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Modulation of broilers' caecal microflora and metabolites in response to a potential probiotic *Bacillus amyloliquefaciens*

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Summary

Studies have found that a dietary supplement of *Bacillus amyloliquefaciens* improved the growth performance, increased the nutrient digestibility of hosts and modulated the intestinal microflora. A total of 360 1-day-old Ross broilers were randomly divided into three treatments: a control group with a basal diet, an antibiotic group with a basal diet and added colistin sulphate, and a probiotics group with a basal diet and added *Bacillus amyloliquefaciens*. The HiSeq high-throughput sequencing analysis of 16S rRNA was used to investigate the differences in birds' caecal microflora, and metabolomics was used to analyse changes in caecal metabolites. Results showed that the supplementation of *Bacillus amyloliquefaciens* significantly improved the BW and ADG compared with the control birds. Results of sequencing indicated that (i) 645, 670, 596 unique operational taxonomic units (OTUs) were found in birds supplemented with *Bacillus amyloliquefaciens* on day 7, 21 and 42, separately, (ii) due to the diversity and relative abundance of the birds' caecal microflora, the OTUs of the caecal microflora clustered according to age and treatment, except on day 42, (iii) among the six predominate families (Ruminococcaceae, Lachnospiraceae, Enterobacteriaceae, Erysipelotrichaceae, Lactobacillaceae and Rikenellaceae), the supplementation of *Bacillus amyloliquefaciens* significantly increased Enterobacteriaceae on day 42, (iv) *Bacillus amyloliquefaciens* increased the relative abundance of Faecalibacterium and Ruminococcus on day 21, increased the Faecalibacterium and Blautia and decreased the Ruminococcus on day 42. The metabolomics of caecal metabolites showed that the dietary *Bacillus amyloliquefaciens* changed the caecal metabolites involved of amino acid metabolism and glyceride metabolism, and the antibiotics changed the caecal metabolites that were related to carbohydrates and amino acid metabolism on day 21.

KEYWORDS

Bacillus amyloliquefaciens, broiler chickens, caecal microflora, growth performance, metabolites

1 | INTRODUCTION

Recently, Bacillus sp. have been used as one of the most widely potential probiotics in poultry, which functions as a substitution of antibiotic growth promoter (Murugesan, Romero, & Persia, 2014). The beneficial characteristics of Bacillus sp. include the ability to survive and germinate in the gastrointestinal tract (Barbosa, Serra, La Ragione, Woodward, & Henriques, 2005) as well as the secretion of protease, amylase and lipase (Santoso, Tanaka, Ohtani, & Sakaida, 2001). Previous studies indicated that *Bacillus amyloliquefaciens* (*B. amyloliquefaciens*) is closely related to *Bacillus subtilis*, which produces extracellular enzymes, including α-amylases, cellulase, metalloproteases and proteases, that enhance the digestibility and absorption of nutrients in the gut (Gangadharan, Sivaramakrishnan, Nampoothiri, Sukumaran, & Pandey, 2008).

High-throughput sequencing technologies have been widely used to investigate the composition of intestinal microbial communities and provided an understanding of microbial ecology that can be leveraged in a series of trials (Langille et al., 2013). Choi, Kim, and Cha (2014) studied the spatial heterogeneity of the microbial community in different gastrointestinal tracts of broilers by high-throughput pyrosequencing. Meanwhile, the caecum is an important gastrointestinal tract for recycling urea, water regulation and carbohydrate fermentation that influences intestinal health and nutrition (Stanley, Hughes, & Moore, 2014; Waite & Taylor, 2014). Metabolomics is one of the most useful approaches to characterise the metabolites of animals (Alistair & Dove, 2013). Therefore, this study was conducted to investigate the effects of *B. amyloliquefaciens* on growth performance, the composition of caecal microflora and the changes of caecal metabolites in broilers based on the metabolomics and high-throughput sequencing.

2 | MATERIALS AND METHODS

2.1 | Birds and design

All the following procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China). A total of 360 1-d-old birds (Ross 308) obtained from a local hatchery (Charoen Pokphand Group, Haining, China) were randomly divided into three treatments, with six replicates per treatment and 20 birds per replicate.

Birds treated as control group (Cont) were fed a basal diet with no addiction, and the antibiotic group (Anti) was fed a basal diet containing 20 mg colistin sulphate/kg. The *B. amyloliquefaciens* group (BAF) was fed a basal diet containing 10^9cfu *B. amyloliquefaciens*/kg. Through the whole test, water and feed were provided ad libitum. The room temperature was kept for 37°C at the first week and gradually decreased to 25°C at a rate of 2.5°C/week. The birds were raised in cages and fed for 42 days. The basal diet based on NRC (1994) requirements, and the ingredients and nutrient composition is listed in Table 1.

TABLE 1 Ingredients and nutrient levels of the basal experimental diet[a] (air-dry basis)

Ingredients	Content (%)
Corn	61.50
Soybean meal	27.50
Fish meal[b]	5.00
Soybean oil[c]	2.00
Vitamin–mineral Premix[d]	4.00
Total	100.00
Nutrient levels	
DE (Mcal/Kg)	3.00
CP, %	21.00
Lys, %	1.22
Met + Cys, %	0.98
Ca, %	1.00
AP, %	0.47

[a]Nutrient level of the diets was based on NRC (1994).
[b]Crude protein content is 62.5%, and ME is 2.79 Mcal/kg.
[c]Metabolisable energy is 8.8 Mcal/kg.
[d]Supplied per kilogram of diet: vitamin A (retinyl acetate), 1,500 IU; cholecalciferol, 200 IU; vitamin E (DL-α-tocopheryl acetate), 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 μg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine, 1.5 mg; pyridoxine, 3.0 mg; Fe, 80 mg; Zn, 40 mg; Mn, 60 mg; I, 0.18 mg; Cu, 8 mg; Se, 0.15 mg.

2.2 | Strains

The probiotic, *B. amyloliquefaciens* (CGMCC 9384) was provided by Zhejiang Huijia Biological Technology Ltd., Anji, China. After fermentation (37°C, 48 hr) and drying, the strain was granulated and used at a concentration of approximately 1.7×10^{10} CFU/g.

2.3 | Growth performance

The body weights of birds were recorded for each cage to calculate the growth performance weekly. Records of feed consumed in each pen were kept for calculating the feed conversion ratio (FCR) at the end of study.

2.4 | Caecal sample collection

On day 1, 7, 14, 21 and 42, three birds (with a similar weight) per cage were euthanised by cervical dislocation and slaughtered. Immediately after the broilers were dissected, the content of the caecum was obtained through manual extrusion. All samples were placed immediately into sterile plastic tubes. To reduce variation between individuals, the caecal contents of six birds were pooled into one biological sample. Overall, 30 samples were used for high-throughput sequencing (on day 1, 7, 21 and 42), and 33 samples were used for metabolite detection (on day 1, 7, 14 and 21 with six biological samples on day 1). All the samples were snap-frozen in liquid nitrogen and stored at −80 until analysis.

2.5 | High-throughput sequencing

The microbial genomic DNA was isolated from the caecal content samples using the PowerFecal® Fecal DNA Kit (MoBio Laboratories, Inc, USA), and the HiSeq 2500 platform (Illumina, San Diego, CA) was used for samples' sequencing. The V3 hypervariable regions of 16S rRNA were amplified by PCR using the barcoded fusion primers F primer: GATCCTACGGGAGGCAGCA, R primer: GCTTACCGCGGCTGCTGGC. The PCR conditions were as follows: initial denaturation at 95°C for 5 min, 25 cycles of 95°C for 60 s, 50°C for 60 s and 72°C for 60 s and then final extension at 72°C for 7 min. The quality, concentration of DNA were assessed on a FAST QC FLASH v1.2.7 (http://ccb.jhu.edu/software/FLASH/), which the chimeric check and classification of non-chimeric sequences were conducted using the RDP CLASSIFIER according to Wang, Garrity, Tiedje, and Cole (2007). Data analysis was performed with the resulting chimera-free FASTA file using QIIME 1.8 software.

RDP CLASSIFIER software and a GREENGENES database were used to note the species (DeSantis et al., 2006; Edgar, 2010). Then, sequences were clustered into operational taxonomic units (OTUs) based on similarities at 97% (similar at the species level) using UCLUST v1.2.22. The microbial community structures were compared using the FAST-UNIFRAC tool. Changes in bacterial abundance were compared using an ANOVA and a two-tailed Student's *t* test to find the significance of OTU estimates.

2.6 | GC-MS-based metabolite profiling of caecal content

The metabolite detection followed the methods of Nie et al. (2015). Briefly, the caecal content was homogenised with L-2-Chlorophe and blended with the chilled extraction liquid (methanol and chloroform). After centrifugation, the supernatant was transferred into the silylated GC vial., which bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane was injected into. Finally, the solution was shaken for 1 hr at 70°C and cooled to room temperature.

The Agilent 7890A GC system (Agilent, USA), LECO Chroma TOF PEGASUS 4D system (LECO, USA) and Agilent DN-5MS (30 m × 250 μm × 0.25 μm, J&W Scientific, Folsom, CA, USA)were used to separate the derivative metabolites. The initial oven temperature was held at 50°C for 1 min and then ramped to 330°C at a rate of 10°C/min 21 and held for 5 min. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. Electron impact ionisation (−70 eV) was utilised, and mass data were collected in a full-scan mode at 85–600 m/z and 20 spectra/s. The metabolites were identified by comparison with the NIST 2005 database version 2.0 (FairCom, USA).

2.7 | Metabolite data analysis

The resulting three-dimensional data table was entered in the SIMCA-P+ version 13.0 software package for multivariate statistical analysis. The significantly altered metabolites were validated by a Student's *t* test using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Both the variable importance in the projection (VIP) values >1.5 and $p < .05$ (Student's *t* test) were used to select metabolites within the control and antibiotics or BAF birds.

3 | RESULTS

3.1 | Growth performance

Compared with the control treatment, the supplementation of *B. amyloliquefaciens* significantly increased the BW of birds on day 14, 35 and 42, and the antibiotic supplementation significantly increased the BW of birds on day 14 and 35, while no differences were found between the BAF and Anti birds on day 7, 14, 28, 35 and 42 (Table 2). Additionally, the dietary *B. amyloliquefaciens* significantly increased the ADG of birds compared with control birds from days 1–14 to days 1–42. As for FCR, both BAF and Anti birds showed higher value ($p < .05$) than the control birds, while no statistical differences were found between the BAF and the others throughout the whole study.

3.2 | High-throughput sequencing of caecal microflora

Venn diagrams were constructed to depict shared and unique OTUs (sequences <4,000) among the groups examined at each time-point

TABLE 2 Effects of *Bacillus amyloliquefaciens* on the growth performance of broilers[1]

		Treatments				
Item	Age	Cont	Anti	BAF	SEM	*p*-value
BW (g)	D7	121.7^{b}	135.2^{a}	127.2^{ab}	2.396	.059
	D14	304.8^{b}	348.1^{a}	361.7^{a}	8.844	.013
	D21	645.3^{ab}	640.3^{b}	679.7^{a}	8.165	.088
	D28	875.2	950.4	972.2	20.6	.129
	D35	1,221^{b}	1,458^{a}	1,460^{a}	40.18	.004
	D42	1,718^{b}	1,921^{ab}	1,953^{a}	47.52	.089
ADG (g)	d 1-14	18.9^{b}	22.0^{a}	22.9^{a}	0.651	.018
	d 15-28	43.6	45.9	46.5	1.612	.768
	d 29-42	60.2	69.3	70.0	3.361	.438
	d 1-42	40.0^{b}	44.8^{ab}	45.5^{a}	1.132	.084
FCR	d 1-21	1.76^{a}	1.51^{b}	1.53^{b}	0.107	.059
	d 21-42	1.97	1.84	1.85	0.097	.382
	d 1-42	2.04	1.98	2.01	0.122	.874

Means in the same row with different superscript letters differ significantly ($p < .05$).
[1]Each mean represents six birds. Cont = birds were fed a basal diet, Anti = birds were fed a basal diet supplemented with 20 mg colistin sulphate/Kg, BAF = birds were fed a basal diet supplemented with 10^{9} cfu *B. amyloliquefaciens*/Kg.

(Figure 1). On day 7, 21 and 42, 835, 1,458 and 1,772 OTUs were shared among the three treatments respectively. Furthermore, 645, 670 and 596 OTUs were found only in BAF birds compared with the control and antibiotic birds on day 7, 21 and 42 respectively. According to the weighted Unifrac UPGMA of broilers' caecal microflora (Figures 2, S1 and S2), the dietary *B. amyloliquefaciens* changed the structure and diversity of caecal microflora in broilers.

Figure 3 showed the six predominate families are Ruminococcaceae, Lachnospiraceae, Enterobacteriaceae, Erysipelotrichaceae, Lactobacillaceae and Rikenellaceae. Compared with the control birds, the supplementation of BAF significantly caused an increase in the relative abundance of Enterobacteriaceae on day 42. The antibiotic supplementation significantly increased the relative abundance of Erysipelotrichaceae on day 7 and the Lachnospiraceae on day 21 and decreased the Ruminococcaceae and Erysipelotrichaceae on day 21 compared with the control and BAF birds. Meanwhile, the relative abundance of Ruminococcaceae on day 21 and 42 and Rikenellaceae on day 42 was numerically increased by dietary BAF compared with the control birds.

Figure 4 shows that the eight predominate genera of caecal microflora in the broilers included Lactobacillus, Dorea, Blautia, Ruminococcus, Morella, Oscillospira and Faecalibacterium. On day 42, the dietary *B. amyloliquefaciens* increased the relative abundance of genus Faecalibacterium and Blautia ($p < .05$), whereas both antibiotic and *B. amyloliquefaciens* decreased the relative abundance of genus Ruminococcus compared with control birds ($p < .05$). On day 21, antibiotic supplementation significantly increased the relative abundance of Dorea, while *B. amyloliquefaciens* increased the relative abundance of Faecalibacterium (16.4%) and Ruminococcus (12.6%) compared with the control and antibiotic birds. Compared with control birds on day 7, the dietary *B. amyloliquefaciens* increased the relative abundance of Morella (10.9%, $p < .05$) and Oscillospira (10.2%) and decreased the Ruminococcus. In contrast, at the level of family, the antibiotics significantly increased the relative abundance of Lachnospiraceae (30%) and decreased the Ruminococcaceae (35.4%) and Erysipelotrichaceae (0.7%) compared with the control and BAF birds on day 21, and the BAF-supplemented birds had the highest relative abundance of Ruminococcaceae (48.5%) and Erysipelotrichaceae (1.6%) among the groups. On day 42, the relative abundance of Rikenellaceae in birds fed *B. amyloliquefaciens* (10.9%) and antibiotics (12.0%) were significantly higher than control birds (8.5%).

3.3 | Caecal metabolites

Combined with PCA scores about the broilers' caecal metabolites, we found that the three treatments were clustered with each other on different time-points (Figure 5). The results suggested that the dietary BAF and antibiotics changed the caecal metabolites. The metabolite data showed that supplementation with antibiotics significantly increased the concentration of maltotriose and monopalmitin caecal content in broilers and decreased oleic acid, sophorose, threitol, 2-deoxyerythritol, palmitoleic acid, 6-methyl-previtamin D and 5α-cholestan-3-one compared with the control birds on day 21, which was mainly related to carbohydrate and amino acid metabolism (Table 3). The supplementation

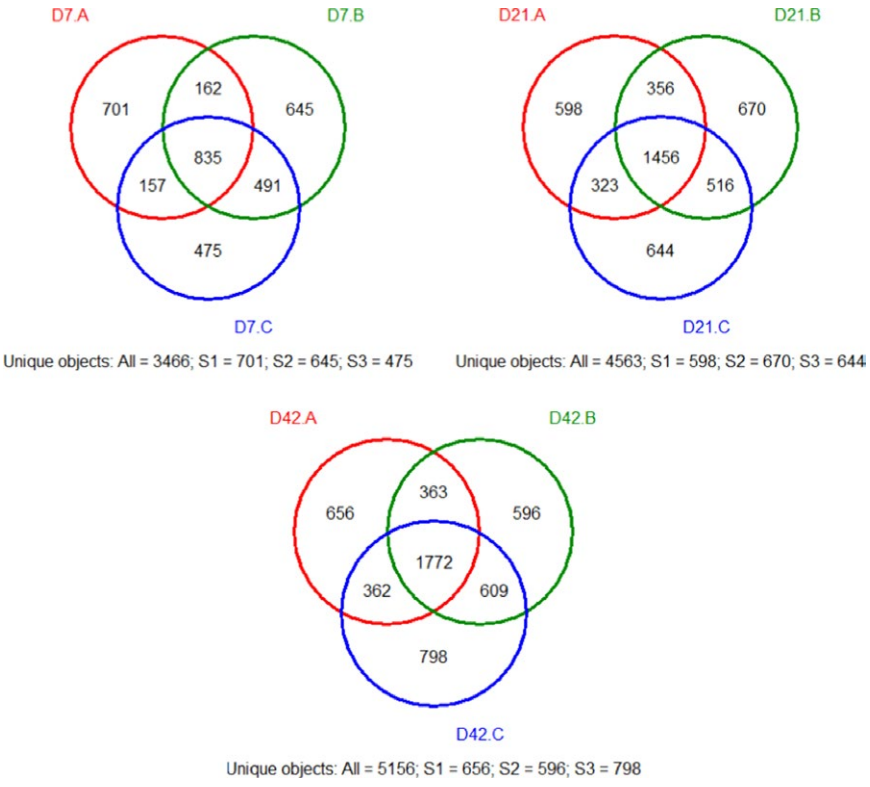

FIGURE 1 Operational taxonomic unit venn diagram of the broilers' caecal microflora D7. A, B, C represents OUT of antibiotic, *B. amyloliquefaciens* and control broilers' caecal microflora on day 7 respectively; D21. A, B, C represents OUT of antibiotic, *B. amyloliquefaciens* and control broilers' caecal microflora on day 21 respectively; D42. A, B, C represents OUT of antibiotic, *B. amyloliquefaciens* and control broilers' caecal microflora on day 42 respectively

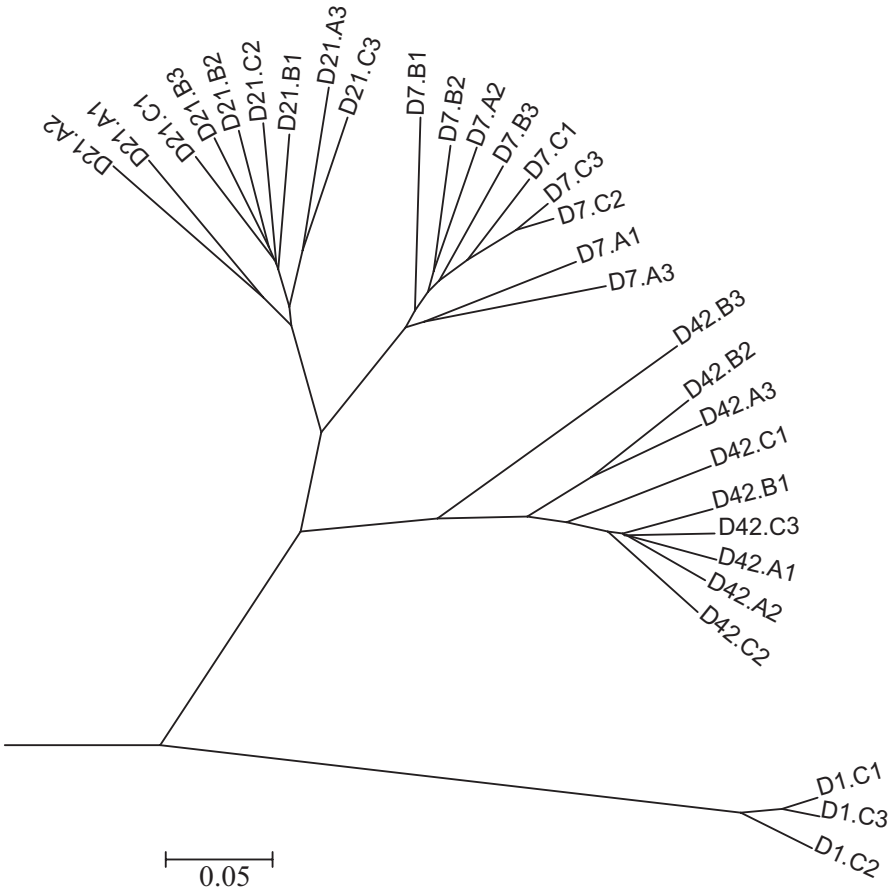

FIGURE 2 The UPGMA (weighted UniFrac) of broilers' caecal microflora D7. A, B, C represents OUT of antibiotic, *B. amyloliquefaciens* and control broilers' caecal microflora on day 7 respectively; D21. A, B, C represents OUT of antibiotic, *B. amyloliquefaciens* and control broilers' caecal microflora on day 21 respectively; D42. A, B, C represents OUT of antibiotic, *B. amyloliquefaciens* and control broilers' caecal microflora on day 42 respectively

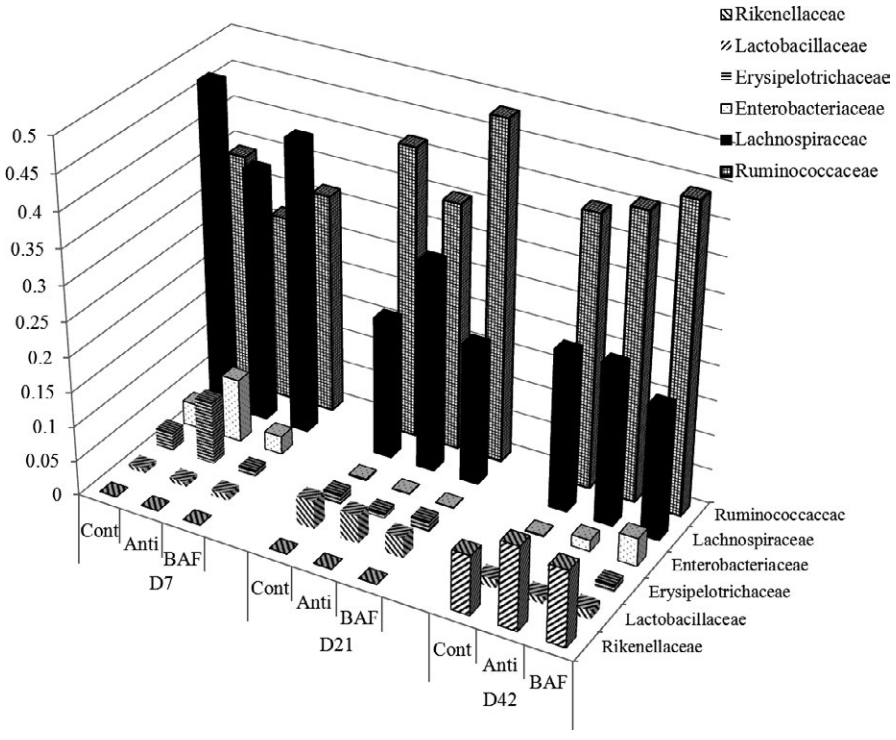

FIGURE 3 Relative abundance of the broilers' caecal microflora in level family Each mean represents six birds. Cont = birds were fed a basal diet; Anti = birds were fed a basal diet supplemented with 20 mg colistin sulphate/Kg; BAF = birds were fed a basal diet supplemented with 10^9 cfu *B. amyloliquefaciens*/Kg

of *B. amyloliquefaciens* significantly increased the concentration of gentiobiose, quinic acid, 3,7,12-trihydroxycoprostane, N-ethylglycine, glycine, N-acetyl-D-galactosamine, 5-hydroxyindole-3-acetic acid and diglycerol as well as decreased the N-acetyl-β-D-mannosamine and 4-aminobutyric acid on day 21 (Table 4), which involved the amino acid and glyceride metabolism.

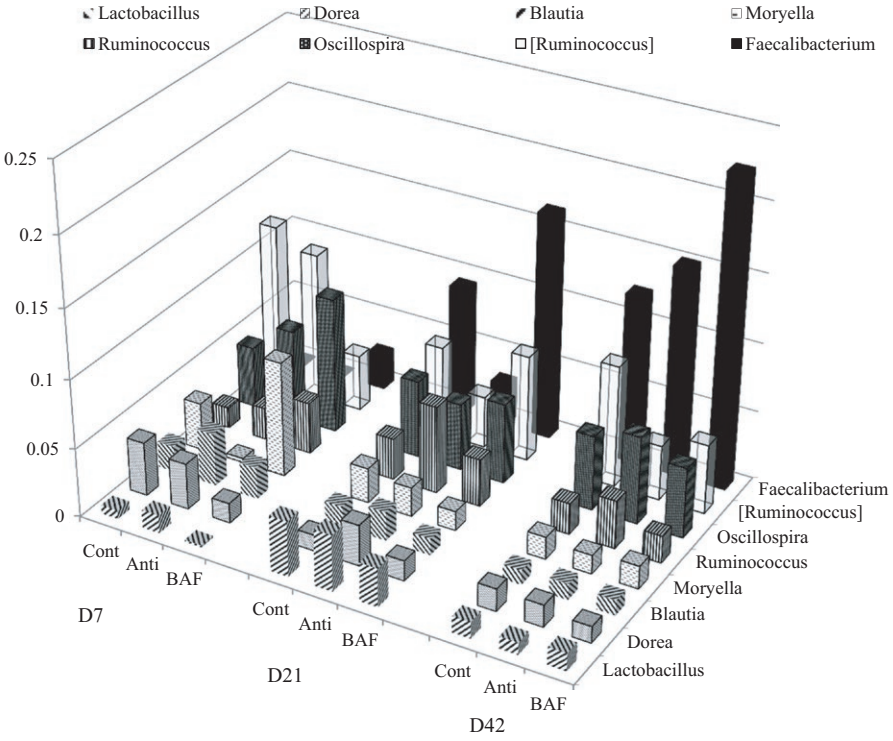

FIGURE 4 Relative abundance of the broilers' caecal microflora in level genus Each mean represents six birds. Cont = birds were fed a basal diet; Anti = birds were fed a basal diet supplemented with 20 mg colistin sulphate/Kg; BAF = birds were fed a basal diet supplemented with 10^9 cfu *B. amyloliquefaciens*/Kg

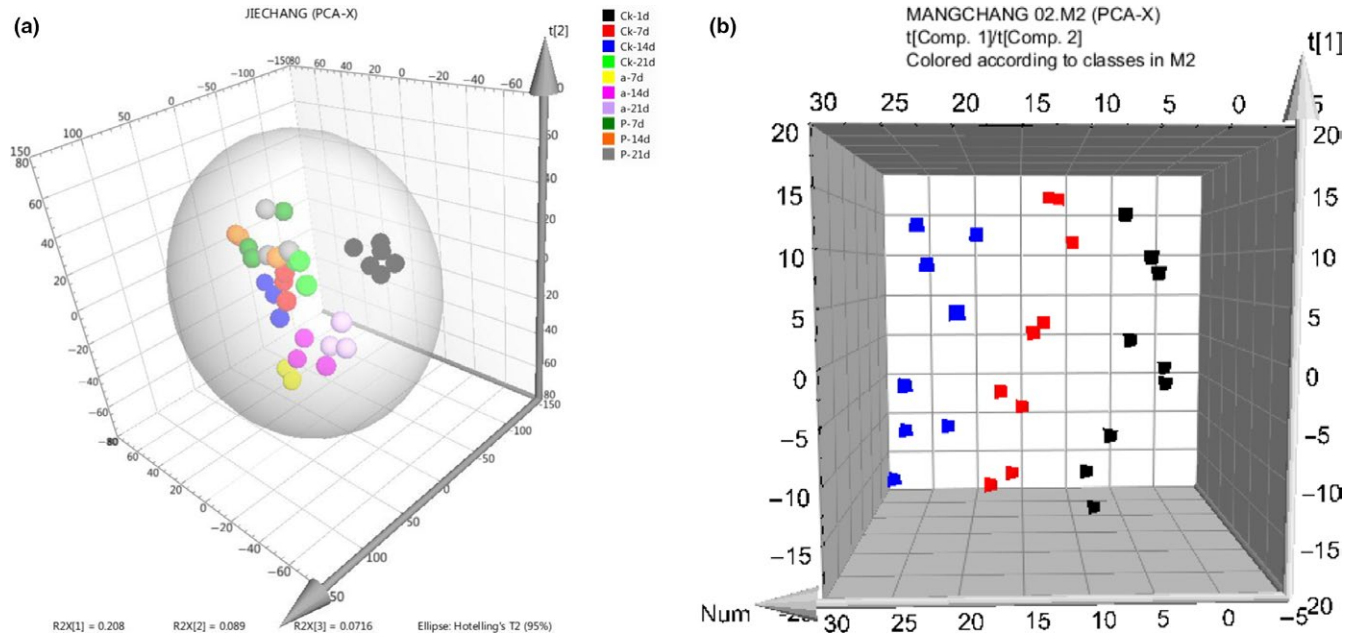

FIGURE 5 (a) Total PCA scores plot about the caecal metabolites in broilers; (b) PCA scores about the metabolites of differential broilers' caecum from day 7 to 21 D1-Cont represents control birds on day 1, D14-Anti represents Anti birds on day 14, D21-BAF represents BAF birds on day 21. Black grid represents control birds, blue grid represents antibiotic birds and red grid represents BAF birds

4 | DISCUSSION

Studies about the application of probiotics have been performed in livestock for decades, and the Bacillus sp. have been confirmed to promote the feed conversion ratio of livestock (EFSA, 2010). Bacillus-based probiotics have recently shown tremendous promise because of their capacity to form spores, which are known to withstand harsh environmental stress and the influence of pH, nutrients and other relevant factors in the gastrointestinal tracts of animals (Shivaramaiah et al., 2011). Particularly, strains of Bacillus can secrete a wide range of enzymes, and enzyme activities (such as amylase, trypsin and lipase) play a key role in the digestion of nutrients in poultry (Cartman,

TABLE 3 Differential caecal metabolites between the control and antibiotics broilers[a] on day 21

Metabolites	Mean of control	Mean of antibiotics	Trend	VIP	*p*-value
Oleic acid	0.003	3.5×10^{-12}	↓	3.599	.032
Sophorose	4.99×10^{-5}	3.0×10^{-12}	↓	2.043	.016
Threitol	4.82×10^{-5}	3.5×10^{-12}	↓	2.897	.027
2-Deoxyerythritol	6.96×10^{-5}	3.5×10^{-12}	↓	2.961	.029
Palmitoleic acid	0.0001	2.93×10^{-5}	↓	1.108	.032
6-Methyl-previtamin D	0.0001	3.47×10^{-6}	↓	2.184	.010
5α-Cholestan-3-one	8.19×10^{-5}	3.21×10^{-7}	↓	2.239	.044
Maltotriose	2.82×10^{-12}	5.92×10^{-5}	↑	2.975	.006
1-Monopalmitin	2.46×10^{-5}	0.0001	↑	2.125	.036

[a]Control = birds were fed a basal diet, Antibiotics = birds were fed a basal diet supplemented with 20 mg colistin sulphate/Kg.

TABLE 4 Differential caecal metabolites between the control and *Bacillus amyloliquefaciens* broilers[a] on day 21

Metabolites	Mean of control	Mean of BAF	Trend	VIP	*p*-Value
4-Aminobutyric acid	2.82×10^{-12}	0.0002	↑	3.244	.045
Gentiobiose	0.0002	0.0008	↑	1.332	.019
Quinic acid	4.32×10^{-5}	0.0001	↑	1.157	.043
3,7,12-Trihydroxycoprostane 2	2.74×10^{-6}	1.52×10^{-5}	↑	1.936	.032
N-Ethylglycine	0.003	0.012	↑	1.587	.018
Glycine	0.009	0.039	↑	1.779	.001
N-Acetyl-D-Galactosamine	0.0003	0.0004	↑	1.769	.019
5-Hydroxyindole-3-Acetic acid	1×10^{-12}	2×10^{-4}	↑	1.583	.001
Diglycerol	0.011	0.003	↓	1.581	.020
N-Acetyl-β-D-Mannosamine	0.003	0.0004	↓	1.571	.003

[a]Control = birds were fed a basal diet; BAF = birds were fed a basal diet supplemented with 10^9 cfu *B. amyloliquefaciens*/Kg.

La Ragione, & Woodward, 2008; Nitsan, Ben-Avraham, Zipora, & Nir, 1991). Additionally, publications have declared there is a positive effect of dietary *B. amyloliquefaciens* on growth performance as well as modulating intestinal health by decreasing the *E. coli* count, the emission of ammonia and hydrogen sulphide in broilers (Ahmed et al., 2014; Lei et al., 2015). According to our study, supplementation with *B. amyloliquefaciens* improved the BW and ADG of broilers. Similarly, previous studies reported that supplementation with *B. amyloliquefaciens* improved the growth performance and feed efficiency of broilers (Mallo, Gracia, Honrubia, & Sedano, 2010).

It is well known that the gut microbiota is a complex ecosystem that has a symbiotic relationship with hosts. Alterations in the composition of gut microflora changed the complement of genes for specific metabolic pathways, which reinforced the host metabolism (Turnbaugh et al., 2006). Recently, high-throughput sequencing technologies have been widely used to analyse the composition and dynamics of the core gut microbiome in chickens. Choi et al. (2014) analysed the hypervariable V1-V3 region of the bacterial 16S rRNA gene about the chicken gut microbiota using a 454 pyrosequencing approach. Furthermore, the present study showed that the Firmicutes was the predominant phylum within the caecum at all time-points and groups agreed to other studies (Danzeisen, Kim, Isaacson, Tu, & Johnson, 2011; Jozefiak et al., 2010). Jumpertz et al. (2011) reported that the increase in faecal Firmicutes was related to the nutrient absorption, whereas an increase in Bacteroidetes decreased the nutrient absorption.

On day 7, 21 and 42, the specific OTUs of the control birds' caecal microflora are approximately 13.7%, 14.1% and 15.5%, and the three different treatments shared approximately 24.1%, 31.9%, 34.4% of the total OTUs, separately, which indicated that supplementation with *B. amyloliquefaciens* and antibiotics dramatically changed the OTUs composition of caecal microbial composition at the start phase for broilers. On day 21, antibiotic supplementation significantly increased the relative abundance of Dorea, while *B. amyloliquefaciens* increased the predominate genus of Faecalibacterium and Ruminococcus compared with control and antibiotic birds. On day 42, the dietary *B. amyloliquefaciens* increased the relative abundance of genus Faecalibacterium and Blautia, while both antibiotic and *B. amyloliquefaciens* decreased the relative abundance of genus Ruminococcus than control birds. Rubio et al. (2015) reported that diets supplemented with additives influenced the intestinal microbiota composition of broiler chickens. Additionally, the use of anticoccidial combined with the antibiotics (virginiamycin or tylosin) significantly caused several genus-level enrichments and depletions, including enrichments in transport system

genes, type I fimbrial genes and type IV conjugative secretion system genes (Danzeisen et al., 2011).

Other studies have showed that, for hosts, the composition and proportion of individual intestinal micro-organisms as well as metabolites are continuously changing (Ponnusamy, Choi, Kim, Lee, & Lee, 2011). The high-throughput sequencing data showed that the diets with added *B. amyloliquefaciens* and antibiotics have changed the caecal microflora in broilers. Therefore, the change of the predominant community may be correlated with the change of metabolites in the intestinal tract. No study has been conducted to investigate the caecal metabolites changed by dietary *B. amyloliquefaciens* based on metabolomics in broilers until now. The PCA results of three treatment groups were separated from each other at each time-point, which indicated that supplementation of *B. amyloliquefaciens* and antibiotics dramatically changed the metabolites of caecal contents in broilers. Data showed that the supplementation of antibiotics significantly increased the concentration of maltotriose and monopalmitin and decreased the oleic acid, sophorose, threitol, 2-deoxyerythritol, palmitoleic acid, 6-methyl-previtamin D and 5α-cholestan-3-one in the broilers' caecum contents on day 21. By searching the metabolite pathway in the Kyoto Encyclopedia of Genes and Genomes (KEGG), it is related to carbohydrate digestion and absorption, primary bile acid biosynthesis and fatty acid metabolism. The supplementation of *B. amyloliquefaciens* significantly increased the concentration of gentiobiose, quinic acid, 3,7,12-trihydroxycoprostane, N-ethylglycine, glycine, N-acetyl-D-galactosamine, 5-hydroxyindole-3-acetic acid and diglycerol as well as decreased the N-acetyl-β-D-mannosamine and 4-aminobutyric acid on day 21. The change is associated with amino acid metabolism as well as amino sugar and nucleotide sugar metabolism, glycerolipid metabolism, primary bile acid biosynthesis, linoleic acid metabolism and phenylpropanoid biosynthesis. In addition, the antibiotic supplementation only increased the concentration of lactose, maltose, while the BAF supplementation increased the concentrations of threonine, monopalmitin, galactinol and allose on day 7. Previously, a study by Hong et al. (2010) assessed the effects of lactic acid probiotics on the metabolic profiling of the faecal contents of mice and found that the levels of faecal short-chain fatty acids were increased by supplementation with probiotics. However, no direct relationship was found within the changes of broilers' caecal microflora and metabolites in the present study, and more tests are needed in the future.

In summary, results showed that the dietary *B. amyloliquefaciens* improved the growth performance of birds in the early stage and changed the metabolites and bacterial community structures of caecal content.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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