




Effects of *Saccharomyces cerevisiae* hydrolysate on growth performance, immunity function, and intestinal health in broilers

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ABSTRACT The current study was performed to explore the effects of dietary supplementation of *Saccharomyces cerevisiae* hydrolysate (SCH) on growth performance, immune function, and intestinal health in broiler chicken. A total of 300 Ross 308 male broilers (1-day-old) were randomly assigned to 2 dietary treatments including a basal diet (control group), and a basal diet supplemented with SCH feed additive (500 mg/kg in starter and grower phase, and 250 mg/kg in finisher phase). Each treatment had 6 replicates with 25 birds each. The results showed that the addition of SCH promoted growth during d 15 to 28 ($P < 0.05$). Although the addition of SCH had no significant effect on the intestinal relative indexes, it significantly increased the jejunum villus height (VH) and the ratio of villus height to crypt depth (VCR) of jejunum, and decreased the crypt depth (CD) of ileum ($P < 0.05$). Furthermore, SCH addition significantly downregulated the mRNA expression of immunomodulatory genes (TNF- α , IL-1 β ,

and IL-6), and upregulated the tight junction genes (ZO-1 and Claudin-1) ($P < 0.05$). High throughput sequencing analysis of bacterial 16S rRNA revealed that dietary SCH supplementation altered cecum microbiota. Alpha diversity analysis showed that a higher bacterial richness in cecum of broilers fed with SCH. The composition of cecum microbiota regulated by SCH addition was characterized by an increased abundance of Firmicutes and a reduced abundance of Bacteroidetes. At the genus level, dietary SCH resulted in a decrease of *Bacteroides* and an increase of short-chain fatty acids (SCFA)-producing bacteria including *Lactobacillus* and *Faecalibacterium*. Taken together, dietary SCH supplementation can stimulate the growth of broilers by regulating the intestinal immunity and barrier function, and improving the intestinal morphology, which may be related to the enhancement of bacterial diversity and the changes of intestinal microbial composition.

Key words: *Saccharomyces cerevisiae* hydrolysate, growth performance, microbiota, broilers

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INTRODUCTION

In the context of antibiotic free livestock production, studies on feed additives as antibiotic substitutes have gained more interest. The main concern for the evaluation of antibiotic alternative is to assess their efficacy on animal health and immune response, as well as animal productivity (Caly et al., 2015; Salahaen et al., 2017). Among the alternatives, yeast hydrolysate is a

promising product, which are usually obtained by liquid fermentation with *Saccharomyces cerevisiae*, and then concentrated or dried after autolysis or hydrolysis catalyzed by exogenous enzymes. *Saccharomyces cerevisiae* hydrolysate (SCH) generally contains abundant nucleotides, B-vitamins, amino acids, and yeast cell wall polysaccharides (such as β -glucan and mannan). As an important component of SCH, nucleotides have a lot of benefits in improving growth performance, regulating immune function, and repairing the gastrointestinal tract of animals (Sauer, 2010; Superchi et al., 2012), and β -glucan and mannan oligosaccharide were generally used as prebiotics to regulate the immune response (Fadl et al., 2020). Meanwhile, SCH have been considered as one of the effective alternatives to antibiotic growth promoters (AGP) in animals, due to their

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ability to improve intestinal nutrients digestibility (Samarasinghe et al., 2004), promote the intestinal health (Fu et al., 2019), enhance disease resistance (Bu et al., 2019), and relieve inflammatory responses (Bu et al., 2020). The specific benefits of SCH on growth performance of poultry have already been described in considerable studies (Awaad et al., 2011; Santovito et al., 2018; Perricone et al., 2022). However, it is not clear how SCH leads to the improvement of growth performance and modulates the body health in broilers. One hypothesis for the mechanism of action of SCH could be related to an improved intestinal resistance to external injuries or a possible role in the modulation of intestinal microbiota (Khalid et al., 2021).

As the first line of defense against the external environment, the intestinal mucosa is firstly damaged when the inflammatory reaction occurs, which would negatively impact the digestion and absorption of nutrients (Bai et al., 2018). Intestinal microbiota plays an important role in digestion, barrier, and immune function of broilers, contributing to subsequent improvement in the growth performance (Pan and Yu, 2014; Pandit et al., 2018). It is widely accepted that AGP boost animal growth mainly through reducing pathogenic bacteria, increasing beneficial bacteria, and modulating gastrointestinal microbiota (Diarra and Malouin, 2014; Gadde et al., 2017). However, information regarding the effect of SCH on the intestinal microbiota in broilers is still limited. Modulation of immune status and intestinal microbiota may provide a new avenue to explain the potential effects of dietary SCH on growth performance and intestinal health.

Therefore, the purpose of this study was to investigate the action by which dietary SCH improve gut immunity and intestinal health, which would lead to the observed improved performance as result.

MATERIALS AND METHODS

Saccharomyces Cerevisiae Hydrolysate

The *Saccharomyces cerevisiae* hydrolysate (I-Care, batch number: 210210; production date: February 2021) used in this experiment was obtained from Prosol S.p.A (Madone, Italy), and added to broiler feed at a prescribed level (the optimal additive dosage has been determined in previous studies). The active ingredients presented in SCH supplement were 38% of crude protein, 4.9% of glutamic acid, 3.5% of nucleotides, 23% of β -glucans, and 15% mannan oligosaccharide. The MSDS and COA of I-Care can be found in Supplementary Materials.

Birds and Management

A temperature-controlled house ($33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for one-day-old chicks and $21^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for chickens after 4 wk old) was provided for this experiment during the whole rearing stage. Birds for the trial were allocated to 4-tier cages with 25 hens per cage (cage size:

$180\text{ cm} \times 120\text{ cm} \times 45\text{ cm}$), and the bottom of each cage was covered with mesh plastic litter. A total of 12 replicates of the 2 treatments were randomly distributed into 12 cages in the chicken house. Each cage is equipped with a feeding trough and 4 nipple drinkers. Chicks were provided with water and feed ad libitum. All birds remained in good health during the feeding period.

Experimental Design and Diets

A total of 300 one-day-old male Ross 308 broiler chicks purchased from a local hatchery were randomly allocated into 2 treatment groups that were fed corn-soybean meal basal diets (control group) and the basal diet supplied with SCH (I-Care, Prosol S.p.A, Italy). Each treatment contained 6 replicates of 25 chicks/cage. The addition amount of SCH was 500 mg/kg in starter & grower diet (d 1–28) and 250 mg/kg in finisher diet (d 29–42). The basal diets (Table 1) were formulated to meet National Research Council (1994).

This study was approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences, Beijing (approval No. FRI-CAAS-20210903). All the management for broilers was performed in accordance with the guidelines of

Table 1. The ingredient composition of basal diet and nutrient levels.

Items	Starter diet (1–14 d)	Grower diet (15–28 d)	Finisher diet (29–42 d)
Corn	54.87	59.12	57.90
Soybean meal (46 CP)	25.85	20.05	18.65
Corn gluten meal (60 CP)	4.00	4.50	5.00
Cottonseed meal	3.00	3.00	3.65
Rapeseed meal	2.50	2.80	3.00
Wheat middlings	2.00	2.00	2.50
Soybean oil	3.35	4.65	5.58
Dicalcium phosphate	1.55	1.25	0.98
Limestone	1.50	1.35	1.45
Salt	0.25	0.20	0.20
<i>DL</i> -Methionine	0.25	0.21	0.20
<i>L</i> -lysine.HCl	0.35	0.32	0.30
<i>L</i> -threonine	0.05	0.02	0.01
Vitamin Premix ¹	0.02	0.02	0.02
Mineral Premix ²	0.20	0.20	0.20
Choline chloride (50%)	0.10	0.10	0.10
Sodium bicarbonate	0.15	0.20	0.25
Phytase	0.01	0.01	0.01
Total	100.00	100.00	100.00
Nutrient levels ³			
AME (MJ/kg)	12.35	12.97	13.18
Crude protein, %	22.00	21.00	20.00
Calcium, %	1.00	0.90	0.85
Available phosphorus, %	0.40	0.35	0.30
Lysine, %	1.25	1.10	1.05
Methionine, %	0.57	0.52	0.50
Methionine+cystine, %	0.90	0.81	0.80
Threonine, %	0.81	0.72	0.68
Tryptophan, %	0.25	0.23	0.19

¹The vitamin premix supplied the following per kg of complete feed: vitamin A, 12,500 IU; vitamin D₃, 2,500 IU; vitamin K₃, 2.65 mg; vitamin B₁, 2 mg; vitamin B₂, 6 mg; vitamin B₁₂, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg.

²The mineral premix supplied the following per kg of complete feed: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; I, 0.35 mg, Se, 0.15 mg.

³Calculated composition.

Table 2. Primer sequences used for the real-time PCR analysis.

Name	Sequence 5'-3'	GenBank number
β -actin	F: 5'-TTGGTTTGTCAAGCAAGCGG-3' R: 5'-CCCCACATACTGGCACTTT-3'	NM_205518.1
TNF- α	F: 5'-TGTGTATGTGCAGCAACCCGTAGT-3' R: 5'-GGCATTGCAATTTGGACAGAAGT-3'	NM_204267
IL-1 β	F: 5'-GCTCTACATGTCGTGTGTGATGAG-3' R: 5'-TGTCGATGTCCCGCATGA-3'	NM_204524
IL-6	F: 5'-TCTGTTTCGCCTTTCAGACCTA-3' R: 5'-GACCACCTCATCGGGATTTAT-3'	AJ309540
IFN- γ	F: 5'-CTCCCGATGAACGACTTGAG-3' R: 5'-CTGAGACTGGCTCCTTTTCC-3'	NM_205149.2
ZO-1	F: 5'-CTCAGGTGTTTCTCTTCTCCTC-3' R: 5'-CTGTGGTTTCATGGCTGGATC-3'	XM_413773.4
Claudin-1	F: 5'-ACAACATCGTGACGCGCCA-3' R: 5'-CCCGTCACAGCAACAAACAC-3'	NM_001013511.2
Occludin	F: 5'-GCAGATGTCCAGCGTTACTAC-3' R: 5'-CGAAGAAGCAGATGAGGCAGAG-3'	NM_205128.1

raising Ross 308 broilers (Delezie et al., 2012). The experiment lasted for 42 d, divided into starter (day 1–14), grower (day 15–28), and finisher (d 29–42) stages.

Growth Performance

The birds and feed were weighed by pen at 0, 14, 28, and 42 d post-hatch for determination of growth performance, including body weight (**BW**), average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**). Mortalities and postmortem weight were recorded daily for the calculation of mortality, body weight gain, and mortality-corrected FCR.

Intestinal Relative Index and Morphology

On d 14, 28, and 42, 6 birds per treatment (1 bird per cage) were scarified and intestinal tissues were collected to analyze relative index of intestinal weight and morphology. Before that, birds had been fasted for 12 h. The relative index of intestinal weight was calculated according to the weight of duodenum, jejunum, ileum, cecum, and the BW of birds. Intestinal relative index = intestinal segment weight of empty (g) / body weight (g) \times 100%. Meanwhile, the middle segments of duodenum, jejunum and ileum (about 2 cm) were collected and fixed in 10% neutral buffered formalin. After wash, dehydration, and clarification, the samples were embedded in paraffin. The serial sections with a thickness of 5 μ m were placed on a glass slide for dewaxing, hydration, and staining. The villus height (**VH**) and crypt depth (**CD**) were measured by NIKON DS-U3 image processing and analyzing system (NIKON ECLIPSE CI, Tokyo, Japan). The ratio of villus height/crypt depth (**VCR**) was calculated.

Real-Time Quantitative PCR

On d 28 and 42, jejunum and ileum mucosa of 6 birds from each treatment group were collected to analyze the

gene expression levels. Total RNA of jejunum and ileum mucosa samples was extracted by Trizol reagent (Thermo Fisher Scientific, Wilmington, DE), and the purity and concentration of total RNA was determined by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Then, 1 μ g RNA of each sample was used to reverse-transcribe into cDNA using TransScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) following the manufacturer's guidelines. The PowUp SYBR Master Mix (Thermo Scientific) was used to carry out real-time quantitative polymerase chain reaction (**qRT-PCR**) on a QuantStudio 5 real-time PCR Design & Analysis system (Applied Biosystems, Foster City, CA). Each sample was measured in duplicate. Primers sequences used in this study were shown in Table 2. The relative mRNA expression levels were normalized to avian β -actin by the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001).

DNA Extraction and PCR Amplification of 16S rRNA Gene Sequences

On d 42, microbial DNA was extracted from 300 mg terminal ileum content samples taken from 6 birds from each treatment group using the E.Z.N.A Soil DNA Kit (Omega Bio-tek, Norcross, GA) according to manufacturer's instructions. The hypervariable region V3–V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp 9700 PCR thermocycler (ABI, CA). The PCR reaction conditions were: initial denaturation at 95 °C for 2 min, followed by 25 cycles consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 5 min at 72 °C. According to the manufacturer's instructions, amplicons were extracted and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA). The excess primer dimers and dNTPs were removed. Purified amplicons were pooled in equal amounts and paired-end sequenced (2 \times 250 bp) throughput analysis was performed at

Shanghai Majorbio Bio-Pharm Technology Co., Ltd., using the Illumina MiSeq platform. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database.

Statistical Analysis

Data analysis of growth performance, intestinal relative index, intestinal morphology, gene expression level and differential species identified were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC). The replicate (each cage) was considered the experimental unit for growth performance and the bird represented the experimental unit for intestinal samples. Data were analyzed using one-way ANOVA and means were separated using Duncan's multiple range test. Differences were considered statistically significant at $P < 0.05$. Data were expressed as Means \pm SD.

For microbiota profiling, raw pair-end sequences were demultiplexed and quality-filtered using The Quantitative Insights Into Microbial Ecology (QIIME, version 1.17; Edgar, 2010). Sequences with length shorter than 150 bp, average Phred scores lower than 20 were filtered through (Dai et al., 2020). The effective reads were clustered into operational taxonomic units (OTUs) based on the 97% similarity. Classification of OTUs at various taxonomic levels were implemented using the Greengenes database. The rarefaction curves and α -diversity analysis were calculated using QIIME (Caporaso et al., 2010). Beta-diversity was estimated using principal coordinate analysis (PCoA) and partial least squares discriminant analysis (PLS-DA). Correlations were analyzed using spearman correlation with the pheatmap package ($P < 0.05$).

RESULTS

Growth Performance

The effects of SCH on growth performance of broilers at different growing phases are shown in Table 3. There's a clear trend that SCH increased BW, ADG and ADFI during the starter phase (1–14 d). Similarly, during finisher phase (29–42 d), dietary SCH supplementation tended to increase BW, ADG, and improve FCR. In particular, dietary SCH significantly increased broiler BW at 28 d ($P < 0.05$), and partially increased ADG for grower phase (15–28 d) ($P < 0.10$). The FCR did not differ, although SCH group had numerically lower FCR ($P > 0.05$). In general, feeding diet supplemented with SCH tended to improve the performance of broilers.

Intestinal Relative Index

Table 4 shows the effect of dietary SCH supplemental on intestinal relative index of broilers. On the whole, there was no significant difference in the relative index of duodenum, jejunum, and ileum between the 2 groups ($P > 0.05$), but the relative index of duodenum and

Table 3. Effect of dietary SCH supplementation on growth performance of 1 to 42-day-old broilers.

Item ²	Control	SCH ¹	P value
Starter (1–14 d)			
BW at d 14 (g)	440.6 \pm 17.2	461.7 \pm 23.8	0.110
ADG (g)	26.8 \pm 1.2	28.3 \pm 1.6	0.095
ADFI (g)	33.6 \pm 2.1	35.1 \pm 0.5	0.133
FCR	1.256 \pm 0.051	1.243 \pm 0.067	0.715
Grower (15–28 d)			
BW at 28 d (g)	1,366.3 \pm 59.8 ^b	1,434.6 \pm 28.4 ^a	0.042
ADG (g)	65.5 \pm 4.2	69.22 \pm 2.01	0.074
ADFI (g)	101.4 \pm 7.7	106.4 \pm 3.3	0.176
FCR	1.551 \pm 0.068	1.538 \pm 0.047	0.718
Finisher (29–42 d)			
BW at 42 d (g)	2,579.8 \pm 105.9	2,688.7 \pm 128.1	0.140
ADG (g)	89.3 \pm 6.5	92.0 \pm 7.5	0.513
ADFI (g)	158.4 \pm 12.4	159.6 \pm 11.4	0.863
FCR	1.775 \pm 0.105	1.736 \pm 0.060	0.446
Whole phase (1–42 d)			
ADG (g)	57.7 \pm 2.4	60.5 \pm 3.2	0.112
ADFI (g)	92.8 \pm 6.2	95.7 \pm 5.2	0.407
FCR	1.608 \pm 0.068	1.581 \pm 0.037	0.402
Mortality (%)	4.74 \pm 3.70	3.57 \pm 2.26	0.523

¹The basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH.

²Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; FCR, feed conversion ratio.

^{a,b}Means within a row with no common superscript differ significantly ($P < 0.05$). Data is presented in mean \pm SD (n = 6).

Table 4. Effect of dietary SCH supplementation on intestinal relative index of 14, 28, and 42-day-old broilers.

Item	Control	SCH ¹	P value
14 d			
Duodenum (%)	1.51 \pm 0.11	1.54 \pm 0.13	0.650
Jejunum (%)	2.48 \pm 0.20	2.54 \pm 0.36	0.748
Ileum (%)	1.74 \pm 0.14	1.86 \pm 0.33	0.428
28 d			
Duodenum (%)	1.09 \pm 0.19	1.16 \pm 0.40	0.696
Jejunum (%)	2.28 \pm 0.21	2.79 \pm 0.77	0.146
Ileum (%)	2.03 \pm 0.39	2.33 \pm 0.58	0.318
42 d			
Duodenum (%)	0.70 \pm 0.18	0.72 \pm 0.19	0.831
Jejunum (%)	1.21 \pm 0.22	1.35 \pm 0.29	0.384
Ileum (%)	0.88 \pm 0.15	0.81 \pm 0.22	0.528

¹The basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH. Data is presented in mean \pm SD (n = 6).

jejunum in SCH group was numerically higher than the control group at 14, 28, and 42 d of age. In addition, there is a trend that the relative index of ileum increased at 14 and 28 d of age by the SCH diet ($P < 0.05$).

Intestinal Morphology

The effects of basal diet supplemented with SCH on the VH, CD, and VCR of duodenum, jejunum, and ileum are shown in Table 5. At 14 d of age, compared with the control group, dietary SCH supplementation tended to increase the VH and VCR of the 3 intestinal segments, and reduce the CD of three intestinal segments ($P > 0.05$). At 28 and 42 d of age, dietary SCH significantly increased the VH and VCR of jejunum ($P < 0.05$). Furthermore, at 42 d of age, dietary SCH

Table 5. Effect of dietary SCH supplementation on intestinal morphology of 14, 28, and 42-day-old broilers.

Item ²		Control	SCH ¹	P value
14 d				
Duodenum	VH (μm)	1,216.05 \pm 219.16	1,265.14 \pm 269.96	0.737
	CD (μm)	168.51 \pm 21.90	160.24 \pm 25.91	0.564
	VCR	7.18 \pm 0.52	7.85 \pm 0.59	0.067
Jejunum	VH (μm)	1,004.34 \pm 126.19	1,065.50 \pm 144.05	0.452
	CD (μm)	155.67 \pm 23.95	151.77 \pm 24.08	0.785
	VCR	6.49 \pm 0.69	7.05 \pm 0.51	0.144
Ileum	VH (μm)	586.96 \pm 69.53	647.69 \pm 59.55	0.135
	CD (μm)	140.62 \pm 24.20	136.89 \pm 16.44	0.762
	VCR	4.29 \pm 0.88	4.76 \pm 0.52	0.284
28 d				
Duodenum	VH (μm)	1,492.25 \pm 142.91	1,614.37 \pm 129.39	0.152
	CD (μm)	192.07 \pm 33.64	182.92 \pm 33.48	0.604
	VCR	8.01 \pm 0.96	8.89 \pm 0.65	0.096
Jejunum	VH (μm)	1,132.98 \pm 114.54 ^b	1,291.81 \pm 124.78 ^a	0.045
	CD (μm)	161.53 \pm 15.59	158.04 \pm 20.00	0.743
	VCR	7.02 \pm 0.41 ^b	8.24 \pm 0.91 ^a	0.013
Ileum	VH (μm)	663.32 \pm 78.23	705.53 \pm 69.28	0.346
	CD (μm)	153.90 \pm 16.67	141.97 \pm 9.51	0.159
	VCR	4.40 \pm 1.05	5.00 \pm 0.71	0.276
42 d				
Duodenum	VH (μm)	1,600.01 \pm 213.03	1,693.67 \pm 315.11	0.640
	CD (μm)	193.04 \pm 24.12	185.76 \pm 32.33	0.731
	VCR	8.29 \pm 0.31	9.11 \pm 0.63	0.057
Jejunum	VH (μm)	1,317.16 \pm 123.97 ^b	1,498.97 \pm 80.24 ^a	0.020
	CD (μm)	185.47 \pm 25.98	167.07 \pm 12.57	0.184
	VCR	7.15 \pm 0.46 ^b	8.99 \pm 0.41 ^a	<0.001
Ileum	VH (μm)	952.92 \pm 85.76	1078.36 \pm 167.15	0.141
	CD (μm)	161.57 \pm 9.58 ^a	144.65 \pm 11.81 ^b	0.022
	VCR	5.89 \pm 0.33	7.45 \pm 0.97	0.005

¹The basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH.

²Abbreviations: CD, crypt depth; VH, villus height; VCR, the ratio of villus height to crypt depth.

^{a,b}Means within a row with no common superscript differ significantly ($P < 0.05$). Data is presented in mean \pm SD (n = 6).

increased the VCR and reduced the CD of ileum significantly ($P < 0.05$). On the whole, feeding diet supplemented with SCH reduced the CD of the duodenum, jejunum and ileum, increase the VH and VCR to a certain extent, and effectively improved intestinal morphology.

The mRNA Expression Levels of Immunomodulatory Genes and Tight Junction Protein

The mRNA expression levels of immunomodulatory genes and tight junction protein in chicken jejunum mucosa are shown in Figure 1. At 28 d of age, compared to the control group, the TNF- α and IL-6 mRNA expressions in jejunum significantly decreased in the SCH groups ($P < 0.05$, Figures 1A and 1C). Diet supplemented with SCH significantly downregulated the mRNA expression of TNF- α and IL-1 β at 42 d of age ($P < 0.05$, Figures 1A and 1B). Furthermore, the ZO-1 and Claudin-1 mRNA expressions in jejunum of SCH group had a significant increase compared to the control group ($P < 0.05$, Figures 1E and 1F). There was no significant difference in the Occludin mRNA expression between the 2 groups ($P > 0.05$). In general, dietary addition of SCH reduced the expression level of intestinal inflammatory factors, increase the expression level of tight junction protein, alleviate the intestinal inflammatory

response of broilers, and play an important role in immune regulation.

Cecum Microbiota Analysis by 16S rRNA

After filtering, an average of 53,524 reads per sample was obtained. First, sequencing depths were examined by plotting the rarefaction curve for richness and the numbers of shared OTUs. Most of the samples reached plateaus, indicating that sampling depth was adequate. Bacterial α -diversity in cecum microbiota was estimated using Shannon, Simpson, and Chao indices of diversity and richness. As shown in Figure 2, there was no significant difference in Shannon, Simpson, or Ace indices between the 2 groups ($P > 0.05$), but Chao index in SCH group was significantly higher than control group ($P < 0.05$, Figure 2C).

Beta-diversity analysis was performed to compare the overall microbial profiles of 2 groups as displayed in Figure 3. PCoA analysis was performed to present a holistic perception of the microbiota via weighted Uni-Frac distance metric. Results for PCoA showed that the visual separation effect of microbial samples was not significant between the 2 groups (Figure 3A), but PLS-DA plot defined groups where samples from different groups occupied distinct positions (Figure 3B), which indicated that the microbiota compositions were dissimilar within the two groups.

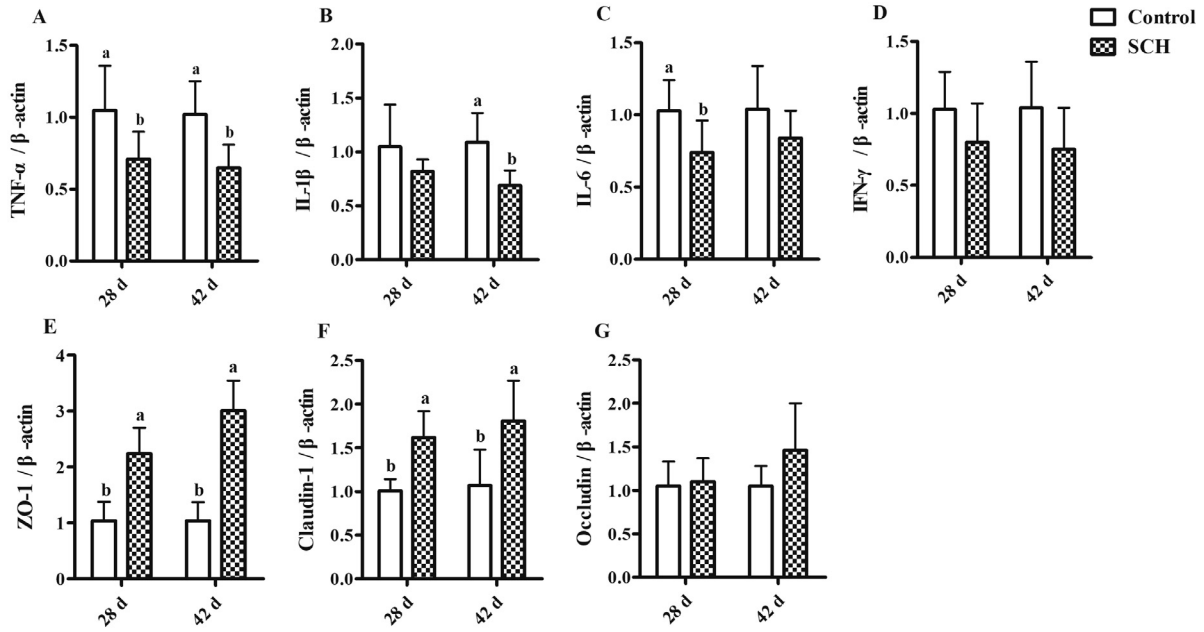


Figure 1. The mRNA expression level of immunomodulatory genes and tight junction protein in chicken jejunum mucosa at 28 and 42 d. (A–D) were relative immunomodulatory genes mRNA expression, (E–G) were relative tight junction protein mRNA expression. Control, control group with the basal diets; SCH, the basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH. ^{a, b} Means within a row with no common superscript differ significantly ($P < 0.05$).

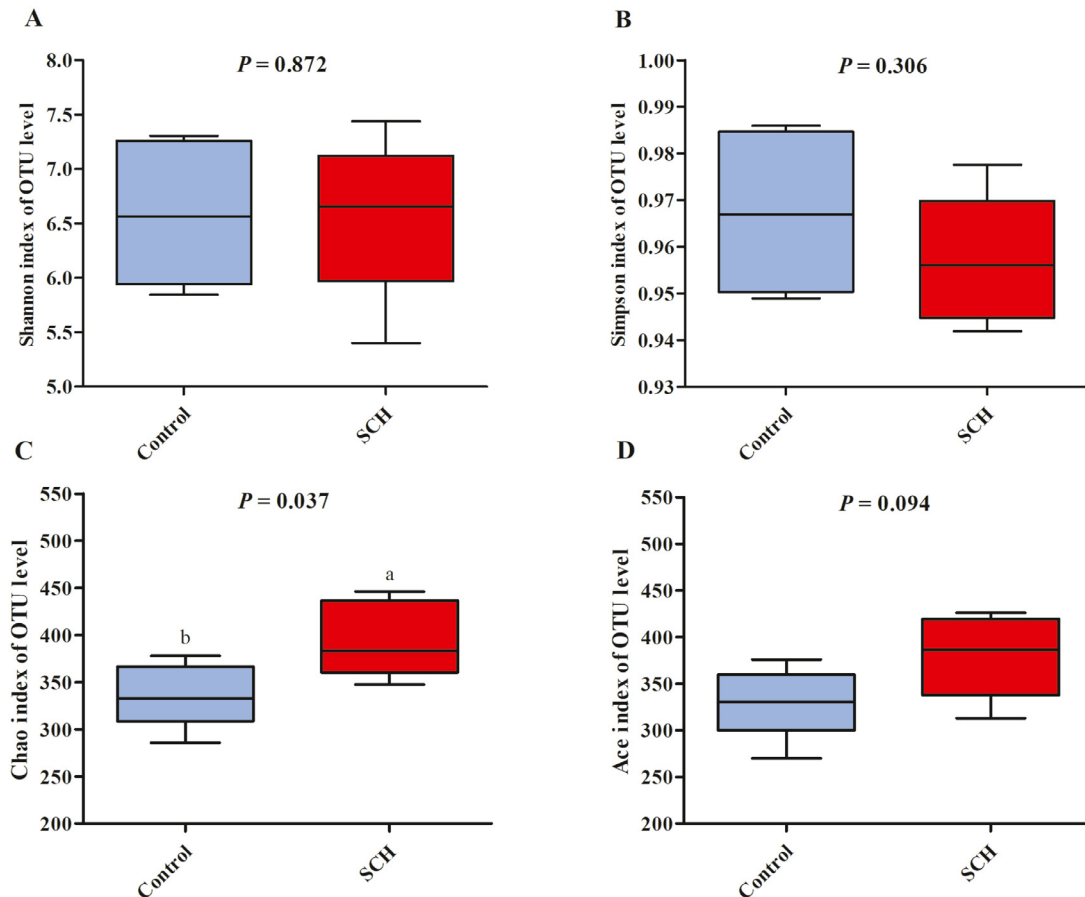


Figure 2. Effects of dietary supplementation with SCH on the cecum microbial α -diversity of broilers on day 42. A–D were Shannon, Simpson, Chao, and Ace index of OTU level results respectively. Control, control group with the basal diets; SCH, the basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH. ^{a, b} Means within a row with no common superscript differ significantly ($P < 0.05$).

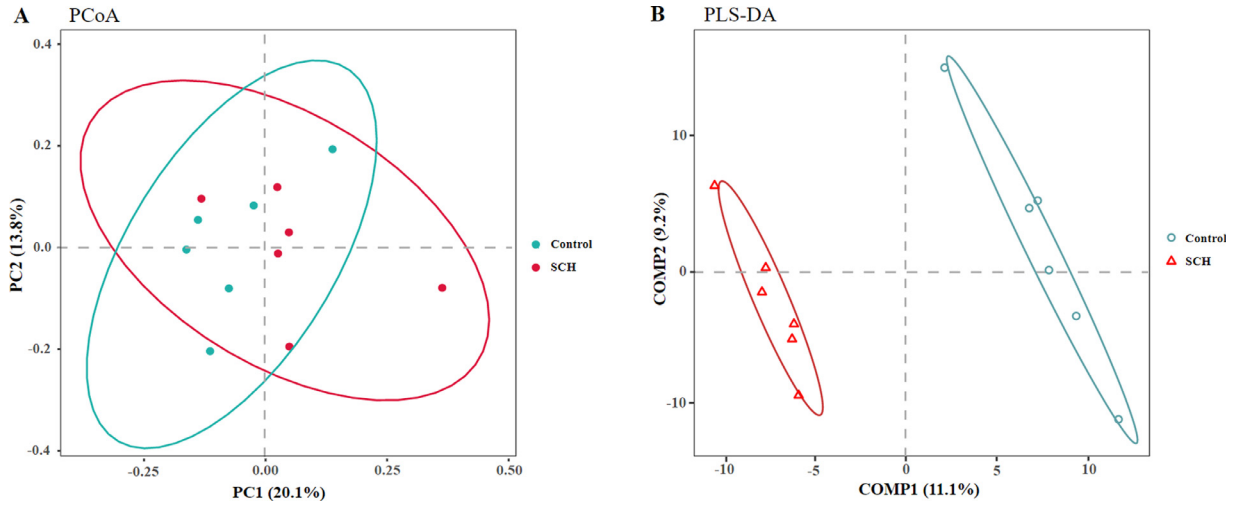


Figure 3. Effects of dietary supplementation with SCH on cecum microbial β -diversity of broilers on d 42. (A) principal coordinate analysis (PCoA) based on based on weighted UniFrac distance calculated from OTU abundance matrix; (B) partial least squares discriminant analysis (PLS-DA); Analysis of similarities (ANOSIM) of weighted UniFrac distances. Control, control group with the basal diets; SCH, the basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH.

To assess the differences induced by SCH in the cecum microbiota, taxonomic compositions were analyzed at phyla and genus levels in Figure 4. At the phylum level, Bacteroidetes and Firmicutes are 2 dominant bacteria phyla of cecum. Broilers fed with SCH diet were characterized by higher relative abundance of Firmicutes

(60.41%: 50.26%) and lower abundance of Bacteroidetes (32.42%: 42.80%) compared with the control group, thus leading to a higher ratio of Firmicutes to Bacteroidetes ratio (Figure 4A). Compared with control group at the genus level, *Lactobacillus* and *Faecalibacterium* in SCH group were increased by 2.80- and 3.86-fold

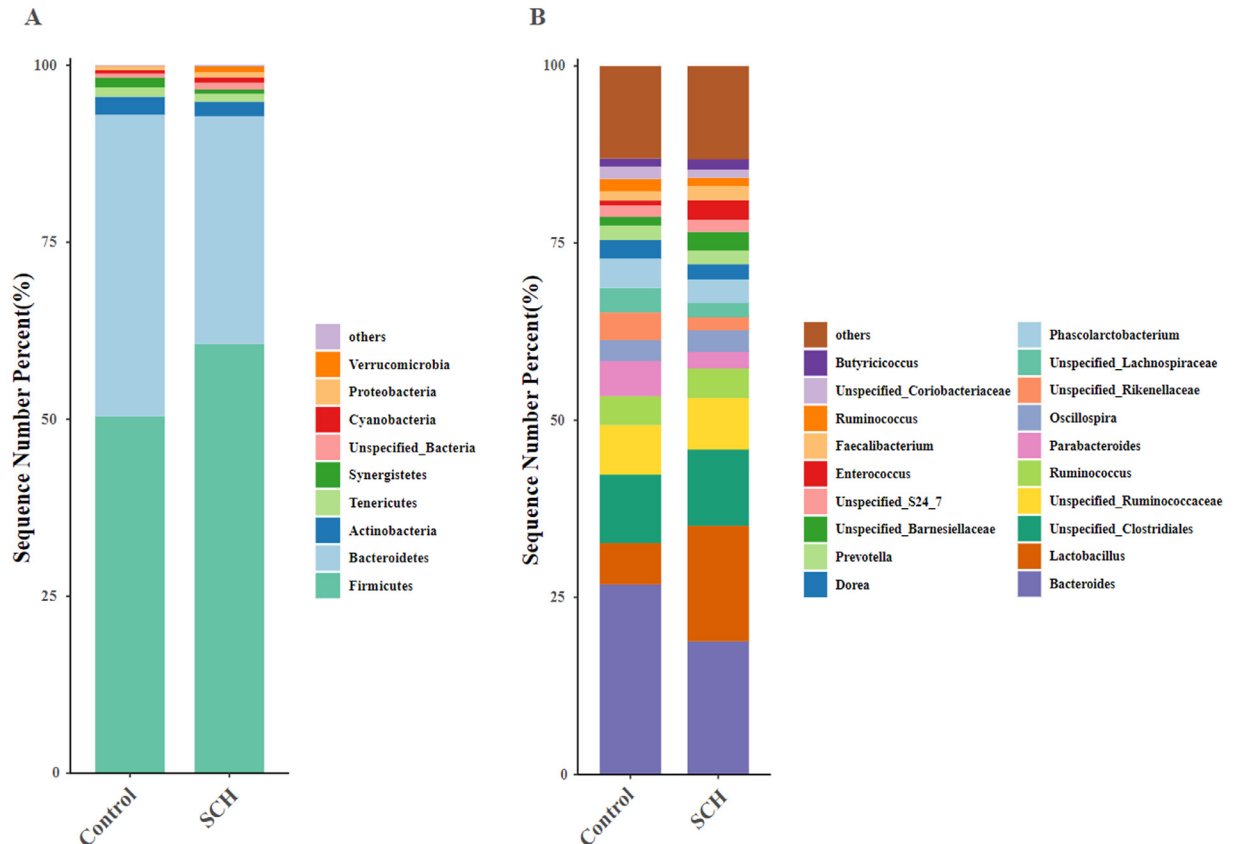


Figure 4. Compositions of cecum microbiota and differential species identified in broilers at phylum and genus level on d 42. (A, B) were the bacterial community compositions at phylum and genus level, respectively. Control, control group with the basal diets; SCH, the basal diets supplied with 500 mg/kg (d 0–28) and 250 mg/kg (d 29–42) SCH.

(16.31%:5.83% and 2.73%:0.71%, respectively), meanwhile *Bacteroides* was decreased by 1.42-fold (19.10%:27.13%, Figure 4B).

DISCUSSION

Considerable studies have documented that the dietary SCH supplementation improved growth performance of animals (Gao et al., 2008; Afsharmanesh et al., 2010; Superchi et al., 2012). In the current study, our data showed that dietary SCH supplementation increased BW, ADG, and ADFI of broilers during the starter and grower phase. It is worth noting that during the starter, grower and overall period, the SCH group enhanced ADG by 5.6, 5.8, and 5.0%, and enhanced BW by 4.8, 5.0, and 4.3%, respectively. Similarly, positive results were observed on growth performance of broilers fed diets with yeast hydrolysate (Li et al., 2016; Wang et al., 2017). One explanation for the growth promotion effects of SCH may be that the yeast-derived additive or its components could improve the anti-inflammatory effect in animals as previously reported (Salinas-Chavira et al., 2018). In addition, it was reported that SCH could improve the growth performance by increasing the VH, reducing intestinal pH, regulating intestinal microbes, increasing the secretion of auxiliary digestive enzymes, and improves nutrient absorption (Zhang et al., 2014). The main reason for the lack of improvement in growth performance in the current study may be related to the reduction of SCH addition in finisher stage.

Our results showed that feeding SCH only slightly improved the relative index of the jejunum, the finding was in accordance with previous studies (Singh et al., 2017). SCH supplementation may have a trophic effect on jejunum and ileum compared to duodenum as proved by the increased VH and VCR observed in the current study, which was consistent with previous observation (Zhang et al., 2005). The improvement of intestinal morphology suggested an ameliorated intestinal nutrient digestibility and absorption capacity and might further contribute to subsequent enhancement in the growth performance (Montagne et al., 2003). Whereas some others documented that no positive effects on intestinal morphology of broilers fed diet supplemented with hydrolyzed yeast products was observed (Baurhoo et al., 2009; Reisinger et al., 2012), which may be related to the composition of yeast hydrolysate and the farming conditions. Different farming conditions, such as farm environment, breeding mode, litter and so on, may have a certain impact on the microbial diversity and community in the intestinal of broilers, which will further affect the intestinal development and growth performance (Gupta, et al., 2021; Xiao et al., 2021). Therefore, the actual application effect of the SCH needs to be evaluated according to its composition and the conditions of the applied farm.

The size of the gap between intestinal epithelial cells is mainly controlled by tight junction proteins, including occludin, claudin, and zonula occludens (ZO) families

(Tang et al., 2015). The intestinal barrier regulated by tight junction proteins performs the crucial role of defense against the passage of pathogens and antigens into the intestinal epithelium (Broom, 2018). Claudins and occludin are transmembrane proteins that are responsible for regulating the size of the intercellular space (Suzuki, 2013). ZO-1 is present in intestinal epithelial cells and can be attached to claudins and occludin to increase the stability of tight junction (Bauer et al., 2010). Our results showed that the mRNA expression of ZO-1 and Claudin-1 in SCH group was higher than control group, which were consistent with previous studies (Chuang et al., 2021a), indicating that broiler chickens fed with SCH had more stable intestinal environment. Therefore, it can be inferred that SCH may improve the integrity of the intestinal epithelium, thereby creating a more friendly gut environment, which could help to resist pathogen infection.

The most important regulator of inflammation is the IL family (Awaad et al., 2011). As one of the most important members of the IL family, IL-1 β mediates many pathways involved in apoptosis or inflammation (Gabay et al., 2010). Previous study reported that IL-1 β is inhibited by β -glucan (Municio et al., 2013), which is abundant in SCH. Important pro-inflammatory cytokines TNF- α and IL-1 β can activate macrophages that regulate cell death and inflammation. IFN- γ is associated with infection and high concentration of IFN- γ may contribute to autoimmune disease (Ivashkiv and Donlin, 2014). The reduction of the pro-inflammatory cytokine related to inflammatory response can decrease energy loss and improve the cell survival rate (Lee et al., 2017). The above results showed that SCH could exert an anti-inflammatory effect by inhibiting the excessive expressions of IL-1 β , IL-6, and TNF- α , which was consistent with previous study (Superchi et al., 2012).

The alterations in intestinal microbiota may substantially affect the intestinal barrier function and inflammation reaction (Liu et al., 2020; Desai et al., 2016). In order to better understand the connection between intestinal barrier and gut microflora, the cecum content which contains the most detailed information regarding chicken gut microbiota was analyzed with 16s rRNA methodology (Pourabedin and Zhao, 2015). Similar to the previous reports (Chuang et al., 2021b), data from analyses of α -diversity and β -diversity corroborate the initial hypothesis that SCH improved microbial richness and altered microbiota structure to a certain extent. The diversity of the intestinal tract microbiota community is believed to have a positive effect on the productivity of the bird (Janczyk et al., 2009).

In the current study, higher Firmicutes-to-Bacteroidetes ratio at the phylum level demonstrated that SCH changed cecum microbiome composition of birds. The abundance of Firmicutes has been proved to be positively correlated with energy and nutrient absorption, while the increase in fecal Bacteroidetes is associated with poor nutrient digestibility (Turnbaugh et al., 2006; Jumpertz et al., 2011), which indicated that the higher ratio of Firmicutes to Bacteroidetes may improve

nutrient digestibility and lead to greater body weight (Paola et al., 2010; Singh et al., 2013). Therefore, the increase in abundance of Firmicutes accompanied by the decrease in abundance of Bacteroidetes may contribute to the nutrient utilization of broilers.

Further analyses revealed more differential species at various taxonomic levels between the 2 groups. At the genus level, there was an increased abundance of *Lactobacillus* in cecum of birds fed SCH. As one of the main genera in the chicken gut, *Lactobacillus* can protect the intestinal barrier by antagonizing pathogens (Serivin, 2004). At the same time, the lactic acid produced by the fermentation of *Lactobacillus* could be used by butyric acid producers, thereby increasing the digestibility of nutrients and improving intestinal morphology. The enrichment of *Faecalibacterium* in SCH addition group also proved this possible pattern. *Faecalibacterium* was one of the most abundant symbiotic bacteria in the colon of healthy people and an important butyrate-producing bacteria in the intestine. Furthermore, *Faecalibacterium* also plays an important role in the intestine of broilers. It can quickly adhere to the intestinal mucosa, inhibit pathogenic bacteria from adhering to the intestinal tract through the exclusion effect, and form the intestinal barrier, so as to protect the intestinal health (Eckhaut et al., 2011; Liu et al., 2019). Previous studies had confirmed that the abundance of *Bacteroides* is positively correlated with the expression of IL-1 β and TNF- α in birds (Wang et al., 2019), which may disrupt the epithelial barrier function (Matthias et al., 2003). In this study, dietary SCH addition triggered a decrease in the abundances of *Bacteroides* and the improvement of intestinal barrier function.

In summary, alteration of the bacterial phylotypes indicated that SCH can improve the immune response and intestinal barrier function by regulating the cecum microbiota, such as supporting commensal lactic acid bacteria and diminishing the detrimental bacteria (Wilson et al., 2005; Yang et al., 2008), which can help to maintain the intestinal morphology and improve the production performance of broilers.

CONCLUSIONS

The overall results revealed that dietary SCH supplementation in broilers could improve growth performance, intestinal morphology and barrier function, while regulating intestinal inflammation, which might be attributed to the enhancement of bacterial richness and alteration of microbial composition, particularly the enrichment of SCFAs-producing bacteria. The understanding of the regulatory role of SCH on intestinal health and growth performance in poultry production warrants further study.

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DISCLOSURES

All authors approve the submission of this manuscript and declare no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102237.

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