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Dietary polyunsaturated fatty acids effect on cecal microbiome profile of maturing broiler chicken

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ABSTRACT

Diet has been reported to impact the diversity and function of gut microbiota. Our study investigated the effect of dietary fat types on cecal microbial composition and predicted function in broiler chickens at days 41 and 55 of age. Four dietary fat sources were evaluated and compared to a control dietary fat source of poultry fat. These were for two diets rich in omega-3 polyunsaturated fatty acids (PUFA) - fish oil and flaxseed oil, a diet rich in long-chain saturated fatty acid (SFA) - lard, and a diet rich in medium-chain saturated fatty acid - coconut oil. At day 55, broiler chickens fed a PUFA-rich diet maintained cecal microbial diversity while broiler chickens fed a SFA-rich diet exhibited a significant reduction in diversity compared to the control diet-fed chickens. More specifically, PUFA intake was associated with elevated levels of microbial carbohydrate metabolizing capability, contributing to efficient energy utilization and enhanced short-chain fatty acid production capability. In contrast, SFA-rich diets lowered abundances for key microbial families like *Lachnospiraceae* and *Bifidobacteriaceae* hampering nutrient digestibility and pathogen resistance. The microbiomes for chickens fed lard and coconut oil diets showed a significant reduction in SCFA-producing microbial taxa abundance while the microbial functional profile indicated reduced carbohydrate metabolism. Our findings underscore the contrasting effects of SFA-rich fat and PUFA-rich fat on the cecal microbiota of broiler chickens. The results suggest that incorporating PUFA-rich dietary fats into broiler feed may offer potential benefits by modulating the cecal gut microbiota toward outcomes associated with elevated carbohydrate utilization without hampering nutrient digestibility and pathogen resistance.

Introduction

Broiler chickens are among the fastest-growing livestock and serve as a major source of affordable animal protein worldwide (Global Chicken Market Size by Production, 2024). Dietary fats are a crucial component of poultry nutrition, helping to meet the birds' high energy demands and improving meat quality. Commonly used fats in poultry feed include animal fats such as lard, tallow, and poultry fat and vegetable oils like soybean, maize, and flaxseed oil (Baião and Lara, 2005; Ravindran et al., 2016). These fats vary in their fatty acid composition and can influence the animal's metabolic, inflammatory, and disease state via gut microbiome-mediated mechanisms (Caesar et al., 2015; Schoeler et al., 2023). The gastrointestinal (GI) tract of chicken harbors trillions of bacteria, whose composition changes dynamically with age and environmental exposure (Apajalahti and Kettunen, 2006; Kubasova et al., 2019; Mandal et al., 2020; Pan and Yu, 2014). It is well established that a balanced growth of beneficial gut microbes promotes pathogen resistance, efficient nutrient digestion and absorption, enhanced gut integrity, and improved immunity (Kogut, 2019; Carrasco et al., 2019). These fats also enhance gut microbial diversity and gut health (Nain et al., 2012; Schoeler et al., 2023). Different types of saturated fats have distinct effects on broiler health. For instance, the inclusion of lard in poultry feed has been linked to an enrichment of pathogenic *Clostridium perfringens* in the gut (Knarreborg et al., 2002). However, coconut oil, which is rich in medium-chain fatty acids, is considered beneficial due to its efficient absorption and oxidation, leading to improved antioxidant status, lipid profiles, immune responses, and growth performance in broiler chickens (Attia et al., 2020; Ferreira et al., 2014). Additionally, the inclusion of coconut oil or other sources of medium-chain fatty acids in broiler feed has been shown to improve intestinal villus histology and enrich cecal members of the *Ruminococcaceae* family and *Faecalibacterium* species. Such alterations in gut microbial composition can also

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affect microbial functions. Saturated fat diets have been found to have specific effects on carbohydrate and amino acid metabolism (Wu et al., 2021). Therefore, maintaining a balanced gut microbiome requires a thorough understanding of how different dietary fat sources affect gut microbial architecture and host physiology. Most research on dietary fats in broiler chickens has primarily focused on improving the nutritional quality of edible products and evaluating broiler performance. However, it is essential to assess these physiological effects in the context of gut microbial composition and functional dynamics, considering their potential health benefits for the host. Therefore, the present study simultaneously examined the effects of dietary PUFA-rich flaxseed oil and fish oil, and SFA-rich lard and coconut oil on broiler cecal microbial composition using shotgun metagenomic sequencing across day 41 and day 55 ages of the broilers. Our previous attempt to study the effect of PUFA / MUFA (Monounsaturated Fatty Acid)/ SFA rich fat sources on broiler cecal microbial composition using 16S rRNA gene sequencing reported a favorable microbial shift for PUFA fats (Jadhav et al., 2024). The present study further explores this effect and, in addition, investigates microbiome function.

Materials and methods

Animals and dietary treatment

Animal care and use procedures were approved by the Institutional Animal Care and Use Committee of North Carolina Agricultural and Technical State University (IACUC #20-004.0) before the commencement of the study. The five different diets analyzed for this study each contained their specific dietary fat source at a 3 % level: conventional corn-soybean meal (SBM) control diet containing poultry fat (CN), and then also corn-SBM basal into which lard (LA), coconut oil (CC), fish oil (FI), or flaxseed oil (FL) were added. The manufacture and fatty acid composition of each dietary fat source are as described in Jadhav et al. (2024). Chicken husbandry and sampling procedures were described in detail by Omaliko et al. (2024). In summary, Chicks were given starter diets (as crumbled pellets) from one-day-old to 3 weeks (days 1-21, Supplementary Table 1) followed by a grower diet until 6 weeks (days 22-41, Supplementary Table 2) and then a finisher diet fed from 6 weeks to 8 weeks (days 42-56, Supplementary Table 3). At days 41 and 55 of age, chickens were randomly taken from each treatment group (one bird per replicate) and were humanely euthanized using CO2 asphyxiation. For day 41 and day 55, the cecal content of five and ten chickens were sampled respectively for each diet regarding subsequent shotgun metagenomic sequencing and analysis (except for the fish diet at day 41, for which there were only four samples processed).

DNA extraction and shotgun metagenomic sequencing

For genomic DNA extraction, 200 mg of cecal contents was utilized. The frozen cecal content was slowly thawed on ice and DNA extraction was carried out using the QIAamp PowerFecal Pro DNA Kit (Qiagen Inc., United States), following the manufacturer's protocol (Emami et al., 2020; Lu et al., 2020). This protocol includes mechanical (bead beating) and enzymatic steps for maximum recovery of microbial genomic DNA. The extracted DNA was assessed for quality (A260/A280 and A260/A230) and quantity using the DeNovix DS-11 Series instrument and QuantiFluor® dsDNA System (Promega) respectively. The extracted DNA was stored at −20°C until further use. 74 cecal samples were sequenced for shotgun sequencing using Illumina NextSeq2000 run in 2 × 150 bp paired end reads utilizing P3 reagents. Sequencing was done by Argonne National Laboratory.

Bioinformatics analysis

The raw sequencing data obtained from the sequencing center ranged from 925,688 – 41,959,446 reads per sample. Raw reads were initially quality controlled using the FastQC v0.12.1 tool. This was followed by processing through the KneadData v0.12.0 tool to trim off the adapter and low-quality region. KneadData was also used to remove reads aligning with the chicken genome (breed: Ross). Read pairs that had a length lower than 75 bases were also discarded. For taxonomic classification, the processed high-quality paired reads were classified using Kraken2 version 2.1.3 (Lu et al., 2022). Kraken2 was run to map reads against the pre-build NCBI standard plus RefSeq reference database (released Jan. 2024) containing genomes from archaea, bacteria, viruses, protozoa, and fungi with a 0.1 confidence threshold. Kraken2 report files generated for all samples were combined to create a biom file using kraken-biom utility. Further microbial analysis was carried out using R version 4.4.1 and Phyloseq package version 1.48.0 (McMurdie and Holmes, 2013). Taxonomic abundances were normalized to the central log ratio were used for statistical analysis that includes the non-parametric Kruskal–Wallis test followed by Dunn's test for multiple comparisons. Taxonomic counts having two or more related short reads and present in more than 5 % of samples were retained for analysis. For a potential functional classification, HUMAnN3 (version 3.9) was used (Beghini et al., 2021). The concatenated metagenomic paired-end sequences were run through the HUMAnN3 pipeline with default parameters. The full ChocoPhlAn pangenome database (release 2019.01) was used for functional pathway abundance and coverage determination, whereas the UniRef90 database (release 2021.03) was used for gene family abundance determination. Unstratified or community-level pathway and gene family data were used for the analysis. The output pathway (MetaCyc) and gene family abundance files for each sample were joined and then normalized to counts per million (cpm). Finally, gene families were regrouped for the study of functional subsystems based on community-level GO (Gene Ontology) and normalized to counts per million before analysis.

Statistical analysis

For exploratory taxonomic analysis, data were transformed to relative abundance and analyzed using R version 4.4.1 and Phyloseq package version 1.48.0. Venn diagrams were made using VennDiagram version 1.7.3 package. Alpha diversity of the samples was calculated for Shannon and Simpson diversity indices using the Phyloseq package function estimate richness. The significant effect of dietary treatments on central log-ratio transformed abundances for each taxa between ≥ 2 groups were studied using the Kruskal-Wallis rank sum test with Dunn's multiple comparison test. The p values were corrected for multiple testing by Benjamini-Hochberg correction to control the false discovery rate, considering p adjusted ≤ 0.05 as significant. The OTU level principal coordinate analysis (PCoA) was performed using Aitchison and Bray-Curtis distance measures with the ecodist R package. Beta diversity was estimated using the Aitchison distance to analyze compositional differences among the treatment groups using non-parametric multivariate ANOVA based on dissimilarities (adonis2) with 999 permutations package used vegan (version 2.6-4). Pairwise comparison for the compositional difference between the groups estimated using pairwise Adonis package (version 0.4.1) and pairs with p adj < 0.25 considered significant. Taxonomic differential abundance analysis was performed using CLR-normalized data using the non-parametric Kruskal-Wallis rank sum test followed by the Dunn test with Benjamini-Hochberg adjustment. Significance was for p-adjusted < 0.05. Microbiome Multivariable Associations with Linear Models (MaAsLin2 version 1.18.0) analysis method with Benjamin–Hochberg correction method (Mallick et al., 2021) was used to study differential abundance for pathways and GO terms, while keeping the control group as a reference. For MaAsLin2 results, $p < 0.05$ and $q < 0.25$ were considered significant.

Results and discussion

Body weight, feed consumption, and feed conversion ratio for all the

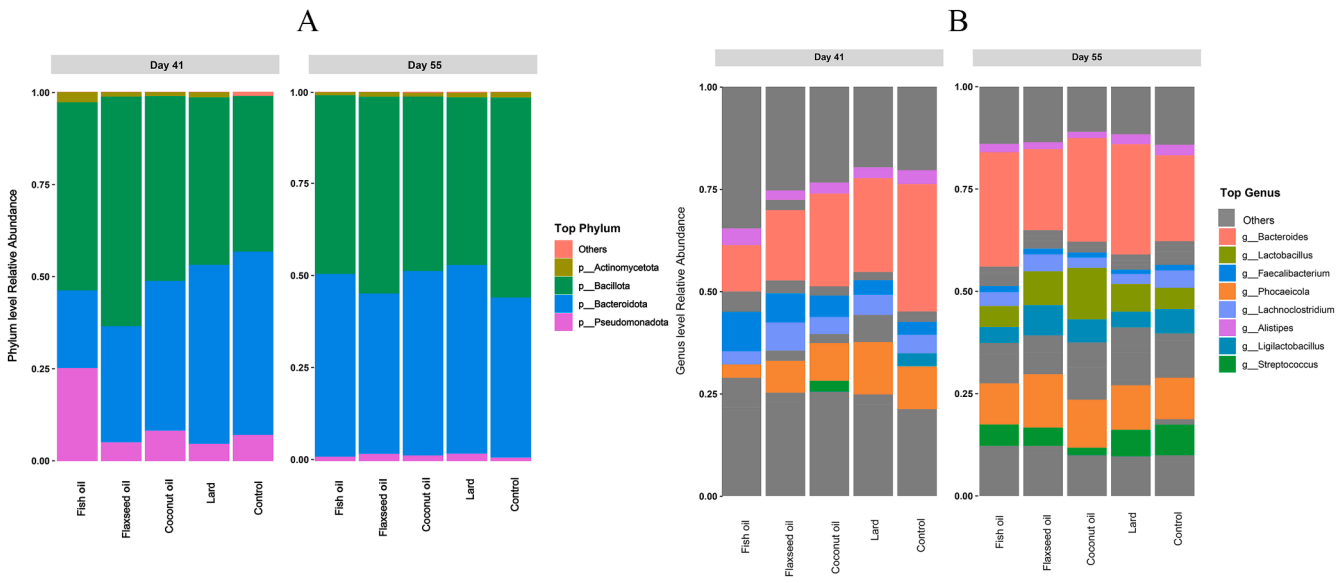

Fig. 1. Relative abundance plots displaying cecal microbial profile for day 41 and day 55 old broilers across the treatment groups. (A) Relative abundance plot for high abundant phylum; (B) Relative abundance plot for high abundant genus; The left panel profiles Day 41: Fish oil ($n = 4$), Flaxseed oil ($n = 5$), Coconut oil ($n = 5$), Lard ($n = 5$), and Control ($n = 5$); The right panel profiles Day 55: Fish oil ($n = 10$), Flaxseed oil ($n = 10$), Coconut oil ($n = 10$), Lard ($n = 10$), and Control ($n = 10$).

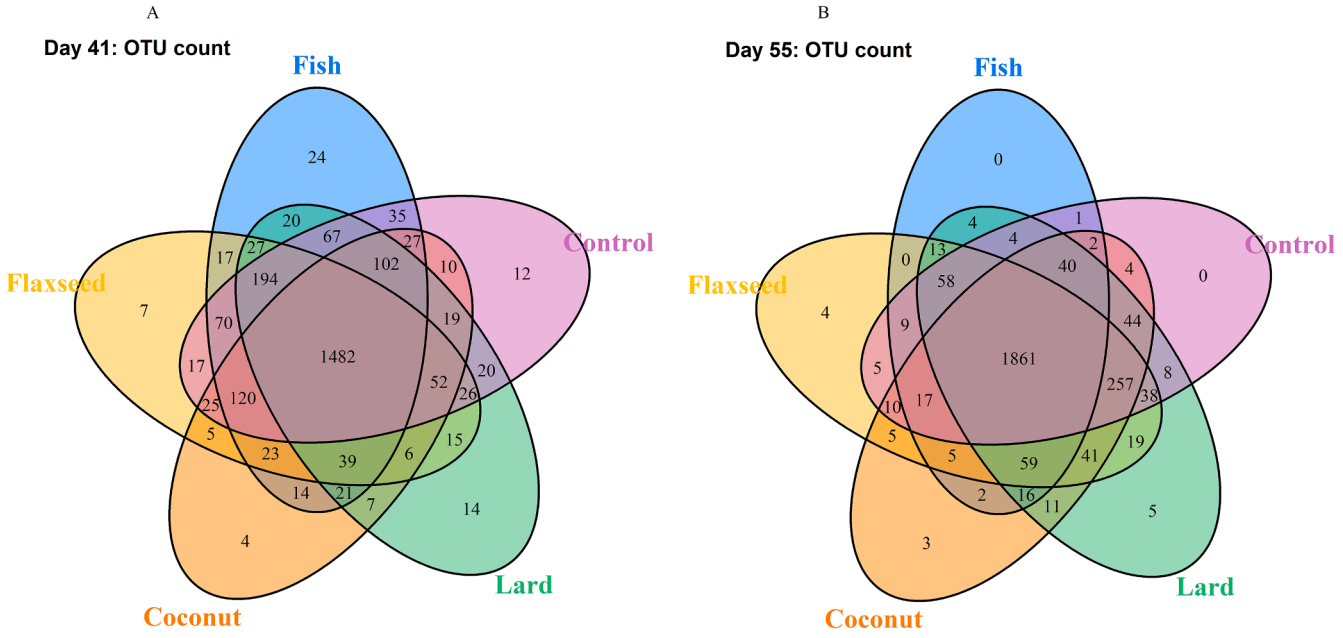

Fig. 2. Venn diagram showing shared and unique OTU taxa across dietary treatment groups. (A) Day 41 with 1482 (58.7 %) shared OTUs across treatment groups with the fish oil group having the highest number of unique OTUs; (B) Day 55 with 1861 (73.1 %) shared OTUs across treatment groups having fewer unique treatment group OTUs found overall in comparison to Day 41.

treatment groups are described in Supplementary Table 4. There was no difference in body weight, body weight gain, and feed conversion ratio among treatment and control diet fed chickens.

Cecal microbial diversity was predominantly composed of the bacterial kingdom, accounting for 8040 OTUs (91.9 %). In contrast, other microbial groups, including viruses, archaea, and eukaryotes, contributed 310 taxa (3.5 %), 273 taxa (3.1 %), and 127 taxa (1.4 %), respectively.

Cecal bacterial community profile

We examined the distribution of different bacterial taxonomic ranks between day 41 and day 55 across treatment groups. At both time points, the cecal bacterial community was dominated by the phylum *Bacillota* (*Firmicutes*) and *Bacteroidota* (*Bacteroidetes*). These were followed by the phylum *Pseudomonadota* (*Proteobacteria*) (Fig. 1A). Across the treatment groups, 630 bacterial families were identified, with the *Bacteroidaceae* family dominating, followed by *Lactobacillaceae, Lachnospiraceae, Oscillospiraceae*, and *Tannerellaceae* (Supplementary Fig. 1A and B). Additionally, 1905 unique genera and 5802 unique bacterial species were identified across the 74 chicken cecal samples. Relative abundance levels for the top genus are shown in Fig. 1B. The highlighted set of top genera between day 41 and day 55 chicken cecal microbes indicates increased relative abundance for *Lactobacillus, Ligilactobacillus*,

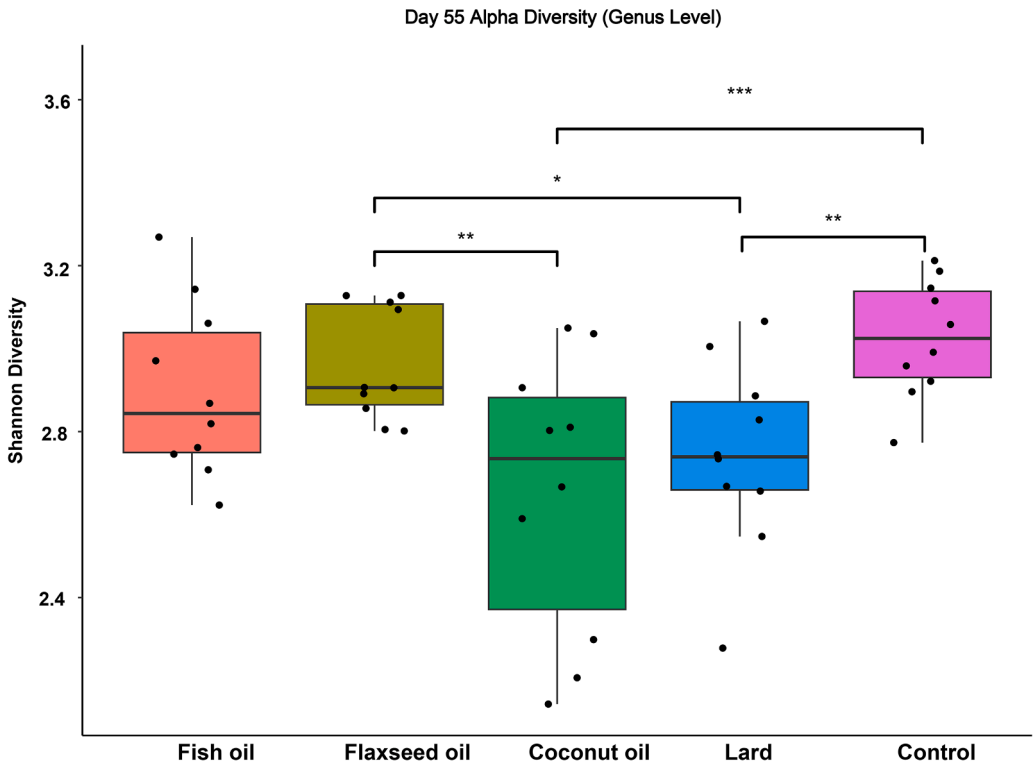

Fig. 3. Day 55 genus-level alpha diversity across treatment diets. Shannon indices were calculated and tested for statistical significance using Kruskal-Walli's rank sum test ($p = 0.0055$) followed by the Dunn test with Bonferroni adjustment. p adj: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001.

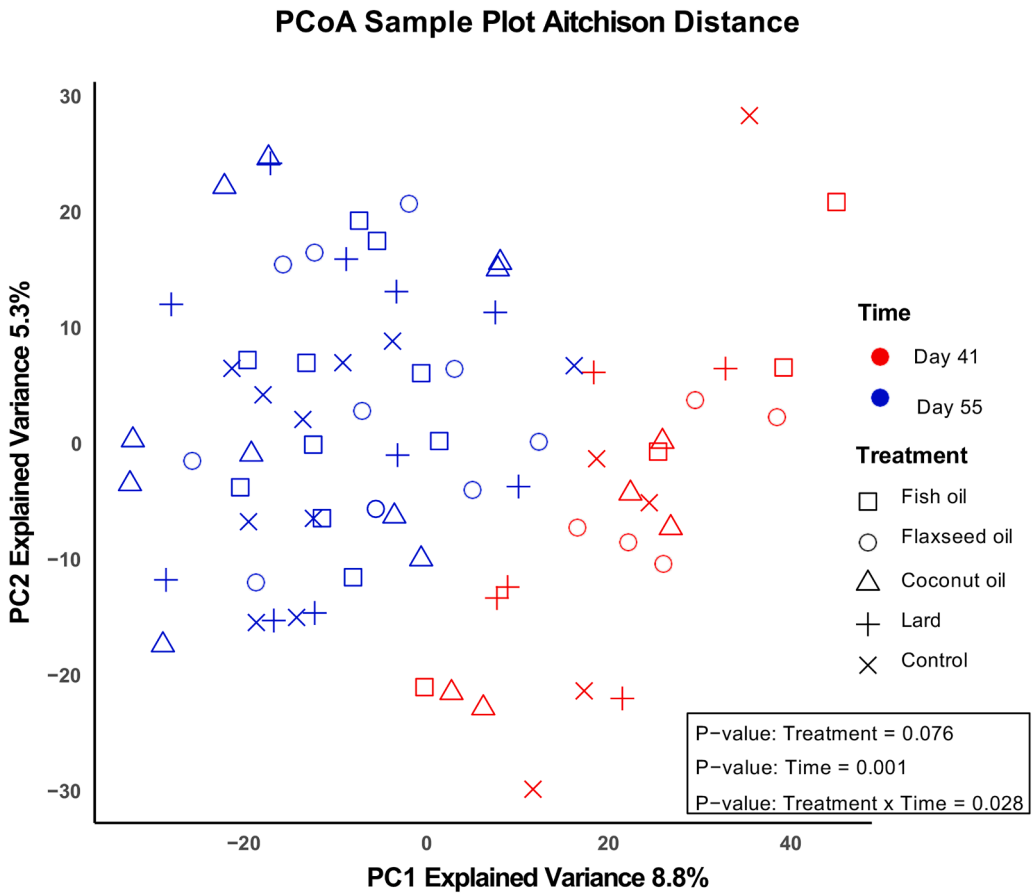

Fig. 4. Principal coordinate analysis using Aitchison distances. Beta diversity is depicted by principal-coordinate analysis (PCoA) at the OTU level. Each dot represents one sample from each treatment group.

Table 1
Permutational multivariate analysis of variance (PERMANOVA).

Factors	Degree of freedom	Aitchison distance	
		pseudo-*F*	*p*-value[a]
Treatment	4 (CC, CN, FI, FL, LA)	1.13	0.075†
Time	1 (day 41, day 55)	6.58	0.001***
Treatment × Time	4 (CC, CN, FI, FL, LA)	1.19	0.015*

CC: Coconut oil; CN: Control; FI: Fish oil; FL: Flaxseed oil; LA: Lard.
[a] *p* value.
* ≤ 0.05 ** ≤ 0.01; ≤ 0.001.
† $p \leq 0.10$.

and *Streptococcus* species on day 55 compared to day 41. We then explored the relationship between all OTU taxa and their treatment diet specificity using Venn diagram (Fig. 2). 58.7 % of OTUs on day 41 and 73.1 % of OTUs on day 55 were shared across all treatment groups. Additionally, treatment-specific taxa were found to decrease on day 55 compared to day 41 (Fig. 2). In addition, the OTU level Venn diagram comparison between day 41 and day 55 indicates more unique OTUs between treatment groups for day 41 compared to day 55, while day 55 has a high number of shared taxa to be found between treatments.

The age of the chicken is a known factor that influences gut microbial composition (Awad et al., 2016; Shang et al., 2018). As a chicken age, the gut microbiome undergoes notable shifts to support heightened energy metabolism and more efficient nutrient processing (Li et al., 2022; Lu et al., 2003; Yang et al., 2022) in addition to some stabilization of microbial diversity (Lan et al., 2005; Xiao et al., 2021). Our study observed a distinct shift in the cecal microbial community structure between days 41 and 55, including for increased abundance of the *Lactobacillaceae* family on day 55 indicating some progression towards a more stabilized microbial state. This shift in microbial composition is attributed to the age as well as the change in dietary regime from the grower diet to the finisher diet phase. The finisher diet fed between day 42 to day 56 has a lower protein content compared to the grower's diet fed between day 21 to day 42. This change in the dietary phase may reflect reduced abundance for protein-utilizing microbial species from *Clostridiaceae* and *Erysipelotrichaceae* family at day 55. However, this effect of diet on microbial composition may be subtle compared to age due to what was found for dietary fat (i.e., distinct from protein) as demonstrated by qualitative and quantitative contrasts specific to dietary fat types across the ages evaluated.

Alpha diversity analysis

Alpha diversity was calculated using Shannon and Simpson indices to evaluate species richness and evenness among the treatment groups for samples at days 41 and 55 at the OTU level. Statistical analysis of the OTU-level and genus-level for day 41 samples across the treatment groups showed no significant difference in diversity (Supplementary Fig. 2A and Supplementary Fig. 3). Statistical analysis of the OTU-level and genus-level for day 55 samples across the treatment groups did however show a significant difference in diversity for genus-level (Supplementary Fig. 2B, Fig. 3, and Supplementary Fig. 4). A posthoc Dunn test identified a significant decrease in Shannon diversity in coconut oil and lard-fed chickens in comparison with the control group (Fig. 3). Overall, day 55 alpha diversity results indicate a decrease in diversity for the SFA-rich fat sources lard and coconut oil. Alpha diversity measures the diversity of microbes within a single sample. In our study on day 55, we observed a reduction in genus-level Shannon and Simpson diversity between groups fed diets having lard and coconut oil versus groups fed control and flaxseed oil-based diets. Previous research has demonstrated similar effects for how diets high in SFA reduce microbial richness and diversity, while PUFA diets tend to maintain or increase microbial diversity (Birkeland et al., 2023; Wolters et al., 2019). A study conducted on mice also found that coconut oil intake leads to a reduction in bacterial diversity (López-Salazar et al., 2021).

Table 2
Day 55 significant OTU-level adonis pairwise comparisons of microbial compositions, calculated on Aitchison distances.

	Pairs	Df	Sums of Sqs	*F* model	R^2	*p*-value	*p* adj
1	Fish oil vs Control	1	2975	1.23	0.06	0.021 *	0.21
2	Coconut oil vs Control	1	3508	1.41	0.07	0.019 *	0.19
3	Fish oil vs Coconut oil	1	3705	1.47	0.07	0.011 *	0.11
4	Flaxseed oil vs Coconut oil	1	3490	1.42	0.07	0.024 *	0.24
5.	Fish oil vs Lard	1	3341	1.35	0.07	0.012 *	0.12

* < 0.05; ** < 0.01; *** <0.001.

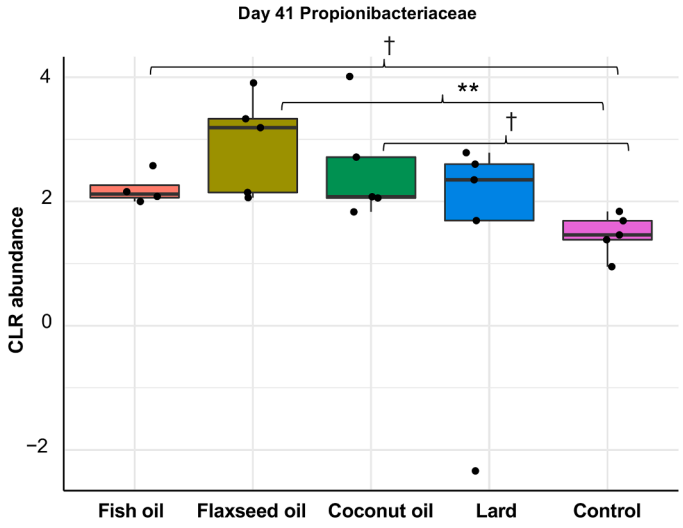

Fig. 5. *Propionibacteriaceae* abundance in day 41 cecal samples. Abundance was tested for statistical significance using the Kruskal-Wallis rank sum test ($p = 0.036$) followed by the Dunn test with Benjamini-Hochberg adjustment. *p* adj value: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, †$p \leq 0.10$.

Beta diversity analysis

Beta diversity analysis studies differences between microbial composition between groups were performed using principal coordinate analysis (PCoA) based on Bray-Curtis and Aitchison distances. PCoA showed distinct clustering of samples for day 41 and day 55 indicating the difference in microbial compositions (Fig. 4). There was no clustering observed as per dietary treatment at either time point. To further investigate the effect of treatment diet on cecal microbial composition, we performed OTU-level PERMANOVA analysis across factors of treatment, time point (age), and treatment-time interaction (Table 1). There was a significant effect of age (day 41 and day 55) on microbial compositions across the treatment groups ($p = 0.001$). The interaction of dietary treatment and time was also found to be significant ($p = 0.015$). Dietary treatment had a less distinct effect on cecal microbial compositions compared to time points ($p = 0.075$). PERMANOVA analysis of Aitchison distances specific to day 41 across the treatment group was insignificant ($p = 0.39$), while a significant effect of treatment was observed for day 55 samples ($p = 0.004$, $R^2 = 0.099$, $F = 1.23$).

To further investigate this effect in day 55 samples, a pairwise comparison of Aitchison distances between the treatments and control group was conducted using pairwise adonis. At day 55, a significant cecal microbial compositional difference was observed for five group

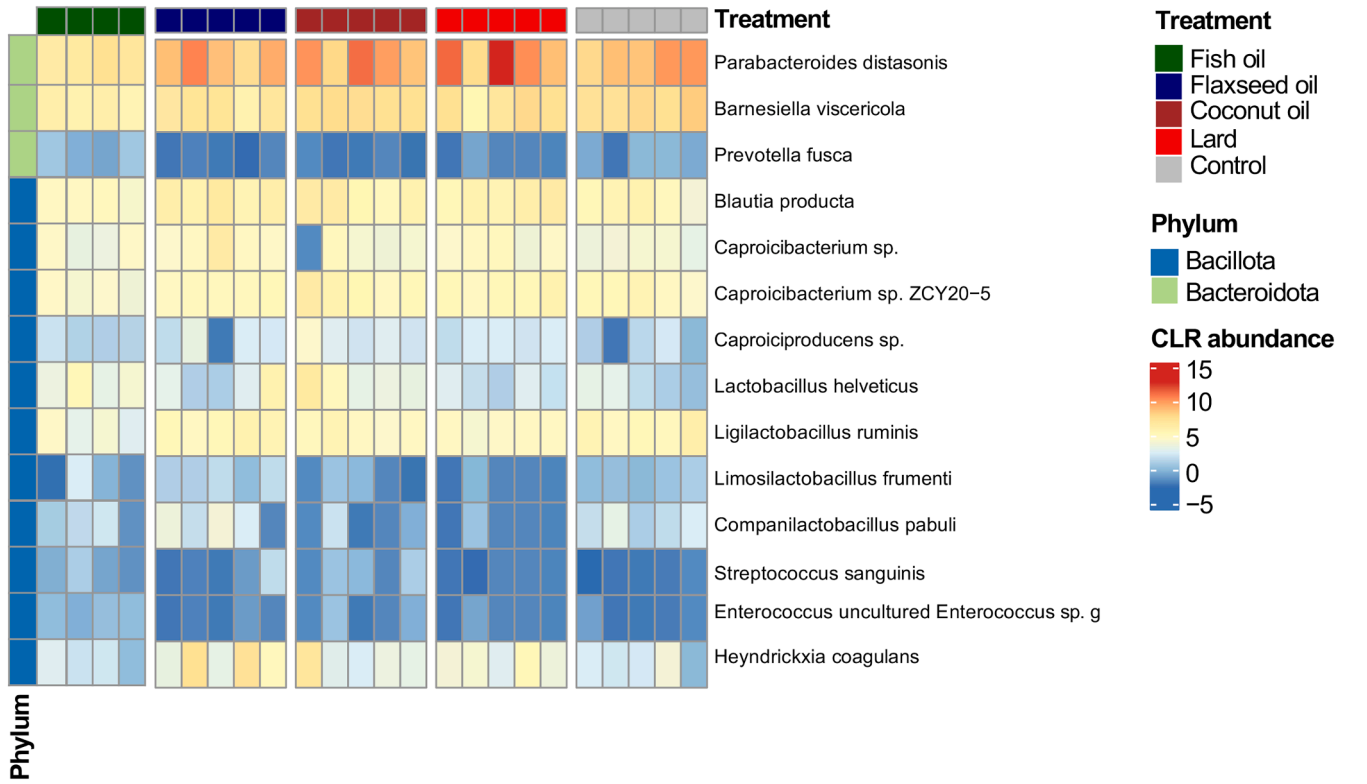

Fig. 6. Heatmap indicating abundances for the taxa differing significantly between the treatment groups and control group for day 41 chickens.

pairs that mainly involved chickens fed with fish oil, control, and coconut oil groups. Table 2 shows all significant day 55 pairs. As indicated in Table 2, day 55 fish oil and coconut oil-fed chickens have significant microbial compositional differences compared to the control-fed group. The other significantly varying pairs involve fish and flaxseed oil-fed chickens (PUFA-rich diets) in comparison to SFA-rich fat-fed groups, coconut oil, and lard.

Differentiating taxa results for day 41

We studied microbial community shift across higher to lower taxonomic levels at both ages. We did not find phylum-level microbial abundance differences to be significant for chickens fed with the treatment diets at day 41. For family-level microbial taxa difference, at day 41, chickens fed with flaxseed oil showed significant enrichment in *Propionibacteriaceae* family abundance compared to the control-fed group (Fig. 5). OTU level taxonomic analysis for day 41 chickens identified differentially abundant taxa between treatment-fed and control groups. Fig. 6 indicates all significant taxa abundances across the treatment groups. Bacterial taxa like *Blautia producta, Heyndrickxias coagulans* (formerly *Bacillus coagulans*), and unknown species from *Caproicibacterium* were found to enrich flaxseed-fed chickens while *Lactobacillus helveticus*, Enterococcus uncultured sp., enriched in fish oil fed chickens compared to the control group (p adj < 0.05; Supplementary Fig. 5A and B). *Lactobacillus helveticus, Caproiciproducens* unknown sp. enriched in the coconut-fed chickens, and *Heyndrickxias coagulans* enriched in lard-fed chickens compared to the control group (p adj $<$ 0.05).

Day 41 chickens supplemented with PUFA-rich flaxseed oil enriched for some gut health- promoting microbes. This included increased abundance for the *Propionibacteriaceae* family capable of producing SCFA (Short Chain Fatty Acid)-propionic acid, which contributes to intestinal mucosa development, anti-inflammatory effect, immunomodulation, and pathogen resistance (Martínez et al., 2016; Lin et al., 2012; L Liu et al., 2021a). SCFA-propionic acid also stimulates the growth of beneficial gut bacteria like *Bifidobacteria* species (Begunova et al., 2019). Flaxseed supplementation was also found to promote *Blautia producta*, an SCFA-producer (*Lachnospiraceae* family), and *Heyndrickxias coagulans* (*Bacillaceae* family). Both species are known for their probiotic, anti-inflammatory, and anti-bacterial properties (Zhang et al., 2021). SCFA is a microbial metabolite essential to maintain gut barrier integrity, immune response, pathogen-resistant and overall physiology of chickens (Jadhav et al., 2022). *B. producta* has been specifically found to restrict the growth of *C. perfringens* and vancomycin-resistant *Enterococci* in the gut, thus preventing the spread of antibiotic-resistant microbes (Liu et al., 2021b). These findings are supported by Gao et al. (2020), reporting an increased abundance of *B. producta* in high-fat diet mice models supplemented with α-linolenic Acid. In the case of coconut oil and lard-fed chickens, when each compared to the control group, increased abundances for probiotic microbial taxa were found for *Lactobacillus helveticus* and *Heyndrickxias coagulans* respectively. However, there was no adverse effect in terms of elevated pathogenic taxa or depletion of beneficial taxa found for lard and coconut oil-fed groups on cecal microbiota observed in comparison to the control group for day 41 chickens.

Differentiating taxa results for day 55

Differentiating taxa for day 55 at the phylum, family, and OTU levels gave the following results. There was no change observed in the phylum-level microbial relative abundances in chickens fed with dietary treatment and control group. However, at the bacterial family level (Fig. 7), the abundance of the *Oscillospiraceae* family (formally known as *Ruminococcaceae*, and which is associated with fiber degradation and SCFA production) increased in birds fed with fish oil in comparison to the control and other treatment groups (Fig. 7A). Other bacterial families like *Coprobacillaceae* (p adj $= 0.054$), *Eubacteriaceae* (p adj $= 0.081$), *Proteinivoraceae* (formerly *Anaerobrancaceae*) (p adj $= 0.004$) abundance also increased in fish oil-fed chickens compared to the control group. On the other hand, relative abundances for *Lachnospiraceae* and *Bifidobacteriaceae* families decreased in the lard-fed birds compared to the control group. While the cecal bacterial abundance of the

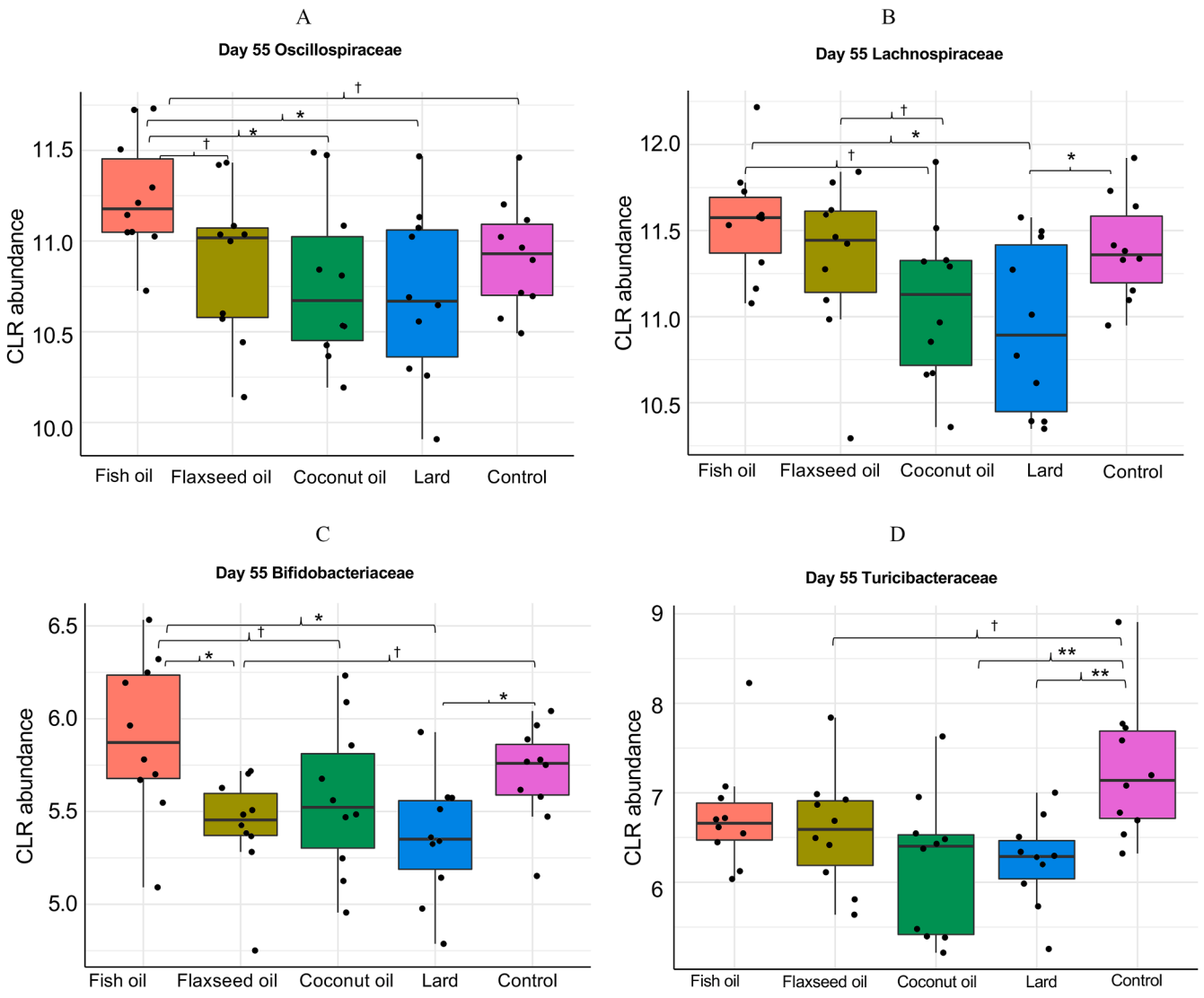

Fig. 7. Box plot of CLR abundance distribution of selected taxa at family level on day 55 of treatment A) *Oscillospiraceae* B) *Lachnospiraceae* C) *Bifidobacteriaceae* D) *Turicibacteraceae*. Significance values were calculated by the Kruskal-Wallis test for treatment groups, followed by Dunn's multiple comparisons and adjusted for false discovery rate using the Benjamini-Hochberg correction. p adj: * $\leq$ 0.05, ** $\leq$ 0.01, *** $\leq$ 0.001, †$p \leq 0.10$.

Peptostreptococcaceae family were increased in the lard (p adj = 0.020) and coconut oil-fed (p adj = 0.031) groups compared to the control group. The *Turicibacteraceae* family was also observed to decrease in all treatment groups except fish oil-fed chickens compared to the control group.

OTU-level microbial changes in abundance were examined as an effect of dietary treatment. Fig. 8 displays the abundances of the bacterial taxa that varied differently between the treatment and control group chickens. Most of these taxa belong to the *Bacillota* phylum. Fig. 9 further indicates a comparison between significant cecal bacterial taxa abundances between the treatment-fed chickens and the control group. On day 55, chickens fed with PUFA-rich fat fish oil showed cecal enrichment for several *Bacteroides* species and *Faecalibacterium* sp. compared to control chickens while supplementing SFA-rich fat coconut oil showed a decrease in abundance level for several cecal microbial taxa belonging to the family *Oscillospiraceae, Faecalibacterium duncaniae, Acutalibacter muris*, and *Caproicibacterium* species compared to the control group. Coconut oil supplementation enriched for some species from the *Bacteroides* genus including bile-tolerant *Bacteroides thetaiotaomicron* and two species from the *Lactobacillus* genus (sp. *sakei* and sp. ESL0680).

Our study demonstrates that on day 55, the microbial composition of the fish oil and coconut oil-fed chickens significantly differed from the control group as indicated by PERMANOVA analysis. The differences in microbial composition likely arose from the distinct fatty acid profiles of the dietary fat. The fish oil diet is rich in PUFAs, particularly omega-3 docosahexaenoic acid (DHA), while the coconut oil diet is high in SFAs, mainly medium-chain fatty acids like lauric acid. The control group's fat source (poultry fat) contains a combination of saturated and unsaturated fatty acids. At day 55, fish oil supplementation to chickens encouraged cecal microbial members from the *Oscillosparaceae* family compared to the control group (p adj = 0.07). The *Oscillosparaceae* family (*Ruminococcaceae*) is known for SCFA production through complex carbohydrate fermentation. This bacterial family has an important role in resistant starch degradation and thus contributes to nutrient digestion (Pal et al., 2021). Other less abundant and lesser-known microbial families, like *Coprobacillaceae, Eubacteriaceae*, and *Proteinivoraceae* were found to be enriched in the fish oil-supplemented chickens compared to the control, generally adding to the level of cecal microbial diversity.

In addition, fish oil-supplemented chickens showed increased abundances in eight *Bacteroides* species – *B. faecium, B. luhongzhouii, B. finegoldii, B. zhangwenhongii, B.* M10, *B.* D2, *B.* unknown, and *Phocaiecola* (*Bacteroides*) *vulgatus. Bacteroides* are well-known for fiber degradation, producing SCFAs primarily acetate as a catabolic byproduct

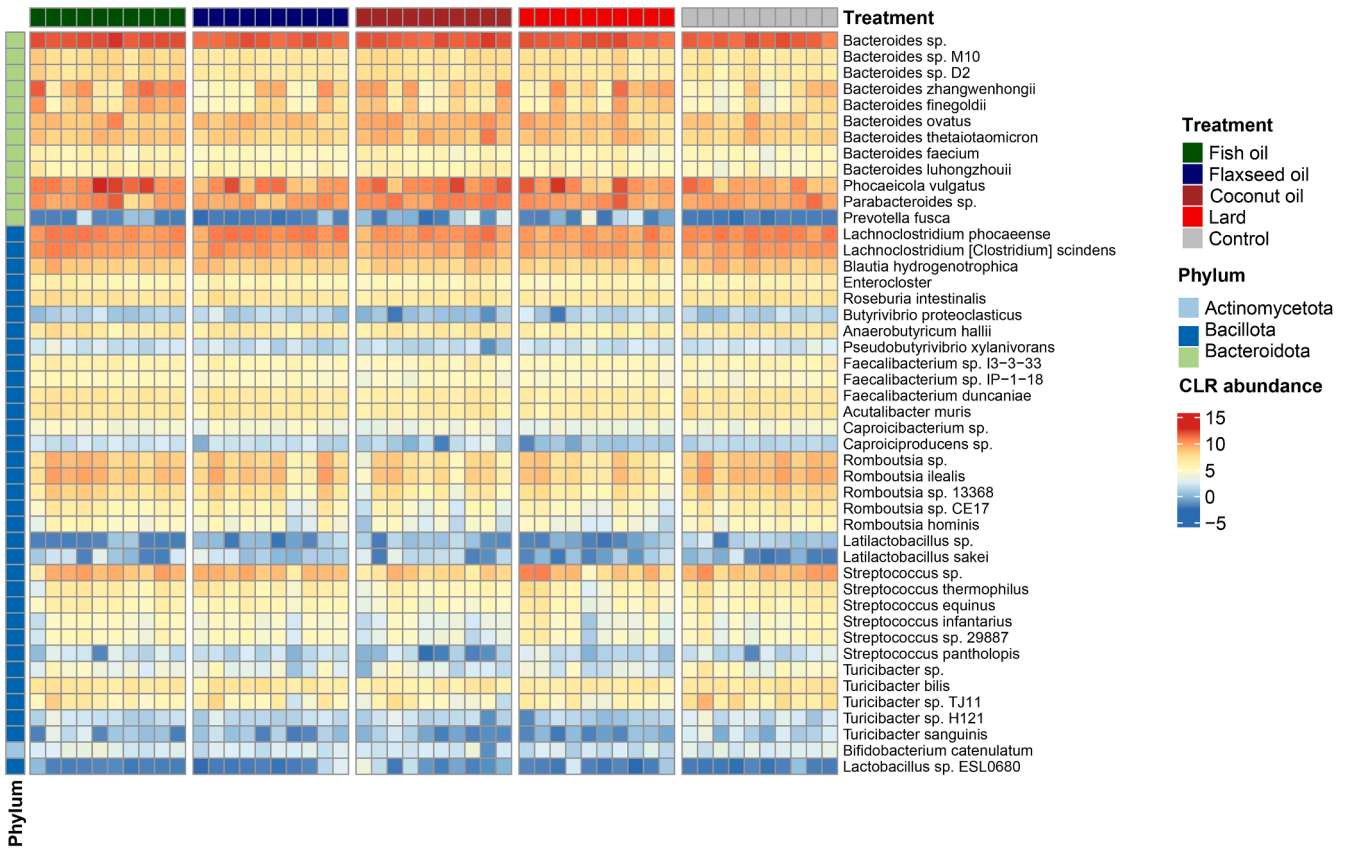

Fig. 8. Heatmap indicating abundances of the taxa differing significantly between the treatment groups and control group day 55 chickens. Significance values were calculated by the Kruskal-Wallis test for treatment groups, followed by Dunn's multiple comparisons and adjusted for false discovery rate using the Benjamini-Hochberg correction.

(Rios-Covian et al., 2017). SCFAs production and *Bacteroides* species capability to transform primary bile acids to secondary bile acids have a critical role in inhibiting pro-inflammatory cytokines, promoting gut health by resisting pathogen colonization and maintaining gut integrity (Shin et al., 2024; Zafar and Saier, 2021). Moreover, acetate produced as the microbial catabolic end product is cross-fed to other butyrate-producing gut microbial species (den Besten et al., 2013; Rios-Covian et al., 2017). These cross-fed bacterial species include members of the *Oscillosparaceae* family and *Faecalibacterium* species observed to be enriched in the fish oil-fed group. The microbial metabolite butyrate is known to be an energy source for the enterocytes, playing an important role in maintaining membrane integrity and developing host immunity (Tian et al., 2020). *Faecalibacterium* species have been found to exhibit anti-inflammatory effects through the production of extracellular vesicles (EVs) and microbial anti-inflammatory molecules (MAM) contributing to gut health (Martín et al., 2023). Additionally, enrichment of these probiotic bacterial taxa in the fish oil-fed group is proposed to be because of increased intestinal alkaline phosphatase (IAP) levels. This increase is likely driven by resolvin E1, a bioactive derivative of omega-3 fatty acids, which has been shown to stimulate IAP production in intestinal epithelial cells. IAP plays a crucial role in detoxifying bacterial endotoxins, thereby reducing gut inflammation and promoting a more favorable environment for the colonization and growth of probiotic microbes. (Arita et al., 2005; Campbell et al., 2010). The enrichment of these probiotic taxa may therefore indicate improved metabolic functions, SCFA production capability, and anti-inflammatory benefits in the fish oil-fed chickens compared to the control group. These changes were not observed for flaxseed-based diets, although a significant reduction was identified for two *Turicibacter* species (Supplementary Fig. 6).

In the case of SFA-rich coconut oil feeding to chickens increased the abundance of *Bacteroides, Parabacteroids*, and *Lactobacillus* species than the control group. In the enriched *Bacteroides* species, some are the same species as enriched in the fish oil-fed group. In addition, there is an increased abundance of *Bacteroides thetaiotaomicron*. This *Bacteroides* species utilizes glycans in the gut mucus layer and thus also can influence the host immune system. However, *B. thetaiotaomicron* is also known to thrive in bile-rich environments (Zocco et al., 2007). An increased abundance of *B. thetaiotaomicron* might indicate towards altered gut bile composition in the coconut-fed chickens. Other *Bacteroides* species increase in abundance in the coconut oil group indicates towards protective effect of the lauric acid. These results are backed up by other studies where the supplementation of coconut oil has increased abundance for *Bacteroides* and *Lactobacillus* species (Wu et al., 2021; Patrone et al., 2018).

Feeding coconut oil or lard to the chickens were found to lower the abundance of gut health-promoting bacterial species compared to the control group. Some of the common species that were reduced in both coconut oil and lard-fed chickens include *Faecalibacterium duncaniae* (also known as *F. prausnitzii*, primary SCFA, and lactic acid producer), *Roseburia intestinalis* (SCFA producer, primary xylan degrader, having anti-inflammatory properties), five species from *Romboutsia* (positively associated with chicken immunity) (Ali et al., 2022). Apart from these common taxa, there are SCFA producers that were found to be reduced in the coconut oil-fed group compared to the control including *Butyrivibrio proteoclasticus* and *Pseudobutyrivibrio xylanivorans*. Both species degrade the complex carbohydrate xylan that is common in the plant-based feed ingredients in poultry diets (Ali et al., 2022). Lard-fed chickens had lower abundance levels for *Faecalibacterium* sp., *Anaerobutyricum hallii, Blautia hydrogenotrophica, Bifidobacterium catenulatum, Romboutsia ilealis*, and *Latilactobacillus* species than the control group. Most of these are SCFA producers, while *B. catenulatum* is known for

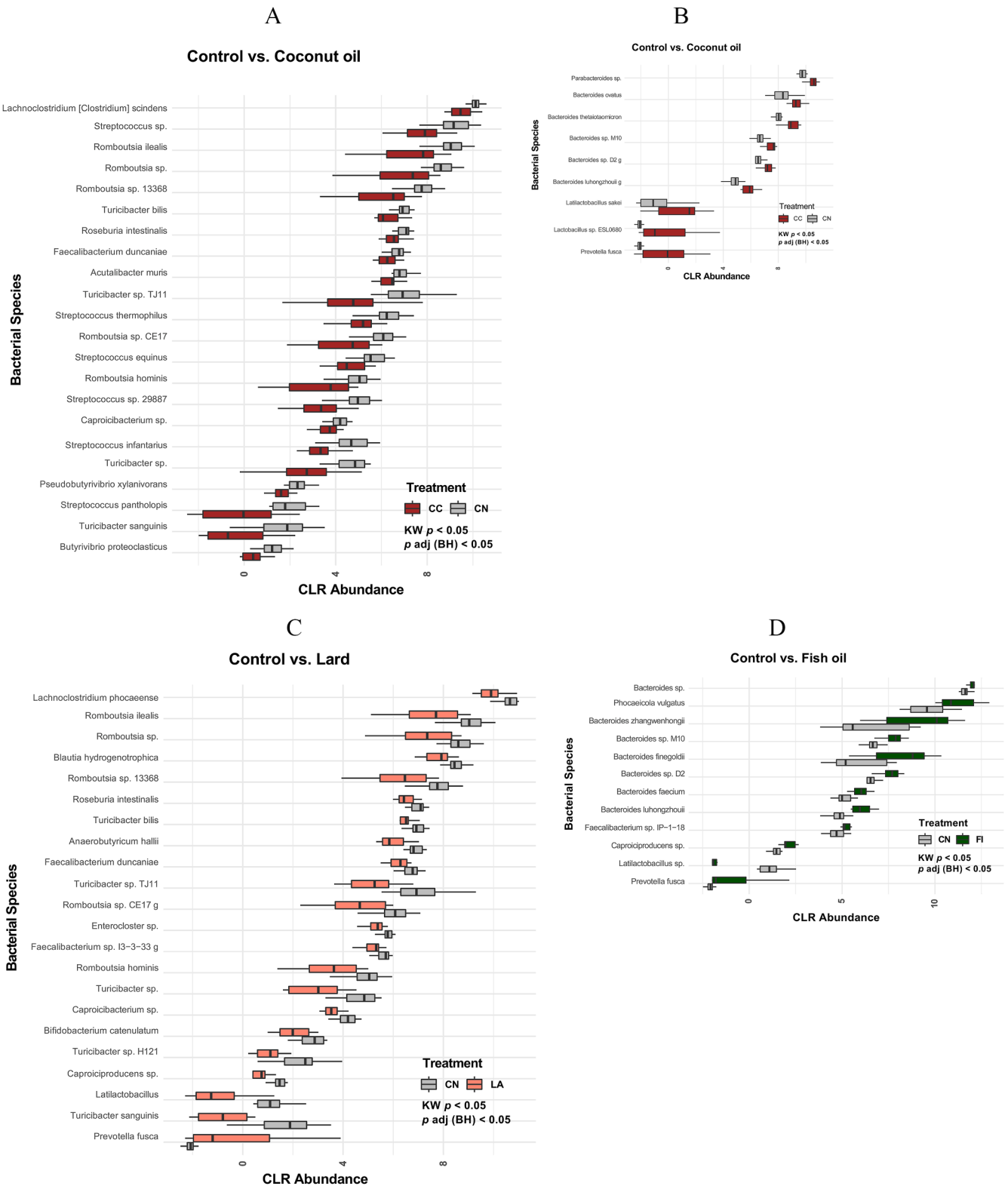

Fig. 9. Box plot of CLR abundance distribution of selected taxa at species level on day 55 of treatment. (A) Bacterial species depleted in the coconut fed chickens as compared to the control group; (B) Bacterial species enriched in the coconut fed chickens as compared to the control group; (C) Bacterial species depleted in the lard fed chickens (except *Prevotella fusca*) as compared to the control group; (D) Bacterial species enriched in the fish oil fed chickens (except *Latilactobacillus* sp.) as compared to the control group. Significance values were calculated by the Kruskal-Wallis test for treatment groups, followed by Dunn's multiple comparisons and adjusted for false discovery rate using the Benjamini-Hochberg correction. p adj: * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, †$p \leq 0.10$.

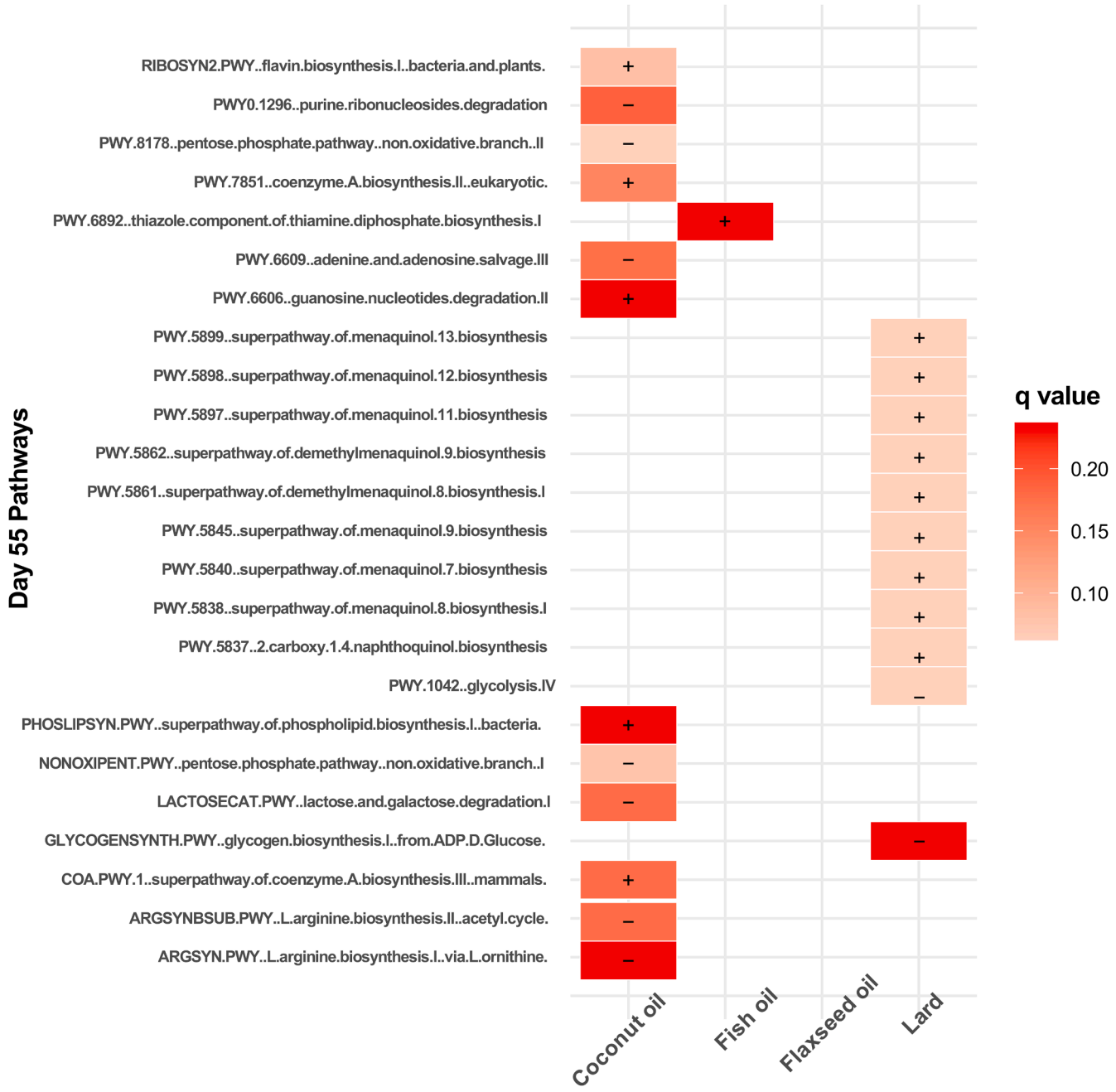

Fig. 10. Significant differentially abundant pathways at day 55 across the treatment groups compared to the control-fed chickens. Pathways were identified using MaAsLin 2 model with $p < 0.05$, $q < 0.25$. The +, - signs indicate positive and negative correlations respectively in comparison to the control group.

improving gut barrier function and *Romboutsia ilealis* is known for its immunomodulatory effect (Song et al., 2024). Similar to our results, a reduction in *Faecalibacterium* species abundance is observed in Lützhøft et al. (2024) high fat mice model study. Moreover, both coconut oil and lard-fed chickens showed a reduced abundance for the *Turicibacteraceae* family compared to the control group. *Turicibacteraceae* species play a role in gut health by modulating the immune system and contributing to SCFA production, which supports gut barrier function (Bello et al., 2024; Liu et al., 2022; Maki and Looft, 2022).

This reduction in the key health-promoting bacteria in coconut oil and lard-fed chickens may be due to the antimicrobial effect of the bile in the gut. A diet high in saturated fat stimulates the discharge of bile acids in the colon while reducing its reabsorption compared to a low-fat diet (Ye et al., 2022; Murakami et al., 2016). Bile components are known to have bactericidal properties and are enhanced by a higher proportion of unconjugated bile acids. The gut microbes play an important role in this bile acid transformation. A balanced gut bile composition is crucial for maintaining gut microbial homeostasis as well. Intestinal bile affects bacterial metabolism including membrane damage, disrupting amino acids, nucleotide, and carbohydrate metabolism making it bactericidal (Devkota and Chang, 2015; Yokota et al., 2012). Thus, high bile levels in the gut expose both the pathogenic and commensal microbes to harsh conditions exerting a selective pressure (Sistrunk et al., 2016). Bile acids, notably cholic acids and deoxycholic acids, observed to restrict the growth of gram-positive anaerobic gut microbes compared to aerobic gram-negative including members of the *Clostridia* class, *Ruminococcaeae* (*Oscillospiraceae*) family, and *Bifidobacterium* species (Folch et al., 1971; Tian et al., 2020; Kurdi et al., 2006). Reduced abundance of key species in the gut for the lard and coconut-fed group along with enrichment of bile tolerant *Bacteroides* species in the coconut-fed group

Table 3
Day 41: significant differentially abundant GO term identified in treatment diet fed chickens using MaAsLin2 compared to the control group.

GO Term	Group Differential	*p* val	*q* val[a]	coef[b]
Succinate dehydrogenase activity	Enriched in Fish	8.22×10^{-6}	0.07	59.31
Phosphorelay response regulator activity	Enriched in Fish	2.01×10^{-5}	0.07	96.33
UDP-N-acetylglucosamine diphosphorylase activity	Enriched in Fish	1.25×10^{-5}	0.07	42.07
3-oxoacyl-[acyl carrier protein] reductase NADPH activity	Enriched in Fish	2.35×10^{-5}	0.07	95.99
2-hydroxy-3-oxopropionate reductase activity	Enriched in Fish	9.62×10^{-6}	0.07	85.91
Transferase activity transferring phosphorus containing groups	Enriched in Fish	1.77×10^{-5}	0.07	88.76
L-threonine catabolic process to propionate	Enriched in Fish	3.65×10^{-5}	0.09	27.53
Hydroxylamine reductase activity	Depleted in Fish	0.000102	0.20	−37.08
Diaminopimelate dehydrogenase activity	Depleted in Fish	0.000125	0.22	−57.18
Peptide transport	Enriched in Fish	0.000147	0.23	284.22
3-deoxy-7-phosphoheptulonate synthase activity	Enriched in Fish	0.000168	0.24	131.20

[a] *q*: adjusted for multiple testing. Benjamin-Hochberg false discovery rate.
[b] Coef: Coefficient indicating the effect size.

compared to the control are consistent with the above explanation.

Microbial functional analysis

Regarding microbial functional analysis, we found that 95.7 % of filtered reads did not map to the MetaCyc pathway database. A total of 449 pathways were identified across all day 41 and day 55 samples. Further analysis of normalized pathway abundances on day 41 across treatments using MaAsLin2 revealed no significant differences. However, on day 55, MaAsLin2 identified 26 pathways that were significantly differentially abundant across treatment groups compared to the control ($p < 0.01$, $q < 0.25$).

Most of these pathways belonging to the coconut oil and lard-fed chickens showed altered association with the control group. Nine pathways related to the menaquinol biosynthesis (PWY-5837, PWY-5838, PWY-5840, PWY-5845, PWY-5861, PWY-5862, PWY-5897, PWY-5898, PWY-5899) were found to be enriched in the lard fed group compared to control fed chickens (Fig. 10). Although, glycolysis (PWY-1042) and glycogen synthesis pathways were less represented in the lard-fed group compared to the control group. In the case of coconut oil-fed chickens, there was less representation of gut microbial pathways participating in carbohydrate metabolisms such as the pentose phosphate pathway (non-oxidative branch I&II) (NONOXIPENT.PWY; PWY.8178) and the lactose and galactose degradation I (LACTOSECAT.PWY) pathway. In the case of nucleotide metabolism, there was less representation of the adenine and adenosine salvage III (PWY.6609), purine ribonucleosides degradation (PWY0.1296), and amino acid metabolism-related pathways like l-arginine biosynthesis I&II. The coconut oil-fed chicken cecal microbes were found to enrich the guanosine nucleotides degradation II (PWY.6606) pathway, the superpathway of phospholipid biosynthesis I (bacteria) (PHOSLIPSYN.PWY), and the flavin biosynthesis I (bacteria and plants) (RIBOSYN2.PWY) pathway when compared to the control-fed chickens.

Mapping of normalized gene family count against gene ontology terms identified a total of 5236 GO terms for day 41 and day 55 samples. GO term abundances for day 41 and day 55 were analyzed for differential abundance using MaAsLin2. For day 41, twelve GO terms showed significant differential abundance between the treatment group and the control-fed chickens. Table 3 details all significant GO terms for day 41 chickens except one with a missing GO description. In the case of day 55 chickens, 386 GO terms were identified as significantly differing across the treatment diet-fed chickens compared to the control group ($p < 0.01$, $q < 0.25$) (Fig. 12). In significant GO terms compared to the control chicken, the coconut oil-fed chicken cecal microbes exhibited the most substantial changes, followed by the lard group (Fig. 11). When compared with the control chickens, the functional profile of PUFA-rich fish and flaxseed oil-fed chicken cecal microbiomes showed fewer changes (i.e., fewer GO terms) compared to the SFA-rich dietary treatments, coconut oil, and lard. Fig. 12 displays how GO term enrichment for cecal microbiomes at day 55 varies for the treatment diet-fed groups for each in comparison to the control group. Among these GO terms, long-chain fatty acid acyl carriers protein ligase activity, acetyl-CoA: oxalate CoA-transferase, and 2-ketobutyrate formate-lyase activity were found to be enriched in the flaxseed oil-fed chicken cecal microbiomes while cellulose catabolic function was found to be enriched for

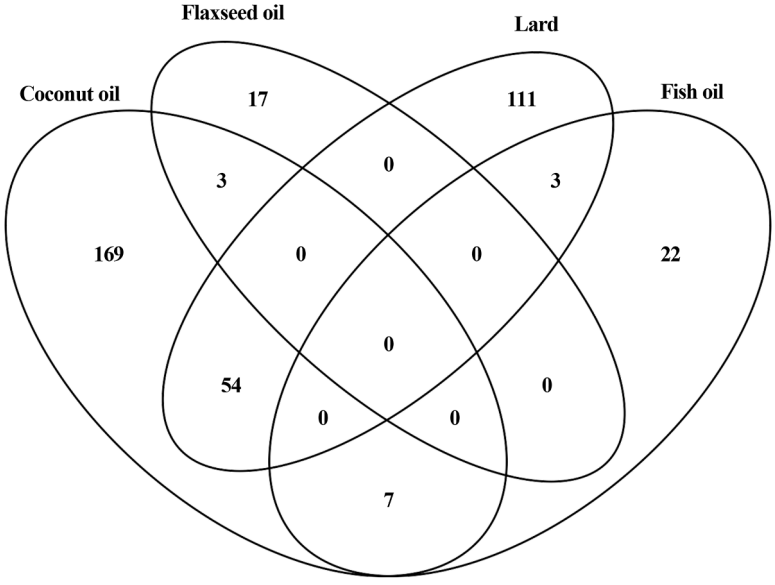

Fig. 11. The Venn diagram shows shared and unique significantly abundant day 55 chicken cecal microbial GO terms across dietary treatment groups compared to control group chickens.

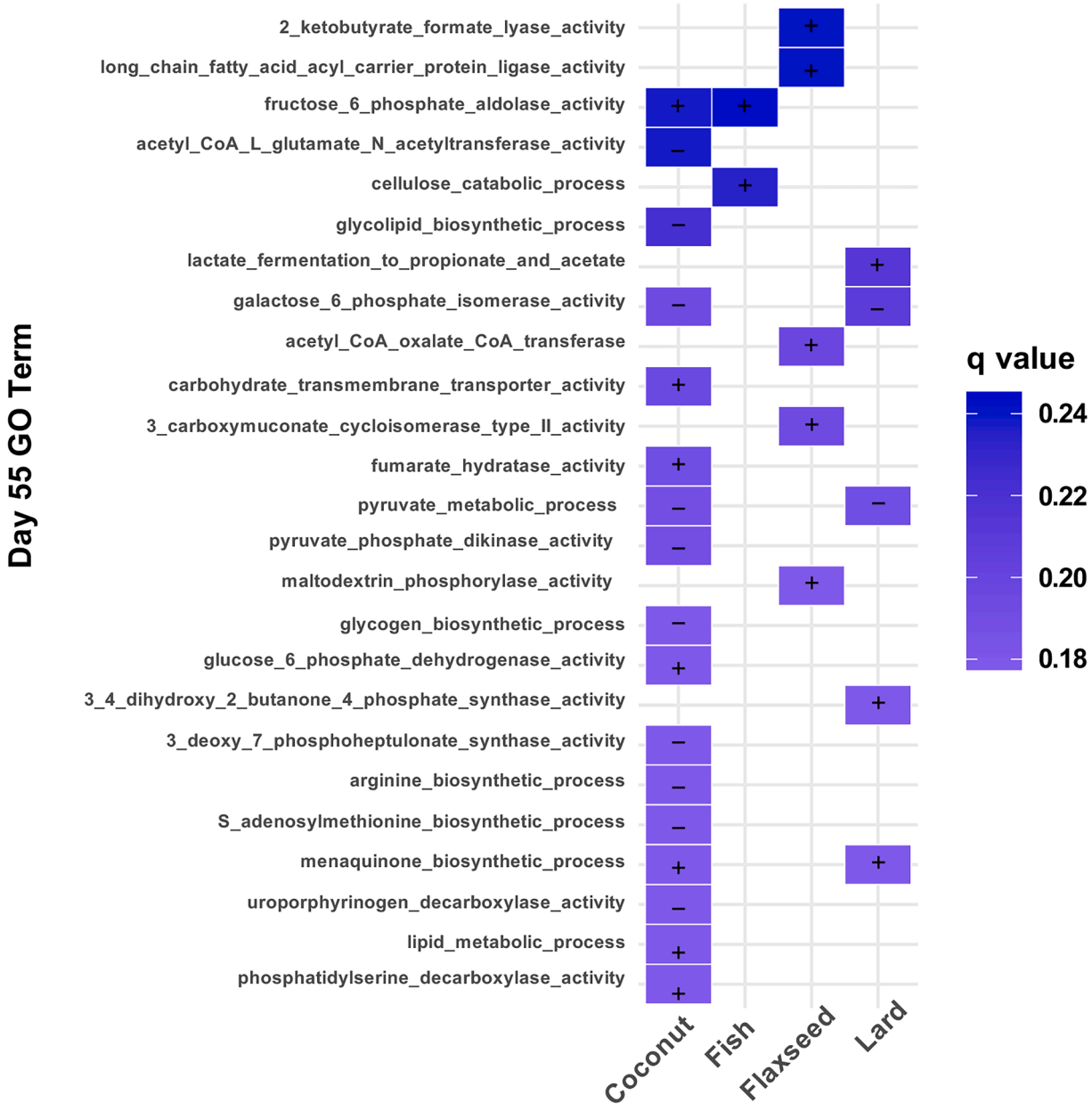

Fig. 12. Significant differentially abundant Day 55 chicken cecal microbial GO terms across the treatment fed groups compared to control fed chickens. GO terms were identified using the MaAsLin 2 model compared to control chickens with $p < 0.05$, $q < 0.25$. The +, - signs indicate positive and negative correlations respectively in comparison to the control group.

the cecal microbiomes of fish oil-fed chickens. The lipid metabolic process was found to be abundant within the microbiomes of coconut oil-fed chickens. The cecal microbiomes of lard-fed chickens showed an increased abundance for lactate fermentation to propionate and acetate GO terms compared to the control group.

For day 55, we found more dynamic cecal microbial functional changes in the lard and coconut oil-fed chickens compared to the control group. For the coconut oil-fed chicken cecal microbes, we observed a mixed/complex functional profile compared to the control. It suppresses many microbial biosynthetic pathways involved in carbohydrate and nucleotide synthesis but enriches (GO terms) the lipid metabolic process. This suggests a microbial functional shift towards lipid utilization while limiting other metabolic activities. This microbial functional profile in coconut oil-fed chickens supports the earlier reduction in the abundance of gut health-promoting SCFA-producing gut microbes compared to the control group. At day 55, the functional profile of lard-fed chicken cecal microbiomes exhibited lower representation for the central metabolic pathway glycolysis as well as reduced glycogen biosynthesis when compared to the control group, indicating hampered carbohydrate metabolism. The lard group cecal microbiome was enriched for menaquinol (vitamin K2) biosynthesis pathways as well as taxa capable of synthesizing menaquinol like *Corynebacterium variabile* and *Rothia* sp. SD9660Na. Menaquinol-related microbiome metabolism has been previously observed in high-fat diet mice model supplemented with probiotics (Vu et al., 2022). This latter finding specific to lard in chickens is novel and would benefit from further investigation. The lard-fed chickens were further observed to enhance the gut bacterial ability to ferment lactate to propionate and acetate, although we did not find microbial taxa enrichment linking to this function. We observed significant microbial functional changes in the flaxseed oil-fed group compared to the control chickens, particularly in the promotion of microbial pathways related to fatty acid metabolism and energy

production. Additionally, we found that the microbiomes of fish oil-fed chickens had elevated cellulose catabolic processes compared to the control group. This microbial function in fish oil-fed chickens relates to the increased cecal abundance of *Bacteroides* species known to utilize cellulose (Forsberg et al., 1981).

Overall, the functional shifts in gut microbiota were found to be highly diet-specific, with microbiomes for fish oil-fed chicken gut enriched for carbohydrate metabolism, cecal microbiomes for coconut oil-fed group having reduced carbohydrate and biosynthetic activities, and the cecal microbiomes for lard-fed chickens having reduced carbohydrate metabolism while supporting menaquinol (vitamin K2) biosynthesis.

Conclusion

The study highlights how different dietary fat sources induce significant alterations in the chicken cecal microbiome abundances by day 55, with PUFA-rich diets (fish and flaxseed oil) promoting a more favorable gut microbial profile compared to SFA-rich diets (lard and coconut oil). Additionally, the fish and flaxseed oil fed chickens maintained gut microbial diversity while SFA rich fat fed chickens showed reduced diversity compared to the control. PUFA-rich diet, mainly fish oil supplementation of chicken, demonstrated beneficial effects on cecal microbes by enriching SCFA-producing, probiotic, anti-inflammatory taxa which is linked to improved gut health and metabolic function. By contrast, the microbiomes of SFA-rich lard and coconut oil-fed chickens exhibited depletion of key microbial families, and species and reduced microbial diversity, which may compromise gut function by impairing immune system management, nutrient degradation, and defense mechanisms against pathogens.

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Ethics declarations

The animal studies were approved by the Institutional Animal Care and Use Committee of North Carolina Agricultural and Technical State University (IACUC #20-004.0). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Supplementary Fig. 1. Relative abundance plots displaying cecal microbial profile for day 41 and day 55 old broiler across the treatment groups (A) Relative abundance plot for high abundant top family (B) Important families highlighted in color and less important as gray in a relative abundance plot for high abundant top family. The left panel profiles Day 41: Fish oil ($n = 4$), Flaxseed oil ($n = 5$), Coconut oil ($n = 5$), Lard ($n = 5$), Control ($n = 5$). The right panel profiles Day 55: Fish oil ($n = 10$), Flaxseed oil ($n = 10$), Coconut oil ($n = 10$), Lard ($n = 10$), Control ($n = 10$)

Declaration of competing interest

All authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105167.

Data availability

All sequence data supporting the findings of this study were deposited in NCBI's repository (SRA) under accession number PRJNA1189501 and are available at the following https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1189501.

References

Ali, Q., Ma, S., La, S., Guo, Z., Liu, B., Gao, Z., Farooq, U., Wang, Z., Zhu, X., Cui, Y., Li, D., Shi, Y., 2022. Microbial short-chain fatty acids: a bridge between dietary fibers and poultry gut health—a review. Anim. Biosci. 35 (10), 1461. https://doi.org/10.5713/ab.21.0562.

Apajalahti, J., Kettunen, A., 2006. Microbes of the chicken gastrointestinal tract. Avian Gut Funct. Health Dis. 28, 107–123. https://doi.org/10.1079/9781845931803.0124.

Arita, M., Yoshida, M., Hong, S., Tjonahen, E., Glickman, J.N., Petasis, N.A., Blumberg, R.S., Serhan, C.N., 2005. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis. Proceed. Natl. Acad. Sci. 102 (21), 7671–7676. https://doi.org/10.1073/pnas.0409271102.

Attia, Y.A., Al-Harthi, M.A., Abo El-Maaty, H.M., 2020. The effects of different oil sources on performance, digestive enzymes, carcass traits, biochemical, immunological, antioxidant, and morphometric responses of broiler chicks. Front. Veterin. Sci. 7, 529514. https://doi.org/10.3389/FVETS.2020.00181/BIBTEX.

Awad, W.A., Mann, E., Dzieciol, M., Hess, C., Schmitz-Esser, S., Wagner, M., Hess, M., 2016. Age-related differences in the luminal and mucosa-associated gut microbiome of broiler chickens and shifts associated with Campylobacter jejuni infection. Front. Cell. Infect. Microbiol. 6 (NOV), 218440. https://doi.org/10.3389/FCIMB.2016.00154/BIBTEX.

Baião, N., Lara, L., 2005. Oil and fat in broiler nutrition. Brazil. J. Poult. Sci. 7 (3), 129–141. https://doi.org/10.1590/S1516-635X2005000300001.

Beghini, F., McIver, L.J., Blanco-Míguez, A., Dubois, L., Asnicar, F., Maharjan, S., Mailyan, A., Manghi, P., Scholz, M., Thomas, A.M., Valles-Colomer, M., Weingart, G., Zhang, Y., Zolfo, M., Huttenhower, C., Franzosa, E.A., Segata, N., 2021. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bobakery 3. ELife 10. https://doi.org/10.7554/ELIFE.65088.

Begunova, A.V., Rozhkova, I.V., Zvereva, E.A., Glazunova, O.A., Fedorova, T.V., 2019. Lactic and propionic acid bacteria: the formation of a community for the production of functional products with bifidogenic and hypotensive properties. Appl. Biochem. Microbiol. 55, 660–669. https://doi.org/10.1134/S0003683819060048.

Bello, S., McQuay, S., Rudra, B., Gupta, R.S., 2024. Robust demarcation of the family Peptostreptococcaceae and its main genera based on phylogenomic studies and taxon-specific molecular markers. Int. J. Systemat. Evolution. Microbiol. 74 (2), 006247. https://doi.org/10.1099/ijsem.0.006247.

Birkeland, E., Gharagozlian, S., Valeur, J., Aas, A.M., 2023. Short-chain fatty acids as a link between diet and cardiometabolic risk: a narrative review. Lipid. Health Dis. 22 (1), 1–13. https://doi.org/10.1186/S12944-023-01803-5, 2023 22:1.

Caesar, R., Tremaroli, V., Kovatcheva-Datchary, P., Cani, P.D., Bäckhed, F., 2015. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. Cell Metabol. 22 (4), 658–668. https://doi.org/10.1016/j.cmet.2015.07.026.

Campbell, E.L., MacManus, C.F., Kominsky, D.J., Keely, S., Glover, L.E., Bowers, B.E., 2010. Resolvin E1-induced intestinal alkaline phosphatase promotes resolution of inflammation through LPS detoxification. Proc. Natl. Acad. Sci. U. S. A. 107, 14298–14303. https://doi.org/10.1073/pnas.091473010.

Devkota, S., Chang, E.B., 2015. Interactions between diet, bile acid metabolism, gut microbiota, and inflammatory bowel diseases. Digest. Dis. 33 (3). https://doi.org/10.1159/000371687.

den Besten, G., Lange, K., Havinga, R., van Dijk, T.H., Gerding, A., van Eunen, K., Müller, M., Groen, A.K., Hooiveld, G.J., Bakker, B.M., Reijngoud, D.J., 2013. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. Am. J. Physiol.-Gastrointest. Liver Physiol. 305 (12), G900–G910. https://doi.org/10.1152/ajpgi.00265.2013.

Diaz Carrasco, J.M., Casanova, N.A., Fernández Miyakawa, M.E., 2019. Microbiota, gut health and chicken productivity: what is the connection? Microorganisms 7 (10), 374. https://doi.org/10.3390/microorganisms7100374.

Emami, N.K., Calik, A., White, M.B., Kimminau, E.A., Dalloul, R.A., 2020. Effect of probiotics and multi-component feed additives on microbiota, gut barrier and immune responses in broiler chickens during subclinical necrotic enteritis. Front. Veterin. Sci. 7, 572142. https://doi.org/10.3389/fvets.2020.572142.

Ferreira, L., Lisenko, K., Barros, B., Zangeronimo, M., Pereira, L., Sousa, R., 2014. Influence of medium-chain triglycerides on consumption and weight gain in rats: a

systematic review. J. Anim. Physiol. Anim. Nutrit. 98 (1), 1–8. https://doi.org/10.1111/JPN.12030.
Floch, M.H., Gershengoren, W., Elliott, S., Spiro, H.M., 1971. Bile acid inhibition of the intestinal microflora—a function for simple bile acids? Gastroenterology 61 (2), 228–233.
Forsberg, C.W., Beveridge, T.J., Hellstrom, A., 1981. Cellulase and xylanase release from Bacteroides succinogenes and its importance in the rumen environment. Appl. Environ. Microbiol. 42 (5), 886–896. https://doi.org/10.1128/aem.42.5.886-896.1981.
Global Chicken Market Size by Production, 2024. Consumption market & volume, import, export and company analysis 2024-2032. Retrieved October 18, 2024, from. https://www.researchandmarkets.com/reports/5740684/global-chicken-market-size-production.
Gao, X., Chang, S., Liu, S., Peng, L., Xie, J., Dong, W., Tian, Y., Sheng, J., 2020. Correlations between α- linolenic acid-improved multitissue homeostasis and gut microbiota in mice fed a high-fat diet. Msystems 5 (6), 10–1128. https://doi.org/10.1128/msystems.00391-20.
Jadhav, V.V., Fasina, Y., Omaliko, P.C., Han, J., Harrison, S.H., 2024. Effect of dietary fat source on the composition of the cecal microbiome in maturing broiler chicken. Front. Microbiol. 15, 1462757. https://doi.org/10.3389/fmicb.2024.1462757.
Jadhav, V.V., Han, J., Fasina, Y., Harrison, S.H., 2022. Connecting gut microbiomes and short chain fatty acids with the serotonergic system and behavior in Gallus gallus and other avian species. Front. Physiol. 13, 1035538. https://doi.org/10.3389/fphys.2022.1035538.
Knarreborg, A., Simon, M., R. E.-A. and, & 2002, undefined, 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Am. Soc. Microbiol. 68 (12), 5918–5924. https://doi.org/10.1128/AEM.68.12.5918-5924.2002.
Kogut, M.H., 2019. The effect of microbiome modulation on the intestinal health of poultry. Anim. Feed Sci. Technol. 250, 32–40. https://doi.org/10.1016/j.anifeedsci.2018.10.008.
Kubasova, T., Kollarcikova, M., Crhanova, M., Karasova, D., Cejkova, D., Sebkova, A., Matiasovicova, J., Faldynova, M., Pokorna, A., Cizek, A., Rychlik, I., 2019. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS ONE 14 (3). https://doi.org/10.1371/JOURNAL.PONE.0212446.
Kurdi, P., Kawanishi, K., Mizutani, K., Yokota, A., 2006. Mechanism of growth inhibition by free bile acids in lactobacilli and bifidobacteria. J. Bacteriol. 188, 1979–1986. https://doi.org/10.1128/JB.188.5.1979-1986.2006.
Lan, Y., Verstegen, M.W.A., Tamminga, S., Williams, B.A., 2005. The role of the commensal gut microbial community in broiler chickens. World's. Poult. Sci. J. 61 (1), 95–104. https://doi.org/10.1079/WPS200445.
Li, M.H., Meng, J.X., Wang, W., He, M., Zhao, Z.Y., Ma, N., Lv, Q.B., Qin, Y.F., Geng, H.L., Zhao, Q., Ni, H.B., Zhang, X.X., 2022. Dynamic description of temporal changes of gut microbiota in broilers. Poult. Sci. 101 (9), 102037. https://doi.org/10.1016/J.PSJ.2022.102037.
Lin, H.V., Frassetto, A., Kowalik Jr., E.J., Nawrocki, A.R., Lu, M.M., Kosinski, J.R., Hubert, J.A., Szeto, D., Yao, X., Forrest, G., Marsh, D.J, 2012. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PloS One 7 (4), e35240. https://doi.org/10.1371/journal.pone.0035240.
Liu, L., Li, Q., Yang, Y., Guo, A., 2021a. Biological function of short-chain fatty acids and its regulation on intestinal health of poultry. Front. Veterin. Sci. 8, 736739. https://doi.org/10.3389/fvets.2021.736739.
Liu, P., Gao, M., Liu, Z., Zhang, Y., Tu, H., Lei, L., Wu, P., Zhang, A., Yang, C., Li, G., Sun, N., Zhang, K., 2022. Gut microbiome composition linked to inflammatory factors and cognitive functions in first-episode, drug-naive major depressive disorder patients. Front. Neurosci. 15, 800764. https://doi.org/10.3389/fnins.2021.800764.
Liu, X., Mao, B., Gu, J., Wu, J., Cui, S., Wang, G., Zhao, J., Zhang, W., Chen, W., 2021b. Blautia—a new functional genus with potential probiotic properties? Gut Microb. 13 (1), 1875796. https://doi.org/10.1080/19490976.2021.1875796.
López-Salazar, V., Tapia, M.S., Tobón-Cornejo, S., Díaz, D., Alemán-Escondrillas, G., Granados- Portillo, O., Noriega, L., Tovar, A.R., Torres, N., 2021. Consumption of soybean or olive oil at recommended concentrations increased the intestinal microbiota diversity and insulin sensitivity and prevented fatty liver compared to the effects of coconut oil. J. Nutrit. Biochem. 94, 108751. https://doi.org/10.1016/J.JNUTBIO.2021.108751.
Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69 (11), 6816–6824. https://doi.org/10.1128/AEM.69.11.6816-6824.2003.
Lu, J., Rincon, N., Wood, D.E., Breitwieser, F.P., Pockrandt, C., Langmead, B., Salzberg, S.L., Steinegger, M., 2022. Metagenome analysis using the Kraken software suite. Nat. Protocol. 17 (12), 2815–2839. https://doi.org/10.1038/s41596-022-00738-y, 2022 17:12.
Lu, M., Li, R.W., Zhao, H., Yan, X., Lillehoj, H.S., Sun, Z., Oh, S.T., Wang, Y., Li, C., 2020. Effects of Eimeria maxima and Clostridium perfringens infections on cecal microbial composition and the possible correlation with body weight gain in broiler chickens. Res. Veterin. Sci. 132, 142–149. https://doi.org/10.1016/J.RVSC.2020.05.013.
Lützhøft, D.O., Bækgård, C., Wimborne, E., Straarup, E.M., Pedersen, K.M., Swann, J.R., Pedersen, H.D., Kristensen, K., Morgills, L., Nielsen, D.S., Hansen, A.K., Bracken, M. K., Cirera, S., Christoffersen, B.Ø., 2024. High fat diet is associated with gut microbiota dysbiosis and decreased gut microbial derived metabolites related to metabolic health in young Göttingen Minipigs. Plos One 19 (3), e0298602. https://doi.org/10.1371/journal.pone.0298602.
Maki, J.J., Looft, T., 2022. Turicibacter bilis sp. nov., a novel bacterium isolated from the chicken eggshell and swine ileum. Int. J. Systemat. Evolution. Microbiol. 72 (1), 005153. https://doi.org/10.1099/ijsem.0.005153.
Mallick, H., Rahnavard, A., McIver, L.J., Ma, S., Zhang, Y., Nguyen, L.H., Tickle, T.L., Weingart, G., Ren, B., Schwager, E.H., Chatterjee, S., Thompson, K.N., Wilkinson, J. E., Subramanian, A., Lu, Y., Waldron, L., Paulson, J.N., Franzosa, E.A., Bravo, H.C., Huttenhower, C., 2021. Multivariable association discovery in population-scale meta-omics studies. PLOS Comput. Biol. 17 (11), e1009442. https://doi.org/10.1371/JOURNAL.PCBI.1009442.
Mandal, R.K., Jiang, T., Wideman Jr, R.F., Lohrmann, T., Kwon, Y.M, 2020. Microbiota analysis of chickens raised under stressed conditions. Front. Veterin. Sci. 7, 482637. https://doi.org/10.3389/fvets.2020.482637.
Martín, R., Rios-Covian, D., Huillet, E., Auger, S., Khazaal, S., Bermúdez-Humarán, L.G., Sokol, H., Chatel, J.M., Langella, P., 2023. Faecalibacterium: a bacterial genus with promising human health applications. FEMS Microbiol. Rev. 47 (4), fuad039. https://doi.org/10.1093/femsre/fuad039.
Martínez, E.A., Babot, J.D., Lorenzo-Pisarello, M.J., Apella, M.C., Chaia, A.P., 2016. Feed supplementation with avian Propionibacterium acid propionic contributes to mucosa development in early stages of rearing broiler chickens. Benefic. Microb. 7 (5), 687–698. https://doi.org/10.3920/BM2016.0077.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8 (4), e61217. https://doi.org/10.1371/journal.pone.0061217.
Murakami, Y., Tanabe, S., Suzuki, T., 2016. High-fat diet-induced intestinal hyperpermeability is associated with increased bile acids in the large intestine of mice. J. Food Sci. 81 (1), H216–H222. https://doi.org/10.1111/1750-3841.13166.
Nain, S., Renema, R.A., Zuidhof, M.J., Korver, D.R., 2012. Effect of metabolic efficiency and intestinal morphology on variability in n-3 polyunsaturated fatty acid enrichment of eggs. Poult. Sci. 91 (4), 888–898. https://doi.org/10.3382/PS.2011-01661.
Omaliko, P.C., Ferket, P.R., Ogundare, T.E., Apalowo, O.O., Enenya, I.G., Iwuozo, O.C., Han, J., Fasina, Y.O., 2024. Impact of dietary fat types on expression levels of dopamine and serotonin transporters in the ileum of broiler chickens. Poult. Sci. 103 (11), 104114. https://doi.org/10.1016/j.psj.2024.104114.
Pal, D., Naskar, M., Bera, A., Mukhopadhyay, B., 2021. Chemical synthesis of the pentasaccharide repeating unit of the O-specific polysaccharide from Ruminococcus gnavus. Carbohydr. Res. 507, 108384. https://doi.org/10.1016/j.carres.2021.108384.
Pan, D., Yu, Z., 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microb. 5 (1), 108–119. https://doi.org/10.4161/GMIC.26945.
Patrone, V., Minuti, A., Lizier, M., Miragoli, F., Lucchini, F., Trevisi, E., Rossi, F., Callegari, M.L., 2018. Differential effects of coconut versus soy oil on gut microbiota composition and predicted metabolic function in adult mice. BMC Genom 19, 1–17. https://doi.org/10.1186/s12864-018-5202-z.
Ravindran, V., Tancharoenrat, P., Zaefarian, F., Ravindran, G., 2016. Fats in poultry nutrition: digestive physiology and factors influencing their utilisation. Anim. Feed Sci. Technol. 213, 1–21. https://doi.org/10.1016/j.anifeedsci.2016.01.012.
Rios-Covian, D., Salazar, N., Gueimonde, M., de los Reyes-Gavilan, C.G., 2017. Shaping the metabolism of intestinal Bacteroides population through diet to improve human health. Front. Microbiol. 8 (MAR). https://doi.org/10.3389/FMICB.2017.00376/FULL.
Schoeler, M., Ellero-Simatos, S., Birkner, T., Mayneris-Perxachs, J., Olsson, L., Brolin, H., Loeber, U., Kraft, J.D., Polizzi, A., Martí-Navas, M., Puig, J., Moschetta, A., Montagner, A., Gourdy, P., Heymes, C., Guillou, H., Tremaroli, V., Fernández-Real, J.M., Forslund, S.K., Caesar, R., 2023. The interplay between dietary fatty acids and gut microbiota influences host metabolism and hepatic steatosis. Nat. Commun. 14 (1), 1–16. https://doi.org/10.1038/s41467-023-41074-3, 2023 14:1.
Shang, Y., Kumar, S., Oakley, B., Kim, W.K., 2018. Chicken gut microbiota: importance and detection technology. Front. Veterin. Sci. 5 (OCT), 420757. https://doi.org/10.3389/FVETS.2018.00254/BIBTEX.
Shin, J.H., Tillotson, G., MacKenzie, T.N., Warren, C.A., Wexler, H.M., Goldstein, E.J.C., 2024. Bacteroides and related species: the keystone taxa of the human gut microbiota. Anaerobe 85, 102819. https://doi.org/10.1016/J.ANAEROBE.2024.102819.
Sistrunk, J.R., Nickerson, K.P., Chanin, R.B., Rasko, D.A., Faherty, C.S., 2016. Survival of the fittest: how bacterial pathogens utilize bile to enhance infection. Clin. Microbiol. Rev. 29 (4), 819–836. https://doi.org/10.1128/cmr.00031-16.
Song, B., Sun, P., Kong, L., Xiao, C., Pan, X., Song, Z., 2024. The improvement of immunity and activation of TLR2/NF-κb signaling pathway by Romboutsia ilealis in broilers. J. Anim. Sci. 102, skae286. https://doi.org/10.1093/jas/skae286.
Tian, Y., Gui, W., Koo, I., Smith, P.B., Allman, E.L., Nichols, R.G., Rimal, B., Cai, J., Liu, Q., Patterson, A.D., 2020. The microbiome modulating activity of bile acids. Gut Microb 11 (4), 979–996. https://doi.org/10.1080/19490976.2020.1732268.
Vu, V., Muthuramalingam, K., Singh, V., Hyun, C., Kim, Y.M., Unno, T., Cho, M., 2022. Effects of β-glucan, probiotics, and synbiotics on obesity-associated colitis and hepatic manifestations in C57BL/6J mice. Eur. J. Nutrit. 1–15. https://doi.org/10.1007/s00394-021-02668-z.
Wolters, M., Ahrens, J., Romaní-Pérez, M., Watkins, C., Sanz, Y., Benítez-Páez, A., Stanton, C., Günther, K., 2019. Dietary fat, the gut microbiota, and metabolic health – A systematic review conducted within the MyNewGut project. Clin. Nutrit. 38 (6), 2504–2520. https://doi.org/10.1016/J.CLNU.2018.12.024.
Wu, Y., Zhang, H., Zhang, R., Cao, G., Li, Q., Zhang, B., Wang, Y., Yang, C., 2021. Serum metabolome and gut microbiome alterations in broiler chickens supplemented with lauric acid. Poult. Sci. 100 (9), 101315. https://doi.org/10.1016/J.PSJ.2021.101315.

Xiao, S.S., Mi, J.D., Mei, L., Liang, J., Feng, K.X., Wu, Y.B., Liao, X.D.i, Wang, Y., 2021. Microbial diversity and community variation in the intestines of layer chickens. Animals 11 (3), 840. https://doi.org/10.3390/ANI11030840.

Yang, M., Shi, L., Ge, Y., Leng, D., Zeng, B., Wang, T., Jie, H., Li, D., 2022. Dynamic changes in the gut microbial community and function during broiler growth. Microbiol. Spectr. 10 (4). https://doi.org/10.1128/spectrum.01005-22.

Ye, Z., Xu, Y.J., Liu, Y., 2022. Different typical dietary lipid consumption affects the bile acid metabolism and the gut microbiota structure: an animal trial using Sprague-Dawley rats. J. Sci. Food Agricult. 102 (8), 3179–3192. https://doi.org/10.1002/jsfa.11661.

Yokota, A., Fukiya, S., Islam, K.S., Ooka, T., Ogura, Y., Hayashi, T., Hagio, M., Ishizuka, S., 2012. Is bile acid a determinant of the gut microbiota on a high-fat diet? Gut microbes 3 (5), 455–459. https://doi.org/10.4161/gmic.21216.

Zafar, H., Saier, M.H., 2021. Gut Bacteroides species in health and disease. Gut Microbe. 13 (1), 1–20. https://doi.org/10.1080/19490976.2020.1848158.

Zhang, B., Zhang, H., Yu, Y., Zhang, R., Wu, Y., Yue, M., Yang, C., 2021. Effects of Bacillus coagulans on growth performance, antioxidant capacity, immunity function, and gut health in broilers. Poult. Sci. 100 (6), 101168. https://doi.org/10.1016/j.psj.2021.101168.

Zocco, M.A., Ainora, M.E., Gasbarrini, G., Gasbarrini, A., 2007. Bacteroides thetaiotaomicron in the gut: molecular aspects of their interaction. Digest. Liver Dis. 39 (8), 707–712. https://doi.org/10.1016/j.dld.2007.04.003.