Effect of a novel strain of *Lactobacillus brevis* M8 and tea polyphenol diets on performance, meat quality and intestinal microbiota in broilers

Xiaozhuo Zou\(^{a,b,c}\), Rong Xiao\(^d\), Huali Li\(^e\), Ting Liu\(^{a,b,c}\), Yong Liao\(^{a,b,c}\), Yuanliang Wang\(^{a,b,c}\), Shusong Wu\(^f\) and Zongjun Li\(^{a,b,c}\)

\(^a\)Hunan Provincial Key Laboratory of Food Science and Biotechnology, Changsha, China; \(^b\)National Research Center of Engineering Technology for Utilization of Functional Ingredients from Botanicals, Changsha, China; \(^c\)College of Agricultural and Biological Technology, Hunan University of Humanities, Science and Technology, Loudi, China; \(^d\)Hunan Institute of Animal and Veterinary Science, Changsha, China; \(^e\)The United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan

**ABSTRACT**

A 56-day experiment was conducted with 480 yellow-feathered broilers to determine the effects of *Lactobacillus brevis* M8 (LB) and tea polyphenols (TP) on their growth performance, meat quality and gut microbiota. Broilers were assigned randomly to eight groups: CTL (negative control); LB (1.0 \(\times\) 10⁸ cfu kg⁻¹ BW); TP (0.06 g kg⁻¹ BW); LB + 0.06 g kg⁻¹ BW TP; MLB (1.0 \(\times\) 10⁸ cfu kg⁻¹ BW + 0.06 g kg⁻¹ BW TP); HLB (0.03 g kg⁻¹ BW TP + 1.0 \(\times\) 10⁸ cfu kg⁻¹ BW LB); and HTP (0.09 g kg⁻¹ BW TP + 1.0 \(\times\) 10⁸ cfu kg⁻¹ BW LB). All groups were divided among three experiments: Exp. 1 (CTL, LB, MLB); Exp. 2 (CTL, LLB, MLB, HLB); Exp. 3 (CTL, LTP, MLB, HTP). Compared with the control, significantly decreased body weights (BW) of LB and TP groups were observed during a period of 56 days (\(p < .05\)). The feed conversion ratio decreased in LB, TP and MLB groups at 14, 21 and 42 days, respectively (\(p < .05\)). Contents of lysine, leucine, glutamic acid, arginine and aspartic acid in muscles of the MLB group were higher than in the LB group (\(p < .05\)). Denaturing gradient gel electrophoresis demonstrated that LB, TP or the combination of TP and LB diets increased species of *Lactobacillus reuteri*, uncultured *Bacteroides* sp. and *L. crispatus*. In summary, dietary supplementation with 1.0 \(\times\) 10⁸ cfu kg⁻¹ BW LB and 0.06 g kg⁻¹ BW TP (single and in combination) improved meat quality and promoted beneficial bacteria in the intestinal tract with an increased colonisation of *Lactobacillus* and *Bacteroides*, but did not enhance growth performance.

**Introduction**

The public has expressed growing concern over possible residual effects of antibiotics and the development of drug resistant bacteria (Panda et al. 2008), which has led researchers to seek a safe and effective alternative to antibiotics in animals’ diets. Probiotics were initially introduced as an alternative to antibiotics and subsequently became an area of great research interest. *Bacillus subtilis* (Jeong and Kim 2014) and lactic acid bacteria (LAB) are the most commonly used candidate bacteria; others include *Enterococcus faecium* (Cao et al. 2013) and *Clostridium butyricum* (Zhang et al. 2014). LAB are generally recognised as safe according to the United States Food and Drug Administration (FDA). Some strains of *Lactobacillus* are probiotics, that are used as additives in poultry feed to provide particular benefits, such as improving growth performance (Nakphaichit et al. 2011; Chen, Chen et al. 2016), preventing enteric pathogen infection (Menconi et al. 2011), enhancing digestive enzyme activity (Li et al. 2015), regulating intestinal microbiota composition (Nakphaichit et al. 2011; Chen, Chen et al. 2016), activating immune responses (Wang L et al. 2015) and ameliorating inflammation (Cao et al. 2012) in chicks. A variety of LAB have demonstrated to be beneficial to human and animal health, and therefore, they have been used as dietary supplements or antibiotic substitutes (Harata et al. 2016; Kumar et al. 2016).

Green tea is rich in polyphenols such as epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate and epicatechin (Khan and Mukhtar 2013). Tea
polyphenols (TPs) have proven to have multiple biological properties, including antimicrobial (Lee et al. 2009; Kumar et al. 2016), anti-oxidative (Wang, Li, et al. 2015; Klimczak and Gliszczynska-Świgło 2016), anti-inflammatory (Deng et al. 2010; Li et al. 2015) and anti-obesity properties (Xu et al. 2015; Chen, Liu, et al. 2016). TPs are frequently used as an animal feed supplement to improve growth performance, digestion and meat quality in the food industry. Previous studies have reported that TPs can prevent laying hens from experiencing the adverse effects of vanadium on egg quality, and liver antioxidant stress, as well as shorten the recovery time (Yuan, Zhang, et al. 2016). Additionally, supplementation with 1000 mg kg⁻¹ TP increases the short-chain fatty acid production to affect caecum microbiota ecology and protect duodenal cells from excess apoptosis caused by vanadium in laying hens (Yuan, Wang, et al. 2016). Cow supplementation with TPs might be useful to improve milk yield and prevent fatty liver syndrome (Winkler et al. 2015). An appropriate level of dietary TP supplementation can increase host resistance by reducing *Haemonchus contortus* burden and weight loss in sheep (Zhong et al. 2014) and diminish negative effects on meat quality, such as the antioxidant activity and meat colour in lambs (Zhang et al. 2015).

In our previous study, the novel *Lactobacillus brevis* strain M8 (LB) was isolated from fresh milk, and displayed the hallmark features of adhesion, showed good survivability at low pH values, and was tolerant to high bile concentrations. Moreover, it showed anti-microbial activity against gram-negative bacteria, such as *Escherichia coli* and *Salmonella* spp. (Xiao et al. 2011). Recent research suggests that co-dietary LB and TP feed can improve serum biochemical parameters and digestive enzyme activities in broiler chickens (Li et al. 2015). Thus, the effects of TPs and *L. brevis* M8 on growth performance, meat quality and intestinal microbiota in yellow-feathered broilers were investigated in the current study.

**Materials and methods**

**Diet preparations**

The *L. brevis* strain M8 (LB) carrying surface layer proteins was selected from fresh milk and collected in the China General Microbiological Culture Collection Center (Beijing, China; accession number: CGMCC 30022). LB, after being grown in de Man, Rogosa and Sharpe (MRS) broth (Difco, Detroit, MI), was cultivated at 37°C until the viable count which was determined with the dilution flat plate counting method exceeded $1.0 \times 10^{10}$ cfu mL⁻¹. Then sucked the bacterial cell suspension was transferred to a sterile centrifuge tube, centrifuged at 4000×g, for 5 min, resuspended with an appropriate amount of normal saline, adjusted to a concentration of $1.0 \times 10^{6}$ cfu mL⁻¹, $1.0 \times 10^{8}$ cfu mL⁻¹ or $1.0 \times 10^{10}$ cfu mL⁻¹, and stored at 4°C prior to oral gavage. Normal saline was used as a control.

TP (≥98%) was purchased from Xuhuang Biological Technology Co., Ltd. (Xi’an, China) and dissolved in distilled water. Contents of 1000 mg of TP included 69.8 mg of caffeine, 495 mg of (-)-EGCG, 112 mg of (-)-epicatechin gallate, 100 mg of (-)-epicatechin, 78 mg of (-)-epigallocatechin and 96 mg of (-)-gallocatechin gallate according to high-performance liquid chromatography-ultraviolet (HPLC-UV) analysis (Wang et al. 2011). Specific amount of TP was dissolved in sterile water immediately prior to oral gavage.

**Experimental birds**

The experimental protocols (registration number: 201205061) used in this study were approved by the Hunan Agricultural University Animal Care and Use Committee (Changsha, China). A total of 480 one-day-old yellow-feathered broiler chicks (weighing 41.70 ± 0.35 g) were purchased from the Hunan Animal Husbandry and Veterinary Research Institute (Changsha, China). Broiler chicks were assigned randomly to eight groups, with six replicate cages with 10 birds per cage in a completely randomised design. All birds were raised in wire-floored cages in an environmentally controlled room with continuous light and had access to feed and water *ad libitum*. Chicks had a continuous light regimen during the entire experimental period. Temperature at day 0 was 33°C and decreased 5°C every week until a temperature of 23°C was reached.

**Diets and experimental design**

Birds received a basal diet (Table 1). Diets were prepared according to the Nutrient Requirement of Poultry (NRC 1994). All diets were formulated in mash form without antibiotics or coccidiostats. Treatments were as follows: the negative control group (CTL); $1.0 \times 10^{6}$ cfu kg⁻¹ BW LB group (LB); 0.06 g kg⁻¹ BW TP group (TP); and $1.0 \times 10^{8}$ cfu kg⁻¹ BW LB +0.06 g kg⁻¹ BW TP group (MLB) were assigned to Exp. 1 to investigate the effects of moderate dosages of LB or/and TP. In Exp. 2, the CTL, $1.0 \times 10^{6}$ cfu kg⁻¹ BW LB +0.06 g kg⁻¹ BW TP group (LLB), MLB group, and $1.0 \times 10^{10}$ cfu kg⁻¹ BW LB +0.06 g kg⁻¹ BW TP group (HLB) were selected to
investigate the effects of different dosages of LB combined with 0.06 g kg\(^{-1}\) BW TP, and Exp. 3, containing the groups of CTL, LTP, MLB and HTP, was designed to investigate the effects of different dosages of TP combined with 1.0 \(\times\) \(10^8\) cfu kg\(^{-1}\) BW LB. The experiments lasted for 56 days.

**Growth performance parameters**

Chickens were weighed, and feed consumption was measured individually on days 1, 7, 14, 21, 28, 35, 42, 49 and 56 to determine BW. The feed weight ratio was calculated to determine the feed conversion by the following formula: 

\[
R = \frac{\text{consumed feed weight (kg)}}{\text{body weight gain (kg)}}.
\]

**Meat quality properties**

Drip loss of broiler meat was calculated using the following formula: 

\[
\text{Drip loss} = \left(\frac{\text{initial weight of fresh chicken meat – weight of refrigerated fresh chicken meat at 56 d}}{\text{initial weight of fresh chicken meat}}\right) \times 100.
\]

The content of free amino acids in chicken breast muscle was analysed with an automatic amino acid analyser (L-8900, Hitachi, Tokyo, Japan). We put 20 mg of chicken breast meat homogenate samples (accurate to 0.1 mg) in a tube for hydrolysis using 6 mol L\(^{-1}\) HCl and phenol. Then the tube was filled with nitrogen, hydrolysed at 110°C for 24 h and cooled before sample injection.

**Sampling and sample processing procedures**

We dissected 10 cm segments of the upper part of the ileocecal junction. The contents of the ileum from three broilers were aseptically collected and pooled. Then approximately 200 mg of faeces was collected in a 2 mL sterile tube. The ileocecal contents were immediately frozen at \(-80\) °C until use. The frozen samples were kept at 4°C for 12 h before isolating genomic DNA.

**Genomic DNA extraction**

Genomic bacterial DNA was extracted using a commercial kit (QIAamp DNA Stool Mini Kit, Hilden, Germany). The extraction was carried out according to the manufacturer's instructions. The kit has proven to be an efficient method for extracting DNA out of intestinal content samples (Xu et al. 2011). The purity and concentration of DNA were determined by a UV-2600/2700 ultraviolet and visible spectrophotometer (Shimadzu, Kyoto, Japan). The ratio of the absorbance at 260 and 280 nm was calculated to be 1.82 ± 0.04. The ratios indicated a low level of protein contamination. All DNA samples were stored at \(-20\) °C for later analysis.

**PCR amplification and perpendicular DGGE analysis**

The V3 region of the 16S rRNA gene (positions 339–524 in the *E. coli* gene) was amplified by primers: GC341F (5’-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGCAGCAG-3’), and 517R (5’-GTGCCAGC(A/C)GCCGCGG-3’). The PCR reaction mixtures were prepared according to a previous study (Wang, Xu, et al. 2015). DGGE was performed using a Dcode Mutation Detection System (Bio-Rad Laboratories Inc., Hercules, CA) as described by the manufacturer. The PCR amplicons were electrophoresed in 8% (wt/vol) polyacrylamide gels (acrylamide: bisacrylamide: acrylamide was 37.5:1) with a 40–70% gradient of denaturant, which increased in the direction of electrophoresis (100% corresponding to 7 M urea and 40% (w v\(^{-1}\)) deionised formamide). Bacterial PCR products (V3 regions of 16S rRNA) were loaded in each lane, and electrophoresis was performed in 1 × TAE buffer at 60 °C under 65 V for 15 h. Gels were stained with ethidium bromide and viewed by a UV image analysis system (Bio-Rad Laboratories Inc., Hercules, CA).

### Table 1. Ingredient and nutrient composition of the basic diet.

| Item | Basal diet |
|------|------------|
| Ingredient |            |
| Bean pulp, % | 39.69 |
| Corn, % | 48.80 |
| Fish meal, % | 2.00 |
| Cottonseed meal, % | 3.00 |
| Corn gluten meal, % | 1.80 |
| Calcium hydrophosphate, % | 1.55 |
| Soya-bean oil, % | 1.00 |
| Salt, % | 0.22 |
| Methionine, % | 0.16 |
| Vitamins, % | 0.03 |
| Premix, % | 0.65 |
| Mountain flour, % | 1.10 |
| Analysed nutrient content |        |
| Metabolic energy, ME, Mcal/kg | 12.55 |
| Crude protein, % | 18.0 |
| Crude ash, % | 7.0 |
| Crude fibre, % | 5.0 |
| Calcium, % | 1.2 |
| Total phosphorus, % | 0.55 |
| Methionine, % | 0.45 |

*The premix provided the following per kg of diet: retinol acetic acid ester, 2.90 mg; folic acid, 1.96 mg; pantothenic acid, 15.71 mg; riboflavin, 8.1 mg; nicotinic acid, 39.9 mg; biotin, 0.33 mg; copper, 10.1 mg; iron, 72.5 mg; zinc, 89.9 mg; manganese, 101 mg; selenium, 0.45 mg.*
The DGGE fingerprint map was generated by the unweighed pair group method with arithmetic mean (UPGMA) analysis using NTSYSpc analysis software (version 2.1, Setauket, NY). The Shannon–Wiener index ($H_0$) was calculated by the following formula:

$$H_0 = - \sum P_i \ln P_i$$

where $P_i$ is defined as the proportion of each band (or species) to the total bands (or species) in the sample (Liu et al. 2012).

**Identification of bacteria by cloning and sequencing**

Representative bands were excised from DGGE gels and placed into 1.5 mL micro-centrifuge tubes and stored at $-20^\circ$C before being immersed in diffusing buffer. DNA was recovered from the solution using a QIAEX II Gel Extraction Kit (Qiagen, Shanghai, China). DNA sequencing was accomplished by a commercial facility (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd., Shanghai, China), and prokaryotic ribosomal RNA gene sequences were obtained from the GenBank database (www.ncbi.nlm.nih.gov/BLAST). The detailed cloning and sequencing protocols used were the same as those described by Xu et al. (2011).

**Statistical analysis**

The data are expressed as means ± standard deviation (SD). After being tested for normal distribution with the Kolmogorov–Smirnov test, experimental values of growth performance muscle quality were analysed by Duncan’s multiple range comparison test between any two groups. Comparisons were performed using SPSS statistical software, version 17.0 (SPSS Inc., Chicago, IL).

### Results

#### Body weights (BW) of broilers

The effects of LB and TP on the BW of broilers during days 28–56 are shown in Table 2. In Exp. 1, BW of broilers in the LB, TP and MLB groups at 28, 35, 42 and 49 days, especially at 56 days ($p < .05$), were lower than in the control group. The BW gain was remarkably lower in the MLB group than in the LB and TP groups at 35 days ($p < .05$). In Exp. 2, the BW gain of broilers in the LLB group was higher than in the MLB and HLB groups at 28 ($p < .05$), 35 ($p < .05$) and 42 days, but lower at 49 and 56 days. In Exp. 3, the BW gain of broilers in the MTP group was the lowest at 28 ($p < .05$), 35 ($p < .05$), 42 ($p < .05$) and 56 ($p < .05$) days.

#### The feed-weight ratio

The effects of LB and TP on feed-weight ratios of broilers were significantly different in the eight groups during days 14–56 (Table 3). In Exp. 1, the ratios of broilers in the control group were significantly lower than in the other groups at 14, 21 ($p < .05$), 28, 35, 42 ($p < .05$), 49 and 56 days. The ratio of day 14 broilers in the MLB group was lower than in the LB group but higher than the TP group ($p < .05$). The ratio in the MLB group was higher than in the LB and TP groups at 35 and 49 days ($p < .05$). In Exp. 2, the feed-weight ratio in the MLB group was lower than in the LLB and HLB groups at 21, 28, 49 and 56 days.

### Table 2. Effects of *Lactobacillus brevis* M8 (LB) and tea polyphenol (TP) on the body weight (BW) of broilers ($x \pm SD$).

| Group | Days | Exp. 1 | Exp. 2 | Exp. 3 |
|-------|------|--------|--------|--------|
|       | 28   | 35     | 42     | 49     | 56     |
| CTL   | 444.77 ± 43.30bc | 780.62 ± 142.99c | 988.63 ± 156.83cd | 1284.36 ± 161.02b | 1801.68 ± 227.90c |
| LB    | 359.43 ± 44.37ab | 731.79 ± 170.22bc | 910.30 ± 232.15c | 1093.18 ± 316.23a | 1703.66 ± 189.65b |
| TP    | 426.07 ± 63.65b  | 688.54 ± 146.32b  | 809.25 ± 103.97b  | 1211.41 ± 166.16b | 1609.48 ± 214.67a |
| MLB   | 327.69 ± 42.46a  | 575.95 ± 86.79a   | 737.17 ± 83.00a   | 1094.19 ± 104.00a | 1597.77 ± 200.57a |
|       | Exp. 2 |        |        |        |        |
| CTL   | 444.77 ± 43.30c  | 780.62 ± 142.99c  | 988.63 ± 156.83c  | 1284.36 ± 161.02c | 1801.68 ± 227.90c |
| LLB   | 436.55 ± 70.41c  | 695.55 ± 114.70ab | 799.92 ± 66.93ab  | 1048.54 ± 103.75a | 1439.77 ± 289.18a |
| MLB   | 327.69 ± 42.46a  | 518.85 ± 108.30ab | 737.17 ± 83.00a   | 1094.19 ± 104.00ab| 1597.77 ± 200.57b |
| HLB   | 399.73 ± 39.31b  | 575.95 ± 86.79a   | 737.17 ± 83.00a   | 1094.19 ± 104.00ab| 1597.77 ± 200.57b |
|       | Exp. 3 |        |        |        |        |
| CTL   | 444.77 ± 43.30c  | 780.62 ± 142.99c  | 988.63 ± 156.83c  | 1284.36 ± 161.02c | 1801.68 ± 227.90c |
| LTP   | 427.08 ± 40.75b  | 714.72 ± 100.17b  | 917.75 ± 98.62b   | 1150.47 ± 170.30ab| 1842.31 ± 373.52b |
| MLB   | 327.69 ± 42.46a  | 575.95 ± 86.79a   | 737.17 ± 83.00a   | 1094.19 ± 104.00a | 1597.77 ± 200.57a |
| HTP   | 461.80 ± 42.12bc | 686.48 ± 84.06b   | 928.31 ± 88.84b   | 1277.82 ± 101.18c | 2051.64 ± 509.97d |

a,b,c,dDifferent letters indicate significant differences among diets within Exp. ($p < .05$).
days ($p < .05$), but significantly higher than in the LLB and HLB groups at 14, 35 and 42 days ($p < .05$). In Exp. 3, the ratio of broilers in the MTP group was higher than in the LTP and HLP groups at 14 ($p < .05$), 21 and 56 days ($p < .05$), but lower than the two groups at 28 ($p < .05$), 42 and 49 days in a dosage independent manner.

**Drip loss of cold fresh chicken**

The results of drip loss evaluation of cold fresh chicken are shown in Table 4. Broilers fed a diet with LB ($p < .05$), TP ($p < .05$) or LB combined with TP had a lower drip loss compared with the control group. The drip loss values were lower in the LLB and HLB groups than in the MLB group ($p < .05$), as well as lower in the LTP and HLP groups ($p < .05$).

**Amino acid content**

The results of amino acid content of broiler chicken meat are shown in Table 5. In Exp. 1, middle dosages of LB and TP increased the content of essential and non-essential amino acids. The contents of lysine (Lys), leucine (Leu) and aspartic acid (Asp) were significantly higher in the MLB group compared with LB ($p < .05$), while glutamic acid (Glu) and arginine (Arg) contents were significantly higher than in both the LB and TP groups ($p < .05$). The contents of isoleucine (Ile) and threonine (Thr) were also higher in the MLB group than in the LB or TP groups, but not significantly. In Exp. 2, the Lys, Ile, Asp and Arg contents in the MLB group ($p < .05$) were significantly higher than in the LLB or HLB groups. The different dosages of LB combined with the middle dosages of TP improved the content of Glu remarkably, with a significant difference among the MLB, LLB and HLB groups ($p < .05$). In Exp. 3, the contents of Leu and Asp in broiler chicken meat in the HTP group were appreciably higher than those in the MTP or LTP groups; however, the Glu content was significantly higher than in the LTP group only ($p < .05$). No significant differences among the groups were observed in the other types of amino acids.

**Intestinal microbiota**

The primary bacterial fingerprints of the control group and the LB and TP diet groups are shown in Figure 1. A clear difference of the diet on gut microbiota of broilers at day 56 was found. The bands of the seven experimental groups were significantly richer than that of the control group (Figure 1(A) and Table 6), which showed that the bacterial diversity and richness were affected by supplementing with LB and/or TP.
In Exp. 1, the number of bands and the Shannon–Wiener index were highest by feeding both middle doses of LB and TP among the four groups (Table 6). Otherwise, some individual bands were unique to the LB group (Figure 1(A)). Sequencing and BLAST analysis of bands p, q and r against GenBank data revealed the closest relatives as *Lactobacillus reuteri*, uncultured *Bacteroides* sp. and uncultured bacteria, respectively, which had a 100% similarity rate (Table 7). The bands of the LB group did not match those of the control group according to UPGMA analysis (in Exp. 1 shown in Figure 2).

All of the faecal samples from both Exp. 2 and Exp. 3 had a similar number of bands and Shannon–Wiener indexes, though the number of bands and the index value of the MLB group were slightly higher than the four other compound groups (Table 6). In addition, according to UPGMA analysis, the bands of the MLB group did not match those of the LB and TP groups or of the four other compound groups (Figure 2). This finding suggested that the bacterial composition and richness were markedly affected by MLB, but not significantly affected by the dosage of LB combined with TP.

Additionally, the main bands from 1 to x, except for bands p and q, appeared in combination groups (i.e. LLB, MLB, HLB, LTP and HTP), which were all derived from uncultured bacteria with a high similarity, above 98% (Figure 1 and Table 6). However, the optical density (OD) values of the domain bands were higher in groups LLB, MLB, HLB, LTP and HTP than in groups LB and TP (Figure 1(A)), revealing that the major bacterial populations in the gut were increased by supplementation with LB and TP together rather than separately.

A new band (d) appeared in the TP, MLB, HLB, LTP and HTP groups simultaneously, but not in the LB and LLB groups, indicating that the middle dose of TP separately or in combination with a low dose LB may not increase the diversity of *Pseudomonas veronii* in the gut. Furthermore, a clear fingerprint of *Lactobacillus crispatus* (band h, with a 99% similarity) was apparent in the seven groups (Figure 1(A)). In the control group, the quantity of *L. crispatus* was present at low levels, with almost no clearly visible band; however, the intensity of the

### Table 5. Effects of *Lactobacillus brevis* M8 (LB) and tea polyphenols (TP) on amino acid content of broiler chicken meat within 56 days (x ± SD).

| Groups | Lys (mg/g) | Val (mg/g) | Met (mg/g) | Ile (mg/g) | Leu (mg/g) | Phe (mg/g) | Thr (mg/g) |
|--------|------------|------------|------------|------------|------------|------------|------------|
| Exp. 1 |            |            |            |            |            |            |            |
| CTL    | 18.3 ± 0.37b | 9.1 ± 0.010 | 9.6 ± 0.018 | 8.9 ± 0.25a | 16.9 ± 0.041b | 2.2 ± 0.006 | 9.6 ± 0.27a |
| LB     | 17.3 ± 0.68a | 8.7 ± 0.027 | 9.3 ± 0.08 | 8.5 ± 0.24a | 16.1 ± 0.054a | 2.2 ± 0.02 | 9.1 ± 0.38b |
| TP     | 18.1 ± 0.40ab | 9.2 ± 0.039 | 9.5 ± 0.01 | 8.9 ± 0.33a | 16.7 ± 0.040a | 2.3 ± 0.01 | 9.4 ± 0.33a |
| MLB    | 18.9 ± 0.66b | 9.3 ± 0.48 | 9.8 ± 0.17 | 9.2 ± 0.26ab | 17.5 ± 0.063b | 2.3 ± 0.08 | 9.9 ± 0.29abc |
| Exp. 2 |            |            |            |            |            |            |            |
| CTL    | 18.3 ± 0.37a | 9.1 ± 0.010 | 9.6 ± 0.02 | 8.9 ± 0.25 | 16.9 ± 0.04b | 2.2 ± 0.01 | 9.6 ± 0.27 |
| LLB    | 17.9 ± 1.06b | 8.9 ± 0.66 | 9.3 ± 0.40 | 8.7 ± 0.54 | 16.5 ± 1.00b | 2.3 ± 0.16 | 9.3 ± 0.54 |
| MLB    | 18.9 ± 0.07b | 9.3 ± 0.48 | 9.8 ± 0.17 | 9.2 ± 0.2 | 17.5 ± 0.063b | 2.3 ± 0.08 | 9.9 ± 0.29 |
| HLB    | 18.1 ± 0.01a | 8.8 ± 0.10 | 9.5 ± 0.02 | 8.7 ± 0.05 | 16.6 ± 0.08a | 2.2 ± 0.05 | 9.4 ± 0.11 |
| Exp. 3 |            |            |            |            |            |            |            |
| CTL    | 18.3 ± 0.37 | 9.1 ± 0.01 | 9.6 ± 0.02 | 8.9 ± 0.25 | 16.9 ± 0.04a | 2.2 ± 0.01 | 9.6 ± 0.27 |
| LTP    | 18.6 ± 0.01 | 9.3 ± 0.14 | 9.6 ± 0.04 | 9.1 ± 0.07 | 17.2 ± 0.04a | 2.2 ± 0.05 | 9.7 ± 0.04 |
| MLB    | 19.9 ± 0.65 | 11.6 ± 0.59 | 8.3 ± 0.37 | 8.6 ± 0.13 | 34.4 ± 0.87a | 6.1 ± 0.19 | 6.8 ± 1.05 |
| HLB    | 19.0 ± 0.14b | 11.0 ± 0.16 | 8.3 ± 0.68 | 8.3 ± 0.13 | 32.9 ± 0.55b | 6.0 ± 0.25 | 6.4 ± 1.27 |

| Asp | Ala | Gly | Ser | Glu | Pro | His |
|-----|-----|-----|-----|-----|-----|-----|
| Exp. 1 | 19.4 ± 0.34b | 11.3 ± 0.31 | 8.2 ± 0.17 | 8.4 ± 0.18 | 33.1 ± 0.96b | 6.0 ± 0.06 | 6.9 ± 0.01 |
| LB | 18.4 ± 0.68a | 11.0 ± 0.43 | 8.5 ± 0.04 | 8.0 ± 0.30 | 31.6 ± 1.71a | 5.9 ± 0.04 | 6.3 ± 0.52 |
| TP | 19.2 ± 0.73b | 11.3 ± 0.53 | 8.5 ± 0.55 | 8.3 ± 0.47 | 32.6 ± 1.47b | 6.0 ± 0.07 | 6.5 ± 0.16 |
| MLB | 19.9 ± 0.65b | 11.6 ± 0.59 | 8.3 ± 0.37 | 8.6 ± 0.13 | 34.4 ± 0.87a | 6.1 ± 0.19 | 6.8 ± 1.05 |
| Exp. 2 | 19.4 ± 0.34a | 11.3 ± 0.31 | 8.2 ± 0.17 | 8.4 ± 0.18 | 33.1 ± 0.96b | 6.0 ± 0.06 | 6.9 ± 0.01 |
| LTP | 18.9 ± 1.21a | 11.2 ± 0.75 | 8.2 ± 0.56 | 8.1 ± 0.48 | 31.9 ± 1.75a | 5.8 ± 0.36 | 7.1 ± 0.95 |
| MLB | 19.9 ± 0.65b | 11.6 ± 0.59 | 8.3 ± 0.37 | 8.6 ± 0.13 | 34.4 ± 0.87a | 6.1 ± 0.19 | 6.8 ± 1.05 |
| HLB | 19.0 ± 0.14a | 11.0 ± 0.16 | 8.3 ± 0.68 | 8.3 ± 0.13 | 32.9 ± 0.55b | 6.0 ± 0.25 | 6.4 ± 1.27 |
| Exp. 3 | 19.4 ± 0.34b | 11.3 ± 0.31 | 8.2 ± 0.17 | 8.4 ± 0.18 | 33.1 ± 0.96b | 6.0 ± 0.06 | 6.9 ± 0.01 |
| LTP | 19.8 ± 0.02a | 11.6 ± 0.01 | 8.6 ± 0.36 | 8.4 ± 0.10 | 33.7 ± 0.02a | 6.0 ± 0.13 | 7.1 ± 0.43 |
| MLB | 19.9 ± 0.65a | 11.6 ± 0.59 | 8.3 ± 0.37 | 8.6 ± 0.13 | 34.4 ± 0.87b | 6.1 ± 0.19 | 6.8 ± 1.05 |
| HTP | 20.3 ± 0.16ab | 12.0 ± 0.25 | 8.6 ± 0.11 | 8.9 ± 0.04 | 35.5 ± 0.24b | 6.1 ± 0.08 | 7.4 ± 0.27 |

a,b,c Different letters indicate significant differences among diets within Exp. (p < .05).
uncultured bacteria represented by band k in the control group was remarkably superior to that of the seven other experimental groups. These results suggest that dietary supplementation with LB or TP may have multiple effects on gut bacterial diversity.

Discussion

Growth performance

The Chinese yellow-feathered broiler chicken is known for being physically fit and providing good meat quality. Prebiotics, probiotics and nutrients are used as dietary supplements to improve their growth performance, intestinal microbiota, as well as immune response (Yeoman et al. 2012; Pan and Yu 2014).

We found that feeding with a middle dose of LB and TP separately or together can reduce the BW gain (within 28–56 days) and feed conversion (within 14–56 days) (Tables 2 and 3), which can affect the performance of the broilers. Zhang et al. (2016) reported that the ratio of total dry matter intake to average BW, which is negatively correlated with the feed conversion rate, was higher with *L. plantarum* and *Bacillus subtilis* treatment compared with the control in calves. However, some other studies have shown *Lactobacillus* to have the opposite result. For example, the supplementation of 0.25% yeast with bacteriocin and *Lactobacillus* cultures resulted in a significantly better feed conversion rate than that of the control group during days 1–21 for broilers (Chen, Chen, et al. 2016), and supplementing with 11 *Lactobacillus* strains alone or in combination with isomalto-oligosaccharides enhances the performance of broiler chickens (Mookiah et al. 2014). Certainly, green tea extracts that primarily contain polysaccharides, polyphenols and caffeine have protective effects against obesity (Chen, Liu, et al. 2016). These findings show that feeding with LB and TP can potentially promote weight loss, which suggests that improved broiler growth performance is related to species differences.

TPs can suppress the formation and accumulation of fat and thus promote its decomposition to prevent obesity by lowering the concentration of serum total cholesterol, triglycerides and low density lipoprotein cholesterol, and by increasing high-density...
lipoprotein cholesterol (Li et al. 2015; Wu et al. 2016). *Lactobacillus* spp. as the dominant genus can regulate the dynamic equilibrium of intestinal flora for maintaining the host health, and are involved in the digestion of complex carbohydrates not digested by the host and in the degradation of lipids and simple sugars (Drissi et al. 2016). A previous study revealed that supplementing with TP or LB results in lower lipoprotein cholesterol (Li et al. 2015; Wu et al. 2016). The growth performance of chickens, such as chest muscle rate, daily gain, feed conversion rate and disease resistance, has been improved greatly by modern farming techniques (Havenstein et al. 2003). However, the pH value and water holding capacity appear to have been negatively affected and the flavour, storage and processing properties of meat have also decreased with the high productivity of broiler chickens (Rance et al. 2002). Drip loss reduces the nutritional value of meat, its flavour and its tenderness (Barbut 2009), and the pH value is related to the drip loss (Pelicano et al. 2003). Therefore, attempts to improve the physico-chemical, sensory and nutritional characteristics of chicken have gained wide attention. There was no significant difference in pH values, which were maintained between 6.31 and 6.74 in the eight groups (data not shown). Table 4 shows the results of drip loss of chest muscles. As expected, the LB and TP dietary supplements individually or synergistically decreased drip loss, except for the middle dose of the LB culture and TP suspension diets (in the MLB group of Exp. 1). This result shows that feed with LB and TP improves the water holding capacity of broiler chicken meat, and there is a synergistic effect between the two oral diets.

Feed with LB and TP increased the content of a variety of amino acids, including free Lys, Leu, Asp, Glu, Arg, Ile and Thr (Exp. 1 as shown in Table 5). Different doses of TP combined with the middle dosage of LB effectively elevated the content of free Lys, Ile, Asp and Arg without a significant dose-dependent relationship. Statistical analysis indicated that the contents of Phe and Thr did not differ among the groups. These observations suggest that LB and TP diets may increase some of the essential and delicate flavour produced by amino acids and could improve the nutrition and taste of meat. It is notable that feeding with

### Table 6. Total number of bands and diversity index of gut microbiota of the ileum content from yellow-feathered broilers at 56 days detected by DGGE.

| Item | Groups of Exp. 1 | Groups of Exp. 2 | Groups of Exp. 3 |
|------|-----------------|-----------------|-----------------|
| Number of bands | CTL | LB | TP | MLB |
| CTL | 19 | 23 | 20 | 26 | 19 | 22 | 26 | 24 | 19 | 24 | 26 | 22 |
| Shannon-Wiener index (H') | 2.88 | 3.05 | 2.94 | 3.16 | 2.88 | 3.01 | 3.16 | 3.07 | 2.88 | 3.09 | 3.16 | 3.03 |

### Table 7. The 16S ribosomal RNA gene sequences of the major DNA bands from the ileum content of yellow-feathered broilers at 56 days by DGGE and sequencing analysis.

| Band number | Closest sequence relative | Accession number in NCBI | Sequence similarity, % |
|-------------|---------------------------|--------------------------|------------------------|
| a | Uncultured bacterium JN021908.1 | 100 |
| b | Uncultured bacterium EU844327.1 | 95 |
| c | Uncultured bacterium FJ511840.1 | 100 |
| d | Uncultured bacterium HQ824944.1 | 100 |
| e | Uncultured bacterium JN021908.1 | 99 |
| f | Uncultured bacterium HQ792289.1 | 99 |
| g | Uncultured bacterium HQ309038.1 | 99 |
| h | *Lactobacillus crispatus* KF684066.1 | 99 |
| i | Uncultured bacterium GU006036.1 | 100 |
| j | Uncultured bacterium EU776793.1 | 99 |
| k | Uncultured bacterium JF259598.1 | 99 |
| l | Uncultured bacterium JQ248088.1 | 100 |
| m | Uncultured bacterium HG326752.1 | 99 |
| n | Uncultured bacterium JX851710.1 | 100 |
| o | Uncultured bacterium HG326752.1 | 98 |
| p | *Lactobacillus reuteri* KF207601.1 | 100 |
| q | Uncultured Bacteroides sp. AY597130.1 | 100 |
| r | Uncultured bacterium KC304719.1 | 100 |
| s | Uncultured bacterium HQ785539.1 | 98 |
| t | Uncultured bacterium JX096236.1 | 100 |
| u | Uncultured bacterium JX851710.1 | 100 |
| v | Uncultured bacterium AM229600.1 | 98 |
| w | Uncultured bacterium JN021918.1 | 100 |
| x | Uncultured bacterium FJ508900.1 | 100 |
| y | Uncultured bacterium JN021908.1 | 100 |
| z | Uncultured bacterium HG326752.1 | 99 |
| \(0\) | Uncultured bacterium JX851710.1 | 100 |
| \(1\) | Uncultured bacterium HG326752.1 | 99 |
| \(2\) | Uncultured bacterium JX851710.1 | 100 |
| \(3\) | Uncultured bacterium HG326752.1 | 99 |
| \(4\) | Uncultured bacterium JX851710.1 | 100 |
| \(5\) | Uncultured bacterium HG326752.1 | 99 |
| \(6\) | Uncultured bacterium JX851710.1 | 100 |
| \(7\) | Uncultured bacterium HG326752.1 | 99 |
| \(8\) | Uncultured bacterium JX851710.1 | 100 |
| \(9\) | Uncultured bacterium HG326752.1 | 99 |

*^Determined by sequence comparison using Basic Local Alignment Search Tool (BLAST) analysis.*

*Band numbers correspond to those in Figure 1.*

*NCBI: National Center for Biotechnology Information.*
the middle dose of TP alone rather than combining it with LB could enhance the tenderness of meat because of the lower drip loss, suggesting that TP may degrade the myofibrillar protein myosin in accordance with research by Zhao et al. (2013). The composition of feed and supplements directly determines the quality of the chicken. Feeding with TP can improve meat quality in many respects, including pH value and amino acid composition (Zhong et al. 2015), which benefit from its antioxidant activity.

**Intestinal microbiota**

Dietary probiotic *Lactobacillus* spp. supplements influence growth performance, meat quality and excreta microflora in broilers. LB is possibly involved in turning complex carbohydrates into the metabolites lactose, acetic acid, ethanol, short-chain fatty acids and inhibine, which could improve nutrient digestion and meat quality in broilers. Variable microbiota inhabiting the gastrointestinal (GI) tract of broiler chickens plays an essential role in pathogen exclusion, nutrient digestion and absorption, and immune system development for maintaining conditioned microbiota and a healthy host (Yeoman et al. 2012). Previous studies have demonstrated that various factors related to age (Zhu et al. 2002), diet (Pan and Yu 2014) and feed additives, such as vitamins (Augustin et al. 2008), amino acids (Hayat et al. 2015), prebiotics (Kim et al. 2011) and probiotics (Jeong and Kim 2014; Chen, Chen, et al. 2016) can impact the GI microbiota of chickens with respect to diversity and composition. The effect of probiotics,

![Figure 2. Cluster analysis using the unweighed pair group method with arithmetic mean (UPGMA) method based on the Dice coefficient for the band pattern in Figure 1. The markers CTL refer to the control group.](image-url)
including *Lactobacillus* spp., *B. subtilis* and yeast, on poultry growth performance has previously been studied for either single strains or multiple strains; however, dietary supplementation influences GI tract performance directly or indirectly via the alteration of microbiota (Nakphaichit et al. 2011).

In the current study, the birds in all seven treatment groups had similar bacteria categories but significant differences in gut richness (Figure 1). Interestingly, feeding with the middle dosage of LB culture generated unique bands, that were due to *L. reuteri*, uncultured *Bacteroides* sp. and uncultured bacteria, respectively (Table 7). Nevertheless, *L. crispatus* (band h, with a 99% similarity) was apparent in the seven dietary supplementation groups compared with the control diet (Figure 1). *L. reuteri* KUB-AC5, derived from chicken intestine, regulated ileum microbiota in the growing stage, so as to enrich potentially beneficial Lactobacilli and suppress Proteobacteria (Nakphaichit et al. 2011). The increased species and proportion of probiotics leads to a rise in digestive enzyme activity, which promotes catabolism and weight loss. Genus *Bacteroides* sp. was unique to the five compound groups (band q in Figure 1(A)), and a previous study indicated that the gut commensal *Bacteroides acidifaciens* is able to prevent obesity in mice (Yang et al. 2016), which may be the reason why body weight gain was decreased after supplementation with both LB and TP in diets of broilers.

The results suggested that the different feeds in our study gave rise to new dominant species (i.e. *L. reuteri*, uncultured *Bacteroides* sp. and uncultured bacteria) outside of the typical *L. crispatus*. The richness of dominant microbacteria, which were derived from uncultured bacteria with a high similarity of over 98%, may strengthen the broilers that were supplemented with both the middle dose of LB and TP for 56 days (Figure 1 and Table 7). In addition, the results were encouraging in that chicken intestinal pathogenic bacteria such as *E. coli* and *Salmonella* spp. were not found.

**Conclusions**

Dietary supplementation with 1.0 × 10^8 cfu kg^-1.8W LB and 0.06 g kg^-1.8W TP respectively or in combination can improve meat quality and increase intestinal levels of beneficial microbiota, but not promote growth performance, of Chinese yellow-feathered broilers.

**Disclosure statement**

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