RESEARCH ARTICLE

# Composition and function of intestinal microorganisms in different intestinal sections of Liangfenghua chickens of China based on high-throughput sequencing technology

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**Liangfenghua chicken is a meat variety selected by hybridization of local yellow feather chickens in China and is the first new poultry variety approved by Chinese government. However, there have been no relevant reports on the structure of intestinal microbial bacteria in Liangfenghua chicken. The purpose of this research was to study the composition and function of intestinal microflora in different parts of Liangfenghua chicken by 16SrDNA high-throughput sequencing technology. The results showed that the flora of the cecum was all clustered together, and the flora between the ileum, jejunum, and duodenum were crossed. The cecal samples clustered more concentratedly, and the microbial composition of the samples within the group was more uniform. At the phylum level, microorganisms in the four intestinal sections involved a total of 19 phyla, mainly *Firmicutes* and *Bacteroidetes*. The relative abundance of *Firmicutes* in duodenum, jejunum, and ileum was more than 84%, which was significantly higher than that in cecum (41.05%), while the relative abundance of *Bacteroidetes*, synergized and unclassified in duodenum, jejunum, and ileum was close to 0%, which was significantly lower than that in cecum ($P < 0.05$). At the genus level, the microorganisms in the four intestinal sections involved 229 genera, 40 genera with high relative abundance, of which 20 had significant differences between groups. The relative abundance of *Lactobacillus* in duodenum (group D), ileum (group I) and jejunum (group J) was more than 80%, which was significantly higher than that in cecum (group C) (3.8%). The relative abundance of *Bacillus* in group I was significantly higher than that in groups J and C ($P < 0.05$), which was also higher than that in group D, but without significant difference. The relative abundance of 18 genera including *Bacteroides*, unclassified, *Alistipes*, and *Barnesiella* was significantly higher in group C than in groups D, I, and J ($P < 0.05$). There were significant differences in microbial composition in different intestinal sections of Liangfenghua chicken, and the microbial diversity in cecum was higher than that in duodenum, jejunum, and ileum. The dominant bacteria in the upper intestine were *Firmicutes* and *Lactobacillus*, and the relative abundance of *Bacillus jejuni* was higher than that in other sections. The dominant bacteria in cecum were *Firmicutes* and *Bacteroides*, and the dominant bacteria were rumen cocci, *Clostridium*, *Bacteroides*, which had digestive function, produce short chain fatty acids, and were conducive to the immune system. This study laid a foundation for the feed formulation and feeding management of Liangfenghua chicken in the replacement period.**

**Keywords:** chicken; intestinal microorganisms; high-throughput sequencing technology.

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## Introduction

The poultry industry is an important animal husbandry industry for the production of human-edible meat and eggs. At present, most chickens are produced by large-scale and intensive large commercial enterprises. Liangfenghua chicken is a meat variety selected by hybridization of local yellow feather chickens in China and is the first new poultry variety approved by Chinese government. Liangfenghua chicken combines the fast growth characteristics of white broiler chickens with the delicate meat quality. Liangfenghua chicken also demonstrates cold tolerance, stress resistance, and disease resistance, which fully embodies the characteristics of landraces and is an ideal model for the study of high-quality meat varieties.

The effects of dietary nutrition, host genetic background, environment, *etc*. on the intestinal microbial communities' structure are different among chicken individuals. The differences in host genotypes can lead to the difference of gastrointestinal bacteria in animals, and the gastrointestinal tract of each animal body contains its own unique set of microbial bacteria [1]. Host genotype has a great influence on the composition and diversity of animal gastrointestinal flora [2]. Due to the lack of statistics of individual number and time point number, the temporal and spatial succession law of chicken intestinal microflora has not been fully explained [3]. At present, the gut metagenomes of seven chicken breeds including Hy-Line Variety Brown, Cobb 500, Ross 308, Arbor Acres broiler, Local yellow-feather chickens, yellow dwarf chicken, and Guangxi local chicken have been reported in China [4]. However, there is no relevant report on the structure of intestinal microbial bacteria in Liangfenghua chicken. Liangfenghua chicken entered the replacement period from 60 days old, and the replacement period played a decisive role in chicken weight and meat quality. The purpose of replacement period is to increase the intake level of nutrients in broilers, so as to maximize their growth rate and achieve rapid fattening. However, during the rapid fattening period of Liangfenghua chicken, the lack of scientific and reasonable feed formula leads to the increase of feed waste and breeding cost, and then, affects the economic income of Liangfenghua chicken breeding enterprises. This study provided a comprehensive overview by analyzing the different intestinal flora structures of Liangfenghua chicken in the replacement period to lay the foundation for the feed formula and feeding management of precision feeding of Liangfenghua chickens entering in the replacement period.

## Materials and methods

### Sample collection

Six healthy 60-day-old (start changing materials and enter the growing period) commercial Liangfenghua chickens weighing 2.70 kg were obtained from Zhumadian Golden Sun Animal Husbandry Co. Ltd, Zhumadian, Henan, China. The temperature of the house was constant, and the chickens were free to drink water and fed by 511 broiler feed (Zhumadian Huazhong Zhengda Co., Ltd, Zhumadian, Henan, China). The chickens were brought back to the sterile laboratory for sample collection with the approval of the local animal protection organization of Animal Ethics Committee of Zhumadian City, Henan Province, China, which is part of the Chinese Center for Animal Disease Control and Prevention. After head dislocation, the duodenum (group D), jejunum (group J), ileum (group I), and cecum (group C) including the intestinal contents were collected and immediately frozen in liquid nitrogen, and then stored at -80°C. for future extraction of metagenomic DNA.

### Genomic DNA extraction

Total community genomic DNA extraction was performed from each sample by using a E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. The concentration of DNA was measured by using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) to ensure that adequate amount of high-quality genomic DNA had been

extracted.

**16S rRNA gene amplification by polymerase chain reaction (PCR)**

PCR was performed immediately after the genomic DNA extraction to amplify the V3–V4 hypervariable amplicon region of the bacterial 16S rRNA gene by using KAPA HiFi Hot Start Ready Mix (2×) (TaKaRa Bio Inc., Osaka, Japan). Two universals bacterial 16S rRNA gene amplicon PCR primers were employed as the forward primer (CCT ACG GGN GGC WGC AG) (N stands for A, C, G, or T; W stands for A or T) and reverse primer (GAC TAC HVG GGT ATC TAA TCC) (H stands for A, C, or T; V stands for A, C, or G) [5]. The reaction was set up as total volume of 30 µL with 2 µL of microbial DNA (10 ng/µL), 1 µL of each forward and reverse primers (10 µM), 15 µL of 2× KAPA HiFi Hot Start Ready Mix. The PCR was performed by using ABI 9700 Thermal Cycler (Applied Biosystems, New York, NY, USA) with denaturing at 95°C for 3 min, followed by first 5 cycles of denaturing at 95°C for 30 s, annealing at 45°C for 30 s, elongation at 72°C for 30 s, then 20 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR products were checked by using electrophoresis in 1% (w/v) agarose gels in TBE buffer (Tris, boric acid, EDTA) stained with ethidium bromide (EB) and visualized under UV light.

**16S gene library construction, quantification, and sequencing**

AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA) were used to purify the free primers and primer dimer species in the amplicon product. Samples were delivered to Sangon BioTech (Shanghai, China) for library construction using a universal Illumina adaptor and index. Before sequencing, the DNA concentration of each PCR product was determined by using Qubit® 2.0 Green double-stranded DNA assay (Thermo Fisher Scientific, Waltham, MA, USA) and the DNA was quality controlled by using Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA). Depending on coverage needs, all libraries were pooled for one run. The amplicons from each reaction mixture were pooled in equimolar ratios based on their concentrations. Sequencing was performed by using Illumina MiSeq system (Illumina, San Diego, CA, USA) according to the manufacturer's instructions.

**Sequence data processing**

After sequencing, data were collected as follows: (1) The two short Illumina readings were assembled by PEAR (v 0.9.6) software (https://cme.hits.org/exelixis/web/software/pear/) according to the overlap. The fastq files were processed to generate individual fasta and qual files, which could then be analyzed by standard methods. (2) Sequences containing ambiguous bases and any longer than 480 base pairs (bp) were dislodged and those with a maximum homopolymer length of 6 bp were allowed. The sequences that were shorter than 200 bp were removed. (3) All identical sequences were merged into one. (4) Sequences were aligned according to a customized reference database. (5) The completeness of the index and the adaptor was checked, and all index and the adaptor sequences were removed. (6) Noise was removed by using the Pre. cluster tool (https://prinseq.sourceforge.net/). Chimeras were detected by using Chimera UCHIME (https://www.drive5.com/usearch/manual/uchime_algo.html). All of the software was in the mothur package (http://mothur.org/). The effective sequences of each sample were submitted to the RDP Classifier (http://rdp.cme.msu.edu/) again to identify archaeal and bacterial sequences.

**Statistical analysis**

Species richness and diversity statistics including coverage, Chao1, Ace, Simpson, and Shannon were also calculated by using mothur (http://mothur.org/). The differences among different groups were analyzed with one-way Analysis of Variance (ANOVA).

## Results

**Table 1.** Analysis of bacterial diversity.

| Samples | OTUs | ACE | Chao1 | Shannon | Coverage | Simpson |
|---|---|---|---|---|---|---|
| D | 231.33±66.07$^{b}$ | 762.46±236.35 | 460.93±147.37$^{b}$ | 1.64±0.42$^{b}$ | 0.997 | 0.33±0.14$^{a}$ |
| J | 232.00±57.65$^{b}$ | 735.80±223.35 | 498.79±127.63$^{b}$ | 1.57±0.38$^{b}$ | 0.997 | 0.34±0.14$^{a}$ |
| I | 246.83±106.99$^{b}$ | 761.95±213.78 | 521.01±184.80$^{b}$ | 1.39±0.31$^{b}$ | 0.997 | 0.43±0.14$^{a}$ |
| C | 653.33±99.31$^{a}$ | 799.66±123.61 | 772.27±119.85$^{a}$ | 3.91±0.35$^{a}$ | 0.997 | 0.07±0.02$^{b}$ |

Notes: D: duodenum; J: jejunum; I: ileum; C: cecum.

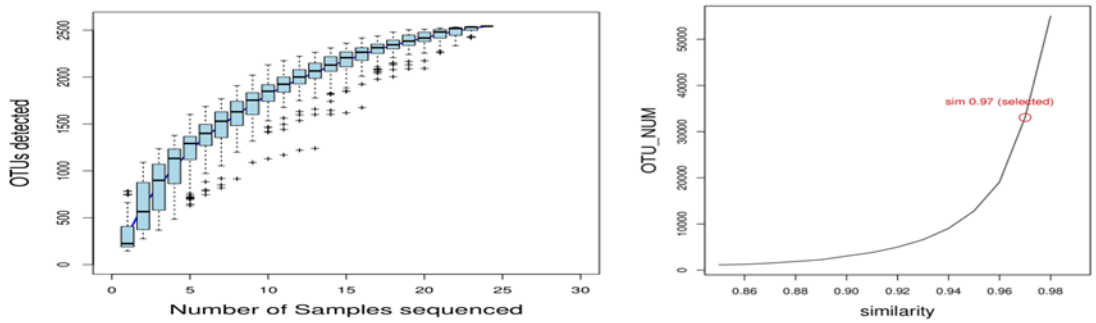


**Figure 1.** Species accumulation curves and the relationship between cluster similarity and OUT number.

**Sequencing data analysis**

A total of 1,363,855 16S rRNA gene sequences were obtained from 24 samples of 4 different sections including duodenum, jejunum, ileum, and cecum of 6 chickens. After quality control, removal of chimeras and sequences outside the target region, a total of 1,129,750 16S rRNA gene sequences were retained for analysis including 295,876 sequences from duodenum with an average length of 426 bp, 296,401 sequences from jejunum with an average length of 428 bp, 281,551 sequences from ileum with an average length of 428 bp, and 255,922 sequences from cecum with an average length of 417 bp.

**Alpha diversity analysis**

Operational taxonomic units (OTUs) were analyzed by bioinformatic statistical analysis at a similar level of 97%. The sequence coverage, observed OTUs, species diversity and richness were shown in Table 1, which demonstrated the differences in microbial abundance in various sections of intestine. The total numbers of OTUs, Chao1, and Shannon in group C were significantly higher than that in the other three groups ($P$ < 0.05), while Simpson was significantly lower than that in the other three groups ($P$ < 0.05). The results indicated that group C had the highest gut microbial richness, and groups D, J, and I had lower gut microbial abundance.

The species accumulation curve was plotted according to OUTs, which reached asymptote (Figure 1) at 97% similarity, and the dilution curve gradually tended to be flat with the coverage rate of each sample sequence reaching more than 99%. The results showed that the sample size was sufficient, the sequencing data were representative, and the test results could be reflected scientifically and objectively.

**Beta diversity analysis**

**(1) Clustering analysis:**

Hierarchical clustering analysis was performed according to the beta diversity distance matrix to construct a tree structure by using R vegan package (https://www.r-project.org/). The beta diversity distance matrix was calculated from the OTU abundance of each sample. The results showed that each sample in groups D, J, and I was on the same branch. There was a cross in the microbial composition of the samples in each

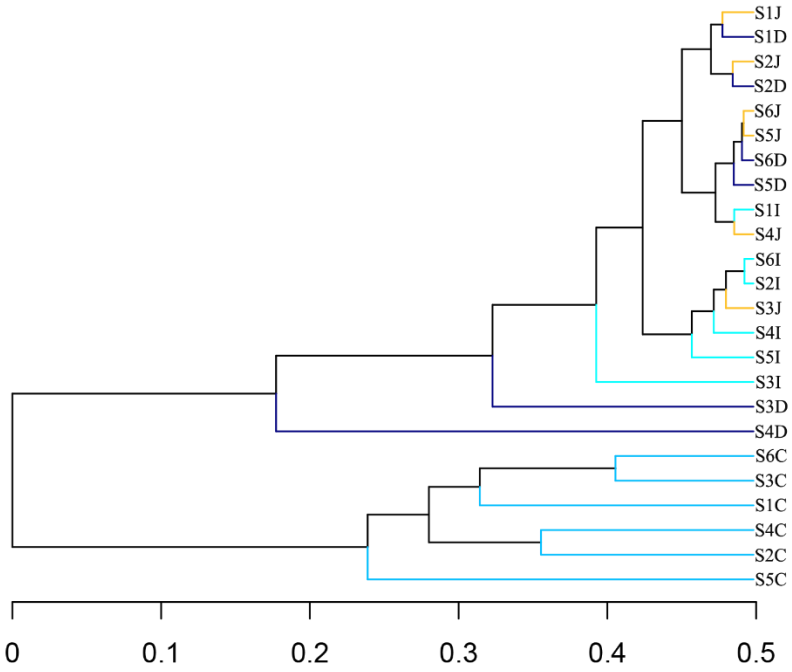


**Figure 2.** Clustering diagram of intestinal microbial species in different parts of Liangfenghua chicken.

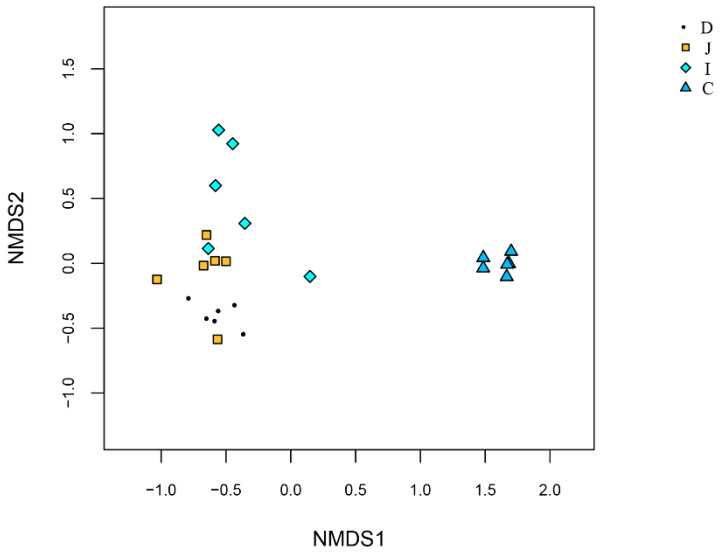


**Figure 3.** Non-metric multidimensional scale (NMDS) analysis in different intestinal sections of Liangfenghua chicken.

group with high similarity, while group C was on the other branch. There was some difference in the samples in the group C, but under the same branch. However, the microbial composition in group C was significantly different from the other three groups (Figure 2).

**(2) Nonmetric multidimensional scaling (NMDS) analysis:**

NMDS analysis was performed on the genus level flora of 4 different sections and showed that the flora in the cecum section was all clustered together, while there was a crossover between

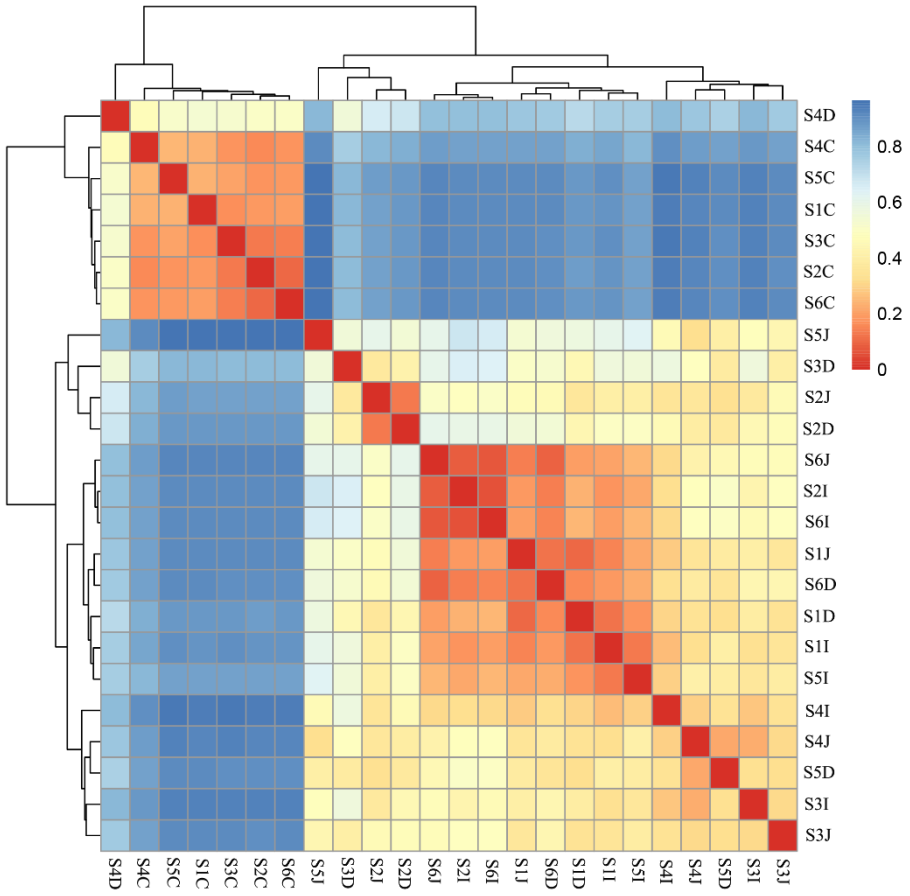


**Figure 4.** Heat map of intestinal microbial distance matrix in different sections of Liangfenghua chicken.

the ileum, jejunum, and duodenum. The results indicated that there were little differences among ileum, jejunum, and duodenum, and there were significant differences between these three sections and cecum in the structure and composition of intestinal microorganisms (Figure 3). In addition, the clustering of samples in the group D and group M was relatively concentrated indicating that the microbial composition of samples in the group was highly homogeneous.

**(3) Analysis of the differences between groups**

The distance matrix between samples was calculated and the results were drawn into a heat map (Figure 4). The distance matrices of the samples in groups D, J, and I were between 0 - 0.7, 0 - 0.6, and 0 - 0.8, respectively. However, the distance matrix of the samples in the group C was between 0 - 0.2. The results indicated that the intra group difference of groups D, J, and I was greater than that of group C, while the sample uniformity of group C was higher than the others. Anosim analysis was used to further analyze the differences between the groups. Comparing the groups D, J, and I to group C, the values of R were 1.000. There were significant differences between group C and the other groups ($P < 0.01$) (Table 2). It again showed that there was difference between the composition of microorganisms in the groups D, J, I, and that in group C.

**Evolutionary analysis of microbial species in different intestinal parts**

The species annotation results of intestinal samples from 4 different sections were used to construct a microbial species classification tree (Figure 5), which showed that the bacterial

**Table 2.** Difference analysis of intestinal microorganisms in different sections of Liangfenghua chicken.

| Comparison | ANOSIM statistic R | *P* Value | permutations |
|---|---|---|---|
| D-J | 0.026 | 0.307 | 999 |
| D-I | 0.180 | 0.048 | 999 |
| D-C | 1.000 | 0.003 | 999 |
| J-I | 0.165 | 0.107 | 999 |
| J-C | 1.000 | 0.003 | 999 |
| I-C | 1.000 | 0.002 | 999 |
| D-J-I-C | 0.457 | 0.001 | 999 |

Notes: D: duodenum; J: jejunum; I: ileum; C: cecum.

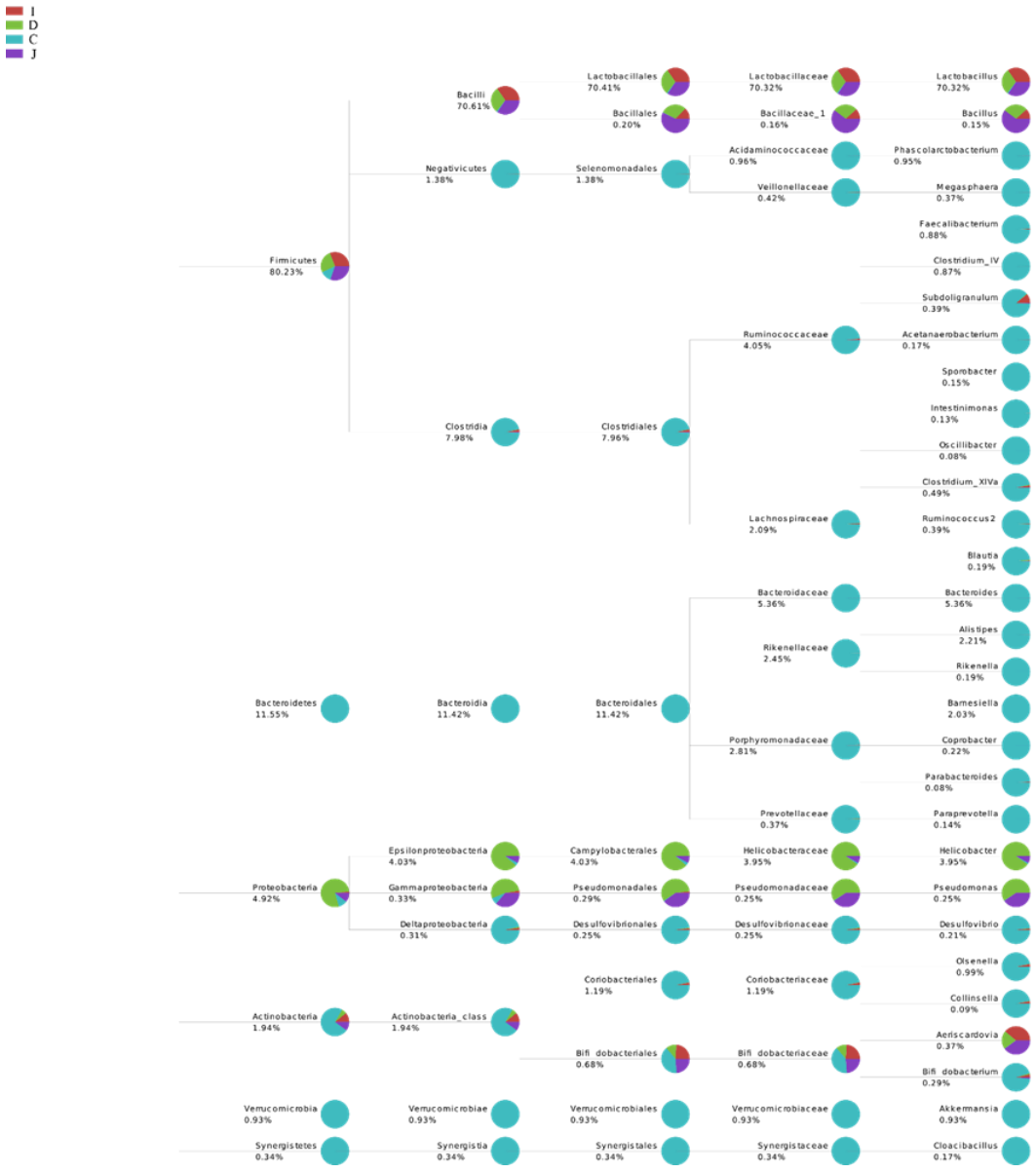


**Figure 5.** Evolutionary taxonomic tree of microbial species in different intestinal sections of Liangfenghua chicken.

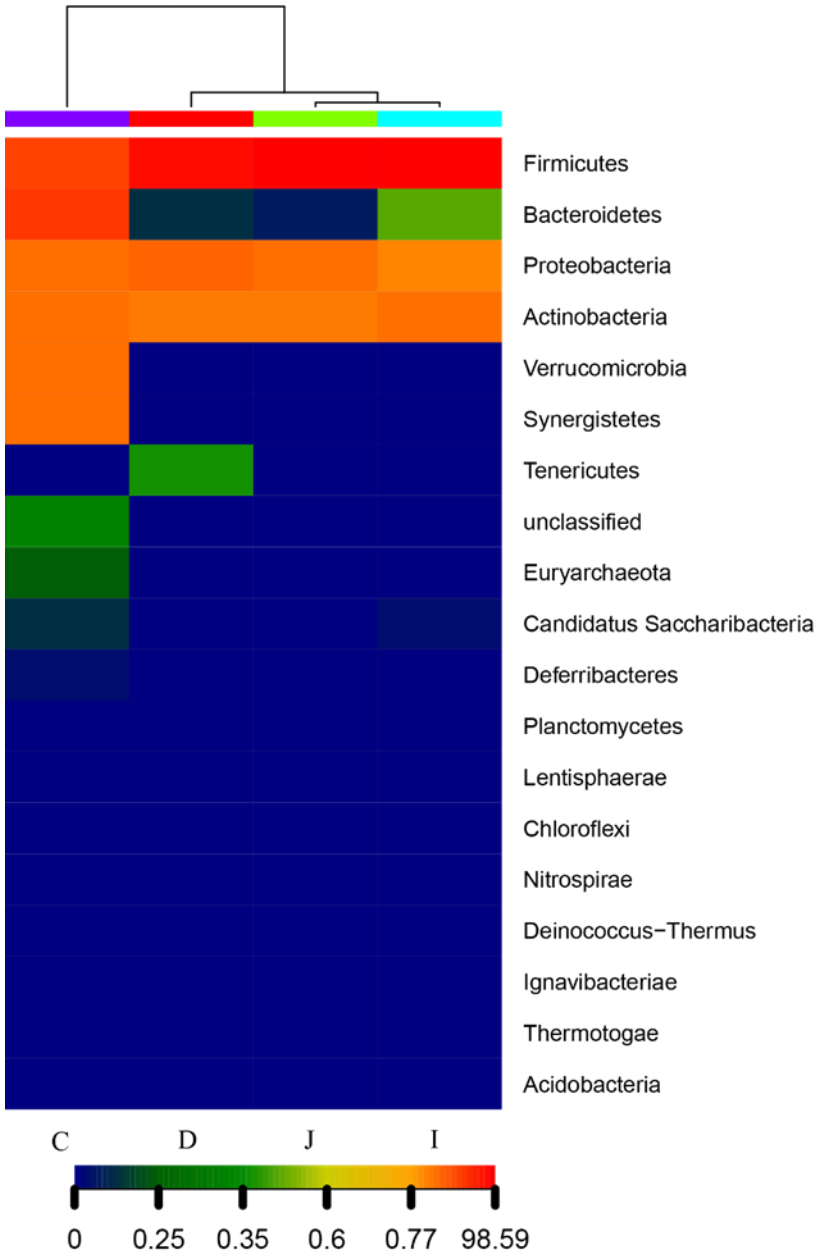


**Figure 6.** Bacteria phyla distributions.

species of the 4 groups of samples were mainly distributed in *Firmicutes* and *Bacteroidetes*. *Bacteroidetes*, *Verrucomicrobia*, and *Synergistetes* were only distributed in group C, and the microbial diversity of the samples was higher. Groups D, J, and I were mainly *Firmicutes* and *Proteobacteria*. The relative abundance of *Bacillus* in *Firmicutes* was more than 70% of the total bacteria. There were significant differences in the composition of intestinal microbiota phyla level and genus level in different sections.

**Community structure analysis**

At the phylum level, there was total of 19 phyla involved in the 4 sections of intestinal microbes. In order to facilitate the observation of the relationship between samples and species composition, a sample clustering tree and

**Table 3.** Bacteria phyla distributions.

| Phylum | D | I | J | C | *P* value |
|---|---|---|---|---|---|
| *Firmicutes* | 84.05[a] | 98.69[a] | 97.14[a] | 41.05[b] | <0.001 |
| *Bacteroidetes* | 0.02[b] | 0.14[b] | 0.01[b] | 46.03[a] | <0.001 |
| *Synergistetes* | 0.00[b] | 0.01[b] | 0.00[b] | 1.33[a] | <0.001 |
| unclassified | 0.00[b] | 0.00[b] | 0.00[b] | 0.11[a] | 0.003 |

Notes: D: duodenum; J: jejunum; I: ileum; C: cecum.

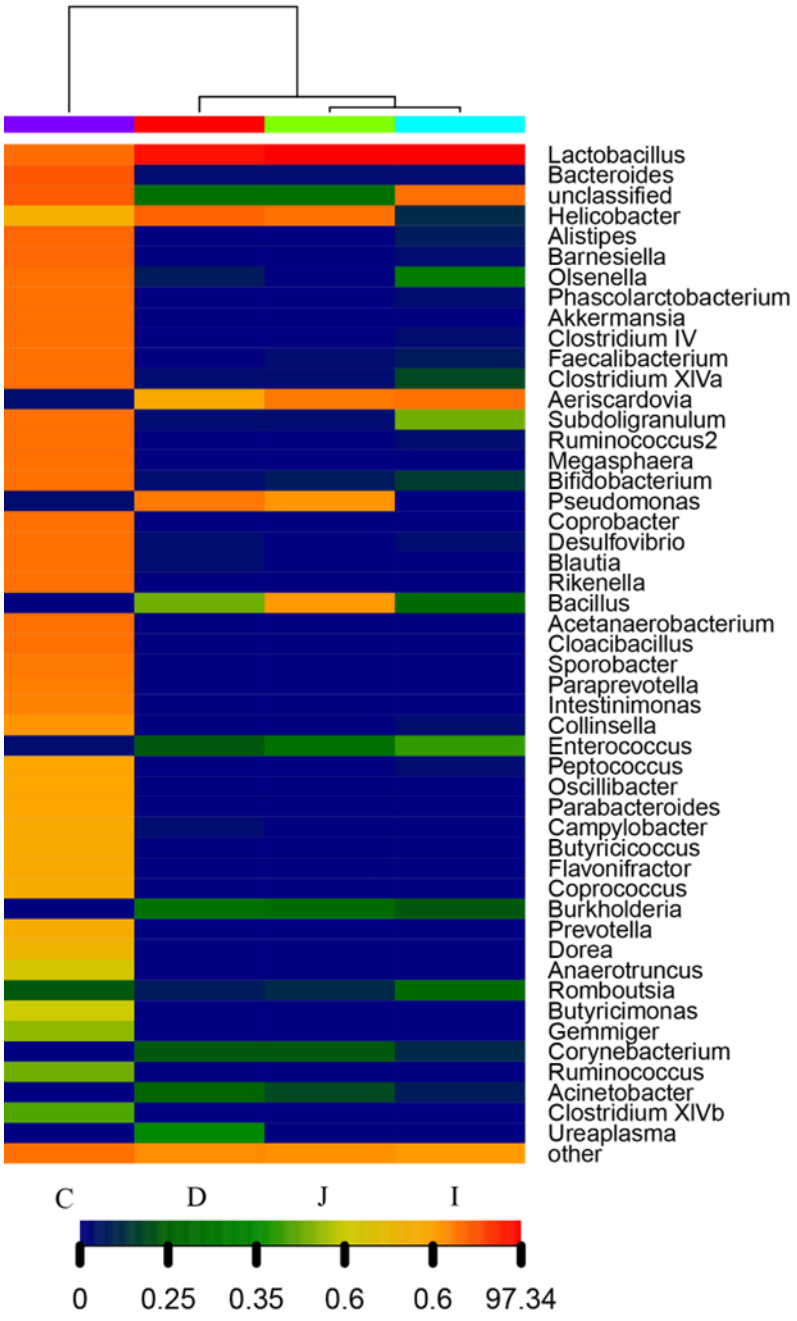


**Figure 7.** Bacteria genus distributions.

species relative abundance heat map was constructed based on the species abundance of each sample (Figure 6). The results showed that *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Synergites* had high relative abundance (> 0.77%). There were significant differences among groups in *Firmicutes*, *Bacteroidetes*, *synergites*, and unclassified (Table 3). The relative abundance of *Firmicutes* in groups D, J, and I was more than 84%, which was significantly higher than that in group C (41.05%), while the relative abundance of *Bacteroidetes*, *Synergites*, and unclassified in groups D, J, and I was close to 0, which was significantly lower than that in group C ($P < 0.05$). The relative abundance of *Bacteroidetes* in group C was about 460 times higher than that in the other three groups.

At the genus level, the 4 sections of intestinal microorganisms involved 229 genera. The samples were clustered, and the relative abundance heat map was constructed (Figure 7). There were 40 genera with high relative abundance (> 0.6%), of which 20 had significant differences between groups (Table 4). The relative abundance of *Lactobacillus* in groups D, J, and I all reached more than 80%, which was significantly higher than that in the group C (3.8%). The relative abundance of *Bacillus* in group I was significantly higher than that in the groups of J and C ($P < 0.05$), and higher than that in the group D, However, there was no significant difference observed.

The relative abundance of 18 genera including *Bacteroides*, unclassified, *Alistipes*, *Barnesiella*, *Phascolarctobacterium*, *Faecalibacterium*, *Clostridium Xlva*, *Subdoligranulum, Ruminococcus* 2, *Bifidobacterium*, *Coprobacter*, *Blautia*, *Rikenella*, *Acetanerobacterium*, *Cloacibacillus*, *Sporobacter*, *Integranimonas*, and *Collinella* in group C was significantly higher than that in groups D, J, and I ($P < 0.05$). The relative abundance of group C was between 0.37-21.4, but the relative abundance of the other three groups was only between 0-0.2 except that unclassified was 0.9.

## Discussion

The intestinal microbial diversity is very important for chickens to adapt to the growth environment and stabilize production performance [6, 7]. However, due to the short residence time of chyme, the number and diversity of microorganisms in chicken intestinal tract are significantly lower than those in vertebrates, and even the dilution curve analysis of single regional samples or single intestinal segment samples cannot reach the gene number of 9.0 M and high saturation [8]. Therefore, it is of great significance to study the complete microflora of chicken intestinal samples from different breeds, regions, and intestinal segments. In this study, 16S rDNA technology was used to study the structure and composition of intestinal microflora in 4 sections including duodenum (D), jejunum (J), ileum (I), and cecum (C) of Liangfenghua chicken. It was found that the intestinal microflora diversity and species richness of cecum were higher than that in duodenum, jejunum, and ileum. One previous report has shown that poultry digestive tract microorganisms were composed of a large number of Gram-positive bacteria with *Bacteroides*, *Firmicutes*, and *Proteobacteria* as the dominant flora. Among which, *Firmicutes* had the highest relative abundance [9]. Similarly, Wei, *et al*. analyzed the 16S rRNA sequences of 3,184 chicken intestinal microbes in the public database and found that the abundance of *Firmicutes* (70%), *Bacteroidetes* (12.3%), and *Proteobacteria* (9.3%) accounted for more than 90% of all sequences [10]. This study showed that, at the phylum level, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were the dominant phyla in different intestinal sections of Liangfenghua chickens, which was consistent with previous studies. Xiao, *et al*. studied 16S rRNA genes in chickens and found that the microbes in duodenum, jejunum, and ileum sections were more monomorphic, while the microbial diversity was higher in the cecum section [11]. In this study, the relative abundance of *Firmicutes* reached more than 97% in the jejunum and ileum, 84% in the duodenum, but

**Table 4.** Contents of bacteria of different genera in different intestinal parts.

| Genus | D | J | I | C | *P* value |
|---|---|---|---|---|---|
| *Lactobacillus* | 83.60$^{a}$ | 96.45$^{a}$ | 97.44$^{a}$ | 3.80$^{b}$ | <0.01 |
| *Bacteroides* | 0.01$^{b}$ | 0.01$^{b}$ | 0.01$^{b}$ | 21.40$^{a}$ | <0.01 |
| unclassified | 0.09$^{b}$ | 0.09$^{b}$ | 0.75$^{b}$ | 19.79$^{a}$ | <0.01 |
| *Alistipes* | 0.00$^{b}$ | 0.00$^{b}$ | 0.02$^{b}$ | 8.81$^{a}$ | <0.01 |
| *Barnesiella* | 0.00$^{b}$ | 0.00b$^{b}$ | 0.01$^{b}$ | 8.12$^{a}$ | <0.01 |
| *Phascolarctobacterium* | 0.00$^{b}$ | 0.00$^{b}$ | 0.01$^{b}$ | 3.77$^{a}$ | <0.01 |
| *Faecalibacterium* | 0.00$^{b}$ | 0.01$^{b}$ | 0.02$^{b}$ | 3.50$^{a}$ | <0.01 |
| *Clostridium XlVa* | 0.02$^{b}$ | 0.01$^{b}$ | 0.05$^{b}$ | 1.89$^{a}$ | <0.01 |
| *Subdoligranulum* | 0.01$^{b}$ | 0.01$^{b}$ | 0.17$^{b}$ | 1.39$^{a}$ | 0.02 |
| *Ruminococcus2* | 0.00$^{b}$ | 0.00$^{b}$ | 0.01$^{b}$ | 1.54$^{a}$ | <0.01 |
| *Bifidobacterium* | 0.02$^{b}$ | 0.02$^{b}$ | 0.04$^{b}$ | 1.08$^{a}$ | <0.01 |
| *Coprobacter* | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.89$^{a}$ | <0.01 |
| *Blautia* | 0.01$^{b}$ | 0.00$^{b}$ | 0.01$^{b}$ | 0.76$^{a}$ | <0.01 |
| *Rikenella* | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.75$^{a}$ | 0.02 |
| *Bacillus* | 0.18$^{ab}$ | 0.37$^{a}$ | 0.07$^{b}$ | 0.00$^{b}$ | 0.01 |
| *Acetanaerobacterium* | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.68$^{a}$ | <0.01 |
| *Cloacibacillus* | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.68$^{a}$ | <0.01 |
| *Sporobacter* | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.61$^{a}$ | <0.01 |
| *Intestinimonas* | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.53$^{a}$ | <0.01 |
| *Collinsella* | 0.00$^{b}$ | 0.00$^{b}$ | 0.01$^{b}$ | 0.37$^{a}$ | <0.01 |

Notes: D: duodenum; J: jejunum; I: ileum; C: cecum.

only 41.05% in the cecum. The relative abundance of *Bacteroidetes* was close to 0 in the duodenum, jejunum, and ileum, but reached 46.03% in the cecum with significant differences between the duodenum, jejunum, and ileum and cecum groups for the two phyla. The results showed that the microbial species in the duodenum, jejunum, and ileum of Liangfenghua chicken were relatively single and concentrated in *Firmicutes*, while the microbial diversity in the cecum was high, mainly distributed in *Firmicutes* and *Bacteroidetes*.

Chicken intestinal microflora has important physiological significance for host health and production performance, mainly in host nutrient absorption, immune system development regulation, immune barrier, and detoxification [12-15]. It has been found that the main microorganisms in the duodenum, jejunum, and ileum of birds are lactic acid bacteria (35%), while the main microbial genera in the cecum include middle *alistipes*, *Bacteroides*, *butyricoccus*, *faecalibacterium*, *Lactobacillus*, and *odoribacter* [11]. Under the experimental conditions, the microorganisms in different intestinal sections of Liangfenghua chicken involved 229 genera at the genus level. The relative abundance of *Lactobacillus* in duodenum, jejunum, and ileum was more than 80%, which was significantly higher than that in the cecum (3.8%) and was consistent with the previous research results. The genus *Lactobacillus* is the most abundant genus in the upper segment of the intestine and ileum with eight *lactobacillus* species identified in chicken gastrointestinal sections [16]. Biagi, *et al*. pointed out that *Lactobacillus* was one of the important probiotics in the animal intestinal microenvironment, which could participate in regulating the balance between microorganisms and improving the body's immunity and disease resistance [17]. The metabolites and whole cells of *Lactobacillus* and *Bifidobacterium* can activate B cells through M cells, and then secrete IgA [17]. The cooperative symbiotic relationship between intestinal microbiota and animals promotes the development of the animal immune system [17]. In this study, lactic acid bacteria in the upper

segment of Liangfenghua chickens occupied an absolute advantage, while the relative abundance in the cecum was lower than that in the other three sections, which might relate to the digestion mode of chickens and the effect of microorganisms on the upper intestinal segment. The feed is digested by pancreatic juice, intestinal juice, bile, and various enzymes in the duodenum, while the unabsorbed nutrients in the duodenum rapidly enter the cecum for microbial fermentation and digestion. The presence of microorganisms in the upper segment of chickens plays more roles in improving immunity and the growth of enterocytes. The relative abundance of *Bacillus* within the jejunum was higher than that in the duodenum, ileum, and cecum. It has been shown that, after feeding broilers with *Bacillus* subtilis in the form of spores, the spores have partially begun to germinate in the duodenum, and in the jejunum. The spores basically germinated as vegetative bodies, and the number of vegetative bodies reached the maximum. the spores also germinated as vegetative bodies in the ileum. The results of this study were consistent with previous findings that *Bacillus* stayed in the chicken intestine [18, 19]. The results were also consistent with the residence law of *Bacillus* found in the intestines of chickens.

The gut microbiota plays an important role in nutrition, metabolism, physiology, and immunity processes, and is composed of a large number of bacteria, which are diverse and have synergistic effect in using and breaking down nutrient resources [20]. The cecum is a section of massive microbial fermentation, detoxification of hazardous substances, and protection against pathogenic bacterial colonization with the major bacterial species of *Clostridium*, *Ruminococcus*, and *Bacillus* [21]. The genera *Bacteroides*, *Ruminococcus*, anaerobic clubbing, *Alistipes* can all produce short chain fatty acids in the gut and reduce and ameliorate intestinal inflammation [22, 23]. Sheng, *et al*. indicated that *Rumen Clostridium* increased the decomposition rate of cellulose and lignin in feed [24]. Goodrich, *et al* suggested that increasing the abundance of *Christensen* in the gut was beneficial to organism health [25, 26]. In this study, the relative abundance of other bacterial genera except *Lactobacillus* in the above microbes was higher in the cecum than in other sections of the intestine, which indicated that Liangfenghua chickens could not only digest and absorb using pancreatic juice, intestinal fluid, bile, and enzymes, but also digest and absorb harder cellulose and so on by microbes. Then, the secretion of short chain fatty acids (SCFA) through microbial synthesis could be absorbed passively [27]. The indirect activation of acid proteases, amylases, lipases, and cellulases leads to increased protein and energy utilization. It can also produce various vitamins, amino acids, and unknown growth factors, *etc*. to promote the healthy growth of animals.

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