Differential effects of *Bacillus subtilis*– and *Bacillus licheniformis*–fermented products on growth performance, intestinal morphology, intestinal antioxidant and barrier function gene expression, cecal microbiota community, and microbial carbohydrate-active enzyme composition in broilers

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ABSTRACT This study investigated the differential effects of *Bacillus subtilis*–fermented products (**SFPs**) and *Bacillus licheniformis*–fermented products (**LFPs**) on the growth performance, intestinal morphology, intestinal gene expression, cecal microbiota community, and microbial carbohydrate-active enzyme composition of broilers. In total, 160 one-day-old unsexed Arbor Acres broiler chicks were randomly allocated to 4 treatment groups (with 8 replicates per group and 5 chicks per replicate): control (**CON**), enramycin (**ENM)**, SFP, and LFP groups, which were fed a basal diet, basal diet supplemented with 10 mg/kg ENM, basal diet supplemented with 10^8 colony-forming units (**CFU**) of *B. subtilis* spores per gram of feed, and basal diet supplemented with 10^8 CFU of *B. licheniformis* spores per gram of feed, respectively. LFP treatment resulted in a higher ($P < 0.01$) body weight at the age of 35 d and higher average daily gain over 15 to 35 ($P < 0.05$) and 1 to 35 ($P < 0.05$) d of age than did the CON and SFP treatments. The average villus heights in the jejunum were longer ($P < 0.05$) in the LFP group than those in the SFP group. Moreover, the LFP group exhibited a higher jejunal barrier function gene expression (mucin 2, zonula occludens-1, and occludin) and cecal antioxidant gene expression (superoxide dismutase) than did the CON group ($P < 0.05$). A principal coordinate analysis of cecal microbiota and carbohydrate-active enzyme composition demonstrated distinct clustering among the groups. *Lactobacillus crispatus* abundance in the cecal digesta was higher ($P < 0.01$) in the SFP group than in the CON and LFP groups. Finally, microbial glycosyltransferase 2 gene expression in the cecal digesta was higher in the LFP group than in the CON and ENM groups. In conclusion, LFPs can improve the growth performance, increase intestinal barrier function and antioxidant gene expression, and modulate cecal microflora composition and carbohydrate-active enzyme composition of broilers. The overall effect of LFPs on growth promotion in broilers was superior to that of SFPs.

Key words: *Bacillus licheniformis*, *Bacillus subtilis*, broiler, carbohydrate-active enzyme, microbiota

INTRODUCTION

Antibiotic growth promoters (**AGPs**) are commonly used to prevent infection and improve growth performance in poultry. However, in poultry, antibiotic overuse and misuse have contributed to an increased risk of antibiotic resistance (Marshall and Levy, 2011). Many countries have restricted the use of AGPs as feed additives for enhancing growth in poultry production. Therefore, determining the effective alternatives to AGPs that can maintain the health status and improving the growth performance of the broilers are imperative.

Probiotics have gained considerable attention as an alternative to AGPs in poultry feeds. Among all probiotics, *Bacillus* species are the most commonly used probiotics in poultry production because of their ability to form endospores, which allows them to survive in harsh environments (with low pH, high bile salt levels, and high heat) and germinate in the gastrointestinal tract of poultry (Ramlucken et al., 2020; Bahaddad et al., 2022). *Bacillus* species can produce antimicrobial substances against enteric pathogens and provide extracellular enzymes, which then reduce infection risk and enhance nutrient utilization (Ramlucken et al., 2020). *Bacillus subtilis* and *Bacillus licheniformis* strains are widely used as probiotics for broilers. Studies have demonstrated that *B. subtilis* or *B. licheniformis*

supplementation can prevent enteric diseases and increase growth performance in broilers (Grant et al., 2018; Aljumaah et al., 2020; Zhao et al., 2020).

The effects of *B. subtilis* and *B. licheniformis* on broiler performance have been studied (Gong et al., 2018; Musa et al., 2019; Xu et al., 2021). *B. subtilis* and *B. licheniformis* supplementation at similar concentrations are similar in their effects on growth and necrotic enteritis prevention (Gong et al., 2018; Musa et al., 2019). By contrast, *B. licheniformis* supplementation produces results superior to those after *B. subtilis* supplementation in terms of growth performance, immunity, and cecal short-chain fatty acid levels in broilers (Xu et al., 2021). Because these findings in the literature have been inconsistent, the differences in the effects of *B. subtilis* and *B. licheniformis* supplementation on broiler performance warrants clarification.

Commercial probiotics, including *B. subtilis* and *B. licheniformis*, are mainly produced by submerged fermentation. Submerged fermentation is relatively expensive due to high cost of required media and instruments compared with solid-state fermentation. Solid-state fermentation only requires simple feed ingredients (bran or soybean meal) as the substrate and very less instrument, capital investment, and energy expenditure (Yafetto, 2022). Due to the environmentally friendly and low-cost process, solid-state fermentation has the potential of being a possible alternative to submerged fermentation for probiotic production. However, the production of probiotics as feed additives using solid-state fermentation is still not widely used in animal production.

Our previous studies have demonstrated that supplementation with *Bacillus* sp.-solid-state fermentation products (*B. subtilis*−fermented products [**SFPs**] and/or *B. licheniformis*−fermented products [**LFPs**]) can improve growth performance, alleviate inflammation, and modulate gut microbiota in broilers (Cheng et al., 2018, 2021a, 2021b; Chen and Yu, 2020, 2021; Yu et al., 2021; Chang and Yu, 2022). However, the differential effects of SFPs and LFPs in broilers from multiple perspectives are required. An integrated analysis of the gut microbiota and microbial carbohydrate-active enzyme gene composition, as affected by the 2 fermented products, is required to investigate the underlying mechanism. *B. subtilis* and *B. licheniformis* produce extracellular enzymes and secondary metabolites to differing extents (Sumi et al., 2015; Elshaghabee et al., 2017); thus, the mechanisms underlying their antimicrobial effects may differ (Tran et al., 2022). Therefore, we hypothesized that SFPs and LFPs differentially regulate growth performance, intestinal morphology, intestinal gene expression, cecal microbiota community, and microbial carbohydrate-active enzyme gene composition in broilers.

In the current study, we investigated the differential effects of a SFP and LFP on the performance, intestinal morphology, and intestinal gene expression in broilers. We also performed an integrated analysis of changes in gut microbiota and microbial carbohydrate-active enzyme gene composition in response to SFP and LFP treatment through metagenomic sequencing in broilers.

MATERIALS AND METHODS

Ethics Statement

The protocols used in the present study were reviewed and approved by the Institutional Animal Care and Use Committee of National Ilan University (109-26).

Birds and Diets

B. subtilis (BCRC 14718) and *B. licheniformis* (ATCC 12713) were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The SFP and LFP preparation methods used in this study have been detailed elsewhere (Cheng et al., 2021a ; Chen and Yu, 2021). The SFPs and LFPs were produced by solid-state fermentation. The detailed composition of SFPs and LFPs is listed in Table 1. In the fermented products used in this study, the *B. subtilis* and *B. licheniformis* spore concentrations were 3.8×10^9 and 4.4×10^9 colony-forming units (**CFU**)/g, respectively. In total, 160 one-day-old unsexed Arbor Acres broiler chicks, with an average body weight of 41.9 ± 0.13 g, were obtained from a local commercial hatchery and randomly allocated to 4 treatment groups (40 chicks per group): 1) control (**CON**) group (basal diet alone), 2) enramycin (**ENM**) group (basal diet supplemented with 10 mg/kg ENM), 3) SFP (basal diet supplemented with 10^8 CFU *B. subtilis* spore/g of feed), and 4) LFP (basal diet supplemented with 10^8 CFU *B. licheniformis* spore/g of feed). Each group had 8 replicates with 5 birds per replicate. The birds were reared in stainless-steel cages and given ad libitum access to feed and water during the experiment. Nutrient levels in basal diets were formulated to meet the National Research Council (1994) nutrient requirements of broiler chickens (Table 2). The experimental diets were given for 35 d, including starter (d 1−14) and growth (d 15−35) phases. A 20:4 h light−dark photoperiod was applied during the experimental period. Ambient temperature was maintained at 33°C at the age of 1 to 3 d and then gradually reduced by 2°C per wk to a final temperature of

Table 1. Composition of *Bacillus* species−fermented products.

	Bacillus subtilis −fermented products	*Bacillus licheniformis* −fermented products
Analyzed value, g kg^{-1}		
Crude protein	276	290
Crude fat	40	42
Carbohydrate	545	527
Ash	64	63
Water	75	78
Calorie, kcal/kg	3644	3646
Spore, CFU/g	3.8×10^9	4.4×10^9

Table 2. Basal diet compositions.

Item	d 1−14	d 15−35
Ingredient, g kg^{-1}, as fed basis		
Corn, yellow	554.2	607.3
Soybean meal, 36.7% CP	355.2	315.3
Fish meal, 60% CP	39.9	36.3
Vegetable oil	35.2	30.2
Limestone	15.2	12.7
Salt	3.0	3.0
Monocalcium phosphate	9.2	7.8
Mineral premix[1]	2.0	2.0
Vitamin premix[2]	2.0	2.0
DL-methionine, 98%	2.0	2.0
L-lysine, 98%	1.0	0.6
Choline chloride	0.5	0.5
Calculated value[3], g kg^{-1}		
Dry matter	889.0	887.0
Crude protein	221.6	206.3
Analyzed calcium	10.2	8.7
Analyzed total phosphorus	6.9	6.3
Lysine	11.2	9.5
Methionine + Cystine	8.5	7.6
ME, kcal/kg	3081.1	3057.2

[1]Supplied per kilogram of diet: 32 mg of Mn (MnSO$_4$·H$_2$O), 16 mg of Fe (FeSO$_4$·7H$_2$O), 24 mg of Zn (ZnO), 2 mg of Cu (CuSO$_4$·5H$_2$O), 800 μg of I (KI), 200 μg of Co (CoSO$_4$), and 60 μg of Se.
[2]Supplied per kilogram of diet: 5232 IU of vitamin A, 800 IU of vitamin D, 8.3 IU of vitamin E, 2.2 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.
[3]Calculated according to tables of feed composition and nutritive values in Taiwan.

approximately 24°C. The broilers were vaccinated with Newcastle disease and infectious bronchitis vaccines through nose drops on experiment d 4 and 14, respectively. Body weight was measured weekly, and feed intake was measured daily. Average daily gain, average daily feed intake, and feed conversion ratio were calculated for d 1 to 14, 15 to 35, and 1 to 35 intervals. Mortality was recorded through visual observations daily.

Sample Collection

Birds were euthanized through carbon dioxide inhalation on d 35 and immediately dissected. Two broilers per replicate were chosen randomly, and their intestinal segments (i.e., duodenum, jejunum, ileum, and cecum) were collected for intestinal morphology and gene expression analysis, and cecal digesta collected from each bird were pooled for metagenomic sequencing. The details of intestinal segment sampling locations were provided in our previous study (Cheng et al., 2021a).

Intestinal Morphology

The collected intestinal segments were fixed in 4% buffered formaldehyde for 24 h, dehydrated in ethanol, equilibrated in xylene, embedded in paraffin wax, and cut into 5-μm-thick sections. The sections were then mounted on glass slides, stained with hematoxylin and eosin, and examined under a microscope coupled with a digital camera (Olympus, Tokyo, Japan). Ten well-oriented, distinct villi were identified on each slide and measured for villus height, crypt depth, and their ratio.

Intestinal Gene Expression

Total RNA was extracted from the small intestine and cecum by using the acid guanidinium thiocyanate−phenol−chloroform extraction method. cDNA synthesis was performed using the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA). The expression of genes (catalase [CAT], superoxide dismutase [SOD], mucin 2 [MUC2], zonula occludens-1 [ZO-1], occludin [OCLN], and claudin [CLDN]) was measured on a CFX connect PCR detection system (Bio-Rad, Hercules, CA). The cDNA was amplified using the iQ SYBR Green Supermix kit (Bio-Rad, Hercules, CA), with initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The sequence of specific oligonucleotide primers for quantitative reverse transcription-PCR is listed in Table 3. The mRNA expression of each genes using the $2^{-\Delta\Delta Ct}$ method were calculated after normalizing to it to the 18S rRNA gene.

Metagenomic Sequencing

Procedures for metagenomic sequencing and bioinformatics analysis have been detailed in our previous study (Chen and Yu, 2022a). In brief, total genomic DNA from the cecal digesta was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA). Metagenomic DNA paired-end libraries were prepared using the Illumina Nextera XTLibrary Preparation Kit (Illumina, San Diego, CA). Sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina). After sequencing, the raw reads were cleaned by removing adaptor sequences and low-quality reads using Trimmomatic (version 0.38). The reads that mapped to chicken, human, maize, soybean, wheat, and fish genomes were filtered out using Bowtie2 (version 2.3.4.1). For each sample, the clean reads

Table 3. Primer sequences for quantitative reverse transcription-PCR.

Gene[1]	Primer sequences (5′-3′)
CAT	F: ACCAAGTACTGCAAGGCGAA
	R: TGAGGGTTCCTCTTCTGGCT
SOD	F: AGGGGGTCATCCACTTCC
	R: CCCATTTGTGTTGTCTCCAA
MUC2	F: GCCTGCCCAGGAAATCAAG
	R: CGACAAGTTTGCTGGCACAT
ZO-1	F: CCGCAGTCGTTCACGATCT
	R: GGAGAATGTCTGGAATGGTCTGA
OCLN	F: GTAGTCGGGTTCGTTTCC
	R: GACCTGATTGCCTAGAGTGT
CLDN	F: GATTTACTCCTACGCTGTGAC
	R: CACAAAGATGGCTATTAGTCCC
18S rRNA	F: ATAACGAACGAGACTCTGGCA
	R: CGGACATCTAAGGGCATCACA

[1]CAT = catalase; SOD = superoxide dismutase; MUC2 = mucin 2; ZO-1 = zonula occludens-1; OCLN = occludin; CLDN = claudin.

were assembled using MEGAHIT (version 1.1.3), and gene prediction was performed on contigs >500 bp by using Prodigal (version 2.6.3). The unaligned sequences from each assembly were compared for removing redundancy using CD-HIT (version 4.6.6). The taxonomic assignment of sequences was conducted using DIA-MOND (version 0.9.22.123). Protein sequences of open reading frames predicted in the contigs that carried carbohydrate-active enzyme-like open reading frames were annotated using the DIAMOND against the Carbohydrate-Active enZYmes Database. Bioinformatics analysis of microbiota and carbohydrate-active enzyme genes was implemented in MicrobiomeAnalyst (Dhariwal et al., 2017), followed by a comprehensive analysis in R (version 0.84). Fisher's alpha index and the Shannon index were used to evaluate the richness and evenness of the microbial and carbohydrate-active enzyme gene compositions, respectively. Heatmap analysis and principal coordinate analysis (**PCoA**) were used to evaluate the overall differences in bacterial community and carbohydrate-active enzyme gene composition. The results of correlation analyses were visualized using R.

Statistical Analysis

Individual cages were considered replicates, each replicate was defined as an experimental unit. The data were tested for normality using the Shapiro−Wilk test. One-way analysis of variance with Tukey's honestly significant difference test was used for analyzing differences. Significance was indicated at $P \leq 0.05$. PCoA based on Bray−Curtis dissimilarity metrics was used to determine similarity in microbial community and carbohydrate-active enzyme gene composition. Spearman's correlation coefficient was calculated for correlations between growth performance and microbial

carbohydrate-active enzyme gene expression. All statistical analyses were performed using SAS software (version 9.4, 2012; SAS Institute, Cary, NC).

RESULTS

Growth Performance, Intestinal Morphology, and Intestinal Gene Expression

The effects of SFP and LFP treatments on broiler growth performance is summarized in Table 4. Compared with CON and SFP treatments, LFP treatment resulted in increased ($P \leq 0.01$) body weight at 35 d of age. The LFP group had higher average daily gain at 15 to 35 ($P \leq 0.05$) and 1 to 35 ($P \leq 0.05$) d of age than did the CON and SFP groups. No significant between-group differences were identified in the ADFI or feed conversion ratio during the experimental period. Compared with CON treatment, LFP treatment resulted in increased ($P \leq 0.05$) mortality during the experimental period. The effects of SFP and LFP treatments on broiler intestinal morphology are summarized in Table 5. The average villus heights in the duodenum were higher ($P \leq 0.05$) in the LFP group than in the ENM group. The LFP group had higher ($P \leq 0.05$) average villus heights in the jejunum compared with the SFP group. No significant between-group differences in ileal morphology were identified. The effects of SFP and LFP treatments on broiler intestinal gene expression are summarized in Table 6. CAT and SOD mRNA expression was lower in the duodenum in the LFP group than in the SFP group ($P \leq 0.01$ and $P \leq 0.001$, respectively). $MUC2$ expression in the duodenum was lower ($P \leq 0.05$) in the CON group than in the ENM group. Moreover, $OCLN$ mRNA expression in the duodenum was lower ($P \leq 0.001$) in the LFP group than in the other groups,

Table 4. Effects of fermented products on broiler growth performance.

Item	Treatment[1]				SEM[2]	P-value[3]
	CON	ENM	SFP	LFP		
Body weight (g/bird)						
1 d	41.9	41.9	41.9	42.0	0.02	0.336
35 d	1712.4[b]	1782.1[ab]	1731.0[b]	1908.0[a]	22.02	0.003
Average daily gain (g/d/bird)						
1−14 d	24.5	25.7	25.0	25.0	0.36	0.640
15−35 d	64.0[b]	66.2[ab]	64.0[b]	70.6[a]	0.86	0.011
1−35 d	48.2[b]	50.0[ab]	48.3[b]	52.4[a]	0.55	0.019
Average daily feed intake (g/d/bird)						
1−14 d	28.5	29.5	28.2	28.3	0.34	0.564
15−35 d	105.6	104.2	102.7	110.7	1.57	0.313
1−35 d	74.8	74.4	72.9	77.7	0.99	0.382
Feed conversion ratio						
1−14 d	1.16	1.15	1.13	1.16	0.01	0.677
15−35 d	1.66	1.58	1.61	1.57	0.03	0.648
1−35 d	1.55	1.49	1.51	1.48	0.02	0.621
Mortality rate (%)						
1−35 d	2.5[b]	12.5[ab]	5.0[ab]	15.0[a]	1.78	0.030

[1]CON = Basal diet; ENM = Basal diet supplemented with enramycin; SFP = Basal diet supplemented with *B. subtilis*−fermented products; LFP = Basal diet supplemented with *B. licheniformis*−fermented products.

[2]SEM = standard error of means.

[3]P-value was obtained by ANOVA.

[a-b]Means in the row without common superscripts are significantly different ($P \leq 0.05$). Mean value representing results from 8 replicate cages per treatment (n = 8).

Table 5. Effects of fermented products on broiler intestinal morphology.

Item		CON	ENM	SFP	LFP	SEM[2]	P-value[3]
				Treatment[1]			
Duodenum	Villus height (μm)	1833.9[ab]	1698.8[b]	1860.9[ab]	1981.5[a]	35.50	0.029
	Crypt depth (μm)	145.6	148.6	129.1	169.4	8.95	0.730
	Villus height: crypt depth	12.7	12.3	14.8	14.4	0.70	0.541
Jejunum	Villus height (μm)	907.3[ab]	870.6[ab]	809.1[b]	946.2[a]	18.61	0.048
	Crypt depth (μm)	128.1	123.6	104.1	121.1	5.17	0.398
	Villus height: crypt depth	7.3	7.6	7.9	8.0	0.33	0.840
Ileum	Villus height (μm)	711.0	677.5	718.5	718.0	14.67	0.750
	Crypt depth (μm)	107.6	115.1	105.6	124.1	4.46	0.471
	Villus height: crypt depth	6.7	6.1	7.0	5.9	0.26	0.410

[1]CON = Basal diet; ENM = Basal diet supplemented with enramycin; SFP = Basal diet supplemented with *B. subtilis*−fermented products; LFP = Basal diet supplemented with *B. licheniformis*−fermented products.

[2]SEM = standard error of means.

[3]P-value was obtained by ANOVA.

[a-c]Mean values in the row without identical superscripted letters are significantly different ($P \leq 0.05$). Mean value representing results from 4 replicate cages per treatment (n = 4).

and *CAT* mRNA expression in the jejunum was lower ($P \leq 0.01$) in the LFP group than in the CON and LFP groups. By contrast, *SOD* expression in the jejunum was higher ($P \leq 0.05$) in the LFP and SFP groups than in the ENM group. Moreover, *MUC2* and *ZO-1* mRNA expression in the jejunum was higher in the LFP group than in the CON group ($P \leq 0.001$ and $P \leq 0.05$), and *OCLN* expression in the jejunum was higher ($P \leq 0.05$)

Table 6. Effect of fermented products on broiler intestinal gene expression.

Item	Gene[2]	CON	ENM	SFP	LFP	SEM[3]	P-value[4]
				Treatment[1]			
Duodenum	*CAT*	1.02[ab]	1.08[ab]	1.53[a]	0.43[b]	0.13	0.009
	SOD	1.08[b]	0.63[c]	1.38[a]	0.95[b]	0.07	<0.001
	MUC2	1.17[a]	0.76[b]	0.81[ab]	0.79[ab]	0.06	0.033
	ZO-1	1.00	1.08	0.99	1.08	0.03	0.583
	OCLN	0.98[a]	1.13[a]	0.77[b]	0.98[a]	0.04	<0.001
	CLDN	1.05	0.59	0.93	0.83	0.07	0.088
Jejunum	*CAT*	0.73[b]	1.78[ab]	2.78[a]	0.35[b]	0.11	0.002
	SOD	1.00[ab]	0.40[b]	1.17[a]	1.14[a]	0.11	0.018
	MUC2	1.01[b]	1.07[b]	0.91[b]	2.45[a]	0.17	<0.001
	ZO-1	0.90[b]	1.98[ab]	1.38[ab]	2.23[a]	0.17	0.016
	OCLN	0.92[b]	2.09[ab]	2.38[a]	2.29[a]	0.20	0.015
	CLDN	1.19[b]	1.80[b]	2.86[a]	1.45[b]	0.17	<0.001
Ileum	*CAT*	1.11[b]	0.68[b]	1.90[a]	0.57[b]	0.13	<0.001
	SOD	1.05	2.04	3.06	2.35	0.33	0.198
	MUC2	1.23	1.10	1.40	1.00	0.07	0.164
	ZO-1	1.00[b]	1.97[a]	1.21[b]	1.53[ab]	0.12	0.006
	OCLN	1.04[ab]	1.34[ab]	0.92[b]	1.50[a]	0.08	0.015
	CLDN	0.84	1.70	1.55	1.61	0.18	0.320
Cecum	*CAT*	0.98[b]	2.11[a]	1.53[ab]	1.75[ab]	0.14	0.009
	SOD	1.00[b]	1.59[ab]	2.26[ab]	2.89[a]	0.26	0.049
	MUC2	0.90[ab]	0.96[ab]	0.71[b]	1.22[a]	0.06	0.016
	ZO-1	0.89[b]	1.47[a]	0.91[ab]	1.10[ab]	0.08	0.035
	OCLN	0.80[b]	1.46[a]	1.34[a]	0.82[b]	0.083	<0.001
	CLDN	1.15[b]	1.43[ab]	2.01[a]	0.97[ab]	0.12	0.007

[1]CON = Basal diet; ENM = Basal diet supplemented with enramycin; SFP = Basal diet supplemented with *B. subtilis*−fermented products; LFP = Basal diet supplemented with *B. licheniformis*−fermented products.

[2]*CAT* = catalase; *SOD* = superoxide dismutase; *MUC2* = mucin 2; *ZO-1* = zonula occludens-1; *OCLN* = occludin; *CLDN* = claudin.

[3]SEM = standard error of means.

[4]P-value was obtained by ANOVA.

[a-c]Mean values in the row without identical superscripted letters are significantly different ($P \leq 0.05$). Mean value representing results from 5 replicate cages per treatment (n = 5) except *SOD* in duodenum and *CAT* in cecum (n = 4)

in the LFP and SFP groups than in the CON group. Compared with the other treatments, SFP treatment induced ($P \leq 0.001$) *CLDN* mRNA expression in the jejunum and *CAT* mRNA expression in the ileum. *ZO-1* expression in the ileum was higher ($P \leq 0.01$) in the ENM group than in the CON and SFP groups. Compared with SFP treatment, LFP treatment induced ($P \leq 0.05$) *OCLN* mRNA expression in the ileum. *CAT* expression in the cecum was higher ($P \leq 0.01$) in the ENM group than in the CON group. Compared with CON treatment, LFP treatment induced ($P \leq 0.05$) *SOD* mRNA expression in the cecum. *MUC2* expression in the cecum was higher ($P \leq 0.05$) in the LFP group than in the SFP group. Compared with CON treatment, ENM treatment induced ($P \leq 0.05$) *ZO-1* mRNA expression in the cecum. *OCLN* expression in the cecum was higher ($P \leq 0.001$) in the ENM and SFP groups than those in the other groups. Compared with CON treatment, SFP treatment induced ($P \leq 0.01$) *CLDN* mRNA expression in the cecum.

Cecal Microbiota Composition

The microbial species richness (i.e., Fisher's alpha) in the cecal digesta was lower ($P \leq 0.05$) in the ENM, SFP, and LFP groups than in the CON group (Figure 1A). The microbial species evenness (Shannon) in the cecal digesta was lower ($P \leq 0.05$) in the ENM group than in the other groups (Figure 1A). The PCoA results indicated a clear difference in bacterial community composition between the groups (Figure 1B). Figure 1C presents the heatmap indicating the 35 most abundant bacteria in the cecal digesta of broilers at species level. Some bacterial clusters, such as those including *Massilicoli timonensis*, *Bacteroides vulgatus*, and *Bacteroides sartorii* were specifically identified in the CON group. Specific bacterial clusters including *Faecalibacterium prausnitzii*, *Faecalibacterium sp. An121*, *Faecalibacterium sp. An58*, and *Faecalibacterium sp. An192*, were identified only in the ENM group. Some bacterial clusters, such as those including *Alistipes communis*, *Blautia sp. An81*,
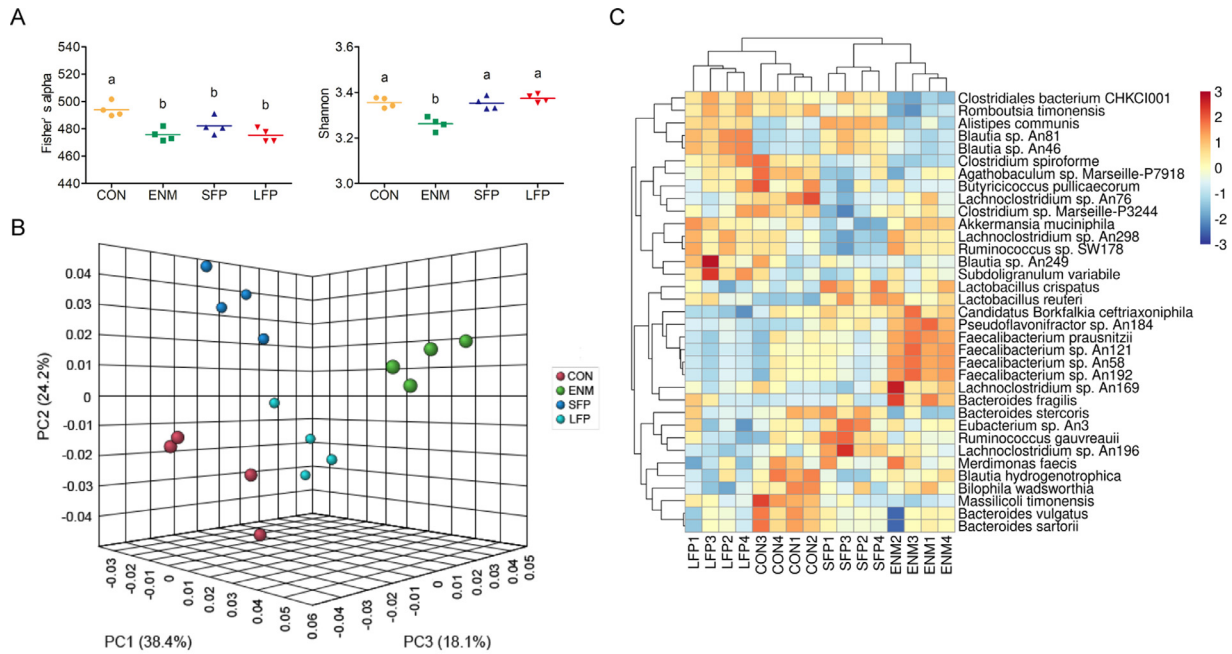
Figure 1. Microbial community structures in cecal digesta for comparison. (A) Scatter plot of bacterial alpha diversity in the CON, ENM, SFP, and LFP groups. Each bar represents mean ± standard deviation (n = 4). Different superscripts indicate significant differences between groups. (B) PCoA of the cecal bacterial communities in the CON, ENM, SFP, and LFP groups (n = 4). (C) Heatmap of microbial species abundance in cecal digesta. Abundance distribution of 35 dominant species (Y axis) across all samples (X axis) is displayed (n = 4). The values are normalized using the Z-score. Abbreviations: CON, basal diet; ENM, basal diet supplemented with enramycin; LFP, Basal diet supplemented with *B. licheniformis*−fermented products; PCoA, principal coordinate analysis; SFP, Basal diet supplemented with *B. subtilis*−fermented products.

and *Blautia sp. An46*, partially overlapped between the LFP and SFP groups. Specific bacterial clusters, such as those including *Lactobacillus crispatus*, *Ruminococcus gauvreauii*, and *Lachnoclostridium sp. An196*, were identified only in the SFP group. Similar bacterial clusters, such as those including *Clostridiales bacterium CHKCI001* and *Romboutsia timonensis*, were identified in the CON, LFP, and SFP groups. The effects of SFP and LFP treatments on the bacterial taxonomy in the cecal digesta of broilers are summarized in Table 7. At the phylum level, the abundance of the phylum Firmicutes was higher ($P \leq 0.05$) in the ENM group than in the CON group, whereas that of the phylum Bacteroidetes was lower ($P \leq 0.01$) in the ENM group than those in the CON and SFP groups. At the genus level, the abundance of the genus *Bacteroides* was lower ($P \leq 0.05$) in ENM or LFPs than those in the CON group, whereas that of the genera *Faecalibacterium* and *Lachnoclostridium* was higher ($P \leq 0.01$) in the ENM group than in the SFP and LFP groups. Compared with the other groups, the SFP group demonstrated higher ($P \leq 0.001$) abundance of the genus *Lactobacillus* but lower ($P \leq 0.001$) abundance of the genus *Clostridium*. Compared with the other groups, the SFP and LFP groups demonstrated higher ($P \leq 0.001$) abundance of the genus *Blautia* and the CON and LFP groups demonstrated higher ($P \leq 0.05$) abundance of the genus *Erysipelatoclostridium*. At the species level, the SFP group had the highest abundance of *Bacteroides stercoris* and *R. gauvreauii*, whereas the ENM group demonstrated the highest ($P \leq 0.05$) abundance of *Candidatus Borkfalkia ceftriaxoniphila*, *Faecalibacterium sp. An58*, and *F.*

prausnitzii. The abundance of *Lachnoclostridium sp. An76* and *Blautia hydrogenotrophica* was lower ($P \leq 0.05$) in the SFP and LFP groups than in the CON group. The abundance of *C. bacterium CHKCI001* was lower ($P \leq 0.001$) in the ENM group than in the other groups. The abundance of *L. crispatus* was higher ($P \leq 0.01$) in the SFP group than in the CON and LFP groups. The abundance of *B. vulgatus* was higher ($P \leq 0.01$) in the CON group than in the ENM and LFP groups. The SFP demonstrated a lower ($P \leq 0.01$) abundance of *Clostridium sp. Marseille-P3244* than did the CON group. The abundance of *Clostridium spiroforme* was higher ($P \leq 0.01$) in the CON and LFP groups than in the ENM group. The abundance of *Lactobacillus reuteri* was higher ($P \leq 0.01$) in the ENM and SFP groups than in the CON group. The abundance of *R. timonensis* was higher ($P \leq 0.001$) in the LFP group than in the ENM and SFP groups, and that of *Akkermansia muciniphila* was higher ($P \leq 0.01$) in the ENM and LFP groups than in the SFP group. SFP and LFP treatments led to the highest ($P \leq 0.001$) abundance of *Blautia sp. An81*.

Microbial Carbohydrate-Active Enzyme Gene Composition in the Cecal Digesta

The between-group differences in the richness of microbial carbohydrate-active enzyme gene were nonsignificant (Figure 2A). By contrast, the evenness of microbial carbohydrate-active enzyme genes in the cecal digesta was higher ($P \leq 0.05$) in LFP group than in the other groups (Figure 2A). The PCoA results exhibited a clear

Table 7. Effects of fermented products on bacterial taxonomy in broiler cecal digesta.

Item	Treatment[1]				SEM[2]	P-value[3]
	CON	ENM	SFP	LFP		
Phylum (%)						
Firmicutes	74.39[b]	78.90[a]	75.95[ab]	77.83[ab]	0.62	0.025
Bacteroidetes	20.27[a]	15.80[b]	19.44[a]	17.04[ab]	0.60	0.010
Genus (%)						
Bacteroides	18.14[a]	14.25[b]	15.84[ab]	14.46[b]	0.52	0.011
Faecalibacterium	6.83[b]	10.71[a]	6.72[b]	5.76[b]	0.52	<0.001
Lachnoclostridium	5.75[ab]	5.93[a]	5.18[c]	5.29[bc]	0.10	0.003
Lactobacillus	4.75[c]	6.86[ab]	8.72[a]	5.51[bc]	0.45	<0.001
Blautia	4.80[b]	5.19[b]	6.58[a]	6.79[a]	0.24	<0.001
Clostridium	2.14[a]	2.04[a]	1.88[b]	2.08[a]	0.03	<0.001
Ruminococcus	1.74	1.69	1.90	1.76	0.04	0.246
Erysipelatoclostridium	2.01[a]	1.19[b]	1.42[ab]	1.85[a]	0.11	0.011
Species (%)						
Bacteroides stercoris	2.19[ab]	0.94[b]	2.69[a]	1.45[ab]	0.25	0.039
Lachnoclostridium sp. An76	2.41[a]	1.93[ab]	1.63[b]	1.81[b]	0.09	0.003
Clostridiales bacterium CHKCI001	2.50[a]	1.29[b]	2.59[a]	2.98[a]	0.18	<0.001
Lactobacillus crispatus	0.69[b]	0.88[ab]	1.11[a]	0.64[b]	0.06	0.005
Bacteroides vulgatus	0.72[a]	0.53[b]	0.56[ab]	0.51[b]	0.03	0.008
Merdimonas faecis	0.73	0.73	0.71	0.65	0.01	0.156
Ruminococcus gauvreauii	0.63[ab]	0.60[b]	0.84[a]	0.54[b]	0.04	0.010
Lachnoclostridium sp. An169	0.73	0.92	0.68	0.70	0.04	0.047
Candidatus Borkfalkia ceftriaxoniphila	0.64[b]	0.87[a]	0.72[ab]	0.41[c]	0.05	<0.001
Blautia hydrogenotrophica	0.60[a]	0.50[ab]	0.42[b]	0.43[b]	0.02	0.003
Clostridium sp. Marseille-P3244	0.59[a]	0.54[ab]	0.47[b]	0.55[ab]	0.02	0.009
Clostridium spiroforme	0.70[a]	0.30[b]	0.42[ab]	0.70[a]	0.06	0.009
Lactobacillus reuteri	0.50[b]	0.90[a]	1.01[a]	0.79[ab]	0.06	0.003
Romboutsia timonensis	0.53[ab]	0.18[c]	0.36[bc]	0.58[a]	0.05	<0.001
Faecalibacterium sp. An58	0.43[b]	0.67[a]	0.40[b]	0.35[b]	0.03	<0.001
Akkermansia muciniphila	0.31[ab]	0.54[a]	0.15[b]	0.55[a]	0.05	0.004
Faecalibacterium prausnitzii	0.43[b]	0.59[a]	0.42[b]	0.38[b]	0.02	<0.001
Blautia sp. An81	0.41[b]	0.43[b]	0.56[a]	0.65[a]	0.03	<0.001

[1]CON = Basal diet; ENM = Basal diet supplemented with enramycin; SFP = Basal diet supplemented with *B. subtilis*−fermented products; LFP = Basal diet supplemented with *B. licheniformis*−fermented products.

[2]SEM = standard error of means.

[3]P-value was obtained by ANOVA.

[a-c]Mean values in the row without identical superscripted letters are significantly different ($P \leq 0.05$). Mean value representing results from 4 replicate cages per treatment (n = 4).

separation of microbial carbohydrate-active enzyme gene composition among the groups (Figure 2B). Figure 2C presents a heatmap showing the 35 most abundant microbial carbohydrate-active enzyme genes in the cecal digesta. The glycoside hydrolase genes *GH51, GH97, GH20,* and *GH92* were identified only in the CON group, whereas *GH77, GH1,* and *GH13_20* were identified only in the ENM group. The carbohydrate esterase 10 gene (*CE10*) was identified only in the SFP group. *GH73* and the glycosyltransferase (GT) 8 gene (*GT8*) were identified in the ENM and SFP groups. The carbohydrate-active enzyme genes *GH31, GH18, GT2,* and *GH36* were identified in the LFP group. The effects of SFP and LFP treatments on the microbial carbohydrate-active enzyme genes in the cecal digesta of broilers are summarized in Table 8. *GT2* abundance was higher ($P \leq 0.01$) in the LFP group than in the CON and ENM groups. *GT4* and *GH1* abundance were the highest ($P \leq 0.01$ and $P \leq 0.001$, respectively) in the ENM group, and *CE10* abundance was the highest ($P < 0.001$) in the SFP group. *GH2* abundance was higher ($P \leq 0.01$) in the LFP group than in the CON and ENM groups, that of *GH3* was higher ($P \leq 0.05$) in the ENM group than in the CON group, that of *CE4* was higher ($P \leq 0.05$) in the LFP group than in the ENM and SFP groups, that of *GH25* was higher ($P \leq$

0.01) in the ENM and SFP groups than in the CON group, and that of *GT51* was higher ($P \leq 0.01$) in the ENM group than in the CON and SFP groups. By contrast, *CE1* abundance was lower ($P \leq 0.001$) in the ENM group than in the other groups. *GH20* and *GH28* abundance was higher ($P \leq 0.05$ and $P \leq 0.01$) in the CON group than in the LFP group, and that of *GH31* was higher ($P \leq 0.001$) in the ENM and LFP groups than in the CON and SFP groups. The between-group differences in the total microbial carbohydrate-active enzyme gene numbers in the cecal digesta were nonsignificant.

Taxonomic Assignment of Microbial GT2

At the genus level, the richness of the taxonomic assignment of microbial *GT2* was lower ($P \leq 0.05$) in the ENM group than in the CON and SFP groups (Figure 3A). By contrast, the evenness of the taxonomic assignment of microbial *GT2* was higher ($P \leq 0.05$) in the LFP group than in the CON and SFP groups (Figure 3A). The PCoA results demonstrated a clear separation of taxonomic assignment of microbial *GT2* composition at the genus level among the groups (Figure 3B). Figure 3C presents a heatmap
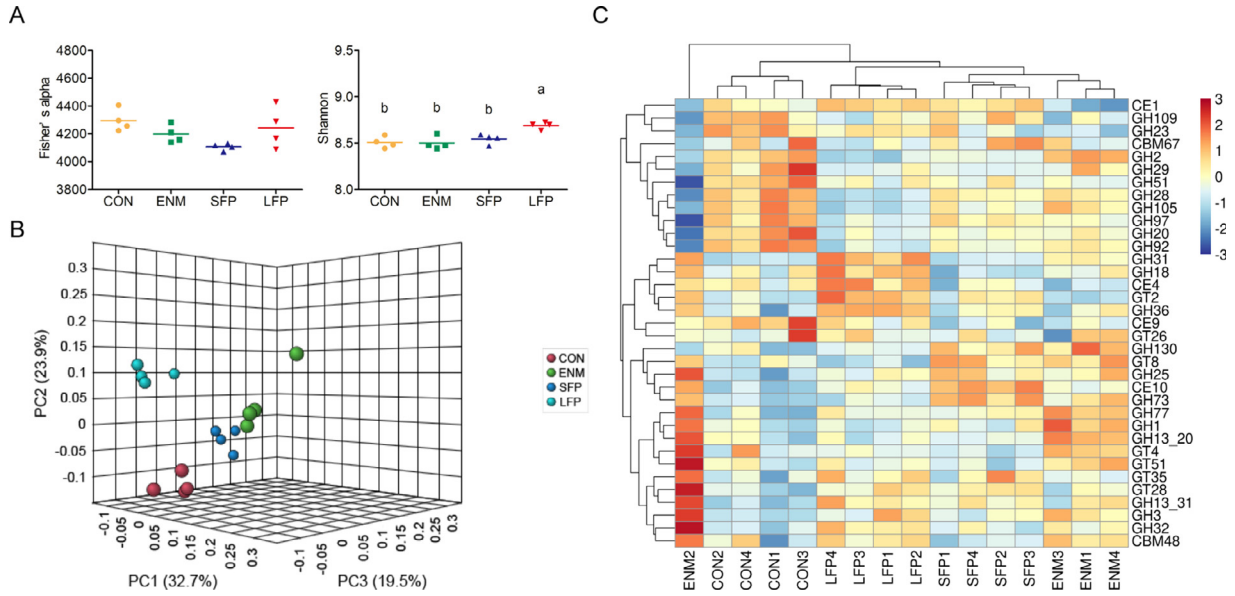
Figure 2. Comparison of microbial carbohydrate-active enzyme gene structure in the cecal digesta. (A) Scatter plot of alpha diversity of microbial carbohydrate-active enzyme gene composition in the CON, ENM, SFP, and LFP groups. Each bar represents mean ± standard deviation (n = 4). Different superscripts indicate significant differences between groups. (B) PCoA of the microbial carbohydrate-active enzyme gene composition in the CON, ENM, SFP, and LFP groups (n = 4). (C) Heatmap of microbial carbohydrate-active enzyme gene abundance in the cecal digesta. Abundance distribution of 35 dominant microbial carbohydrate-active enzyme genes (Y-axis) across all samples (X-axis) is displayed (n = 4). The values are normalized using the Z-score. Abbreviations: CON, basal diet; ENM, basal diet supplemented with enramycin; LFP, Basal diet supplemented with *B. licheniformis*−fermented products; PCoA, principal coordinate analysis; SFP, Basal diet supplemented with *B. subtilis*−fermented products.

demonstrating the dominant taxonomic assignment of microbial *GT2* at the genus level. The genera *Paenibacillus*, *Mediterraneibacter*, and *Bacteroides* exhibited the highest proportions of *GT2* in the CON group; the genera *Faecalibacterium*, and *Neglecta* had the highest proportions of *GT2* in the ENM group; the genera *Lactobacillus*, *Anaeromassilibacillus*, and *Roseburia* exhibited the highest proportions of *GT2* in the SFP group;

and the genera *Pseudoflavonifractor*, *Turicibacter*, and *Bacillus* demonstrated the highest proportions of *GT2* in the LFP group. Figure 3D presents the results of analysis of the correlation between the dominant carbohydrate-active enzyme genes in the cecal digesta and broiler growth performance. All detected microbial carbohydrate-active enzyme genes, except *CE1* and *GH2*, were positively correlated with average daily gain and

Table 8. Effects of fermented products on dominant carbohydrate-active enzyme gene in broiler cecal digesta.

Gene[2] (%)	CON	ENM	SFP	LFP	SEM[3]	P-value[4]
			Treatment[1]			
GT2	13.64[b]	13.75[b]	14.07[ab]	14.61[a]	0.12	0.006
GT4	6.80[b]	7.00[a]	6.72[b]	6.78[b]	0.03	0.006
GH2	4.54[a]	4.51[a]	4.37[ab]	4.27[b]	0.04	0.008
GH3	3.10[b]	3.40[a]	3.19[ab]	3.28[ab]	0.04	0.014
CE10	2.63[c]	2.85[b]	3.04[a]	2.79[bc]	0.04	<0.001
CE4	2.69[ab]	2.59[b]	2.60[b]	2.86[a]	0.04	0.018
GH1	1.91[b]	2.30[a]	1.99[b]	1.92[b]	0.05	<0.001
GH25	1.91[b]	2.12[a]	2.09[a]	2.01[ab]	0.02	0.009
GT51	1.83[b]	1.98[a]	1.80[b]	1.85[ab]	0.02	0.009
CE1	1.69[a]	1.54[b]	1.74[a]	1.77[a]	0.02	<0.001
GH20	1.78[a]	1.57[b]	1.62[ab]	1.57[b]	0.03	0.014
GH28	1.71[a]	1.50[ab]	1.57[ab]	1.39[b]	0.03	0.008
GH31	1.38[b]	1.50[a]	1.40[b]	1.57[a]	0.02	<0.001
GT28	1.37	1.44	1.44	1.43	0.01	0.234
GH29	1.46	1.36	1.33	1.34	0.02	0.067
	CON	ENM	SFP	LFP	SEM	P value
Total abundance[5]	164299	158394	150967	154980	4638.6	0.811

[1]CON = Basal diet; ENM = Basal diet supplemented with enramycin; SFP = Basal diet supplemented with *B. subtilis*−fermented products; LFP = Basal diet supplemented with *B. licheniformis*−fermented products.

[2]GT = glycosyltransferase; GH = glycoside hydrolase; CE = carbohydrate esterase.
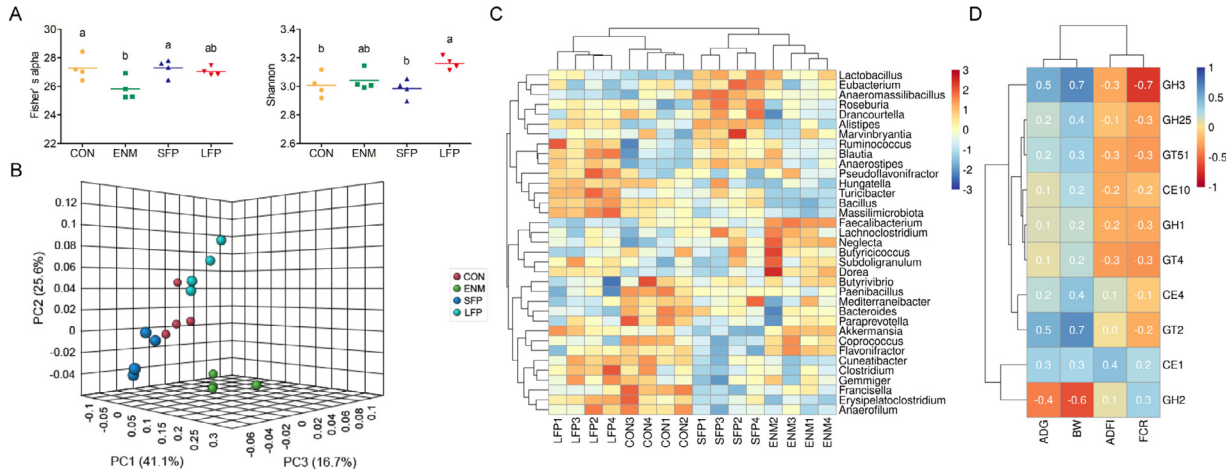
[3]SEM = standard error of means.

[4]P-value was obtained by ANOVA.

[5]Total carbohydrate-active enzyme gene abundance.

[a-c]Mean values in the row without identical superscripted letters are significantly different ($P \leq 0.05$). Mean value representing results from 4 replicate cages per treatment (n = 4).

Figure 3. Taxonomic assignment of microbial *GT2* at the genus level in the cecal digesta for comparison. (A) Scatter plot of alpha diversity of taxonomic assignment of microbial *GT2* in the CON, ENM, SFP, and LFP groups. Each bar represents mean ± standard deviation (n = 4). Different superscripts indicate significant differences between groups. (B) PCoA of the taxonomic assignment of microbial GT2 gene in the CON, ENM, SFP, and LFP groups (n = 4). (C) Heatmap of abundance of taxonomic assignment of microbial GT2 gene in the cecal digesta. Abundance distribution of 35 dominant genera (Y-axis) across all samples (X-axis) is displayed (n = 4). The values are normalized using the Z-score. (D) Results of Spearman's correlation analysis between dominant carbohydrate-active enzyme genes and growth performance. Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CON, basal diet; ENM, basal diet supplemented with enramycin; FCR: feed conversion ratio; LFP, Basal diet supplemented with *B. licheniformis*—fermented products; PCoA, principal coordinate analysis; SFP, Basal diet supplemented with *B. subtilis*—fermented products.

body weight and negatively correlated with feed conversion ratio. The average *GT2* abundance was positively correlated with average daily gain and body weight and negatively correlated with feed conversion ratio. By contrast, the average *GH2* abundance was negatively correlated with average daily gain and body weight.

DISCUSSION

Few studies have studied the effects of *B. subtilis* and *B. licheniformis* on the growth performance of broilers (Gong et al., 2018; Musa et al., 2019; Xu et al., 2021). Supplementation with *B. subtilis* (1×10^5 CFU/g) or *B. licheniformis* (1×10^5 CFU/g) exhibit similar daily body weight gain in broilers (Gong et al., 2018). *B. subtilis* (2×10^9 CFU/g) and *B. licheniformis* (2×10^9 CFU/g) have been noted to demonstrate necrotic enteritis preventive (or partially preventive) effects similar to those of antibiotics in broilers (Musa et al., 2019). In the present study, compared with SFP treatment (1×10^8 CFU/g), LFP treatment (1×10^8 CFU/g) improved body weight at 35 d of age and led to average daily gain at 15 to 35 and 1 to 35 d of age in broilers. This observation agrees with the results of Xu et al. (2021); the authors observed that at identical concentration of supplementation (1.5×10^6 CFU/g), *B. licheniformis* more effectively promoted growth in broilers than did *B. subtilis*. Furthermore, dietary inclusion of *B. licheniformis* (3×10^6 CFU/g) improved body weight gain and reduced feed conversion ratio compared with *B. subtilis* (6.6×10^6 CFU/g) in broilers (Arif et al., 2021). It has been demonstrated that probiotic-mediated mucus production and secretion by intestinal goblet cells are crucial for the maintenance of intestinal epithelial barrier function (Ohland and Macnaughton, 2010). An intact intestinal epithelial barrier can protect the host from pathogens,

prevent gut microbiota dysbiosis, and improve subsequent growth. This study also demonstrated that LFP supplementation promoted *MUC2* expression in the jejunum and cecum in broilers. PCoA on cecal microbiota and microbial carbohydrate-active enzyme gene composition demonstrated a clear separation of the 4 main groups, indicating gut microbiota composition was modulated in response to LFP treatment. Previous study has shown that *B. licheniformis* is more effective in degrading the protein of soybean meal than is *B. subtilis* (Dai et al., 2022). This result indicates that *B. licheniformis* exhibits higher proteolytic enzyme activity, which may increase the nutrient utilization in the gastrointestinal tract and subsequent growth performance in broilers. Taken together, these results indicated that at identical concentration of supplementation, LFPs lead to superior growth performance than do SFPs. The growth promoting effects of LFPs in broilers may be mediated through the formation of an effective physical barrier in the gut through increased mucin production, competitive exclusion of enteric pathogens via cecal microbiota alteration, and promotion of nutrient utilization by highly efficient microbial proteolytic enzyme activity. LFP supplementation unexpectedly increased the mortality of broilers in the present study. There is no clear explanation for this results and more work will be needed in future to further investigate the effects of different concentration of LFP on the mortality of broilers.

On ingestion, spores from several *Bacillus* species can germinate and grow in the small intestinal tract of broilers (Cartman et al., 2008). Therefore, many hypotheses have been proposed for the mechanism through which *Bacillus* sp. regulate gut microbiota in broilers, such as the competitive exclusion of pathogens, production of antimicrobial peptides, and secretion of extracellular enzymes or secondary metabolites (Ramlucken et al., 2020). It has been reported that dietary

supplementation with *Bacillus* sp.-based probiotics has positive effects on the modulation of intestinal microbiota in broilers (Luise et al., 2022). *B. subtilis* supplementation increases the abundance of beneficial bacteria and decreases the abundance of pathogens in broiler intestines (Bilal et al., 2021; Šimunović et al., 2022). Dietary supplementation with *B. licheniformis* promotes *Lactobacillus* sp. growth and prevents pathogen colonization in broiler intestines (Lin et al., 2017; Xu et al., 2018). Our previous studies, we have demonstrated that dietary supplementation with SFPs reverses dysbiosis of cecal microbial community in broilers by using inflammatory models (Chen and Yu, 2021, 2022a). Dietary LFP inclusion leads to the modulation of microbial composition in broiler intestines through an increase in *Lactobacillus* sp. abundance (Chen and Yu, 2020; Cheng et al., 2021b). In the current study, we found that LFP supplementation reduced species richness in the cecal digesta of broilers. However, our previous study demonstrated that LFP supplementation increased species richness and evenness in the cecal digesta of broilers (Chen and Yu, 2022a). Furthermore, cecal bacterial abundance at the genus level differed between the results of the 2 studies. For instance, the abundance of *Lactobacillus* sp. in the cecal digesta of broilers increased in our previous study, but in the present study, the abundance of *Lactobacillus* sp. remained unchanged. This inconsistency may be due to the differences in dietary *B. licheniformis* spore concentrations used in the 2 studies. Our previous studies have demonstrated that SFP supplementation can modulate the cecal microbiota of broilers in lipopolysaccharide or dextran sulfate sodium-induced inflammatory models (Chen and Yu, 2021, 2022b). In the present study, similar to LFP supplementation, SFP supplementation reduced species richness in the cecal digesta of broilers. LFP and SFP supplementation consistently reduced the abundance of *Lachnoclostridium* sp. and increased the abundance of *Blautia* sp. By contrast, compared with LFP supplementation, SFP supplementation specifically increased *Lactobacillus* sp. abundance but reduced that of *Clostridium* sp. It has been demonstrated that *B. subtilis* and *B. licheniformis* differentially produce extracellular enzymes, antimicrobial peptides, and secondary metabolites (Velmurugan et al., 2009; Sumi et al., 2015; Elshaghabee et al., 2017). These microbe-derived substances also play a critical role in modulating gut microbiota composition (Liu et al., 2022). Thus, the differences in the effects of SFP and LFP supplementation on the cecal microbiota may be due to the differences in the production of extracellular enzymes, antimicrobial peptides, and secondary metabolites (Velmurugan et al., 2009; Sumi et al., 2015; Elshaghabee et al., 2017). These results indicated that at identical supplementation concentrations, SFPs and LFPs have a distinct effect on the regulation of cecal microbiota composition in broilers.

In the present study, the functional annotation of bacterial genome revealed that *GT2* was the most dominant microbial carbohydrate-active enzyme gene in the cecal digesta of broilers and differentially regulated among groups. GTs catalyze the transfer of a glycosyl group from activated sugar donors to a diverse range of acceptors, such as lipids, proteins, antibiotics, xenobiotics, and phytotoxins (Bowles et al., 2005). GT-mediated glycosylation can alter the stability, chemical properties, and bioactivity of its acceptors (Bowles et al., 2005). Glycosylation also can occur on microbe-derived substances, such as mycotoxins. Mycotoxins can be glycosylated, generating modified mycotoxins with decreased toxicity (Li et al., 2020). Mycotoxins, which are toxic fungal secondary metabolites, constitute a serious problem for the gut health and growth of poultry. Mycotoxin contamination commonly occurs in the field as well as during animal feed processing and storage (Yang et al., 2019). LFP supplementation increased microbial *GT2* expression in the cecal digesta, and broiler growth performance was positively correlated with microbial *GT2* abundance, implying that microbial *GT2* might regulate growth-related factors or their activity in the intestines of broilers through glycosylation. Whether LFP-mediated increase in microbial *GT2* expression contributes to detoxification of mycotoxins through glycosylation remain to be confirmed. Differential carbohydrate-active enzyme gene distribution was also observed between the effects of SFP and LFP treatments because of the dominance of different bacterial taxa. These findings thus demonstrated that SFPs and LFPs not only differentially modulate cecal microbiota composition but also alter microbial carbohydrate-active enzyme gene levels in the cecal digesta of broilers. Whether alterations in the composition of microbial carbohydrate-active enzyme genes, particularly *GT2*, or their expression has a beneficial effect on gut health and growth of broilers warrant further investigation.

In conclusion, LFP supplementation improves the body weight and average daily gain, promotes jejunal and cecal intestinal barrier function and antioxidant gene expression, modulates cecal microbiota composition, and increases microbial glycosyltransferase 2 gene expression in broilers. Specifically, LFP promoted broiler growth more effectively than did SFP. This finding provides an improved understanding of the benefits of using LFPs as an antibiotic alternative in poultry feeds

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102670.

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