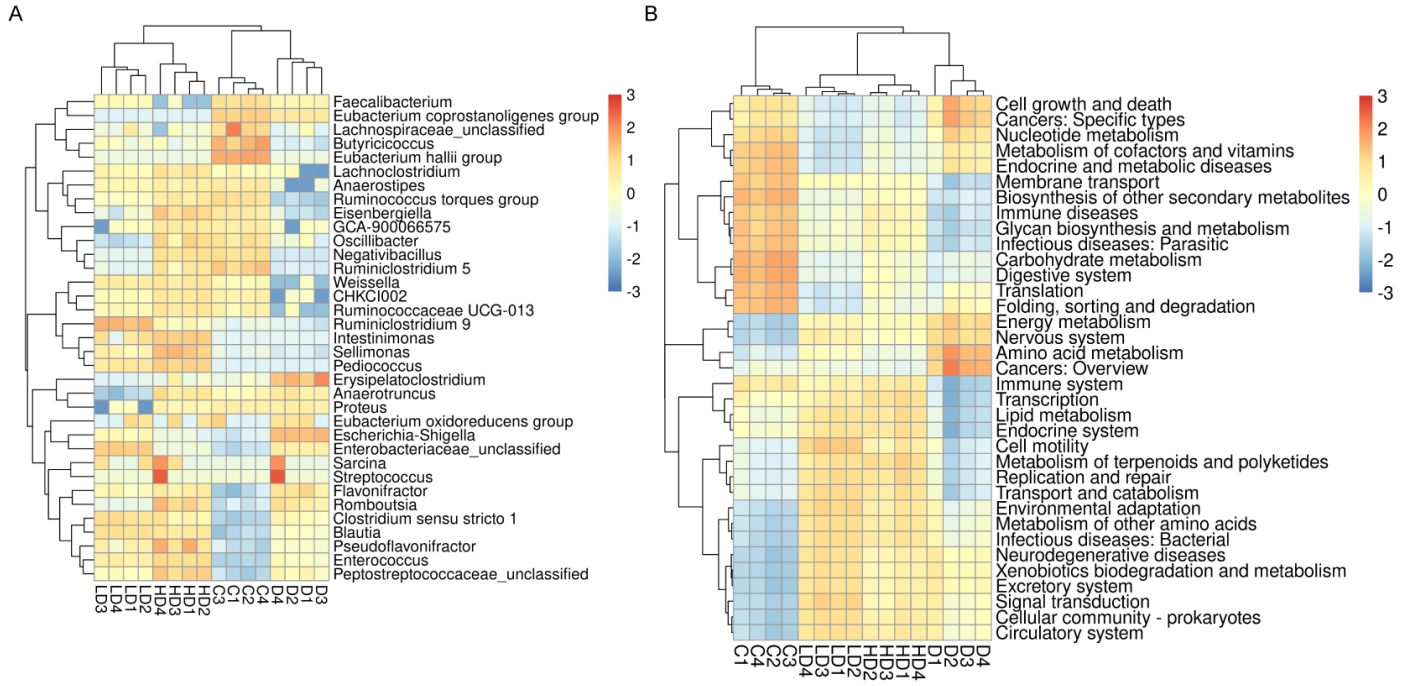

Figure 2 – Heat map of bacterial abundance distribution and microbial functions in the cecal digesta of broilers under dextran sulfate sodium challenge. (A) Species abundance heat map showing the abundance distribution of the dominant 35 genera of cecal digesta of broilers under DSS challenge. Samples from non-DSS challenge group (C), DSS challenge only group (D), DSS challenge plus 0.5 mL/L GLE group (LD), and DSS challenge plus 1 mL/L GLE group (HD) is plotted on the X-axis (n = 4), and the Y-axis represents the genus. (B) Differences in microbial functions based on KEGG functional categories in the cecal digesta of broilers under DSS challenge. Samples from C, D, LD, HD is plotted on the X-axis (n = 4), and the Y-axis represents the microbial functions.

the other groups. Cell growth and death, nucleotide metabolism, metabolism of cofactors and vitamins, and endocrine and metabolic disease's functions were reduced in the LD and HD groups compared to the other groups. DSS challenges (D, LD, and HD groups) increased neurodegenerative diseases, xenobiotics biodegradation and metabolism, and excretory system functions compared to the C group. The functional prediction of cecal microbiota is presented in Table 7. The results demonstrated that the immune system and translation were increased, and xenobiotics biodegradation and metabolism were decreased in the LD group compared with the other groups ($p \leq 0.001$). The metabolism of cofactors and vitamins was promoted, and infectious diseases and parasitic diseases were reduced in the D group compared with the other groups ($p \leq 0.05$). Transport and catabolism, substance dependence, and energy metabolism were decreased, and lipid metabolism, folding, sorting, and degradation were increased in the LD and HD groups compared with the other groups ($p \leq 0.001$).

DISCUSSION

Establishing a healthy intestinal microbial composition can prevent inflammation and provide optimal nutrient utilization for broiler growth (Pourabedin & Zhao, 2015). Host and environmental factors affect the intestinal microbiota of broilers (Kers *et al.*, 2018). Intestinal inflammation disrupts the balance and diversity of the intestinal microbiota, resulting in intestinal dysbiosis (Lupp *et al.*, 2007; Lobionda *et al.*, 2019). In poultry, it has been demonstrated that DSS challenge induces intestinal inflammation, leading to diarrhea, necrotic enteritis, and body weight loss (Menconi *et al.*, 2015; Kuttappan *et al.*, 2016; Nii *et al.*, 2020). In this study, inflammatory

Table 7 – Differences in microbial functions within the cecal digesta of broilers under dextran sulfate sodium challenge based on Kyoto Encyclopedia of Genes and Genomes functional categories.

	Relative abundance (%)					
	C[1]	D	LD	HD	SEM	*p*-value
Immune system	21.0[2,c]	21.1[c]	22.7[a]	22.0[b]	0.19	≤ 0.001
Xenobiotics biodegradation and metabolism	11.7[a]	10.7[b]	10.5[c]	10.9[b]	0.12	≤ 0.001
Metabolism of cofactors and vitamins	9.7[b]	10.2[a]	9.3[c]	9.4[c]	0.10	≤ 0.001
Transport and catabolism	6.4[a]	5.9[b]	4.5[c]	4.8[c]	0.20	≤ 0.001
Substance dependence	5.4[a]	5.6[a]	4.7[b]	4.7[b]	0.11	≤ 0.001
Lipid metabolism	4.2[b]	4.2[b]	4.6[a]	4.7[a]	0.06	≤ 0.001
Folding, sorting and degradation	3.8[c]	4.1[b]	4.6[a]	4.5[a]	0.08	≤ 0.001
Translation	3.7[c]	4.8[b]	5.3[a]	4.9[b]	0.15	≤ 0.001
Energy metabolism	3.0[a]	2.9[b]	2.5[c]	2.7[c]	0.05	≤ 0.001
Infectious diseases: Parasitic	3.0[ab]	2.9[b]	3.0[ab]	3.0[a]	0.01	0.03

[1]C = Basal diet; D = Basal diet plus dextran sulfate sodium challenge; LD = Basal diet plus 0.5 mL/L GLE and DSS challenge; HD = Basal diet plus 1.0 mL/L GLE and DSS challenge.

[2]Data are mean values of 4 replicates (n = 4).

[a-c]Means of a row with no common superscript are significantly different ($p \leq 0.05$).

gene expression in the small intestine was induced in the DSS challenge-only group, which is in agreement with a previous study (Nii *et al*., 2020). Studies have indicated that GLE polysaccharides have anti-inflammatory and immunomodulatory effects in DSS-treated mice (Wei *et al*., 2018). Our previous studies demonstrated that GLE supplementation in drinking water regulates the immune system of broilers (Chen & Yu, 2020; Chuang & Yu, 2022). Here, we further demonstrated that GLE supplementation (0.5 and 1 mL/L) reduced intestinal inflammation-associated gene expression in broilers under DSS challenge. In the microbiota analysis, the richness of bacterial species in the cecal digesta of broilers was reduced in the DSS challenge-only group. This observation agrees with the results of Guo *et al*. (2021), who observed that DSS challenge decreased the richness of bacterial species in the feces of mice (Guo *et al*., 2021). Research has demonstrated that *G. lucidum* can modulate the intestinal microbiota, which may be associated with the prevention of metabolic syndromes (Chang *et al*., 2015; Chen *et al*., 2020; Sang *et al*., 2021). Our previous study showed that GLE supplementation in drinking water reduced the richness and evenness of fecal microbiota in broilers (Chen & Yu, 2020). In this study, the richness of bacterial species was reduced in the cecal digesta of broilers under DSS challenge but reversed to normal levels when 1 mL/L of GLE was supplied in drinking water. Supplementation with GLE at 1 mL/L increased the evenness of bacterial species in the cecal digesta of DSS-treated broilers, implying that GLE may differentially regulate bacterial diversity in different physiological statuses (health and inflammation) of broilers. Taken together, our findings are consistent with those of other studies concluding that GLE induces an anti-inflammatory response and regulates gut microbial diversity in broilers under DSS challenge.

The dominant gut microbial phylum in broilers is Firmicutes, which is associated with nutrient digestion and absorption (Hou *et al*., 2016). The phylum Firmicutes has been reported to exhibit anti-inflammatory effects and reduce the abundance of Firmicutes associated with inflammatory bowel disease (Baxter *et al*., 2014; Natividad *et al*., 2015; Magne *et al*., 2020). The phylum Proteobacteria includes a wide variety of opportunistic pathogenic genera, and its increased abundance of the phylum Proteobacteria correlates with gut dysbiosis (Shin *et al*., 2015). Here, we demonstrated that the abundance of the phylum Firmicutes was reduced and that of the phylum Proteobacteria was increased in the DSS challenge-only group. These results suggest that DSS challenge can disrupt the balance of the cecal microbiota composition in broilers. A previous study showed that the abundance of the phylum Firmicutes increased and the abundance of the phylum Proteobacteria decreased in the small intestine and cecum of DSS-treated rats in response to *G. lucidum* polysaccharide treatment (Xie *et al*., 2019). Here, GLE supplementation also increased the abundance of Firmicutes and reduced the abundance of Proteobacteria in the cecal digesta of broilers under DSS challenge, which is in agreement with a previous study (Xie *et al*., 2019). At the genus level, the abundance of the genera *Escherichia-Shigella* and *Erysipelatoclostridium* specifically increased in the DSS challenge-only group in the present study. It has been suggested that the genus *Escherichia-Shigella*, a group of opportunistic pathogenic bacteria, can destroy intestinal structure and exert pro-inflammatory activities through the production of virulence factors

(Kaur & Ganguly, 2003; Aminshahidi *et al*., 2017). Members of the *genus Erysipelatoclostridium* are considered opportunistic pathogens and are associated with metabolic syndrome and gout (Shao *et al*., 2017; Zhao *et al*., 2019). These results indicated that DSS challenge may increase the abundance of pathogens in the gut of broilers. *G. lucidum* polysaccharides increase short-chain fatty acid-producing bacteria and reduce pathogens in the small intestine and cecum of DSS-treated rats (Xie *et al*., 2019). *G. lucidum* exhibits antimicrobial activity against pathogenic species of bacteria, such as *Escherichia coli* (Quereshi *et al*., 2010). Our previous study also demonstrated that GLE can induce antimicrobial peptide gene expression in broilers (Chen & Yu, 2020). Here, we demonstrated that the genus *Escherichia-Shigella* abundance was reduced in the cecal digesta of DSS-treated broilers in response to GLE supplementation. This observation is in agreement with the results of Xie *et al*. (2019), who observed that *G. lucidum* polysaccharide supplementation decreased the abundance of the genus *Escherichia-Shigella* in the small intestine and cecum of DSS-treated rats. In addition, the abundances of the genera *Ruminiclostridium_9* and *Sellimonas* were specifically elevated in the cecal digesta of DSS-treated broilers in response to GLE supplementation. It has been suggested that members of the genus *Ruminiclostridium_9*, a group of short-chain fatty acid-producing bacteria, can break down polysaccharides (Ravachol *et al*., 2016). Members of the genus *Sellimonas* express several genes involved in host nutrient transport and energy production (Muñoz *et al*., 2020). These findings demonstrate that DSS challenge not only induces the intestinal inflammatory response of broilers but also causes cecal microbiota imbalance, resulting in a profound increase in the pathogen community in the gut. GLE supplementation normalized the cecal microbiota of broilers under the DSS challenge. Whether this modification of gut microbiota caused by GLE has a direct impact on broiler growth remains to be confirmed.

In the present study, the cecum length of GLE-treated broilers under DSS challenge did not recover by the end of the experiment (14 days). This may explain why growth performance was not ameliorated in GLE-treated broilers under DSS challenge during the starter phase. The survival rate was increased in DSS-treated broilers in response to GLE supplementation (0.5 and 1 mL/L). DSS-induced inflammation-associated gene expression in the gut of broilers was also inhibited in GLE-treated broilers under a DSS challenge. The microbial diversity (species richness) in the cecal digesta of GLE-treated broilers (1 mL/L) had returned to normal levels. Furthermore, DSS-induced pathogens (genera *Escherichia-Shigella* and *Erysipelatoclostridium*) in the cecal digesta were reduced by GLE. Therefore, the beneficial effects of GLE on the growth performance of DSS-treated broilers may be observed after an extended feeding period.

The present study indicates that GLE supplementation alleviates DSS-induced chronic gut inflammation and microbial dysbiosis of broilers. However, caution must be taken when generalizing the results of the present small sample size to a larger population. For increasing the precision of GLE effects, an experiment using a large sample size may be needed in the future.

CONCLUSION

We demonstrated for the first time that GLE supplementation can normalize the cecal microbial community of broilers under DSS challenge during the starter phase by increasing the number of beneficial bacteria and decreasing the number of pathogens.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology [grant numbers MOST 109-2313-B-197-001] and Chung Cheng Agriculture Science and Social Welfare Foundation [grant numbers 111GC002] in Taiwan.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AL-Zuhariy MTB, Hassan WH. Hepatoprotective and immunostimulatory effect of *Ganoderma*, *Andrographolide* and *Turmeric* against Aflatoxicosis in broiler chickens. International Journal of Poultry Science 2017;16:281-7.

Aljumaah MR, Alkhulaifi MM, Abudabos AM, Aljumaah RS, Alsaleh AN, Stanley D. *Bacillus subtilis* PB6 based probiotic supplementation plays a role in the recovery after the necrotic enteritis challenge. PLoS One 2020;15(6):e0232781.

Alkhulaifi MM, Alqhtani AH, Alharthi AS, Al Sulaiman AR, Abudabos AM. Influence of prebiotic yeast cell wall extracts on growth performance, carcase attributes, biochemical metabolites, and intestinal morphology and bacteriology of broiler chickens challenged with *Salmonella typhimurium* and *Clostridium perfringens*. Italian Journal of Animal Science 2022;21:1190-9.

Aminshahidi M, Arastehfar A, Pouladfar G, Arman E, Fani F. Diarrheagenic *Escherichia coli* and *Shigella* with high rate of extended-spectrum beta-lactamase production:two predominant etiological agents of acute diarrhea in Shiraz, Iran. Microbial Drug Resistance 2017;23:1037-44.

Baxter NT, Zackular JP, Chen GY, Schloss PD. Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. Microbiome 2014;17:20.

Chang CJ, Lin CS, Lu CC, Martel J, Ko YF, Ojcius DM, et al. *Ganoderma lucidum* reduces obesity in mice by modulating the composition of the gut microbiota. Nature Communications 2015;6:7489.

Chen HS, Tsai YF, Lin S, Lin CC, Khoo KH, Lin CH, et al. Studies on theimmuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi)polysaccharides. Bioorganic & Medicinal Chemistry 2004;12:5595-601.

Chen HW, Yu YH. Effect of *Ganoderma lucidum* extract on growth performance, fecal microbiota, and bursal transcriptome in broilers. Animal Feed Science and Technology 2020;267:114551.

Chen M, Xiao D, Liu W, Song Y, Zou B, Li L, et al. Intake of *Ganoderma lucidum* polysaccharides reverses the disturbed gut microbiota and metabolism in type 2 diabetic rats. International Journal of Biological Macromolecules 2020;155:890-902.

Chen JY, Yu YH. *Bacillus subtilis*-fermented products ameliorate the growth performance, alleviate intestinal inflammatory gene expression, and modulate cecal microbiota community in broilers during the starter phase under dextran sulfate sodium challenge. Journal of Poultry Science 2022;59:260-71.

Chuang KB, Yu YH. *Ganoderma lucidum* extract regulates gut morphology and microbial community in lipopolysaccharide-challenged broilers. Brazilian Journal of Poultry Science 2022;24:1-10.

Cör D, Knez Ž, KnezHrnčič M. Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of *Ganoderma lucidum* terpenoids and polysaccharides: a review. Molecules 2018;23(3):649.

Diaz Carrasco JM, Casanova NA, Fernández Miyakawa ME. Microbiota, gut health and chicken productivity: what is the connection? Microorganisms 2019;7:374.

Guo C, Guo D, Fang L, Sang T, Wu J, Guo C, et al. *Ganoderma lucidum* polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon. Carbohydrate Polymers 2021;267:118231.

Håkansson Å, Tormo-Badia N, Baridi A, Xu J, Molin G, Hagslätt ML, et al. Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice. Clinical and Experimental Medicine 2015;15:107-20.

Hou Q, Kwok LY, Zheng Y, Wang L, Guo Z, Zhang J, ET AL. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. Scientific Reports 2016;6:37376.

Kaur T, Ganguly NK. Modulation of gut physiology through enteric toxins. Molecular and Cellular Biochemistry 2003;253:15-19.

Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Stegeman JA, Smidt H. Host and environmental factors affecting the intestinal microbiota in chickens. Frontiers in Microbiology 2018;9:235.

Khan M, Chand N, Naz S, Khan RU. Dietary tea tree (*Melaleuca alternifolia*) essential oil as alternative to antibiotics alleviates experimentally induced Eimeria tenella challenge in Japanese quails. Journal of Animal Physiology and Animal Nutrition; 2022. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jpn.13719.

Kuttappan VA, Vicuña EA, Faulkner OB, Huff GR, Freeman KA, Latorre JD, et al. Evaluation of changes in serum chemistry in association with feed withdrawal or high dose oral gavage with dextran sodium sulfate- (DSS-) induced gut leakage in broiler chickens. Poultry Science 2016;95:2565-9.

Laroui H, Ingersoll SA, Liu HC, Baker MT, Ayyadurai S, Charania MA, et al. Dextran sodium sulfate (DSS) induces colitis in mice by forming nano-lipocomplexes with medium-chain-length fatty acids in the colon. PLoS One 2012;7:e32084.

Lee KW, Ho Hong Y, Lee SH, Jang SI, Park MS, Bautista DA, Ritter GD, et al. Effects of anticoccidial and antibiotic growth promoter programs on broiler performance and immune status. Research in Veterinary Science 2012;93(2):721-8.

Liu T, Ma Q, Zhao L, Jia R, Zhang J, Ji C, et al. Protective effects of sporoderm-broken spores of *Ganderma lucidum* on growth performance, antioxidant capacity and immune function of broiler chickens exposed to low level of Aflatoxin B1. Toxins 2016;8:278.

Lobionda S, Sittipo P, Kwon HY, Lee YK. The role of gut microbiota in intestinal inflammation with respect to diet and extrinsic stressors. Microorganisms 2019;7:E271.

Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007;2:204.

Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, Balamurugan R. The Firmicutes/Bacteroidetes ratio:a relevant marker of gut dysbiosis in obese patients? Nutrients 2020;12:1474.

Mao T, Water J van de, Keen C, Stern J, Hackman R, Gershwin M. Twomushrooms, *Grifolafrondosa* and *Ganoderma lucidum*, can stimulatecytokine gene expression and proliferation in human T lymphocytes.International Journal of Immunotherapy 1999;15:13-22.

Menconi A, Hernandez-Velasco X, Vicuña EA, Kuttappan VA, Faulkner OB, Tellez G, et al. Histopathological and morphometric changes induced by a dextran sodium sulfate (DSS) model in broilers. Poultry Science 2015;94:906-11.

Muñoz M, Guerrero-Araya E, Cortés-Tapia C, Plaza-Garrido A, Lawley TD, Paredes-Sabja D. Comprehensive genome analyses of *Sellimonas intestinalis*, a potential biomarker of homeostasis gut recovery. Microbial Genomics 2020;6:mgen000476.

Natividad JM, Pinto-Sanchez MI, Galipeau HJ, Jury J, Jordana M, Reinisch W, et al. Ecobiotherapy rich in Firmicutes decreases susceptibility to colitis in a humanized gnotobiotic mouse model. Inflammatory Bowel Diseases 2015;21(8):1883-93.

Nii T, Bungo T, Isobe N, Yoshimura Y. Intestinal inflammation induced by dextran sodium sulphate causes liver inflammation and lipid metabolism disfunction in laying hens. Poultry Science 2020;99:1663-77.

Ogbe AO, Mgbojikwe LO, Owoade AA, Atawodi SE, Abdu PA. The effect of a wild mushroom (*Ganoderma lucidum*) supplementation of feed on the immune response of pullet chickens to infectious bursal disease vaccine. Electronic Journal of Environmental, Agricultural and Food Chemistry 2008;7:2844-55.

Ogbe AO, Atawodi SE, Abdu PA, Sannusi A, Itodo AE. Changes in weight gain, faecal oocyst count and packed cell volume of *Eimeria tenella*-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract. Journal of the South African Veterinary Association 2009;80:97-102.

Pourabedin M, Zhao X. Prebiotics and gut microbiota in chickens. FEMS Microbiology Letters 2015;362:fnv122.

Quereshi S, Pandey AK, Sandhu SS. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. People's Journal of Scientific Research 2010;3:9-14.

Ishaq R, Chand N, Khan RU, Saeed M, Laudadio V, Tufarelli V. Methanolic extract of neem (*Azadirachta indica*) leaves mitigates experimentally induced coccidiosis challenge in Japanese quails. Journal of Applied Animal Research 2022;50(1):498-503.

Ravachol J, Philip P de, Borne R, Mansuelle P, Maté MJ, Perret S, et al. Mechanisms involved in xyloglucan catabolism by the cellulosome-producing bacterium *Ruminiclostridiumcellulolyticum*. Scientific Reports 2016;6:22770.

Sang T, Guo C, Guo D, Wu J, Wang Y, Wang Y, et al. Suppression of obesity and inflammation by polysaccharide from sporoderm-broken spore of *Ganoderma lucidum* via gut microbiota regulation. Carbohydrate Polymers 2021;256:117594.

Shao T, Shao L, Li H, Xie Z, He Z, Wen C. Combined signature of the fecal microbiome and metabolome in patients with gout. Frontiers in Microbiology 2017;8:268.

Shen B, Wang J, Guo Y, Gu T, Shen Z, Zhou C, et al. Dextran sulfate sodium salt-induced colitis aggravates gut microbiota dysbiosis and liver injury in mice with non-alcoholic steatohepatitis. Frontiers in Microbiology 2021;2:756299.

Shin N, Whon TW, Bae J. Proteobacteria:microbial signature of dysbiosis in gut microbiota. Trends in Biotechnology 2015;33:496-503.

Sofyan A, Angwar M, Herdian H, Istiqomah L, Febrisiantosa A, Julendra H, et al. Performance enhancement and immunity profile of broiler treated feed additive containing lactic acid bacteria and *Ganoderma lucidum*. Media Peternakan 2012;35:201-6.

Suresh G, Das RK, Kaur Brar S, Rouissi T, Avalos Ramirez A, Chorfi Y, et al. Alternatives to antibiotics in poultry feed:Molecular perspectives. Critical Reviews in Microbiology 2018;44:318-35.

Wei B, Zhang R, Zhai J, Zhu J, Yang F, Yue D, et al. Suppression of Th17 cell response in the alleviation of dextran sulfate sodium-induced colitis by *Ganoderma lucidum* polysaccharides. Journal of Immunology Research 2018;2018:2906494.

Wickramasuriya SS, Park I, Lee K, Lee Y, Kim WH, Nam H, et al. Role of physiology, immunity, microbiota, and infectious diseases in the gut health of poultry. Vaccines 2022;10(2):172.

Wu Y, Wang D. A new class of natural glycopeptides with sugar moiety-dependent antioxidant activities derived from *Ganoderma lucidum* fruiting bodies. Journal of Proteome Research 2009;8(2):436-42.

Xie J, Liu Y, Chen B, Zhang G, Ou S, Luo J, et al. *Ganoderma lucidum*polysaccharide improves rat DSS-induced colitis by altering cecal microbiota and gene expression of colonic epithelial cells. Food & Nutrition Research 2019;63:1559.

Yan Y, Kolachala V, Dalmasso G, Nguyen H, Laroui H, Sitaraman SV, et al. Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. PLoS One 2009;4(6):e6073.

Yang M, Wang X, Guan S, Xia J, Sun J, Guo H, et al. Analysis of triterpenoids in *ganoderma lucidum* using liquid chromatography coupled with electrospray ionization mass spectrometry. Journal of the American Society for Mass Spectrometry 2007;18(5):927-39.

Zhao Y, Li K, Luo H, Duan L, Wei C, Wang M, et al. Comparison of the intestinal microbial community in ducks reared differently through high-throughput sequencing. BioMed Research International 2019;2019:9015054.