

Research Paper

CHARACTERIZATION OF MICROBIAL DIVERSITY AND FATTY ACID COMPOSITION IN THE INTESTINE OF HEALTHY DOMESTIC DUCKS

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ARTICLE HIGHLIGHTS

- Healthy duck gut shows rich bacterial diversity dominated by Firmicutes.
- Duck intestine is enriched in saturated fatty acids compared to the feed.
- Short chain fatty acids in duck intestine greatly exceed those in the feed.
- Genera *Lactobacillus* and *Ruminococcus* are key markers of duck gut health.
- Palmitic, stearic, and vaccenic acids emerge as major intestinal lipids.

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ABSTRACT

Fatty acids exhibit antibacterial properties and serve as metabolic substrates for intestinal bacteria, shaping the composition of the microbiota and the synthesis of microbial metabolites. This study aimed to analyze the duck intestinal microbiota community and its fatty acid profile. This study utilized 25 healthy domestic ducks (*Anas platyrhynchos*) sourced from an intensive laying farm in Central Java, Indonesia. An exploratory observational research design was employed to analyze data obtained from this study. The intestinal bacteria of duck samples were analyzed using Next-Generation Sequencing (NGS), and fatty acid components using Gas Chromatography–Mass Spectrometry (GC-MS). Duck feed samples were analyzed for nutritional composition using proximate analysis and fatty acid composition using GC-MS. The Simpson and Shannon indices revealed rich microbial diversity in the duck intestine. The abundance of bacteria was dominated by several phyla, such as Firmicutes (82.58%), Actinobacteria (12.25%), and Bacteroidetes (4.27%). The Firmicutes/Bacteroidetes ratio was 19.32, with the intestine containing greater proportion of saturated fatty acids (74.32%) compared to unsaturated fatty acids (33.62%). Additionally, the concentration of short-chain fatty acids (SCFAs) in the duck's intestine was 15.72 times greater than that found in the feed. Microorganisms, including Bacteroides (2.88%), *Faecalibacterium* (0.16%), *Prevotella* (0.08%), *Butyrivibrio* (0.67%), *Ruminococcus* (2.2%), *Oscillospira* (0.02%), and *Mucispirillum* (0.018%) are genera that produce SCFA. Bacteria capable of synthesizing medium-chain fatty acids (MCFA) were identified, namely *Ruminococcaceae* UGC-005 (1.85%) and *Acinetobacter* (0.005%). Bacteria producing saturated long-chain fatty acids (LCFA) were identified, namely *Lactobacillus* (12.12%), *Prevotella* (0.08%), and *Alistipes* (0.13%). Bacteria capable of producing polyunsaturated fatty acids (PUFA) metabolites including conjugated linoleic acid (CLA) and conjugated linolenic acid (CLnA) were identified, namely *Propionibacterium* (0.004%), *Lactobacillus* (12.12%), and *Bifidobacterium* (0.21%). In summary, the intestinal microbiota of healthy ducks exhibits considerable diversity and a high diversity index. Various bacterial genera contribute to the production of SCFA, MCFA, and LCFA, as well as the conversion of PUFA into CLA and CLnA metabolites. This baseline microbiota–fatty acid profile can guide microbiota-informed feed optimization and candidate probiotic selection to support SCFA production, gut health, and productivity, and it provides a reference for monitoring dysbiosis and evaluating dietary interventions in intensive duck production systems.

Keywords: LCFA, MCFA, microbiota, PUFA, SCFA

INTRODUCTION

Ducks are a group of waterfowl whose part of life requires water habitats. The characteristic of waterfowl is their genetic superiority, shown by their better body resistance compared to land fowl. The immune system that is built in the intestinal microhabitat of ducks does not cause "negative effects" for ducks, even though pathogenic microorganisms are in their intestines. This raises the suspicion that the resistance mechanism in ducks is mediated by the diversity of microorganisms/microbiota in the intestines. Microbiota consists of a diverse bacterial community, and its abundance and composition offer insights into the host's metabolic state, nutrient absorption, and modulation of the immune system (Thaïss *et al.* 2016; Hou *et al.* 2022). Symbiotic microorganisms provide protection against pathogen colonization and overgrowth of native pathogens. These intricate mechanisms encompass competitive interactions among microbes and the stimulation of the host's immune defense mechanism (Caballero-Flores *et al.* 2023). Furthermore, gut microbiota, influenced by diet, plays a crucial role in regulating the host's metabolism and impacting immune responses (Zheng *et al.* 2020).

Gut microbiota is essential for intestinal health and immune function, metabolizing dietary fiber through fermentation and decomposition to produce short-chain fatty acids (SCFAs), which serve as an energy source for the host (Bridgman *et al.* 2017; Joseph *et al.* 2017). Short-chain fatty acids serve as key regulators of intestinal homeostasis and immunity by activating innate lymphoid cells (ILCs) and enhancing IL-22 production in CD4⁺ T cells (Yang *et al.* 2020). SCFAs also act as inhibitors of certain pathogens. Lactic acid bacteria break down carbohydrates into lactic acid, leading to a reduced environmental pH that suppresses the growth of *Clostridium perfringens*, *Salmonella typhimurium*, and *Escherichia coli* (van Zyl *et al.* 2020). Previously, an *in vivo* study has also identified an inverse relationship between SCFA levels, specifically acetate, butyrate, and propionate, and the abundance of Enterobacteriaceae in the colon (Sorbara *et al.* 2019). Additionally, certain commensal bacteria produce bacteriocins, a molecule that selectively inhibits the growth of specific bacterial species (Lajis 2020).

The predominant SCFAs, accounting for 95% of microbial metabolic by products in animals, include acetate, butyrate, and propionate (Ohira *et al.* 2017). These SCFAs are absorbed and

distributed to human tissues, serving as substrates for lipogenesis and cholesterol synthesis, or even energy production through gluconeogenesis (He *et al.* 2020). Research indicates that gut microbiota influences lipid metabolism through various mechanisms, with dietary lipids impacting host physiology via microbial interactions. Lipase enzymes break down fat precursors into free fatty acids, most of which are absorbed in the small intestine. However, some fatty acids reach the colon, where they directly shape gut microbiota composition (Schoeler & Caesar 2019).

Fatty acids exhibit antibacterial properties and serve as metabolic substrates for intestinal bacteria, thereby shaping the microbiota composition and influencing microbial metabolite production. Their antibacterial effect occurs through the disruption and dissolution of bacterial cell membranes (Jackman *et al.* 2016; Shilling *et al.* 2013). Bacteria also can utilize fatty acids as an energy source, while intestinal microbes also contribute to the biosynthesis of saturated fatty acids. The metabolism of long-chain saturated fatty acids has been shown to promote *Lactobacillus* growth and strengthen intestinal barrier integrity (Chen *et al.* 2015). This study examined the gut microbiota composition in ducks and analyzed their fatty acid profile. Understanding microbial communities in the gastrointestinal tract of healthy ducks is crucial for predicting microbiota shifts in response to dietary changes and physiological adaptations. Identifying key markers of intestinal microbiome stability can facilitate the development of alternatives to antibiotic growth promoters (AGPs) for modern poultry farming (Hodak *et al.* 2023).

MATERIALS AND METHODS

This observational study explored duck intestinal contents by taking samples from an intensive egg-laying duck farm in Central Java, Indonesia. We selected 25 local domestic ducks (*Anas platyrhynchos domesticus* Linnaeus, 1758), based on the inclusion criteria, including: 1) an egg-laying breed variety; 2) kept in intensive caging; 3) three months old; and 4) had not received antibiotics or other medications for at least a month before sampling. The ducks received a normal basal diet of SNI 01-3910-2006—compliant commercial layer pellets blended with rice bran and corn. The ration was supplemented with soybean meal or minced snail for protein and balanced with limestone or dicalcium phosphate plus a vitamin–mineral premix. The selected ducks were considered clinically healthy based

on the criteria of having bright–alert–responsive behavior; normal basal feed and water intake during the preceding 48 hours; absence of clinical signs (no nasal/ocular discharge, coughing, open-mouth breathing, diarrhea, lameness, skin/feather lesions, or neurologic signs); clean vent and normal fecal consistency; body weight within $\pm 10\%$ of the age-matched flock mean; and no palpable coelomic distension. A veterinarian performed the brief physical examination on all candidates; any ducks failing one or more criteria were excluded.

This was an observational farm-based study. No invasive procedures, surgery, or euthanasia were performed by the authors. Ducks were managed under routine husbandry practices at the farm, and sampling or handling was conducted briefly and under veterinary supervision to minimize stress and maintain animal welfare.

Ethical Statement

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Intestinal Content Extraction and Processing

The selected ducks were terminated following standard breeder procedures, then the gut contents were aseptically extracted and placed into sterile falcon tube. Duck samples were excluded from the study if endoparasites were found in their intestines, because this could have a negative impact on gut anatomy and physiology.

Samples of intestinal contents from twenty-five ducks were then divided randomly into five groups. The intestinal contents from each group were thoroughly combined and homogenized with a vortex mixer before being stored in 3 mL microtubes. The samples were then frozen at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis of microbial composition through metagenomic sequencing via Next-Generation Sequencing (NGS) and for fatty acid profiling using Gas Chromatography–Mass Spectrometry (GC-MS).

Duck feed samples were taken at random in the amount of 1 kg to be analyzed for nutritional composition using proximate analysis and fatty acid composition was analyzed using GC-MS. Each analysis was carried out 3 times.

Microbiome DNA Isolation and Profiling

Bacterial DNA was isolated from the samples using the QIAamp DNA Stool Mini Kit (Qiagen, San Diego, California, USA) in accordance with the manufacturer's protocol to ensure optimal DNA yield and purity. The characterization of gut microbiota diversity and abundance was conducted by targeting the V3-V4 hypervariable region of the 16S rRNA gene, a widely used marker for microbial community profiling (Yarza *et al.* 2014).

The amplification was performed using the Illumina HiSeq 2500 sequencing platform, employing the forward primer 338F (5'-GGACTACHVGGGTWTCTAAT-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which specifically anneal to conserved regions flanking the targeted gene segment (Holm *et al.* 2019). These primers were designed with an additional barcode sequence of eight bases unique to each sample for accurate identification during sequencing.

The polymerase chain reaction (PCR) was conducted in a final reaction volume of 20 μL , consisting of 0.5 μL (5 U/ μL) Easy Taq DNA polymerase (TransGen Biotech. Co. LTD), 2 μL of $10\times$ Easy Taq buffer, 2 μL of 0.25 mmol/L deoxynucleotide triphosphates (dNTPs), 0.2 $\mu\text{mol/L}$ of each primer, 10 ng of DNA template, and molecular-grade double-distilled water (ddH_2O) to complete the volume.

The amplification program with polymerase chain reaction (PCR) was $94\text{ }^{\circ}\text{C}$ denaturation for 3 minutes, followed by 27 cycles (denaturation at $95\text{ }^{\circ}\text{C}$ for 30 seconds, annealing at $55\text{ }^{\circ}\text{C}$ for 30 seconds, extension at $72\text{ }^{\circ}\text{C}$ for 45 seconds) and final extension at $72\text{ }^{\circ}\text{C}$ for 10 minutes. Following successful amplification, a metagenomic sequencing approach was applied to analyze microbial community composition and structure in the duck gut environment.

Operational Taxonomic Unit (OTU) Cluster and Species Annotation

Uparse software (version 7.0.1001, available at <http://drive5.com/uparse/>) was used to analyze nucleotide sequences. Then, the sequences exhibiting a similarity of 97% or higher were grouped into the same operational taxonomic unit (OTU).

Taxonomic classification was facilitated by extracting representative sequences from each OTU, then subjected to further annotation. Species

identification was performed according to the GreenGenes database (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>), utilizing the Ribosomal Database Project (RDP) Classifier (version 2.2, accessible at <http://sourceforge.net/projects/rdp-classifier/>). This classification approach enabled taxonomic assignment of each representative sequence based on established hierarchical criteria.

Sequencing Data Analysis

Metagenomic analysis of the 16S rRNA gene sequencing data was conducted using the QIIME2 software package (version 2019.4; Caporaso *et al.* 2010). Paired-end files were demultiplexed with the demux plugin, and quality control was executed on each sample using the Dada2 plugin (Callahan *et al.* 2016). Five diversity indices were produced from this analysis, including observed OTUs, Shannon, Simpson, Chao1, and ACE. Taxonomic and species assignments were determined using the GreenGenes 13_8 99% OTU database (McDonald *et al.* 2012). Heatmaps were generated using the Heatmap plugin, and taxonomic bar plots were created in Microsoft Excel 2010.

Gas Chromatography Mass Spectrometry Analysis

Gas chromatography mass spectrometry (GC-MS) was employed to evaluate the fatty acid components present in duck feed and its intestinal contents. Twenty grams of each sample were dried at 30 °C for three days to reduce excessive water content. Subsequently, 250 mg portions of the dried samples were taken and crushed using a mortar and pestle. The material was wrapped in filter paper and placed in a Soxhlet extractor. To extract fatty acid components, the sample was dissolved in 250 mL of n-hexane. The extraction was carried out following a modified procedure based on Eneroth *et al.* (1968) and Batta *et al.* (2002), with each sample underwent 12 cycles.

The extract was evaporated at 68 °C for six hours until a constant weight was achieved, then injected into the GC-MS. Analysis was performed using a Shimadzu QP2010S with an Agilent HP-5 column (30 m length, 0.25 mm internal diameter) and helium as the carrier gas. The mass spectrometer operated under electron ionization (EI) at 70 eV, with the ion source temperature maintained at 250 °C. Compound identification was conducted through a library search using the Shimadzu NIST/WILEY+ mass spectral database.

Feed Proximate Composition Analysis

The proximate composition of the duck feed was determined according to the Association of Official Analytical Chemists (AOAC 2016) method. Each proximate component type was analyzed in triplicate, specifically, crude protein was determined using the Kjeldahl method. Subsequently, total fat was measured via acid hydrolysis with a Soxhlet extractor at 60 °C until a constant weight was achieved. Ash content was measured using the gravimetric method by incinerating the sample at 550 °C until a constant weight was achieved. Total carbohydrates content was calculated as the sum of starch (AOAC 996.11), free sugars (e.g., AOAC 982.14/2001.05), and total dietary fiber (AOAC 991.43 or 2009.01), all on a [dry-matter/as-fed] basis.

High Performance Liquid Chromatography (HPLC) Analysis

Analysis of carbohydrate components in the duck feed was carried out using the HPLC method. The analysis was conducted in triplicate. First, the duck feed was evaporated to reduce its water content to 20%. Then, 0.5 mL of feed was ground with a mortar and placed into a threaded tube. Organic material hydrolysis was performed by adding 10 mL of 6 N HCl, followed by incubation in a water bath at 100 °C for 24 hours. The solution was then cooled to room temperature and evaporated, leaving 1 mL of concentrate. After dilution with 0.01 N HCl to a final volume of 10 mL, the solution was filtered.

Five standard sugar solutions (0.1, 0.5, 1, 2.5, and 5 µg/mL) were prepared in distilled water to a total volume of 5 mL. The solution was stabilized using an orthophthalaldehyde (OPA) reagent with citrate buffer (pH 3.25). Both the OPA reagent and mobile phase solution (eluent) were filtered through a polytetrafluoroethylene (PTFE) membrane and degassed. Standard and sample solutions were also filtered through a cellulose nitrate membrane before HPLC injection.

Each sample or standard solution was mixed 1:1 with the OPA reagent, homogenized using a vortex, degassed, and loaded into a syringe. The analysis was performed using a Shimadzu LC-20AT HPLC equipped with an SPD-20A detector (RF-20A Fluorescence Detector) under the following conditions: sodium citrate buffer (pH 3.25), 450 nm wavelength, and a run time of 30 minutes.

RESULTS AND DISCUSSION

Based on the amplification step, an average of 252,305 sequence reads was obtained from pooled intestinal samples. These reads were grouped into operational taxonomic units (OTUs). An average of 188,874 OTUs was recorded in the duck intestine, and an average of 1,164.6 species was observed (Table 1).

The values of Shannon and Simpson indices indicated a high microbial diversity in the duck intestine. The average Shannon index observed in this study was 5.145 ± 1.47 , which is consistent with the values reported for other poultry. Previous studies have reported moderate Shannon index values for duck intestinal content, ranging from 4.00 to 5.00 (Guo *et al.* 2019; Wen *et al.* 2019), whereas in mammals, it typically ranges from 7.00 to 9.00 (Cotozzolo *et al.* 2020; Xie *et al.* 2021).

The abundance-based coverage estimator (ACE) and Chao1 values of bacteria in duck's intestines were 619,277 and 609,072 respectively. Bacterial abundance was dominated by three of the 19 phyla identified, namely Firmicutes (82.58%), Actinobacteria (12.25%) and Bacteroidetes (4.27%). The relative abundance of 16 other bacterial phyla was less than 1%. Unassigned bacteria only accounted for only 0.006% of the abundance within the community (Table 2).

The dominance of these three phyla in the intestines of ducks in this study is in line with previous research on geese (*Cygnus cygnus*) (Susanti

et al. 2020), turkeys (*Meleagris gallopavo*) (Miller *et al.* 2021), Muscovy ducks (*Cairina moschata*) (Xiao *et al.* 2021), and chickens (Clavijo *et al.* 2022).

Bacteria of the Firmicutes phylum play a crucial role in carbohydrate fermentation process and lipid metabolism (Bourdon *et al.* 2019), by producing several enzymes that degrade dietary fibers or polysaccharide into small molecules (Pham *et al.* 2019). In intensively reared ducks, the small-intestinal Firmicutes-to-Bacteroidetes (F/B) ratio was 19.32, higher than previously reported in chickens (Susanti *et al.* 2022), geese (Susanti *et al.* 2020), and Muscovy ducks (Susanti *et al.* 2021) in Indonesia. A higher F/B ratio typically reflects a lower abundance of Bacteroidetes relative to Firmicutes, a pattern correlated with low fiber intake (Méndez-Salazar *et al.* 2018). Furthermore, fiber-enriched poultry feed has been shown to increase Bacteroidetes, thereby lowering the F/B ratio (Magne *et al.* 2020).

The composition of gut microbiota, characterized by a high abundance of Firmicutes correlated with increased nutrient absorption, whereas high abundance of Bacteroidetes linked to decreased nutrient absorption (Jumpertz *et al.* 2011). Based on the proximate analysis, the duck feed contained higher carbohydrates (20.91%) than protein (17.83%) and fat (3.65%) (Table 3). On the other hand, polysaccharides were more abundant than monosaccharides (Table 4), it was assumed that small-molecule polysaccharides were digested and absorbed in the small intestine.

Table 1 Summary statistics and diversity indices

Sequencing results and diversity index	Value
Sequencing number	252,305 $\pm$ 17,807.51
OTUs (operational taxonomic unit)	188,874 $\pm$ 12,597
Observed species	1,164.6 $\pm$ 58.45
Shannon index	5.145 $\pm$ 1.47
Simpson index	0.883 $\pm$ 0.09
Chao1	609,072 $\pm$ 64,272.76
ACE (abundance-based coverage estimator)	619,277 $\pm$ 68,525.85
Goods_coverage	0.998 $\pm$ 0.00

Table 2 Average absolute and relative abundance of the bacterial phyla in the intestines of ducks

Phylum	Average absolute number	Relative abundance (%)
Firmicutes	155,969.4±20,430.3	82.579
Actinobacteria	23,128.4±17,940	12.245
Bacteroidetes	8,072.4±6,773	4.274
Proteobacteria	1,108.4±560	0.586
Tenericutes	120.2±16.1	0.064
Saccharibacteria	44.0±7.2	0.023
Thermomicrobia	65.0±7.8	0.034
Acidobacteria	120.4±2.9	0.064
Gemmatimonadetes	63.4±3.2	0.034
Fusobacteria	33.2±4.8	0.018
Deferribacteres	34.0±3.3	0.018
Chloroflexi	33.2±1.5	0.018
Nitrospirae	26.4±1.4	0.014
Spirochaetes	16.0±9.8	0.008
Latescibacteria	23.2±4.7	0.012
Cyanobacteria	2.4±0.3	12.71x10 ⁻⁴
Verrucomicrobia	1.0±0.0	2.12x10 ⁻⁴
Planctomycetes	1±0.0	6.35x10 ⁻⁴
Zixibacteria	1±0.5	3.18x10 ⁻⁴
Others	11.6±6.0	0.006

Table 3 Results of proximate analysis of duck feed

Nutritional content	Amount (%)
Water	9.90 ± 0.90
Ash	25.81 ± 2.17
Protein	17.83 ± 1.72
Carbohydrates	20.91 ± 1.37
Fat	3.65 ± 0.84
Other compounds	21.90 ± 1.39

Table 4 The carbohydrate content in duck feed

Carbohydrate	Content (μmol/g)
Monosaccharide	
Arabinose (C5)	12.64 ± 0.85
Galactose (C6)	33.13 ± 2.13
Glucose (C6)	26.44 ± 1.69
Mannose (C6)	19.75 ± 1.31
Rhamnose (C6)	21.27 ± 1.29
Xylose (C5)	16.55 ± 1.04
Total	129.77 ± 8.29
Polysaccharide	
Trehalose (disaccharide)	62.96 ± 3.75
Raffinose (trisaccharide)	56.09 ± 3.54
Stachyose (tetra saccharide)	79.38 ± 5.16
Total	198.43 ± 12.44

The dominant class of bacteria in the phylum Firmicutes were Bacilli (62.57%), Clostridia (15.58%), and Erysipelotrichia (4.42%). The Actinobacteria phylum was dominated by class unidentified-Actinobacteria (11.90%) and Coriobacteria (0.26%). The phylum Bacteroidetes was dominated by class Bacteroidia (4.26%), Gammaproteobacteria (0.26%), Alphaproteobacteria (0.17%) and Deltaproteobacteria (0.12%) (Fig. 1).

The eight genera of bacteria dominating duck intestine was *Streptococcus* (31.62%), *Lactobacillus* (12.12%), *Corynebacterium_1* (8.20%), *Bacillus* (7.11%), *Weissella* (4.12%), *Enterococcus* (3.45%), *Rombutsia* (2.94%), *Bacteroides* (2.88%). The prevalence of other genera was less than 2.8%, including *Prevotella* (0.08%), *Alistipes* (0.13%), *Propionibacterium* (0.004%), and *Bifidobacterium* (0.21%) (Table 5).

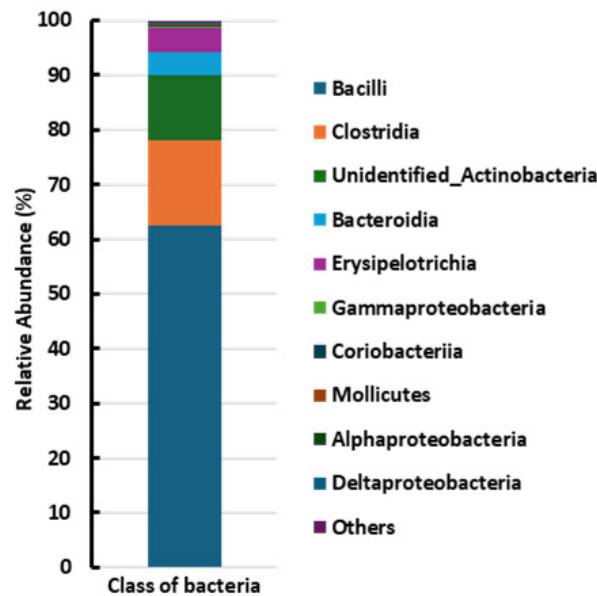


Figure 1 Relative abundance of the top ten bacterial classes in the gut of healthy duck

Table 5 Relative abundance of genera bacteria in intestinal of duck

Genera of bacteria	%
<i>Streptococcus</i>	31.62
<i>Lactobacillus</i>	12.12
<i>Corynebacterium_1</i>	8.2
<i>Bacillus</i>	7.11
<i>Weissella</i>	4.12
<i>Enterococcus</i>	3.45
<i>Rombutsia</i>	2.94
<i>Bacteroides</i>	2.88
<i>Subdoligranulum</i>	2.64
<i>Pediococcus</i>	2.07
<i>Ruminococcaceae</i> UGC-005	1.51
<i>Butyricicoccus</i>	0.67
<i>Bifidobacterium</i>	0.21
<i>Faecalibacterium</i>	0.16
<i>Ruminococcus</i>	0.14
<i>Alistipes</i>	0.13
<i>Prevotella</i>	0.08
<i>Oscillospira</i>	0.02
<i>Mucispirillum</i>	0.018
<i>Acinetobacter</i>	0.006
<i>Propionibacterium</i>	0.004
Others	19.9

Streptococcus, *Lactobacillus*, and *Enterococcus* genera play a role in carbohydrate metabolism, especially the absorption and conversion of monosaccharides (Wessels 2022). The *Lactococcus* genus is also involved in carbohydrate and lipid metabolism (Maki *et al.* 2019). In ducks, *Bacteroides* modulates immune system and enhancing intestinal mucosal barrier against pathogenic agent, like virus and bacteria (Wexler & Goodman 2017) by producing SCFA, including butyrate and propionate acid (Yang *et al.* 2018). The intestinal lipid composition was influenced by multiple sources, such as dietary lipids secreted by host's intestinal cells

and those produced by resident bacteria, and undigested fats. Among these, host-derived lipid byproducts including intestinal secretions, bile, and shed epithelial cells, are rich in cholesterol and phospholipids (Gérard 2020).

Saturated fatty acids in the intestine were 1.41-fold higher than in the feed. In total, 17 distinct saturated fatty acids were detected in the feed (5 SCFA, 5 medium-chain fatty acids-MCFA, 7 long-chain fatty acids-LCFA), whereas 15 were identified in the intestine (6 SCFA, 5 MCFA and 4 LCFA) were identified in the intestine (Table 6).

Table 6 Composition of various saturated fatty acids found in duck feed and gut content

Fatty Acids	Feed (%)	Intestine (%)	Balance	Fold
SCFA				
Acetic acid (C2)	0.25 ± 0.00	27.86 ± 2.04	27.61	111.56
Butyric acid (C4)	0.72 ± 0.01	6.36 ± 0.34	5.64	8.84
Isobutyric acid (C4)	0.00 ± 0.00	1.39 ± 0.23	1.39	-
Isovaleric acid (C5)	0.51 ± 0.47	1.98 ± 0.19	1.47	3.87
Propionic acid (C3)	0.54 ± 0.01	12.10 ± 0.46	11.57	22.45
Valeric acid (C5)	1.04 ± 0.02	1.41 ± 0.23	0.37	1.36
Subtotal SCFA	3.36	52.90	49.54	15.72
MCFA				
Heptanoic acid (C7)	0.85 ± 0.01	1.62 ± 0.43	0.77	1.91
Hexanoic acid (C6)	0.30 ± 0.17	1.78 ± 0.29	1.48	5.88
Octanoic acid (C8)	0.67 ± 0.01	1.92 ± 0.79	1.25	2.88
Nonanoic acid (C9)	0.32 ± 0.01	1.44 ± 0.51	1.11	4.46
Decanoic acid (C10)	0.72 ± 0.01	1.18 ± 0.59	0.46	1.65
Subtotal MCFA	2.56	6.16	3.60	2.41
LCFA				
Lauric acid (C12)	23.79 ± 0.38	0.84 ± 0.29	-22.95	0.04
Myristic acid (C14)	7.49 ± 0.12	1.16 ± 0.26	-6.33	0.15
Pentadecanoic acid (15)	0.01 ± 0.03	0.00 ± 0.00	-0.01	0.00
Palmitic acid (C16)	10.70 ± 0.17	9.93 ± 0.84	-0.77	0.93
Heptadecanoic acid (C17)	0.50 ± 0.01	0.00 ± 0.00	-0.50	0.00
Stearic acid (C18)	3.97 ± 0.06	3.33 ± 0.27	-0.63	0.84
Arachidic acid (C20)	0.16 ± 0.14	0.00 ± 0.00	-0.16	0.00
Subtotal LCFA	46.61	15.26	-31.35	0.33
Total	52.54	74.32	21.79	1.41

Notes: SCFA= short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid

Comparative analysis showed that the concentration of SCFAs in the intestinal tract was approximately 15.72 times compared in the feed (Table 6). While five types of SCFA that were detected in feed, six were identified in the intestine. Isobutyric acid was absent in feed but was detected in small amounts (1.39%) in the intestine. Among the SCFAs, acetic acid showed the most significant increase in the intestine, rising 111.56 times compared to propionic acid (22.45-fold), butyric acid (8.84-fold), isovaleric acid (3.87-fold) and valeric acid (1.36-fold). Valeric acid was the most abundant SCFA in the feed (1.04%) but exhibited the smallest increase in the intestine (1.36-fold) compared to other SCFA.

High levels of SCFAs in the intestine play a crucial role in maintaining host metabolic homeostasis. Produced by commensal bacteria, SCFAs, including acetate, butyrate, and propionate, help prevent pathogen overgrowth and colonization while also mediating inflammation control (Wei *et al.* 2016; Chen *et al.* 2019). Specifically, butyrate is the main anti-inflammatory modulator among other SCFAs. Chen and Vivetta (2020) reported that butyric acid plays a role in reducing acute inflammation caused by pathogenic bacteria by inhibiting the growth of pathobionts, increasing the integrity of the mucosal barrier, encouraging the dominance of obligate anaerobic bacteria, and reducing the availability of oxygen in the intestine. Conversely, butyrate appears to boost the activity of M2 macrophages and regulatory T cells—both essential for moderating immune responses—while simultaneously reducing the extent to which neutrophils infiltrate tissues.

Within the gastrointestinal tract, a diverse community of microbes converts dietary fibers into a range of SCFAs through processes such as fermentation and decomposition. Animal intestinal microbes primarily convert polysaccharide substrates, especially fiber, into SCFAs, with acetate, propionate, and butyrate comprising approximately 95% of these metabolic products (Ohira *et al.* 2017). Most SCFAs are generated in the cecum (Yang *et al.* 2018), where a diverse microbial community plays a pivotal role. Notably, bacteria from the genera *Bacteroides*, *Faecalibacterium*, *Prevotella*, and *Butyrivibrio* (Zhang *et al.* 2015), along with *Ruminococcus*, *Oscillospira*, and *Mucispirillum* (Yang *et al.* 2020), have been recognized as key SCFA producers in this region. For instance, *Bacteroides* are typically associated with acetate

production, while *Faecalibacterium* is well-known for its role in butyrate synthesis (LeBlanc *et al.* 2017; Ohira *et al.* 2017).

In our study, the identified relative abundances were 2.88% for *Bacteroides*, 0.16% for *Faecalibacterium*, 0.08% for *Prevotella*, 0.67% for *Butyrivibrio*, 0.14% for *Ruminococcus*, 0.02% for *Oscillospira*, and 0.018% for *Mucispirillum* (Table 5). Additionally, these bacteria possess genes involved in the butyrate metabolism, such as butyryl-CoA, butyrate kinase, and acetate CoA-transferase, which are crucial for maintaining butyrate production within the microbiome (Yang *et al.* 2020).

The concentration of MCFAs in the intestine was 2.41 times higher in the feed (Table 6). In particular, hexanoic acid increased by 5.88-fold, the largest boost among the MCFAs examined. Other acids also raised significantly, with nonanoic acid increasing 4.46-fold, octanoic acid 2.88-fold, heptanoic acid 1.91-fold, and decanoic acid 1.65-fold. These increases suggest in situ microbial synthesis of MCFAs in the gut. Previous research has reported that certain microorganisms, including strains like *Ruminococcaceae* CPB6 (Zhu *et al.* 2015), *Acinetobacter* spp. (Kucek *et al.* 2016), and *Clostridium kluyveri* (Wang *et al.* 2018a), can synthesize MCFAs through a carbon chain elongation pathway (Angenent *et al.* 2016). Consistent with these findings, our study identified the presence of *Ruminococcaceae* UGC-005 (1.51%) and *Acinetobacter* (0.006%) (Table 5).

Key enzymes known as coenzyme A transferases (CoATs) are essential for the elongation process of these carbon chains. For example, Yang *et al.* (2021) showed that *Ruminococcaceae* CPB6 is capable of extending carbon chains to produce caproic acid (C6). Caproic acid formation involves different pathways: it can be synthesized from lactate (serving as the electron donor) combined with C2-C4 carboxylic acids (acting as electron acceptors), or from heptanoic acid (C7) paired with C3-C5 carboxylic acids, respectively (Zhu *et al.* 2017; Wang *et al.* 2018).

The profile of LCFA in the intestine exhibited an overall reduction, measuring 0.33-fold in comparison to the feed (Table 6). Seven types of LCFA were detected in the feed, while four types of LCFA were identified in the intestine. The feed primarily contained three types of LCFA: lauric acid (23.79%), followed by palmitic acid (10.70%), and myristic acid (7.49%). Meanwhile, pentadecanoic acid, heptadecanoic and arachidic acid were not

detected in the intestine. Furthermore, the data in Table 6 indicate that the detection of saturated fatty acids in the intestine diminishes as the carbon chain length increases.

The low levels of saturated LCFA in the intestine may be due to the fact that LCFA is widely used by bacteria to form outer membrane vesicles (OMV). Outer membrane vesicles are particles measuring 20 - 250 nm which are composed of proteins, hydrolytic enzymes, lipopolysaccharides, DNA and RNA (Schwechheimer & Kuehn 2015). OMV *Bacteroides* is able to modulate immunity and tolerance against intestinal commensal bacteria (Kulkarni & Jagannadham 2014). It was further reported that OMV production by *Bacteroides fragilis* was influenced by the concentration of palmitic acid, while OMV of *Bacteroides thetaiotaomicron* was influenced by the dose of palmitoleic acid (Tafti *et al.* 2019). Moreover, some bacteria such as *Prevotella*, *Lactobacillus*, and *Alistipes* can produce saturated LCFA (Zhao *et al.* 2018).

Previous study reported that saturated LCFA contributes to increased colonic motility thereby facilitating defecation in mice (Zhao *et*

al. 2018). In another investigation, researchers observed that microbial components, most notably lipopolysaccharide (LPS), can influence the movement of the gastrointestinal tract by activating the signaling pathway associated with toll-like receptor 4 (TLR4) (Anitha *et al.* 2012).

Meanwhile, the proportion of unsaturated fatty acids in the intestine was observed to be (0.95-fold) lower than that in the feed. Fourteen types of unsaturated fatty acids (5 mono-unsaturated fatty acid-MUFA, 9 poly-unsaturated fatty acid-PUFA) were detected in the feed, whereas ten types (4 MUFA, 6 PUFA) were found in the intestine (Table 7). The contribution of commensal bacteria to the lipid profile in the intestine comes from dead bacterial cells, which contain many phospholipids, sphingolipids, and saturated and unsaturated fatty acids. According to current scientific estimates, bacteria are responsible for producing approximately 25% to 33% of the lipids present in feces. This implies that a significant portion of the excreted lipids originate from microbial sources rather than being solely derived from diet or the host (Gerard 2020).

Table 7 Composition of unsaturated fatty acids in duck feed and intestines

Fatty acids	NSF	Feed (%)	Intestinal (%)	Balance	Fold
MUFA					
Eicosenoic acid	20:1, n-9	0.70 ± 0.01	0.00 ± 0.00	-0.70	0.00
Erucic acid	22:1, n-9	0.20 ± 0.18	0.00 ± 0.00	-0.20	0.00
Myristoleic acid	14:1, n-5	0.20 ± 0.00	0.59 ± 0.32	0.40	3.02
Oleic acid	18:1, n-9	17.62 ± 0.28	11.30 ± 0.58	-6.32	0.64
Palmitoleic acid	16:1, n-7	3.08 ± 0.05	1.81 ± 0.20	-1.28	0.59
Vaccenic acid	18:1, n-7	0.00 ± 0.00	1.66 ± 0.21	1.66	-
Subtotal MUFA		21.81	15.36	-6.45	0.70
PUFA					
Arachidonic acid	20:4, n-6	0.97 ± 0.02	2.52 ± 0.16	1.55	2.60
Linoleic acid	18:2, n-6	4.93 ± 0.08	11.15 ± 0.58	6.21	2.26
α Linolenic acid	18:3, n-3	2.11 ± 0.03	2.24 ± 0.25	0.12	1.06
γ Linolenic acid	18:3, n-6	0.44 ± 0.01	0.79 ± 0.30	0.35	1.80
Stearidonic acid	18:4, n-3	0.79 ± 0.01	0.00 ± 0.00	-0.79	0.00
Eicosapentaenoic acid	20:5, n-3	2.38 ± 0.04	0.00 ± 0.00	-2.38	0.00
Eicosatetraenoic acid	20:4, n-6	0.62 ± 0.01	0.00 ± 0.00	-0.62	0.00
Eicosadienoic acid	20:2, n-6	0.28 ± 0.16	0.00 ± 0.00	-0.28	0.00
Docosahexaenoic acid	22:6, n-3	0.88 ± 0.01	0.00 ± 0.00	-0.88	0.00
Dihomo γ linolenic acid (DGLA)	20:3, n-6	0.00 ± 0.00	0.69 ± 0.31	0.69	-
Docosatetraenoic acid	22:4, n-6	0.00 ± 0.00	0.88 ± 0.29	0.88	-
Subtotal PUFA		13.41	18.26	4.85	1.36
Total		35.21	33.62	-1.6	0.95

Notes: NSF = numerical structural formula; MUFA = mono-unsaturated fatty acid; PUFA = poly-unsaturated fatty acid

The total amount of PUFA in the intestine (18.26%) was higher than MUFA (15.36%). Meanwhile in feed, MUFAs (21.81%) were more abundant than PUFA (13.41%). Overall, only five types of unsaturated fatty acids were found in higher composition in the intestine compared to the feed, namely myristoleic acid (3.02-fold), α linolenic acid (1.06-fold), γ linolenic acid (1.8-fold), linoleic acid (2.26-fold) and arachidonic acid (2.6-fold). Unsaturated fatty acids that were absent from the feed but detected at low levels in the intestine were vaccenic acid, dihomo- γ -linolenic acid (DGLA), and docosatetraenoic acid. In contrast, eicosenoic, erucic, stearidonic, eicosapentaenoic (EPA), eicosatetraenoic (arachidonic), eicosadienoic, and docosahexaenoic (DHA) acids were present in the feed but not detected in the intestine (Table 7).

Certain intestinal bacteria can enzymatically convert PUFAs into beneficial metabolites like conjugated linolenic acid (CLnA) and conjugated linoleic acid (CLA), both of which contribute to overall health. Key bacterial producers of these PUFA metabolites in humans and animals include *Propionibacterium*, *Lactobacillus*, and *Bifidobacterium* (Salsinha *et al.* 2018). Conjugated linoleic acid has been determined against cancer, obesity, and atherosclerosis properties (Basak & Duttaroy 2020), while CLnA has anti-cancer activity (Ren *et al.* 2020). Study of Druart *et al.* (2014) reported that the cecal production of CLA metabolites was higher in rats fed sunflower oil (rich in n-6 PUFA), while CLnA metabolite products were higher in those fed flaxseed oil (rich in n-3 PUFA).

This study found that the composition of saturated fatty acids in the intestine (74.32%) was higher than unsaturated fatty acids (33.62%) (Tables 6 and 7). The predominance of saturated fatty acids in the duck intestinal profile most likely reflects preferential host uptake of MUFA/PUFA, leaving a larger fraction of saturated fatty acid unabsorbed in the lumen, rather than a true deficit of unsaturated fats (Zhang *et al.* 2025). Furthermore, unsaturated fatty acids are generally emulsified, hydrolyzed, and absorbed more efficiently than saturated fatty acids in poultry, its make intestinal tract often becomes relatively enriched in saturated fats after digestion (Sycho 2016).

This finding is biologically important because a higher residual saturated fatty acid load can alter the lipid substrates reaching distal gut regions and, in turn, help shape microbial community structure

and fermentation capacity. Previous study reported that improving lipid utilization (through blending saturated with unsaturated fats to enhance emulsification) could reduce residual saturated fats in digesta and potentially support a more favorable microbiota–metabolite profile (Oketch *et al.* 2023).

Several long-chain unsaturated fatty acids present in the feed were no longer detected in intestinal contents, whereas vaccenic acid, dihomo- γ -linolenic acid, and docosatetraenoic acid appeared de novo; in parallel, and arachidonic acid increased (Table 7). This pattern is expected from three processes. First, diet-derived long-chain MUFA/PUFA are efficiently absorbed in proximal intestine after micellar solubilization and re-esterification in enterocytes, so they often vanish from distal digesta, explaining the disappearance of 20–22-carbon UFAs (Oketch *et al.* 2023). Second, the n-6 elongation/desaturation pathway (FADS/ELOVL) can convert fatty acid with medium unsaturated chain (from 18:2 n-6 to 18:3 n-6) and also long-unsaturated chain (from 20:3 n-6 and 20:4 n-6 to 22:4 n-6), consistent with the emergence of DGLA and 22:4 n-6 and the rise of AA in the lumen (from endogenous secretions/cell turnover) (Nematbakhsh *et al.* 2021). Third, microbiota-mediated modification of unsaturated fatty acids, including isomerization/partial hydrogenation, can yield 18:1 n-7 (vaccenic acid), which explains its appearance despite absence in the feed (well-documented in ruminants and observed in non-ruminant models) (Toral *et al.* 2024).

The loss of EPA/DHA from digesta most plausibly indicates efficient absorption rather than depletion; conversely, the presence of DGLA and higher AA signals active essential-FA metabolism that may influence mucosal eicosanoid tone (e.g., DGLA-derived PGE₁ vs AA-derived series-2 prostanoids) (Oketch *et al.* 2023). Because functional impact depends on tissue deposition and fermentation context, future work should quantify cecal SCFAs and plasma/egg-yolk FA profiles, and relate them to performance indices (e.g., feed conversion, growth) to determine biological significance (Schoeler *et al.* 2023).

In non-ruminant poultry, a higher saturated fraction in intestinal digesta alongside lower luminal MUFA/PUFA is expected when long-chain unsaturated fatty acids are efficiently absorbed proximally. The absorbed MUFA/PUFA are then exported and deposited in tissues and eggs rather than remaining in the lumen. This pattern

is consistent with normal lipid physiology in birds and with trials showing that dietary unsaturated fats enrich egg-yolk fatty acids (Oketch *et al.* 2023). Elevated SCFAs from cecal fermentation are beneficial for gut function, strengthening tight junctions and mucus, and modulating immunity (e.g., up-regulating anti-inflammatory pathways including IL-10), which together improve resistance to pathogens (Liu *et al.* 2022). In poultry specifically, SCFAs contents are linked to better gut barrier integrity and performance (Ali *et al.* 2022) and reduce lipid accretion or favorably influence lipid metabolism (Yang *et al.* 2024).

Previous study showed that excessive intake of SCFA production can lead to enteritis, indicating the need for optimal levels to maximize benefits without adverse effects (Liu *et al.* 2022). Additionally, the synergistic effects of SCFAs with other dietary components, could further enhance their efficacy in promoting poultry growth, health, and meat quality (Szabó *et al.* 2023; Hao *et al.* 2021). Because functional impact depends on SCFA quantity and proportions and on tissue or yolk fatty-acid profiles, future studies should quantify cecal SCFAs and relate intestinal, plasma, and yolk FA data to performance outcomes including feed conversion, growth, or egg metrics.

This study did not test associations between microbiota and fatty-acid profiles and performance outcomes, including feed conversion ratio, average daily gain/body weight, nor did it quantify tissue SCFAs or conjugated PUFA directly; thus, functional inferences remain indirect. Future work should (i) integrate longitudinal sampling with targeted SCFA and CLA/CLnA quantification, (ii) apply pathway-resolved omics (shotgun metagenomics or metatranscriptomics) to verify lipid-modifying and fermentative genes, and (iii) model the joint effects of feed composition, key taxa (e.g., *Lactobacillus*, *Ruminococcaceae* UGC-005), and fatty-acid signatures on performance metrics. Controlling for potential confounders (age, housing, prophylactics, and antibiotics) will strengthen causal interpretation.

CONCLUSION

This study provided a comprehensive insight into the gut microbiota and fatty acid profiles of healthy ducks. The bacterial community was predominantly composed of family Firmicutes, which are associated with a diet rich in carbohydrates and a notable Firmicutes/Bacteroidetes ratio. The

intestinal lipid profile was characterized by a high concentration of saturated fatty acids, and several bacterial genera were linked to the production of SCFAs, MCFAs, LCFAs, and PUFA-derived metabolites, such as CLA and CLnA, highlighting the metabolic capacity of the gut microbiota in lipid processing. These findings enhance our understanding of the role of gut microbes in lipid metabolism and may inform future research on duck nutrition and health.

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REFERENCES

- Ali Q, Ma S, La S, Guo Z, Liu B, Gao Z, ..., Shi Y. 2022. Microbial short-chain fatty acids: A bridge between dietary fibers and poultry gut health-a review. *Anim Biosci* 35(10):1461. DOI: 10.5713/ab.21.0562
- Angenent LT, Richter H, Buckel W, Spirito CM, Steinbusch KJ, Plugge CM, ..., Hamelers HVM. 2016. Chain elongation with reactor microbiomes: open-culture biotechnology to produce biochemicals. *Environ Sci Technol* 50(6):2796-2810. DOI: 10.1021/acs.est.5b04847.
- Anitha M, Vijay-Kumar M, Sitaraman SV, Gewirtz AT, Srinivasan S. 2012. Gut microbial products regulate murine gastrointestinal motility via toll-like receptor 4 signaling. *Gastroenterology* 143: e1004 (1006–1016). DOI: 10.1053/j.gastro.2012.06.034.
- AOAC International. 2016. Official methods of analysis of AOAC international. 20th Edition. Gaithersburg (US): AOAC International.
- Basak S, Duttaroy AK. 2020. Conjugated linoleic acid and its beneficial effects in obesity, cardiovascular disease, and cancer. *Nutrients* 12(7):1913. DOI: 10.3390/nu12071913.
- Batta AK, Salen G, Batta P, Tint GS, Alberts DS, Earnest DL. 2002. Simultaneous quantitation of fatty acids, sterols and bile acids in human stool by capillary gas-liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 775(2):153–161. DOI: 10.1016/S1570-0232(02)00289-1.
- Bourdon C, Lelijveld N, Thompson D, Dalvi PS, Gonzales GB, Wang D, ..., Nyirenda MJ. 2019. Metabolomics in plasma of Malawian children 7 years after surviving severe acute malnutrition: "ChroSAM" a cohort study. *EBioMedicine* 45:464–472. DOI: 10.1016/j.ebiom.2019.06.041.
- Bridgman SL, Azad MB, Field CJ, Haqq AM, Becker AB, Mandhane PJ, ..., Kozyrskyj AL. 2017. Fecal short chain fatty acid variations by breastfeeding status in infants at 4 months: Differences in relative versus absolute concentrations. *Front Nutr* 4(11):1–12. DOI: 10.3389/fnut.2017.00011.

- Caballero-Flores G, Pickard JM, Núñez G. 2023. Microbiota-mediated colonization resistance: Mechanisms and regulation. *Nat Rev Microb* 21(6):347–360. DOI: 10.1038/s41579-022-00833-7
- Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holman SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 13(7):581–583. DOI: 10.1038/nmeth.3869.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, ..., Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7(5):335–336. DOI: 10.1038/nmeth.f.303.
- Chen J, Vitetta L. 2020. The role of butyrate in attenuating pathobiont-induced hyperinflammation. *Immune Netw* 20(2):e15(1–18). DOI: 10.3390/nu11051026.
- Chen J, Zhao KN, Vitetta L. 2019. Effects of intestinal microbial-elaborated butyrate on oncogenic signaling pathways. *Nutrients* 11(5):1026(1–26). DOI: 10.3390/nu11051026
- Chen P, Torralba M, Tan J, Embree M, Zengler K, Starkel P, ..., Schnabl B. 2015. Supplementation of saturated long-chain fatty acids maintains intestinal eubiosis and reduces ethanol-induced liver injury in mice. *Gastroenterol* 48(1):203–214.e16. DOI: 10.1053/j.gastro.2014.09.014.
- Clavijo V, Morales T, Vives-Flores MJ, Muñoz AR. 2022. The gut microbiota of chickens in a commercial farm treated with a Salmonella phage cocktail. *Sci Rep* 12(1):991. DOI: 10.1038/s41598-021-04611-4.
- Cotozzolo E, Cremonesi P, Curone G, Menchetti L, Riva F, Biscarini F, ..., Brecchia G. 2020. Characterization of bacterial microbiota composition along the gastrointestinal tract in rabbits. *Animals* 11(1):31. DOI: 10.3390/ani11010031.
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, ..., Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72(2):5069–5072. DOI: 10.1128/AEM.03006-05.
- Druart C, Neyrinck AM, Vlaeminck B, Fievez V, Cani PD, Delzenne NM. 2014. Role of the lower and upper intestine in the production and absorption of gut microbiota-derived PUFA metabolites. *PLoS ONE* 9(1):e87560. DOI: 10.1371/journal.pone.0087560.
- Eneroth P, Hellstrom K, Sjoval J. 1968. A method for quantitative determination of bile acid in human feces. *Acta Chem Scand* 22(6):1720–1744. DOI: 10.3891/acta.chem.scand.22-1729
- Fu X, Liu Z, Zhu C, Mou H, Kong Q. 2019. Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria. *Crit Rev Food Sci Nutr* 59: S130–S152. DOI: 10.1080/10408398.2018.1542587.
- Gerard P. 2020. The crosstalk between the gut microbiota and lipids. *OCL-Oilseeds and fats Crops and Lipids* 27(70):1–7. DOI: 10.1051/ocl/2020070.
- Guo B, Li D, Zhou B, Jiang Y, Bai H, Zhang Y, ..., Chen G. 2019. Comparative characterization of bacterial communities in geese consuming of different proportions of ryegrass. *PLoS One* 14(10):e0223445. DOI: 10.1371/journal.pone.0223445.
- Hao Y, Ji Z, Shen Z, Wu Y, Zhang B, Tang J, ..., Xie M. 2021. Effects of total dietary fiber on cecal microbial community and intestinal morphology of growing White Pekin duck. *Front Microbiol* 12:727200. DOI: 10.3389/fmicb.2021.727200.
- He J, Zhang P, Shen L, Niu L, Tan Y, Chen L, ..., Zhu L. 2020. Short-chain fatty acids and their association with signalling pathways in inflammation, glucose and lipid metabolism. *Int J Mol Sci* 21(17):6356. DOI: 10.3390/ijms21176356.
- Hodak CR, Bescucci DM, Shamash K, Kelly LC, Montina T, Savage PB, Inglis GD. 2023. Antimicrobial growth promoters altered the function but not the structure of enteric bacterial communities in broiler chicks ± microbiota transplantation. *Animals* 13(6):997. DOI: 10.3390/ani13060997.
- Holm JB, Humphrys MS, Robinson CK, Settles ML, Ott S, Fu L, ..., Ravel J. 2019. Ultrahigh-throughput multiplexing and sequencing of >500 basepair amplicon regions on the illumina HiSeq 2500 platform. *mSystems* 4(1):e00029-19. DOI: 10.1128/mSystems.00029-19.
- Hou J, Xiang J, Li D, Liu X, Pan W. 2022. Gut microbial response to host metabolic phenotypes. *Front Nutr* 9:1019430. DOI: 10.3389/fnut.2022.1019430.
- Jackman JA, Yoon BK, Li D, Cho NJ. 2016. Nanotechnology formulations for antibacterial free fatty acids and monoglycerides. *Molecules* 21(3):305. DOI: 10.3390/molecules21030305.
- Joseph J, Depp C, Shih PB, Cadenhead KS, Schmid-Schonbein G. 2017. Modified mediterranean diet for enrichment of short chain fatty acids: Potential adjunctive therapeutic to target immune and metabolic dysfunction in schizophrenia? *Front Neurosci* 11:155 (1–16). DOI: 10.3389/fnins.2017.00155.
- Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. 2011. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr* 94(1):58–65. DOI: 10.3945/ajcn.110.010132.
- Kucek LA, Nguyen M, Angenent LT. 2016. Conversion of L-lactate into n-caproate by a continuously fed reactor microbiome. *Water Res* 93:163–171. DOI: 10.1016/j.watres.2016.02.018.
- Kulkarni HM, Jagannadham MV. 2014. Biogenesis and multifaceted roles of outer membrane vesicles from Gram-negative bacteria. *Microbiol* 160(Pt 10):2109–2121. DOI: 10.1099/mic.0.079400-0.
- Lajis AFB. 2020. Biomanufacturing process for the production of bacteriocins from Bacillaceae family. *Bioresour Bioprocess* 7(1):8. DOI: 10.1186/s40643-020-0295-z.
- LeBlanc JG, Chain F, Martin R, Bermudez-Humaran LG, Courau S, Langella P. 2017. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb Cell Fact* 16(79):1–10. DOI: 10.1186/s12934-017-0691-z.
- Lin J, Hunkapiller AA, Layton AC, Chang YJ, Robbins KR. 2013. Response of intestinal microbiota to antibiotic growth promoters in chickens. *Foodborne Pathog Dis* 10(4):331–337. DOI: 10.1089/fpd.2012.1348.

- Liu L, Li Q, Yang Y, Guo A. 2021. Biological function of short-chain fatty acids and its regulation on intestinal health of poultry. *Front Vet Sci* 8:736739. DOI: 10.3389/fvets.2021.736739.
- McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, ... Hugenholtz P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 6(3):610–618. DOI: 10.1038/ismej.2011.139.
- Magne F, Gotteland M, Gauthier L, Zazueta A, Pessoa S, Navarrete P, Balamurugan R. 2020. The Firmicutes/Bacteroidetes ratio: A relevant marker of gut dysbiosis in obese patients? *Nutrients* 12(5):1474. DOI: 10.3390/nu12051474.
- Maki KA, Diallo AF, Lockwood MB, Franks AT, Green SJ, Joseph PV. 2019. Considerations when designing a microbiome study: Implications for nursing science. *Biol Res Nurs* 21(2):125–141. DOI: 10.1177/1099800418811639.
- Méndez-Salazar EO, Ortiz-López MG, de Los Angeles Granados-Silvestre M, Palacios-González B, Menjivar M. 2018. Altered gut microbiota and compositional changes in Firmicutes and Proteobacteria in Mexican undernourished and obese children. *Front Microbiol* 9:2494. DOI: 10.3389/fmicb.2018.02494.
- Miller EA, Winfield B, Weber BP, Flores-Figueroa C, Munoz-Aguayo J, Huisinga J, Johnson TJ. 2021. Convergence of the turkey gut microbiota following cohabitation under commercial settings. *J Anim Sci Biotechnol* 12(1):59(1–10). DOI: 10.1186/s40104-021-00580-4.
- Nematbakhsh S, Pei CP, Selamat J, Nordin N, Idris LH, Razis AFA. 2021. Molecular regulation of lipogenesis, adipogenesis and fat deposition in chicken. *Genes* 12(3):414. DOI: 10.3390/genes12030414.
- Ohira H, Tsutsui W, Fujioka Y. 2017. Are short chain fatty acids in gut microbiota defensive players for inflammation and atherosclerosis? *J Atheroscler Thromb* 24(7):660–672. DOI: 10.5551/jat.RV17006.
- Oketch EO, Wickramasuriya SS, Oh S, Choi JS, Heo JM. 2023. Physiology of lipid digestion and absorption in poultry: An updated review on the supplementation of exogenous emulsifiers in broiler diets. *J Anim Physiol Anim Nutr* 107(6):1429–1443. DOI: 10.1111/jpn.13859.
- Pham TPT, Raoult D, Million M. 2019. IGF1 levels in children with severe acute malnutrition after nutritional recovery: A good predictor for children's long-term health status. *EBioMedicine* 45:9–10. DOI: 10.1016/j.ebiom.2019.07.011.
- Ren Q, Yang B, Zhu G, Wang S, Fu C, Zhang H, ..., Chen W. 2020. Antiproliferation activity and mechanism of c9, t11, c15-CLNA and t9, t11, c15-CLNA from *Lactobacillus plantarum* ZS2058 on colon cancer cells. *Molecules* 25(5):1225. DOI: 10.3390/molecules25051225.
- Salsinha AS, Pimentel LL, Fontes AL, Gomes AM, Rodríguez-Alcalá LM. 2018. Microbial production of conjugated linoleic acid and conjugated linolenic acid relies on a multienzymatic system. *Microbiol Mol Biol Rev* 82(4):e00019-18. DOI: 10.1128/mmbr.00019-18.
- Schoeler M, Caesar R. 2019. Dietary lipids, gut microbiota and lipid metabolism. *Rev Endocr Metab Disord* 20(4):461–472. DOI: 10.1007/s11154-019-09512-0.
- Schoeler M, Ellero-Simatos S, Birkner T, Mayneris-Perxachs J, Olsson L, Brolin H, ..., Caesar RC. 2023. The interplay between dietary fatty acids and gut microbiota influences host metabolism and hepatic steatosis. *Nat Com* 14(1):5329. DOI: 10.1038/s41467-023-41074-3.
- Schwechheimer C, Kuehn MJ. 2015. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. *Nat Rev Microbiol* 13(10):605–619. DOI: 10.1038/nrmicro3525.
- Shilling M, Matt L, Rubin E, Visitacion MP, Haller NA, Grey SF, Woolverton CJ. 2013. Antimicrobial effects of virgin coconut oil and its medium-chain fatty acids on *Clostridium difficile*. *J Med Food* 16(12):1079–1085. DOI: 10.1089/jmf.2012.0303.
- Sorbara MT, Dubin K, Littmann ER, Moody TU, Fontana E, Seok R, ..., Pamer EG. 2019. Inhibiting antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular acidification. *J Exp Med* 216(1):84–98. DOI: 10.1084/jem.20181639.
- Susanti R, Christijanti W. 2022. Effects of production system on the gut microbiota diversity and IgA distribution of Kampung chickens, Indonesia. *Biodiversitas* 23(2):1082–1090. DOI: 10.13057/biodiv/d230252.
- Susanti R, Christijanti W, Yuniastuti A. 2021. Immunohistochemical distribution of Immunoglobulin-A in relation to the intestinal microbiota of *Cairina moschata* (Muscovy) duck. *J Phys Conf Ser* 1918:052004. DOI: 10.1088/1742-6596/1918/5/052004.
- Susanti R, Yuniastuti A, Sasi FA, Dafip M. 2020. Metagenomic analysis of intestinal microbiota in geese from different farming systems in Gunungpati, Semarang, Indonesia. *J Biotechnol* 25(2):76–83. DOI: 10.22146/ijbiotech.53936.
- Sychov MY. 2016. The digestibility of nutrients at different levels and sources of lipids in duck diets. *Ukr J Ecol* 6(1):361–374.
- Szabó RT, Kovács-Weber M, Zimborán Á, Kovács L, Erdélyi M. 2023. Effects of short-and medium-chain fatty acids on production, meat quality, and microbial attributes—a review. *Molecules* 28(13):4956. DOI: 10.3390/molecules28134956.
- Tafti ZSM, Moshiri A, Marvasti FE, Tarashi S, Khalili SFS, Motahhary A, ..., Siadat SD. 2019. The effect of saturated and unsaturated fatty acids on the production of outer membrane vesicles from *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*. *Gastroenterol Hepatol Bed Bench* 12(2):155–162.
- Thaiss CA, Levy M, Korem T, Dohnalová L, Shapiro H, Jaitin DA, ..., Elinav E. 2016. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* 167(6):1495–1510.e12. DOI: 10.1016/j.cell.2016.11.003.
- Toral PG, Hervás G, Frutos P. 2024. Invited review: Research on ruminal biohydrogenation—Achievements, gaps in knowledge, and future approaches from the perspective of dairy science. *J Dairy Sci* 107(12):10115–10140. DOI: 10.3168/jds.2023-24591

- van Zyl WF, Deane SM, Dicks LMT. 2020. Molecular insights into probiotic mechanisms of action employed against intestinal pathogenic bacteria. *Gut Microbes* 12(1):e1831339(1–25). DOI: 10.1080/19490976.2020.1831339.
- Vital M, Howe AC, Tiedje JM. 2014. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *MBio* 5(2):e00889. DOI: 10.1128/mBio.00889-14.
- Wang H, Li X, Wang Y, Tao Y, Lu S, Zhu X, Li D. 2018a. Improvement of n-caproic acid production with *Ruminococcaceae* bacterium CPB6: Selection of electron acceptors and carbon sources and optimization of the culture medium. *Microb Cell Fact* 17(1):99–108. DOI: 10.1186/s12934-018-0946-3.
- Wang Y, Li B, Dong H, Huang X, Chen R, Chen X, ..., Zhang B. 2018b. Complete genome sequence of *Clostridium kluyveri* JZZ applied in Chinese strong-flavor liquor production. *Curr Microbiol* 75(11):1429–1433. DOI: 10.1007/s00284-018-1539-4.
- Wei Y, Yang J, Wang J, Yang Y, Huang J, Gong H, ..., Chen D. 2016. Successful treatment with fecal microbiota transplantation in patients with multiple organ dysfunction syndrome and diarrhea following severe sepsis. *Crit Care* 20(1):332. DOI: 10.1186/s13054-016-1491-2.
- Wen C, Yan W, Sun C, Ji C, Zhou Q, Zhang D, ..., Yang N. 2019. The gut microbiota is largely independent of host genetics in regulating fat deposition in chickens. *ISME J* 13(6):1422–1436. DOI: 10.1038/s41396-019-0367-2.
- Wessels AG. 2022. Influence of the gut microbiome on feed intake of farm animals. *Microorganisms* 10(7):1305. DOI: 10.3390/microorganisms10071305.
- Wexler AG, Goodman AL. 2017. An insider's perspective: *Bacteroides* as a window into the microbiome. *Nat Microbiol* 2:17026. DOI: 10.1038/nmicrobiol.2017.26.
- Xiao X, Fu Z, Li N, Yang H, Wang W, Lyu W. 2021. Modulation of the intestinal microbiota by the early intervention with *Clostridium butyricum* in muscovy ducks. *Antibiotics* 10(7):826. DOI: 10.3390/antibiotics10070826.
- Xie F, Xu L, Wang Y, Mao S. 2021. Metagenomic sequencing reveals that high-grain feeding alters the composition and metabolism of cecal microbiota and induces cecal mucosal injury in sheep. *mSystems* 6(5):e0091521. DOI: 10.1128/mSystems.00915-21.
- Yang H, Xiao Y, Gui G, Li J, Wang J, Li D. 2018. Microbial community and short-chain fatty acid profile in gastrointestinal tract of goose. *Poult Sci* 97(4):1420–1428. DOI: 10.3382/ps/pex438.
- Yang H, Lyu W, Lu L, Shi X, Li N, Wang W, Xiao Y. 2020. Biogeography of microbiome and short-chain fatty acids in the gastrointestinal tract of duck. *Poult Sci* 99(8):4016–4027. DOI: 10.1016/j.psj.2020.03.040.
- Yang J, Qin K, Sun Y, Yang X. 2024. Microbiota-accessible fiber activates short-chain fatty acid and bile acid metabolism to improve intestinal mucus barrier in broiler chickens. *Microbiology Spectrum* 12(1):e02065-23. DOI: 10.1128/spectrum.02065-23.
- Yang Q, Guo S, Lu Q, Tao Y, Zheng D, Zhou Q, Liu J. 2021. Butyryl/Caproyl-CoA:Acetate CoA-transferase: Cloning, expression and characterization of the key enzyme involved in medium-chain fatty acid biosynthesis. *Biosci Rep* 41(8):BSR20211135. DOI: 10.1042/BSR20211135.
- Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, ..., Cong Y. 2020. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat Commun* 11(1):4457. DOI: 10.1038/s41467-020-18262-6.
- Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, ..., Rosselló-Móra R. 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 12(9):635–645. DOI: 10.1038/nrmicro3330.
- Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, ..., Zhang H. 2015. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J* 9(9):1979–1990. DOI: 10.1038/ismej.2015.11.
- Zhang WX, Tian G, Zhang KY, Bai SP, Ding XM, Wang JP, ..., Zeng QF. 2025. Effects of dietary supplementation with oleic acid on growth performance, dietary fat utilization, serum and intestinal lipid metabolic parameters, and enterocyte lipid droplet metabolism in Pekin ducks. *Poult Sci* 104(5):105035. DOI: 10.1016/j.psj.2025.105035.
- Zhao L, Huang Y, Lu L, Yang W, Huang T, Lin Z, ..., Bian Z. 2018. Saturated long-chain fatty acid-producing bacteria contribute to enhanced colonic motility in rats. *Microbiome* 6:107. DOI: 10.1186/s40168-018-0492-6.
- Zheng D, Liwinski T, Elinav E. 2020. Interaction between microbiota and immunity in health and disease. *Cell Res* 30:492–506. DOI: 10.1038/s41422-020-0332-7.
- Zhu X, Tao Y, Liang C, Li X, Wei N, Zhang W, ..., Bo T. 2015. The synthesis of n-caproate from lactate: a new efficient process for medium-chain carboxylates production. *Sci Rep* 5:14360–14369. DOI: 10.1038/srep14360.
- Zhu X, Zhou Y, Wang Y, Wu T, Li X, Li D, Tao Y. 2017. Production of high-concentration n-caproic acid from lactate through fermentation using a newly isolated *Ruminococcaceae* bacterium CPB6. *Biotechnol Biofuels* 10:102–114. DOI: 10.1186/s13068-017-0788-y.