

Characterisation of gut microbiota in early chicken development

B. Wu, Y. Bai, Y. Guan, Ch. Lou, Z. Chen and Hua Li*

Foshan University, School of Life Science and Engineering,
Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding, Foshan 528000, China

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* Corresponding author:
e-mail: okhuali@fosu.edu.cn

ABSTRACT. The gut microbiota plays a crucial role in nutrient absorption, health maintenance and protecting the host against pathogen invasion. Studies have shown that the microbial composition and diversity of gut microbiota during early life can influence later growth and development. While specific pathogen-free (SPF) experimental animals are considered standard in biomedical, veterinary, and production research fields, little is known about the composition and diversity of the gut microbiota during the early developmental stages of these birds. Therefore, the present study aimed to explore the structure and changes of the gut microbiota during the early life of SPF chickens. Faecal DNA was sampled from randomly selected chickens, followed by high-throughput sequencing of the V3-V4 region of the 16S rRNA gene, at 11, 13, 15, and 17 days of age, designated as experimental groups D11, D13, D15, and D17. The sequencing results indicated that day 17 of life may be a turning point for gut microbiota colonisation, exhibiting a notable 51.98% increase in the number of operational taxonomic units compared to 15 days old birds. Analysis of faecal bacterial community compositions across the four age groups of SPF chickens showed dominance of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* in groups D11, D13, and D15, while *Firmicutes*, *Proteobacteria*, and *Cyanobacteria* were dominant in group D17. Additionally, group D17 had the highest number of bacterial genera and greatest diversity among the four groups tested. This study systematically elucidated the structure and dynamics of the gut microbiota during early life of chickens and provided a benchmark for future research on chicken gut microbiota.

Introduction

The digestive tract of animals is a complex microbial ecosystem, characterised by a dynamic symbiotic relationships between the host microbiota and the external environment (Johnson and Versalovic, 2012). The gut microbiota plays a crucial role in the health, disease, and production of animal hosts (Putignani et al., 2014). Beneficial microbial communities contribute to maintaining normal physiological functions, while harmful microbial populations can disrupt the composition of the gut microbiota, potentially leading to disease occurrence (DiGiulio et al., 2008).

From the bird's birth, the gut microbiota undergoes continuous evolution, with early life stages being a critical period for establishing the gut ecosystem. Adult chickens harbour a large number of microorganisms in their gut, comprising over 1000 different species, resulting in high diversity and relative abundance (Chambers and Gong, 2011). Although each section of the chicken's digestive tract contains varying types and number of microorganisms, the caecum stands out for its highest bacterial density, with approximately 1011 microorganisms per g (net weight) of faeces. Therefore, the caecum is an important organ for studying the intestinal microflora of chickens. Zhu et al. (2002)

analysed the microbiota in the caecum of chickens at different ages using molecular biology techniques and found that the predominant species in one-day-old chicken caecum was *Enterococcus*, with several other bacteria detected. However, the microbial community structure changed significantly within the first week of chicken's life. Analysis of the caecal microbiota in 6-week-old chickens showed that the family *Clostridiaceae* accounted for the largest proportion (65.0%), followed by the genera *Clostridium*, *Lactobacillus*, and *Bacteroides*. As chickens grew, the microbiota in the caecum became more diverse and displayed differences in the community structure, indicating a gradual evolution from a transient, simple composition to a complex and balanced microflora (Zhu et al., 2002). It is well known that the critical period for establishing the chicken gut microbiota occur in the early stages after hatching, characterised at first by a low diversity and high instability, making it susceptible to changes from external factors such as the gut environment (Hooper et al., 2000; Medvecky et al., 2018). Therefore, the initial post-hatch development period is a critical phase, leaving a narrow window for the establishment of a permanent microbial population (Baldwin et al., 2018). The settlement of the first microbiota in the gastrointestinal tract is particularly important for the health and productivity of chickens, as demonstrated by the use of antibiotics and probiotics to promote growth in poultry farming (Zhou et al., 2012; Stanley et al., 2014). A growing body of evidence suggests that the gut microbiota plays a critical role in the maturation of the immune system and disease prevention during the neonatal, childhood, and adult life stages (He et al., 2023).

Extensive research efforts have been directed towards understanding the influence of gut microbiota on the growth, development, and productivity of broilers and layers (Feng et al., 2020; Gan et al., 2020; Xu et al., 2023). However, there is little literature on the evolution of gut microbiota, especially the early-life microbiota, in specific pathogen-free (SPF) chickens, which are standard laboratory animals used in life science and medical research. These birds exhibit high sensitivity to various pathogenic microorganisms and are utilised as high-standard raw materials for the development of human and poultry biological products. SPF chickens not only reduce the interference of unrelated pathogens, but also establish an animal infection system for analysing the occurrence and changes of diseases. High-throughput sequencing of the 16S rRNA gene allows to explore more complex microbial communities in animals. In this study, high-throughput sequencing of the 16S

rRNA gene V3-V4 region in SPF chicken faeces was performed using the Illumina platform. The aim was to investigate the initial development and changes of the intestinal flora in SPF chickens during early growth stages, elucidate the mechanisms of bacterial community colonisation, and provide a reference for the establishment of a permanent microbial community in early life. This research holds significant importance for the more precise regulation of microbial community structure, prevention of intestinal diseases, and promotion of overall health.

Material and methods

Animals

In this experiment, 72 8-day-old SPF chickens (36 male and 36 female), with an average weight of 64–65 g and good health status were randomly selected. The birds were purchased from Xinxing Dahua Agricultural and Poultry Egg Co., Ltd., and housed for the duration of the feeding experiment at the Animal Experiment Base of Foshan University. Ethical approval for the experiment was obtained from the university's Institutional Animal Ethics Committee (FOSU 2022-191).

Sample collection

Prior to commencing the experiment, a thorough disinfection process was conducted, including fumigation of the enclosed housing units (50 × 35 × 45 cm), cages, feeders, and drinkers. Upon arrival, newly hatched chicks were individually weighed and stratified based on their body weights. Birds with significantly lower or higher body weights were excluded from the study. All chickens were fed a non-medicated conventional maize-soybean meal diet with free access to feed and water. The photoperiod was regulated in the range of 14–20 h per day. The diet composition is shown in Table 1.

A total of 72 pathogen-free SPF chickens were randomised into 4 groups, with 18 chickens in each group. Three cages were set up for each group, with 6 chickens per cage. Faecal samples were collected from each group on days 11, 13, 15, and 17 after hatching, designated as D11, D13, D15, and D17. Two faecal samples were randomly collected from each cage, resulting in 6 samples sent for sequencing from each age group. Chickens were allowed to defecate freely in sterile cages, and stool samples were collected within 1 min using a 5 ml centrifuge tube. The samples were carefully labelled and promptly stored at –80 °C for future analysis.

Table 1. Composition and proportions of nutrients in the basal diet, %

Ingredients	days 1–21
Maize	66.8
Soybean meal	26.4
Fish meal	2.0
Meat meal	1.0
CaHPO ₄	1.5
Limestone	1.0
NaCl	0.3
Premix ¹	1.0
Total	100.00
Chemical composition	
ME/(MJ/kg) ²	13.51
crude protein	19.87
P	0.80
Ca	1.10
metionine	0.31
lysine	1.03

¹ provided per kg of diet: IU: vit. A₁ 2500, vit. D₃ 3000, vit. E 20; mg: vit. B₁ 3.5, vit. B₂ 8.5, vit. B₁₂ 25, riboflavin 7.5, pantothenic acid 18.5, biotin 0.25, Fe 100, Zn 50, Cu 8, Mn 30, I 0.3; ² ME – metabolizable energy, calculated

DNA extraction

Total bacterial DNA was extracted and purified from stool samples according to the kit manufacturer's instructions, followed by 16S rRNA gene high-throughput sequencing.

16S rRNA gene sequencing

Following the extraction of total DNA from the samples, PCR amplification, purification, quantification, and normalisation were performed to generate sequencing libraries according to the standardised operating procedure of Beijing Biomarker Technologies Co., Ltd. The primers used were as follows: forward primer (F): 5'ACTCCTACGGGAGGCAGCA3', reverse primer (R): 5'GGACTACHVGGGTWTCTAAT3'. PCR conditions for the primary amplification were as follows: initial denaturation at 95 °C for 2 min; followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, with a final extension at 72 °C for 7 min. For the secondary amplification, the conditions were as follows: initial denaturation at 98 °C for 30 s, followed by 25 cycles of denaturation at 98 °C for 10 s, annealing at 65 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were separated on 1.8% agarose gels at a voltage of 120 V for 40 min, and subsequently the target fragments were cut out and purified. Qualified libraries were sequenced on an Illumina Novaseq 6000 platform (Illumina, San Diego, USA), and the results were stored in the FASTQ

format. Raw reads obtained by sequencing were filtered using Trimmomatic v0.33 software (Bolger et al., 2014). Cutadapt 1.9.1 software (Martin, 2011) was used to identify and remove primer sequences to obtain clean reads. Clean reads of each sample were merged by overlapping using Usearch v10 software (Edgar, 2010), and the length of the merged data was filtered according to the length range of different regions. UCHIME v4.2 software (Edgar et al., 2011) was used to identify and remove chimeric sequences, resulting in the final effective reads. Information analysis involved feature clustering (OTUs, ASVs), diversity analysis, differential analysis, correlation analysis, and functional prediction analysis. Usearch v10 software (Edgar, 2010) was used to cluster reads at a similarity level of 97.0% and obtain OTUs. Feature sequences were taxonomically annotated using the Silva.138 reference database and naive Bayesian classifier to obtain information on the species classification corresponding to each feature. Community composition of each sample was subsequently statistically analysed at various taxonomic levels (phylum, class, order, family, genus, and species) using QIIME2 v2020.6 to generate species abundance tables. R tools were used to draw community structure diagrams at different taxonomic levels for each sample.

Statistical analysis

Alpha diversity indices, including the Shannon index, Chao1 index, and Simpson index were evaluated using QIIME2 2020.6 software (Bolyen et al., 2019). The data in the table were plotted as box plots using R scripts to visually display differences in alpha diversity between individual sample groups. Beta diversity analysis was performed using QIIME2 2020.6 software to compare the similarity of species diversity between different samples. Principal component analysis (PCA) analysis was employed to illustrate the differences between multiple datasets using a two-dimensional coordinate system; PCA analysis plots were generated using R software. Additionally, the Lefse package in Python was used to analyse bacterial taxa that showed statistical differences between individual groups. Distance matrices based on different algorithms (binary, bray, weighted, unweighted) were constructed to generate sample heat maps using R, so that differences between pairwise samples could be intuitively observed from colour gradients. Statistical analysis was performed using IBM SPSS Statistics 24 (Chicago, USA). Student's t-test was used to validate the significance of the observed differences. Differences were considered significant at $P < 0.05$.

Results

Sequencing and OTU clustering

A total of 960 365 reads were obtained from faecal samples, and 958 291 clean reads were generated after quality control and assembly of paired-end reads. Each sample produced at least 79 623 clean reads, with an average of 79 858 clean reads. Figure 1A shows the changing trend in out characteristics in different age groups of chickens. The number of OTUs in each group is listed in

Table 2. To assess changes in intestinal microbiota diversity with chicken age, rarefaction curves were plotted, and alpha diversity indices were calculated. The rarefaction curve gradually saturated as the number of samples increased (Figure 1B), indicating adequate assessment of bacterial phenotypic diversity and microbial community diversity at the 97% threshold across the 12 samples. Moreover, the curve showed an upward shift along the y-axis with age, indicating a gradual increase in gut microbiota diversity over time.

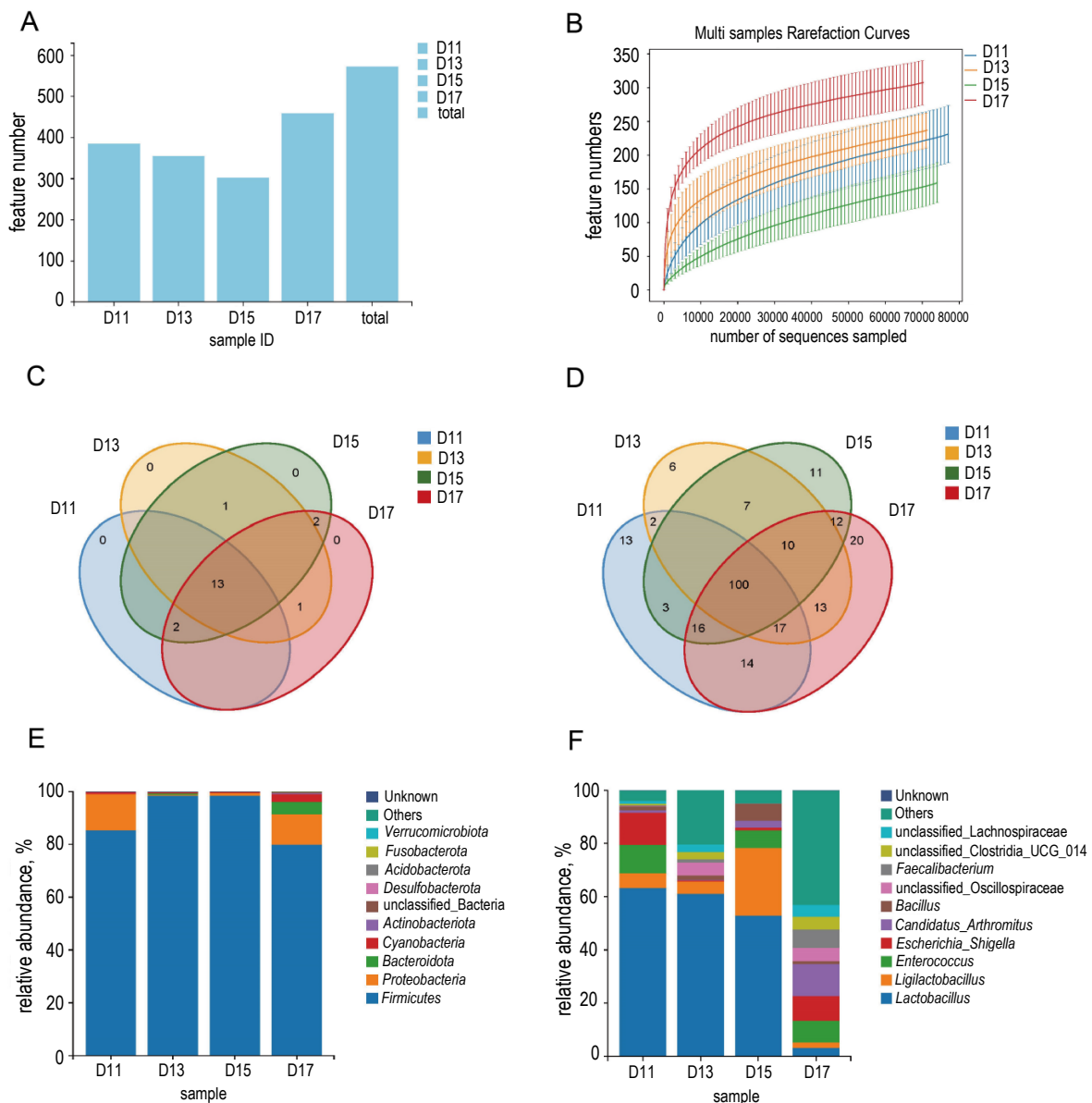


Figure 1. (A) Number of operational taxonomic units (OTUs) in faecal samples in different age groups (B). Dilution curves of faecal samples in different age groups. Venn Diagram for species classification of faecal samples of different age groups. (C) Phylum level. (D) Species level. Histogram of species classification of faecal samples in different age groups. (E) Most abundant species at the phylum level. (F) Most abundant species at the species level. (C & D) Venn Diagrams of microbial groups in faecal samples for different age groups at the phylum and species levels, respectively (E & F) Histogram chart representing microbial groups in faecal samples in different age groups at the phylum and species levels, respectively (D11) – day 11, (D13) – day 13, (D15) – day 15, (D17) – day 17

Note: B: The x-axis represents randomly selected sequencing reads, and the y-axis represents the number of features obtained based on the sampled reads. Each curve represents a sample and is marked with a different colour.

Table 2. Number of operational taxonomic units

Group	Number of OTUs
D11	385
D13	355
D15	302
D17	459

D11 – SPF chicken faecal samples were analysed at day 11, D13 – SPF chicken faecal samples were analysed at day 13, D15 – SPF chicken faecal samples were analysed at day 15, D17 – SPF chicken faecal samples were analysed at day 17; OTUs – operational taxonomic unit

Comparison of the intestinal flora in SPF chickens in different age groups

In the four age groups, a total of 18 phyla and 237 genera were annotated, with 13 phyla and 100 genera showing high abundance (Figures 1C,D). At the phylum level, the most abundant were bacteria in the following order: *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* (Figure 1E). The phylum *Firmicutes* was dominant in groups D11, D13, and D15, accounting for over 80% of the total abundance. Besides *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* were also present in group D17 (Figure 2A).

At the genus level, differences in bacterial composition were evident between the four age groups. In group D11, the three dominant genera included *Lactobacillus*, *Escherichia-Shigella*, and *Enterococcus*. In group D13, the most abundant genera were

Lactobacillus, *Ligilactobacillus*, and *unclassified Oscillospiraceae*. In group D15, *Lactobacillus* and *Ligilactobacillus* remained the dominant genera, but were joined by *Enterococcus*, while in group D17, *Candidatus_Arthromitus*, *Escherichia-Shigella*, and *Enterococcus* emerged as the most abundant genera (Figure 1F). Moreover, the highest number of genera and greatest microbial diversity was determined in group D17 (Figure 2B).

Changes in intestinal flora diversity in SPF chickens across different age group

Alpha diversity

The α -diversity indices, including Chao1, ACE, Shannon, and Simpson indices were utilised to estimate the complexity of gut microbiota. Chao1 and ACE indices were applied to evaluate species richness, while Shannon and Simpson indices were employed to assess species diversity (Table 3). There was a significant difference in species richness between groups D15 and D17 ($P < 0.05$) (Figure 2C). As the age progressed, the number of bacteria in the gut also gradually increased, and eventually a stable microbial community was established. Correspondingly, Simpson and Shannon indices demonstrated an increase with age, with a significant difference in species diversity recorded between groups D11, D15, and D17 ($P < 0.05$) (Figure 2D).

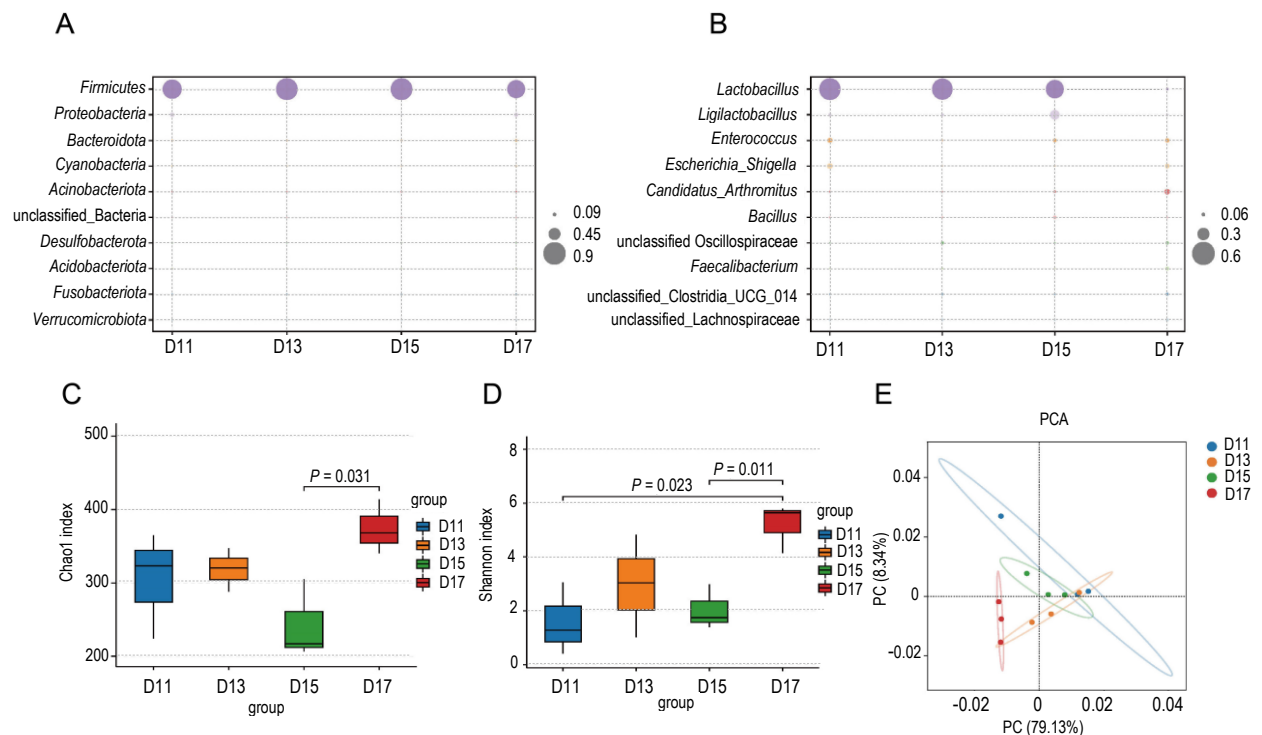


Figure 2. Taxonomic bubble maps of faecal samples of different age groups at the genus level. (A) Most abundant species at the phylum level. (B) Most abundant species at the species level. Alpha diversity analysis in faecal samples of different age groups. (C) Chao1 index boxplot. (D) Shannon index boxplot. (E) PCA analysis of faecal samples of different age groups. (D11) – day 11, (D13) – day 13, (D15) – day 15, (D17) – day 17

Table 3. Alpha diversity index of faecal samples at different days of age

Group	Chao1	ACE	Simpson	Shannon
D11	304.0196	341.2091	0.383766667	1.582966667
D13	318.3769	338.8989333	0.5929	2.964066667
D15	242.8333333	303.5014667	0.6267	2.0381
D17	373.9312333	391.9097	0.932466667	5.194566667

D11 – SPF chicken faecal samples were analysed at day 11, D13 – SPF chicken faecal samples were analysed at day 13, D15 – SPF chicken faecal samples were analysed at day 15, D17 – SPF chicken faecal samples were analysed at day 17; Chao1 – Chao1 index, one of the measures of species richness; ACE – abundance-based coverage estimator, an index used to estimate the number of species contained in a community; Simpson – Simpson index, one of the indices used to estimate the diversity of microorganisms in a sample; Shannon – Shannon-Wiener index was used to estimate the diversity of microorganisms in a sample

Beta diversity

PCA analysis revealed that samples from groups D13, D15, and D17 clearly clustered together, indicating a high degree of similarity in the composition of bacterial communities of these group. On the other hand, samples derived from group D11 exhibited greater dispersion, suggesting some level of variability between individuals within this group. The confidence ellipses for samples from group D17 did not overlap with those from groups D11 and D13, indicating a clear clustering and differences in gut microbiota composition between these groups (Figure 2E).

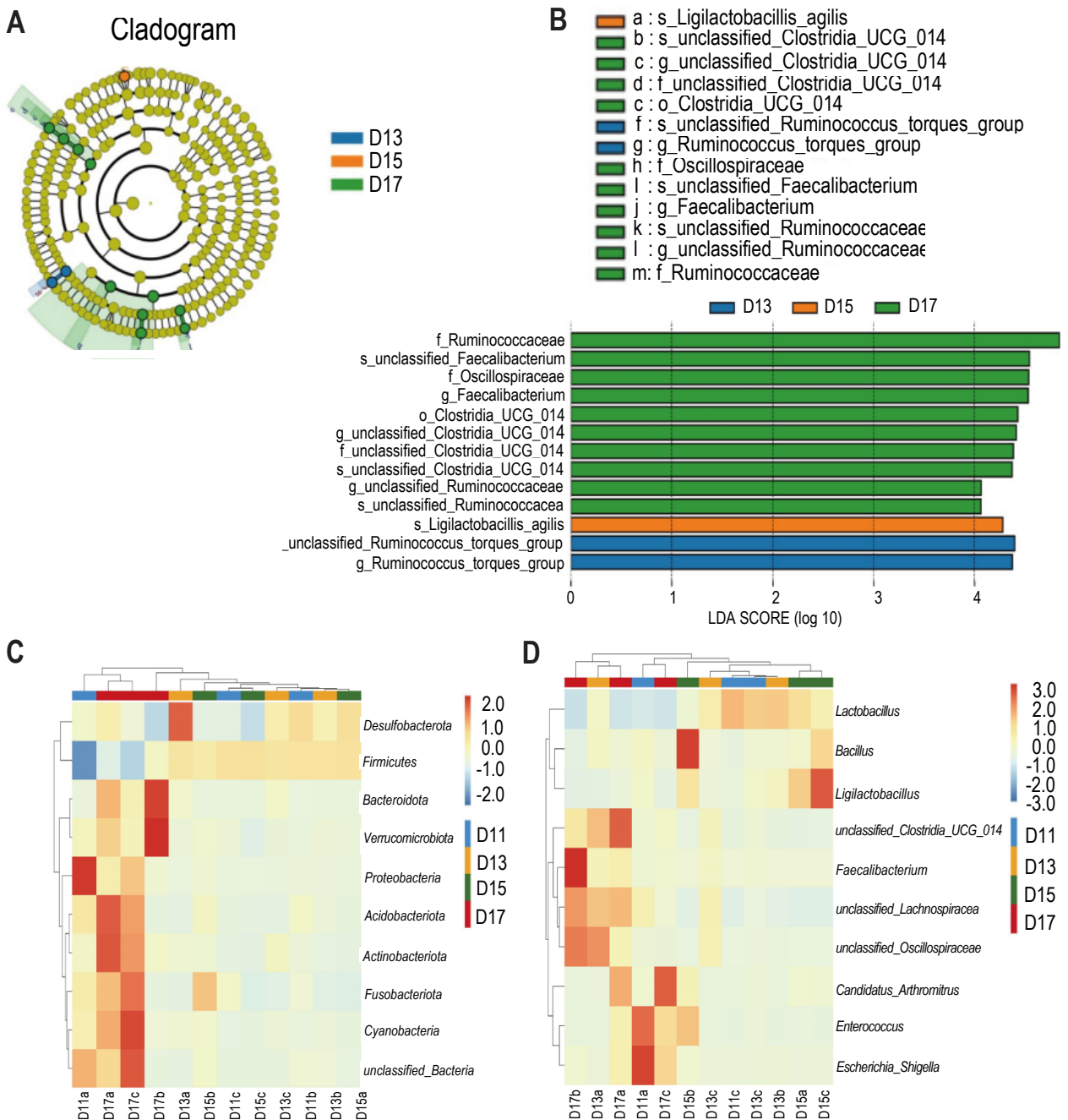


Figure 3. (A) LfSe analysis of the evolutionary branching diagram. (B) Histogram of LDA value distribution. (C) Heat map of species abundance at the phylum level. (D) Heat map of species abundance at the species level. (D11) – day 11, (D13) – day 13, (D15) – day 15, (D17) – day 17

Changes in the abundance of the intestinal flora in SPF chickens across different age groups

Linear discriminant analysis effect size (LEfSe) was used to compare the differential microbiota in the faeces of chickens from individual age groups. The results showed that the abundance of *Ruminococcus torques* group was highest in group D13 group, while *Ligilactobacillus agilis* was most abundant in group D15. In addition, the abundance of *Oscillospiraceae*, the order *Clostridia* (UCG014), along with its families, genera, and species, *Faecalibacterium* and its species, and *Ruminococcaceae* and its families, genera, and species were most numerous in group D17 (Figures 3A,B). Heatmap analysis showed an increase in the abundance of *Verrucomicrobiota*, *Bacteroidota*, and *Cyanobacteria* (Figures 3C, 4A–C), while a decline of *Firmicutes*, *Desulfobacterota*, and *Proteobacteria* was observed with age (Figures 4D,F). Furthermore, the relative abundance of *Cyanobacteria* in group D17 showed significant differences compared to groups D13 and D15 ($P < 0.05$), while the relative

was low, and this difference was also statistically significant compared to groups D11 and D13.

The heatmap representing the microbiota in group D17 revealed predominantly high abundance of most bacteria, indicating that the 17th day of age marks a critical turning point in the microbiota composition of SPF chickens.

Discussion

Although many studies have investigated changes in the gut microbiota and microbial composition of poultry subjected to various treatments (Mohd Shaufi et al., 2015; Donaldson et al., 2017), there has been limited research analysing the community structure of early gut microbiota in SPF chickens. The present study investigated SPF chicken faecal samples at 11 days (D11), 13 days (D13), 15 days (D15), and 17 days (D17) of age. The findings showed that group D17 had the highest number of OTUs (459). Alpha and beta diversity analysis revealed that the number of bacteria in the gut

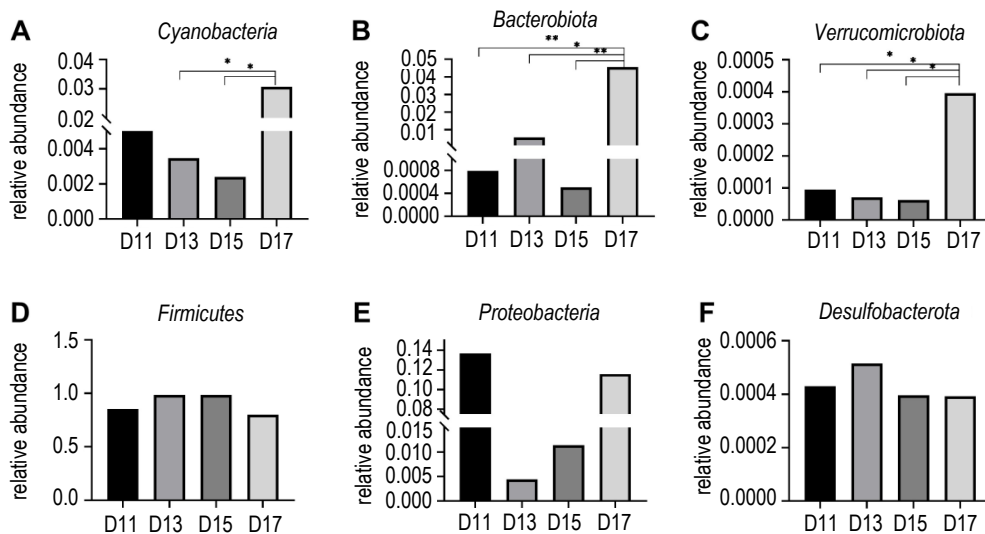


Figure 4. Histogram of the relative microbial abundance in chickens of different ages at the phylum level (* = $P < 0.05$, ** = $P < 0.01$). (D11) – day 11, (D13) – day 13, (D15) – day 15, (D17) – day 17

abundance of *Verrucomicrobiota* and *Bacteroidota* differed significantly from the other three groups ($P < 0.05$). At the genus level, there was an increase in the abundance of *Bacillus*, *Ligilactobacillus*, and *Faecalibacterium* populations (Figure 3D, 5A,C), while a decrease was observed in *Lactobacillus*, *Escherichia*, *Shigella*, and *Enterococcus* in group D17 (Figures 5D,F). The relative abundance of *Ligilactobacillus* and *Bacillus* in group D15 was high, and the difference was significant, while the relative abundance of *Lactobacillus* in group D17

gradually increased with age, leading to the establishment of a stable bacterial community. Moreover, the PCA plot showed that the D17 samples had non-overlapping confidence ellipses compared to samples from groups D11 and D13, indicating significant clustering and differences in gut microbiota composition. Similarly, LEfSe analysis and the heat map of species abundance validated the above findings. Overall, the early gut microbiota of SPF chickens underwent dynamic changes, with bacterial abundance and diversity increasing with age, resulting

in a denser bacterial community. These results were consistent with findings from studies conducted on commercial broilers (Takeshita et al., 2021) and laying hens (Videnska et al., 2014).

After comparing the microbial community compositions in faecal samples of SPF chickens from different age groups, it was found that the abundance of *Verrucomicrobiota*, *Bacteroidota*, and *Cyanobacteria* increased as dominant phyla, while the counts of *Firmicutes*, *Desulfobacterota*, and *Proteobacteria* decreased as inferior phyla. This trend was consistent with findings from studies on the systemic developmental composition of the gut microbiota of commercial laying hens. Specifically, from hatching to the end of the production cycle, the microbiota in the faeces of laying hens was primarily composed of *Firmicutes* and *Proteobacteria*, with *Actinobacteria* becoming dominant in the laying and post-laying stages (Joat et al., 2021). *Firmicutes* play a crucial role in the degradation of cellulose and other complex polysaccharides, and produce volatile fatty acids and other organic acids that can serve as an energy source for chickens (Bao et al., 2019). *Actinobacteria*, on the other hand, decompose polysaccharides and proteins to produce short-chain fatty acids, amino acids, and amides, as well as other metabolites. Certain species of *Actinobacteria* are known to produce lactic acid and other beneficial substances, which exert antimicrobial and immune-regulating effects, contributing to maintaining the stability and functionality of the gut microbiota. In addition, *Actinobacteria* can also participate in cholesterol metabolism, and some species exhibit inhibitory effects on the growth of intestinal pathogens (Barka et al., 2016). Fluctuations in the relative abundance of *Actinobacteria* and *Firmicutes* in the human gut microbiota have been associated with environmental factors (De Filippo et al., 2010), obesity (Ley et al., 2006), pregnancy (Chavoya-Guardado et al., 2022), and other physiological changes within the body. An increase in the abundance of *Actinobacteria* may potentially enhance the intestinal immune response and disease resistance in chickens, thereby reducing the risk of diseases. Overall, in the early stages, *Firmicutes* and *Proteobacteria* play crucial roles in establishing a relatively healthy gut microbiota, facilitating food digestion, and providing necessary nutrients (Joat et al., 2023). As chickens mature and their diet evolves to include more easily digestible nutrients such as proteins and carbohydrates, *Actinobacteria* may thrive due to their ability to efficiently utilise these compounds. This increase in their proportion helps maintain the balance and stability of the gut microbiota, thereby contribut-

ing to the health and development of chickens. This changing trend is crucial for ensuring the optimal health and development of chickens.

In the gut microbiota of commercial broiler chickens, the abundance of *Bacillus*, *Ligilactobacillus* and *Faecalibacterium* increased at the genus level, while the abundance of *Lactobacillus*, *Escherichia Shigella*, and *Enterococcus* decreased with age. The most common bacteria found in the gut microbiota of commercial broiler chickens include the genera *Lactobacillus*, *Bifidobacterium*, and *Escherichia* (Bolton, 1965; Wielen et al., 2002; Yeoman et al., 2012; Saxena et al., 2016). *Bacillus* is recognized for its role in promoting gut health, increasing the growth rate and body weight of poultry, and enhancing the immune system, and thus resistance to bacterial infections (Additives et al., 2019; Bilal et al., 2021; Larsberg et al., 2023). *Ligilactobacillus* is believed to contribute to maintaining gut microbiota balance, enhancing intestinal mucosal barrier function, alleviating inflammation, and supporting the immune system (Moretti et al., 2022). *Faecalibacterium* can produce short-chain fatty acids such as butyrate and propionate, which are beneficial for maintaining intestinal health and reducing mitigating inflammation in the digestive tract (Wang et al., 2016); it has been observed that the abundance of these bacteria changes with age (Xi et al., 2019). An increase in the abundance of these beneficial genera can enhance the immune system, digestive absorption capacity, and productivity of SPF chickens, thereby offering multiple advantages for their health.

The observations revealed notable shifts in the abundance of specific bacterial genera in the gut microbiota of 11–15-day-old SPF chickens, contrasting with a significant increase in 17-day-old SPF chickens. This change is accompanied by a substantial alteration in the abundance of key bacterial genera, with the greatest reduction observed in *Lactobacillus* population, whose size decreased from 63% to 3%. These results underscore the 17th day of life as a pivotal moment in shaping the early microbial composition in SPF chickens. In contrast, Van der Wielen et al. (2002) observed minimal changes in the abundance of the gut microbiota in the duodenum and jejunum in broiler chickens at 11 days of age (Wielen et al., 2002). Amit-Romach et al. (2004) used a primer probe to demonstrate fluctuation in the chicken microbiota occurring from day 4 to 25 (Amit-Romach et al., 2004). In goats, Zhuang et al. (2020) identified early microbial changes in the jejunum and colon occurring between days 14 and 28 of age. Only one study has investigated the timing

of microbial changes in the gut of SPF chickens, which also occurred between the 14th and 28th day of life; however, no interim sampling was performed in that study (Xi et al., 2019).

In summary, the large fluctuations in the abundance of key bacterial phyla such as *Bacteroidota* and *Firmicutes* during the initial stages of gut microbiota development suggest that the 17th day of life is a critical turning point in the microbial composition of SPF chickens.

The colonisation of gut microbiota is a dynamic process that reaches a state of equilibrium over time, and is influenced by various factors, including environment, genetics, sex, and administered medications, which may cause differences in gut microbiota composition between individuals. In this study, we analysed the composition and changes of the gut microbiota in SPF chickens under normal rearing conditions. However, samples were collected randomly, and thus factors such as genetics, sex, and social hierarchy in the population could affect the dynamic process of gut colonisation by microbiota (Kers et al., 2018; Zeng et al., 2022).

Conclusions

The gut microbiota plays a significant role in overall health of the host, and its establishment during early life is crucial for subsequent microbial composition. Specific pathogen-free (SPF) chickens are indispensable in biomedical research and other fields. This study revealed that the period of first 17 days of life of SPF chickens is crucial for gut microbiota colonisation. Significant changes in the composition, diversity, and abundance of gut microbiota are evident during this phase, laying a foundation for the regulation of gut microbiota in early chick life and providing a theoretical basis for further investigations into gut microbiota dynamics.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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