

## RESEARCH ARTICLE

### Multi-Omics Comparing Analysis of Chicken from Different Altitudes

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#### ARTICLE HISTORY (26-882)

Received: July 23, 2026  
Revised: September 23, 2026  
Accepted: September 25, 2026  
Published online: September 28, 2026

#### Key words:

Chicken  
Microbiota  
Multi-omics  
Biomarkers  
Metabolites

#### ABSTRACT

Chicken is a popular and important food poultry; however, little is known about the intestinal microbiota and metabolite differences between Xizang chicken (LH) and flat area chicken (ZJ). Hence, we performed this study to compare the microbiota structure and metabolites of chicken from different altitudes via multi-omics sequencing. Results showed that there were 567 614 filtered sequences in bacterial microbiota and 610 966 filtered sequences in fungal microbiota. The indexes of Pieloue ( $P < 0.05$ ) and Simpson ( $P < 0.05$ ) in bacterial microbiota of the LH group were significantly higher than those in ZJ group. Bacteria biomarkers of *Ezakiella*, *Peptococcus*, *Faecalicoccus*, *Limosilactobacillus*, *Ligilactobacillus*, *Clostridium* T, *Akkermansia* and fungi biomarker of *Pichia* were found between the two chicken groups. There were 260 differential metabolites discovered between higher and lower altitude chickens. Our results may give novel insights into future Xizang chicken breeding and understanding their plateau adaptability.

**To Cite This Article:** Wang H, Xian L, Da P, Wu Y, Su Z, Zang L, Wang H, Zhu Y and Feng J, 2026. Multi-omics comparing analysis of chicken from different altitudes. Pak Vet J, 46(9): 2293-2302. <http://dx.doi.org/10.29261/pakvetj/2026.220>

#### INTRODUCTION

Chicken is popular and important food, which provide nutritious meat and egg products (Aljohani and Zaman, 2024). There is an increasing trend of chicken meat demanding due to its healthy low fat and high protein meat (Katemala *et al.*, 2022; Mazhar *et al.*, 2025). Compared with commercial fast-growing chicken, native or local chicken has better taste and texture, which is more popular and acceptable (Jayasena *et al.*, 2015). Xizang chicken is a rare Chinese plateau bird breed, which lives on the harsh high altitude (2200-4100m) regions with hypothermia and hypoxia and strong radiation conditions (Du *et al.*, 2022). Xizang chicken is usually found in farming regions of Xigaze, Lhasa, Shannan and Nyingchi of Xizang (Yuan *et al.*, 2022), and there were about 1.6 million Xizang Chicken in 2020 with 453 400 birds slaughtered in this province. This small body size chicken is economically important for highland people by providing high protein, rich essential amino acids meat and eggs (Zhaxi *et al.*, 2021). Besides that, Xizang chicken is also famous for its medicinal value (Qie *et al.*, 2023).

The intestinal microbiota is commonly of consisting of billions of microorganisms like various of bacteria,

fungi, virus and etc. (Huang *et al.*, 2023; Zhu *et al.*, 2025), which is confirmed to be able to regulate health and disease by influencing the substance metabolism, epithelial mucosa homeostasis, immune function of host (Zhang *et al.*, 2020). Those microbes usually produce multiple metabolites by digesting diets and those metabolites are the executor during the crosstalk between host and microbiota (Rooks and Garrett, 2016). For instance, the widely known bacteria generated short-chain fatty acids could support the function and growth of epithelial cells (Zhang *et al.*, 2023). The microbiome is dynamic and can be affected by many factors like diet changes, environmental aspects, lifestyle, health or ill condition, and antibiotic or other drugs ingesting (El-Sayed *et al.*, 2021). The microbiota of animals and people affected by altitude, an important factor discussed in earlier studies (Liu *et al.*, 2024; Ma *et al.*, 2025; Deng *et al.*, 2026). However, little is known about the intestinal microbiota and metabolite differences between Xizang chicken and flat area chicken. Hence, we performed this study to compare the microbiota structure and metabolites of chicken from different altitudes via multi-omics sequencing.

## MATERIALS AND METHODS

**Samples collection:** Total of six fresh rectal content samples were collected from local chicken farms from Lhasa, Xizang (n=3, LH1-3) with average altitude 3650 m and average temperature 7.4°C, and Jining, Shandong (n=3, ZJ1-3) with average altitude 35-40 m and average temperature 13.3-14.1°C in August 2025.

### Microbiota sequencing chicken from different regions:

Rectal content samples from Lhasa and Jining were used for DNA extraction by employing EasyPure® Stool Genomic DNA Kit (TransGen Biotech, Beijing). Then 1.2% agarose gel electrophoresis and spectrophotometer of NanoDrop 2000 (Thermo Fisher Scientific, USA) were utilized for quantity and quality detection of DNA products of chicken (Wirajana *et al.*, 2024; Ali *et al.*, 2026). The 16S rRNA V3-4 region and ITS V1 region were amplified by using primary pairs of 338F/806R (5'-ACTCCTACGGGAGGCAGCAG-3', 5'-GGACTACHVGGGTWTCTAAT-3') and ITS1/ITS2 (5'-CTTGGTCATTTAGAGGAAGTAA-3', 5'-GCTGCGTTCTTCATCGATGC-3') (Gao *et al.*, 2022; Xu *et al.*, 2025), respectively. At last, all those PCR generations from chicken were sent for double pair-end 250 bp sequencing by piloting MiSeq sequencer at Bioyi Biotechnology Co., Ltd (Wuhan, China).

### Bioinformatics analysis of chicken from Lhasa and Jining:

The obtained raw reads of chicken from Lhasa and Jining were proceeded by QIIME2 (2023) and R packages (v4.4) according to their illustration. First, all the raw sequences were sent to demultiplex and primers cut via demux plugin and cutadapt plugin. Then those sequences were proceeded for quality filtering, denoising, merging and chimera removing by using DADA2 plugin (Kim *et al.*, 2023). Second, the non-singleton amplicon sequence variants (ASVs) of chicken were aligned through mafft (Rozewicki *et al.*, 2019) and which were further used for bacteria and fungi taxonomy assignment via classify-sklearn naïve Bayes taxonomy classifier in feature-classifier plugin against the Greengenes 2 and UNITE database (<https://unite.ut.ee/>), respectively (Viana *et al.*, 2023). The ASVs between chicken from Lhasa and Jining was examined via Venn diagram by utilizing R package (Yue *et al.*, 2021). Third, the microbiota richness, evenness and diversity compared between chicken from Lhasa and Jining were compared by alpha-diversity metrics evaluation, which include Chao1, Faith's PD, Simpson, Good's coverage etc. by QIIME2 (Chen *et al.*, 2024). The microbiota structure comparing between chicken from Lhasa and Jining was also performed by employing Jaccard metrics and Bray-Curtis metrics and visualized by principal component analysis (Li *et al.*, 2019), principal coordinate analysis (Morsy *et al.*, 2025), unweighted pair-group method with arithmetic means hierarchical clustering (Tan *et al.*, 2020) and nonmetric multidimensional scaling (Ho *et al.*, 2024). Fourth, the differentially abundant taxa across groups were revealed by linear discriminant analysis effect size and T-test (Liu *et al.*, 2023; Xu *et al.*, 2026). Fifth, the gut microbial functions of chicken from Lhasa and Jining were

predicted and compared through PICRUST2 by aligning against MetaCyc and KEGG databases (Kanehisa *et al.*, 2023; Yi *et al.*, 2025).

### Metabolomics analysis of chicken from Lhasa and Jining:

For metabolite extraction, all chicken samples (100mg per sample) from Lhasa and Jining were mixed separately with an extraction solution (500µL) containing 200µL methanol, 200µL acrylonitrile and 100µL deionized water. After that, it was vortexed for 30 seconds, homogenized at 35Hz for 4minutes, sonicated for 5minutes in a water bath at 4°C, incubated at -40°C for 1hour to precipitate proteins, and then centrifuged at 12000rpm for 15minutes to extract the supernatant. A UHPLC system (Thermo Fisher Scientific, USA) with a Waters ACQUITY UPLC BEH Amide column connected to an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific, USA) was used for the LC-MS/MS study of metabolites.

The mass spectrometry raw data of chicken samples were changed to mzXML format via ProteoWizard (Kessner *et al.*, 2008). Then metabolites of chick were identified by aligning against an in-house library and public databases (HMDB, KEGG) with an identification score  $\geq 0.5$  and a coefficient of variation  $< 0.3$  (Feng *et al.*, 2024). Differential metabolites between chicken samples from Lhasa and Jining were discovered via t-test with fold change  $\geq 2$ , variable importance in projection  $> 1$  and  $P < 0.05$ . Volcano plots were generated using ggplot2 in the R package to visualize discrepant metabolites between the two chicken groups.

**Statistical Analysis:** Statistical analysis of chicken samples from Lhasa and Jining was done by using SPSS (version 27.0).  $P < 0.05$  was regarded as statistically significant, and all data for chickens at various altitudes were presented as mean  $\pm$  standard deviation.

## RESULTS

### Sequence data and alpha diversity comparing analysis

**of chicken from different altitudes:** There were more than 96 400 (LH) and 101 700 (ZJ) raw sequences and 88 000 (LH) and 92300 (ZJ) filtered sequences achieved in the bacterial microbiota of chicks (Table 1), which were aligned with 1768 ASVs, with 159 of them shared by the two different chicken groups (Fig. 1a). The rarefaction curves of all chicken samples reached peak points and stayed as horizontal curves, which illustrated that the current samples were sufficient to present the diversity of chicken microbiota (Fig. 1b). The rank abundance curves in the LH group decreased more gently than in the ZJ group, which indicated higher evenness in those samples (Fig. 1c). The indexes of Pieloue ( $P < 0.05$ ) and Simpson ( $P < 0.05$ ) in group LH were significantly higher than those in group ZJ, while no obvious difference was found in other indexes (Table 3; Fig. 1d).

There were more than 106900 (LH) and 103200 (ZJ) raw sequences, and 97700 (LH) and 89900 (ZJ) filtered sequences achieved in the fungal microbiota of chicks (Table 2), which were aligned with 189 ASVs, with 22 of them shared by the two different chicken groups (Fig. 2a). The rarefaction curves of all chicken samples

arrived at the peak and remained horizontal curves, and it was shown that the current samples were adequate to display the variety of chicken microbiota (Fig. 2b). The rank abundance curves in group LH and ZJ decreased gently, which indicated the high evenness of the current samples (Fig. 2c). None of the alpha diversity indexes showed any discernible differences (Table 4; Fig. 2d).

**Comparison analysis of the microbiota of chicken in different taxa from different altitudes:** In the bacterial microbiota, Firmicutes A (37.09%), Firmicutes D (26.45%) and Actinobacteriota (20.74%) were the dominant phyla in group LH and Firmicutes A (52.33%), Firmicutes D (29.78%), and Bacteroidota (9.70%) were the main phyla in group ZJ (Fig. 3a). Clostridia 258483 (37.09%), Bacilli (26.45%) and Actinomycetia (15.29%) were the main classes in group LH, and Clostridia 258483 (52.33%), Bacilli (29.78%) and Bacteroidia (9.70%) in group ZJ (Fig. 3b). At the order level, Lactobacillales (21.35%), Peptostreptococcales (13.60%) and Actinomycetales (9.14%) were the prime orders in group LH and Peptostreptococcales (50.81%), Lactobacillales (28.64%) and Bacteroidales (9.42%), the dominating orders in group ZJ (Fig. 3c). At the family level, Lactobacillaceae (15.08%), Peptostreptococcaceae 256921 (12.66%), and Mycobacteriaceae (6.26%) were

the primary families in group LH and Peptostreptococcaceae 256921 (49.85%), Lactobacillaceae (26.01%) and Marinilabiliaceae (9.28%) were main families in group ZJ (Fig. 3d). At genera level, *Ligilactobacillus* (12.90%), *Romboutsia* B (10.94%) and *Corynebacterium* (7.42%) were the main genus in group LH and *Romboutsia* B (49.75%), *Ligilactobacillus* (27.93%) and JC017 (10.35%) were the prime genus in group ZJ (Fig. 3e).

**Table 1:** Bacteria sequencing data of chicken from different altitudes

| Samples | Input  | Filtered | Denosed | Merged | Non-chimeric | Non-singleton |
|---------|--------|----------|---------|--------|--------------|---------------|
| LH1     | 109965 | 100329   | 99420   | 95426  | 83394        | 83374         |
| LH2     | 103325 | 94100    | 93078   | 90382  | 87984        | 87867         |
| LH3     | 96472  | 88011    | 86911   | 83337  | 80884        | 80868         |
| ZJ1     | 108396 | 99287    | 98679   | 97377  | 94192        | 94180         |
| ZJ2     | 104276 | 93488    | 92745   | 91197  | 86863        | 86827         |
| ZJ3     | 101758 | 92399    | 91851   | 91359  | 88451        | 88319         |

**Table 2:** Fungi sequencing data of chicken from different altitudes

| Samples | Input  | Filtered | Denosed | Merged | Non-chimeric | Non-singleton |
|---------|--------|----------|---------|--------|--------------|---------------|
| LH1     | 106957 | 97763    | 97718   | 92534  | 87827        | 87827         |
| LH2     | 122264 | 108281   | 108068  | 102343 | 93161        | 93160         |
| LH3     | 132941 | 120788   | 120718  | 119360 | 106759       | 106759        |
| ZJ1     | 127267 | 114343   | 114081  | 112354 | 99715        | 99715         |
| ZJ2     | 105880 | 79855    | 79710   | 75743  | 71277        | 71277         |
| ZJ3     | 103217 | 89936    | 89727   | 88813  | 78720        | 78720         |

**Table 3:** Alpha-diversity analysis of bacterial microbiota of chicken from different altitudes

| Samples | Chao1            | ACE              | Faith pd    | Goods coverage    | Observed species | Pielou e          | Shannon          | Simpson           |
|---------|------------------|------------------|-------------|-------------------|------------------|-------------------|------------------|-------------------|
| LH1     | 265.555555555556 | 267.174557297456 | 12.86982963 | 0.999921899406435 | 265              | 0.582188646604729 | 4.68653043237767 | 0.897869049811842 |
| LH2     | 515.65625        | 517.554185084778 | 36.84278464 | 0.999908882640841 | 515              | 0.671991335580676 | 6.0535859812284  | 0.951118741866318 |
| LH3     | 699              | 699              | 36.08917211 | 1                 | 699              | 0.792843492485618 | 7.49169601301521 | 0.984824881032212 |
| ZJ1     | 199.4            | 201.059372934185 | 11.95427673 | 0.999908882640841 | 198              | 0.393326084595901 | 3.00082496736173 | 0.605232294219426 |
| ZJ2     | 313.04347826087  | 313.539971812716 | 39.93832035 | 0.999973966468812 | 313              | 0.442300892839313 | 3.66668273765303 | 0.745160546018928 |
| ZJ3     | 130.103448275862 | 131.045161290323 | 32.72528268 | 0.999960949703218 | 130              | 0.42142534783053  | 2.95940379819943 | 0.730844839582102 |

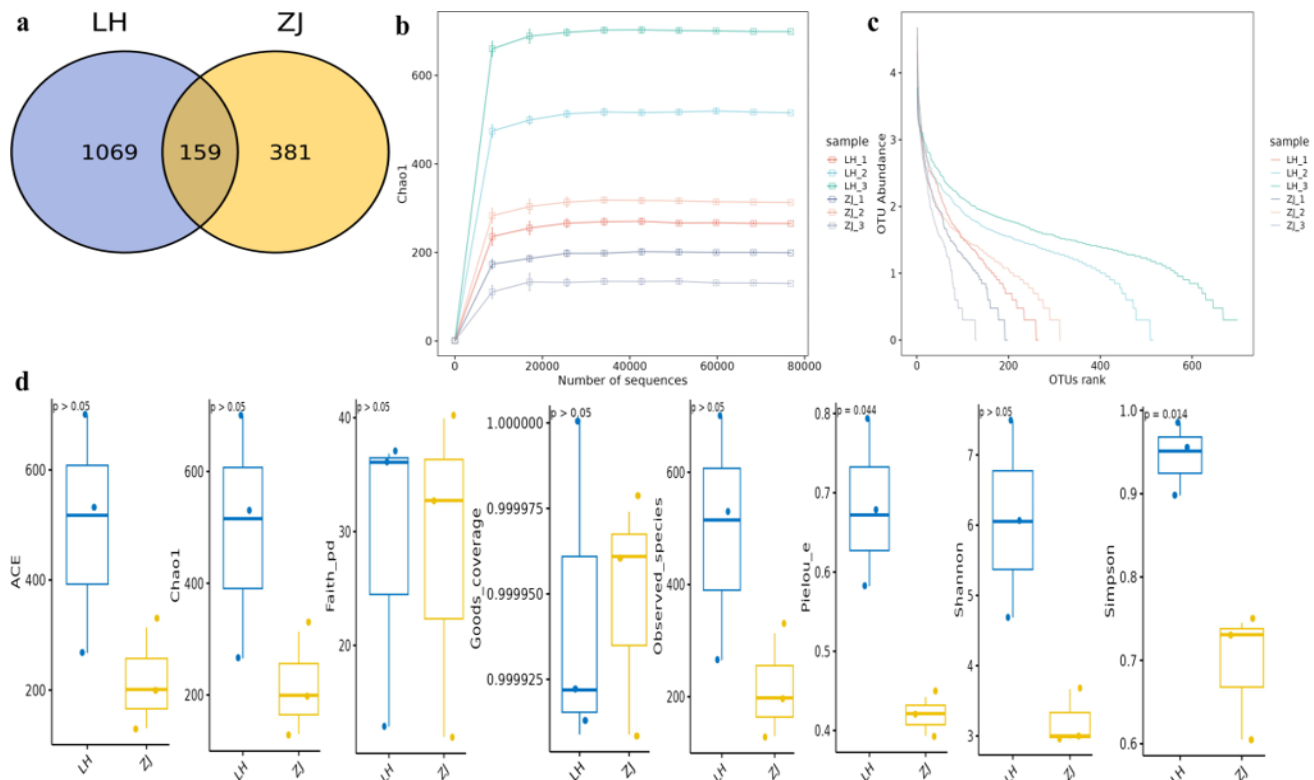


**Fig. 1:** Venn diagram and alpha-diversity analysis of bacterial microbiota of chicken from different altitudes. (a) Venn diagram, (b) Rarefaction curve, (c) Rank abundance curve, (d) Index.

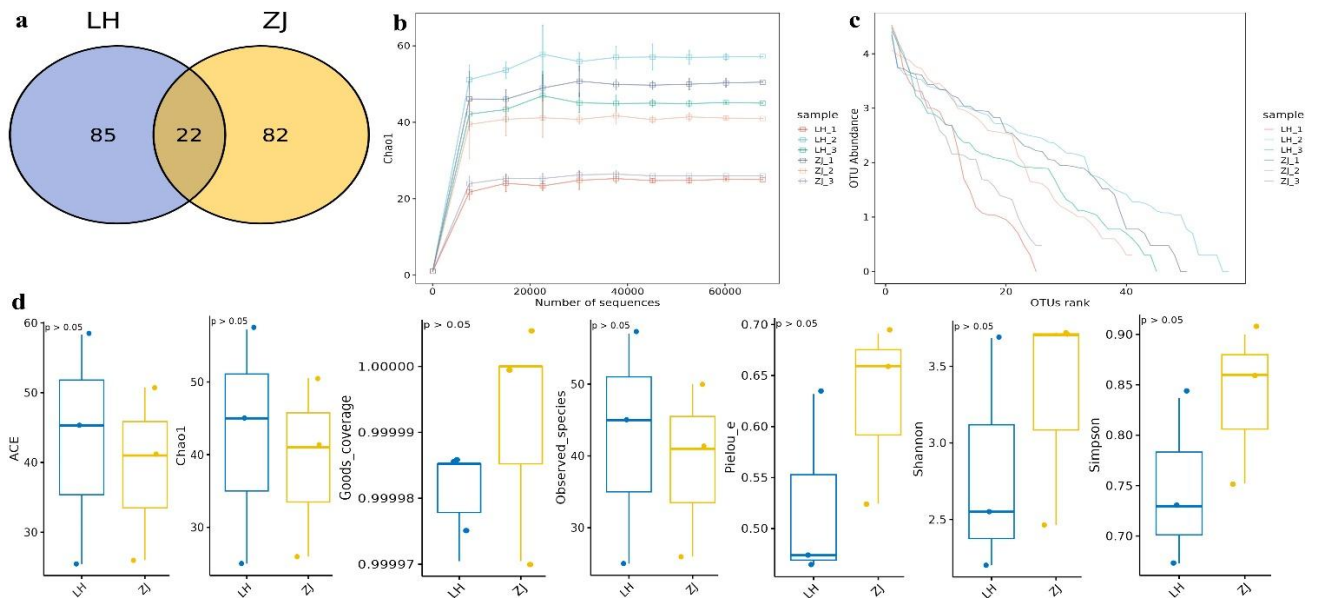


**Fig. 2:** Venn diagram and alpha-diversity analysis of fungal microbiota of chicken from different altitudes. (a) Venn diagram, (b) Rarefaction curve, (c) Rank abundance curve, (d) Index.

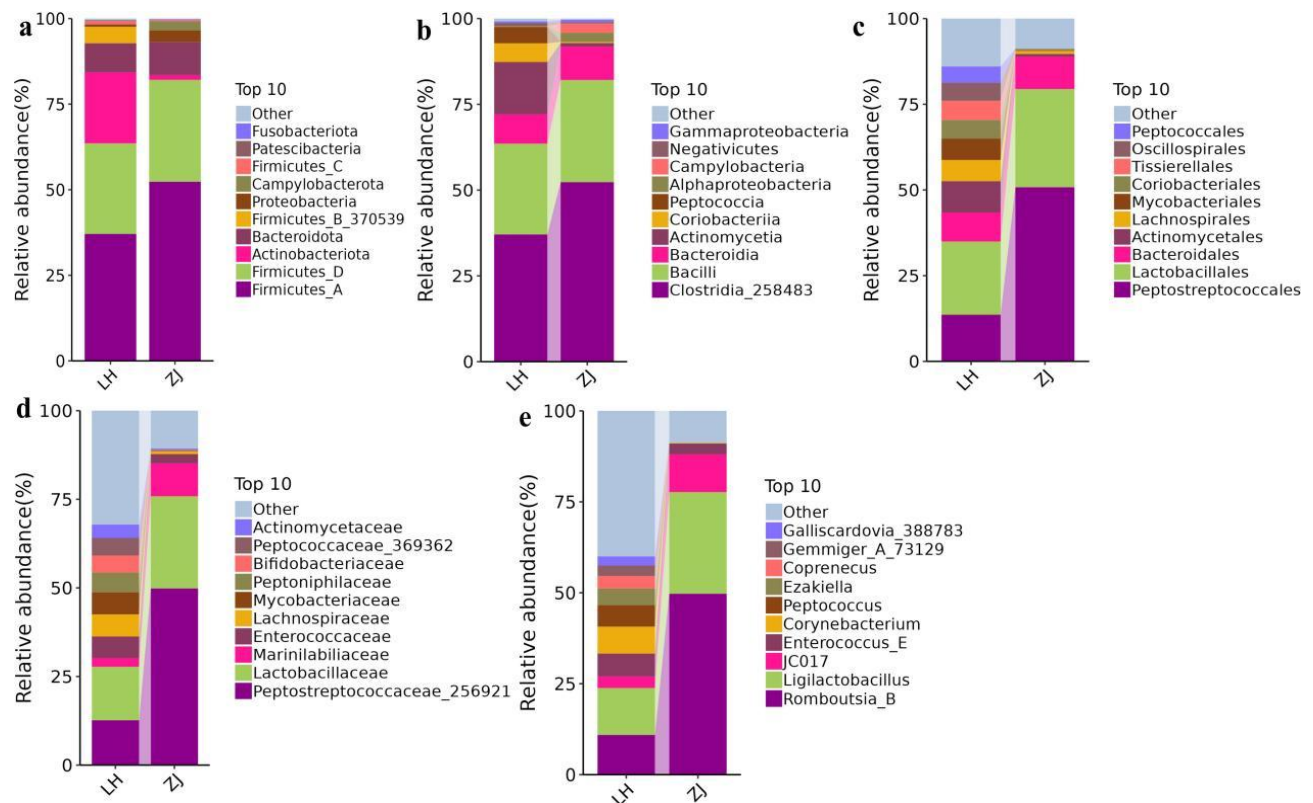


**Fig. 3:** Comparison analysis of bacterial microbiota of chicken from different altitudes. (a) Phylum, (b) Class, (c) Order, (d) Family, (e) Genera.

**Table 4:** Alpha-diversity analysis of fungal microbiota of chicken from different altitudes

| Samples | Chao1 | ACE              | Good's Coverage   | No of species | Observed | Pielou e          | Shannon          | Simpson           |
|---------|-------|------------------|-------------------|---------------|----------|-------------------|------------------|-------------------|
| LH1     | 25    | 25.454561042524  | 0.999985231787101 | 25            |          | 0.473893961680181 | 2.20069540724537 | 0.672686175675031 |
| LH2     | 57.2  | 58.3307479224377 | 0.999970463574203 | 57            |          | 0.631875758218294 | 3.68566180030426 | 0.836860530899001 |
| LH3     | 45    | 45.3054205809308 | 0.999985231787101 | 45            |          | 0.464393613644302 | 2.55038150500819 | 0.7296678410819   |
| ZJ1     | 50.5  | 50.729537503364  | 0.999970463574203 | 50            |          | 0.659297370584707 | 3.7209795458767  | 0.859924147542118 |
| ZJ2     | 41    | 41               | 1                 | 41            |          | 0.691345780962778 | 3.70392097468139 | 0.899745401684594 |
| ZJ3     | 26    | 26               | 1                 | 26            |          | 0.524359574217901 | 2.46472056924137 | 0.752268434676383 |

In the fungal microbiota, Ascomycota and Mucoromycota were the primary phyla in both LH (98.79%, 1.19%) and ZJ groups (92.06%, 5.11%) (Fig. 4a). At the class level, Eurotiomycetes (35.75%), Sordariomycetes

(33.81%) and Dothideomycetes (17.42%) were the dominating classes in group LH and Eurotiomycetes (43.91%), Sordariomycetes (30.68%) and Saccharomycetes (12.60%) the primary classes in group ZJ (Fig. 4b).

Eurotiales (35.75%), Hypocreales (33.80%) and Dothideales (17.24%) were the primary orders in group LH and Eurotiales (42.79%), Hypocreales (26.80%) and Saccharomycetales (12.60%) in group ZJ (Fig. 4c). Aspergillaceae (35.76%), Nectriaceae (28.62%) and Saccotheciaceae (17.24%) were the primary families in group LH and Aspergillaceae (42.79%), Nectriaceae (25.87%) and Saccharomycetales fam Incertae sedis (7.98%) remained the prime families in group ZJ (Fig. 4d). *Fusarium* (29.64%), *Penicillium* (22.12%) and *Aureobasidium* (18.63%) were the dominating genus in group LH, and *Penicillium* (28.20%), *Fusarium* (26.33%) and *Paecilomyces* (11.95%) were the staple genus in group ZJ (Fig. 4e).

**Exploring microbiota biomarkers of chicken from different altitudes:** Beta-diversity analysis of bacterial microbiota showed that there was obviously no group distance between group LH and ZJ via analysis of PCA

(Fig. 5a), PCoA (Fig. 5b), NMDS (Fig. 5c), UPGMA (Fig. 5d), and Permanova (Fig. 5e). LefSe showed that the genus of *Ezakiella*, *Peptococcus*, *Faecalicoccus* and *Limosilactobacillus* were observably higher in group LH ( $P < 0.05$ ), while *Ligilactobacillus*, *Clostridium* and *Akkermansia* were markedly higher and significant in ZJ group (Fig. 7). Test showed that Actinobacteriota in group LH was markedly higher than in group ZJ ( $P < 0.05$ ) (Fig. 9a). The abundance of *Corynebacterium*, *Carnobacterium* A 320743 and *Alloprevotella* was slightly greater in group LH, while *Mailhella* was more in group ZJ at genus level (Fig. 9b). Beta-diversity analysis of fungal microbiota showed no apparent group distance between LH and ZJ groups via PCA analysis (Fig. 6a), PCoA (Fig. 6b), NMDS (Fig. 6c), UPGMA (Fig. 6d) and Permanova (Fig. 6e). LefSe showed that *Pichia* was significantly higher in group LH than in ZJ group ( $P < 0.05$ ) (Fig. 8).

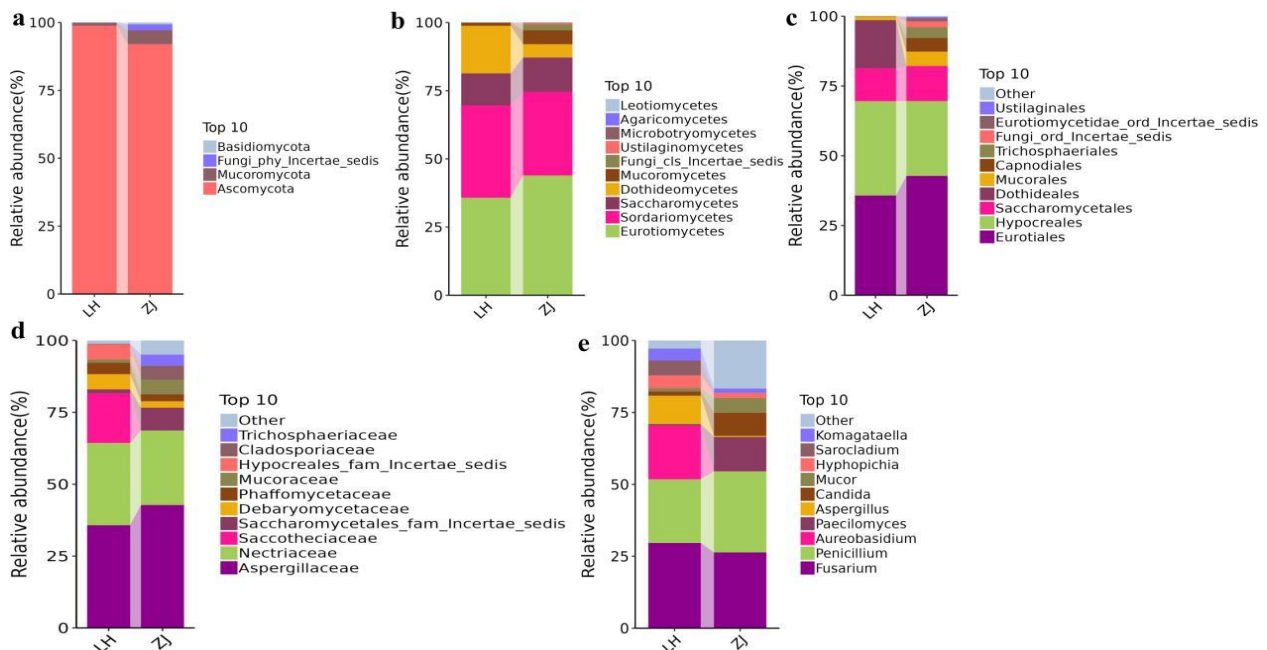


**Fig. 4:** Comparison analysis of fungal microbiota of chicken from different altitudes. (a) Phylum, (b) Class, (c) Order, (d) Family, (e) Genera.

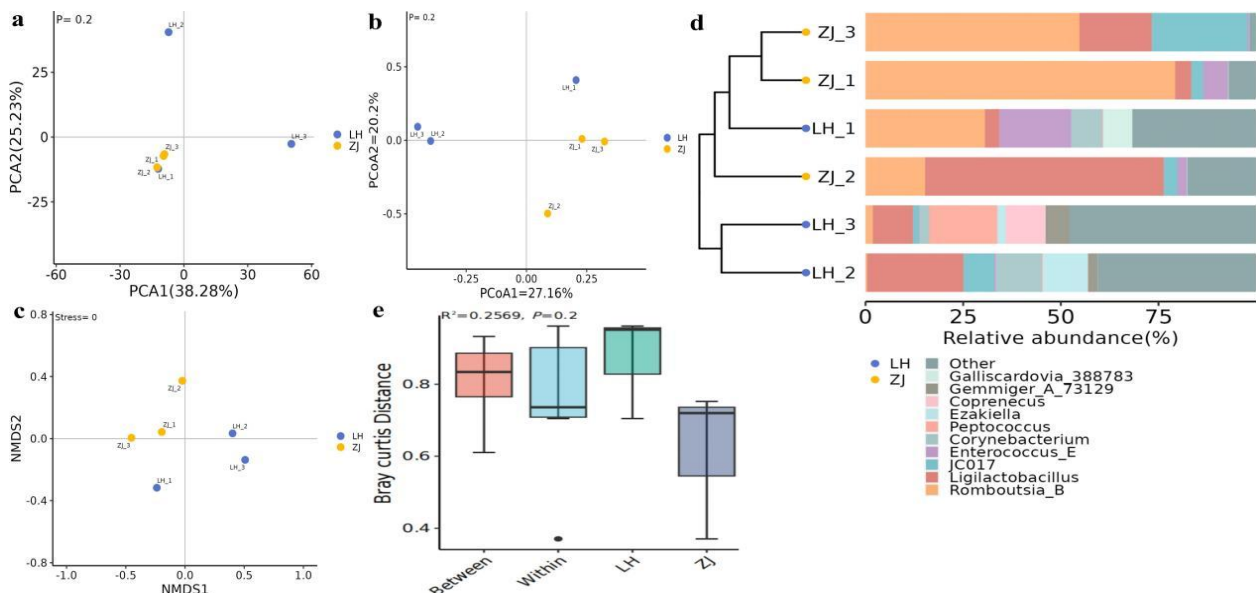


**Fig. 5:** Beta-diversity analysis of bacterial microbiota of chicken from different altitudes. (a) PCA, (b) PCoA, (c) NMDS, (d) UPGMA, (e) Permanova.

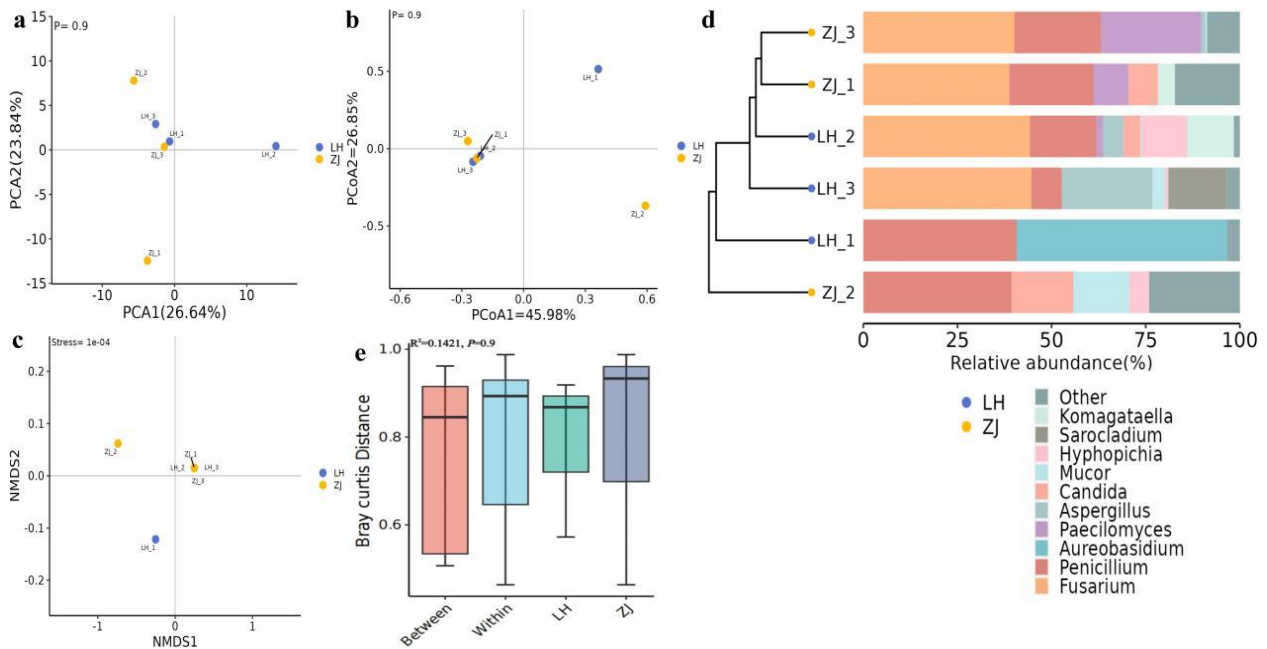


**Fig. 6:** Beta-diversity analysis of fungal microbiota of chicken from different altitudes. (a) PCA, (b) PCoA, (c) NMDS, (d) UPGMA, (e) Permanova.

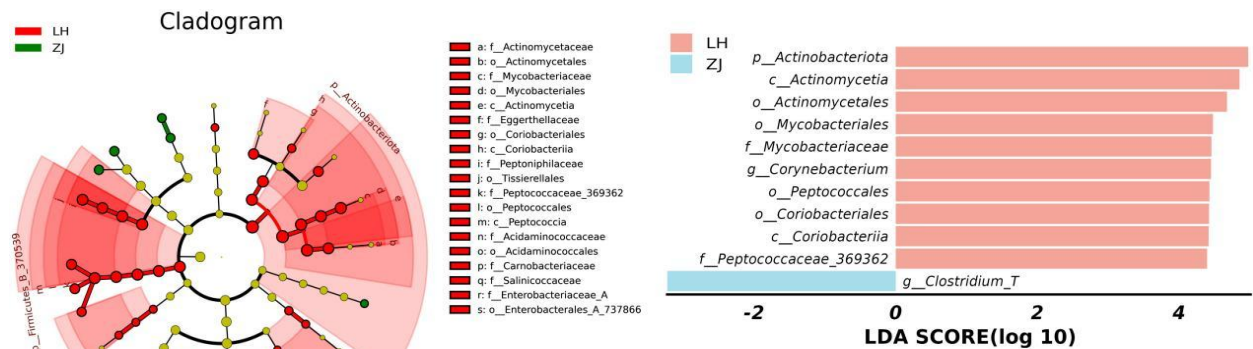


**Fig. 7:** Exploring bacterial biomarkers of chicken from different altitudes via LEfSe.

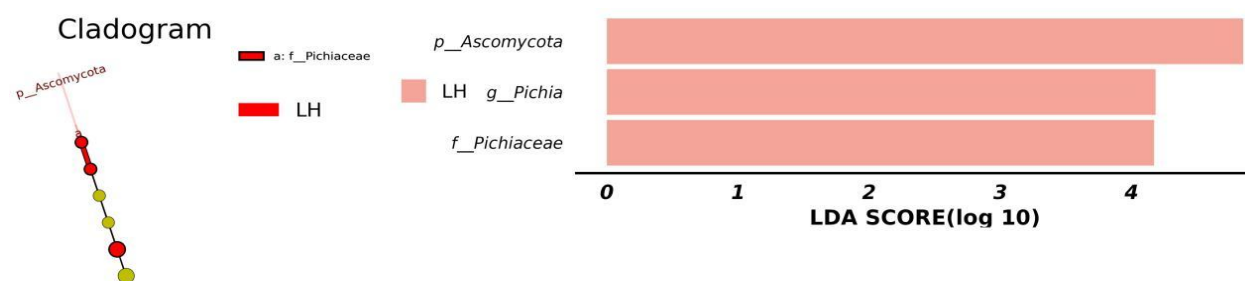


**Fig. 8:** Exploring fungal biomarkers of chicken from different altitudes via LEfSe.

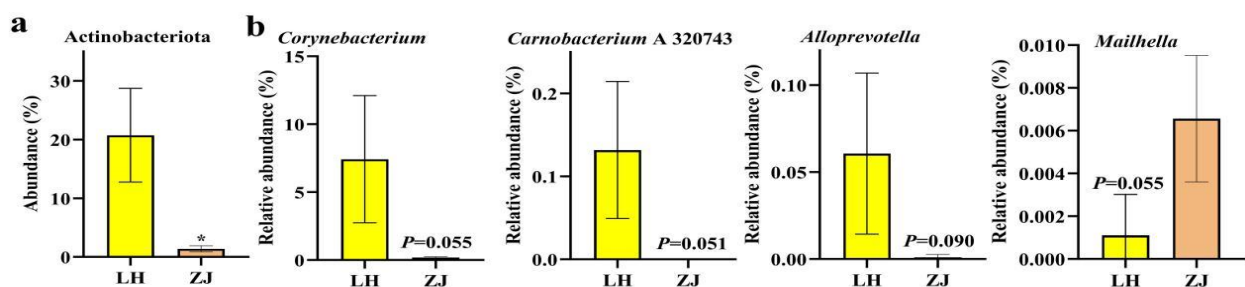


**Fig. 9:** Exploring bacterial biomarkers of chicken from different altitudes via a t-test. Significance is presented as \*P<0.05; data were depicted as the mean ± SEM (n = 3). (a) Phylum, (b) Genera.

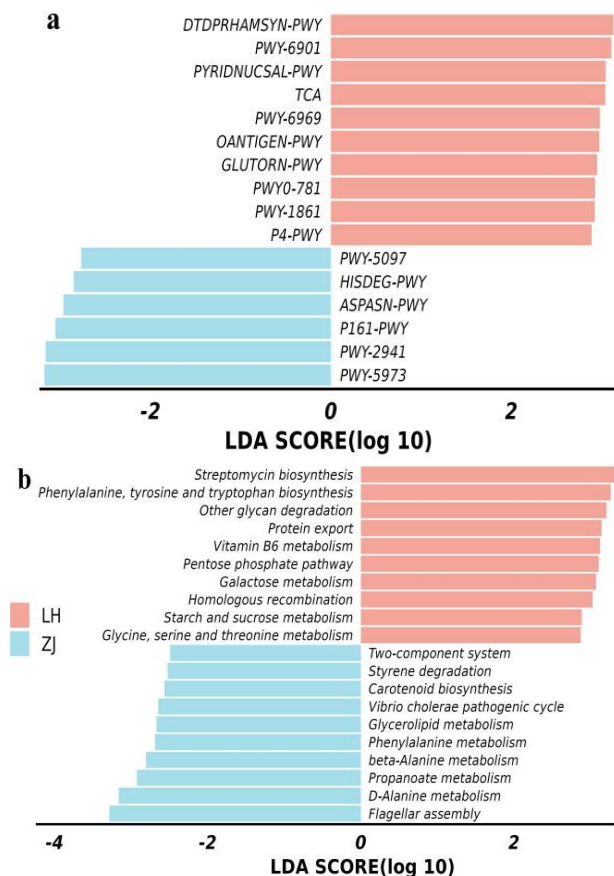


**Fig. 10:** Bacterial function comparison analysis of MetaCyc and KEGG pathways of chicken from different altitudes.

**Microbiota function comparison analysis of chicken from different altitudes:** MetaCyc comparing analysis showed that the abundance of pathways of DTDPRHAMSYN-PWY (dTDP-L-rhamnose biosynthesis I) ( $P < 0.05$ ), PWY-6901 (superpathway of glucose and xylose degradation) ( $P < 0.05$ ), PYRIDNUCSAL-PWY (NAD salvage pathway I) ( $P < 0.05$ ), TCA (TCA cycle I) ( $P < 0.05$ ), PWY-6969 (2-oxoglutarate: ferredoxin oxidoreductase) ( $P < 0.05$ ), OANTIGEN-PWY (superpathways of menaquinol-11 biosynthesis) ( $P < 0.05$ ), GLUTORN-PWY (superpathways of menaquinol-11 biosynthesis) ( $P < 0.05$ ), PWY0-781 (aspartate superpathways) ( $P < 0.05$ ), PWY-1861 (formaldehyde assimilation II) ( $P < 0.05$ ) and P4-PWY (superpathways of L-lysine, L-threonine and L-methionine biosynthesis I) ( $P < 0.05$ ) were significantly higher in group LH, while PWY-5097 (L-lysine biosynthesis VI) ( $P < 0.05$ ), HISDEG-PWY (L-histidine degradation I) ( $P < 0.05$ ), ASPASN-PWY (superpathway of L-aspartate and L-asparagine biosynthesis) ( $P < 0.05$ ), P161-PWY (acetylene degradation) ( $P < 0.05$ ), PWY-2941 (L-lysine biosynthesis II) ( $P < 0.05$ ) and PWY-5973 (cis-vaccenate biosynthesis) ( $P < 0.05$ ) were observably higher in group ZJ (Fig. 11a). KEGG pathways comparing analysis found that Streptomycin biosynthesis, Phenylalanine metabolism, Other glycan degradation, protein export, vitamin B6 metabolism, Pentose and glucuronate interconversions, Galactose metabolism, Homologous recombination, Starch and sucrose metabolism and Glycine, serine and threonine metabolism were significantly higher in group LH, while two-

component system, Styrene degradation, Carotenoid biosynthesis, Vibrio cholerae pathogenic cycle, Glycerolipid metabolism, Phenylalanine metabolism, beta-Alanine metabolism, Propanoate metabolism, D-Alanine metabolism and Flagellar assembly were observably higher and significant in group ZJ (Fig. 11b).

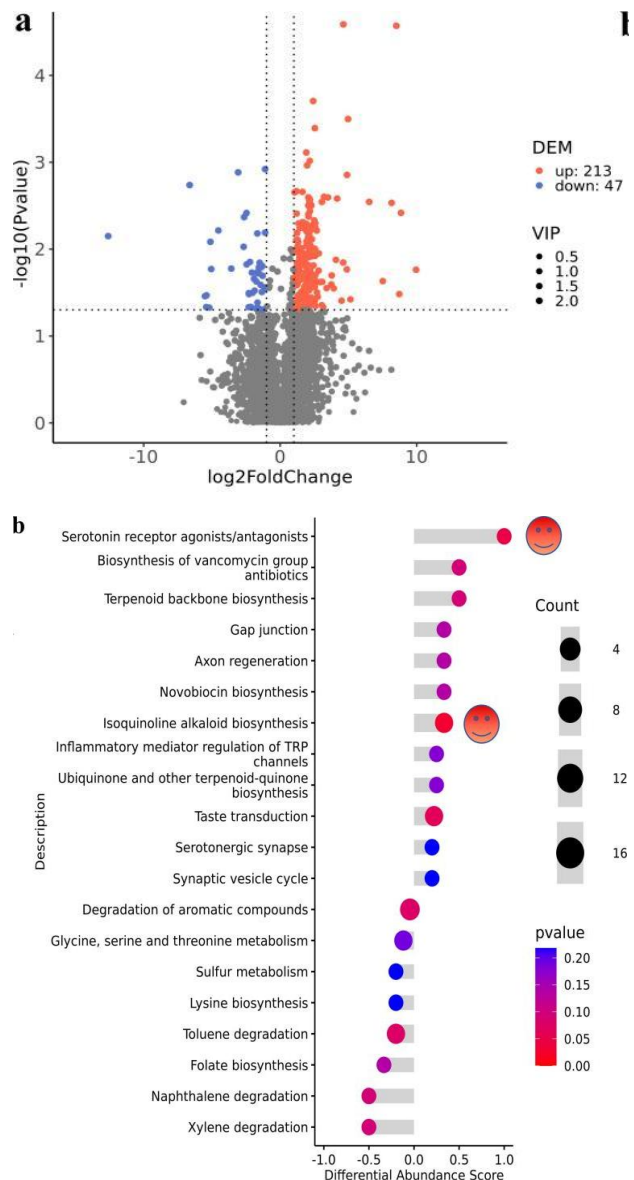


**Fig. 11:** Metabolites comparison analysis of chicken from different altitudes. (a) Volcano plot of differential metabolites, (b) KEGG.

**Metabolites comparing analysis of chicken from different altitudes:** There were 260 differential metabolites between group LH and ZJ by Volcano plot (Fig. 11a), with Ggstop ( $P < 0.01$ ), Docosaheptaenoic acid ( $P < 0.05$ ), Sodium (4-methoxybenzoxy) acetate ( $P < 0.05$ ) and Eburicoic acid ( $P < 0.05$ ) clearly higher in group LH, while 3-(2,3-Dihydrobenzo(b)furan-5-yl) propanoic acid ( $P < 0.0001$ ), 2,3-DI-Phytanyl-glycerol ( $P < 0.01$ ), Adenosine 5-O-(3-thiotriphosphate) ( $P < 0.01$ ) and Phosphiphen ( $P < 0.05$ ) were significantly higher in group ZJ. KEGG showed that Isoquinoline alkaloid biosynthesis ( $P < 0.05$ ) and Serotonin receptor agonists/antagonists ( $P < 0.05$ ) in group LH was obviously higher than in group ZJ (Fig. 11b).

## DISCUSSION

The Chicken meat consumption in China has increased rapidly in the past decade, with more than 15.46 million tons in 2020 (Cui *et al.*, 2022). Therefore, highly effective breeding of chicken is of great importance and meaningful, especially in plateau regions with limited food resources.

In this study, we performed 16S rRNA and ITS amplicon sequencing of chicken from Lhasa and Jining and obtained 567614 filtered sequences in bacterial microbiota and 610966 filtered sequences in fungal microbiota (Table 1, Table 2). The indexes of Pielou and Simpson in bacterial microbiota of group LH were significantly higher than in group ZJ (Table 3; Fig. 1d), which was partially in line with yaks and mice in different altitudes (Zhu *et al.*, 2024; Li *et al.*, 2025). The relative abundance of Firmicutes A (37.09%) in group LH was lower than that in group ZJ (52.33%), while Actinobacteriota (20.74%) in group LH was higher than in group ZJ (1.39%), in the bacterial microbiota. Firmicutes is one of the dominating phyla in the host intestinal microbiota, which is highly associated with the conversion of fiber and cellulose into short-chain fatty acids (SCFAs) (Kim *et al.*, 2023). The lower abundance of Firmicutes in chicken in LH group may explain the lower feed conversion ratio in Tibetan chicken. Actinobacteriota is related to maintaining the intestinal barrier homeostasis and immune function (Zhu *et al.*, 2024); the abundance of Actinobacteriota in Xizang chicken may enhance the immunity of this bird at high altitude. The ratio of Firmicutes/Bacteroidota was 8.13 and 8.51 in group LH and ZJ, respectively, which may indicate a slight microbiota change between group LH and ZJ (Kim *et al.*, 2023). In the fungal microbiota, though Ascomycota and Mucoromycota were the main phyla in chicken in group LH and ZJ, while Ascomycota was slightly higher in LH group (98.79% vs 92.06%), and Mucoromycota was slightly higher in ZJ group (5.11% vs 1.19%). A previous study found Mucoromycota was responsible for selenium usage and could promote the colonization of eukaryotic microbes (Li *et al.*, 2023), the higher abundance of which may contribute to the microbiota homeostasis of ZJ chicken. LEfSe showed that genus of *Ezakiella*, *Peptococcus*, *Faecalicoccus* and *Limosilactobacillus* were observably higher in group LH, while *Ligilactobacillus*, *Clostridium* T and *Akkermansia* were markedly higher in group ZJ (Fig. 7). Among them, the abundance of *Ezakiella* was associated with dietary factors (Zeng *et al.*, 2025). The difference in this genus between group LH and ZJ may be caused by the feed differences. *Peptococcus* is related to higher intestinal inflammation (Sun *et al.*, 2022), the abundance of this genus in LH chicken may be associated with a harsh plateau environment. *Faecalicoccus* is a representative genus of the butyrate-generating family of Erysipelotrichaceae, especially in the poultry cecum (Liu *et al.*, 2021), *Limosilactobacillus* is a lactic acid/lactate producing genus (Li *et al.*, 2023), the greater abundance of these two genera may enhance the plateau adaptability of Xizang Chicken. *Ligilactobacillus* is a widely known probiotic genus, which could promote growth and improve host health (Zhang *et al.*, 2022), *Akkermansia* is

also a probiotic genus, which could relieve inflammation in animals (Panzetta and Valdivia, 2024), the higher abundance of those two genera in low altitude chicken may promote the growth of chicken in group ZJ. The microbiota difference between group LH and ZJ also affected their metabolites, with 260 differential metabolites (Fig. 11a), which later affected microbiota function (Fig. 11b). Among them, Eburicoic acid could inhibit inflammatory response (Jin *et al.*, 2024), the higher abundance of Eburicoic acid in Xizang chicken may be associated with their inflammatory levels caused by the harsh higher altitude environment.

This study has several limitations. First, the relatively small sample size ( $n = 3$  per group) limits statistical power and the generalizability of the findings. Second, because chickens were collected from geographically distinct regions, differences in diet, management practices, climate, host genetics, and environmental exposure may also have contributed to the observed microbiome and metabolomic variations. Therefore, the observed differences should be interpreted as associations rather than effects attributable solely to altitude. Finally, although differential taxa and metabolites were identified using statistical thresholds, future studies should include larger cohorts, controlled experimental designs, and multiple-testing correction to improve analytical robustness.

**Conclusions:** In summary, this study compares the bacterial and fungal gut microbiota and metabolomic profiles of chickens from high and low altitude regions. Distinct bacterial biomarkers, including *Ezakiella*, *Peptococcus*, *Faecalicoccus*, *Limosilactobacillus*, *Ligilactobacillus*, *Clostridium* T, and *Akkermansia*, as well as the fungal biomarker *Pichia*, were identified, together with 260 differential metabolites between the two groups. These findings provide valuable insights into the gut microbial composition and metabolic characteristics of chickens from different geographical regions and may contribute to future breeding strategies for Xizang chickens. However, because the chickens originated from different geographical locations with potential differences in management, diet, climate, genetics, and other environmental factors, the observed microbiome and metabolomic variations cannot be attributed solely to altitude. Moreover, the relatively small sample size limits the generalizability of the findings. Therefore, larger studies under controlled experimental conditions are needed to clarify the specific effects of altitude on the chicken gut microbiome and metabolome.

**Data availability:** In the NCBI Sequence Read Archive, the available raw data for yaks are under the consent numbers PRJNA1481692 and PRJNA1481694.

**Ethical approval:** All animal-related experiments were approved by the ethics committee of Xizang Academy of Agricultural and Animal Husbandry Sciences (XZAAA2025060201).

**Authors contribution:** HW, JF did the research and methodology; Solutions, reagents, lab supplies, and analytical instruments are produced by HW, LX, PD, YW, ZS, LZ, HW, YZ, JF. HW prepared draft; HW, JF did

paper writing, review and revision; JF did supervision and visualization; The final text was reviewed and approved by all authors.

**Funding:** The work was supported by the National Key Research and Development Program of China (2022YFD1600901) and the work was supported by the Central Financial Guidance for Local Projects of the Shandong Provincial Department of Science and Technology (YDZX2023002) and the earmarked fund for CARS-39-S17, and the Research and Application of High-Yield Cellulase *Bacillus subtilis* Preparations in the Diet of Xizang Chickens (XZKNKY-XMSTC-2025-006).

**Acknowledgement:** None.

## REFERENCES

- Ali M, Xu C, Mahmood MS, et al., 2026. Mechanistic insights into restoration of gut barrier and microbiota homeostasis by allicin and Qingchang Huashi formula in *Cryptosporidium parvum*-infected immunosuppressed murine model. *Microbial Pathogenesis* 217:108559. <https://doi.org/10.1016/j.micpath.2026.108559>
- Aljohani ASM and Zaman MA, 2024. Evaluation of anticoccidial activity of ethanolic extract of clove in broiler chicken. *Pakistan Veterinary Journal* 44(3): 757-762. <http://dx.doi.org/10.29261/pakvetj/2024.250>
- Chen Q, Palanisamy V, Wang R, et al., 2024. Salmonella -induced microbiome profile in response to sanitation by quaternary ammonium chloride. *Microbiology Spectrum* 12:e2323-e2346. <https://doi.org/10.1128/spectrum.02346-23>
- Cui B, Wang L D, Chen X, et al., 2022. Chicken meat taste preferences, perceived risk of human infection with avian influenza virus, and self-reported chicken meat consumption in China. *Preventive Veterinary Medicine* 203:105658. <https://doi.org/10.1016/j.prevetmed.2022.105658>
- Deng M, Sun L, Yu C, et al., 2026. Altitude drives the divergence of rumen fungal communities between cattle and yak in Yunnan plateau. *Microbial Pathogenesis* 213:108358. <https://doi.org/10.1016/j.micpath.2026.108358>
- Du X, Li F, Kong F, et al., 2022. Altitude-adaption of gut microbiota in Tibetan chicken. *Poultry Science* 101:101998. <https://doi.org/10.1016/j.psj.2022.101998>
- El-Sayed A, Aleya L and Kamel M, 2021. Microbiota's role in health and diseases. *Environmental Science and Pollution Research* 28:36967-36983. <https://doi.org/10.1007/s11356-021-14593-z>
- Feng Y, Shi YH, Wang T, et al., 2024. Quercetin ameliorates cognitive impairment in app/psI mice via gut microbiota modulation and tryptophan metabolism restoration. *Pakistan Veterinary Journal* 45(4): 1698-1709. <http://dx.doi.org/10.29261/pakvetj/2025.335>
- Gao M, Yu A, Chen M, et al., 2022. Airborne fungi and human exposure in different areas of composting facilities. *Ecotoxicology and Environmental Safety* 243:113991. <https://doi.org/10.1016/j.ecoenv.2022.113991>
- Ho C W, Chen P Y, Liao Y, et al., 2024. Uncovering the microbiome landscape in sashimi delicacies. *Scientific Reports* 14:5454. <https://doi.org/10.1038/s41598-024-55938-1>
- Huang Y, Zhang P, Han S, et al., 2023. Lactoferrin Alleviates Inflammation and Regulates Gut Microbiota Composition in H5N1-Infected Mice. *Nutrients* 15:3362. <https://doi.org/10.3390/nu15153362>
- Jayasena D D, Jung S, Kim S H, et al., 2015. Endogenous functional compounds in Korean native chicken meat are dependent on sex, thermal processing and meat cut. *Journal of the Science of Food and Agriculture* 95:771-775. <https://doi.org/10.1002/jsfa.6882>
- Jin R, Zhao Z, Zhang Q, et al., 2024. An anti-inflammatory active ingredients in *Poriae cutis*: Screening based on network pharmacology. *Die Pharmazie-An International Journal of Pharmaceutical Sciences* 79:72-81. <https://doi.org/10.1691/ph.2024.3647>
- Kanehisa M, Furumichi M, Sato Y, et al., 2023. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research* 51:D587-D592. <https://doi.org/10.1093/nar/gkac963>
- Katemala S, Molee A, Thumanu K, et al., 2022. A comparative study of meat quality and vibrational spectroscopic properties of different chicken breeds. *Poultry Science* 101829.
- Kessner D, Chambers M, Burke R, et al., 2008. ProteoWizard: open source software for rapid proteomics tools development. *Bioinformatics* 24:2534-2536. <https://doi.org/10.1093/bioinformatics/btn323>
- Kim J, Cui Y, Lee H, et al., 2023. Microbiome dataset of bacterial and fungal communities in anthosphere of twelve different wild plants in South Korea. *Data in Brief* 50:109470. <https://doi.org/10.1016/j.dib.2023.109470>
- Kim J, Zhang S, Zhu Y, et al., 2023. Amelioration of colitis progression by ginseng-derived exosome-like nanoparticles through suppression of inflammatory cytokines. *Journal of Ginseng Research* 47:627-637. <https://doi.org/10.1016/j.jgr.2023.01.004>
- Kim JE, Tun HM, Bennett D C, et al., 2023. Microbial diversity and metabolic function in duodenum, jejunum and ileum of emu (*Dromaius novaehollandiae*). *Scientific Reports* 13:4488. <https://doi.org/10.1038/s41598-023-31684-8>
- Li L, Qu L, Li T, 2023. The effects of Selenohomolanthionine supplementation on the rumen eukaryotic diversity of Shaanbei white cashmere wether goats. *Scientific Reports* 13:13134. <https://doi.org/10.1038/s41598-023-39953-2>
- Li L, Wang F, Liu Y, et al., 2019. Intestinal microbiota dysbiosis in children with recurrent respiratory tract infections. *Microbial Pathogenesis* 136:103709. <https://doi.org/10.1016/j.micpath.2019.103709>
- Li S, Zhu W, Guo R, et al., 2025. Changes in gut microbiota of gallstone mice at different altitudes based on 16S rDNA sequencing. *Frontiers in Microbiomes* 4:1618718. <https://doi.org/10.3389/frmbi.2025.1618718>
- Li Y, Pu L, Li Y, et al., 2023. Design, synthesis and evaluation of a myricetin and nobiletin hybrid compound for alleviating hyperuricemia based on metabolomics and gut microbiota. *RSC Advances* 13:21448-21458. <https://doi.org/10.1039/D3RA03188H>
- Liu D, Chen D, Xiao J, et al., 2024. High-altitude-induced alterations in intestinal microbiota. *Frontiers in Microbiology* 15:1369627. <https://doi.org/10.3389/fmicb.2024.1369627>
- Liu H, Yan C, Hao C, et al., 2023. Dynamic changes in intestinal microbiota and metabolite composition of pre-weaned beef calves. *Microbial Pathogenesis* 175:105991. <https://doi.org/10.1016/j.micpath.2023.105991>
- Liu Y, Lin Q, Huang X, et al., 2021. Effects of dietary ferulic acid on the intestinal microbiota and the associated changes on the growth performance, serum cytokine profile, and intestinal morphology in ducks. *Frontiers in Microbiology* 12:698213. <https://doi.org/10.3389/fmicb.2021.698213>
- Ma X, Duan C, Wang X, et al., 2025. Human gut microbiota adaptation to high-altitude exposure: longitudinal analysis over acute and prolonged periods. *Microbiology Spectrum* 13:e2916-e2924. <https://doi.org/10.1128/spectrum.02916-24>
- Mazhar F, Geng Q, Zhao Z, et al., 2025. Chicken primordial germ cells (pgcs) formation, regulation, and fate determination under omics approaches: a comprehensive scientific review. *Pakistan Veterinary Journal* 45(2): 475-487. <http://dx.doi.org/10.29261/pakvetj/2025.187>
- Morsy Y, Shafie N S, Mostafa M, et al., 2025. Integrative analysis of gut microbiota and metabolic pathways reveals key microbial and metabolomic alterations in diabetes. *Scientific Reports* 15:30686. <https://doi.org/10.1038/s41598-025-09328-w>
- Panzetta M E, Valdivia R H, 2024. Akkermansia in the gastrointestinal tract as a modifier of human health. *Gut Microbes* 16:2406379. <https://doi.org/10.1080/19490976.2024.2406379>
- Qie M, Li Y, Hu X, et al., 2023. A new and effective method to trace tibetan chicken by amino acid profiling. *Foods* 12:876. <https://doi.org/10.3390/foods12040876>
- Rooks MG, Garrett WS, 2016. Gut microbiota, metabolites and host immunity. *Nature Reviews Immunology* 16(6):341-352. <https://doi.org/10.1038/nri.2016.42>
- Rozewicki J, Li S, Amada KM, et al., 2019. MAFFT-DASH: integrated protein sequence and structural alignment. *Nucleic Acids Research* 47:W5-W10. <https://doi.org/10.1093/nar/gkz342>
- Sun L, Guo L, Xu G, et al., 2022. Quercetin reduces inflammation and protects gut microbiota in broilers. *Molecules (Basel, Switzerland)* 27:3269. <https://doi.org/10.3390/molecules27103269>
- Tan G, Hu M, Li X, et al., 2020. High-throughput sequencing and metabolomics reveal differences in bacterial diversity and

- metabolites between red and white sufu. *Frontiers in Microbiology* 11:758. <https://doi.org/10.3389/fmicb.2020.00758>
- Viana M C, Curty G, Furtado C, et al., 2023. Naso-opharyngeal microbiome from breast cancer patients diagnosed with COVID-19. *Frontiers in Microbiology* 13:1074382. <https://doi.org/10.3389/fmicb.2022.1074382>
- Wirajana IN, Ariantari NP, Shyu DJH, et al., 2024. Prokaryotic communities profiling of Indonesian hot springs using long-read Oxford Nanopore sequencing. *BMC Research Notes* 17:286. <https://doi.org/10.1186/s13104-024-06941-2>
- Xu C, He Q, Zhu Z, et al., 2025. Propolis improves intestinal barrier function against *Cryptosporidium parvum* via NLRP6 inflammasome. *Mbio* 16:e231725. <https://doi.org/10.1128/mbio.02317-25>
- Xu C, Ji Y, Sha Q, et al., 2026. Novel inhibitors against *Cryptosporidium parvum* through blocking its energy metabolism. *PLoS Neglected Tropical Diseases* 20(10): e0014742. <https://doi.org/10.1371/journal.pntd.0014742>
- Yi P, Li T, Xu L, et al., 2025. The differential gut microbiota and their MetaCyc pathways in IBRV infected Angus calves. *Frontiers in Microbiology* 16:1588341. <https://doi.org/10.3389/fmicb.2025.1588341>
- Yuan J, Li S, Sheng Z, et al., 2022. Genome-wide run of homozygosity analysis reveals candidate genomic regions associated with environmental adaptations of Tibetan native chickens. *BMC Genomics* 23:91. <https://doi.org/10.1186/s12864-021-08280-z>
- Yue T, Chen S, Zhu J, et al., 2021. The aging-related risk signature in colorectal cancer. *Aging* 67:7330-7349.
- Zeng Q, Zhu J, Hu Y, et al., 2025. The critical role of diet, exercise, and sleep in shaping the gut microbiota of children with idiopathic short stature: a Retrospective study. *Frontiers in Immunology* 16:1566722. <https://doi.org/10.3389/fimmu.2025.1566722>
- Zhang M, Li D, Yang X, et al., 2023. Integrated multi-omics reveals the roles of cecal microbiota and its derived bacterial consortium in promoting chicken growth. *Msystems* 8:e823-e844. <https://doi.org/10.1128/msystems.00844-23>
- Zhang P, Liu J, Xiong B, et al., 2020. Microbiota from alginate oligosaccharide-dosed mice successfully mitigated small intestinal mucositis. *Microbiome* 8:112. <https://doi.org/10.1186/s40168-020-00886-x>
- Zhang Y, Jiang F, Yang B, et al., 2022. Improved microbial genomes and gene catalog of the chicken gut from metagenomic sequencing of high-fidelity long reads. *Gigascience* 11:giac116. <https://doi.org/10.1093/gigascience/giac116>
- Zhaxi C, Zhao S, Zhang T, et al., 2021. Stable isotopes verify geographical origin of Tibetan chicken. *Food Chemistry* 358:129893. <https://doi.org/10.1016/j.foodchem.2021.129893>
- Zhu Y, Wang J, Cidan Y, et al., 2024. Gut microbial adaptation to varied altitudes and temperatures in Tibetan Plateau Yaks. *Microorganisms* 12:1350. <https://doi.org/10.3390/microorganisms12071350>
- Zhu Y, Lu S, Cidan Y, et al., 2025. Protective Effects of Traditional Chinese Herbal Medicine Formulas (TCHMFs) Via Influencing Anti-Oxidative Capacity, Inflammatory Mediators, and Gut Microbiota in Weaned Yaks. *Pakistan Veterinary Journal* 45(1): 184-194. <http://dx.doi.org/10.29261/pakvetj/2025.114>