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Article

Effects of Probiotic *Lactopantibacillus plantarum* on Growth Performance, Antioxidant Capacity, Immune Function, and Cecal Microbiota in Broiler Chicken

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Simple summary: With the ban on the antibiotics added to feed as growth promoters, numerous probiotics were developed as an alternative to antibiotics to be added to the rations. Although a considerable number of studies have shown that *L. plantarum* has positive effects on intestinal health, more evidence is needed to establish whether *L. plantarum* plays an efficient role in replacing antibiotics as growth promoter in broiler feeding. Therefore, the present work evaluated the effects of *L. plantarum* on the growth performance, oxidation resistance, immune response, intestinal microbiota, and short chain fatty acid profiles of broilers and compared with those of antibiotic growth promoters.

Abstract: This research is aimed to investigate the effects of *Lactopantibacillus plantarum* (*L. plantarum*) on growth performance, oxidation resistance, immunity, as well as cecal microbiota in broilers. This work classed three hundred and sixty 1-day-old broilers as three groups randomly, including control (CON, basal diet), antibiotic (ANT, 75 mg kg⁻¹ chlortetracycline added into basal diet), or probiotic group (LP, 5×10⁸ CFU kg⁻¹ *Lactopantibacillus plantarum* HJLP-1 contained within basal diet). Animal were then fed for 42 days, and each group consisted of 15 broilers for 8 replicates. Compared with CON, *L. plantarum* supplementation significantly improved the average weight daily gain (AWDG) ($P < 0.05$) while reducing feed-to-gain ratio over the entire supplemental period ($P < 0.05$). Birds fed *L. plantarum* had marked lower serum ammonia and xanthine oxidase levels ($P < 0.05$) than those in ANT and CON. Significant improvements ($P < 0.05$) of superoxide dismutase, catalase, serum IgM and IgY contents in broilers fed *L. plantarum* were also observed when compared those in CON and ANT groups. Both *L. plantarum* and antibiotics decreased the pro-inflammatory factor IL-1 β level significantly ($P < 0.05$), while only *L. plantarum* promoted the anti-inflammatory factor IL-10 level dramatically in the serum ($P < 0.05$) compared with CON. The *L. plantarum* dramatically ($P < 0.05$) increased acetic acid and butyric acid concentrations in the cecal contents, when compared to those in CON and ANT. Among the differences revealed by 16S rRNA analysis, *L. plantarum* markedly improved the community richness of cecal microbiota. At the genus level, butyric acid-producing bacteria *Ruminococcus* and *Lachnospiraceae* were found in higher relative abundance in samples of *L. plantarum* treated birds. In conclusion, dietary *L. plantarum* supplementation promoted the growth and health of broilers, likely by inducing a shift in broiler gut microbiota toward short-chain fatty acid (SCFA)-producing bacteria. Therefore, *L. plantarum* has tremendous potential as an alternative for antibiotics in poultry breeding.

Keywords: *Lactopantibacillus plantarum*; broiler; growth performance; immune function; cecal microbiota.

1. Introduction

Broiler chickens are one of the main sources of animal protein for human consumption. To maximize the production, intensive indoor systems are employed by the commercial poultry producers [1]. However, the intensively reared broiler chickens encounter the simultaneous action of

multiple stressors from the housing environment, which causes high mortality of broilers from common infectious or metabolic diseases [2]. Conventional poultry farms use antibiotics for treatment, prophylaxis, and growth promotion, which give the rise of antibiotic resistance pathogens. The emergence and spread of antibiotic resistance affect animal and human health and become a global concern [3,4]. Therefore, many countries have made efforts to limit antibiotics application into feeds. In 2020, antibiotics added to feed as growth promoters were completely banned in China.

With the ban on antibiotics, an increasing number of probiotics are being developed as an alternative to antibiotics to be added to the rations [5,6]. Suitable probiotics can improve the intestinal flora, enhance the absorption of nutrients by livestock and poultry, strengthen immunity, and reduce the impact on farm animal health and growth induced by feeding environment [7].

Lactopantibacillus plantarum is a homofermentative, aerotolerant, Gram-positive bacterium that produces both isomers of lactic acid as the main end-product of carbohydrate fermentation [8]. It can be obtained in numerous sources, such as human feces human breast milk, fermented food products and cheese. [9-13]. Evaluating probiotic properties of *L. plantarum* revealed its antioxidant and immune-enhancing effects [14]. *L. plantarum* is suggested to have an important effect on gastrointestinal disorders and diarrhea prevention [15,16]. In broiler chickens, feeding *L. plantarum* can improve growth performance, promote gut microbial homeostasis, prevent pathogen infection, and reduce the intestinal barrier injury caused by toxins. [17-19]. However, as a considerable number of studies have shown that *L. plantarum* has positive effects on intestinal health, more evidence is needed to establish whether *L. plantarum* plays an efficient role in replacing antibiotics as growth promoter in broiler feeding. Therefore, the present work evaluated whether *L. plantarum* affected broilers in terms of the growth performance, oxidation resistance, immune response, intestinal microbiota, and short chain fatty acid profiles of broilers and compared with those of antibiotic growth promoters.

2. Material and Methods

2.1. Experimental animals and design

A total of 360 Avian chickens (one-day-old) were individually weighed and randomly assigned into three groups: (1) control group (CON) fed basal diet; (2) antibiotic group (ANT) fed the base diet supplemented with 75 mg/ kg of chlortetracycline; (3) probiotic group (LP) fed the basal diet plus 5×10⁸ CFU/ kg of *L. plantarum* HJLP-1 supplied by Vegamax Biotechnology Co. Ltd. (Huzhou, China). Each treatment 15 birds of 8 replicates in each pen. Dietary formulations met or exceeded the daily growth needs of broiler chickens (NRC, 1994; Table 1). The feeding experiment lasted for 42 d. Animals had free access to water and feed. Continuous light, suitable temperature and air quality for white feather broiler breeding were maintained in the coop.

Table 1. Raw material composition and nutritional level of basic dietary (air-dry basis).

Items	1-21d	22-42d
Ingredients(%)		
Corn	61.80	65.60
Soybean meal	22.50	17.55
Extruded soybean	8.45	10.00
Import fish meal	3.00	3.00
CaHPO ₄	1.66	1.45
Limestone	1.10	1.00
NaCl	0.32	0.30
DL- methionine	0.16	0.10
L- lysine	0.01	
Premix ¹	1.00	1.00
Total	100.00	100.00
Nutrition levels		

Metabolizable energy (MJ/kg)	12.45	12.70
Crude protein	21.00	19.20
Lysine	1.15	0.95
Methionine	0.54	0.44
Calcium	0.99	0.89
Available phosphorus	0.53	0.49

¹Premix is provided for feed per kg: VA 1500 IU, VB₁ 1.5 mg, VB₆ 3.0 mg, VB₁₂ 0.01 mg, VD₃ 200 IU, VE 10 IU, VK 0.5 mg, Biotin 0.15 mg, D-pantothenic acid 10 mg, Folic acid 0.5 mg, Nicotinic acid 30 mg, Trace elements Cu, Fe, Zn, Mn, Se, I are 8 mg, 80 mg, 40 mg, 60 mg, 0.15 mg, 0.18 mg respectively.

2.2. Growth performance

The weights of broilers from each pen were taken on days 1, 21 and 42 for evaluating average weight daily gain (AWDG). Meanwhile, the remaining feed was weighed for calculating the average daily feed intake (ADFI) together with feed to gain ratio (F/G).

2.3. Sample collection

Eight broilers from every group were randomized to collect samples on day 42 (one broiler from each replicate). Left -wing venous blood collection was completed in the 5-ml sterilized tubes and centrifugated at 3,000 × g and 4 °C for 10-min period for obtaining serum. After blood collection, the broilers were sacrificed. Cecal contents were aseptically removed. Each sample was preserved under -80 °C prior to subsequent analyses.

2.4. Measurements of biochemical parameters in serum

Serum samples were screened for biochemical parameters about metabolites, antioxidants, immunoglobulin and cytokines using the corresponding diagnostic kits obtained from the Nanjing Angle Gene Bioengineering Co., Ltd. (Nanjing, China).

2.5. Assessment of cecal microflora contents by 16S rRNA sequencing

Cecal microbial genomic DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany). Then, 338F-806R region (V3-V4 hypervariable region, 468 base pairs) of the bacterial 16S rRNA was amplified and sequenced on Illumina MiSeq platform (Illumina, San Diego, USA). Finally, the composition and abundance of the caecum microflora were determined by alpha and beta diversity analysis.

2.6. Short chain fatty acid concentration analysis

The concentration of cecal short chain fatty acid (SCFA) was estimated by headspace sampler gas chromatography (Agilent Technologies, USA) using the method of Thanh et al. [20]. The 0.5 g sample was dissolved in 1 mL water. After centrifugation at 12500 g for 5 min, the supernatant was extracted and mixed with 25% phosphoric acid. The concentration of SCFAs was determined by an Agilent Technologies 7890A Network System equipped with a 30 m × 0.32 mm × 1.8 μm column (DB-624) and flame ionization detector.

2.7. Statistical Analysis

One-way analysis of variance (ANOVA) was performed using GraphPad Prism 8.2.1 software (GraphPad Prism Inc., USA), followed by Tukey's method for comparing differences. Results were expressed as means with standard error of mean (SEM). Statistical significance was set at *P* < 0.05. Analysis of bacteria community data was conducted using the cloud majorbio platform (<http://cloud.majorbio.com/>).

3. Results

3.1. Growth performance

Table 2 displays growth performance analysis. In comparison with control, both *L. plantarum* HJLP-1 and antibiotics treatments improved the body weight (BW) significantly ($P < 0.05$) during the start period (day 1 to 21). While during the finisher period (days 21 to 42), only LP group showed remarkably elevated BW ($P < 0.05$). Accordingly, during the start period, the birds fed *L. plantarum* HJLP-1 or antibiotics had higher average weight daily gain (ADWG) than birds in CON group ($P < 0.05$). In the finisher period and during the whole supplementation period (days 1-42), LP group had markedly elevated ADWG compared with ANT and CON groups ($P < 0.05$). During the start period, supplementation with antibiotics decreased the F/G compared with the LP and CON groups ($P < 0.05$). Whereas in the finisher period and during the whole supplementation period, broilers fed with *L. plantarum* HJLP-1 had close F/G ratio to those of ANT group and obviously decreased relative to CON group ($P < 0.05$).

Table 2. Effects of dietary supplementation of *L. plantarum* on growth performance of broilers.

Items	CON	ANT	LP	SEM	P-value
BW,g					
1 d	38.29	39.52	38.32	0.401	0.361
21 d	732.08 ^b	778.24 ^a	800.13 ^a	8.945	0.001
42 d	1875.86 ^b	1926.47 ^b	2124.36 ^a	40.63	0.019
ADG (g/d)					
1-21 d	33.54 ^b	35.26 ^a	36.31 ^a	0.361	0.001
22-42 d	55.48 ^b	56.47 ^b	64.85 ^a	1.764	0.048
1-42 d	44.51 ^b	45.87 ^b	52.57 ^a	1.205	0.005
ADFI,g					
1-21 d	54.02 ^b	54.42 ^{ab}	59.84 ^a	0.935	0.008
22-42 d	118.19	111.66	118.63	3.432	0.677
1-42 d	80.00	78.62	86.05	1.516	0.098
F:G					
1-21 d	1.61 ^{ab}	1.54 ^b	1.64 ^a	0.017	0.036
22-42 d	2.13 ^a	1.98 ^{ab}	1.83 ^b	0.046	0.023
1-42 d	1.80 ^a	1.72 ^{ab}	1.70 ^b	0.018	0.067

In the same row with different superscripts differ significantly ($p < 0.05$). Pen was regarded as the experimental unit. n=8 per treatment. Abbreviations: CON represents broilers fed a basal diet; ANT represents the broilers fed a basal diet supplemented with 75 mg/kg chlortetracycline; LP represents the broilers fed a basal diet and *L. plantarum*. F: G means feed-to-gain ratio.

3.2. Serum metabolite index

Effects of *L. plantarum* HJLP-1 on the metabolite index in broiler serum are shown in Table 3. Although either *L. plantarum* HJLP-1 or antibiotic supplementation significantly decreased the serum ammonia (NH₃) level ($P < 0.05$) relative to CON group, the serum ammonia levels of broilers fed *L. plantarum* HJLP-1 dramatically decreased compared with broilers fed antibiotics ($P < 0.05$). serum urea nitrogen (UN) and uric acid (UA) contents were not significantly different. Furthermore, birds in the LP group had lower serum xanthine oxidase (XOD) levels than birds in CON and ANT groups ($p < 0.05$).

Table 3. Effects of *L. plantarum* on metabolite index in serum of broilers.

Items	CON	ANT	LP	SEM	P-value
NH ₃ (μmol/L)	10.83 ^a	9.03 ^b	6.54 ^c	0.490	0.001

BUN (mmol/L)	0.31	0.29	0.23	0.016	0.136
UA (μmol/L)	147.84	129.88	116.41	6.584	0.148
XOD (U/L)	3.83 ^a	4.30 ^a	3.10 ^b	0.168	0.005

In the same row with different superscripts differ significantly ($p < 0.05$). Individual broiler was regarded as the experimental unit. $n=8$ per treatment. Abbreviations: CON represents broilers fed a basal diet; ANT represents the broilers fed a basal diet supplemented with 75 mg/kg chlortetracycline; LP represents the broilers fed a basal diet and *Lactobacillus plantarum*; BUN means blood urea nitrogen; UA means urea acid; XOD means xanthine oxidase.

3.3. Antioxidant index in serum

Effects of *L. plantarum* HJLP-1 on the antioxidant index in serum are shown in Table 4. Addition of *L. plantarum* HJLP-1 significantly improved the SOD and CAT levels in serum ($P < 0.05$). MDA and GSH-PX contents were not significantly different across diverse groups.

Table 4. Effects of *L. plantarum* on antioxidant index in serum of broilers.

Items				SEM	P-value
	CON	ANT	LP		
GSH-PX (U/mL)	6.90	7.83	8.37	0.591	0.617
SOD (U/mL)	12.28 ^b	12.96 ^{ab}	13.22 ^a	0.155	0.026
CAT (U/mL)	9.98 ^b	10.79 ^{ab}	11.80 ^a	0.304	0.039
MDA (U/mL)	8.12	7.56	7.91	0.199	0.534

In the same row with different superscripts differ significantly ($p < 0.05$). Individual broiler was regarded as the experimental unit. $n=8$ per treatment. Abbreviations: CON represents broilers fed a basal diet; ANT represents the broilers fed a basal diet supplemented with 75 mg/kg chlortetracycline; LP represents the broilers fed a basal diet and *Lactobacillus plantarum*; GSH-PX means glutathione peroxidase; SOD means superoxide dismutase; CAT means catalase; MDA means malonaldehyde.

3.4. Immunoglobulin and cytokines index in serum

As shown in Table 5, both *L. plantarum* HJLP-1 addition markedly elevated serum IgM and IgY contents in broilers ($P < 0.05$) compared with that in CON and ANT groups. Compared with CON, *L. plantarum* HJLP-1 supplementation reduced pro-inflammatory factor IL-1 β levels significantly ($P < 0.05$) and promoted anti-inflammatory factor IL-10 expression dramatically ($P < 0.05$). Antibiotics also markedly reduced IL-1 β levels ($P < 0.05$), but had no effect on IL-10 levels. In addition, the pro-inflammatory factor TNF- α level in the serum of broilers fed *L. plantarum* HJLP-1 remarkably decreased compared with broilers fed antibiotics ($P < 0.05$).

Table 5. Effects of *L. plantarum* on serum immunoglobulins and immune response cytokines of broilers.

Items				SEM	P-value
	CON	ANT	LP		
IgA ng/mL	168.11	177.93	176.81	4.327	0.626
IgY ng/mL	1.49 ^c	1.79 ^b	2.07 ^a	0.067	0.001
IgM μg/mL	3.09 ^c	3.61 ^b	4.26 ^a	0.143	0.001
IL-1 β pg/mL	94.19 ^a	83.02 ^b	83.02 ^b	1.900	0.011
IL-6 pg/mL	394.04	393.77	387.22	4.480	0.801
IL-10 pg/mL	17.43 ^b	20.82 ^b	28.43 ^a	1.272	0.001
TNF- α pg/L	38.74 ^{ab}	40.23 ^a	37.35 ^b	0.568	0.114
INF- β pg/mL	150.78	149.55	145.68	4.573	0.905

In the same row with different superscripts differ significantly ($p < 0.05$). Individual broiler was regarded as the experimental unit. $n=8$ per treatment. Abbreviations: CON represents broilers fed a

basal diet; ANT represents the broilers fed a basal diet supplemented with 75 mg/kg chlortetracycline; LP represents the broilers fed a basal diet and *Lactobacillus plantarum*.

3.5. Microflora structure in the cecal contents

As shown in Figure 1(A-E), *L. plantarum* supplementation induced some changes in microflora structure in the cecal digesta of broilers. Venn diagram demonstrated that the unique Operational Taxonomic Units (OTUs) of the LP group were 166, much higher than those of CON (39) and ANT (24) groups (Figure 1A). According to genus relative abundance-based principal component analysis (PCA), microbiota in the LP group clearly separated from the CON and ANT groups (Figure 1B). Furthermore, *L. plantarum* supplementation markedly improved the community richness, as evidenced by the significantly increased ACE and Chao indices, compared to other groups. ($P < 0.05$) (Figure 1C). Samples from ANT and LP groups had higher proportion of *Firmicutes* and lower ratio in *Bacteroidetes* than those from CON group, at the level of phylum (Figure 1D). Results of linear discrimination analysis coupled with effect size (LEfSe) indicated *Lactoplantibacillus plantarum* supplementation mainly enriched *f_Ruminococcaceae* and *unclassified_f_Lachnospiraceae*; antibiotic supplementation enriched *Alistipes* and *Oscillospirales*, whereas *Bacteroides*, *Bacteroidaceae* and *Barnesiellaceae* were enriched at genus level of control broilers (Figure 1E).

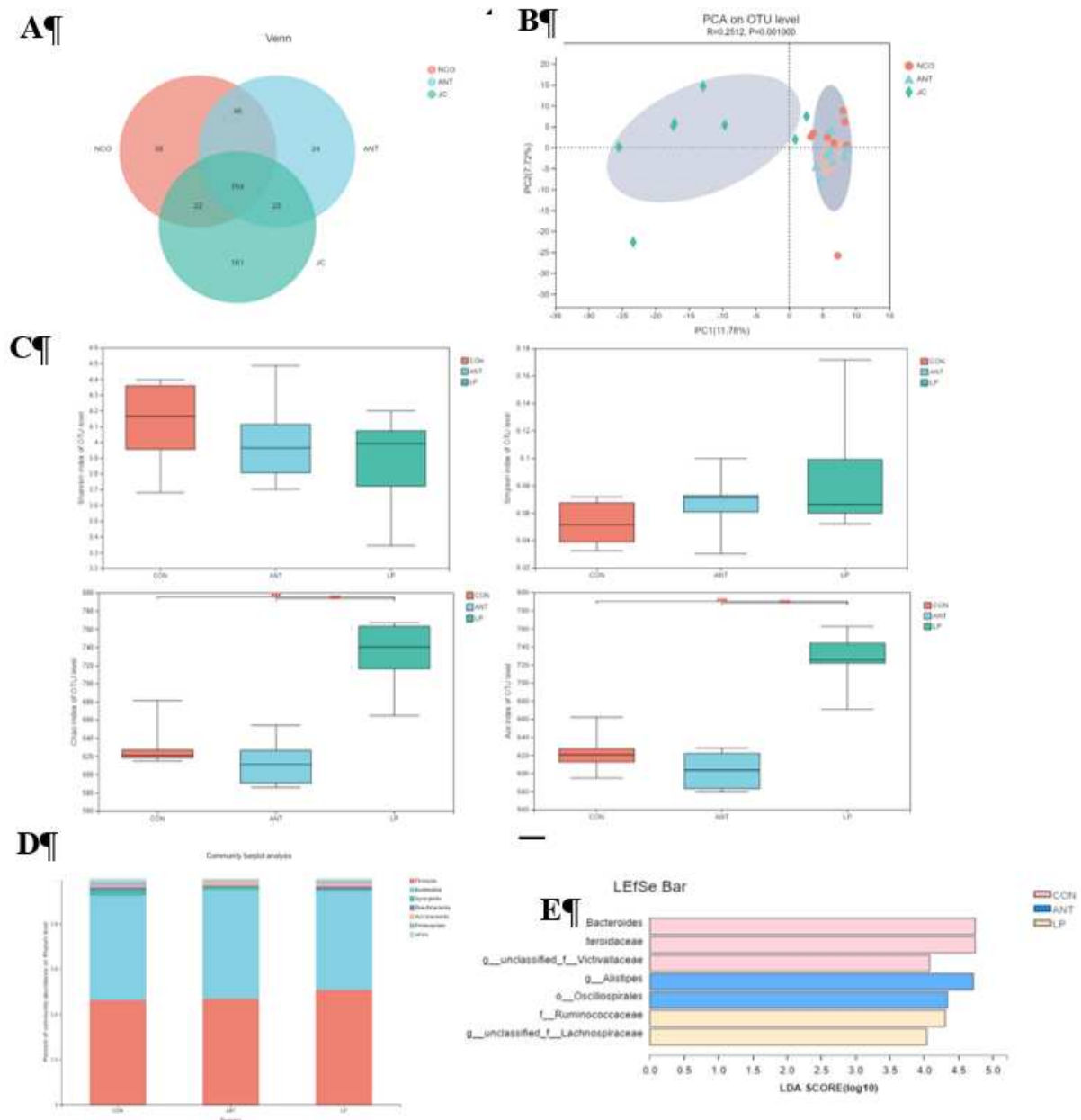


Figure 1. Analysis of the composition of the fecal microbiota. (A) The Venn diagram summarizing the numbers of common and unique observed taxonomic units (OTUs) in the microflora community in the cecum contents of broiler chicken. (B) Principal component analysis (PCA). (C) Shannon, Simpson, ACE, and chao indices reflecting alpha diversity. (D) Microbiota composition at the phylum level. (E) Histogram of LDA scores for taxonomic biomarkers identified by LefSe. LDA scores ($\log_{10}$) > 4 indicate enriched taxa in cases. Significance was determined using one-way ANOVA. * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

3.6. Short chain fatty acid concentrations

Figure 2 displays SCFA results. Butyric acid and acetic acid within the cecal contents of broilers fed *L. plantarum* HJLP-1 dramatically increased ($P < 0.05$) compared with ANT and CON. There was no significant modification in propionic acid concentration among the three treatment groups.

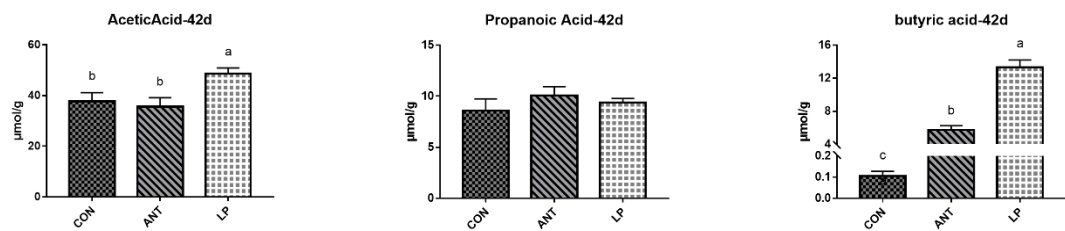


Figure 2. Effects of *Lactobacillus plantarum* on SCFAs in broilers. Values are presented as mean $\pm$ SEM. Individual broiler was regarded as the experimental unit. n=8 per treatment. Different superscripts differ significantly ($p<0.05$). Abbreviations: CON represents broilers fed a basal diet; ANT represents the broilers fed a basal diet supplemented with 75 mg/kg chlortetracycline; LP represents the broilers fed a basal diet and *Lactobacillus plantarum*.

3.7. Link between cecal bacteria community and short chain fatty acid

Figure 3 shows the spearman correlation heatmap of the top 50 genera and short chain fatty acid. During the digestion, *f_Ruminococcaceae*, *Alistipes* and *unclassified_f_Lachnospiraceae* were significantly positively correlated with butyric acid production, whereas, *Bacteroides* had a significant negative correlation.

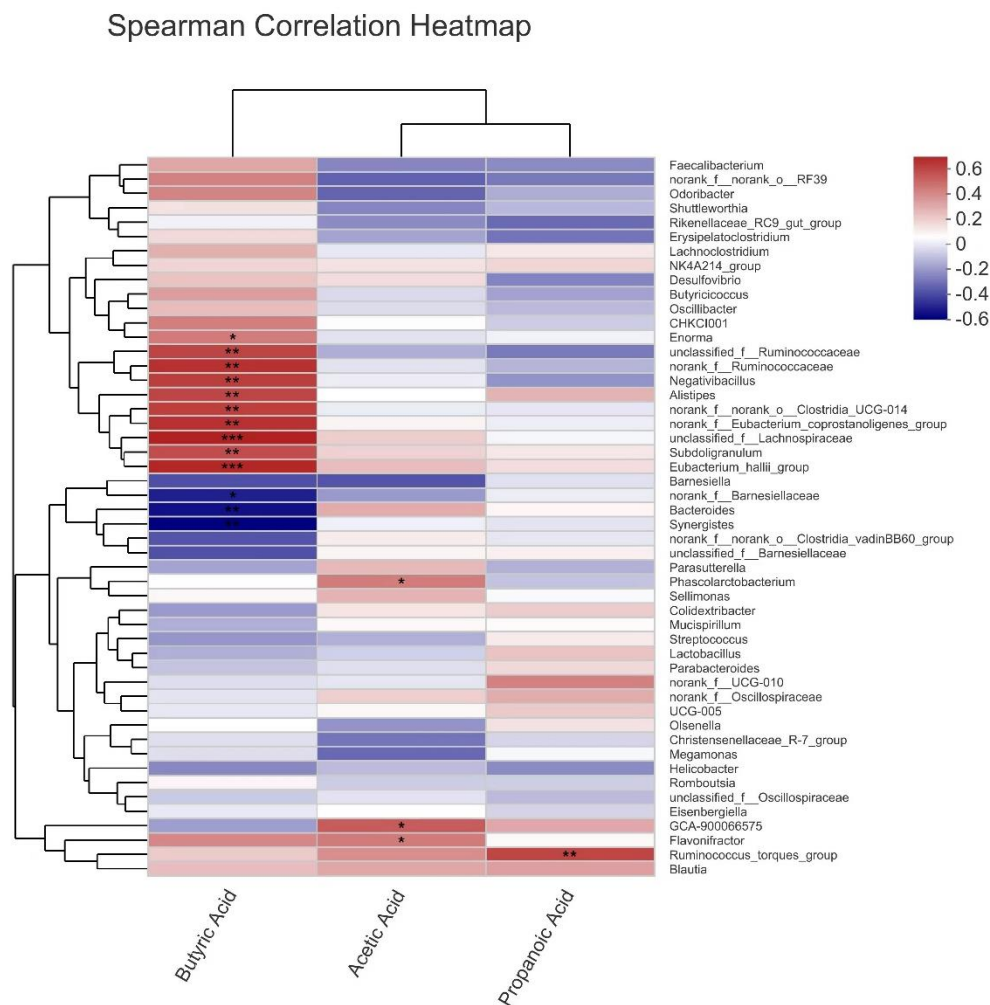


Figure 3. Correlation heatmap of the top 50 genera and SCFAs in broilers. Significance was determined using one-way ANOVA. * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$.

4. Discussion

Probiotics are widely reported to promote broiler health and development [21-23]. *Lactopantibacillus plantarum* has also been shown to promote growth in broilers [24-26]. Consistent with these previous reports, in this study, supplementation with *L. plantarum* HJLP-1 increased the BW of broilers without influencing feed intake (FI), resulting in reduced F/G ratio (improved feed conversion) over the entire supplemental period to a level equivalent to or lower than that under antibiotics. Therefore, the increased ADG might be attributed to the improvement in nutrient utilization. These beneficial effects on growth performance indicate that *L. plantarum* HJLP-1 could be an effective substitute for antibiotics in poultry diets.

Serum uric acid (UA), urea nitrogen (UN), and ammonia levels have been considered as indicators to evaluate the utilization of amino acid in broilers [27]. Elevated serum indexes can cause disruptions in amino acid and nucleotide metabolism, ultimately affecting antioxidant capacity [28] and immune function of the body [29]. In this study, *L. plantarum* HJLP-1 supplementation reduced serum UA, UN, ammonia ($P < 0.05$), and XOD ($P < 0.05$), compared with CON and ANT. Hence, *L. plantarum* HJLP-1 may have a positive effect on amino acid utilization in broiler diets. XOD represents a critical enzyme responsible for UA production; The enhanced XOD activity can assist in generating UA with producing reactive oxygen species (ROS) [30].

Malondialdehyde, T-SOD, CAT, and GSH-Px represent major factors adopted for assessing oxidative status of the enzymatic system. As the lipid peroxidation production MDA can damage physical cell activity and alter biological membrane function, which has been frequently adopted to be the biomarker for oxidative injury. In contrast, SOD protects cells from free radicals by catalysing the conversion of superoxide into oxygen and oxygen peroxide (H_2O_2). Later, CAT and GSH-PX can convert the obtained hydrogen peroxide to water for protecting cells against damage due to oxygen stress [31]. In vitro studies revealed that some *L. plantarum* fermentate potently reduce and scavenge free radicals [32,33]. In vivo studies have shown that *L. plantarum* colonizes the intestinal tract, which has an important effect on protecting against free radicals. Li et al. (2021) [34] found that feeding *Lactopantibacillus plantarum* KSFY06 at high concentrations can inhibit the decrease of oxidation associated enzymes and complexes in serum and liver caused by liver injury effectively. Izuddin et al. (2020) [35] reported that dietary with *Lactopantibacillus plantarum* supplementation in diet of post weaning lambs can improve antioxidants levels in serum and rumen, as well as, upregulate antioxidant activities in liver and rumen barrier function. Similar to these studies, adding *L. plantarum* HJLP-1 in diet significantly increased serum T-SOD and CAT activities in broilers. Therefore, *L. plantarum* HJLP-1 promoted the antioxidant capacity of broilers mainly by scavenging free radicals.

Serum immunoglobulin, a key indicator for animal's humoral immunity status, represents a defense mechanism against the intrusion of foreign substances into the living body. IgA, IgG (IgY), and IgM represent the critical immunoglobulins with avian species [36,37]. According to our results, supplementation with *L. plantarum* HJLP-1 enhanced broilers immunity through promoting serum IgM and IgY levels. Such result conformed other studies [38], where feeding a postbiotic produced by *L. plantarum* HJLP-1 can improve serum IgM and IgY levels in broilers. Based on the fact that cytokines have essential effects on inflammatory and immune responses, the balance of them exerts a critical effect on resisting infection. Increases in pro-inflammatory factors, like TNF- α , IL-6, and IL-1 β , induce systemic inflammation reaction and tissue damage [39], while IL-10 is the anti-inflammatory and tolerogenic factor blocking pro-inflammatory factor production [40]. Some studies have reported that *L. plantarum* could upregulate anti-inflammatory factors while suppressing pro-inflammatory factors within the intestinal mucus of broilers challenged by toxins or pathogens [18,41]. The results of this study revealed that *L. plantarum* HJLP-1 also decreased serum IL-1 β expression while increasing IL-10 expression, which indicated that supplementation with *L. plantarum* HJLP-1 might be beneficial for attenuating the systemic inflammatory response in broilers.

Numerous studies have concluded that probiotics had positive effects on health, performance, and disease resistant of the hosts, as they can regulate the gut microbial flora balance in hosts by inhibiting the proliferation of pathogenic species and improving the number of beneficial bacteria

[33, 42, 43]. Therefore, the beneficial effects of *L. plantarum* HJLP-1 on broilers in this study may be attributed to its influence on the endogenous commensal microflora and changes in metabolite production by the intestinal bacteria.

SCFAs, especially butyric acid, propionate, and acetic acid represent main bacterial fermentation end products of dietary components, especially indigestible fibers in the intestine and play important roles in maintaining the health of host. Both of acetic acid and lactic acid were detected in postbiotics produced by a variety of *Lactobacillus plantarum* [44]. Acetic acid can contribute to environmental acidification and inhibit the growth of less acidophilic organisms (most pathogenic bacterial species) in the same ecosystem [45]. Butyric acid has been shown to have a critical effect on growth promotion [46,47], oxidative stress reduction [48], immune modulation, anti-inflammatory effects [49], and inhibition of colonization by pathogenic bacteria in broilers [50]. According to our results, the dramatically increased butyric acid and acetic acid concentrations in the cecal contents of broilers fed *L. plantarum* HJLP-1 maybe partly responsible for the beneficial effects of *L. plantarum* HJLP-1 on broilers.

Diverse microorganisms densely colonized in the gastrointestinal tract of poultry have been considered to have vital effects on host health and growth performance [51]. Our results indicate that *L. plantarum* HJLP-1 plays a role in modulating the gut microbiota of broilers. *L. plantarum* can enrich the cecal microbiota communities with unique microbiota, as reflected by significantly increased ACE, Chao richness indices, and abundance of unique OTUs in LP compared to CON and ANT. Compared to the samples of CON group, the decreased Bacteroidetes abundance while increased Firmicutes abundance observed in LP and ANT group samples consider an increase of Firmicutes/Bacteroidetes ratio at the cecal level, which showed positively relation to an increase in BW in broilers [52,53], which may be a possible reason for the growth-promoting effects of *L. plantarum* and antibiotics. Moreover, As indicated by the spearman correlation heatmap, *Ruminococcaceae* and *Lachnospiraceae* that enriched in LP group promote butyric acid production. The *Alistip*, which enriched in ANT group, also have positive correlation on butyric acid production. While the *Bacteroides* that enriched in control group inhibit the butyric acid production. This is probably the main reason for the dramatic increase in butyric acid by *L. plantarum* HJLP-1 and antibiotic.

5. Conclusion

Taken together, *Lactopantibacillus plantarum* HJLP-1 supplementation could improve growth performance and promote the antioxidant capacity and immune function of broilers. These beneficial effects of *L. plantarum* HJLP-1 on broilers may be attributed to its influence on some specific bacterial abundance in the intestinal microbiota, resulting in a dramatic increase in butyric acid and acetic acid content. Therefore, *L. plantarum* HJLP-1 could be an efficient substitute for antibiotics to promote growth and health of broiler in production.

Author Contributions: Caimei Yang and Yinglei Xu designed this experiment and edited manuscript. Shuting Wang, Qing Li performed animal experiments, Shuting Wang and Ruiqiang Zhang collected samples and analyzed them, and Jie Feng guided the implementation of the experiment and revised the article. All authors read and approved the final manuscript.

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Institutional Review Board Statement: The animal care protocol was approved by the Animal Ethics Committee of Zhejiang A&F University (SYXKzhe2016-087).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: None of authors declare a conflict of interest.

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