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Characterization of microbiota and secretory Ig-A in the domestic duck (*Anas platyrhynchos)* small and large intestine

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Abstract. *Susanti R, Utami NR, Yuniastuti A. 2023. Characterization of microbiota and secretory Ig-A in the domestic duck* (Anas platyrhynchos) *small and large intestine. Biodiversitas 24: 2458-2466.* The Microbiome composition could affect the duck's intestines' microenvironment, which may shape the microstructure's anatomy, metabolism, and immune system. Therefore, this study aims to characterize the intestinal microbiome's abundance, diversity, and IgA distribution in ducks' intestines. This study took 15 healthy domestic ducks (*Anas platyrhynchos* Linnaeus, 1758) from an intensive duck farm in Central Java, Indonesia. Five grams of each small and large intestine content were collected aseptically for metagenomic analysis. Then, the intestine organs were taken for immunohistochemistry preparations to depict the IgA distribution. The results showed that the small intestine has a greater bacterial abundance community, with 18 phyla, while only 13 are found in the large intestine. Interestingly, three phyla: Firmicutes, Actinobacteria, and Bacteroidetes, were found dominantly in both organs. However, the comparison of Firmicutes and Bacteroidetes ratio was higher in the small intestine (2357.76) than large intestine (10.64). The IgA distribution in the small and large intestines showed intermediate staining intensity (score: 2.07-2.20) and a final Allred score of 5.0 (positive). Even though dysbiosis microbiome was present in intestines with dominant species *Streptococcus* and *Enterococcus*, it seems no significant IgA over secretion. Understanding duck's gut immune response is important because it is highly tolerant to the pathogens that make them play an important role as an environmental reservoir for pathogenic viruses and bacteria and may potentially comprehend future infectious disease outbreaks.

Keywords: Ducks, intestine, metagenome, microbiota, secretory IgA

INTRODUCTION

Domestic ducks are well adapted to the environment, highly resistant to pathogenic bacteria and viruses, low-fat, and produce high-quality meat that is popular among consumers (Xia et al. 2019; Rohaeni et al. 2021). Domestic ducks contain abundant and complex microbes in their digestive micro-ecosystem, affecting nutrition intake, immunity against pathogens, metabolism, and meat productivity (Zhou et al. 2023). The gastrointestinal ecosystem has a symbiosis between host and resident microorganisms. The intestinal tract is an important part of the body's defense system and the main site for digestion and absorption of feed nutrients (Garro et al. 2018). The microbiome composition plays a vital role in feed digestion and nutrient absorption. Furthermore, it produces essential Short-Chain Fatty Acid (SCFA), boosting the immune system and metabolism rate (Best et al. 2017).

Previous research demonstrates that unique bacteria community structures characterize the gut microbiome in different duck species that could manage and contributes to the duck's growth (Liu et al. 2018; Wei et al. 2020). The gut microbiome mainly conducts a vital physiological function in the small and large intestines (Zhu et al. 2020). In addition, the microbiome secretes SCFA as fermentation products that help water and electrolyte absorption in the large intestine (Skrypnik and Sulibursk a 2018). Currently, most microbiome research focuses on the cecum since it plays a crucial role as a pathogen's reservoir (Tao et al. 2020). In contrast, each intestinal compartment has a distinctive function and physicochemical properties (Elson and Alexander 2015; Khaleel and Atiea 2017) and is inhabited by specific microbial communities (Waite and Taylor 2014).

Different intestine parts have different microbial community structures and play different roles in poultry health and growth (Pan and Yu 2014). The intestinal function of poultry is mainly carried out in the small intestine, and the relative weight, length, and density of the small intestine are important indexes for measuring intestinal development (Yang et al. 2017). On the other hand, the large intestine is an important part that absorbs water, electrolytes, and short-chain fatty acids produced by bacterial fermentation (DiBaise et al. 2008). Different intestinal compartments have different functions and physicochemical characteristics (Stanley et al. 2014; Nakao et al. 2015) and are inhabited by specific microbial communities (Rinttila and Apajalahti 2013).

Intestine mucosa directly contacts the various pathogenic and non-pathogenic microorganisms as a front liner of the defense mechanism; it then limits bacteria movement to penetrate the epithelial cell (Huang 2021). The mucosa layer consists of high-density fluid, macrophages, neutrophils, and Immunoglobulin (Ig)-A. Specifically, IgA is the primary antibody to prevent bacterial infection in the intestines and is secreted by dependent and independent T-cells (Schofield and Palm 2018). The dependent T-cell produces IgA with high

affinity and specificity, which protects the intestinal mucosal surface from bacterial pathogen invasion and colonization. Meanwhile, the independent T-cell produces IgA with low affinity, binds (coats) commensal bacteria, and provides competitive inhibition against pathogenic species (Tezuka and Ohteki 2019). Thus, IgA can be secreted and activated during a steady-state (undisturbed; healthy) or intestinal infection condition. Furthermore, the abundance and diversity of the intestinal microbiome are affected mainly by feed composition and nutrition intake; then, it automatically modulates the host's immune quality in the intestines (Schofield and Palm 2018; Wei et al. 2020).

The growth of an animal depends on its capacity to digest food. The presence of intestinal microbiota will modulate the host's immune response. Therefore, the diversity of microbiome types greatly determines the host's intestinal immune system quality. Unfortunately, information explaining microbiota variation in the small and large ducks' intestines is still very limited. This study aimed to analyze the genomic characteristics of small and large intestinal microbiota and the distribution profile of IgA intestinal in ducks.

MATERIALS AND METHODS

An exploratory observational study was conducted in intensive egg-laying-duck farming in Central Java, Indonesia. Fifteen healthy domestic ducks (*Anas platyrhynchos* Linnaeus, 1758) were collected from the farm with the inclusive criteria, including laid eggs duck, three months old, and antibiotics or drugs-free for a month before sample collection.

Sample preparation

Ducks were sacrificed according to standard breeder procedures; the small and large intestinal content sample was examined. The ducks' sample was excluded from the study when it contained endoparasite because it could adversely affect intestinal anatomy and physiology. The viable intestinal contents from each small and large intestines of each duck have collected aseptically. The small and large intestinal content sample from fifteen ducks was then randomly divided into three groups. The content sample from the same intestinal part of each group was combined thoroughly and mixed until homogenous. Next, Five grams of each small and large intestinal content was used for further metagenomic analysis. The collected sample was homogenized using a vortex and stored in 3 mL microtubes. Then the samples were frozen at -20°C for further analysis by Next-Generation Sequencing (NGS). Two centimeters of each small and large intestine was separated and cleaned thoroughly with sterile distilled water and submerged using 10% formalin solution in phosphate buffer saline. Subsequently, histological preparations were performed from the tissues for detection. At the same time, their secretory IgA was stained by immunohistochemistry.

DNA isolation and Next-Generation Sequencing (NGS) analysis

QIAamp DNA Stool Mini Kit (Qiagen, San Diego, California, US) was used for DNA extraction from intestinal contents samples, following manufacturer protocol. First, the DNA bacteria from stool samples were extracted separately then the isolated DNA from 15 samples was pooling-combined thoroughly. Next, intestinal microbiota diversity and abundance were determined utilizing the 16S rRNA gene marker region V3-V4. The amplification process used Illumina HiSeq 2500 platform for 20 cycles using primer-forward 338F (5′-GGACTACHV GGGTWTCTAAT-3′) and primer-reverse 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Holm et al. 2019), which bound to the barcode, i.e., a sequence of eight bases specific to each sample. Furthermore, metagenomic analysis was used to generate sequencing amplification.

OTU cluster and species annotation

Sequences analysis was performed by Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/). First, sequences with ≥97% similarity were assigned to the same OTUs. Then, the representative sequence for each OTU was screened for further annotation. Finally, species annotation The GreenGene Database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) was used for each representative sequence based on the RDP classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier/) algorithm to annotate taxonomic information.

Sequencing data analysis

Metagenomic analysis of 16S rRNA microbial sequencing data was processed utilizing Quantitative Insights Into Microbial Ecology software package (QIIME2) Ver. 2019.4 (Caporaso et al. 2010). First, the paired-end file was demultiplexed using the demux plugin. Then quality control was performed on every sample using the Dada2 plugin (Callahan et al. 2016). Furthermore, four diversity indexes were used in this study, explained in Table 1.

The taxonomy and species were identified based on Greengenes 13_8 99% OTU database. First, data visualization was performed in the heatmap form that was compiled using the heatmap plugin; then, barplot taxa were collected in Microsoft Excel 2010.

Table 1. Diversity indices of the bacteria community of small and large intestines

Indices	Function in diversity measurement	Reference
Shannon	Measures uncertainty about the identity of species in the sample and its units quantify information.	(Shannon and Weaver 1949)
Simpson	Measures the probability that two individuals randomly selected from a sample belong to the same species	(Simpson 1949)
Chao1	An estimator based on abundance, which estimates the richness of bacterial communities	(Chao 1984)
Observed OTUs	The number of unique Operational Taxonomic Units (OTUs) that are observed in a sample of bacterial communities	(DeSantis et al. 2006)

Table 2. Allred scoring system

Positive cells percentage (A)	Staining intensity (B)	Final score (A + B)
0: no positive cells	0: none	0-2: negative
1: <1% of positive cells	1: weak	3-8: positive
2: 1-10% positive cells	2: intermediate	
3: 11-33% positive cells	3: strong	
4: 34-66% positive cells		
5: ≥ 67		

Sources: Fedchenko and Reifenrath (2014)

High-Performance Liquid Chromatography (HPLC) analyzes sugars

Sugar components analysis of the feed using the HPLC method evaporated again to leave 20% moisture. Then, as much as 0.5 mL of feed was crushed and put into a threaded tube. The next step followed the previous method (Susanti et al. 2021a).

Detection and staining of secretory IgA by immunohistochemistry

Histological samples were fixed for over 15 days in a 4% neutral paraformaldehyde solution. Next, the intestinal organ samples were cut and embedded with paraffin using standard methods. Next, the paraffin-embedded duck intestine was deparaffinized, Blocking Endogenous Enzyme, Antigen Retrieval (Heat-induced epitopes retrieval). Finally, primary antibody staining with goat anti-chicken IgA antibody HRP conjugated (A.30-103P; Bethyl Laboratories Inc) by sequential incubation, according to the previous method (Susanti et al. 2021b).

Observations were conducted to determine the emergence of brown stain antigen (IgA). The bright-field observation method examined each intestinal tissue from five fields of view, four edges, and one middle section. Calculated observations were percentage proportions of positive cells and staining intensity. Immunohistochemistry evaluation using the Allred scoring system provides a range of 0-8 (Koerdt et al. 2014) as a result of combining the percentage of the positive cells (score 0-5) with reaction product intensity (score 0-3). The final Allred score and its category were defined as shown in Table 2.

The immune-histochemistry data was stated in a criteria-based score of the histological appearance to determine IgA distribution. All scores are then summed (Table 2) and used to compare the histological conditions of the small and large intestines. Finally, a qualitative description was conducted to compare and explain the histological appearance of the small and large intestines.

RESULTS AND DISCUSSION

The intestinal tract is a complex ecosystem, which is the place where the symbiotic relationship occurs between the host's cells and microorganisms. The hosts frequently adapt to the bacteria's abundance and diversity by producing IgA as a natural immune response (Pabst and Slack 2020). Hence, understanding IgA characterization and bacteria composition could comprehensively help observe and predict the correlation between bacteria variations with duck's metabolism and immune mechanism against diseases. Recently, most studies of the intestine microbiota have focused on the effect of feed on the large intestine microbiome (Zhao et al. 2017; Cao et al. 2020). This study examines the abundance and diversity of microbiota in small and large intestines and its relation to secretory IgA distribution. The small intestine ecosystem is closely related to digestive health, as this intestine part facilitates most of the nutrient's assimilation and absorption (Best et al. 2017; Volk and Lacy 2017).

Metagenomic microbiota in the small and large intestine

The HPLC analysis shows that the monosaccharide compound in the feed is more varied. That is true, even though the concentration is lower than polysaccharides. At least six monosaccharides were detected in the fed that may naturally appear from the feed composition raw materials (Table 3).

Fed is the most influential factor that affects gut microbiome diversity. Therefore, there is a high probability that the intestinal bacteria of ducks will change depending on the type of feed (Susanti et al. 2021a). Based on the amplification step, there are 137,157 sequence reads obtained from small intestine pooled-sample contents. Each sample's sequence read then was grouped into OTUs that were categorized into 18 phyla in the small intestine, and 13 phyla were identified in the large intestine. Furthermore, several phyla, including Gemmatimonadetes, Chloroflexi, Nitrospirae, Latescibacteria, Planctomycetes, and Zixibacteria, were only found in the small intestine. Meanwhile, Verrucomicrobia was only found in the large intestine.

The average number of OTUs in the small intestine was almost 118,017, while 69,319 in the large intestine. The abundance-base coverage estimator and Chao1 values also denoted that the small intestine microbiome was more abundant than the large intestine. Chao1 value in the small intestine was higher (637.46±97.29) than in the large intestine (597.44±61.05), as well as the abundance-base coverage estimator (ACE) value was higher in the small intestine (662.09±101.62) than in large intestine (600.69±62.86) (Table 4). This data revealed that various bacteria were abundant in the small intestine, then the population decreased in the large intestine.

The microbiome abundance is higher in the large intestine than the small intestine, performed to Shannon, Simpson diversity index, and observed species (Table 2). Alpha diversity of large intestine microbiota varies in various animals. Shannon's previous study showed that the Shannon index ranged from 4-5 in poultry (Guo et al. 2019; Wen et al. 2019) but 7-9 in mammals (Cotozzolo et al. 2020; Xie et al. 2021). The Shannon's index in the current study averaged 6.39±0.56, higher than in other poultry. The high diversity of large intestine microbiota can also be observed in the percentage of another phylum that had not been identified (the others): 53.57% in large intestine and

30.77% in the small intestine. These data indicated that the bacteria community in the small intestine is less diverse than the bacteria community in the large intestine, even though the bacteria abundance is higher in the small intestine. This finding is relevant to previous studies that explain that the bacteria population is more abundant but less diverse in the small intestine than in the large intestine (Yang et al. 2020; Zhu et al. 2020).

Furthermore, dominant phyla Firmicutes, Actinobacteria, and Bacteroidetes were observed in the small and large intestines. Firmicutes are more prevalent in the small intestine. At the same time, the large intestine is dominantly populated by Actinobacteria and Bacteroidetes (Figure 1). The other six phyla are in the small and large intestine: Proteobacteria, Tenericutes, Thermomicrobia, Saccharibacteria, Acidobacteria, Fusobacteria, and Gemmatimonadetes, were found in only <1% of the total observed species.

Furthermore, Figure 2 shows the proportional contribution of dominant bacteria in the small and large intestines from sampling ducks, specifically, Firmicutes, Proteobacteria, Saccharibacteria, Acidobacteria, and Gemmatimonadetes. Meanwhile, phylum Actinobacteria, Bacteroidetes, Tenericutes, Thermomicrobia, and Fusobacteria only contribute >50% of the total microbiome in the large intestine. Interestingly, two main phyla were found at opposite concentrations, where Bacteroidetes were only found in the small intestine, then Gemmatimonadetes were found only in the large intestine.

The higher bacterial density indicated by the dark color explains that the large intestine may have a denser bacteria community than the small intestine (Figure 3). That indicated certain bacteria were more abundant in the population number in the small intestine and less in the large intestine. However, the diversity index performs that bacterium in the large intestine is more diverse (Table 2). Therefore, as a whole, no anomalies were found in this study, i.e., diversity index values did not deviate from one or more other values. All diversity index parameters indicated that the large intestine had a higher diversity of intestinal bacteria than the small intestine.

The domination of Firmicutes, Actinobacteria, and Bacteroidetes is not only found in this study. Several studies also explain similar founding in different species of birds, including turkeys (*Meleagris gallopavo* Linnaeus, 1758) (Craft et al. 2022), Muscovy duck (*Cairina moschata* Linnaeus, 1758) (Lyu et al. 2021), geese (*Cygnus cygnus* Linnaeus, 1758) (Susanti et al. 2020), and broiler chickens (Ross 308) (Xiao et al. 2017). Bacteroidetes dominance in the large intestine in this study following previous studies that Bacteroidetes dominance varied in Pekin duck (*A. platyrhynchos*) 65%, Muscovy (*C. moschata*) 50% (Vasaï et al. 2014a), Shaoxing duck 33% (Zhao et al. 2019), Cherry Valley duck 33.4% (Dai et al. 2018), mule duck 13.27% (Vasaï et al. 2014b). In addition, a high bacteria composition in the large intestine may cause by the undigested natural fiber. That fiber provides long-chain sugar used to metabolize most mutual bacteria (El-Katcha et al. 2021).

All bacteria members of Bacteroidetes are Gram-negative, consisting of anaerobic obligate and aerobic bacteria. Bacteroidetes can be resident in all parts of the gastrointestinal tract because they can adapt to conditions with different pH, nutrients, and oxygen availability. Bacteroidetes' predominance in the large intestine is related to their role as degrading biopolymers (polysaccharides). Genetically, Bacteroidetes have enzymes that degrade polysaccharides from food, such as fiber (cellulose). Also, polysaccharides, such as N-glycans, could be secreted in the gastrointestinal tract (Mckee et al. 2021).

Table 3. Detected sugar content in the feed

Sugar	Sugar category	Concentration (µmol/g)
Xylose	Monosaccharide	16.76±1.106
Arabinose	Monosaccharide	12.80±0.906
Rhamnose	Monosaccharide	21.52±1.367
Glucose	Monosaccharide	26.79±1.769
Galactose	Monosaccharide	33.51±2.223
Mannose	Monosaccharide	19.98±1.363
Total of monosaccharide		131.36
Trehalose	Polysaccharide	63.60±3.960
Raffinose	Polysaccharide	56.67±3.689
Stachyose	Polysaccharide	80.24±5.424
Total of polysaccharide		200.51

Table 4. Summary statistics and diversity indices

Item	Small intestine	Large intestine
Sequencing number	137,128±26.41	106,834±14137.25
OTU	118,016.7±1255.45	69,318.67±10381.17
OTU (value)	118,017±1255	69,319±10381
Observed-species	487±77	573±56
Shannon	3.75±0.24	6.39±0.56
Simpson	0.78±0.02	0.97±0.02
Chao1	637.46±97.29	597.44±61.05
ACE (Abundance-base Coverage Estimator)	662.09±101.62	600.69±62.86
Good's coverage	0.998	0.999

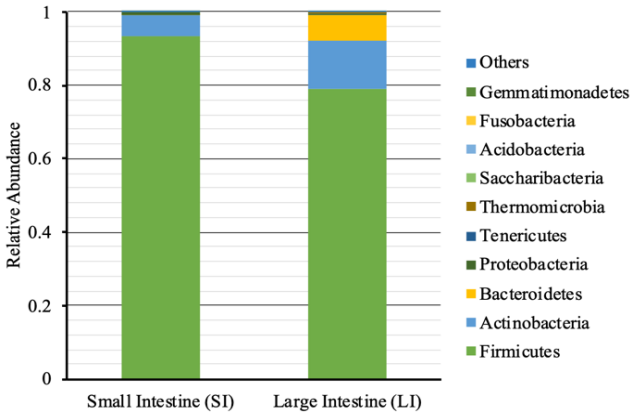


Figure 1. Average spatial distribution of the top 10 phyla in the small and large intestines pooled-sample content of ducks

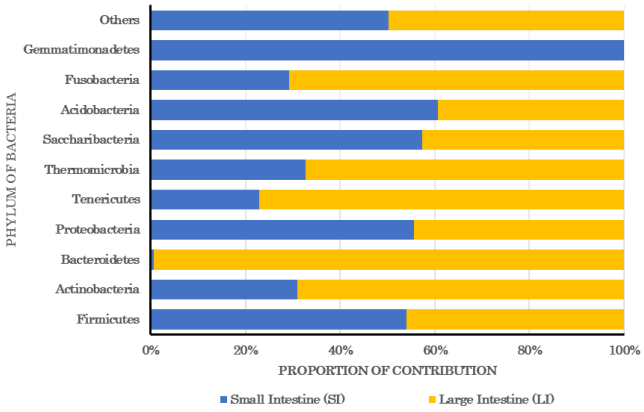


Figure 2. Comparison of the average percentage contribution of each phylum in the small and large intestine

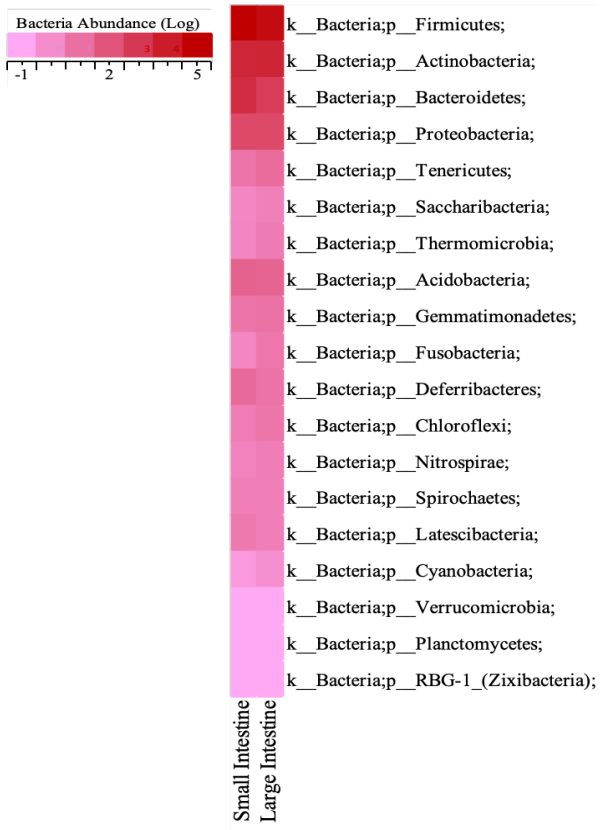


Figure 3. Phylum heatmap distribution of intestinal bacteria density and abundance

The Firmicutes/Bacteroidetes ratio in intestinal tracks is a relevant marker for dysbiosis conditions affected by digested fiber content. Fiber-contained feed proves increasing Bacteroidetes population and lowering Firmicutes/Bacteroidetes ratios (Magne et al. 2020). In this study, polysaccharide composition in the duck feed is higher than monosaccharides (Table 2). However, it is probably being digested into monosaccharides and absorbed in the small intestine. It is proven by the low ratio found in the large intestine that may cause by decreased remaining digested fiber in the large intestine. Further analysis should be conducted to measure the polysaccharides composition and types in small and large intestines to describe gut microbiome-related sugar contents in ducks.

In addition, the various components of feed may the main factor contributes to gut microbiome diversity in this study. The feed composition for the ducks is mainly composed of concentrate, bran, dried rice, and additional feed in the form of palm sugar and molasses. That feed composition produces high monosaccharide content (Table 2). Overfeeding practice increases high monosaccharides consumption that triggers dysbiosis and metabolic inflammation in the intestinal organs (Wei et al. 2020). In addition, it starts glucotoxicity and increases intestinal cell permeability, and loosens junctions, which results in intestinal cell necrosis (Chakaroun et al. 2020; Filippello et al. 2021). Furthermore, dysbiosis is characterized by a decreased population of Segmented Filamentous Bacteria (SFB) such as *Clostridium* and *Candidatus arthromitus*, which is increased risk of obesity, delayed Th17 maturation, and reduced IgA production (Luo et al. 2021; Elsasser 2022). In addition, excessive monosaccharide sugar also decreases SCFA produced by Firmicutes, which play a role in the boost immune system. A low population of Firmicutes allows increasing composition of Proteobacteria and opportunistic pathogenic bacteria that cause leaky gut syndrome (Zhang et al. 2022).

The ducks' gut microbiome and intestinal tract are one of the primary organs for natural immunity that are influenced by the intake of nutrients from feed (Garro et al. 2018). Feed-transit time in the small intestine is short because of fast luminal flow, and the presence of digestive enzymes and bile makes it suitable only for certain bacteria (Yang et al. 2020). Conversely, the ducks' gut microbiome is diverse depending on their rearing management, which affects their physiology (Wang et al. 2018; Zhao et al. 2019).

The metagenomic analysis also reveals 282 total genera were identified in ducks' small and large intestines. However, the number of identified groups in the small intestine is up to 232 genera and 3,294 other is unidentified. Then 204 identified genera and 22,384 unknown genera were found in the large intestine. The top 10 genera were *Streptococcus, Bacillus, Lactobacillus, Corynebacterium, Weissella, Subdoligranulum, Enterococcus, Bacteroides, Faecalitalea, and Romboutsia* (Figure 4). *Streptococcus* and *Lactobacillus* were most abundant in small intestines, accounting for 60.06% and 18.35%, respectively. Nonetheless, these genera were present at a drastically low level in the large intestine (less than 8%) (Table 5). Five genera in the small intestine (*Bacillus, Corynebacterium, Weissella, Enterococcus, Romboutsia*) were in the range of 1.23-4.22%, and the other three genera (*Subdoligranulum, Bacteroides, Faecalitalea*) were in the field of <1%. In the large intestine, no dominant genus was seen. There were seven genera (*Streptococcus, Lactobacillus, Corynebacterium, Weissella, Subdoligranulum, Enterococcus, Faecalitalea*) which were in the range of 5.79-9.61%, two other genera (*Faecalitalea* and *Bacteroides*) were in the field of 1.49-3.1, and the genus *Bacillus* was only 0.04%.

It was discovered that *Corynebacterium, Weissella, Subdoligranulum, Enterococcus, Bacteroides,* and

Faecalitalea were all detected in greater abundance in the large intestine than in the small intestine. For example, Figure 5 shows that the genera *Streptococcus, Bacillus, Lactobacillus,* and *Romboutsia* contributed more than 60% of the total bacteria in the small intestine. On the other hand, the genera *Corynebacterium, Waissella, Subdoligranulum, Enterococcus, Bacteroides,* and *Faecalitalea* dominated the large intestine.

The proportion of genera *Streptococcus*, *Bacillus*, *Lactobacillus*, and *Romboutsia* contributed more (>60%) to the small intestine. At the same time, the genera *Corynebacterium*, *Waissella*, *Subdoligranulum*, *Enterococcus, Bacteroides*, and *Faecalitalea* contributed more (>60%) in the large intestine. In addition, it was reported that Bacteroides could stimulate immune system development, act as differentiators of immunomodulatory functions, and enhance the animal mucosal barrier (Wexler and Goodman 2017). Meanwhile, *Streptococcus, Lactobacillus, Bifidobacterium*, and *Enterococci* can evolve metabolic pathways based on fast absorption and monosaccharides alteration (Wessels 2022). *Lactococcus's* presence in the small intestine is associated with lipid and carbohydrate metabolism (Maki et al. 2019). Resident bacteria in the small intestine are dominated by aerotolerant species, while in the large intestine are dominated by strict aero-intolerant species (Mailhe et al. 2018).

Pathogenic bacteria related to duck health are *R. anatipestifer*, *E. coli*, *Salmonella*, *Streptococcus*, and *Enterococcus* (Volk and Lacy 2017). In this study, *Streptococcus* and *Enterococcus* were detected both in the small and large intestines, as in the study result by Yang et al. (2020) in muscovy duck. Both *Enterococcus* and *Streptococcus* seem to be part of the duck intestine microbiome, with 15-50 % of the population comprising both bacteria (Vasai et al. 2014a). *Streptococcus* and *Enterococcus* were 62.56% in the small intestine, while 16.75% in the large intestine. Interestingly, the high composition of *Streptococcus* and *Enterococcus* was identified in the sample duck microbiome. However, the ducks in this present study were clinically healthy. The presence of pathogenic genera of bacteria needs to be further studied because they do not seem necessarily triggers any infection. Likewise, Figueroa's et al. (2020) research result showed that the intestinal microbiota of ducks could control several pathogenic bacteria and avian influenza virus replication. It makes ducks a natural reservoir for the avian influenza virus while preventing disease outbreaks.

Immunohistochemical observation of secretory IgA in the small and large intestine

The abundance of bacteria community may also contribute to the duck's intestinal anatomy as an interaction result with their microhabitat. Immunohistochemical analysis results of the IgA showed that all intestinal organs described in this study were histologically normal. IgA labeled immune showed brown staining of cytoplasm. These results indicated that IgA was discovered in ducks' small and large intestines, as depicted in Figure 6. The IgA was distributed equally with no significantly different in the small and large intestines. The IgA is massively found in lamina propria, with most cells arranged around the intestinal crypts (Figure 6).

Based on the immunohistochemical analysis, the proportion of positive cells in the small and large intestines was 15.40% (score: 2.93) and 16.48% (score: 2.80), respectively. In addition, both small and large intestines exhibited intermediate staining intensity (score: 2.07-2.20). Therefore, the final Allred score (Sum of positive cells percentage score and staining intensity score) for the small intestine and large intestine was 5.0 (positive) (Table 6).

Table 5. The differential analysis of the ten most abundant genera in the small and large intestines of ducks

Genera	Abundant (%)	
	Small intestine (SI)	Large intestine (LI)
Streptococcus	60.06±5.09	8.20±4.41
Bacillus	1.23±1.42	0.04±0.01
Lactobacillus	18.35±12.93	5.79±3.64
Corynebacterium_1	3.63±1.19	6.66±12.22
Weissella	4.22±3.72	9.27±14.63
Subdoligranulum	0.10±0.12	9.61±9.45
Enterococcus	2.52±0.97	8.73±9.92
Bacteroides	0.02±0.02	3.10±1.81
Faecalitalea	0.07±0.06	6.19±3.79
Romboutsia	2.33±3.41	1.49±1.52
Others	7.47±4.49	40.51±7.32

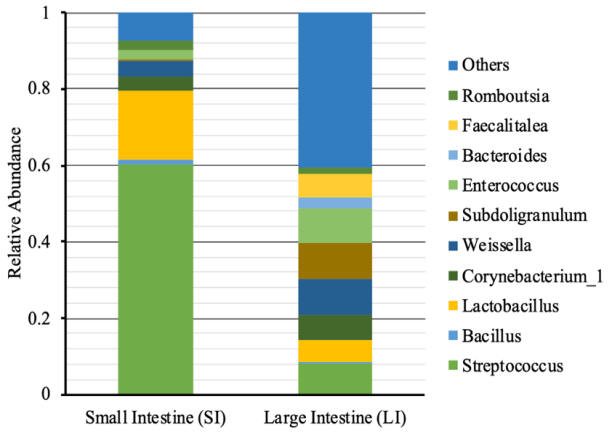


Figure 4. Average spatial distribution of the top 10 genera in the small and large intestines of duck

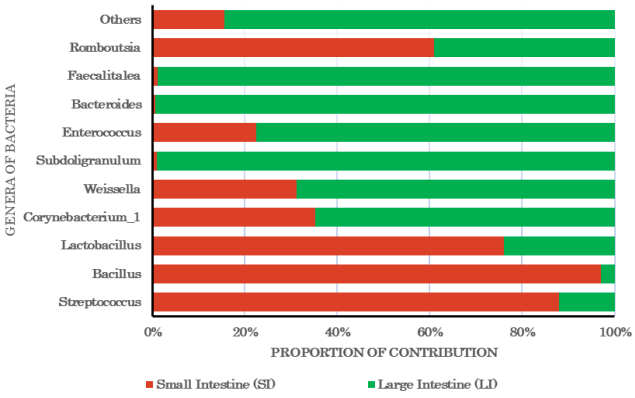
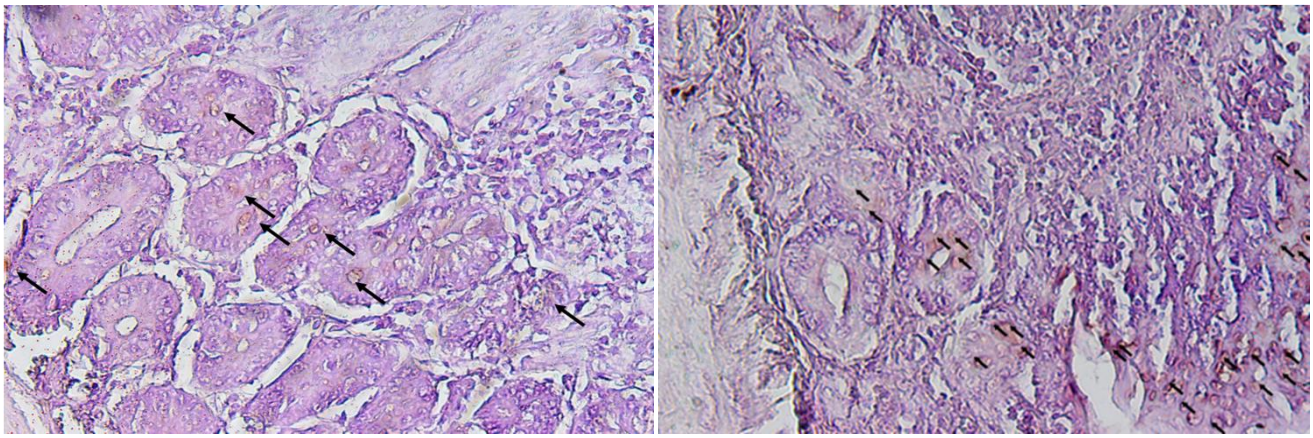


Figure 5. Comparison of the average percentage contribution of the top ten genera in the small and large intestine



A **B**

Figure 6. IgA immune-histochemical staining in the small intestine (A) and large intestine (B) of duck. The IgA (black arrows) were distributed in lamina propria, and some cells were accumulated around the crypts (magnification: 400×)

Table 6. Representation of immunohistochemistry staining intensity, positive cells, and final score of IgA in the small and large intestine

Intestinal section	% Positive cells	Positive cells score	Staining intensity score	Final Allred score
Small intestine	15.40±5.32	2.93±0.29	2.07±0.38 (intermediate)	5.00±0.47 (positive)
Large intestine	16.48±6.31	2.80±0.41	2.20±0.39 (intermediate)	5.00±0.60 (positive)

Furthermore, IgA production is increased if there is a pathogenic infection in the intestinal tract. For example, broiler chickens infected with *Salmonella enteritidis* can cause an increase in IgA production (Pabst and Slack 2020). Methionine deficiency significantly lowers broiler chickens' duodenal IgA levels (Wu et al. 2018). In this study, ducks were clinically healthy, although *Streptococcus* and *Enterococcus* were identified in their intestines. In these ducks' intestines, there was a balance - microbiota composition, and no microorganisms promoted IgA overproduction. IgA response effectively stabilizes symbiotic microorganisms' colonization and resists the invasion of exogenous organisms (Schofield and Palm 2018). Furthermore, IgA is crucial in selecting and stabilizing specific symbionts within the host (Donaldson et al. 2018), highly influencing microbiota composition. This is indicated by dysbiosis due to IgA lack in individuals (Fadlallah et al. 2018). The duck immune system may be tolerant to the presence of infectious agents, and this is relevant to its role as a reservoir for various pathogenic viruses and bacteria (Evseev and Magor 2019; Figueroa et al. 2020).

The intestinal microbiota is isolated from the host by only an epithelial membrane and a mucus layer (Schroeder 2019). Epithelial cells and mucus layer are the initial protection against microbes and the initial tolerance to commensal microbes (Ali et al. 2020). Host-commensal microbe interactions are dynamically controlled and determined by complicated interactions among microbiota, intestinal immunity, metabolites (metabolomics), and nutrients. Host metabolism is enhanced as part of the mutualistic symbiosis between host and commensal microbiota, and healthy intestinal tissue conditions and homeostasis are maintained. IgA response determines commensal species combination colonizing in the intestinal tract. IgA response requires considerable metabolic energy to trigger the production of antibodies by tissue-resident plasma cells (Penny et al. 2022). Microbiota is also needed to produce IgA in the intestine (Robak et al. 2018). A study by Figueroa et al. (2020) showed a decrease in mRNA expression of IgA's heavy chain constant region in antibiotic-treated ducks' intestines. In the intestine, there is also an interaction between enteric viruses and microbiota. Under normal conditions, avian influenza virus replication in duck's intestines decreased compared to ducks treated with antibiotics.

In conclusion, the high sugar content may increase imbalance conditions in duck's intestinal microbiome, characterized by changes in bacterial composition, which is more dominated by *Streptococcus* and *Enterococcus*. However, the condition does not manifest and exhibits chronic inflammatory symptoms in the small and large intestinal organs. It is indicated by the distribution of IgA in the small and large intestines, which is not overexpressed. In addition, the diversity of bacteria is more dominant in the large intestine, which may indicate that the contents of the large intestine still have enough nutrients to support bacterial growth. Therefore, further study should be conducted to holistically identify the host's immune condition to identify specific-related immune cells in the microbiome, including Th17, B cell, neutrophile, and macrophage.

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REFERENCES

Ali A, Tan HY, Kaiko GE. 2020. Role of the intestinal epithelium and its interaction with the microbiota in food allergy. Front Immunol 11: 604054. DOI: 10.3389/fimmu.2020.604054.

Best AA, Porter AL, Fraley SM, Fraley GS. 2017. Characterization of gut microbiome dynamics in developing pekin ducks and impact of management system. Front Microbiol 7: 2125. DOI: 10.3389/fmicb.2016.02125.

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.

Cao Y, Liu H, Qin N, Ren X, Zhu B, Xia X. 2020. Impact of food additives on the composition and function of gut microbiota: A review. Trends Food Sci Technol 99: 295-310. DOI: 10.1016/j.tifs.2020.03.006.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7 (5): 335-336. DOI: 10.1038/nmeth.f.303.

Chakaroun RM, Massier L, Kovacs P. 2020. Gut microbiome, intestinal permeability, and tissue bacteria in metabolic disease: Perpetrators or bystanders?. Nutrients 12 (4): 1082. DOI: 10.3390/nu12041082.

Chao A. 1984. Nonparametric estimation of the number of classes in a population. Scand J Stat 11 (4): 265-270.

Cotozzolo E, Cremonesi P, Curone G, Menchetti L, Riva F, Biscarini F, Marongiu ML, Castrica M, Castiglioni B, Miraglia D, Luridiana S, Brecchia G. 2020. Characterization of bacterial microbiota composition along the gastrointestinal tract in rabbits. Animals 11 (1): 31. DOI: 10.3390/ani11010031.

Craft J, Eddington H, Christman ND, Pryor W, Chaston JM, Erickson DL, Wilson E. 2022. Increased microbial diversity and decreased prevalence of common pathogens in the gut microbiomes of wild turkeys compared to domestic turkeys. Appl Environ Microbiol 88 (5): e0142321. DOI: 10.1128/aem.01423-21.

Dai SJ, Zhang KY, Ding XM, Bai SP, Luo YH, Wang JP, Zeng QF. 2018. Effect of dietary non-phytate phosphorus levels on the diversity and structure of cecal microbiota in meat duck from 1 to 21 d of age. Poult Sci 97 (7): 2441-2450. DOI: 10.3382/ps/pey090.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, 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.

DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. 2008. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc 83 (4): 460-469. DOI: 10.4065/83.4.460.

Donaldson GP, Ladinsky MS, Yu KB, Sanders JG, Yoo BB, Chou WC, Conner ME, Earl AM, Knight R, Bjorkman PJ, Mazmanian SK. 2018. Gut microbiota utilize immunoglobulin a for mucosal colonization. Science 360 (6390): 795-800. DOI: 10.1126/science.aaq0926.

El-Katcha MI, Soltan MA, Shewita R, Abdo SE, Sanad AS, Tufarelli V, Alagawany M, El-Naggar K. 2021. Dietary fiber and lysolecithin supplementation in growing ducks: Effect on performance, immune response, intestinal morphology and lipid metabolism-regulating genes. Animals 11 (10): 2873. DOI: 10.3390/ani11102873.

Elsasser TH. 2022. Immunometabolic considerations with regard to the domestic chicken, *Gallus gallus*. Anim Front 12 (5): 8-10. DOI: 10.1093/af/vfac052.

Elson CO, Alexander KL. 2015. Host-microbiota interactions in the intestine. Dig Dis 33 (2): 131-136. DOI: 10.1159/000369534.

Fadlallah J, Kafsi HE, Sterlin D, Juste C, Parizot C, Dorgham K, Autaa G, Gouas D, Almeida M, Lepage P, Pons N, Chatelier EL, Levenez F, Kennedy S, Galleron N, de Barros JPP, Malphettes M, Galicier L, Boutboul D, Mathian A, Miyara M, Oksenhendler E, Amoura Z, Doré J, Fieschi C, Ehrlich SD, Larsen M, Gorochov G. 2018. Microbial ecology perturbation in human IgA deficiency. Sci Transl Med 10 (439): eaan1217. DOI: 10.1126/scitranslmed.aan1217.

Fedchenko N, Reifenrath J. 2014. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagn Pathol 9: 221. DOI: 10.1186/s13000-014-0221-9.

Figueroa T, Bessière P, Coggon A, Bouwman KM, van der Woude R, Delverdier M, Verheije MH, de Vries RP, Volmer R. 2020. The microbiota contributes to the control of highly pathogenic H5N9 influenza virus replication in ducks. J Virol 94 (10): e00289-20. DOI: 10.1128/JVI.00289-20.

Filippello A, Di Mauro S, Scamporrino A, Malaguarnera R, Torrisi SA, Leggio GM, Di Pino A, Scicali R, Purrello F, Piro S. 2021. High glucose exposure impairs L-cell differentiation in intestinal organoids: Molecular mechanisms and clinical implications. Intl J Mol Sci 22 (13): 6660. DOI: 10.3390/ijms22136660.

Garro C, Brun A, Karasov WH, Caviedes-Vidal E. 2018. Small intestinal epithelial permeability to water-soluble nutrients higher in passerine birds than in rodents. J Anim Physiol Anim Nutr (Berl) 102 (6): 1766-1773. DOI: 10.1111/jpn.12969.

Guo B, Li D, Zhou B, Jiang Y, Bai H, Zhang Y, Xu Q, Zhao W, 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.

Holm JB, Humphrys MS, Robinson CK, Settles ML, Ott S, Fu L, Yang H, Gajer P, He X, McComb E, Gravitt PE, Ghanem KG, Brotman RM, 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.0002919.

Huang FC. 2021. The interleukins orchestrate mucosal immune responses to *Salmonella* infection in the intestine. Cells 10 (12): 3492. DOI: 10.3390/cells10123492.

Khaleel IM, Atiea GD. 2017. Morphological and histochemical study of small intestine in indigenous ducks (*Anas platyrhynchos*). IOSR J Agric Vet Sci 10 (7): 19-27. DOI: 10.9790/2380-1007021927.

Koerdt S, Siebers J, Bloch W, Ristow O, Kuebler AC, Reuther T. 2014. Role of oxidative and nitrosative stress in autogenous bone grafts to the mandible using guided bone regeneration and a deproteinized bovine bone material. J Craniomaxillofac Surg 42 (5): 560-567. DOI: 10.1016/j.jcms.2013.07.027.

Liu Y, Li Y, Feng X, Wang Z, Xia Z. 2018. Dietary supplementation with *Clostridium butyricum* modulates serum lipid metabolism, meat quality, and the amino acid and fatty acid composition of Peking ducks. Poult Sci 97 (9): 3218-3229. DOI: 10.3382/ps/pey162.

Luo Q, Lei X, Xu J, Jahangir A, He J, Huang C, Liu W, Cheng A, Tang L, Geng Y, Chen Z. 2021. An altered gut microbiota in duck-origin parvovirus infection on cherry valley ducklings is associated with mucosal barrier dysfunction. Poult Sci 100 (4): 101021. DOI: 10.1016/j.psj.2021.101021.

Lyu W, Liu X, Lu L, Dai B, Wang W, Yang H, Xiao Y. 2021. Cecal microbiota modulates fat deposition in muscovy ducks. Front Vet Sci 8: 609348. DOI: 10.3389/fvets.2021.609348.

Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa 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.

Mailhe M, Ricaboni D, Vitton V, Gonzalez JM, Bachar D, Dubourg G, Cadoret F, Robert C, Delerce J, Levasseur A, Fournier PE, Angelakis E, Lagier JC, Raoult D. 2018. Repertoire of the gut microbiota from stomach to colon using culturomics and next-generation sequencing. BMC Microbiol 18: 157. DOI: 10.1186/s12866-018-1304-7.

Maki JJ, Klima CL, Sylte MJ, Looft T. 2019. The microbial pecking order: Utilization of intestinal microbiota for poultry health. Microorganisms 7 (10): 376. DOI: 10.3390/microorganisms7100376.

Mckee LS, La Rosa SL, Westereng B, Eijsink VG, Pope PB, Larsbrink J. 2021. Polysaccharide degradation by the Bacteroidetes: Mechanisms and nomenclature. Environ Microbiol Rep 13 (5): 559-581. DOI: 10.1111/1758-2229.12980.

Nakao N, Kaneda H, Tsushima N, Ohta Y, Tanaka M. 2015. Characterization of primary structure and tissue expression profile of

the chicken apical sodium-dependent bile acid transporter mRNA. Poult Sci 94 (4): 722-727. DOI: 10.3382/ps/pev027.
Pabst O, Slack E. 2020. IgA and the intestinal microbiota: The importance of being specific. Mucosal Immunol 13 (1): 12-21. DOI: 10.1038/s41385-019-0227-4.
Pan D, Yu Z. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5 (1): 108-119. DOI: 10.4161/gmic.26945.
Penny HA, Domingues RG, Krauss MZ, Melo-Gonzalez F, Lawson MAE, Dickson S, Parkinson J, Hurry M, Purse C, Jegham E, Godinho-Silva C, Rendas M, Veiga-Fernandes H, Bechtold DA, Grencis RK, Toellner KM, Waisman A, Swann JR, Gibbs JE, Hepworth MR. 2022. Rhythmicity of intestinal IgA responses confers oscillatory commensal microbiota mutualism. Sci Immunol 7 (75): eabk2541. DOI: 10.1126/sciimmunol.abk2541.
Rinttila T, Apajalahti J. 2013. Intestinal microbiota and metabolites-implications for broiler chicken health and performance. J Appl Poult Res 22 (3): 647-658. DOI: 10.3382/japr.2013-00742.
Robak OH, Heimesaat MM, Kruglov AA, Prepens S, Ninnemann J, Gutbier B, Reppe K, Hochrein H, Suter M, Kirschning CJ, Marathe V, Buer J, Hornef MW, Schnare M, Schneider P, Witzenrath M, Bereswill S, Steinhoff U, Suttorp N, Sander LE, Chaput C, Opitz B. 2018. Antibiotic treatment-induced secondary IgA deficiency enhances susceptibility to *Pseudomonas aeruginosa* pneumonia. J Clin Invest 128 (8): 3535-3545. DOI: 10.1172/JCI97065.
Rohaeni ES, Subhan A, Hanifah VW, Bakrie B, Sumantri I. 2021. Effects of feeding alabio ducks with fresh golden snail on egg production and quality. J Hunan Univ Nat Sci 48 (10): 305-313.
Schofield WB, Palm NW. 2018. Gut microbiota: IgA protects the pioneers. Curr Biol 28 (18): R1117-R1119. DOI: 10.1016/j.cub.2018.08.019.
Schroeder BO. 2019. Fight them or feed them: How the intestinal mucus layer manages the gut microbiota. Gastroenterol Rep 7 (1): 3-12. DOI: 10.1093/gastro/goy052.
Shannon CE, Weaver W. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana.
Simpson EH. 1949. Measurement of diversity. Nature 163 (1): 688. DOI: 10.1038/163688a0.
Skrypnik K, Suliburska J. 2018. Association between the gut microbiota and mineral metabolism. J Sci Food Agric 98 (7): 2449-2460. DOI: 10.1002/jsfa.8724.
Stanley D, Hughes RJ, Moore RJ. 2014. Microbiota of the chicken gastrointestinal tract: Influence on health, productivity and disease. Appl Microbiol Biotechnol 98 (10): 4301-4310. DOI: 10.1007/s00253-014-5646-2.
Susanti R, Yuniastuti A, Sasi FA, Dafip M. 2020. Metagenomic analysis of intestinal microbiota in geese from different farming systems in Gunungpati, Semarang. Indones J Biotechnol 25 (2): 76-83. DOI: 10.22146/ijbiotech.53936.
Susanti R, Dafip M, Christijanti W, Yuniastuti A. 2021a. The interaction of intestinal bacteria and feed: A case study of intensive-duck husbandry in Central Java. J Indonesian Trop Anim Agric 46 (2): 154-165. DOI: 10.14710/jitaa.46.2.154-165.
Susanti R, Christijanti W, Yuniastuti A. 2021b. 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.
Tao Z, Zhu C, Xu W, Shi Z, Zhang S, Song W, Liu H, Li H. 2020. *Riemerella anatipestifer* infection affects intestinal barrier structure and immune reactions in the duck cecum. Avian Pathol 49 (6): 572-580. DOI: 10.1080/03079457.2020.1792414.
Tezuka H, Ohteki T. 2019. Regulation of IgA production by intestinal dendritic cells and related cells. Front Immunol 10: 1891. DOI: 10.3389/fimmu.2019.01891.
Vasaï F, Ricaud KB, Bernadet MD, Cauquil L, Bouchez O, Combes S, Davail S. 2014a. Overfeeding and genetics affect the composition of intestinal microbiota in *Anas platyrhynchos* (pekin) and *Cairina moschata* (muscovy) ducks. FEMS Microbiol Ecol 87 (1): 204-216. DOI: 10.1111/1574-6941.12217.
Vasaï F, Ricaud KB, Cauquil L, Daniel P, Peillod C, Gontier K, Tizaoui A, Bouchez O, Combes S, Davail S. 2014b. *Lactobacillus sakei* modulates mule duck microbiota in ileum and ceca during overfeeding. Poult Sci 93 (4): 916-925. DOI: 10.3382/ps.2013-03497.
Volk N, Lacy B. 2017. Anatomy and physiology of the small bowel. Gastrointest Endosc Clin N Am 27 (1): 1-13. DOI: 10.1016/j.giec.2016.08.001.
Waite DW, Taylor MW. 2014. Characterizing the avian gut microbiota: Membership, driving influences, and potential function. Front Microbiol 5: 223. DOI: 10.3389/fmicb.2014.00223.
Wang S, Chen L, He M, Shen J, Li G, Tao Z, Wu R, Lu L. 2018. Different rearing conditions alter gut microbiota composition and host physiology in Shaoxing ducks. Sci Rep 8 (1): 7387. DOI: 10.1038/s41598-018-25760-7.
Wei R, Ye F, He F, Song Q, Xiong X, Yang W, Xu H, Li L, Liu H, Zeng X, Chen L, Han C. 2020. Comparison of overfeeding influence on slaughter performance, small intestinal physiology and microbiota between Gang goose and Tianfu meat goose. J World's Poult Res 10 (2): 348-358. DOI: 10.36380/jwpr.2020.40.
Wen C, Yan W, Sun C, Ji C, Zhou Q, Zhang D, Zheng J, 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.
Wu B, Li L, Ruan T, Peng X. 2018. Effect of methionine deficiency on duodenal and jejunal IgA+ B cell count and immunoglobulin level of broilers. Iran J Vet Res 19 (3): 165-171.
Xiao Y, Xiang Y, Zhou W, Chen J, Li K, Yang H. 2017. Microbial community mapping in intestinal tract of broiler chicken. Poult Sci 96 (5): 1387-1393. DOI: 10.3382/ps/pew372.
Xia WG, Abouelezz KFM, Fouad AM, Chen W, Ruan D, Wang S, Azzam MMM, Luo X, Fan QL, Zhang YN, Zheng CT. 2019. Productivity, reproductive performance, and fat deposition of laying duck breeders in response to concentrations of dietary energy and protein. Poult Sci 98 (9): 3729-3738. DOI: 10.3382/ps/pez061.
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 TL, Liu XC, Liao Q, Wang SL, Zhang CY, Rong GEC, Leng J. 2017. Application advances of metagenome technology in intestinal microorganisms of chicken. Chin Anim Husb Vet Med 44 (3): 659-666. DOI: 10.16431/j.cnki.1671-7236.2017.03.006. [Chinese]
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.
Zhang H, Qin S, Zhang X, Du P, Zhu Y, Huang Y, Michiels J, Zeng Q, Chen W. 2022. Dietary resistant starch alleviates *Escherichia coli*-induced bone loss in meat ducks by promoting short-chain fatty acid production and inhibiting Malt1/NF-κB inflammasome activation. J Anim Sci Biotechnol 13 (1): 92. DOI: 10.1186/s40104-022-00739-7.
Zhao G, Zhou L, Dong Y, Cheng Y, Song Y. 2017. The gut microbiome of hooded cranes (*Grus monacha*) wintering at Shengjin Lake, China. Microbiologyopen 6 (3): e00447. DOI: 10.1002/mbo3.447.
Zhao Y, Li X, Sun S, Chen L, Jin J, Liu S, Song X, Wu C, Lu L. 2019. Protective role of dryland rearing on netting floors against mortality through gut microbiota-associated immune performance in Shaoxing ducks. Poult Sci 98 (10): 4530-4538. DOI: 10.3382/ps/pez268.
Zhou Y, Yan C, Chen D, Zhang C, Zhao X. 2023. Integration of grape-duck production pattern boosts duck behavior, meat quality, fecal microbiota and soil microorganisms. Agriculture 13 (1): 218-235. DOI: 10.3390/agriculture13010218.
Zhu C, Song W, Tao Z, Liu H, Zhang S, Xu W, Li H. 2020. Analysis of microbial diversity and composition in small intestine during different development times in ducks. Poult Sci 99 (2): 1096-1106. DOI: 10.1016/j.psj.2019.12.030.