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Unveiling microbiome diversity and abundance in the ceca and intestine of freshly slaughtered market-sold *kampung* chickens

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Abstract. *Murwani R, Sembiring A, Cahyani NKD, Anggoro AW, Kurniasih EM, Budiharjo A, Ambariyanto A. 2025. Unveiling microbiome diversity and abundance in the ceca and intestine of freshly slaughtered market-sold* kampung *chickens. Biodiversitas 26: 909-919.* The native Indonesian *kampung* chicken is a popular and desirable meat source sold live at traditional markets, offering fresh meat for consumers. The aim of this research was to investigate the microbial diversity of intestines and cecum of freshly sacrificed female *kampung* chickens using genomic sequencing. The DNA from 50 samples of female *kampung* chickens was extracted and amplified by PCR targeting the 16S rRNA V4 region. The PCR products were sequenced, and the taxonomic composition was summarized using the Phyloseq package, with taxa merged at six levels: domain, phylum, class, family, genus, and species. The result showed that the microbiome was dominated by Firmicutes (61.22%), Bacteroidota (20.06%), Actinobacteriota (7.14%), and Proteobacteria (2.81%). *Lactobacillus aviarius* (2.82%) was abundant across the samples. The cecum exhibited more diverse microbiomes than the intestines, with a predominance of Firmicutes. Beneficial bacteria, such as *L. aviarius* (5.26%) and *Bacteroides barnesiae* (3.03%), were dominant in the small intestine and cecum, respectively. A significant portion of the sequences remained unidentified or uncultured (50.95% and 20.61%, respectively). The gut microbiome of market-sold, freshly sacrificed female *kampung* chickens displays remarkably high diversity and richness, characterized by beneficial bacterial abundance, crucial for maintaining the chickens' overall health. These findings strongly support the consumer preference for fresh female *kampung* chicken meat, validating the traditional choice of the local community.

Keywords: *Bacteroides barnesiae*, Firmicutes, indigenous chicken, *kampung* chicken, *Lactobacillus aviaries*

INTRODUCTION

Indigenous chickens constitute a significant source of protein and income in many regions, particularly in rural and lower-income communities, and are often preferred over commercial breeds. Their adaptability to local environments, resilience to diseases, and ability to thrive on minimal resources make them a cost-effective choice. They are typically reared using conventional, cost-effective management practices that are well-suited to the socio-economic conditions of rural areas (Kingori et al 2010; Dessie et al. 2012; Padhi 2016; Sutriyono and Setianto 2019; Abbasi et al. 2021; Aleme 2022). This makes indigenous chickens an invaluable asset for smallholder farmers who depend on them for subsistence and income generation.

In Indonesia, the indigenous *kampung* chicken is notably prevalent and frequently sold at traditional market venues nationwide (Hidayat and Asmarasari 2015; Sutriyono and Setianto 2019; Rahardja 2021). These chickens are highly valued for their adaptability to local environments and are typically reared using conventional, cost-effective management practices. Their regular sale in traditional markets generates income and business opportunities while holding substantial cultural significance. The importance of these chickens as a source of animal protein has increasingly attracted attention from the poultry industry and researchers globally (Yadav et al. 2017; Kpomasse et al. 2023; Xu et al. 2023; Wu et al. 2024). Over recent decades, advancements in research and development have augmented indigenous chicken production, with contemporary studies utilizing direct sequencing techniques to evaluate gut microbiome diversity (Borda-Molina et al. 2018; Shang et al. 2018).

The chicken gut microbiome is essential for digestion, nutrient absorption, and immune system function. It prevents harmful pathogen colonization and produces short-chain fatty acids that support gut health. Consequently, gut microbiome diversity and composition can enhance chicken

health, reduce the risk of foodborne illness, and result in nutrient-rich, high-quality meat for consumers (Yegani and Korver 2008; Pandit et al. 2018; Carrasco et al. 2019; Aruwa et al. 2021; Glendinning et al. 2024; Shen et al. 2024). The composition of the chicken microbiome is influenced by various factors, including feed/diet, genetics, age, and environment (Yudiarti et al. 2012; Wei et al. 2013; Pan and Yu 2014; Hegde et al. 2016; Rubinelli et al. 2017; Clavijo and Flórez 2018; Kers et al. 2018; Susanti and Christijanti 2022). The indigenous *kampung* chickens are raised under different housing and management practices than commercial chickens (Murwani 2008; Murwani and Murtini 2009; Setiadi et al. 2020; Murwani et al. 2022), impacting their gut microbiome. Studies have shown that the composition of the gut microbiome can respond rapidly to dietary changes. This rapid response is due to the dynamic nature of the gut microbiome, which can quickly adapt to new nutrients and environmental conditions (Aruwa et al. 2021; Takeshita et al. 2021; Zou et al. 2022).

The traditional market setting is close to humans, and the chickens are typically housed in bamboo cages with ad libitum feed and drinking water. The chicken is sacrificed on site, the digestive tract is removed, and its content is one of the possible sources of contamination of foodborne pathogens in chicken meat. The traditional market setting, where these chickens are sold live and handled for a short period, is unique and crucial to study as it represents the direct point of consumer access to fresh chicken meat. The handling and sale of live chickens in these settings, such as the type of feed provided during the sales period and handling, can influence the microbiome of the chickens. Despite the importance of these factors, the traditional market settings, including the feed during the sale and their gut microbiomes, have not been extensively studied. Previous research focused on the microbial composition of broiler chicken meat from modern farms (Kim et al. 2019; Li et al. 2020). The aim of this study was to fill the research gap by assessing the microbial diversity of the small intestinal and caecal contents of freshly sacrificed female *kampung* chickens sold in traditional market settings. Investigating the richness and diversity of their gut microbiomes could provide valuable insights into consumer preferences for their meat.

MATERIALS AND METHODS

Animal ethics

This study did not involve animal treatment or intervention that caused harm. The Ethical Committee of the Faculty of Public Health-Universitas Diponegoro approved the protocols of this study (Approval number: 132/EA/KEPK-FKM/ 2022).

Female *kampung* chicken samples

Kampung chickens were collected from traditional markets in Semarang City, the capital of Indonesia's Central Java Province. The live chickens were housed in a bamboo cage (inside the traditional market building) and fed primarily rice bran mixed with a small, variable amount of concentrate, according to the vendor, for practicality. The mixed feed was wetted lightly with drinking water to soften it. Feed and drinking water were given ad libitum. The chickens were housed on-site for a short period, up to a maximum of one week, depending on the demand. The rice bran and commercial concentrate were sampled and analyzed for proximate composition in an accredited laboratory. Five traditional market sampling sites (Group 1 to Group 5) were purposely chosen in Semarang City, Central Java, Indonesia, where *kampung* chickens were sold alive. Therefore, five female *kampung* chickens were sampled randomly from each sampling site, and 25 female *kampung* chicken samples were obtained (Table 1).

The female chicken body weight was lighter than that of the male and consequently cheaper and preferred by consumers. The vendor sacrificed them in situ (on-site) according to standard animal welfare procedures. The abdominal cavity was opened, the whole intestinal tract was removed, put in an iced box, and samples were taken to a clean laboratory bench. First, the intestine attached to the gizzard was separated and tied. Subsequently, the cecum was separated from the small and large intestines at the junction. Its content was squeezed and gently pushed thoroughly into a labeled jar containing absolute ethanol (analytical grade, Merck). Finally, the small intestine was treated similarly, and the content was preserved in absolute ethanol. In the end, 25 caecal and 25 small intestinal content samples (50 samples) were obtained for this study.

DNA extraction and sequencing

10 g of the homogenized sample was extracted using the MO-BIO Powermax® Soil DNA Isolation Kit (MoBio, Carlsbad, USA) according to manufacturing protocol with the addition of 400 µg/mL Proteinase K (Qiagen GmbH, Hilden, Germany). All DNA extracts were purified using MO-BIO PowerClean® DNA Clean-Up Kits (MoBio, Carlsbad, USA) and quantified using Qubit dsDNA HS Kit (Thermo Fisher Scientific, Oregon, USA), run on 1% agarose gel (Bio-Rad Laboratories, USA) in sodium borate buffer (Merck KGaA, Darmstadt, Germany) to check the quality of DNA. Next, to assess microbial diversity, 16S rRNA was amplified using primers 515f and 806r, targeting the V4 region of the 16S rRNA (Caporaso et al. 2012; Walters et al. 2015). Library preparation for 16S follows a single indexing approach where barcodes are on the forward primer of the 515f-806r primers pair to facilitate multiplexing of up to 96 samples per run. PCR was performed in triplicate, using five ng of DNA from each sample. The following PCR conditions were used: initial denaturation at 94°C for 3 min, 35 cycles each at 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s, followed by a final extension step at 72°C for 10 min. PCR products were visualized in 1.2% agarose gel electrophoresis and subsequently cleaned before being sequenced in MiSeq Illumina (Illumina, USA) using a V2 300-cycle kit with 20% PhiX DNA (Callahan et al. 2016) was added to each run to improve data quality.

Table 1. Sampling ID, gut site, and sampling locations

Samples ID	Gut site sampled	Sampling locations
F1_cec	Cecum	Group_1
F2_cec	Cecum	Group_1
F3_cec	Cecum	Group_1
F4_cec	Cecum	Group_1
F5_cec	Cecum	Group_1
L11_sec	Cecum	Group_2
L12_sec	Cecum	Group_2
L13_sec	Cecum	Group_2
L14_sec	Cecum	Group_2
L15_sec	Cecum	Group_2
L21_sec	Cecum	Group_3
L22_sec	Cecum	Group_3
L23_sec	Cecum	Group_3
L24_sec	Cecum	Group_3
L25_sec	Cecum	Group_3
L31_sec	Cecum	Group_4
L32_sec	Cecum	Group_4
L33_sec	Cecum	Group_4
L34_sec	Cecum	Group_4
L35_sec	Cecum	Group_4
L41_sec	Cecum	Group_5
L42_sec	Cecum	Group_5
L43_sec	Cecum	Group_5
L44_sec	Cecum	Group_5
L45_sec	Cecum	Group_5

Samples ID	Gut site sampled	Sampling locations
F1_si	Small intestine	Group_1
F2_si	Small intestine	Group_1
F3_si	Small intestine	Group_1
F4_si	Small intestine	Group_1
F5_si	Small intestine	Group_1
L11_si	Small intestine	Group_2
L12_si	Small intestine	Group_2
L13_si	Small intestine	Group_2
L14_si	Small intestine	Group_2
L15_si	Small intestine	Group_2
L21_si	Small intestine	Group_3
L22_si	Small intestine	Group_3
L23_si	Small intestine	Group_3
L24_si	Small intestine	Group_3
L25_si	Small intestine	Group_3
L31_si	Small intestine	Group_4
L32_si	Small intestine	Group_4
L33_si	Small intestine	Group_4
L34_si	Small intestine	Group_4
L35_si	Small intestine	Group_4
L41_si	Small intestine	Group_5
L42_si	Small intestine	Group_5
L43_si	Small intestine	Group_5
L44_si	Small intestine	Group_5
L45_si	Small intestine	Group_5

Sequence and statistical analysis

For further analysis, forward and reverse FASTQ sequences were demultiplexed and imported into QIIME2 version 2017.8.0 (the Quantitative Insights into Microbial Ecology 2 program, https://qiime2.org/) (Bolyen et al. 2019; Murwani et al. 2024). The DADA2 (Divisive Amplicon Denoising Algorithm 2) software was embedded in QIIME2 to filter quality, trim, de-noise, and merge the data (Bolyen et al. 2019). A feature classifier in QIIME2 trained against the SILVA SSU non-redundant database (https://www.arb-silva.de) was used to assign taxonomy to all the V4 regions of 16S ribosomal sequence variants. Contaminating mitochondrial and chloroplast sequences were filtered out of the resulting feature ASV table. The taxonomic composition of each sample was summarized in the Phyloseq package in R (R development core team), with taxa merged at the six different levels (domain, phylum, class, family, genus, and species).

RESULTS AND DISCUSSION

Feed proximate composition

The *kampung* chickens were mainly fed rice bran, which contained 13.38% moisture, 10.8% ash, 8.51% fat, 8.56% protein, 17.25% crude fiber, and 41.51% carbohydrates. They were also given additional feed in the form of a factory-made concentrate containing 9.5% moisture, 39.24% ash, 4% fat, 25.32% protein, 2.06% crude fiber, and 20.34% carbohydrates.

DNA amplification and microbial diversity

A total of 7,058,879 reads were obtained from the 50 samples of female chicken gut (cecum and small intestine), resulting in 9,380 amplicon sequence variants (ASVs), a molecular equivalent of species. These data only covered the bacterial domain and excluded mitochondria and chloroplast sequences. Sequence reads from the samples range from 80,246 to 205,469, averaging 141,177.6 reads per sample. Data were rarefied evenly with a depth of 80,246 sequences per sample, and the subsequent data were used for all downstream applications. A total of 4,012,300 reads and 8,869 ASVs were obtained after quality filtering and exclusion of chimera. The 16S rRNA amplicon data of chicken gut microbiome were assigned to seven bacterial taxonomic levels (domain, phylum, class, order, family, genus, and species) based on the SILVA database (https://www.arb-silva.de). Downstream analysis only used the bacterial domain and excluded the mitochondrial and chloroplast sequences. Combining the results from all 50 samples, Firmicutes was the dominant phylum in the chicken gut microbiome (61.22%) and also in the sequence reads (ASV=51.2%), followed by Bacteroidota (20.06%), Actinobacteria (7.14%), and Proteobacteria (2.81%) (Table 2 and Figure 1.A).

The single region V4, with its ability to discriminate microbial ASVs up to the genus level in this study (Figure 1), holds great potential for future research in microbial identification. However, at the lowest taxonomic level (species or culturable), almost half of the sequences could not be identified (50.95%) or identified as specific taxa, which were not culturable (uncultured bacterium 20.61%) (Figure 1.F and Table 2). This finding highlights the challenges and inspires hope for further advancements in the field. It was also observed that the abundance of *L. aviarius* was 2.82% in the overall samples.

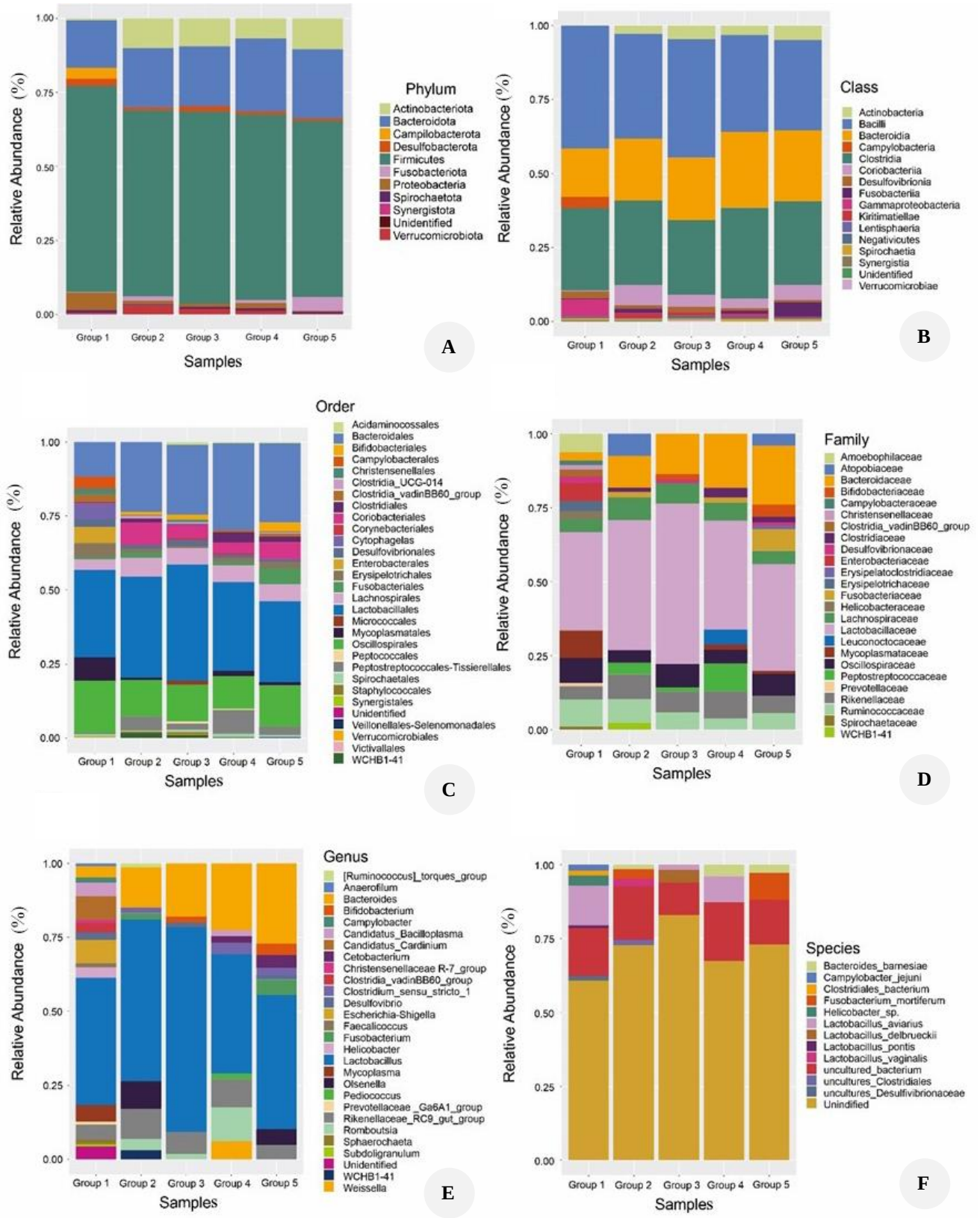

Figure 1. The relative abundance (%) of gut microbiome distribution using V4 region of 16S rRNA amplicon sequencing for female *kampung* chicken. The bar plot is constructed based on A. Phylum, B. Class, C. Order, D. Family, E. Genus, F. Species. The bar plots only show that the phylum, class, and order levels contribute more than 2% of the relative abundance of each sample. Meanwhile, the family, genus, and species levels shown on the bar plot contributed more than 5% of the relative abundance of each sample

Table 2. List of taxa (phyla and species level) and reads abundance of overall 50 samples (25 small intestine and 25 cecum content) of female *kampung* chickens

Taxa	Percentage of reads abundance
Phylum	
Actinobacteriota	7.14
Bacteroidota	20.06
Campilobacterota	0.94
Desulfobacterota	1.89
Firmicutes	61.22
Proteobacteria	2.81
Spirochaetota	1.19
Synergistota	0.51
Verrucomicrobiota	1.70
Fusobacteriota	1.79
Unidentified	0.19
Phyla with reads abundance <2% total	0.56
Species	
Bacteroides barnesiae	1.95
Campylobacter jejuni	0.32
Clostridiales bacterium	0.59
Fusobacterium mortiferum	1.01
Helicobacter sp.	0.46
Lactobacillus aviarius	2.82
Lactobacillus delbrueckii	0.63
Lactobacillus pontis	0.78
Lactobacillus vaginalis	0.77
Unidentified	50.95
uncultured *Clostridiales*	1.32
uncultured_Desulfovibrionaceae	0.40
uncultured_bacterium	20.61
Species with reads abundance <5% total	17.39

Table 3. List of taxa (phyla and species level) and reads abundance from total reads of 25 cecal samples of female *kampung* chickens

Taxa	Percentage of reads abundance
Phylum	
Bacteroidota	29.03
Firmicutes	50.36
Campilobacterota	0.57
Actinobacteriota	7.15
Proteobacteria	2.37
Spirochaetota	1.89
Desulfobacterota	3.09
Fusobacteriota	0.92
Synergistota	0.81
Unidentified	0.24
Verucobacteriota	2.72
Phyla with reads abundance <2% total	0.85
Species	
Bacteroides barnesiae	**3.03**
Campvlobacter jejuni	0.37
Clostridiales bacterium	1.00
Fusobacterium mortiferum	0.80
Helicobacter sp.	0.03
Lactobacillus aviarius	0.37
Lactobacillus delbrueckii	0.12
Lactobacillus pontis	0.27
Lactobacillus vaginalis	0.24
uncultured bacterium	28.74
uncultured *Clostridiales*	1.90
uncultured Desulfovibrionaceae	0.71
Unidentified	40.07
Species with reads abundance <5% total	22.35

Gut microbiome composition in the cecum and small intestine of female *kampung* chicken

Figure 2 and Tables 3 and 4 show that in the cecum and small intestine, Firmicutes was the most abundant phylum (50.36%, 72.08%), followed by Bacteroidota (29.03% and 11.09%) and Actinobacteria (7.15% and 7.12%). Phyla with smaller abundance in the cecum (more than 2% reads abundance) were Desulfobacterota (3.09%), Verrucomicrobiota (2.72%), and Proteobacteria (2.37%), and in the small intestine were Proteobacteria (3.25%) and Fusobacteriota (2.67%). In the phylum Firmicutes, Clostridia (35.4% reads abundance) was the dominant class in the cecum and Bacilli (54%) in the small intestine, with the order Lactobacillales being the most abundant (46%) (Figure 2.C). All taxa in the phylum Bacteroidota consisted of class Bacteroidia in the cecum (29%) and small intestine samples (11%). Gammaproteobacteria was the dominant class from the phylum Proteobacteria in the small intestine and cecum (Figure 2B).

The cecum was predominantly occupied by the orders Bacteroidales (class Bacteroidia, phylum Bacteroidetes) and Oscillospirales (class Clostridia, phylum Firmicutes), accounting for 21% and 4.2% of the reads, respectively (Figure 2.C). This dominance of Bacteroidales and Oscillospirales in the cecum suggests a unique microbial environment in this part of the digestive system. In the small intestine, the order Lactobacillales (class Bacilli, phylum Firmicutes) was the most abundant taxa, comprising 46% of the reads. Additionally, Bacteroidales, Mycoplasmatales, and Oscillospirales were also prominent in the small intestine (Figure 2.C)

The present findings revealed a novel aspect of microbiome diversity, showing that the cecum exhibits a higher diversity than the intestine (Figure 2.A-F). This consistent pattern, despite some variations, is a unique discovery. The prevalence of the genus *Lactobacillus* in the small intestines and *Bacteroides* in the cecum (Figure 2E) further adds to the novelty of this present work. At the species level, *L. aviarius* and *Bacteroides barnesiae* emerged as the dominant species in the small intestine and cecum, respectively (Figure 2, Tables 3 and 4).

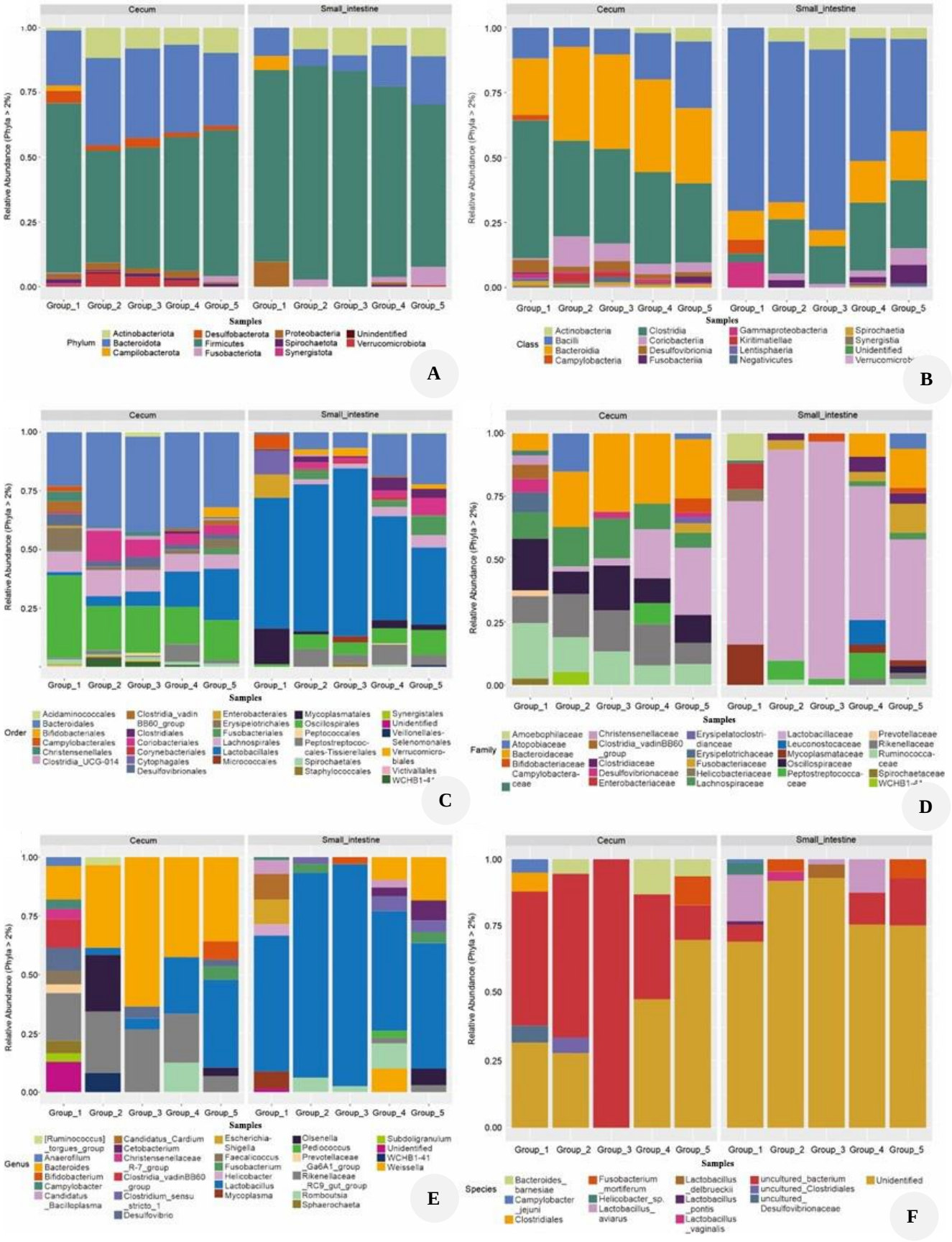

Figure 2. The relative abundance (%) of gut microbiome distributions using V4 region of 16S rRNA amplicon sequencing for female *kampung* chicken cecum and small intestine. The bar plot is constructed based on A. Phylum, B. Class, C. Order, D. Family, E. Genus, F. Species. Phylum, class, and order level contribute more than 2% of the relative abundance of each sample. Family, genus, and species level contribute more than 5% of the relative abundance of each sample

Table 4. List of taxa (phyla and species level) and reads abundance from total reads of 25 small intestinal samples of female *kampung* chickens

Taxa	Percentage of reads abundance
Phylum	
Bacteroidota	11.09
Firmicutes	72.08
Campilobacterota	1.32
Actinobacteriota	7.12
Proteobacteria	3.25
Spirochaetota	0.49
Desulfobacterota	0.70
Fusobacteriota	2.67
Synergistota	0.20
Unidentified	0.15
Verucobacteriota	0.67
Phyla with reads abundance <2% total	0.26
Species	
Bacteroides barnesiae	0.88
Campvlobacter jejuni	0.28
Clostridiales bacterium	0.19
Fusobacterium mortiferum	1.21
Helicobacter sp.	0.89
Lactobacillus aviarius	**5.26**
Lactobacillus delbrueckii	1.15
Lactobacillus pontis	1.30
Lactobacillus vaginalis	1.30
uncultured bacterium	12.47
uncultured *Clostridiales*	0.74
uncultured Desulfovibrionaceae	0.10
Unidentified	61.83
Species with reads abundance <5% total	12.4

Discussion

The *kampung* chicken is an indigenous tropical chicken of Indonesia. It served as a crucial protein source before the modern breed was introduced, becoming increasingly popular and the preferred meat for diverse consumers. The health of these domestic chickens is vital for their productivity, sustainability, and consumer satisfaction. This study aimed to elucidate the crucial role of the gut microbiome composition of *kampung* chickens sold live at traditional markets before sacrifice through genomic sequencing. The results showed that the microbiome was predominantly composed of Firmicutes (61.22%), Bacteroidota (20.06%), Actinobacteriota (7.14%), and Proteobacteria (2.81%).

Comparative studies on the most abundant phyla in the chicken intestinal and caecal microbiome revealed that in broilers, Firmicutes dominated (70%), followed by Bacteroidetes (12.3%) and Proteobacteria (9.3%) (Wei et al. 2013). In dual-purpose local Egyptian breeds, the jejunum microbiome was primarily composed of Firmicutes (45.8%), Proteobacteria (41.5%), Bacteroidetes (8.0%), Actinobacteria (2.9%), and Verrucomicrobia (0.4%), constituting 98.2% of the identified phyla (Abdel-Kafy et al. 2022). Another study indicated higher microbial diversity and richness in indigenous breeds compared to broilers, with variations in dominant phyla. In the indigenous Aseel chicken, the gut was dominated by Bacteroidetes (44%), Firmicutes (43%), Proteobacteria (6%), Actinobacteria (1%), and Cyanobacteria (0.8%), making up 94.8% of the phyla. In the Nicobari breed, Bacteroidetes (53%) was the most dominant, followed by Firmicutes (24%), Proteobacteria (8%), Fusobacteria (5%), Verrucomicrobia (2%), and Cyanobacteria (2%). Conversely, in broilers, Firmicutes dominated (81%), followed by Bacteroidetes (6%), Cyanobacteria (6%), Proteobacteria (4%), and Verrucomicrobia (1%) (Paul et al. 2021).

A study on broiler Cobb-500 and indigenous Omani chickens fed a corn-soybean diet showed varying phylum abundances in different intestinal parts (Al-Marzooqi et al. 2020). Firmicutes dominated the duodenum in broilers (86.3%), followed by Proteobacteria (11.9%). The jejunum had a high abundance of Actinobacteria (54.8%) and Firmicutes (42.4%), while the ileum was dominated by Deinococcus-Thermus (67.8%), followed by Firmicutes (18.6%) and Actinobacteria (11.4%). In Omani chickens, the duodenum, jejunum, and ileum were similarly dominated by Firmicutes (94.2%, 93.8%, and 96%, respectively), followed by Actinobacteria (2.4%, 5.4%, and 3.5%, respectively). Firmicutes were the most dominant phylum in the caecum of both broilers (97%) and Omani chickens (81%). Actinobacteria (27%) was the second most dominant in broilers, while Bacteroidetes (12.4%) and Proteobacteria (4%) were the second most dominant in Omani chickens (Al-Marzooqi et al. 2020).

In the present study, Firmicutes was the most dominant phylum, a finding that has significant implications for the field of microbiology and poultry science. This aligned with the observation in the broiler (Wei et al. 2013) but differed from Indian indigenous chickens, where Bacteroidetes were most dominant (Paul et al. 2021). The findings align more closely with Indonesian *kampung* chickens reared in extensive and semi-intensive systems, where Firmicutes was dominant, followed by Actinobacteria and Bacteroidetes (Susanti and Christijanti 2022). It is also similar to a study by Mootane et al. (2024), where Firmicutes (74%) was the most abundant phylum, followed by Proteobacteria (8%), Actinobacteria (5%), and Bacteroidota (3%). The Firmicutes to Bacteroidetes ratio in the present study was higher in the intestine (72.1% to 11.1%) than in the caecum (50.4% to 29.0%), corresponding with the dominant species in these gut segments (Tables 3 and 4), namely *L. aviarius* in the small intestine and *B. barnesiae* in caecum. The higher Firmicutes abundance in the intestine could be associated with nutrient utilization, a key insight that piques interest for further research.

Variations in phylum abundance and richness across studies were influenced by factors such as diet, sex, genetics/strain, and environmental conditions, including production systems (Wei et al. 2013; Pan and Yu 2014; Hegde et al. 2016; Lee et al. 2017; Rubinelli et al. 2017; Clavijo and Flórez 2018; Kers et al. 2018; Shi et al. 2019; Al-Marzooqi et al. 2020; Paul et al. 2021; Abdel-Kafy et al. 2022; Susanti and Christijanti 2022; Varriale et al. 2022). These studies consistently demonstrated that caecal microbiome diversity exceeds that of the intestine. Additionally, microbiome patterns are linked to the distinct functions of the intestine and cecum.

The intestinal microbiome, a crucial part of the body's defense system, maintains the intestinal lining and protects against pathogenic bacteria through competitive exclusion. This protective role of the microbiome provides a sense of security regarding health. Additionally, the microbiome is involved in modulating the immune system and metabolism of the host (Sommer and Bäckhed 2013; Oakley et al. 2014; Kogut 2019; Aruwa et al. 2021; Proszkowiec 2022).

The current study revealed that *Lactobacillus* was abundant in the female *kampung* chicken small intestine and cecum, with 2.82% of *L. aviarius* in the overall samples. *L. aviarius* (genus *Lactobacillus*, family Lactobacillaceae, phylum Firmicutes) was found throughout the gastrointestinal tract of the broiler and was more abundant in the upper GI tract. *L. aviarius* was the highest in the gizzard, jejunum, and ileum (Mwangi et al. 2010). Two subspecies of *L. aviarius* (subsp. *aviarius* sp. nov., subsp. nov., and subsp. *araffinosus* subsp. nov.) were isolated from the chicken intestine in 1984 (Wang et al. 2014). Both are Gram-positive, non-motile, non-spore-forming, short rods, strictly anaerobic, and homo-fermentative, producing DL-lactic acid. The first sub-strain can use raffinose, an oligosaccharide (trisaccharide) (Fujisawa et al. 1984) found in rice bran (Saunders 1985), providing a substrate for *L. aviarius* to flourish. Dietary supplementation of *L. aviarius* CML352 to chickens can reduce abdominal fat deposition and improve egg quality in late-phase hens (Xu et al. 2022), providing evidence of their advantages to *kampung* chicken.

It was also observed that *kampung* chickens were fed primarily rice bran meal (*dedak*) mixed with a small, arbitrary amount of commercial concentrate for practicality during their short-term sale in the market. As a by-product of rice milling, rice bran is commonly used in higher percentages (ranging from 50% to 80%) due to its cost-effectiveness (Rohaeni et al. 2021). This feed is readily accessible and widely utilized for feeding *kampung* chickens (Akhadiarto 2017; Rohaeni et al. 2021). The high crude fiber content in rice bran meal provides various substrates that promote the growth of beneficial bacteria, such as *Lactobacillus*, while inhibiting pathogenic bacteria like *Salmonella* (Rubinelli et al. 2017). Additionally, numerous studies have demonstrated that non-digestible oligosaccharides found in cereal brans act as prebiotics, modulating gut microbiota, and immune interactions to favor chicken health (Fujisawa et al. 1984; Pourabedin and Zhao 2015; Rubinelli et al. 2017; Teng and Kim 2018; Ricke et al. 2020). Therefore, the discovery of *L. aviarius* dominance in the small intestine (5.26%) of female *kampung* chickens at the point of sale is novel and highlights the significance of beneficial strains, marking a noteworthy and intriguing finding in animal science.

On the other hand, the cecum, a blind sac posterior to the junction of the small intestine and the chicken's rectum, receives the undigested matter from the intestine. Significant amounts of expelled dry matter (18%) and excreted water (17%) make their way into the cecum (Svihus et al. 2013). Water and salt are refluxed into the ceca with monosaccharides, causing water and salt to be reabsorbed in this area. The undigested dry matter, mostly fibers, is a rich substrate for anaerobic bacteria, producing short-chain fatty acids (SCFAs) using the residing microbial enzymes. SCFAs, such as acetate, butyrate, propionate, succinate, and lactate, provide energy for the host and play an essential role in host performance and health (Sergeant et al. 2014). The anaerobic environment of ceca provide a suitable condition for diverse anaerobic bacteria to flourish in addition to intestinal microbes, resulting in more diversity (Lee et al. 2017; Al-Marzooqi et al. 2020). The cecum of the female *kampung* chicken in the present study harbored *B. barnesiae* as the most abundant identified species (3.03%). *B. barnesiae* (genus *Bacteroides*, family Bacteroidaceae, order Bacteroidales, class Bacteroidia, phylum Bacteroidota) was successfully isolated from healthy chickens (Sakamoto et al. 2015). The finding of *B. barnesiae* dominance (3.03%) in the cecum of the female kampung chickens at the sale point is new and benefits the host *kampung* chicken. It is a Gram-negative, non-spore-forming, pleomorphic rod, and strictly an-aerobe bacterium that can utilize various sugars (glucose, lactose, sucrose, maltose, salicin, xylose, cellobiose, mannose, and raffinose) to produce acids. *Bacteroides barnesiae* is also essential in the breakdown of complex molecules into simpler compounds utilized by the chicken and microorganisms themselves, utilization of nitrogenous substances, biotransformation of bile acids and other steroids, and prevention of colonization of the intestine by pathogenic microorganisms (Lan et al. 2006).

The results revealed that most species in the intestine and cecum were unidentified and uncultured, highlighting the importance of collaboration in this field. This is not surprising as numerous studies on the gut microbiota using genomic sequences that target the V4 region of 16s rRNA aligned the obtained sequences with previously known sequences in the database (Wei et al. 2013; Almeida et al. 2019). The study of human gut microbiota found 1,952 uncultured candidate bacterial species (Almeida et al. 2019). Furthermore, some *Lactobacillus* species isolated from the chicken gut are unculturable in MRS agar media (Adhikari and Kwon 2017). A high abundance of uncultured bacteria could be due to the absence of a necessary nutrient in the culture medium, the unsuitability of the culture medium, the production of chemicals by other bacteria in the sample that inhibit the target organism, or the requirement of co-culture with other bacteria.

This study reveals that female *kampung* chickens sold in traditional market environments are primarily fed a diet of rice bran meal supplemented with a small amount of concentrate. Just before being sacrificed on-site, they exhibit a high level of microbial diversity and richness in their gut microbiota. This diversity includes a significant presence of beneficial bacteria, such as *Lactobacillus aviarius* in the small intestine and *Bacteroides barnesiae* in the cecum of chickens. Moreover, the presence of other minor taxa can play roles in maintaining the stability and functionality of the gut ecosystem. One of the functional roles is functional redundancy, where the presence of multiple microbial species can perform similar functions within the ecosystem. If one species is lost or decreases, others can perform the same function, ensuring microbiome stability and resilience (Zhang et al. 2023). This finding of

abundant beneficial bacteria is significant as it underscores the potential health benefits these bacteria confer to the host, marking an interesting discovery in the *kampung* chicken gut microbiome.

In conclusion, genomic sequencing has revealed extensive microbial diversity and richness within the female gut of native Indonesian *kampung* chickens. Analysis of the V4 region of 16S rRNA identified thousands of amplicon sequence variants (ASVs) across various bacterial taxa, with Firmicutes (61.2%), Bacteroidota (20.1%), Actinobacteriota (7.1%), and Proteobacteria (2.8%) being predominant in the female chicken gut. Notably, the cecum exhibited greater microbiome diversity compared to the intestine. The discovery of a high abundance of beneficial bacteria, specifically *B. barnesiae* in the cecum and *L. aviaries* in the intestine, was particularly novel. These findings strongly support the consumer preference for fresh female *kampung* chicken meat, validating the traditional choice of the local community.

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