RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Special Issue: 35-41 (2026)

Correlation between Gut Microbiota Diversity and Body Weight in Muchuan Black-boned Chickens

Wencong Long[1], Yuli Liu[2], Zi Liang[2], Juan Liao[2]

10.18805/IJAR.BF-2137

ABSTRACT

Background: The muchuan black-boned chicken is a nationally protected breed valued for meat and medicine use in China. Slow growth of muchuan black-bone chickens remains a major constraint to its large-scale farming and industrialization. The gut microbiota has been widely recognized as a key factor influencing growth performance. To date, systematic studies linking gut microbiota to body weight in this breed are lacking. Accordingly, this study aimed to reveal the correlation between gut microbiota and body weight and screen weight-related functional bacteria and provides a theoretical basis for improving growth performance *via* intestinal microecological regulation in local chicken breeds.

Methods: A total of 130 180-day-old muchuan black-boned chickens were used. Jejunal digesta samples were collected from high-weight (W) and low-weight (B) groups respectively. The V4 region of the 16S rRNA gene was sequenced by Illumina MiSeq high-throughput sequencing technology, followed by OTU clustering, alpha and beta diversity analysis, principal coordinate analysis (PCoA) and MetaStat analysis to compare the gut microbial diversity and community structure between chickens with different body weights.

Result: A total of 2102 OTUs were obtained, with a similarity of 97%. At the phylum level, the dominant microbiota of muchuan black-boned chickens were *Firmicutes*, *Bacteroidetes* and *Proteobacteria* and the *Firmicutes/Bacteroidetes* (F/B) ratio was higher in group W. Alpha diversity showed that Simpson, Chao1 and ACE indices were higher in group B. PCoA showed clear separation of microbial communities between Group W and Group B. MetaStat analysis revealed that *Caproiciproducens* (*Ruminococcaceae*) was significantly enriched in group W.

Key words: Body weight, Gut diversity, Illumina MiSeq sequencing, Jejunal microbiota, Muchuan black-boned chicken.

INTRODUCTION

The muchuan black-boned chicken is a native protected breed from Muchuan County, Leshan City, Sichuan Province, belonging to the chuanan mountain black-boned chicken lineage. It is a dual-purpose genetic resource with high edible and medicinal importance in China (Yu *et al*., 2018). The breed displays complete black pigmentation in feathers, skin, muscle, bones and viscera, with tender meat rich in iron, zinc, selenium and essential amino acids. It forms the core of the local characteristic livestock industry in Muchuan County (Liao *et al*., 2024). However, due to its genetic characteristics, the muchuan black-boned chicken has a significantly lower adult weight compared to large commercial breeds, which severely restricts its large-scale breeding and industrial development (Yu *et al*., 2019).

Gut microbiota play a key role in nutrient absorption, energy metabolism, immune regulation, intestinal barrier construction and other important physiological processes (Grice and Segre, 2012). The stability and imbalance of community structure directly affect animal growth performance, health status and production efficiency (Sommer and Bäckhed, 2013). Colonizing the gut of sterile chickens with relevant microorganisms aids in the gradual development and maturation of the chickens' immune system, which plays a crucial role in maintaining the chickens' overall health (Taha-Abdelaziz *et al*., 2018). In addition, gut microbes can secrete a large number of digestive enzymes, which can improve the body's digestibility of nutrients, thereby improving production performance (Schokker *et al*., 2015). In recent years, the correlation between gut microbiota and growth traits of poultry has become a research hotspot in the field of animal microecology. Many studies have confirmed that the enrichment of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* can significantly promote the digestion and absorption of nutrients, improve the daily weight gain and body weight of animals, while the excessive

[1]Bamboo Resource Conservation and Utilization Key Laboratory of Sichuan Province, Leshan Normal University, Leshan, Sichuan 614000, China.

[2]Engineering Research Center of Sichuan Province Higher School of Local Chicken Breeds Industrialization in Southern Sichuan, Leshan Normal University, Leshan 614000, China.

Corresponding Author: Juan Liao, Engineering Research Center of Sichuan Province Higher School of Local Chicken Breeds Industrialization in Southern Sichuan, Leshan Normal University, Leshan 614000, China. Email: lsliaojuan@163.com

How to cite this article: Long, W., Liu, Y., Liang, Z. and Liao, J. (2026). Correlation between Gut Microbiota Diversity and Body Weight in Muchuan Black-boned Chickens. *Indian Journal of Animal Research.* **60(Special issue):** 35-41. doi: 10.18805/IJAR.BF-2137.

Submitted: 01-05-2026 **Accepted:** 14-06-2026 **Online:** 27-06-2026

proliferation of harmful bacteria will lead to intestinal dysfunction and inhibit growth and development (Zhang *et al*., 2025; Zhang *et al*., 2023; Fathima *et al*., 2022).

Current research on Muchuan Black-Boned Chicken mainly focuses on the mechanism of melanin synthesis. Systematic research on its intestinal microbial community structure is relatively scarce and only a few studies focus on its gut microbial diversity, analyzing the differences of intestinal microbes of individuals with different skin colors, but almost does not involve the association analysis of intestinal microbes and growth traits (Yu *et al*., 2018; Yu *et al*., 2019; Liao *et al*., 2024). Therefore, the aim of the study was to estimate the correlation between gut microbiota and body weight of Muchan black bone chickens.

MATERIALS AND METHODS

Experimental animals

130 180-day-old healthy black-feathered Muchuan Black-Bone hens were purchased from Sichuan MuChuan Black Phoenix Black-Bone Chicken Industry Co., Ltd. and all chickens were raised under identical feeding and management conditions throughout the breeding process. The experiment was conducted from April 2025 to December 2025.The work was finished at Leshan Normal University.

Sample collection

Each of the 130 Muchuan Black-Bone hens was weighed individually (precision: 0.1 g) and numbered in descending order of body weight. The 3 heaviest hens were selected as the high-body-weight group (W group) and the 3 lightest hens as the low-body-weight group (B group). Under sterile conditions, the jejunal digests of each hen was collected; the samples were sequentially numbered W2, W3, W5 and B1, B3, B5, rapidly frozen in liquid nitrogen and stored in a -80°C freezer upon return to the laboratory.

Total DNA extraction

An appropriate sample was taken, added with lysis buffer containing 2% SDS and incubated at 55~65°C for 30 min Proteinase K (50 μg/mL) was added and digestion was performed at 37°C for 1 h. Two extractions were performed with phenol-chloroform-isopropyl alcohol (25:24:1), the supernatants were collected and 1/10 volume of 3 mol/L NaAc (pH 5.2) and twice the volume of anhydrous ethanol were added. Precipitation was carried out at -20°C for 1 h, followed by centrifugation at 4°C and 12,000 rpm for 10 min to collect the precipitate. The precipitate was washed twice with 70% ethanol, dried and dissolved in TE buffer. The purity and concentration of DNA were determined using 1% agarose gel electrophoresis. An appropriate amount of sample DNA was transferred into a centrifuge tube and diluted with sterile water to 1 ng/μL (Velásquez-Mejía *et al*., 2018).

Library construction and high-throughput sequencing

Using diluted sample genomic DNA as the template, PCR amplification was performed with the 16S rRNA gene V4 region-specific primers 515F (5′-GTGCCAGCMGC CGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), synthesized by Beijing Novogene Biotech Co., Ltd. The PCR products were detected by 2% agarose gel electrophoresis and the target bands were excised and recovered using a gel recovery kit. Library construction was performed using the Ion plus fragment library Kit 48 reactions (Thermo fisher scientific). After quantitative analysis with Qubit and library validation, the constructed libraries were sequenced at Beijing Novogene Biotech Co., Ltd (Liao *et al*., 2024).

Sequencing data processing

The raw data were processed using Cutadapt software (V1.9.1, http://cutadapt.readthedocs.io/en/stable/) to remove low-quality sequences, split samples based on barcodes and eliminate primer sequences (Martin, 2011). Subsequently, alignment with the species annotation database was performed to eliminate chimeras, yielding clean reads (Edgar, 2011). All sample sequences were clustered into OTUs and classified into species using Uparse software (Uparse v7.0.1001,http://drive5.com/uparse/) with 97% similarity, followed by species annotation of representative OTU sequences (Edgar, 2013; Quast *et al*., 2013). Based on the OTU clustering results, alpha diversity and beta diversity indices were calculated, principal coordinate analysis (PCoA) was conducted and MetaStat differential species screening analysis was performed.

Metastats analysis was performed using R software (Version 2.15.3) to conduct permutation tests among groups at each taxonomic level (Phylum, class, order, family, genus, species), generating p-values. These p-values were then adjusted using the benjamini and hochberg false discovery rate method to obtain q-values (White *et al*., 2009). For species showing significant inter-group differences, t-tests were conducted between groups using R software, followed by visualization of the results.

RESULTS AND DISCUSSION

Body weight performance

Statistical analysis revealed that the average body weight of the 130 Muchuan Black-Boned Chickens was 1,757 g, with a coefficient of variation (CV) of 8.76%, indicating a normal distribution. The average body weight was 1,998.5±10.2 g for the high-weight group and 1,337.5±11.5 g for the low-weight group. The independent-sample *t*-test showed a statistically significant difference in body weight between group W and group B ($P<0.01$).

Sequencing data quality control

This study performed high-throughput 16S rRNA gene sequencing on intestinal microbial samples from the muchuan black-boned chicken in groups W and B, yielding a total of 1,623,456 raw reads. As shown in Table 1,

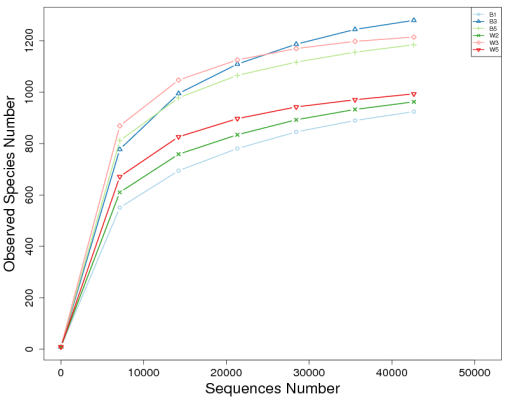
the number of raw reads before quality control ranged from 57,013 to 90,346, while the number of clean reads after quality control ranged from 54,601 to 86,260, with an average sequencing coverage efficiency of 94.57±1.9%. The average sequence length was 252.7 nt, the Q20 value ranged from 74.66% to 88.21% and the GC content remained stable between 52.68% and 53.42%. These results indicated that the sequencing quality was high, the data were valid and reliable and all samples met the requirements for subsequent bioinformatics analysis.

Dilution curve analysis

A certain amount of sequencing data is randomly selected from the samples and the number of OTUs is counted. The dilution curve is constructed by the amount of sequencing data and the number of corresponding species, so as to describe the curve of sample diversity within the group. OTU clustering results showed that a total of 2102 OTUs were obtained with 97% sequence similarity as the threshold and the coverage index of all samples was above 99%. According to Fig 1, as the number of sequences increases, the dilution curve of each sample gradually tends to be gentle and the number of newly detected OTUs decreases. This indicates that the sequencing data volume in this study was adequate and the sequencing depth was sufficient to reflect the species richness within the samples, which ensured the accuracy and reliability of the analysis results (Caporaso *et al*., 2010).

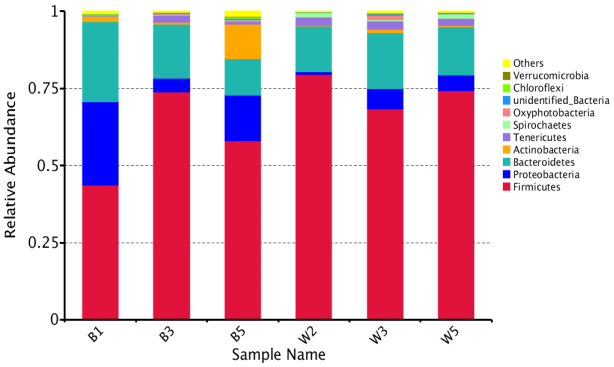
Gut microbiota community composition

The community composition information of each sample at different taxonomic levels was counted to obtain the annotation results of species at the five taxonomic levels of phyla, class, order, family and genus. At the phylum level, the top five in abundance were *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, Actinobacteria and Tenericutes (Fig 2). The abundance of *Firmicutes* was highest, accounting for more than 40% of all samples. This microbial community composition was consistent with the gut microbial structure of Jiangxi local chicken, Tibetan chicken and other local chicken breeds (Li *et al*., 2024), indicating that *Firmicutes* and *Bacteroidetes* are the core groups of poultry intestinal microbes. The average value of *Firmicutes*/*Bacteroidetes* ratio (F/B) in group W was 4.66, which was higher than 3.62 of the group B. The F/B ratio is an important indicator reflecting the relationship between gut microbiota and host energy metabolism. A higher F/B ratio is helpful to improve energy utilization efficiency and promote weight gain (Turnbaugh *et al*., 2006; Sommer and Bäckhed, 2013). The *Firmicutes* phylum can secrete digestive enzymes such as cellulase and amylase to promote the degradation and



Note: The x-axis represents the number of sequencing reads randomly drawn from a given sample, while the y-axis represents the number of OTUs that can be constructed based on this sample size.

Fig 1: Sample dilution curve.



Note: The x-axis shows a single sample; the y-axis shows the relative abundance of each phylum. "Others" represents the total relative abundance of phyla not shown separately.

Fig 2: Relative abundance of jejunal microbiota at the phylum level in high-body-weight (W) and low-body-weight (B) groups.

Table 1: Data preprocessing and quality control statistics.

Sample name	Raw reads	Clean reads	Base (nt)	AvgLen (nt)	Q20	GC%	Effective%
B1	83565	80113	20295404	253	75.12	53.42	95.87
B3	90346	86260	21780677	252	88.21	52.92	95.48
B5	57013	54601	13841429	253	76.17	53.35	95.77
W2	85311	80241	20334064	253	74.66	52.68	94.06
W3	69353	67409	17019308	252	86.22	52.73	97.2
W5	78651	71607	18152858	253	78.91	53.00	91.04

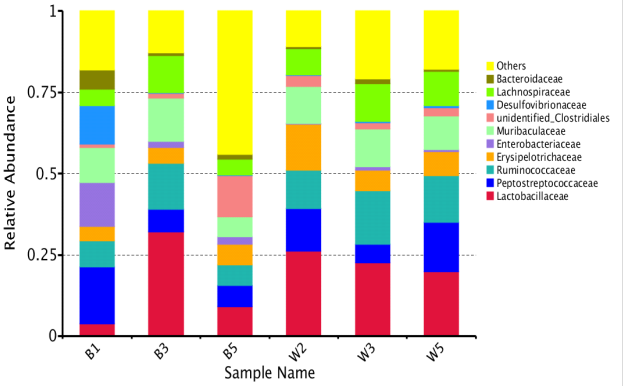
absorption of carbohydrates in feed, thereby improving energy utilization efficiency; while the Bacteroidetes phylum mainly participates in the metabolism of proteins and polysaccharides. An excessively high relative abundance of this phylum may reduce the efficiency of energy conversion, thereby inhibiting weight gain (Sommer and Bäckhed, 2013; Bäckhed *et al*., 2004; Yadav and Jha, 2019).

At the family level, the microbial community structure in group W was relatively stable (Fig 3). The *Lactobacillaceae* and *Ruminococcaceae* families maintained high abundance in each sample in group W and were one of the core dominant families in group W. In contrast, the microbial community structure in group B samples varied greatly among individuals, with the dominant bacterial families varying significantly among individuals. The results showed that the gut microbial community structure of high weight Muchuan Black-Bone chicken was more stable. As a typical beneficial flora in the gut, *Lactobacillaceae* can produce lactic acid, reduce the pH value of the gut, inhibit the proliferation of harmful bacteria such as *Escherichia coli*, promote the repair of intestinal mucosal barrier, improve the efficiency of nutrient absorption and then promote the growth of the host (Pessione, 2012; Zhang *et al*., 2025). Previous research on Muchuan Black-Boned chickens indicated that gut microbiota composition is also associated with skin color (Liao *et al*., 2024). Furthermore, probiotics belonging to *Lactobacillus* strains have been shown to enhance growth performance by improving gut health and nutrient digestibility (Shawky *et al*., 2025; Jinturkar *et al*., 2009). In this study, *Lactobacillaceae* were enriched in the high-weight group, suggesting they may serve as potential probiotics for local chicken breeds.*Ruminococcaceae* can participate in the degradation of dietary fiber and the synthesis of short chain fatty acids (SCFAs). As an important metabolite of intestinal microorganisms, SCFAs can not only provide energy for the host, but also regulate intestinal immune function, improve intestinal health and indirectly affect the growth performance of the host (Louis and Flint, 2009; Canfora *et al*., 2015).

Alpha and beta diversity analysis

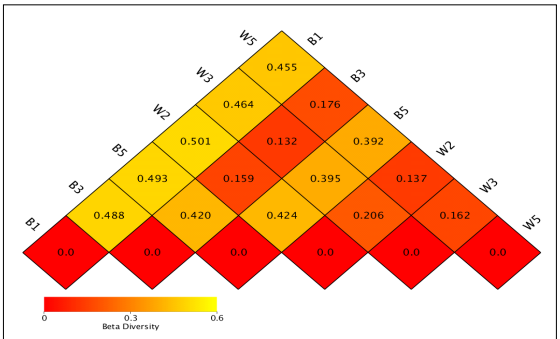
Alpha diversity analysis showed that the Goods coverage index of all samples exceeded 0.996, indicating sufficient sequencing depth (Table 2). The observed species, Shannon index, Simpson index and Goods_coverage did not differ significantly between the B and W groups (P>0.05). However, the Chao1, ACE and PD_whole_tree indices, were significantly higher in the B group than in the W group (P<0.05). These findings demonstrate that samples in group B possess a greater diversity of gut microbial species, higher community richness and more extensive phylogenetic diversity.

In beta diversity studies, the Weighted Unifrac distances among samples ranged from 0.137 to 0.206 in group W (Fig 4),



Note: The x-axis shows individual samples; the y-axis shows the relative abundance of each family. "Others" indicates the total relative abundance of families not individually displayed.

Fig 3: Relative abundance of jejunal microbiota at the family level in high-body-weight (W) and low-body-weight (B) groups.



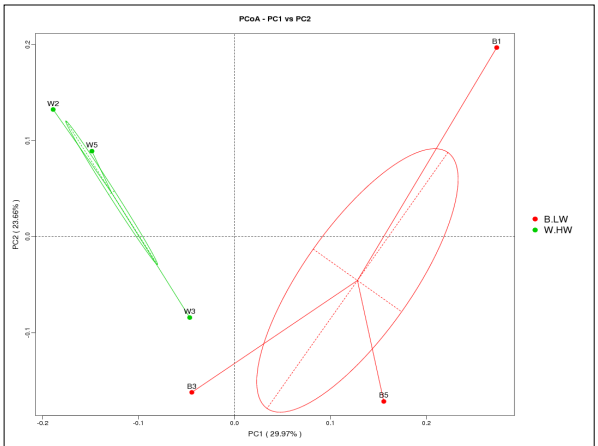
Note: The values in each cell represent the community dissimilarity coefficients between pairs of samples. Lower values (red) indicate higher similarity in microbial community structure; higher values (yellow) indicate greater dissimilarity. A value of 0 indicates that the microbial communities of the two samples are identical (no difference, shown in red).

Fig 4: Heatmap of beta diversity based on weighted unifrac distance.

Table 2: Alpha diversity indices of jejunal microbiota in muchuan black-boned chickens.

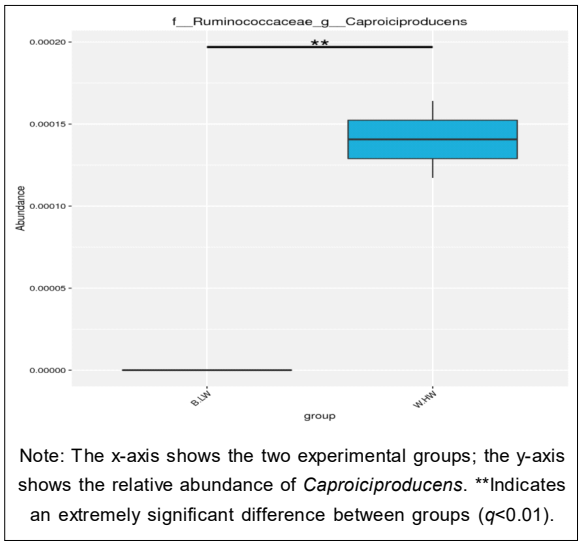
Group	Observed species	Shannon	Simpson	chao1	ACE	Goods coverage	PD whole tree
B	1129.00±186.00[a]	7.38±0.38[a]	0.98±0.01[a]	1246.00±149.00[a]	1245.00±153.00[a]	0.996±0.001[a]	89.02±10.74[a]
W	1056.33±137.42[a]	7.41±0.38[a]	0.98±0.00[a]	1111.78±112.89[b]	1118.50±110.75[b]	0.997±0.001[a]	76.30±13.42[b]

Note: Data are expressed as mean±standard deviation (n = 3); Note: The different lowercase letters in the column headings indicate significant differences between groups (P<0.05), while the same letters indicate no significant differences between groups (P>0.05).



Note: Each point represents one sample. Red= Low-body-weight group (B); Green= High-body-weight group (W). The PC1 and PC2 axes represent the first and second principal components, respectively, with the numerical value in parentheses indicating the proportion of sample variation explained by each principal component. The clustering intensity within the same group reflects intra-group similarity, while the separation degree between different groups reflects inter-group differences.

Fig 5: Principal coordinate analysis (PCoA) of Jejunal gut microbiota communities.



Note: The x-axis shows the two experimental groups; the y-axis shows the relative abundance of *Caproiciproducens*. **Indicates an extremely significant difference between groups (q<0.01).

Fig 6: Relative abundance of *Caproiciproducens* in high-body-weight (W) and low-body-weight (B) groups.

suggesting similar microbial community structures and stable community composition within the group. In contrast, the distances for group B ranged from 0.420 to 0.493, indicating significant variations in microbial community composition and structure among samples, as well as poor intra-group community stability.

Unweighted UniFrac distance was used for PCoA analysis. The results showed that the samples of the W group and B group were significantly separated (Fig 5). The sample points in group W are mainly distributed on the left side of the graph (PC1 negative value area) and the clustering degree of sample points in the group is high, indicating that the similarity of bacterial community structure in group W is high. The sample points in group B are mainly distributed on the right side of the map (PC1 positive area). The sample points in the group are relatively scattered, but there is no obvious overlap with the samples in group W as a whole, indicating obvious differences in gut microbial community structure between the two groups. These results suggested that the differences in body weight were closely related to the changes in gut microbial community structure, which was consistent with the conclusion that gut microbiota could regulate host growth and development (Zhang *et al*., 2022; Yadav and Jha, 2019).

Differential gut microbiota analysis

MetaStat differential species analysis showed that *Caproiciproducens* belonging to the family Ruminococcaceae was extremely significantly enriched in the W group (q<0.01) and was almost absent in the B group (Fig 6). *Caproiciproducens* is mainly involved in the synthesis of short-chain fatty acids such as hexanoic acid, which can provide energy for intestinal epithelial cells, improve intestinal barrier function and enhance energy metabolism efficiency, thereby promoting host weight gain (Esquivel-Elizondo *et al*., 2021). This indicated that *Caproiciproducens* may be a key functional bacterium related to the weight gain of muchuan black-boned chickens, which could be used as a candidate strain for the development of microecological preparations. The results of this study are consistent with the conclusions of many studies on the composition and structure of intestinal microorganisms in livestock and poultry. The enrichment of the rumen cocci family and the related bacterial genera for SCFA synthesis has been confirmed to be positively correlated with growth performance and body weight indicators. Wang *et al*. (2023) discovered in their research on local chicken breeds that the microbiota in the cecum can account for approximately 10.1% of the individual body weight variation. Among them, the SCFA-producing bacterial flora positively regulates growth performance through the fatty acid metabolism pathway. Akram *et al*. (2024) further discovered that *Ruminococcaceae* was enriched in the cecum of high-weight chicken flocks and was significantly correlated with the concentrations of butyric acid and caproic acid as well as the feed conversion efficiency. This study provides a scientific basis for analyzing the microbial mechanism underlying the growth traits of Muchuan Black-Boned Chickens and also offers a candidate target for the development of probiotics for local chicken breeds.

CONCLUSION

According to the previous results it could be concluded showed that *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the dominant phyla of gut microbiota in muchuan

black-boned chickens at the phylum level. The F/B ratio was significantly higher in group W, which suggested that the higher F/B ratio might be positively correlated with the faster growth of host weight. At the family level, the sum of relative abundance of *Lactobacillaceae*, Peptostreptococcaceae and *Ruminococcaceae* in group W accounted for more than 40%. Moreover, the consistency of the microbiota structure within the group is relatively high. Alpha diversity analysis indicated that Group B exhibited higher richness and evenness of gut microbial species. Beta diversity analysis consistently revealed significant differences in the gut microbial community structure between Group W and Group B, suggesting a close correlation between body weight differences and gut microbiota composition.

Differential species analysis revealed that *Caproiciproducens* was significantly enriched in Group W ($P<0.01$). This genus may promote the weight gain of muchuan black-boned chickens by enhancing the host's energy utilization efficiency. The weight-related microbial groups identified in this study, such as *Caproiciproducens* and other body weight-related microbial groups, provide a theoretical reference for future use of probiotics or feeding management methods to adjust the intestinal flora and improve the growth performance of muchuan black-bone chickens.

ACKNOWLEDGEMENT

This project was supported by the Leshan Science and Technology Bureau Project (No. 24YYJC0028).

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures were performed in accordance with the institutional and national guidelines for the care and use of laboratory animals. Ethical approval was granted by the Leshan Normal University.

Conflict of interest

The author declares that they have no conflicts of interest in the research presented in this manuscript.

REFERENCES

Akram, M.Z., Sureda, E.A., Comer, L., Corion, M. and Everaert, N. (2024). Assessing the impact of hatching system and body weight on the growth performance, caecal short-chain fatty acids and microbiota composition and functionality in broilers. *Animal Microbiome.* **6(1):** 41. doi: 10.1186/s42523-024-00331-6.

Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F. and Gordon, J.I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America.* **101(44):** 15718-15723. doi: 10.1073/pnas.0407076101.

Canfora, E.E., Jocken, J.W. and Blaak, E.E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. Nature reviews. *Endocrinology.* **11(10):** 577-591. doi: 10.1038/nrendo.2015.128.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N. *et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods.* **7(5):** 335-336. doi: 10.1038/nmeth.f.303.

Edgar, R.C. (2013). UPARSE: Highly accurate OTU sequencesfrom microbial amplicon reads. *Nature Methods.* **10(10):** 996-998. doi: 10.1038/nmeth.2604.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. and Knight, R. (2011). UCHIME improves sensitivity and speed of chimeradetection. *Bioinformatics*. **27(16):** 2194-2200. doi: 10.1093/bioinformatics/btr381.

Esquivel-Elizondo, S., Bağcı, C., Temovska, M., Jeon, B.S., Bessarab, I., Williams, R.B.H., Huson, D.H. and Angenent, L.T. (2021). The isolate caproiciproducenssp. 7D4C2 produces n-caproate at mildly acidic conditions from hexoses: Genome and rBOX comparison with related strains and chain-elongating bacteria. *Frontiers in Microbiology.* **11:** 594524. doi: 10.3389/fmicb.2020.594524.

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

Grice, E.A. and Segre, J.A. (2012). The human microbiome: Our second genome. *Annual Review of Genomics and Human Genetics.* **13:** 151-170. doi: 10.1146/annurev-genom-090711-163814.

Jinturkar, A.S., Gujar, B.V., Chauhan, D.S. and Patil, R.A. (2009). Effect of feeding probiotics on the growth performance and feed conversion efficiency in goat. *Indian Journal of Animal Research.* **43(1):** 49-52.

Li, J., Li, Y., Xiao, H., Li, W., Ye, F., Wang, L., Li, Y., Wang, C., Wu, Y., Xuan, R., Huang, Y. and Huang, J. (2024). The intestinal microflora diversity of aboriginal chickens in jiangxi province, China. *Poultry Science.* **103(2):** 103198. doi:10.1016/j.psj.2023.103198.

Liao, J., Shen, X., Long, W., Yu, S.G., Wang, G., Du, C.H. and Ling, Z. (2024). Analysis of the difference of intestinal microbes in muchuan black-bone chickens with two skin colors. *Indian Journal of Animal Research*. **58(5):** 753-758. doi: 10.18805/IJAR.BF-1752.

Louis, P. and Flint, H.J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters.* **294(1):** 1-8. doi: 10.1111/j.1574-6968.2009.01514.x.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet Journal*. **17(1)**. https://doi.org/10.14806/ej.17.1.200

Pessione, E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: Lights and shadows. *Frontiers in Cellular and Infection Microbiology.* **2:** 86. doi: 10.3389/fcimb.2012.00086.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research.* **41(Database issue):** D590-D596. doi: 10.1093/nar/gks1219.

Schokker, D., Veninga, G., Vastenhouw, S.A., Bossers, A., de Bree, F.M., Kaal-Lansbergen, L.M., Rebel, J.M. and Smits, M.A. (2015). Early life microbial colonization of the gut and intestinal development differ between genetically divergent broiler lines. *BMC Genomics.* **16(1):** 418. doi: 10.1186/s12864-015-1646-6.

Shawky, M., Rayan, G., Alyousef, Y., Darrag, H., Najib, H. and Mohammed, A. (2025). Potential impacts of dietary *bacillus licheniformis* inclusion on growth performance, serum metabolites, antioxidant profiles and immune status of broiler chickens. *Indian Journal of Animal Research.* **59(Special Issue):** 144-150. doi: 10.18805/IJAR.BF-2004.

Sommer, F. and Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. Nature reviews. *Microbiology.* **11(4):** 227-238. doi: 10.1038/nrmicro2974.

Taha-Abdelaziz, K., Hodgins, D.C., Lammers, A., Alkie, T.N. and Sharif, S. (2018). Effects of early feeding and dietary interventions on development of lymphoid organs and immune competence in neonatal chickens: A review. *Veterinary Immunology and Immunopathology.* **201:** 1-11. doi: 10.1016/j.vetimm.2018.05.001.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R. and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* **444(7122):** 1027-1031. doi: 10.1038/nature05414.

Velásquez-Mejía, E.P., de la Cuesta-Zuluaga, J. and Escobar, J.S. (2018). Impact of DNA extraction, sample dilution and reagent contamination on 16S rRNA gene sequencing of human feces. *Applied Microbiology and Biotechnology.* **102(1):** 403-411. doi: 10.1007/s00253-017-8583-z.

Wang, L., Zhang, F., Li, H., Yang, S., Chen, X., Long, S., Yang, S., Yang, Y. and Wang, Z. (2023). Metabolic and inflammatory linkage of the chicken cecal microbiome to growth performance. *Frontiers in Microbiology.* **14:** 1060458. doi: 10.3389/fmicb.2023.1060458.

White, J.R., Nagarajan, N. and Pop, M. (2009). Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Computational Biology.* **5(4):** e1000352. doi: 10.1371/journal.pcbi.1000352.

Yadav, S. and Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance and health of poultry. *Journal of Animal Science and Biotechnology*. **10:** 2. doi: 10.1186/s40104-018-0310-9.

Yu, S., Wang, G., Liao, J. and Tang, M. (2019). Five alternative splicing variants of the TYR gene and their different roles in melanogenesis in the muchuan black-boned chicken. *British Poultry Science*. **60(1):** 8-14. doi: 10.1080/00071668.2018.1533633.

Yu, S., Wang, G., Liao, J., Tang, M. and Sun, W. (2018). Transcriptome profile analysis of mechanisms of black and white plumage determination in black-bone chicken. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry and Pharmacology.* **46(6):** 2373-2384. doi: 10.1159/000489644.

Yu, S., Wang, G., Liao, J. and Tang, M. (2018). Transcriptome profile analysis identifies candidate genes for the melanin pigmentation of breast muscle in Muchuan black-boned chicken. *Poultry Science*. **97(10):** 3446-3455. doi: 10.3382/ps/pey238.

Zhang, M., Li, D., Yang, X., Wei, F., Wen, Q., Feng, Y., Jin, X., Liu, D., Guo, Y. and Hu, Y. (2023). Integrated multi-omics reveals the roles of cecal microbiota and its derived bacterial consortium in promoting chicken growth. *mSystems.* **8(6):** e0084423. doi: 10.1128/msystems.00844-23.

Zhang, S., Yu, M., Zhao, T., Geng, Y., Liu, Z., Zhang, X. and Yu, L. (2025). Effects of probiotics on gut microbiota in poultry. *AIMS Microbiology.* **11(3):** 754-768. doi: 10.3934/microbiol.2025032.

Zhang, X., Akhtar, M., Chen, Y., Ma, Z., Liang, Y., Shi, D., Cheng, R., Cui, L., Hu, Y., Nafady, A. A., Ansari, A.R., Abdel-Kafy, E.M. and Liu, H. (2022). Chicken jejunal microbiota improves growth performance by mitigating intestinal inflammation. *Microbiome.* **10(1):** 107. doi: 10.1186/s40168-022-01299-8.