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PCA-based metagenomics reveals functional and taxonomic diversity in Kedu chicken gut microbiota

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Abstract. *Pandupuspitasari NS, Sugiharto S, Agusetyaningsih I, Lestari DA, Khan FA, Setiaji A, Raza MA. 2025. PCA-based metagenomics reveals functional and taxonomic diversity in Kedu chicken gut microbiota. Biodiversitas 26: 4035-4041.* The Gastrointestinal Tract (GIT) microbiota plays a central role in poultry health, productivity, and resilience, influencing digestion, nutrient absorption, immune development, and disease resistance. This study provides the first multi-database Principal Component Analysis (PCA)-based metagenomic characterization of the gut microbiota in Kedu chickens (*Gallus gallus* (Linnaeus, 1758)), an indigenous Indonesian breed. Digesta from five GIT segments (crop, gizzard, jejunum, colon, and cecum) of 21 traditionally reared Kedu chickens were sequenced on the Illumina NovaSeq X Plus platform, yielding 1,523,876 unigenes. Functional annotation against Non-Redundant Protein Database (NR), Clusters of Orthologous Groups (COG), Kyoto Encyclopedia of Genes and Genomes (KEGG), Carbohydrate-Active enZYmes Database (CAZy), Antibiotic Resistance Genes Database (ARDB), Comprehensive Antibiotic Resistance Database (CARD), and Virulence Factor Database (VFDB) revealed enrichment of genes involved in metabolism (38.2%), cellular processes (15.7%), environmental information processing (12.6%), and genetic information processing (11.0%). PCA separated GIT segments by functional specialization: The jejunum clustered distinctly (PC1 variance = 26.48% for NR, 30.04% for COG, 67.16% for KEGG) due to nutrient absorption-related functions, while the cecum exhibited the highest diversity and carbohydrate-active enzyme profiles (PC1 = 24.98% for CAZy). ARDB/CARD analysis showed that distal GIT segments (colon, cecum) contained higher densities of antimicrobial resistance genes, and VFDB revealed elevated virulence factor abundance in ileum and cecum. These findings highlight segment-specific microbiota roles, supporting the development of targeted probiotics and feed strategies, and contribute to conserving the functional biodiversity of Kedu chickens in sustainable production systems.

Keywords: Dimensionality reduction, gut microbiota, indigenous chicken, poultry health, principal component

INTRODUCTION

The Gastrointestinal Tract (GIT) of poultry hosts a complex and dynamic microbial ecosystem essential for host health, productivity, and adaptability. These microorganisms influence nutrient digestion, absorption, immune development, disease resistance (Wickramasuriya et al. 2022). A balanced microbiota supports feed efficiency, growth, and resilience against environmental and pathogenic stressors (Adedokun and Olojede 2019), whereas dysbiosis can impair productivity and immunity (Yang and Jobin 2014). Indigenous chicken breeds represent important genetic and cultural resources. Compared with intensively selected commercial broilers and layers, these breeds often possess traits such as disease tolerance, ability to utilize low-quality feed, and distinctive meat and egg quality (Sutopo et al. 2022; Kpomasse et al. 2023). The Kedu chicken, native to Central Java, Indonesia, is valued for cultural significance, adaptability to village systems, and economic potential for smallholders. Despite this, little is known about its gut microbial ecology, limiting opportunities to apply microbiome-based strategies for productivity and conservation.

High-throughput sequencing and functional metagenomics now enable the characterization of microbiota not only in terms of taxonomic composition but also functional potential. By linking microbial genes to pathways, functional metagenomics reveals capabilities related to metabolism, fiber degradation, immune modulation, and pathogen resistance (Choi et al. 2015). Studies on Kampung chickens in Indonesia (Murwani et al. 2025), Thai native chickens (T-Thienprasert et al. 2025), and Ethiopian village chickens (Kassa et al. 2024) have shown unique microbial compositions shaped by management systems and local feed resources. However, no comparable work exists for Kedu chickens. Metagenomic datasets are high-dimensional, requiring statistical methods for pattern recognition. Principal Component Analysis (PCA) is widely used when variables are continuous and normally distributed, reducing noise and revealing dominant trends

(Greenacre et al. 2022). To date, no study has applied a multi-database, PCA-based metagenomic approach to characterize the functional and taxonomic diversity of Kedu chicken GIT microbiota. PCA has effectively clustered poultry gut samples by GIT region, diet, or environment (Zhang et al. 2019). While efficient and interpretable, PCA is limited in detecting non-linear relationships, a gap that can be addressed by methods such as Non-Metric Multidimensional Scaling (NMDS) or Principal Coordinate Analysis (PCoA) (Nguyen et al. 2023).

Using multiple functional annotation databases provides a comprehensive view of microbial capabilities. The CAZy database identifies carbohydrate-active enzymes for fiber degradation; ARDB and CARD detect antimicrobial resistance genes (ARGs); VFDB catalogs virulence factors; and KEGG maps metabolic pathways. Integrating these resources with PCA allows detection of functional specialization across GIT regions and associations between traits of ecological or applied importance. Such knowledge can guide targeted probiotic development, dietary interventions, and breeding programs. Microbial community structure and function vary along the GIT due to differences in pH, nutrient availability, and transit time. Proximal segments such as the crop and gizzard are adapted for initial feed processing, while the jejunum specializes in nutrient digestion and absorption. The cecum acts as a fermentation chamber enriched in fiber-degrading microbes that produce short-chain fatty acids. Mapping these differences in Kedu chickens can reveal adaptations linked to performance under traditional rearing.

This research addresses that gap by sequencing digesta from five GIT segments, annotating genes using seven major databases, and applying PCA to identify key functional and taxonomic patterns. We hypothesize that each segment harbors distinct microbial assemblages and functions reflecting their physiological roles, together forming a unique microbiome signature shaped by the Kedu chicken's genetic background and environment. The objectives of this study are to: (i) profile the taxonomic and functional diversity of Kedu chicken GIT microbiota; (ii) determine segment-specific functional specializations using PCA; (iii) discuss implications for sustainable production, probiotic development, and conservation of this indigenous breed. By establishing a foundational microbiome profile, this work contributes to the conservation and improvement of native poultry genetic resources.

MATERIALS AND METHODS

Ethical approval

The Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 61-06/A-11/KEP-FPP) approved the experimental procedures for this study.

Birds and sampling

Twenty-one healthy adult Kedu chickens (*Gallus gallus* Linnaeus, 1758)), 6-8 months old and weighing 1.8-2.3 kg, were sourced from traditional free-range systems in Kedu Village, Temanggung District, Central Java, Indonesia. Sample size was determined based on statistical power analysis ($\alpha = 0.05$, power = 0.8) and prior poultry microbiome studies to ensure adequate detection of differences among GIT segments. Birds were raised under typical smallholder management, with daytime scavenging and supplemental feeding of mixed grains. Following euthanasia method by cervical dislocation, the GIT was aseptically excised. Digesta samples were collected from five segments crop, gizzard, jejunum, colon, and cecum—using sterile instruments to avoid cross-contamination. Each sample was placed in sterile cryovials, snap-frozen in liquid nitrogen, and stored at -80°C until DNA extraction.

DNA extraction

Microbial DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), following the manufacturer's protocol with bead-beating to enhance lysis of Gram-positive bacteria. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and agarose gel electrophoresis. Only samples with A260/A280 ratios between 1.8 and 2.0 were used for sequencing.

Metagenomic sequencing

DNA was extracted and fragmented to 3500 bp by Covaris M220 (Gene Company Limited, China) to make paired-end library. NEXTFLEX® Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA) was used to construct Paired-end library P Sequencing of paired end library was performed on an Illumina NovaSeq™ X Plus (Illumina Inc., San Diego, CA, USA).

Quality control and assembly

Raw reads were quality-filtered with Fastp v0.20.0 (Chen et al. 2023) using parameters: minimum length 50 bp, quality score Threshold Q20, adapter removal enabled, and polyG trimming. Host-derived reads were removed by mapping to the *Gallus gallus* reference genome (GRCg6a) using Bowtie2 v2.4.4 with default settings. Clean reads were assembled into contigs using MEGAHIT v1.1.2 with a k-mer range of 21-99 and a minimum contig length of 300 bp.

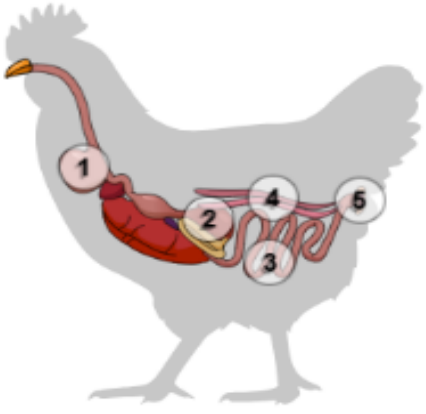

Figure 1. The gastrointestinal tract served as the source for sample collection: 1. Crop, 2. Gizzard, 3. Jejunum, 4. Cecum, 5. Colon

Gene prediction and non-redundant catalog construction

Open Reading Frames (ORFs) were predicted using Prodigal v2.6.3 in metagenomic mode. A non-redundant gene catalog was generated using CD-HIT v4.8.1 at 95% sequence identity and 90% alignment coverage thresholds to remove duplicates.

Taxonomic and functional annotation

The best-hit taxonomy of non-redundant genes was obtained by aligning them against the National Center for Biotechnology Information (NCBI) Non-Redundant Protein Database (NR) database by DIAMOND[6] (http://ab.inf.uni-tuebingen.de/software/diamond/, version 2.0.13) with an e-value cutoff of 1e-5. Similarly, functional annotation of non-redundant genes was obtained for Clusters of Orthologous Groups (COG), Kyoto Encyclopedia of Genes and Genomes (KEGG), Carbohydrate-Active enZYmes Database (CAZy), Antibiotic Resistance Genes Database (ARDB), Comprehensive Antibiotic Resistance Database (CARD), and Virulence Factor Database (VFDB). Taxonomic assignment was performed using a Naïve Bayes classifier in QIIME 2 v2023.7 trained on the SILVA 138 database. SILVA was chosen over Greengenes due to its broader, more up-to-date prokaryotic taxonomy coverage.

Taxonomic assignment

Representative ASVs were taxonomically classified using a Naïve Bayes classifier trained using the SILVA 138 reference database. Taxonomic ranks were identified from the phylum to the genus level, and species-level resolution was attempted. Functional prediction of microbial communities was conducted using PICRUSt2, which infers the gene content from phylogenetic information. The predicted gene families were annotated using the following databases.

i. NR: For broad-spectrum protein sequence annotation and homology-based searches.
ii. COG: The predicted genes classification into functional categories, for example, cellular processes, metabolism, and information storage.
iii. KEGG: To reconstruct the metabolic and signaling pathways relevant to gut microbiota functionality.
iv. CAZy: To annotate enzymes involved in carbohydrate metabolism, which is essential for fiber digestion and host-microbe interactions.
v. ARDB and CARD: To identify predicted genes associated with antibiotic resistance, enabling assessment of Antimicrobial Resistance (AMR) risk within microbial communities.
vi. VFDB: To predict potential virulence genes, facilitating the identification of pathogenic traits among gut bacteria. These annotations provided a functional profile for each sample, allowing ecological interpretation beyond taxonomic composition.

Statistical and multivariate analysis

All statistical analyses were performed in R v4.3.1. Alpha Diversity Indices (Shannon, Simpson, Chao1) and beta diversity metrics (Bray-Curtis dissimilarity, weighted UniFrac) were calculated using the vegan v2.6-4 and phyloseq v1.38.0 packages. Differences among GIT segments were tested with PERMANOVA (999 permutations). Functional and taxonomic abundance data were centered and scaled prior to Principal Component Analysis (PCA) using prcomp in R. PCA visualizations were created using ggplot2 v3.4, with variance explained by each axis reported in figure legends. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Metagenomic sequencing of 105 GIT samples from 21 Kedu chickens produced 302.4 Gbp of high-quality reads, assembled into over 1.5 million non-redundant unigenes. Functional annotation across COG, KEGG, and CAZy databases (Figure 2) revealed that carbohydrate transport/ metabolism, amino acid metabolism, and energy production were dominant categories. The jejunum was enriched in nutrient metabolism and transporter genes, whereas the cecum showed higher representation of fermentation pathways and fiber-degrading enzymes such as glycoside hydrolases (GH43, GH53) and polysaccharide lyases (PL8). Alpha diversity indices were highest in the cecum, followed by the colon, and lowest in the jejunum. Beta diversity analyses confirmed significant community structure differences among segments (PERMANOVA, $p<0.001$), with distal and proximal GIT communities forming distinct clusters.

PCA analyses of taxonomic and functional profiles (Figures 3-9) consistently separated jejunum from distal segments along PC1, reflecting contrasts between nutrient absorption and fermentation-associated functions. Distal segments (cecum, colon) clustered together, characterized by enrichment in short-chain fatty acid production pathways, antimicrobial resistance genes (β-lactamase, tetW, tetQ, ermB), and virulence factors (fimH, lapA). Proximal segments (crop, gizzard) grouped together, dominated by *Lactobacillus* spp. and functions related to initial feed breakdown and acid tolerance. Mantel tests confirmed strong correlations between taxonomic composition and functional capacity, particularly linking Bacteroides abundance with fiber degradation potential in the cecum. These findings indicate clear spatial specialization of the Kedu chicken GIT microbiome, shaped by both physiological environment and functional demands.

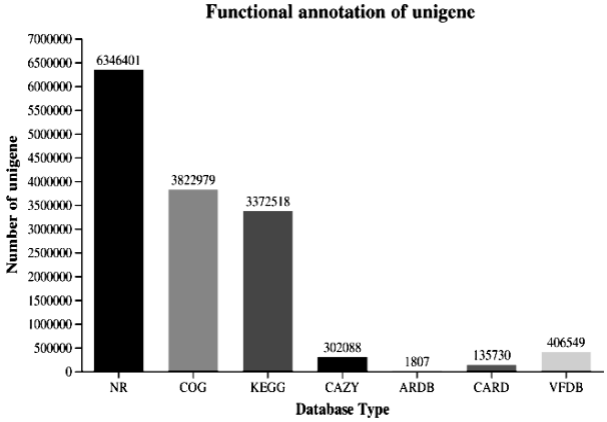

Figure 2. Functional annotation of unigenes in the GIT of Kedu chicken

Discussion

Metagenomic sequencing of 105 GIT samples from 21 Kedu chickens produced 302.4 Gbp of high-quality reads, assembled into over 1.5 million non-redundant unigenes. Functional annotation across COG, KEGG, and CAZy databases (Figure 2) revealed that carbohydrate transport/metabolism, amino acid metabolism, and energy production were dominant categories. The jejunum was enriched in nutrient metabolism and transporter genes, whereas the cecum showed higher representation of fermentation pathways and fiber-degrading enzymes such as Glycoside Hydrolases (GH43, GH53) and Polysaccharide Lyases (PL8). Alpha diversity indices were highest in the cecum, followed by the colon, and lowest in the jejunum. Beta diversity analyses confirmed significant community structure differences among segments (PERMANOVA, $p<0.001$), with distal and proximal GIT communities forming distinct clusters. This study presents the first detailed functional and taxonomic characterization of the gastrointestinal microbiota in Kedu chickens using high-throughput metagenomics combined with a multi-database, PCA-based analytical approach. By integrating annotations from NR, COG, KEGG, CAZy, ARDB, CARD, and VFDB, we obtained a multidimensional view of microbial communities across five GIT segments. Clear proximal-distal differentiation was consistently observed in PCA plots (Figures 3-9) and supported by diversity analyses, aligning with patterns reported in Kampung chickens (Murwani et al. 2025), Thai indigenous breeds (T-Thienprasert et al. 2025), and commercial broilers (Oakley et al. 2014). However, the enrichment of fiber-degrading enzymes and fermentation pathways in distal segments suggests that Kedu chickens possess a microbiome uniquely adapted to the high-fiber, scavenged diets typical of low-input rural systems, a feature that may contribute to their resilience in resource-limited environments (Chowdhury et al. 2023).

Functional annotation results (Figure 2) revealed that carbohydrate transport/metabolism, amino acid metabolism, and energy production pathways dominate the Kedu chicken microbiome. The cecum showed higher prevalence of Glycoside Hydrolases (GH43, GH53) and Polysaccharide Lyases (PL8), reflecting its role in plant fiber degradation. These enzymes, largely contributed by Bacteroides and Ruminococcus, are essential for Short-Chain Fatty Acid (SCFA) production, particularly butyrate, which supports epithelial integrity and modulates inflammation (Kogut 2019). Similar functional profiles have been documented in Ethiopian village chickens (Kassa et al. 2024), suggesting that this trait is conserved across indigenous breeds adapted to fibrous diets. The functional-taxonomic linkages observed here are consistent with metagenomic studies in other rural poultry populations (Sergeant et al. 2014).

The PCA of NR annotations (Figure 3) showed a clear separation of jejunum samples from other segments along PC1, driven by enrichment in nutrient absorption-related functions, while COG-based PCA (Figure 4) highlighted amino acid transport/metabolism and cell wall/membrane biogenesis as major contributors to clustering. KEGG pathway PCA (Figure 5) further supported this separation, with the jejunum enriched in glycolysis, amino acid metabolism, and transporter pathways, while the cecum and colon were enriched in fermentation and methanogenesis pathways. These findings align with work in Thai native chickens showing that jejunal microbiota are specialized for nutrient absorption, whereas cecal microbiota are optimized for energy salvage from fibrous material (T-Thienprasert et al. 2025).

The CAZy-based PCA (Figure 6) highlighted the specialization of distal segments for fiber degradation, with cecum and colon clearly separated from proximal regions along PC1. This mirrors the enzyme abundance trends seen in Figure 2 and is supported by studies linking CAZy enzyme profiles to dietary fiber utilization in poultry (Sergeant et al. 2014; Stanley et al. 2016). ARG-related PCAs from ARDB (Figure 7) and CARD (Figure 8) both clustered distal segments due to higher prevalence of resistance genes, including β-lactamase, tetW, tetQ, and ermB. The VFDB-based PCA (Figure 9) revealed similar clustering patterns, with distal segments enriched in adhesion genes (fimH, lapA) and toxin-associated factors, consistent with patterns seen in free-range chickens exposed to environmental microbial sources (Zhang et al. 2022).

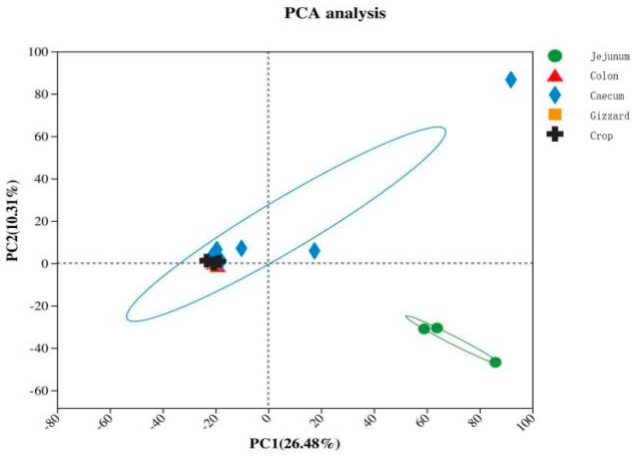

Figure 3. PCA analysis for GIT of Kedu chicken based on Non-Redundant Protein database

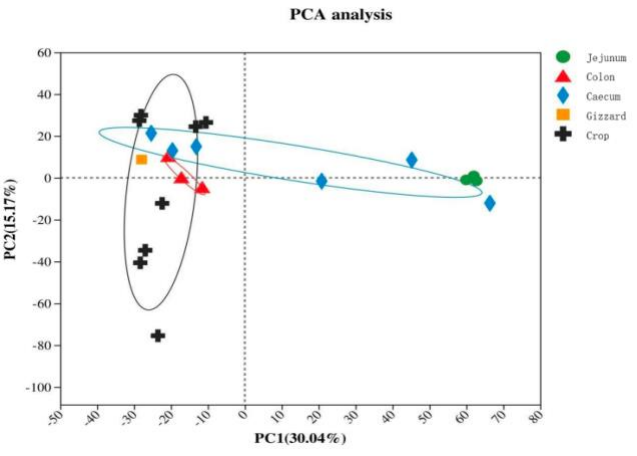

Figure 4. PCA analysis for GIT of Kedu chickens based on clusters of Orthologous groups

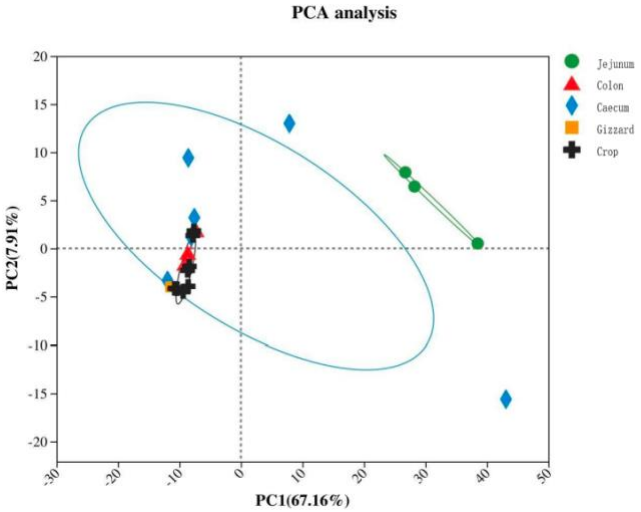

Figure 5. PCA analysis for GIT of Kedu chicken based on Kyoto Encyclopedia of genes and genomes

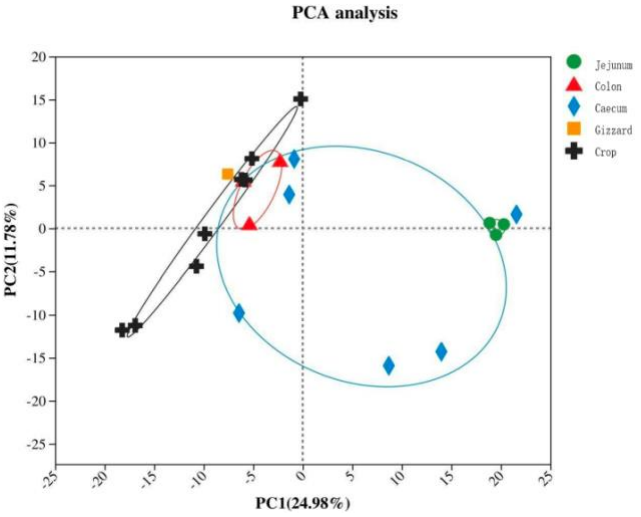

Figure 6. PCA analysis for GIT of Kedu chicken based on Carbohydrate-Active enZYmes database

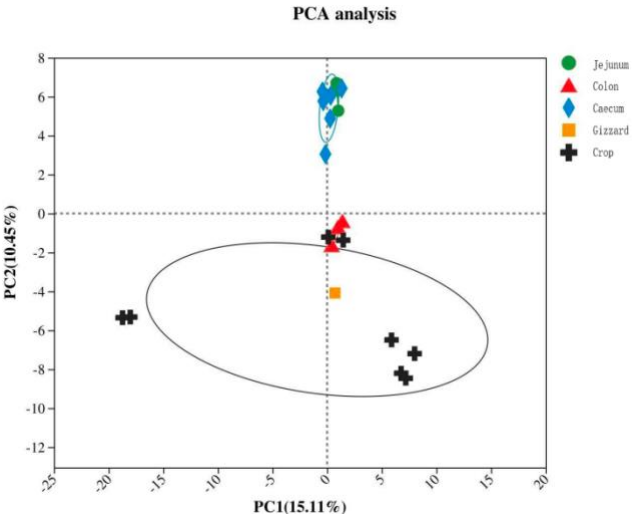

Figure 7. PCA analysis for GIT of Kedu chicken based on Antibiotic Resistance Genes database

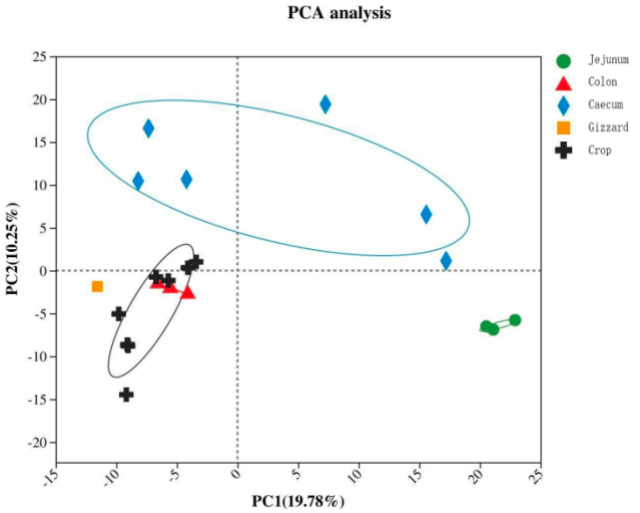

Figure 8. PCA analysis for GIT of Kedu chicken based on Comprehensive Antibiotic Resistance database

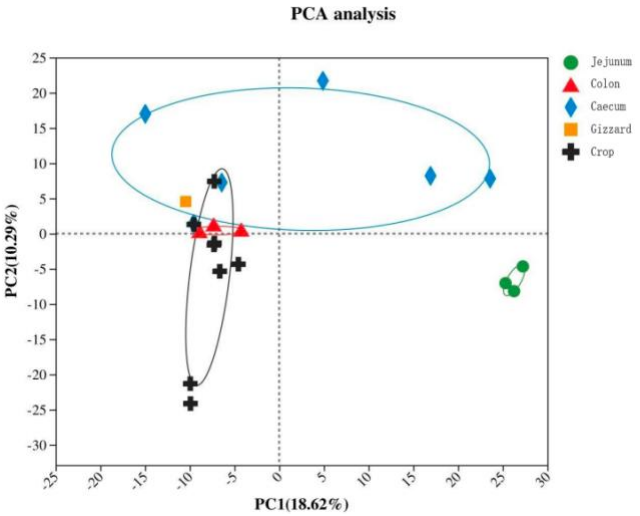

Figure 9. PCA analysis for GIT of Kedu chicken based on Virulence Factor database

Proximal segments such as the crop and gizzard were dominated by *Lactobacillus* spp., associated with acid tolerance, lactic acid production, and bacteriocin synthesis traits advantageous for pathogen suppression and feed efficiency (Bai et al. 2021). The jejunum's profile, as shown in Figures 3-5, was optimized for nutrient absorption, with functional traits converging with those observed in other indigenous breeds (T-Thienprasert et al. 2025). This proximal-distal functional partitioning reflects fundamental physiological differences in digestion and absorption along the avian GIT (Pan and Yu 2014).

The detection of antimicrobial resistance genes and virulence factors in distal GIT segments, particularly evident in Figures 7-9, raises important biosecurity concerns. ARGs in traditionally reared poultry have been reported in multiple countries (Nhung et al. 2017; Zhang et al. 2022), often linked to environmental exposure via contaminated water, soil, or other livestock. This highlights the need for

biosecurity interventions and prudent antimicrobial stewardship even in systems where antibiotic usage is minimal.

From an applied perspective, the traits identified here provide several opportunities for microbiome-informed management. The fiber-degrading potential of the cecal microbiome (Figures 2 and 6) could be enhanced with targeted prebiotics to promote Bacteroides and Ruminococcus. Beneficial *Lactobacillus* from proximal segments (Figures 3-5) could be developed into breed-specific probiotics, as strain-level specificity is important for efficacy (Gadde et al. 2017). Additionally, microbiome insights could inform selective breeding programs that pair favorable host genotypes with beneficial microbial communities, improving productivity while maintaining adaptability (Stanley et al. 2016).

While PCA was effective in revealing major variation patterns across databases (Figures 3-9), limitations exist. In CAZy PCA (Figure 6), PC2 explained only 11.78% of variance, which may result from both biological variability and the linear constraints of PCA. Nonlinear ordination methods such as NMDS, PCoA, or t-SNE could reveal additional ecological gradients (Nguyen et al. 2023). Future studies incorporating these methods, along with longitudinal sampling, will be valuable for assessing microbiome stability over time and under different feeding regimes. By integrating taxonomic profiles, functional annotations, and multivariate analyses, this study establishes a baseline microbiome profile for Kedu chickens and identifies functionally significant traits with relevance to feeding, health management, and conservation. Leveraging these traits could enhance sustainable production and safeguard the genetic and cultural value of this indigenous Indonesian breed (Chowdhury et al. 2023; Kassa et al. 2024). The presence of multiple antimicrobial resistance mechanisms in a traditional breed, such as Kedu chicken, reared under semi-intensive systems with limited antimicrobial regulation, highlights the potential role of native poultry in maintaining and disseminating Antimicrobial Resistance (AMR).

Complementing these findings, PCA based on the VFDB (Figure 9) revealed notable heterogeneity in virulence gene profiles across different GIT regions. Notably, samples from the ileum and cecum showed enriched expression of genes related to adhesion (e.g., fimbriae), invasion, and toxin production, suggesting a higher potential for pathogenicity within these segments. This could be due to favorable environmental conditions within the ileum and cecum that support the colonization and persistence of potentially harmful bacteria. Although the majority of the gut microbiota are likely commensals, the presence of virulence factors necessitates biosecurity vigilance, especially in indigenous poultry systems with limited pathogen control (Fathima et al. 2022). These findings reflect the dual nature of the gut microbiome as both a contributor to the host and potential reservoir for pathogens, similar to observations reported in free-range poultry flocks.

The functional and microbial diversity observed across the GIT segments of Kedu chickens highlights the presence of specialized microbial communities that contribute to distinct physiological functions within each region. In the context of host-microbe interactions, chickens raised in natural, organic environments develop a microbiome that enhances immune responses to pathogens (viruses and bacteria) due to its diverse and functional profile, compared to chickens raised in confined or controlled environments (Kers et al. 2018; Rothrock and Locatelli 2019). For instance, organic feeding has been linked to higher *Enterococcus* populations, which may contribute to competitive exclusion of pathogens (Zhang et al. 2025). Similar patterns are observed in other indigenous breeds: Kampung chickens, another Indonesian native breed, exhibit higher microbial diversity in the cecum compared to the small intestine, reflecting their adaptation to fibrous diets and free-ranging conditions (Murwani et al. 2025). Likewise, indigenous chickens in tropical environments, such as those studied by Kpomasse et al. (2023), demonstrate resilient gut microbiomes that support disease resistance and nutrient utilization under low-input farming systems.

In conclusion, this study delivers the first comprehensive PCA-based metagenomic profile of the Kedu chicken gut microbiome, revealing clear proximal-distal specialization in both taxonomy and function. Proximal segments were dominated by *Lactobacillus* spp. and nutrient absorption pathways, while distal segments, especially the cecum, were enriched in fiber-degrading enzymes, fermentation pathways, antimicrobial resistance genes, and virulence factors. These findings point to practical opportunities for breed-specific probiotic development, targeted prebiotic feeding to enhance fiber utilization, and microbiome-informed breeding strategies. At the same time, the detection of antimicrobial resistance genes emphasizes the need for biosecurity and prudent antibiotic stewardship, even in low-input systems. Together, these insights provide a scientific foundation for improving the sustainability, productivity, and conservation of Kedu chickens.

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REFERENCES

Adedokun SA, Olojede OC. 2019. Optimizing gastrointestinal integrity in poultry: The role of nutrients and feed additives. Front Vet Sci 5: 348. DOI: 10.3389/fvets.2018.00348.

Bai Y, Wang R, Yang Y, Li R, Wu X. 2021. Folic acid absorption characteristics and effect on cecal microbiota of laying hens. Front Vet Sci 8: 720851. DOI: 10.3389/fvets.2021.720851.

Chen L, Bai X, Wang T, Liu J, Miao X, Zeng B, Li D. 2023. Gut microbial diversity analysis of different native chickens and screening of chicken-derived cecal. Animals 13: 3672. DOI: 10.3390/ani13233672.

Choi KY, Lee TK, Sul WJ. 2015. Metagenomic analysis of chicken gut microbiota for improving metabolism and health of chickens-A review. Asian-Australas J Anim Sci 28 (9): 1217-1225. DOI: 10.5713/ajas.15.0026.

Chowdhury MAH, Ashrafudoulla M, Mevo SIU, Mizan MFR, Park SH, Ha SD. 2023. Current and future interventions for improving poultry health and poultry food safety and security: a comprehensive review. Compr Rev Food Sci Food Saf 22 (3): 1555-1596. DOI: 10.1111/1541-4337.13121.

Fathima S, Shanmugasundaram R, Adams D, Selvaraj RK. 2022. Gastrointestinal microbiota and their manipulation for improved growth and performance in chickens. Foods 11 (10): 1401. DOI: 10.3390/foods11101401.

Gadde U, Oh ST, Lee YS, Davis E, Zimmerman N, Rehberger T, Lillehoj HS. 2017. The effects of direct-fed microbial supplementation, as an alternative to antibiotics, on growth performance, intestinal immune status, and epithelial barrier gene expression in broiler chickens. Probiotics Antimicrob Proteins 9 (4): 397-405. DOI: 10.1007/s12602-017-9275-9.

Greenacre M, Groenen PJF, Hastie T, D'Enza AI, Markos A, Tuzhilina E. 2022. Principal component analysis. Nat Rev Methods Primers 2: 100. DOI: 10.1038/s43586-022-00184-w.

Kassa G, Alemayehu D, Andualem B. 2024. Isolation, identification, and molecular characterization of probiotic bacteria from locally selected Ethiopian free range chickens gastrointestinal tract. Poult Sci 103 (2): 103311. DOI: 10.1016/j.psj.2023.103311.

Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Stegeman JA, Smidt H. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. Front Microbiol 9: 235. DOI: 10.3389/fmicb.2018.00235.

Kogut MH. 2019. The effect of microbiome modulation on the intestinal health of poultry. Anim Feed Sci Technol 250: 32-40. DOI: 10.1016/j.anifeedsci.2018.10.008.

Kpomasse CC, Kouame YAE, N'nanle O, Houndonougbo FM, Tona K, Oke OE. 2023. The productivity and resilience of the indigenous chickens in the tropical environments: Improvement and future perspectives. J Appl Anim Res 51 (1): 456-469. DOI: 10.1080/09712119.2023.2228374.

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 (2): 909-919. DOI: 10.13057/biodiv/d260240.

Nguyen TA, He J, Le LT, Bao W, Tran NH. 2023. Federated PCA on Grassmann manifold for anomaly detection in IoT networks. IEEE INFOCOM 2023 - IEEE Conf Comput Commun: 1-10. DOI: 10.1109/INFOCOM53939.2023.10229026.

Nhung NT, Chansiripornchai N, Carrique-Mas JJ. 2017. Antimicrobial resistance in bacterial poultry pathogens: a review. Front Vet Sci 4: 126. DOI: 10.3389/fvets.2017.00126.

Oakley BB, Lillehoj HS, Kogut MH, Kim WK, Maurer JJ, Pedroso A, Lee MD, Collett SR, Johnson TJ, Cox NA. 2014. The chicken gastrointestinal microbiome. FEMS Microbiol Lett 360 (2): 100-112. DOI: 10.1111/1574-6968.12608.

Pan D, Yu Z. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes 5: 108-119. DOI: 10.4161/gmic.26945.

Rothrock Jr MJ, Locatelli A. 2019. Importance of farm environment to shape poultry-related microbiomes throughout the farm-to-fork continuum of pasture-raised broiler flocks. Front Sustain Food Syst 3: 48. DOI: 10.3389/fsufs.2019.00048.

Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW, Pallen MJ. 2014. Extensive microbial and functional diversity within the chicken cecal microbiome. PLoS One 9 (3): e91941. DOI: 10.1371/journal.pone.0091941.

Stanley D, Moore RJ, Wong CH. 2018. An insight into intestinal mucosal microbiota disruption after stroke. Sci Rep 8 (1): 568. DOI: 10.1038/s41598-017-18904-8.

Sutopo S, Lestari DA, Kurnianto E, Setiaji A. 2022. Egg weight, sex and variety effects on body weights and growth ability of Kedu chickens. Adv Anim Vet Sci 10 (5): 1017-1022. DOI: 10.17582/journal.aavs/2022/10.5.1017.1022.

T-Thienprasert NP, Jaithon T, Klomkliew P, Chanchaem P, Suwanasopee T, Koonawootrittriron S, Kovitvadhi A, Chundang P, Pongprayoon P, Kityakarn S, Luksirikul P, Payungporn S. 2025. Comparative analysis of the gut microbiota of Thai indigenous chicken fed house crickets. Animals 15 (7): 1070. DOI: 10.3390/ani15071070.

Wickramasuriya SS, Park I, Lee K, Lee Y, Kim WH, Nam H, Lillehoj HS. 2022. Role of physiology, immunity, microbiota, and infectious diseases in the gut health of poultry. Vaccines 10 (2): 172. DOI: 10.3390/vaccines10020172.

Yang Y, Jobin C. 2014. Microbial imbalance and intestinal pathologies: Connections and contributions. Dis Model Mech 7 (10): 1131-1142. DOI: 10.1242/dmm.016428.

Zhang P, West NP, Chen P-Y, Thang MWC, Price G, Cripps AW, Cox AJ. 2019. Selection of microbial biomarkers using genetic algorithms and principal component analysis. BMC Bioinformatics 20: 413. DOI: 10.1186/s12859-019-3001-4.

Zhang L, Said LB, Diarra MS, Fliss I. 2022. Effects of bacterial-derived antimicrobial solutions on shelf-life, microbiota and sensory attributes of raw chicken legs under refrigerated storage condition. Int J Food Microbiol 383: 109958. DOI: 10.1016/j.ijfoodmicro.2022.109958.

Zhang W, Jian X, Ding S, Chang J, Ji S, Chi Y. 2025. Insights into the gut microbiota characteristics between the organic and traditional feeding chickens based on amplicon and metagenomic sequencing. Front Microbiol 15: 1509461. DOI: 10.3389/fmicb.2024.1509461.