

Article

Gut Microbiome Structural Dynamics in Japanese Quail Across Developmental Stages

Daniela da Silva Gomes ¹, Alexandre Lemos de Barros Moreira Filho ², Wydemberg José de Araújo ³, Gustavo Felipe Correia Sales ¹, Hemilly Marques da Silva ¹, Thalís José de Oliveira ¹, Antonio Venício de Sousa ¹, Celso José Bruno de Oliveira ¹ and Patrícia Emília Naves Givisiez ^{1,*}

¹ Department of Animal Science, Federal University of Paraíba (CCA/UFPB), Areia 58397-000, Brazil

² Department of Animal Science, Federal University of Paraíba (CCHSA/UFPB), Bananeiras 58200-000, Brazil

³ Federal Institute of Paraíba (IFPB), Princesa Isabel 58755-000, Brazil

* Correspondence: patriciagivisiez@gmail.com

Abstract

The cecal microbiota is essential for intestinal health and performance. This study describes the succession patterns of the cecal microbiota in Japanese quail (*Coturnix japonica*) until 42 days of age. Sixty quails were raised using standard conditions and fed corn–soybean meal diets. Cecal contents were sampled from five birds weekly from 7 to 42 days of age and submitted to Illumina 16S rRNA sequencing for metabarcoding analysis. Diversity and functional prediction were carried out with QIIME2, PICRUST2, STAMP and MicrobiomeAnalyst 2.0. *Firmicutes* increased from 50% at 7 days to more than 80% at 42 days, whereas *Bacteroidota* decreased from 45% to 12% in the same period. Alpha diversity progressively increased with age, indicating a richer and more balanced microbiota at later ages. Genera such as *Bacteroides* were predominant in the beginning and later were replaced by *Lachnospiraceae*, *Ruminococcus* and *Faecalibacterium*. These developmental taxonomic features aligned with significant shifts in ten metabolic pathways identified by prediction, revealing a transition from biosynthetic functions to complex carbohydrate metabolism and cell wall biosynthesis. The first seven days are considered a critical window for probiotics intervention, which may favor the establishment of a microbiota that is more stable and beneficial to quail performance.

Keywords: cecal microbiota; *Coturnix japonica*; metabarcoding



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1. Introduction

Quail production has an increasing role in egg and meat production in Brazil [1]. Knowledge about quail intestinal physiology, including host–microbiota interactions, is critical for production optimization and health [2]. Although microbiota composition and function have been studied in other production birds such as broilers, layers and turkeys, information on the microbial dynamics in quails, mainly related to different rearing phases, is scarce.

A detailed comprehension of how intestinal microbiota composition and activity affect quail development, nutrient absorption and response to sanitary challenges is essential. Notably, recent studies have shown that the intestinal microbiota in poultry show significant shifts in structural composition and diversity along rearing phases [3,4]. Such changes are intrinsically associated with the specific physiological changes and nutritional requirements of each phase from post-hatching until market or production age [5].

Birds have a shorter gastrointestinal tract compared to other production animals, and transit time is significantly faster, directly affecting gut microbiota composition and growth [6]. Microbiota development and stability are intrinsically dictated by the host and host–microbiota interaction becomes essential for gut morphology regulation, consequently affecting food digestion and nutrient absorption. The predominant phyla in the avian gut microbiota include *Bacteroidota*, *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Tenericutes* [7,8]. Independently of age or intestinal segment, *Firmicutes*, *Bacteroidota*, *Actinobacteria* and *Proteobacteria* are among the most abundant phyla in layers and turkeys [9,10].

In this sense, the ceca is the main fermentation site in the avian gut. Gram-positive bacteria represent more than 90% of the cecal microbiota [11], and the most predominant phyla are *Firmicutes*, *Bacteroidota* and *Actinobacteria* [12]. The succession of different bacterial groups in the cecum of quails in different ages suggests functional adaptations of the microbiota to host needs at each ontogenetic stage [13,14]. Furthermore, diet emerges as a major factor of microbiota modulation in quails of different ages [15–17]. Understanding these interactions is crucial for quail production improvement. Therefore, this study aim is to investigate the composition and functionality of the intestinal microbiota of Japanese quails in different phases of development.

2. Materials and Methods

All procedures were approved by the Ethics Committee on the Use of Animals (CEUA) of the Biotechnology Center of the Federal University of Paraíba (#6352050821) and are aligned with international guidelines for animal use. These guidelines, particularly those related to the 3Rs (Replace, Reduce, Refine) principles, are reflected in Brazilian regulations such as law no. 11.794/2008. Experimental procedures were carried out at the Center for Human, Social and Agricultural Sciences (CCHSA/UFPB), located in Bananeiras, Paraíba, Brazil, whereas laboratory analyses were carried out at the Center for Agricultural Sciences of UFPB (CCA/UFPB), Areia, Paraíba, Brazil.

One-day-old male and female Japanese quails (*Coturnix japonica*) were obtained from a commercial hatchery. The birds were individually weighed and distributed into five groups of 12 birds ($n = 60$) according to a completely randomized design. Birds were kept in galvanized cages (55 cm length $\times$ 55 cm width $\times$ 40 cm height) with wood shavings. Feed and water were provided ad libitum in tray or trough feeders (according to age) and pressure drinkers. Feeders and drinkers were cleaned daily, and cages were cleaned weekly to change the bedding material. Heating was provided by 250 W light bulbs and environmental temperature was lowered during rearing from 38 °C (day-old birds) to 22 °C (42 d-old birds). Relative humidity was between 65% and 70%, and the light cycle was 24L:0D until day 35 and 15L:9D afterwards. Corn and soybean meal diets were prepared according to Silva and Costa [18] (Table S1). One quail per cage was randomly selected at 7, 14, 21, 28, 35 and 42 days of age for sampling ($n = 5/\text{age}$). Following intraperitoneal injection with thiopental (150 mg/kg), the birds were slaughtered by exsanguination and opened longitudinally. The whole ceca were sampled and snap-frozen in liquid nitrogen, followed by storage at -80 °C until analysis.

The cecal contents were scraped with glass slides and DNA was extracted using a commercial kit (DNeasy PowerSoil, Qiagen, Hilden, Germany). DNA integrity was assessed by 1% agarose gel electrophoresis, followed by quantification using fluorometry (Qubit, ThermoFisher, Waltham, MA, USA) and all samples were standardized to 5 ng DNA/ μL according to Illumina guidelines (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf (accessed on 1 July 2025)). The 16S rRNA V3-V4 amplicon libraries were prepared using primers (F: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN

GGC WGCAG-3'; R: 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATCC-3') and identified using the Nextera XT Index Kit Set A (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The libraries were purified with magnetic beads (AMPure XP, Beckman, Indianapolis, IN, USA), following instructions provided with the kit. Library quality and size were evaluated using a 10,000 bp marker in a 5200 Fragment Analyzer (Agilent, Santa Clara, CA, USA). Paired-end sequencing was performed in a MiSeq using a V2 kit (2 × 250 cycles) following the manufacturer's instructions (Illumina, USA). Demultiplexed reads were obtained in fastq format and processed using QIIME2 [19]. Joined reads were selected by size (>240 bp) according to quality control using the PHRED score ($Q > 20$) and de-replicated using DADA2. Chimeras were verified and removed using UCHIME. Clustering was performed by the De Novo method with 99% similarity to obtain the ASVs (Amplicon Sequence Variants). The mean sequencing depth was 441,161 reads with a coverage of $1000\times$ per sample. The number of ASVs per sample was rarefied based on the sample with the fewest ASVs (3663) (Figure S1). Taxonomic classification was assigned using the Naïve Bayes method on the SILVA 138 database (<https://www.arb-silva.de/> (accessed on 1 March 2025)) with 99% for the V3-V4 region. The ASV table was used to predict the functional potential of the microbiome using PICRUST2 (version 2.4.2). The alpha and beta diversity analyses were performed using MicrobiomeAnalyst 2.0 [20].

Differences in alpha diversity indexes were determined with the Mann–Whitney / Kruskal–Wallis test, and beta diversity was compared with Permutational Multivariate Analysis of Variance using distance matrices based on the Bray–Curtis index. Comparative analyses of multiple groups to determine the differential abundance and the statistical difference in the functional prediction were performed using the STAMP statistical program (<https://beikolab.cs.dal.ca/software/STAMP> (accessed on 1 March 2025)) [21], the Farthest Neighbor method and the ANOVA statistical test with a significance level of 0.05 probability. Pathway analyses were performed by ANOVA with the Games–Howell post hoc test, a 95% confidence interval and the eta-squared effect size, with effect size at 0.05. Multiple-test correction was performed with Storey's FDR.

3. Results

3.1. Relative Abundance

The weekly assessment of the cecal microbiota taxonomic composition in Japanese quails evidenced a predominance of the phylum *Firmicutes* in all ages and increasing relative abundance from 50% at 7 days of age (d) to 80% at 42 d (Figure S2A and Table S2). Conversely, the second most abundant phylum, *Bacteroidota*, showed a significant reduction in relative abundance from 45% to 12% at the same ages. The abundance of the phylum *Proteobacteria* was relatively low in all ages (2%), with only a transitory increase in proportion to 6% at 14 days.

As for genera, *Bacteroides* was the most abundant genus initially and relative abundance decreased significantly with age (Figure S2B and Table S3). On the other hand, the genera *Lachnospiraceae*, *Sellimonas*, *Subdoligranulum*, *Butyricicoccus* and *Ruminococcus_torque_group* showed complex shifts in abundance with age. *Streptococcus* showed a relative abundance peak in 14-day-old quails.

3.2. Microbiota Diversity

At seven days, ASVs and Chao1 indices evidenced low microbial richness. Although richness increased progressively until 42 days, there were no differences between ages (Figure 1, Table S4). The Shannon index, which considers both richness and taxa equitability,

was greater at 42 days compared to that at 7 days ($p = 0.001$). The Simpson index also increased between 7 and 42 days of age ($p = 0.002$).

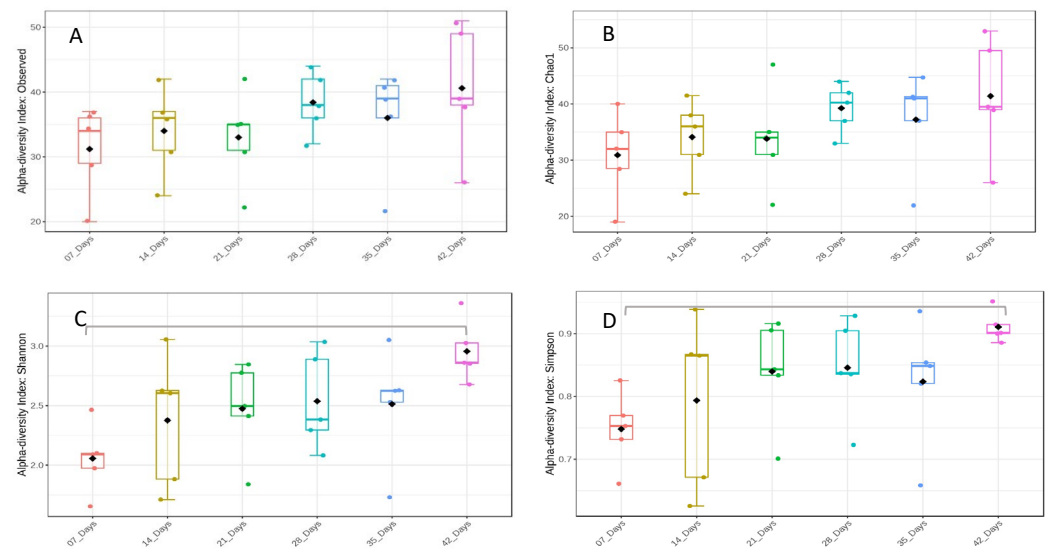


Figure 1. Alpha diversity indices of the cecal microbiota of Japanese quails from 7 to 42 days of age. Differences between 7 and 42 days are indicated. (A) Observed ASVs; (B) Chao1; (C) Shannon ($p = 0.001$); and (D) Simpson ($p = 0.002$).

Beta diversity showed a clear separation across different age groups (Figure 2) according to the main coordinate analysis (PCoA) based on the Bray–Curtis dissimilarity. There were differences between 7 and 14 days ($p = 0.014$), 7 and 35 days ($p = 0.012$), 7 and 42 days ($p = 0.008$), 14 and 42 days ($p = 0.013$) and 28 and 42 days ($p = 0.007$) (Table S4).

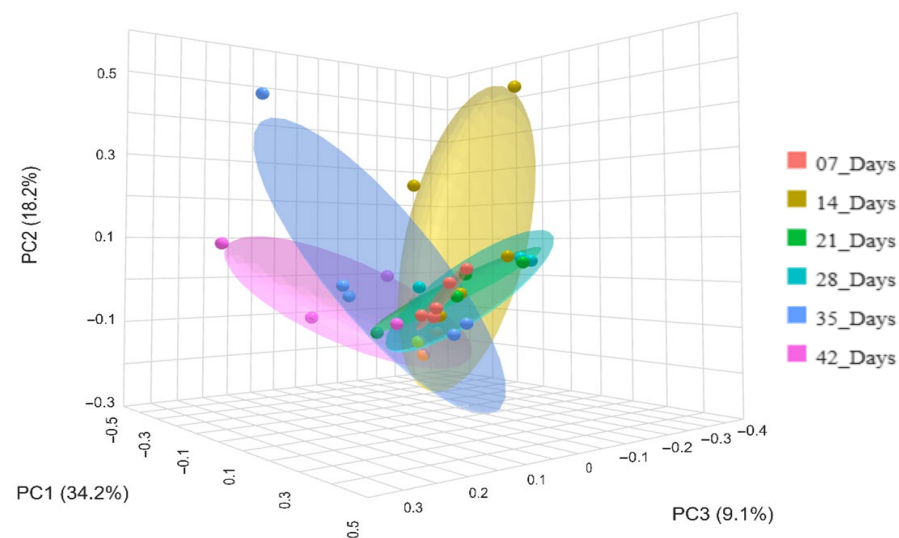


Figure 2. Beta diversity of the cecal microbiota of Japanese quails from 7 days to 42 days (Bray–Curtis).

3.3. Differential Abundance

Significant differential abundances were seen for sixteen bacterial genera (Figure 3A). The abundance of *Bacteroides*, *Enterococcaceae_unclassified*, *Ruminococcus_torques* and *Eggerthella* was greater in samples from 7-day-old quails (Figures S3–S6, respectively). *Bacteroides* relative abundance decreased ($p < 0.05$) between 7 days (ca. 45%) and 42 days (ca. 12%). The relative abundance of *Enterococcaceae_unclassified* was greater ($p < 0.05$) at 7 days compared with that at 21, 28 and 35 days (0.5% vs. <0.1%). *Ruminococcus_torques_group* relative abundance was greater at 7 days compared with that at 14 ($p < 0.02$) and 21 days

($p < 0.05$), phases of microbiota transition and establishment. *Eggerthella* abundance at 7 days (0.3%) was greater than any other ages ($p < 0.01$), with marked decrease and low representativity in later ages.

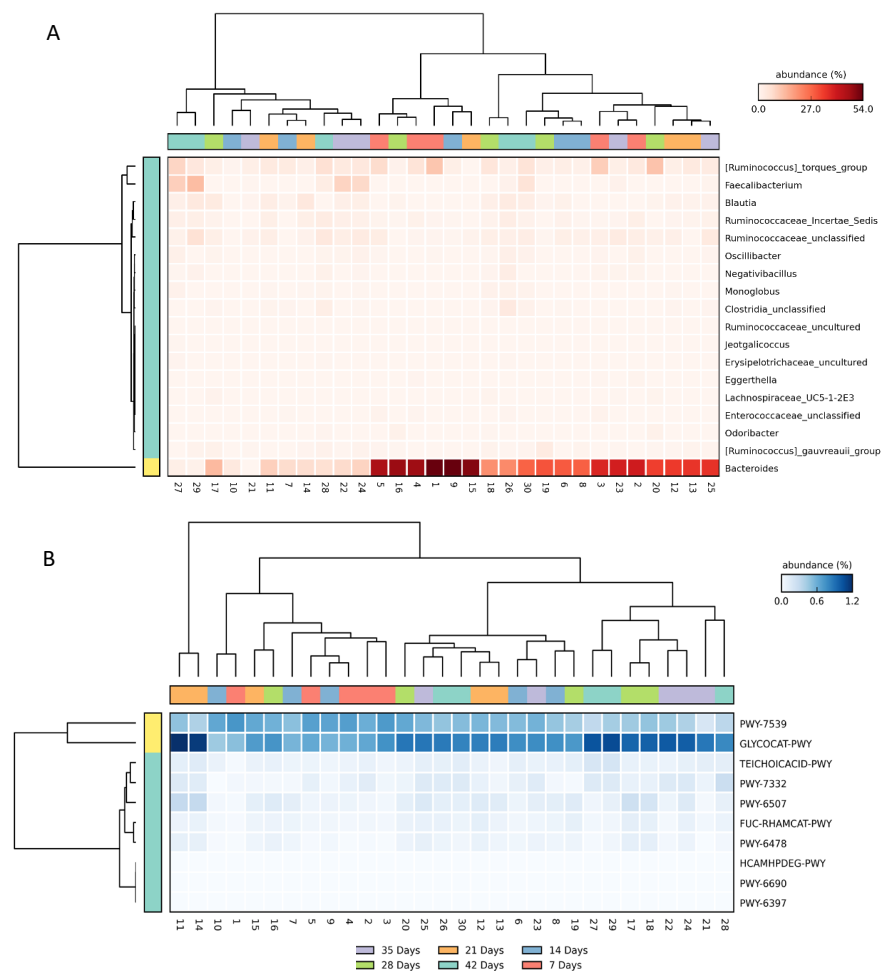


Figure 3. Differential abundance in the cecal microbiota of Japanese quails from 7 days to 42 days of age. (A) Genera. (B) Pathways.

During the rapid growth phase (14–28 days), there was an increase in the relative abundance of *Lachnospiraceae_UCG-1-2E3*, *Ruminococcus_gauvreauii_group* and *Odoribacter* (Figures S7–S9, respectively). *Lachnospiraceae_UCG-1-2E3* abundance was the greatest at 28 d compared with that at 35 ($p < 0.05$), 42 ($p < 0.02$) and 7, 14 and 21 days of age ($p < 0.01$). *Ruminococcus_gauvreauii_group* abundance increased significantly between 7 (0.099%) and 28 days (1.22%; $p < 0.05$). *Odoribacter* abundance also peaked at 28 days compared with that at 7, 14 and 21 days ($p < 0.05$).

At 35 and 42 days, taxa with greater abundances included *Faecalibacterium*, *Blautia*, *Oscillibacter*, *Negativibacillus*, *Monoglobus*, *Ruminococcaceae_incertain_sedis*, *Ruminococcaceae_unclassified*, *Ruminococcaceae_uncultured*, *Clostridia_unclassified*, *Jeotgalicoccus* and *Erysipelotrichaceae_unclassified* (Figures S10–S20). The abundance of *Faecalibacterium* was low but increased significantly, with values at 42 days higher compared with those at other ages ($p < 0.01$). *Blautia* abundance increased significantly ($p < 0.05$) between 7 (ca. 0%) and 42 days (ca. 2%). *Oscillibacter* abundance tended to increase with age, but it was greater at 42 days only when compared to that at 14 and 21 days ($p < 0.05$). *Negativibacillus* and *Monoglobus* abundances increased significantly at 42 days ($p < 0.05$ and $p < 0.01$) compared with those at other ages, when abundance was very low. The relative abundance profiles of the *Ruminococcaceae* family varied significantly with age, with an overall trend of increasing

at later stages but with specific distinct behaviors of different subtypes within this family. *Ruminococcaceae_incertae_sedis* abundance was higher at 42 days compared with that at 28 ($p < 0.02$) and 14 days ($p < 0.01$), respectively. *Ruminococcaceae_uncultured* showed an increase at 42 days compared with that at all other ages ($p < 0.01$). *Ruminococcaceae_unclassified* abundance was also higher in the later quail growth phase. The relative abundance of non-classified bacteria within the group *Clostridia_unclassified* increased between 7 and 42 days (0% vs. 1.4%, $p < 0.05$). *Jeotgalicoccus* showed an increase at 42 days compared with that at all other ages ($p < 0.01$), when abundance was very low. The abundance of *Erysipelotrichaceae_uncultured* was also greater at 42 days compared with that at other ages ($p < 0.01$ for 7, 21 and 28 days and $p < 0.02$ for 14 and 35 days).

Functional prediction analysis identified ten differentially abundant ($p < 0.05$) metabolic pathways according to age (Figures 3B and S21–S30). Pathways with higher abundance in the ceca of 7d old compared to 42 d old birds (Figures S21–S23) included 6-hydroxymethyl-dihydropterin diphosphate biosynthesis III (PWY-7539), 3-phenylpropionate and 3-(3-hydroxyphenyl)propionate degradation (HCAMHPDEG-PWY) and Cinnamate and 3-hydroxycinnamate degradation to 2-hydroxypentadienoate (PWY-6690). At 21 and 28 days of age (Figures S24–S26), pathways with higher abundance compared to those at 7 and 14 days were 4-deoxy-L-threo-hex-4-enopyranuronate (DTHE) degradation (PWY-6507), the superpathway of fucose and rhamnose degradation (FUC-RHAMCAT-PWY) and GDP-D-glycero- α -D-manno-heptose biosynthesis (PWY-6478). The relative abundance of the pathway glycogen degradation I (Glycocat-PWY) was higher at 21, 35 and 42 days compared to that at 7 and 14 days of age (Figure S27). In 42-day-old birds, the pathway Poly (glycerol phosphate) wall teichoic acid biosynthesis (Teichoicacid-PWY), the superpathway of UDP-N-acetylglucosamine-derived O-antigen building block biosynthesis (PWY-7332) and Mycolyl-arabinogalactan-peptidoglycan complex biosynthesis (PWY-6397) showed more relative abundance (Figure S28–S30).

4. Discussion

The initial colonization of the commensal gastrointestinal microbiota is critical for homeostatic maintenance in newly hatched birds. The temporal analysis of Japanese quail cecal microbiota from 7 to 42 days post-hatch revealed distinct phases of microbial community maturation characterized by progressive shifts in taxonomic composition and functional capacity, with the gradual enrichment of species and abundance uniformity. The consistent evolution pattern of microbial richness and the transient dominance peaks observed in intermediate ages highlight the dynamic nature of microbial succession, during which events of selective proliferation or colonization may happen in specific moments [22]. The dendrogram produced with the similarities of bacterial abundance profiles showed the tendency of clustering by sampling day (Figure 3), and the distinct patterns associated with the different quail development phases corroborate the temporal progression in the cecal microbiota composition.

The early post-hatch period (7d) was characterized by significantly reduced alpha diversity and distinct microbial composition, with the dominance of *Bacteroides* and *Enterococcaceae*, indicative of an unstable colonization state with limited functional repertoire. This microbial profile, typically associated with a low abundance of beneficial taxa including *Lactobacillus* and *Bifidobacterium* [23], predisposes birds to increased susceptibility to enteric disorders. Crisol-Martínez et al. [23] demonstrated a positive correlation between elevated *Bacteroides* abundance and disease incidence in quails, recommending probiotic intervention during the initial seven days post-hatch. Given the established positive association between high microbial diversity and host health, conversely with low diversity correlating with pathological states and dysbiosis [24,25], the observed low microbial abundance in

Japanese quails in the early post-hatch period could be associated with compromised gut histological features, including reduced villus height and decreased crypt depth [17]. Furthermore, germ-free Japanese quail showed significantly lower emotional reactivity until 14 days of age, corroborating the existence of an intestinal microbiota–brain axis in birds and the importance of microbiota establishment in bird behavior and well-being [26].

The taxonomic profile aligns with the increased abundance of the folate biosynthesis pathway (PWY-7539) observed at 7 days, as *Bacteroides* species are well-known producers of B-vitamins, including folate (vitamin B9), which are crucial for early post-hatch development [27]. The high abundance of *Bacteroides* in young quail parallels observations in other avian species, where early colonization by Gram-negative bacteria provides essential metabolic functions before the establishment of more complex communities [28].

The concurrent increase of aromatic compound degradation pathways, including 3-phenylpropionate, 3-(3-hydroxyphenyl) propionate degradation (HCAMHPDEG-PWY) and cinnamate degradation (PWY-6690) at 7 days, suggests the active microbial processing of plant-derived phenolic compounds present in the early diet or environment. Although there is a scarcity of studies in avian species, these pathways are typically associated with the breakdown of lignin-derived compounds and other aromatic metabolites, potentially contributing to detoxification processes or the generation of bioactive metabolites that may influence mammal host physiology [29]. The genus *Eggerthella*, with relatively high abundance at 7 days of age, is associated with the metabolism of plant-derived compounds and may contribute to the observed aromatic compound degradation activity [30]. This genus has been previously implicated in autoimmune pathogenesis in immunocompromised poultry through the activation of Th17 cell populations via microbial metabolites, ultimately triggering excessive inflammatory cascades and immune dysfunction [31,32].

The higher abundance of *Ruminococcus torques_group* at 7 days compared to 14 and 21 days suggests its role as an early colonizer. This genus is associated with protein fermentation [29] and may contribute to amino acid metabolism during the initial stages of gut development, supporting the intense protein synthesis requirements in the fast growth phase of birds.

The co-occurrence of *Enterococcaceae* and *Streptococcus* reinforce the transitional nature of the early microbiota, consistent with progressive increases in both microbial richness and evenness at later ages and the gradual expansion of *Firmicutes* as ecosystem stabilization progresses. This gastrointestinal microbiota maturation and associated diversity enhancement reflects multiple developmental factors including metabolic functional transitions, evolving host–microbe immunological interactions, dietary diversification and environmental adaptations [33]. The upregulation of carbohydrate degradation pathways, particularly DTHE degradation (PWY-6507) and fucose and rhamnose degradation (FUC-RHAMCAT-PWY) at 21–28 days coincided with the increased abundance of *Lachnospiraceae*, primary degraders of plant cell wall components for the breakdown of complex polysaccharides [34]. This finding possibly reflects adaptation of the microbiota to processing complex polysaccharides in the feed. The significant increase in *Ruminococcus gauvreauii_group* from 0.099% at 7 days to 1.22% at 28 days represents a 12-fold increase that coincides with enhanced carbohydrate processing capacity. This genus is particularly efficient at degrading recalcitrant plant polysaccharides and likely drove the observed increase in DTHE degradation activity [35]. A similar pattern was observed for *Odoribacter*, also capable of enhanced carbohydrate fermentation, as this genus is associated with the production of short-chain fatty acids from complex carbohydrates [36].

The persistence of GDP-D-glycero- α -D-manno-heptose biosynthesis (PWY-6478) during the 21–28 day period suggests that specific Gram-negative populations remain metabolically active during this transitional phase. This pathway is associated with lipopolysac-

charide biosynthesis and may indicate the presence of potentially pathogenic bacteria that have not yet been outcompeted by beneficial commensals, consistent with the incomplete stabilization of the microbiota during this developmental window [37].

The mature cecal microbiota at 42 days showed a remarkable shift toward Gram-positive, fiber-fermenting bacteria, with significant increases in *Faecalibacterium*, *Blautia*, *Oscillibacter* and multiple *Ruminococcaceae* taxa. This taxonomic transition directly supports the observed elevation of cell wall biosynthesis pathways and enhanced energy metabolism. The emergence of *Faecalibacterium* at 42 days is particularly significant, reflecting the enhanced availability of complex fibrous substrates and establishment of robust butyrate biosynthetic pathways. Butyrate represents a critical metabolite for cellular energetics and microbiota homeostasis. It exerts potent anti-inflammatory effects through the inhibition of nuclear factor- κ B (NF- κ B) signaling, a master regulator of inflammatory responses [38,39]. Furthermore, this short-chain fatty acid reinforces intestinal barrier integrity, particularly tight junction functionality, thereby maintaining selective permeability and preventing the translocation of luminal toxins and pathogenic microorganisms [40]. Butyrate also directly stimulates goblet cell mucin secretion, enhancing the protective mucus barrier [41]. Therefore, *Faecalibacterium* genus is considered a marker for healthy gut microbiomes in both commercial poultry [33,42] and humans [43]. The concurrent elevation of teichoic acid biosynthesis (Teichoicacid-PWY) at 42 days likely reflects the increased abundance *Faecalibacterium* and other Gram-positive beneficial commensals. Moreover, its low initial abundance followed by a significant increase at 42 days suggests that *Faecalibacterium* requires a mature, stable microbial environment to establish successfully. Additional significant butyrate producers include *Oscillibacter* and *Clostridia* species [44].

The *Ruminococcaceae* family encompasses genera including *Blautia*, a recently established genus derived from taxonomic reclassification of select *Ruminococcus* species [45] and *Ruminococcus*, both characterized by robust short-chain fatty acid production capacity. The increased abundance of *Blautia* from essentially zero at 7 days to 2% at 42 days, represents one of the most significant taxonomic shifts observed. *Blautia* species are important butyrate producers and are associated with the degradation of complex carbohydrates [46]. Their establishment in the gut likely contributes to the enhanced glycogen degradation activity (Glycocat-PWY) observed at later ages, as this genus can utilize various carbon sources for energy production. This age-associated abundance increase is supported by studies across multiple species including swine, rodents and broiler chickens [47–49]. Therefore, the results suggest that *Blautia* might not be a primary colonizing genus but rather establishes and proliferates along with microbiota development and dietary diversification. These organisms provide essential energy substrates for enterocytes while modulating inflammatory responses and facilitating dietary cellulose digestion [46,50,51].

The diverse *Ruminococcaceae* populations that increased at 42 days (*Ruminococcaceae_incertain_sedis*, *Ruminococcaceae_uncultured*, *Ruminococcaceae_unclassified*) represent a mature fiber-fermenting community capable of processing complex dietary substrates. *Negativibacillus*, recently characterized within the *Ruminococcaceae* family [52], associates with mature intestinal ecosystems. Despite the recognition of its presence in developed host microbiomes, the comprehensive understanding of its metabolic and ecological functions remains limited [53]. The variation in the relative abundance profiles of the *Ruminococcaceae* family according to age indicate an increase in the abundance of family members that could not be identified at the genus or species levels. The variation may reflect the diversity of many species within the family. For example, since *Ruminococcus_gauvreauui_group* abundance was greater only at 28 d, the absence of other differences suggests a possible importance during microbiota transition from the initial phase to the rapid growth phase.

Monoglobus pectinilyticus, the sole characterized representative of the genus *Monoglobus*, demonstrates specialized pectin degradation capabilities, targeting this complex plant cell wall polysaccharide [54]. The delayed establishment of *Monoglobus* likely reflects progressive dietary complexity introduction, particularly in fiber-rich components requiring specialized enzymatic machinery for efficient catabolism. Optimal polysaccharide degradation efficiency is fundamental for maximizing host dietary energy extraction [55]. Similarly, uncultured *Erysipelotrichaceae* harbor genes encoding pyruvate metabolism enzymes, including ferredoxin oxidoreductase [56] and 4-hydroxyphenylacetate decarboxylase [57]. These enzymatic capabilities suggest active participation in carbon compound degradation and transformation, contributing significantly to energy metabolism [58]. *Jeotgalicoccus* was first reported as an abundant member of the family *Staphylococcaceae* in chicken, turkey and duck house air [59]. Later, this genus was reported in the respiratory tract [60] and gut of broilers [61,62], as well as poultry litter [63], eggshell [64] and excreta or poultry house dust [65]. *Jeotgalicoccus* in the present study was initially low and increased with age in the gut of Japanese quails, suggesting a specific role of this genus in the microbiota of older quails. *Jeotgalicoccus* also increased in the feces of female quails 24 h after being treated with a low dose of trichlorfon residue, evidencing the immediate effects of this widely used organophosphate insecticide in gut microbiota [24]. The apparent wide distribution in the poultry rearing industry may raise some concerns in terms of occupational hazard [59,66]. Furthermore, the increased *Jeotgalicoccus* abundance in dust or the excreta of broilers in low-performance farms [56] warrant further investigation of this genus in quail production systems.

While the scarcity of the literature on quail microbiota limits discussion on practical applications, the taxonomic characterization and functional pathway changes, particularly the shift from biosynthetic functions to complex carbohydrate metabolism and cell wall biosynthesis, seem to reflect universal principles of gut microbiota development across avian species. However, the specific timing and magnitude of these changes may be influenced by factors such as diet composition, housing conditions and genetic background, highlighting the importance of species-specific microbiota research [67]. Furthermore, Lyte et al. [68] propose that Japanese quail should be used as an avian model to understand how the microbiome affects host neuroendocrine system responses to stress. In this sense, our study provides a foundation for future research applications in commercial quail production or exploitation of quails as animal models.

5. Conclusions

The Japanese quail cecal microbiota undergoes systematic maturation from an initially species-poor, potentially pro-inflammatory community toward a stable, functionally diverse consortium enriched in beneficial short-chain fatty acid producers associated with optimal intestinal health. These results support the hypothesis that targeted early interventions, particularly probiotic supplementation, may effectively mitigate initial microbial imbalances and accelerate the establishment of a functionally beneficial microbiota with consequent improvements in zootechnical performance and overall avian health. A comprehensive understanding of these developmental microbial processes is essential for formulating evidence-based nutritional and management strategies that optimize intestinal health and enhance productivity in commercial poultry operations.

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