

Dietary effects of sophorolipids on nutrient bioavailability and intestinal microenvironments in broiler chickens

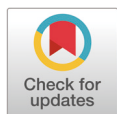
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Abstract

Using antibiotics as growth promoter has been banned in poultry feed industry, thus various researchers try to seek an alternative to replace the growth-promoting antibiotics. In this study, we aimed to evaluate the growth performance via intestinal nutrient utilization and cecal microbial composition of broiler after dietary supplementation with most commonly using antibiotics, zinc bacitracin, and sophorolipid. A total of 180 1-day-old chicks were randomly assigned, and dietary treatment was as follow: CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented diet; and SPL, 250 ppm of sophorolipid supplemented diet. Their growth performance was evaluated and the samples of blood, small intestine, and ileal and cecal digesta were collected for biochemical, histological, and genomic analyses. The body weight and average daily gain of 7-day-old chicks were higher in ZB and those in overall experimental period were improved by ZB and SPL supplementation ($p < 0.05$). Their intestinal characteristics were not affected by dietary treatments in duodenum and ileum. Nonetheless, villus height was increased by SPL supplementation in jejunum ($p < 0.05$). Moreover, dietary SPL supplementation could down-regulate the expression level of pro-inflammatory cytokine, $IL-1\beta$ ($p < 0.05$). mRNA levels of lipid and protein transporters did not differ among the treatments, however, relative expression levels of carbohydrate transporters, $GLUT2$ and $SGLT1$ were increased in broiler chicken's jejunum fed zinc bacitracin and sophorolipid supplemented diets ($p < 0.05$). Dietary zinc bacitracin supplementation could increase the population of *Firmicutes* in phylum level, and the portion of *Turicicacter* in genus level. On the other hands, the portion of *Faecalibacterium* was increased by dietary SPL supplementation compared to the other treatments. Our findings suggest that SPL supplementation improves growth performance through enhanced carbohydrate utilization capacity via improvement of gut morphological status and modulation of the cecal microbial population of broilers.

Keywords: Broiler, Growth, Gut microenvironment, Nutrient transporter, Sophorolipid, Zinc bacitracin

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Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Kwak MJ, Park MY, Whang KY.

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Ethics approval and consent to participate

The project underwent proper ethical standards and the experiments were approved by the Institutional Animal Care and Use Committee of Korea University, Seoul, Korea (KU-2020-0083).

INTRODUCTION

Since the 1950s, antibiotic growth promoters (AGP) have been utilized as supplements to improve broiler productivity in terms of growth performance, including body weight (BW) and feed efficiency (FE) [1]. Nevertheless, concerns over residual or antibiotic resistance have resulted in the restriction of AGP usage in broilers and from early 2006, AGP were banned in many countries, including the United States, European Union members, and Korea [2–4]. Consequently, various alternative feed additives, such as organic acids and phytochemicals, were developed to replace antibiotics as growth promoters but none of them have equaled the efficiency of AGP yet [5–9].

Many studies were conducted to determine how antibiotics enhance broiler growth. Elimination of pathogenic gut microbiota via antibiotic action promotes energy utilization for growth [10]. Antibiotics also modulate gut development by enhancing mucosal immunity, feed digestion, and nutrient absorption [11–13]. However, the mechanisms involved in its growth-promoting effects remain unclear. The recent application of next-generation sequencing technique in animal nutrition has accentuated the capability of antibiotics to regulate gut microbiota, leading to an in-depth understanding of the mechanisms of antibiotic action in growth improvement in animals, as correlations were observed between the microbiota and biological phenotype by microbial sequencing. For example, AGP supplementation improved broiler weight gain and increased the ratio of Firmicutes to Bacteroidetes. Hou et al. distinguished the fecal microbiota composition between obese broilers and a divergently lean line [14]. Despite these studies, the link between the capacity of antibiotics to reduce the prevalence of enteric pathogens and the mechanism behind growth improvement remains obscure [2], which is essentially one of the reasons behind the complexity of developing alternatives for AGP.

In this study, we have focused on the novel glycolipid-type biosurfactant, named Sophorolipid (SPL). Recently, SPL has attracted much attention with its lower toxicity and more biodegradability compared to diverse biological detergents in various industrial fields including hygiene, medicine, and pharmacological fields [15]. SPL also could act as an antimicrobial agent, an immune modulator, a depigment agent, a wound healing accelerator, and a skin fibroblast stimulator [16–18]. Therefore, we conducted an experiment with SPL and zinc bacitracin to validate the possibility of SPL as a valuable replacement for AGP in livestock industry. ZB has been a commonly used antibiotic and it can improve the growth performance of broilers by alteration of intestinal morphology of broilers [19]. We aimed to investigate the effects of dietary supplementation with SPL on growth performance, intestinal morphology, nutrient utilization, and cecal microbiota of broilers.

MATERIALS AND METHODS

Dietary treatment of the broiler chicks

A total of 180 broiler chicks (1-day-old Ross 308) were obtained from a local hatchery. Their basal diets followed the requirements of the National Research Council and involved a three-phase (starter, grower, and finisher) feeding program for 35 days (Table 1). The chicks were randomly assigned to 3 treatment groups with 6 replicates (10 chicks/pen). The experimental groups for growth performance evaluation consisted of three dietary treatments: CON, basal diet; ZB, 100 ppm of ZB supplemented basal diet; SPL, 250 ppm of SPL supplemented basal diet. From days 35 to 38, Cr₂O₃ was added to their diets for subsequent collection of excreta. The chicks were fed and provided with water *ad libitum* and raised under the following environmental conditions:

Table 1. Composition of experimental diets for broiler starter (d 1 to 7), grower (d 8 to 21), and finisher (d 22 to 35) phases

Item	Starter	Grower	Finisher
Ingredient (% [as-is basis])			
Corn	52.45	55.82	58.60
Soybean meal (dehulled)	39.15	35.55	32.05
Soy oil	3.59	4.36	5.33
L-Lysine sulfate (55%)	0.31	0.21	0.21
DL-methionine (98%)	0.38		
L-Methionine (99%)		0.32	0.31
Threonine (98%)	0.10	0.07	0.06
Choline Cl (50%)	0.07	0.07	0.08
MonoCa-P	1.75		
Dicalcium phosphate		1.68	1.60
Limestone	1.54	1.25	1.10
Salt	0.30	0.25	0.25
NaHCO ₃	0.10	0.19	0.19
Vitamin mixture ¹⁾	0.14	0.14	0.12
Mineral mixture ²⁾	0.11	0.11	0.11
Total	100.00	100.00	100.00
Nutrient contents			
Crude protein (%)	23.00	21.50	20.00
AME _n (kcal/kg)	3,000.00	3,100.00	3,200.00
Ca (%)	0.96	0.87	0.81
P (%)	0.78	0.70	0.64
Lysine (%)	1.44	1.29	1.19
Methionine (%)	0.56	0.51	0.48
Cysteine (%)	0.37	0.35	0.33
Threonine (%)	0.97	0.88	0.81

¹⁾Provided per kilogram of complete diet: vitamin A, 6,300 IU; vitamin D, 2,800 IU; vitamin E, 35 mg; vitamin K₃, 1.75 mg; vitamin B₁, 2 mg; vitamin B₂, 6 mg; vitamin B₆, 3 mg; vitamin B₁₂, 13 µg; biotin, 0.1 mg; calcium pantothenic acid, 15 mg; folic acid, 1.5 mg; niacin, 50 mg.

²⁾Provided per kilogram of complete diet: Mn, 100 mg; Cu, 17 mg; Zn, 92 mg; Fe, 50 mg; I, 1.5 mg; Co, 0.15 mg; Se, 0.3 mg. AMEn, nitrogen-corrected apparent metabolizable energy.

gradually decreasing temperature from 32°C to 24°C, average room humidity of 60% to 65%, and 20 h of light and 4 h dark, as instructed in the Ross 308 manual. The methods pertaining to the use of animals in this study were approved by the Institutional Animal Care and Use Committee (IACUC, KU-2020-0083) of Korea University.

Growth performance evaluation and sample collection

The BW and feed intake were measured weekly for 35 days, and thereafter, 2 chicks from each pen were sacrificed with CO₂ gas to collect the blood and small intestine. The weight and length of small intestine from each chick were measured. The duodenum, jejunum, and ileum samples were collected and fixed in 4% formaldehyde for histological analysis. Jejunal tissues were also collected for gene expression analysis and stored at -20°C until further analysis. On day 38, excreta of 2 chicks were collected and pooled per pen and the cecal contents were immediately frozen in dry ice until further analysis.

Serum analysis

Serum from the blood sample was obtained by centrifugation at 12,000×g at 4°C for 15 minutes. Serum glucose, blood urea nitrogen (BUN), triglycerides (TG), and total cholesterol levels were measured using an analytical kit by spectrometric method (Embiel, Gunpo, Korea).

Apparent ileal energy digestibility

After storage, the ileal digesta were thawed, dried at 105°C overnight, and ground prior to the energy digestibility test, which was conducted using a bomb calorimeter. The apparent total tract nutrient digestibility (AD) and apparent metabolizable energy (AME) were calculated using the equations described by Fenton and Fenton as follows [20]:

$$\text{AD (\%)} = 100 - 100 \times [(\text{Cr}_2\text{O}_3 \text{ in diet} \times \text{Nutrient in feces}) / (\text{Cr}_2\text{O}_3 \text{ in feces} \times \text{Nutrient in diet})]$$

$$\text{AME (kcal/kg)} = \text{Gross energy (GE)}_{\text{diet}} - [\text{GE}_{\text{feces}} \times (\text{Cr}_2\text{O}_3 \text{ of diet} / \text{Cr}_2\text{O}_3 \text{ in feces})]$$

Histological analysis of small intestine

The fixed jejunum samples were processed for paraffin embedding, sectioning, and hematoxylin and eosin staining for intestinal morphology analysis. Images were obtained using microscope camera (ToupTek, Hangzhou, Zhejiang, China). Villus height and crypt depth were analyzed at 10× magnification per slide using ImageJ v1.49 (NIH, Bethesda, MD, USA) [21].

RNA extraction and gene expression analysis

Frozen jejunal tissue samples were homogenized in TRIzol reagent (Invitrogen, Waltham, MA, USA) and the total RNA from each sample was obtained according to the manufacturer's protocol. Subsequently, cDNA was synthesized by reverse transcription using a high-capacity cDNA synthesis kit (Applied Biosystems, Waltham, MA, USA). The relative mRNA levels of each gene was determined using StepOnePlus (Applied Biosystems) and normalized to β-actin using 2^{-ΔΔCt} method [22]. The primer sequences used in this study are listed in Table 2.

DNA extraction and 16S rRNA amplicon metagenomic analysis

Microbial DNA was extracted from the cecal samples using QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The DNA samples were used as templates for polymerase chain reaction (PCR) amplification by targeting the V3–V4 hypervariable regions of the bacterial 16S rRNA gene using the primers 341F (5'-TCGT CGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3') and 805R (5'-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAGGACTAC HVGGGTATCTAATCC-3'). Secondary amplification was performed by attaching the barcodes with i5 forward primer (5'-AATGATACGGCGACCACCGAGATCTACAC-XXXXXXXXTCGTCGGCAGCGTC-3') and i7 reverse primer (5'-CAAGCAGAAG ACGGCATACGAGAT-XXXXXXXX-GTCTCGTGGGCTCGG-3'), with X referring to the barcode region. The PCR conditions were as follows: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, polymerization at 72°C for 30 s with 25 cycles for primary PCR, and 8 cycles for secondary PCR. The PCR amplicons were purified using CleanPCR (CleanNA, Waddinxveen, Netherlands) and the quality and size of the products were assessed using 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA). Equal amounts of amplicons from each sample were pooled and subjected to Illumina MiSeq sequencing (Illumina, San Diego, CA, USA) at ChunLab (Seoul, Korea).

Table 2. Oligonucleotide primers used in qRT-PCR analysis

Gene name	Sequence (forward, reverse)	Reference
Housekeeping gene		
GAPDH	F: 5'- CTACACACGGACACTTCAAG -3' R: 5'- GACTACGGGGGTACAAACA -3'	NM_204305
Carbohydrate transporters		
GLUT2	F: 5' - AAAGAGAGTGTCCATCGGGC -3' R: 5' - GGAAGACGGAGATAACCGTGA -3'	NM_207178
SGLT2	F: 5' - TGCTGCGGACATCTCTGTTA -3' R: 5' - TCAGAGCATTCCATTCATACCCTC -3'	NM_001293240
Lipid transporters		
FABP1	F: 5'- ACTGGCTCCAAAGAATGACCAATG -3' R: 5'- TGCTCCGTTGAGTTCCGGTCAC -3'	NM_204192
CD36	F: 5'- GCGATTTGGTTAATGGCACT -3' R: 5'- TCTCCAACATCAATCGGTGA -3'	NM_001030731
DGAT2	F: 5'- AAAAGGGGATGCTGCCTATCT -3' R: 5'- GCTTACGCAGCTCCATCTTCT -3'	FJ868804
FATP4	F: 5'- AGGGATTTGTGAAACTGGCACT -3' R: 5'- CTTTGGGATGGTGATGGGTT -3'	XM_419374.4
Protein transporters		
PepT1	F: 5'- TTTGACCAGCAGGGATCGAG -3' R: 5'- CCAGCAAGGAACATGCCAAC -3'	NM_204365
CAT1	F: 5'- CAAGAGGAAAACCTCCAGTAATTGCA -3' R: 5'- AAGTCGAAGAGGAAGGCCATAA -3'	XM_417116
rBAT	F: 5'- CTTGCAACAGTGAGCTACCCATA -3' R: 5'- TAAAGACGCTGTCTAACCCTCCAA -3'	XM_426125
y+LAT1	F: 5'- AGGGTGTGTGGAGATGTCAG -3' R: 5'- GAAGCTGTGTGAACGACG -3'	XM_418326
Tight junction proteins		
ZO-1	F: 5'- GTCTCCCTACTCTGGGGGAA -3' R: 5'- GAACAGCAAATGCGTGCTCA -3'	XM_413773
CLDN1	F: 5'- AGATCCAGTGCAAGGTGTACG -3' R: 5'- GCTGACAGACCTGCAATGATGA -3'	NM_001013611.2
OCLD	F: 5'- ATGAGACCGACTACACCACG -3' R: 5'- CCACATCCTGGTATTGAGGGC -3'	NM_205128.1
Inflammatory cytokines		
IL-1 β	F: 5'- TGCCTGCAGAAGAAGCCTCG -3' R: 5'- GGAAGGTGACGGGCTCAAAA -3'	NM_204524
TGF- β	F: 5'- ATCCTCAGCAAACCTGCGTCT -3' R: 5'- GCTCTTTGGCCCAATACTCA -3'	NM_001318456.1
IFN- γ	F: 5'- AGCTCCCGATCAACGAC -3' R: 5'- CAGGAGGTCATAAGATGCCA -3'	NM_205149.1

qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT2, glucose transporter 2; SGLT2, sodium-glucose transporter 2; FABP1, fatty-acid binding protein 1; CD36, cluster of differentiation 36; DGAT2, diacylglycerol o-acyltransferase; FATP4, fatty-acid transport protein 4; PepT1, peptide transporter 1; CAT1, cationic amino acid transporter; rBAT, neutral and basic amino acid transport protein; y+LAT1, y + L amino acid transporter 1; ZO-1, zona occludens-1; CLDN1, claudin 1; OCLD, occludin; IL-1 β , interleukin-1 β ; TGF- β , transforming growth factor β ; IFN- γ , interferon- γ .

Microbial community analysis

The raw sequence data were analyzed using mothur v.1.38 [23]. The barcoded fusion primers and low-quality reads with read lengths below 300 bp were eliminated. Subsequently, the sequencing reads were compared using basic local alignment search tool (BLASTN) as a reference to remove chimeric sequences. Microorganisms were taxonomically identified using EzBioCloud database at the species level with a 97% similarity cutoff (ChunLab).

Statistical Analysis

All values are presented as mean \pm SE and were derived from triplicate experiments. Quantitative data were subjected to one-way ANOVA. Multiple comparisons were performed using Tukey's honestly significant difference (HSD) test and the results were considered statistically significant at $p < 0.05$. Analyses were performed using the SAS 9.4 software (SAS Institute, Cary, NC, USA). The Wilcoxon rank-sum test was used to analyze the differences in alpha diversity indices and the taxonomy of microbial populations.

RESULTS

Growth performance and serum analysis

The BW of the chicks improved in ZB and SPL treatments on day 14, and both final BW (day 35) were significantly heavier compared to the CON group. Moreover, average daily gain (ADG) throughout the experimental period (days 1 to 35) also significantly increased in both ZB and SPL groups (Table 3). The final BW improved in ZB (16.54%), and SPL (14.59%) compared to that in the control ($p < 0.05$). Although their average daily feed intake (ADFI) did not differ among the treatments, their ADG significantly increased in ZB and SPL in every growth period except in the control ($p < 0.05$). This resulted in greater FE in ZB and SPL compared to that in the control during the overall period (day 0–35).

The levels of serum glucose, BUN, TG, and total cholesterol marginally differed among the treatments. However, TG levels in ZB and SPL treatment groups were significantly increased compared to that of CON group (Table 4). The concentrations of BUN, total cholesterol and glucose were not affected by dietary AGP and SPL supplementation in 35-day-old broilers.

Apparent total tract energy digestibility and characteristics of small intestine

The AD levels in the chicks did not vary considerably among the treatments (Table 5). The length and weight of each section of the small intestine were measured by histological staining (Table 6). Representative images of jejunum are shown in Fig. 1. The ratio of small intestine weight to BW did not considerably differ among the treatments, however, the intestinal weight per length was significantly lighter compared to the other treatments. Moreover, villus height, crypt depth, and villus height to crypt depth (V:C) ratio in the duodenum and ileum did not substantially differ among the treatments, however, villus height in the jejunum was significantly higher in the SPL treatment group compared to the other treatments. Relative gene expression levels of various nutrient transporters in the jejunum, namely glucose and amino acid transporters and fatty acid binding proteins are summarized in Table 7. The relative transcript levels of nutrient transporters in the jejunum were not differed among the treatments, nevertheless, expression levels of glucose transporters including GLUT2 and SGLT1 were significantly increased in ZB and SPL supplemented diet groups. mRNA expression levels of tight junction proteins were not different among treatments, however, the relative expression level of il-1 β was significantly decreased by dietary SPL supplementation in broiler chickens (Table 8).

Table 3. Growth performance of broiler chickens fed experimental diets for 35 days¹⁾

Treatment	CON ²⁾	ZB	SPL	SEM	p-value
Body weight (g)					
Day 1	45.52	45.46	45.48	0.083	0.962
Day 7	134.18 ^b	151.82 ^a	133.46 ^b	2.778	0.001
Day 21	582.00 ^b	732.00 ^a	702.19 ^a	22.657	0.005
Day 35	2,001.73 ^b	2,332.80 ^a	2,293.79 ^a	50.831	0.004
Average daily gain (g/day)					
Day 1–7	12.67 ^b	15.19 ^a	12.57 ^b	0.395	0.001
Day 8–21	31.99 ^b	41.44 ^a	40.62 ^a	1.519	0.007
Day 22–35	101.41 ^b	114.34 ^a	113.69 ^a	2.127	0.007
Day 1–35	55.89 ^b	65.35 ^a	64.24 ^a	1.452	0.004
Average daily feed intake (g/day)					
Day 1–7	34.65	35.42	34.63	0.245	0.348
Day 8–21	85.00	84.86	91.06	4.240	0.816
Day 22–35	139.20	143.16	148.02	2.735	0.451
Day 1–35	96.61	98.29	102.56	2.414	0.619
Feed efficiency					
Day 1–7	0.366 ^b	0.429 ^a	0.363 ^b	0.010	0.003
Day 8–21	0.380	0.507	0.453	0.022	0.056
Day 22–35	0.730	0.802	0.770	0.014	0.110
Day 1–35	0.580 ^b	0.670 ^a	0.629 ^a	0.015	0.042

¹⁾Mean values represent 6 replicates per treatment; pen is the experimental unit. 10 chicks in 1 pen.

²⁾CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented group; SPL, 250 ppm of sophorolipid supplemented group.

^{a,b)}Different superscripts within the same row significantly differ ($p < 0.05$).

Table 4. Blood biochemical analysis of the broiler chickens fed experimental diets for 35 days¹⁾

Treatment	CON	ZB	SPL	SEM	p-value
Glucose (mg/dL)	222.87	249.26	284.09	12.405	0.136
Blood urea nitrogen (mg/dL)	2.56	2.63	2.42	0.097	0.683
Triglyceride (mg/dL)	10.56 ^b	25.65 ^a	27.34 ^a	2.855	0.016
Total cholesterol (mg/dL)	92.67	83.64	89.50	4.905	0.640

¹⁾Mean values represent 6 replicates per treatment; a chicken is the experimental unit.

²⁾CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented group; SPL, 250 ppm of sophorolipid supplemented group.

^{a,b)}Different superscripts within the same row significantly differ ($p < 0.05$).

Table 5. Energy digestibility of the broiler chickens fed experimental diets for 35 days¹⁾

Treatment	CON ²⁾	ZB	SPL	SEM	p-value
Energy digestibility (%)	79.55	78.45	84.54	1.766	0.640

¹⁾Mean values represent 6 replicates per treatment; a chicken is the experimental unit.

²⁾CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented group; SPL, 250 ppm of sophorolipid supplemented group.

Cecal microbiota community analysis

At the phylum level, all the bacterial sequences were classified into 5 phyla; the ceca were dominated by Firmicutes (70.3%) and Bacteroidetes (26.7%). The population of Firmicutes increased by ZB supplementation, whereas that of Bacteroidetes concomitantly decreased. Consistent with the results of taxonomic composition analysis, the ratio of Firmicutes to Bacteroidetes increased in

Table 6. Characteristics and morphological indexes of small intestine from the broiler chickens fed experimental diets for 35 days¹⁾

Treatment	CON ²⁾	ZB	SPL	SEM	p-value
BW (kg)	2.16	2.50	2.26	0.070	0.119
Length (cm)	166.00	167.17	163.67	4.234	0.949
Weight (g)	52.25	53.78	56.91	2.356	0.737
Relative weight (g/kg BW)	25.03	20.44	24.44	0.837	0.037
Weight/ length (g/cm)	0.33	0.30	0.36	0.008	0.018
Duodenum					
Villus height (μm)	1,101.75	1,168.07	1,262.17	31.747	0.207
Crypt depth (μm)	156.42	167.11	187.71	5.983	0.152
Villus height/crypt depth	7.04	7.01	6.77	0.142	0.743
Jejunum					
Villus height (μm)	933.60 ^b	910.32 ^b	1,127.69 ^a	31.543	0.001
Crypt depth (μm)	86.90	90.61	102.22	2.870	0.063
Villus height/crypt depth	10.84	10.09	11.05	0.235	0.223
Ileum					
Villus height (μm)	372.65	402.39	440.68	21.399	0.471
Crypt depth (μm)	56.39	62.95	60.78	1.593	0.248
Villus height/crypt depth	6.58	6.45	7.21	0.304	0.601

¹⁾Mean values represent 6 replicates per treatment; a chicken is the experimental unit.

²⁾CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented group; SPL, 250 ppm of sophorolipid supplemented group.

^{a,b}Different superscripts within the same row significantly differ ($p < 0.05$).

BW, body weight.

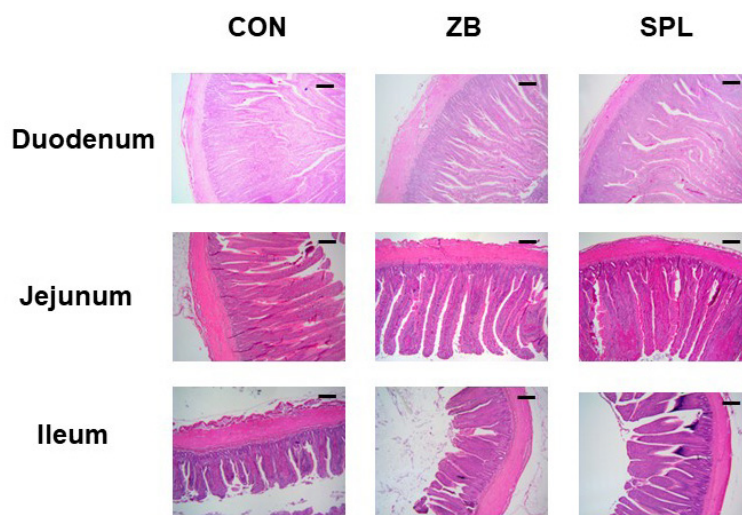


Fig. 1. Representative images of jejunum after H&E staining from broiler chickens. CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented diet; SPL, 250 ppm of sophorolipid supplemented diet, bar = 250 μm.

the ZB-supplemented groups relative to that in the control group. We further analyzed the cecal microbial taxonomy at the genus level (Fig. 2B). Of the 712 genera, the bacterial sequences were classified into 18 genera. The dominant genera in the ceca of the chicks were *Barnesiella* (23.47%) and *Faecalibacterium* (15.38%), which belong to Bacteroidetes and Firmicutes, respectively. The proportion of *Barnesiella*, *Faecalibacterium*, and JN713889 were increased by dietary SPL

Table 7. Relative gene expression levels of nutrient transporters in jejunum of broiler chickens fed experimental diets for 35 days¹⁾

Treatment	CON ²⁾	ZB	SPL	SEM	p-value
Carbohydrate transporter, fold change					
<i>GLUT2</i>	1.00 ^b	3.37 ^a	3.53 ^a	0.438	0.020
<i>SGLT1</i>	1.00 ^b	2.15 ^a	1.98 ^a	0.189	0.017
Lipid transporter, fold change					
<i>FABP1</i>	1.00	0.81	1.11	0.071	0.231
<i>CD36</i>	1.00	2.49	3.13	0.487	0.269
<i>DGAT2</i>	1.00	1.08	1.01	0.066	0.884
<i>FATP4</i>	1.11	1.69	2.05	0.198	0.156
Protein transporter, fold change					
<i>PepT1</i>	1.00	0.62	1.10	0.112	0.201
<i>CAT1</i>	1.00	1.00	0.98	0.054	0.989
<i>rBAT</i>	1.00	0.97	0.96	0.042	0.931
<i>Y+LAT1</i>	1.00	2.15	1.59	0.258	0.221

¹⁾Mean values represent 6 replicates per treatment; a chicken is the experimental unit.

²⁾CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented group; SPL, 250 ppm of sophorolipid supplemented group.

^{a,b}Different superscripts within the same row significantly differ ($p < 0.05$).

GLUT2, glucose transporter 2; SGLT2, sodium-glucose transporter 2; FABP1, fatty-acid binding protein 1; CD36, cluster of differentiation 36; DGAT2, diacylglycerol o-acyltransferase 2; FATP4, fatty-acid transport protein 4; PepT1, peptide transporter 1; CAT1, cationic amino acid transporter; rBAT, neutral and basic amino acid transport protein; y+LAT1, y + L amino acid transporter 1.

Table 8. Relative gene expression levels of tight junction proteins and inflammatory cytokines in jejunum of broiler chickens fed experimental diets for 35 days¹⁾

Treatment	CON	ZB	SPL	SEM	p-value
Tight junction proteins, fold change					
<i>ZO-1</i>	1.00	1.70	1.46	0.198	0.483
<i>OCLD</i>	1.00	0.93	0.96	0.054	0.889
<i>CLDN1</i>	1.00	0.59	0.54	0.094	0.079
Inflammatory cytokine, fold change					
<i>IL-1β</i>	1.00 ^a	0.66 ^{ab}	0.42 ^b	0.096	0.033
<i>TGF-β</i>	1.00	0.70	0.99	0.072	0.130
<i>IFN-γ</i>	1.00	1.19	0.75	0.102	0.174

¹⁾Mean values represent 6 replicates per treatment; a chicken is the experimental unit.

²⁾CON, basal diet; ZB, 100 ppm of zinc bacitracin supplemented group; SPL, 250 ppm of sophorolipid supplemented group.

^{a,b}Different superscripts within the same row significantly differ ($p < 0.05$).

ZO-1, zona occludens-1; OCLD, occludin; CLDN1, claudin-1; IL-1 β , interleukin-1 β ; TGF- β , transforming growth factor- β ; IFN- γ , interferon- γ .

supplementation, and the population of *Romboutsia*, *Blautia*, *Lactonifactor*, and *Turicibacter* was decreased. On the contrary, dietary ZB addition decreased the portion of *Barnesiella*, *Faecalibacterium*, and *Lactonifactor*, but increased those of ZN713389, *Turicibacter* compared to the CON group.

DISCUSSION

This study demonstrated that SPL and ZB supplementation possibly promotes growth and alters the cecal microbiota towards the dominance of Firmicutes relative to Bacteroidetes with upregulated jejunal glucose utilization and upregulated intestinal inflammation. Previous studies

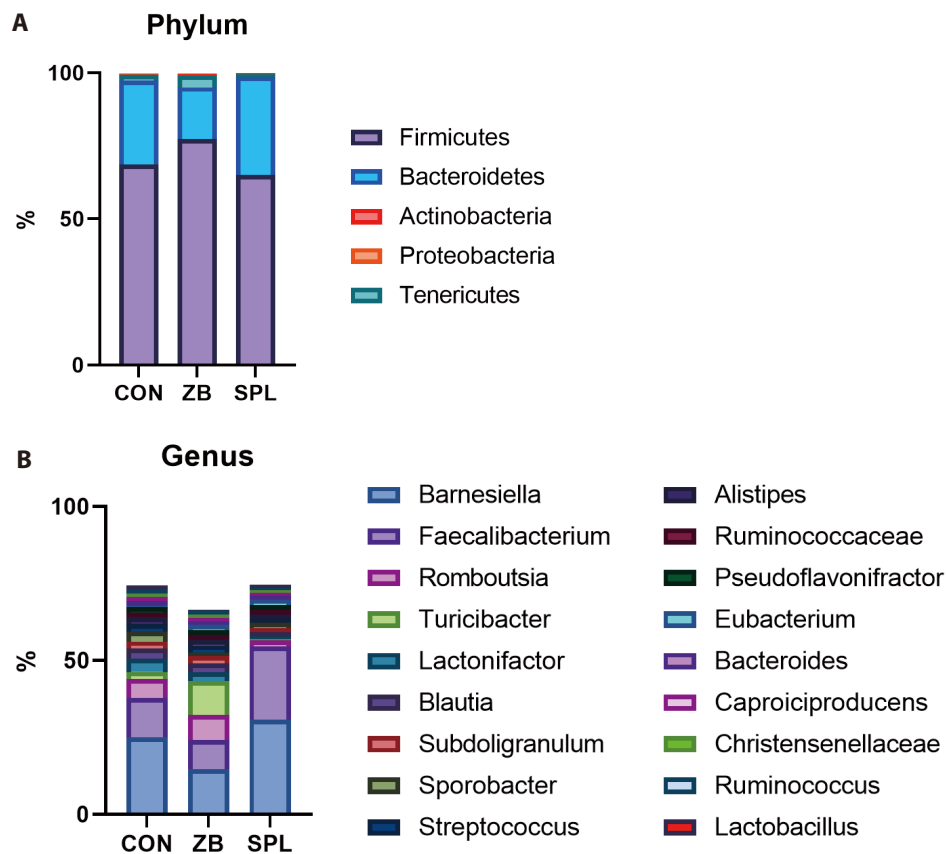


Fig. 2. Cecal microbiota composition in the broiler chickens fed experimental diets supplemented with 100 ppm of zinc bacitracin and 250 ppm of sophorolipid. (ZB) at 0 (CON), 50 (ZB 50), 100 (ZB 100), or 200 (ZB 200) ppm for 35 days. (A) phylum level and (B) genus level. The values represent the mean values of 6 replicates per treatment. One chicken is the experimental unit.

have reported the growth-promoting effects of SPL and ZB in poultry industrial field [11,15]. They commonly proposed that dietary SPL would be able to modify intestinal microbial population in a proper trend to promote gut microenvironments [15]. Particularly, this study indicated that SPL in broilers feed could increase glucose utilization capacity by upregulation of relative expression levels of carbohydrate transporters, and energy utilization including lipid metabolism in liver.

The results of biochemical analyses indicated that dietary supplementation with ZB and SPL did not affect the serum glucose, BUN, and total cholesterol levels in broilers. These data correspond to the findings of Manafi et al. and Attia et al., conversely, the concentration level of TG was increased by dietary addition of SPL in this study [19,24]. We assumed that dietary SPL could increase the carbohydrate uptake in jejunum by increment of expression level of transporters, including GLUT2 and SGLT, and it might lead to boosted lipid biosynthesis in liver metabolism.

We also evaluated whether the improved growth performance by SPL supplementation could be attributed to nutrient utilization and found that SPL supplementation showed increasing tendency in energy digestibility and significantly raised jejunal villus height of the chicks. On the other hands, it remains to be clarified whether antibiotics alter intestinal structure and function. In a study by Manafi et al., energy digestibility improved, and villus height was elevated with 50 ppm of bacitracin methylene disalicylate (BMD) supplementation, but their study differed from ours in terms of experimental duration; they examined the broilers 42 days after supplementation [19].

Nonetheless, our results are consistent with the findings of Miles et al., and Ao et al., as the villus height in their BMD-supplemented treatments did not differ from those of the control groups at 5 weeks [25,26]. Considering the variation in the forms of bacitracin in BMD and ZB, Daghighian et al. compared their corresponding effects on the growth performance of turkeys and found that BMD supplementation at 55 ppm increased BW in female turkeys, relative to those supplemented with the same amount of ZB at 4 weeks [27].

Previous studies have investigated the effects of AGP and SPL on cecal microbiota. We observed that ZB supplementation increased the population of Firmicutes, as opposed to Bacteroidetes, which is consistent with the findings of Gong et al. [11]. Furthermore, there was a remarkable shift in the population of *Barnesiella* at the genus level with a decline in Bacteroidetes. The functional role of *Barnesiella* is yet to be described in detail, but Wang et al. reported a negative correlation between the abundance of *Barnesiella* and the high-fat diet-induced metabolic syndrome phenotype, which is highly associated with increased BW. Some species of *Barnesiella* were found to produce acetate, suggesting that they suppress body fat accumulation [28,29]. Despite the indirect relationship, our results corroborate the potential of *Barnesiella* to attenuate weight gain in broilers.

Our findings indicate that a possible mechanism of SPL and ZB supplementation linked to increased nutrient absorption for growth improvement in broilers. Turnbaugh et al. reported that enhanced energy harvest and nutrient utilization via the modulation of cecal bacteria may be associated with the abundance of Firmicutes, which are consistent with our findings [30]. Numerical increment in the AD levels of SPL-supplemented chicks could be attributed to our experimental period, which was relatively sufficient for microbial populations to modify energy digestibility.

ZB and SPL has proven to be effective against gram-positive bacteria [15,31]. In contrast, we found that the population of *Barnesiella*, a gram-negative bacterium, dramatically decreased as ZB supplementation increased. Given that *Barnesiella* is the largest population of Bacteroidetes at the genus level in the chicken cecal content, ZB supplementation in our study may have largely affected *Barnesiella* among other populations [32]. A similar occurrence was observed in the study by Kim et al., in which tylosin, another antibiotic, decreased the gram-negative bacteria population despite the distinct ability of the antibiotic to target gram-positive bacteria. Further studies are needed to elucidate the role of *Barnesiella* in broiler energy utilization and nutrient metabolism [33].

Overall, the results of our study indicate that optimal dosage of ZB and SPL supplementation promote growth performance and modulate cecal microbial composition. Moreover, SPL supplementation may increase the glucose utilization capacity by upregulation of relative expression level of carbohydrate transporter and gut morphological index and lowering inflammation in jejunum.

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