Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2023 24(11):1014-1026
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2200621

Check for updates

Effects of *Aeriscardovia aeriphila* on growth performance, antioxidant functions, immune responses, and gut microbiota in broiler chickens

Muhammad Zahid FAROOQ[1,2], Xinkai WANG[1], Xianghua YAN[1]✉

[1]*National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, Frontiers Science Center for Animal Breeding and Sustainable Production, College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan 430070, China*
[2]*Department of Animal Sciences, University of Veterinary and Animal Sciences (Jhang Campus), Lahore 54000, Pakistan*

Abstract: *Aeriscardovia aeriphila*, also known as *Bifidobacterium aerophilum*, was first isolated from the caecal contents of pigs and the faeces of cotton-top tamarin. *Bifidobacterium* species play important roles in preventing intestinal infections, decreasing cholesterol levels, and stimulating the immune system. In this study, we isolated a strain of bacteria from the duodenal contents of broiler chickens, which was identified as *A. aeriphila*, and then evaluated the effects of *A. aeriphila* on growth performance, antioxidant functions, immune functions, and gut microbiota in commercial broiler chickens. Chickens were orally gavaged with *A. aeriphila* (1×10^9 CFU/mL) for 21 d. The results showed that *A. aeriphila* treatment significantly increased the average daily gain and reduced the feed conversion ratio ($P<0.001$). The levels of serum growth hormone (GH) and insulin-like growth factor 1 (IGF-1) were significantly increased following *A. aeriphila* treatment ($P<0.05$). Blood urea nitrogen and aspartate aminotransferase levels were decreased, whereas glucose and creatinine levels increased as a result of *A. aeriphila* treatment. Furthermore, the levels of serum antioxidant enzymes, including catalase ($P<0.01$), superoxide dismutase ($P<0.001$), and glutathione peroxidase ($P<0.05$), and total antioxidant capacity ($P<0.05$) were enhanced following *A. aeriphila* treatment. *A. aeriphila* treatment significantly increased the levels of serum immunoglobulin A (IgA) ($P<0.05$), IgG ($P<0.01$), IgM ($P<0.05$), interleukin-1 (IL-1) ($P<0.05$), IL-4 ($P<0.05$), and IL-10 ($P<0.05$). The broiler chickens in the *A. aeriphila* group had higher secretory IgA (SIgA) levels in the duodenum ($P<0.01$), jejunum ($P<0.001$), and cecum ($P<0.001$) than those in the control group. The messenger RNA (mRNA) relative expression levels of *IL-10* ($P<0.05$) and *IL-4* ($P<0.001$) in the intestinal mucosa of chickens were increased, while nuclear factor-κB (*NF-κB*) ($P<0.001$) expression was decreased in the *A. aeriphila* group compared to the control group. Phylum-level analysis revealed Firmicutes as the main phylum, followed by Bacteroidetes, in both groups. The data also found that *Phascolarctobacterium* and *Barnesiella* were increased in *A. aeriphila*-treated group. In conclusion, oral administration of *A. aeriphila* could improve the growth performance, serum antioxidant capacity, immune modulation, and gut health of broilers. Our findings may provide important information for the application of *A. aeriphila* in poultry production.

Key words: Chicken; *Aeriscardovia aeriphila*; Gut health; Microbiome; Immune function

1 Introduction

Antibiotic use in food and animal production has been drastically reduced to preserve antibiotic potency and prevent antimicrobial resistance. Green and safe alternatives to antibiotics such as probiotics and plant extracts have gained increasing attention, and these alternatives are generally recognized as safe substances to enhance the health and performance of animals (Cheng et al., 2014; Buntyn et al., 2016; Mehdi et al., 2018). Probiotics have been shown to improve animal health and performance by influencing the gut microbiota, strengthening the intestinal barrier, and modulating the immune system.

Aeriscardovia aeriphila, also termed *Bifidobacterium aerophilum*, is a Gram-positive anaerobic bacterium that may have beneficial effects on host health (Hirayama et al., 2011). *A. aeriphila* has been demonstrated to exhibit probiotic properties, such as the ability to adhere to intestinal epithelial cells, antimicrobial activity against pathogenic microorganisms,

✉ Xianghua YAN, xhyan@mail.hzau.edu.cn
Xianghua YAN, https://orcid.org/0000-0003-2238-6218

Received Dec. 14, 2022; Revision accepted Apr. 16, 2023;
Crosschecked Aug. 11, 2023; Published online Sept. 22, 2023

© Zhejiang University Press 2023

and resistance to gastrointestinal conditions such as low pH and bile salts (García-Cayuela et al., 2014), promoting a balanced inflammatory response and potentially preventing the onset of certain autoimmune or inflammatory diseases (Ferrario et al., 2016), or even having an impact on weight management possibly by modulating lipid metabolism or influencing satiety signals (Zheng et al., 2020). However, its potential as a probiotic for poultry production remains unexplored. However, there is limited research specifically on the effects of *A. aeriphila* in poultry production, specifically in broiler chickens. We hypothesize that *A. aeriphila* may also have a positive influence on broiler chickens. To test this hypothesis, the present study aims to evaluate the effects of *A. aeriphila* on broiler chicken growth performance, antioxidant functions, immune responses, and gut microbiota composition.

2 Materials and methods

2.1 Bacteria

A. aeriphila was isolated from chicken feces using chopped meat medium-78 and bifidobacterium medium-58 (DSMZ GmbH). The broth was filtered and centrifuged to collect bacteria and then suspended in phosphate-buffered saline (PBS).

2.2 Experimental design and sample collection

A total of 48 1-d-old Arbor Acre commercial broiler chickens ((43.5±0.1) g) were randomly divided into two groups, the control and *A. aeriphila* treatment groups, with six replicates per group and each replicate containing four chickens. In the control group, PBS (1 mL) was given orally for up to 21 d. In the treatment group, *A. aeriphila* (1 mL, 1×10^9 CFU/mL (CFU: colony-forming units)) was orally administered to chickens for 21 d. For sampling, after a 12-h overnight fast, two chickens from each replicate were chosen. The basal corn-soybean meal diets in the manuscript were formulated to meet the chicken's nutrient requirements (China, NY/T 33-2004, 2004), as described in the Arbor Acre broilers diets by Wang et al. (2023). The broilers were housed in 3-layer galvanized iron wire cages in an enclosed space with exhaust blowers for ventilation, with one pen per duplicate. The room temperature was kept at 30–33 ℃ with a temperature decrease of 1 ℃ every 5 d. The relative humidity was maintained at 65%–70%. The provision of artificial light lasted for 23 h. Throughout the trial, food and water were freely given to all the chickens.

Blood samples were collected from the wing vein and then centrifuged at 3000 r/min for 10 min at 4 °C to separate the serum. The serum samples were stored at −80 °C for further analysis. Chickens were humanely euthanized using cervical dislocation. For the analysis of intestinal microbiota, intestinal contents were aseptically collected, placed into sterile centrifuge tubes, and immediately snap-frozen in liquid nitrogen. The small intestine (duodenum, jejunum, and cecum) samples were divided into the appropriate sections and then promptly frozen in liquid nitrogen for further analysis.

2.3 Measurement of growth performance

The body weights and feed consumption were measured at 1 and 21 d of age. The average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated at 21 d of age.

2.4 Serum biochemical index analysis

Serum was examined using a blood chemistry analyzer (Fuji Film Co., Ltd., Tokyo, Japan) to assess blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total cholesterol (TCHO), total protein (TP), albumin (ALB), total bilirubin (TBIL), triglyceride (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). In addition, the growth hormone (GH) and insulin-like growth factor-1 (IGF-1) levels were assessed using commercially available kits (Beijing North Institute of Biological Technology, Beijing, China).

2.5 Serum antioxidant capacity analysis

The levels of malondialdehyde (MDA) and catalase (CAT) and the total antioxidant capacity (T-AOC) were measured in the homogeneous supernatant of the serum using commercial test kits (Nanjing Jiancheng Bioengineering Institute of China, Nanjing, China) following the protocols described by the manufacturers.

2.6 Intestinal immune function analysis

To eliminate tiny particles, samples were centrifuged for 10 min at 12000*g* using the double-antigen sandwich method after being soaked in PBS (100 μL per 10 mg of the sample) and homogenized using a

homogenizer. The levels of immunoglobulin M (IgM), IgA, IgG, serum glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), interleukin-1 (IL-1), IL-4, IL-6, IL-10, transforming growth factor-β (TGF-β), interferon-γ (IFN-γ), and secretory IgA (SIgA) in small intestine contents (duodenum, jejunum, and cecum) were measured using BioAssay™ enzyme-linked immunosorbent assay (ELISA) kits (USBiological Life Sciences, MA, USA).

2.7 qPCR

The animal tissues were transferred into 1.5-mL centrifuge tubes containing TRIzol reagent (Invitrogen, CA, USA). For intestine tissues, the materials were homogenized for 120 s at 4 ℃. Total RNA from the sample was extracted in accordance with the reagent's instructions. A NanoDrop 2000 (Thermo Fisher Scientific, Massachusetts, USA) was used to measure the quantity and quality of RNA in the samples. The generation of complementary DNA (cDNA) from total RNA was performed using a synthesis kit (ABclonal, Wuhan, China). Table 1 shows the creation of primers from GenBank sequences stored on the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov) using basic local alignment search tool (BLAST) and Primer3 (https://primer3.ut.ee). The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). For quantitative real-time polymerase chain reaction (qPCR) on the QuantStudio 6 PCR System (Applied Biosystems, Hammonton, NJ, USA), SYBR Green Supermax (ABclonal) was used with thermocycler conditions of 10 min of 95 ℃ in step 1 and 40 cycles at 95 ℃ for 5 s in step 2 followed by 30 s at 60 ℃. Melting curves between 60 ℃ and 90 ℃ were gathered for IL-2 with step 1 as 3 min of 95 ℃ and step 2 as 40 cycles at 95 °C for 5 s, 20 s at 62 ℃, and 30 s at 72 ℃. The relative expression of messenger RNA (mRNA) was estimated with the $2^{-\Delta\Delta C_T}$ method after the target gene expression was standardized with the mean of the reference genes (Livak and Schmittgen, 2001; Michaelidou et al., 2013).

2.8 Microbial genomic DNA extraction and 16S rDNA sequencing and analysis

DNA kit (QIAamp PowerFecal Pro DNA Kit ID: 51804, QIAGEN, Germany) was used to extract the microbial genomic DNA from the cecal content of chickens. The microbial genomic DNA was extracted according to the procedures from the kit. The V3–V4 regions of bacterial 16S ribosomal DNA (rDNA) were amplified using the 341F (5'-ACTCCTACGGGAG GCAGCAG-3') and 806R (5'-GGACTACHVGGGT WTCTAAT-3') primers. Illumina MiSeq libraries were used for the generation of 2×300 bp reads (BGI, Shenzhen, China). More than 76 000 raw reads from each sample were obtained on average. The low-quality bases were removed from the raw data to splice the paired-end reads to obtain the tags using FLASH software (Magoč and Salzberg, 2011). In each sample, more than 68 000 clean reads and 34 000 tags were acquired on average. Subsequently, the tags were grouped into operational taxonomic units (OTUs) (97% confidence level) using the UPARSE method. Chimeric sequences were eliminated using UCHIME with the Gold database (v4.2.40) (Edgar et al., 2011; Edgar, 2013). The OTU taxonomy was determined using the Ribosomal Database Project (RDP) classifier v.2.2 with 0.6 OTUs as a minimum confidence level. OTUs were trained using the Greengenes database (v201305) through quantitative insights into microbial ecology (QIIME) (v1.8.0) (Caporaso et al., 2010). The diversity (alpha and beta) of OTUs was examined using MOTHUR (v1.31.2) and QIIME (v1.8.0), respectively. Principal component analysis (PCA) of OTUs was conducted using the package "ade4" of R software. Utilizing QIIME 2, phylogenetic beta diversity measurements, such as principal co-ordinates analysis (PCoA), were carried out. Taxonomic biomarkers were found using

Table 1 Primers used for qPCR analysis

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
HMBS	GGCTGGGAGAATCGCATAGG	TCCTGCAGGGCAGATACCAT	Khan et al., 2017
β-actin	CACAGATCATGTTTGAGACCTT	CATCACAATACCAGTGGTACG	de Boever et al., 2008
IL-4	GTGCCCACGCTGTGCTTAC	AGGAAACCTCTCCCTGGATGTC	Lammers et al., 2010
IL-10	CGCTGTCACCGCTTCTTCA	TCCCGTTCTCATCCATCTTCTC	Liu SQ et al., 2018
NF-κB	GCACAACGCCTCTTCACATA	GGCTCAAAGTTCTCAACGTG	NM_001396395

qPCR: quantitative real-time polymerase chain reaction; *HMBS*: hydroxymethylbilane synthase; *IL-4*: interleukin-4; *NF-κB*: nuclear factor-κB.

a study of the linear discriminant analysis (LDA) effect size (LEfSe) with LDA score>2.0 and P<0.05. Key biomarkers were defined with LDA score≥4.0 and P<0.05.

2.9 Statistical analysis

GraphPad Prism 9 was used to conduct significance analyses using two-tailed Student's t-test. The data are presented as mean±standard deviation (SD), and each data point is shown in the graph. P values of <0.05 were considered significant.

3 Results

3.1 Effects of *A. aeriphila* on growth performance of broilers

Our data indicated that oral gavage of *A. aeriphila* significantly increased the ADG (P<0.001, Fig. 1b) compared to the control group, while there was no significant difference in ADFI (P>0.05, Fig. 1c). As a result, the FCR was significantly reduced by *A. aeriphila* treatment (P<0.001, Fig. 1a) compared to the control group. Moreover, *A. aeriphila* treatment resulted in significant increases in the levels of GH (P<0.01, Fig. 1d) and IGF-1 (P<0.05, Fig. 1e) compared to the control group.

3.2 Effects of *A. aeriphila* treatment on serum biochemistry in broilers

The effects of *A. aeriphila* treatment on serum biochemistry are shown in Fig. 2. Notably, *A. aeriphila* treatment led to a significant decrease in the levels of serum AST (P<0.01, Fig. 2b) and BUN (P<0.05, Fig. 2e). Conversely, the levels of serum GLU (P< 0.001, Fig. 2c) and CRE (P<0.05, Fig. 2d) were significantly increased after *A. aeriphila* treatment. On the other hand, no significant differences were observed in the levels of serum TBIL (Fig. 2a), ALB (Fig. 2f), TG (Fig. 2g), TP (Fig. 2h), ALT (Fig. 2i), and TCHO (Fig. 2j) between the control and *A. aeriphila* groups.

3.3 Effects of *A. aeriphila* on antioxidant capacity in broilers

The effects of *A. aeriphila* on antioxidant activity are shown in Fig. 3. Treatment with *A. aeriphila* led to significant increases in serum GSH-Px (P<0.05, Fig. 3a), CAT (P<0.01, Fig. 3b), T-AOC (P<0.05, Fig. 3c), and SOD (P<0.001, Fig. 3d) activity. However, the serum MDA level was not significantly altered by *A. aeriphila* treatment (Fig. 3e). These results suggested that *A. aeriphila* treatment enhanced the antioxidant capacity in broilers.

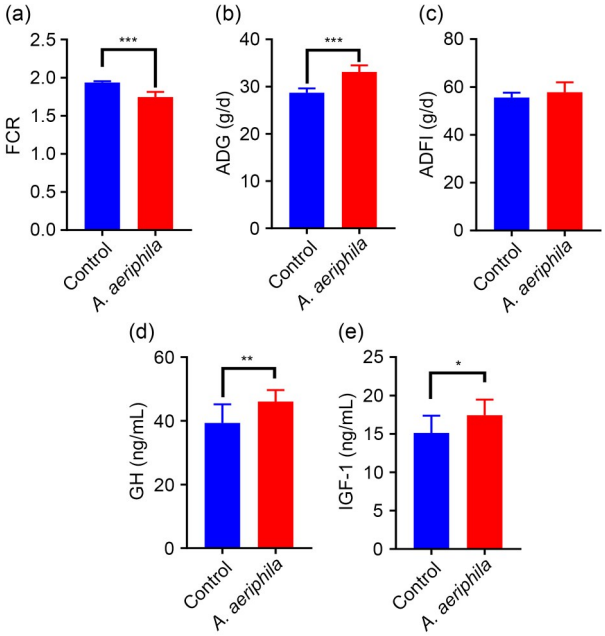

Fig. 1 Effects of *Aeriscardovia aeriphila* on growth performance and the levels of serum hormones in broilers. (a) Feed conversion ratio (FCR) of broilers; (b) Average daily gain (ADG) of broilers; (c) Average daily feed intake (ADFI) of broilers; (d) Serum growth hormone (GH) levels in broilers; (e) Serum insulin-like growth factor-1 (IGF-1) levels in broilers. The data are presented as mean±standard deviation (SD), with n=6 (a–c) and n=10 (d, e), and were evaluated by Student's t-test ($^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001).

3.4 Alterations in the levels of blood immunoglobulins in broilers by *A. aeriphila* treatment

The impact of *A. aeriphila* treatment on immune responses was evaluated and is presented in Fig. 4. Treatment with *A. aeriphila* resulted in significant increases in serum IgA (P<0.05, Fig. 4a), IgG (P<0.01, Fig. 4b), and IgM (P<0.05, Fig. 4c) levels. The level of serum IL-1 (P<0.05, Fig. 4d) was significantly decreased, while the levels of serum IL-4 (P<0.05, Fig. 4e) and serum IL-10 (P<0.05, Fig. 4g) were significantly increased by treatment with *A. aeriphila*. There was no obvious difference in the serum IL-6 (Fig. 4f), TGF-β (Fig. 4h), or IFN-γ (Fig. 4i) level between the control and *A. aeriphila* groups.

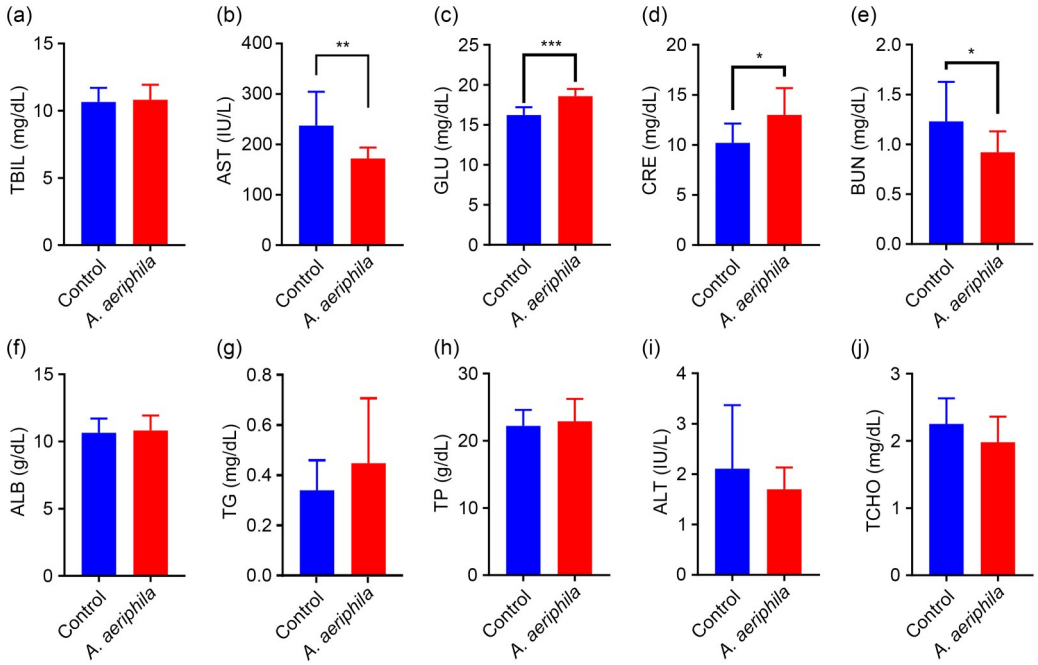

Fig. 2 Serological analyses of the levels of serum total bilirubin (TBIL) (a), aspartate aminotransferase (AST) (b), glucose (GLU) (c), creatinine (CRE) (d), blood urea nitrogen (BUN) (e), albumin (ALB) (f), triglyceride (TG) (g), total protein (TP) (h), alanine aminotransferase (ALT) (i), and total cholesterol (TCHO) (j) in broilers. The data are presented as mean±standard deviation (SD), with n=10, and were evaluated by Student's t-test ($^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001).

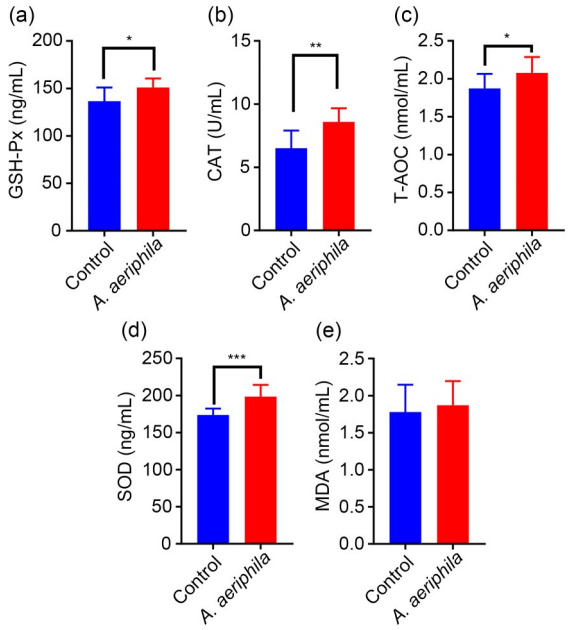

Fig. 3 Effects of *Aeriscardovia aeriphila* on the redox state and antioxidant levels in broilers. The impacts of *A. aeriphila* on the glutathione peroxidase (GSH-Px) (a), catalase (CAT) (b), total antioxidant capacity (T-AOC) (c), and superoxide dismutase (SOD) (d) activity, and the malondialdehyde (MDA) level (e) in broilers. The results are represented as mean±standard deviation (SD), with n=10, and were evaluated by Student's t-test ($^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001).

3.5 Effects of *A. aeriphila* treatment on the immune responses in the intestinal mucosa of broilers

To investigate the impact of *A. aeriphila* treatment on intestinal mucosal immune responses, we measured mucosal immune-related immunoglobulin in the small intestine of chickens. Treatment with 1×10^{9} CFU/mL *A. aeriphila* led to higher production of SIgA in the intestinal fluid of the duodenum (P<0.01, Fig. 5a), jejunum (P<0.001, Fig. 5b), and cecum (P<0.001, Fig. 5c). Furthermore, *A. aeriphila* treatment resulted in significant increases in the mRNA levels of *IL-4* (P<0.05, Fig. 5d) and *IL-10* (P<0.001, Fig. 5e), but a significant decrease in nuclear factor-κB (*NF-κB*) (P<0.001, Fig. 5f). These findings suggested that *A. aeriphila* treatment enhanced intestinal mucosal immune responses in broilers.

3.6 Alterations in gut bacterial diversity of broilers by *A. aeriphila* treatment

A total of 76 000 reads were obtained from each sample after preliminary quality screening, with an average of 68 000 reads per sample and a median read length of 300 bp. The total number of OTUs at a more than 97% similarity level was 34 000. The number of

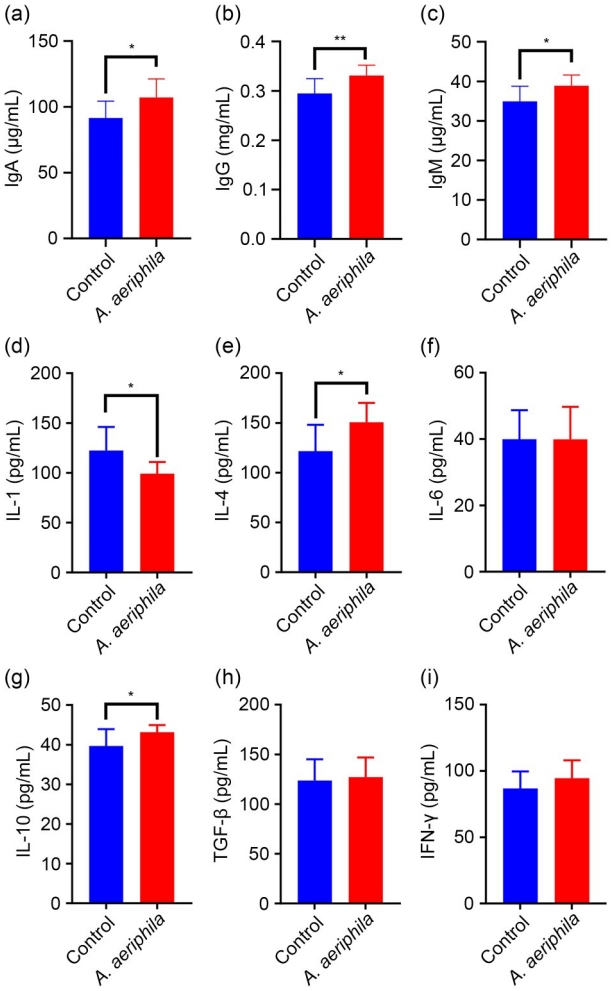

Fig. 4 Effects of oral administration of *Aeriscardovia aeriphila* on the levels of immunoglobulins and cytokine secretion. Levels of serum immunoglobulin A (IgA) (a), IgG (b), IgM (c), interleukin-1 (IL-1) (d), IL-4 (e), IL-6 (f), IL-10 (g), transforming growth factor-β (TGF-β) (h), and interferon-γ (IFN-γ) (i) in broilers. The results are represented as mean±standard deviation (SD), with n=10, and were evaluated by Student's t-test ($^{*}P$<0.05, $^{**}P$<0.01).

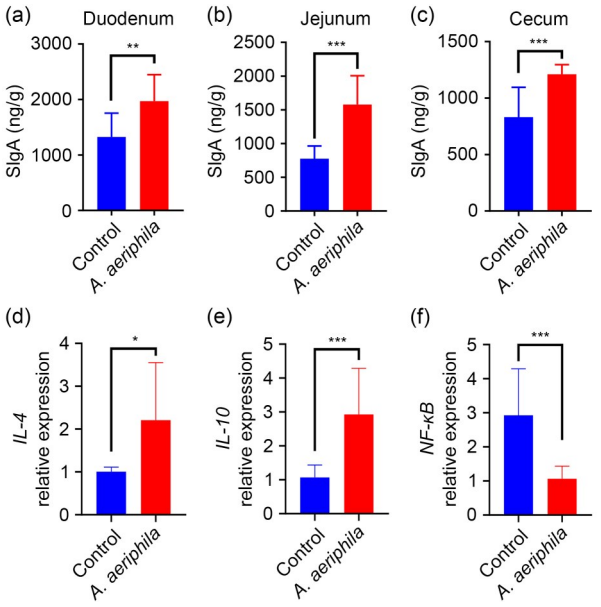

Fig. 5 Levels of intestinal secretory immunoglobulin A (SIgA) and messenger RNA (mRNA) relative expression of interleukin-4 (*IL-4*), *IL-10*, and nuclear factor-κB (*NF-κB*) in the control and *Aeriscardovia aeriphila* groups. The levels of intestinal SIgA in the duodenum (a), jejunum (b), and cecum (c) and the mRNA relative expression of *IL-4* (d), *IL-10* (e), and *NF-κB* (f) in control and *A. aeriphila*-treated broilers. Data are shown as mean±standard deviation (SD), with n=10, and were evaluated by Student's t-test ($^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001).

OTUs in the dilution curve increased parallel to the number of sequences, indicating sufficient sequencing quantity for each sample for the characterization of the gut microbiota composition (Fig. 6a). The steep curve indicated decreased species evenness. The library had an average coverage of 99.91% (Fig. 6b), and the flat tendency of the Shannon dilution curve indicated reasonable sequencing data in this study (Fig. 6c). Most of the indices, i.e., abundance-based coverage estimator (ACE) (Fig. 7a), Chao1 richness (Chao1) (Fig. 7b), coverage (Fig. 7c), evenness (Fig. 7d), Shannon (Fig. 7e), and Simpson (Fig. 7f), reflected the alpha diversity of microbial communities between the control and *A. aeriphila* groups. Alpha diversity indices were not significantly changed by *A. aeriphila*, as evidenced by the Student's t-test (P>0.05). There was a distinct separation between the control and *A. aeriphila* groups in the PCoA analysis, with a lower degree of dispersion among the control samples than among the *A. aeriphila* samples (Fig. 7g). PCo1 explained 20.97%, and PCo2 explained 15.91% of the variance in the PCoA, cumulatively explaining 36.88% of the species composition changes.

3.7 Comparative analysis of gut bacterial taxonomic composition between *A. aeriphila* and control groups

At the phylum level, Firmicutes was identified as the dominant phylum (62.89%), followed by Bacteroidetes (26.38%) in both groups. Notably, the *A. aeriphila* group had a higher Proteobacteria content (6.16%) than the control group (Fig. 8a). At the class level, the *A. aeriphila* group demonstrated an increased abundance of Clostridia (57.04%), Actinobacteria (0.26%), Epsilonproteobacteria (5.06%), and

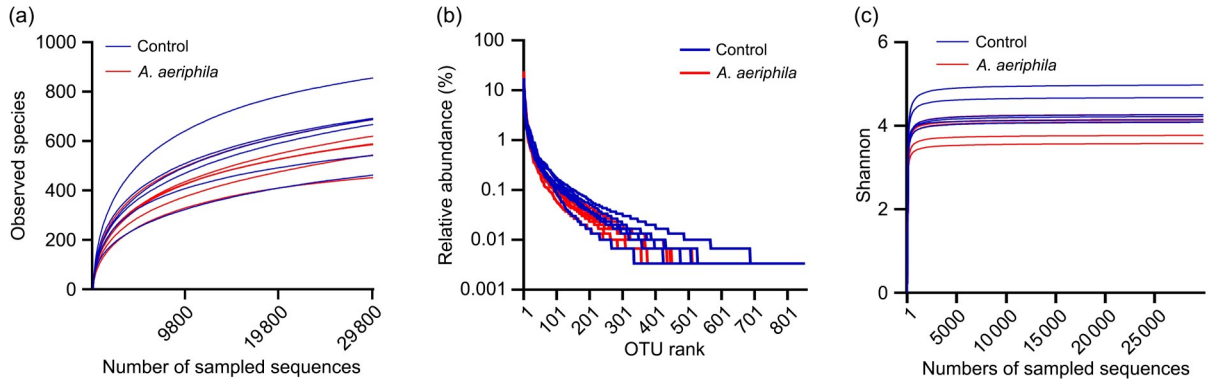

Fig. 6 Average proportion of each 16S sequence read attributed to each taxon in the control and *Aeriscardovia aeriphila* groups. (a) Species dilution curve based on operational taxonomic units (OTUs); (b) Rank curve based on OTUs; (c) Shannon dilution curve.

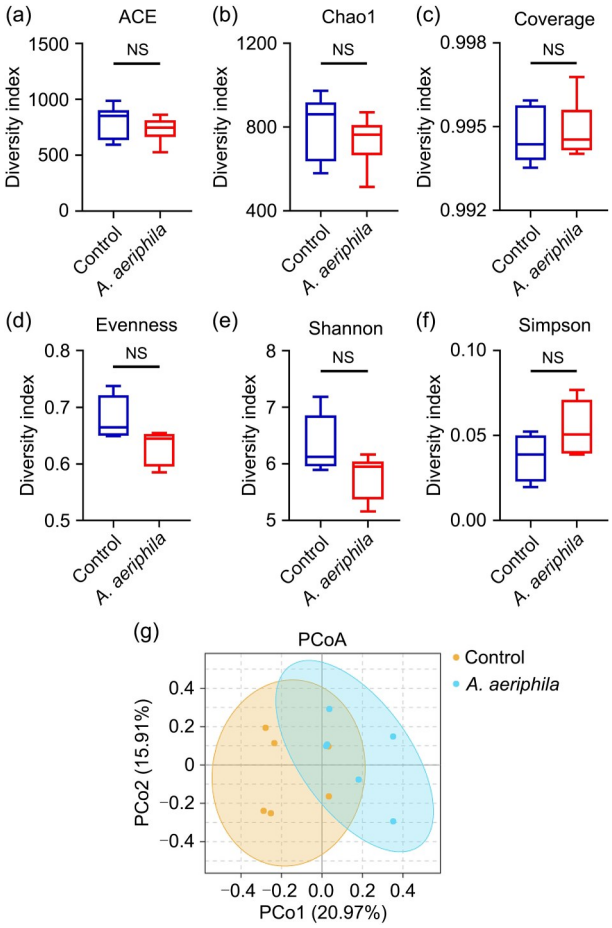

Fig. 7 Alpha and beta diversities of operational taxonomic units (OTUs) in *Aeriscardovia aeriphila* and control groups. (a) Abundance-based coverage estimator (ACE) index; (b) Chao1 richness (Chao1) index; (c) Coverage; (d) Evenness; (e) Shannon index; (f) Simpson index; (g) Principal coordinates analysis (PCoA) of bacterial communities. Data are expressed as mean±standard deviation (SD), with n=10, and were evaluated by unpaired Student's t-test (NS: not significant; P>0.05).

Bacteroidia (25.93%) compared to the control group (Fig. 8b). At the order level (Fig. 8c), the *A. aeriphila* group exhibited a higher abundance of Clostridiales (58.15%), Campylobacterales (4.15%), Bacteroidales (27.54%), and Lactobacillales (1.51%). At the family level, the *A. aeriphila* group showed an increased presence of Ruminococcaceae (33.73%), Lactobacillaceae (1.27%), Helicobacteraceae (5.02%), and Bacteroidaceae (11.36%) compared to the control group (Fig. 8d). Last, at the genus level, the analysis revealed that the relative abundances of *Faecalibacterium* (7.74%), *Lactobacillus* (1.27%), *Barnesiella* (6.56%), and *Alistipes* (7.96%) increased in response to *A. aeriphila* supplementation (Fig. 8e).

3.8 LEfSe analysis and cladogram of the gut bacterial communities in broilers

LEfSe was applied to identify high-dimensional biomarkers between the control and *A. aeriphila* groups, and the cladogram represented different levels of taxonomy showing the significantly different taxa (Fig. 9a). The differentially abundant taxa were determined using the LDA model, which estimated the effect size of each significantly different taxon. LEfSe analysis revealed the abundance of the phyla Proteobacteria, Epsilonproteobacteria, Campylobacterales, and Helicobacteraceae and the genus *Helicobacter* in the *A. aeriphila* group samples (Fig. 9b). At the family level, nine families showed significantly different proportions in the *A. aeriphila* group compared to the control group. Four families, Acidaminococcaceae, Helicobacteraceae, Porphyromonadaceae, and Pasteurellaceae exhibited increased abundance (P<0.05). Conversely, five families, Clostridiales_Incertae_Sedis_XIII,

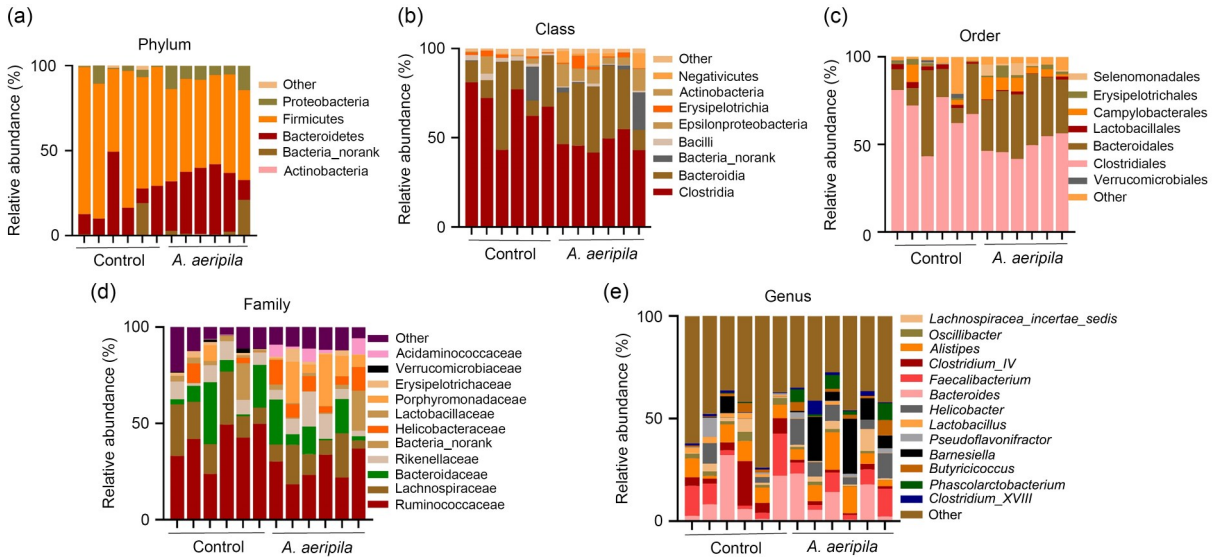

Fig. 8 Taxonomic compositions of gut microbiota in broilers. Gut bacterial taxonomic compositions at the phylum (a), class (b), order (c), family (d), and genus (e) levels.

Verrucomicrobiaceae, Streptococcaceae, Ruminococcaceae, and Lactobacillaceae, displayed decreased abundance in the *A. aeriphila* group compared to the control group (Fig. 9c). Genus-level analysis presented more information revealing the overabundance of the genera *Barnesiella* and *Phascolarctobacterium* in *A. aeriphila*. However, the genera *Lactobacillus*, *Clostridium IV*, *Streptococcus*, *Akkermansia*, and *Oscillibacter* were abundant in the control group (Fig. 9d).

4 Discussion

Recent research has shown that probiotics, such as *Bacillus* spp., *Lactobacillus* spp., and *Saccharomyces* spp., could improve the growth performance of livestock (Cao et al., 2019; Lokapirnasari et al., 2019; Massacci et al., 2019). Increasing evidence supports the idea that dietary *Bacillus subtilis* or *Bacillus licheniformis* could improve hen growth performance, especially in light of the growing interest in probiotics and their use in animal research (Bader et al., 2012; Liu et al., 2012; Chen and Yu, 2020). In line with this research, our findings showed that oral gavage of *A. aeriphila* enhanced growth performance by increasing the ADG and ADFI, and decreasing the FCR during the feeding phase. A previous study demonstrated that *Bacillus coagulans*, a probiotic, improves growth-related indicators in chickens and could boost chicken immunity and reduce intestinal inflammation. GH, 3,5,3'-triiodothyronine, thyroxine, and IGF-1 are the main hormones needed to sustain adequate growth in chickens (Scanes, 2009). In the current study, serum GH and IGF-1 levels were determined. The results demonstrated that the *A. aeriphila* group had higher levels of serum GH and IGF-1 than the control group. Our findings support the judgments of Beckman et al. (1998), who discovered a strong and positive association between the mean plasma IGF-1 levels and the mean growth rates of young Chinook salmon. Supplementation of *B. coagulans* in broiler diets alleviated necrotic enteritis-caused loss in body weight gain, as previously described (Khalique et al., 2020). Similarly, our research findings revealed that oral gavage of *A. aeriphila* bacteria into Arbor Acre broiler chickens for 21 d increased broiler body weight, ADG, and FCR considerably compared to the control group.

Oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, can lead to oxidative damage in broiler chickens. This damage can manifest as lipid peroxidation, protein oxidation, and DNA damage, ultimately impairing cellular function and contributing to various pathological conditions, including growth retardation, immunosuppression, and reduced meat quality (Surai et al., 2017). Antioxidant enzymes are crucial components of the animal defense system against oxidative damage caused

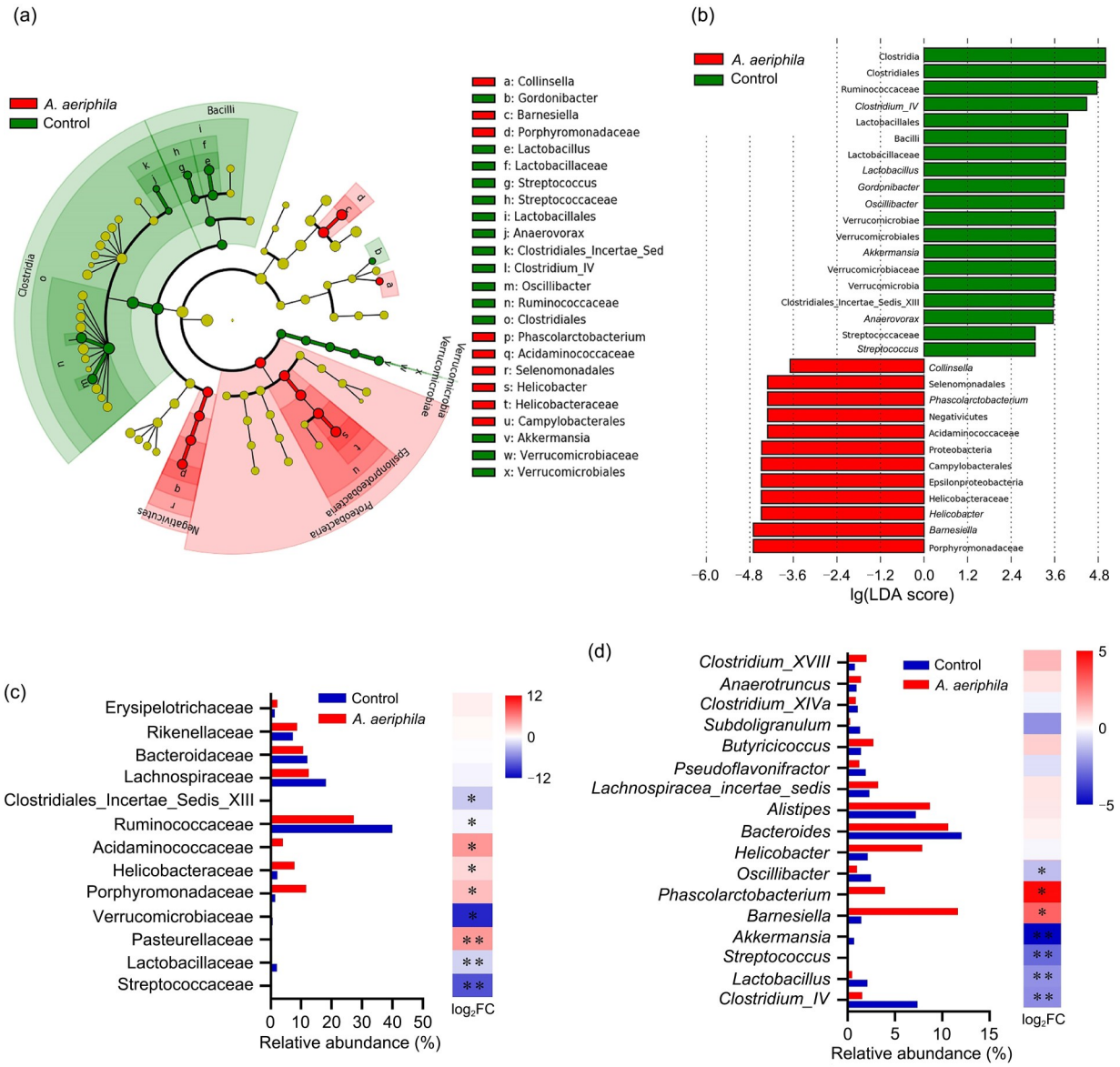

Fig. 9 Taxonomic differences between the control and *Aeriscardovia aeriphila* groups. (a) Cladogram based on the linear discriminant analysis (LDA) effect size (LEfSe) showing differentially abundant taxonomic clades with an LDA score of >4.0 between the *A. aeriphila* and control groups. Biomarker taxa are heighted by colored circles, and the circle diameter is relative to the abundance of biomarkers. (b) LEfSe between *A. aeriphila* (red) and control (green). (c, d) Boxplots representing the average proportion of each 16S sequence read attributed to each taxon at the family level (c) and at the genus level (d) (blue, control samples; red, *A. aeriphila* samples). Data are represented as mean±standard deviation (SD), with *n*=10, and were evaluated by unpaired Student's *t*-test (* *P*<0.05, ** *P*<0.01). FC: fold change.

by an imbalance between ROS production and the antioxidant defense system. The primary enzymes in the antioxidant system, including GSH-Px, SOD, and CAT, play key roles in scavenging ROS (Liu et al., 2020). In the present study, broilers were administered *A. aeriphila* orally, which led to increases in GSH-Px, SOD, T-AOC, and CAT activity, indicating that broiler antioxidant capacity might be enhanced. *A. aeriphila*, as a member of *Bifidobacteria*, is capable of producing short chain fatty acids (SCFAs) (Russell et al., 2011). Among these SCFAs, butyric acid has been demonstrated to stimulate the synthesis of endogenous antioxidants, which may explain how *A. aeriphila* improved antioxidant enzymes directly. We found no statistically significant changes in MDA levels when *A. aeriphila* bacteria were administered orally to broilers, which may be because the dosage and duration of *A. aeriphila* supplementation in our study might not

have been sufficient to elicit a noticeable effect on MDA levels. Consistent with our findings, *B. coagulans* supplementation in the diet dramatically increased the serum SOD and CAT activity in broilers and noticeably decreased the serum MDA content, as previously reported (Lin et al., 2014). Thus, *A. aeriphila* may have a role in protecting broilers from oxidative stress-induced damage, which is a significant factor in how well it can enhance meat and poultry production. The levels of serum immunoglobulins (IgA, IgG, and IgM) were increased following *A. aeriphila* treatment in this study. IgA, IgM, and IgY are important immunological components in birds, and immunoglobulins have a protective function in vivo. IgM was involved in the process of acute infection, whereas IgA was involved in the mucosal immune response, as previously described (Bian et al., 2016). In line with this, *A. aeriphila* treatment significantly increased the levels of SIgA in broiler (duodenum, jejunum, and cecum) intestinal contents. Similarly, the *A. aeriphila* group showed significantly higher levels of relative expression of *IL-10* and *IL-4* mRNAs and a markedly reduced level of *NF-κB* mRNA in the intestinal mucosa of chickens, suggesting decreased intestinal inflammation. Additionally, we discovered that the levels of serum IL-10, IL-4, and IL-1 were higher than those of the pro-inflammatory molecules IL-6, TGF-β, and IFN-γ in the broilers treated with *A. aeriphila*. According to the aforementioned findings, *A. aeriphila* bacteria may facilitate the regulation of immunological responses. These findings support the following judgments: chickens exhibit immunoregulation for *B. coagulans*, according to previous studies (Panda et al., 2005; Bai et al., 2013).

ALT and AST are enzymes primarily found in the liver, and their levels in serum can be used to assess liver function and possible damage (Thapa and Walia, 2007). TP and ALB are indicators of the nutritional and metabolic status of broilers, with TP reflecting the total concentration of serum proteins and ALB being the main protein in serum, playing an important role in maintaining oncotic pressure and transporting various molecules (Livingston et al., 2020). TBIL is a breakdown product of heme and an indicator of liver function and bile metabolism (Birbrair et al., 2013). In the present study, serum concentrations of ALT, TP, ALB, and TBIL were not significantly affected, while there was a significant decrease in AST enzyme following *A. aeriphila* supplementation. These results concur with those of Djouvinov et al. (2005), who discovered that probiotic treatment had no impact on chicken TP concentrations. A significant increase (P<0.05) in the serum GLU concentration of the treatment group was observed in our investigation compared to the control group. This increase could be attributed to a modest improvement in lactose absorption and gluconeogenesis, as stated by Das et al. (2005). While Alaqaby et al. (2014) claimed that broilers fed probiotics showed no changes in blood GLU levels, our results differed from their findings. Our findings showed that there was a significant difference in the levels of BUN and CRE in the *A. aeriphila* group compared to the control group. BUN and plasma CRE were associated with the functionality and health of the kidneys. Concerning the effect of probiotic supplementation on serum TCHO and TG, a nonsignificant effect was found between *A. aeriphila* and the control group. Similar findings were made by Panda et al. (2006), who discovered that dietary supplementation with probiotics comprising *Lactobacillus sporogenes* at 100 mg/kg diet significantly decreased the levels of blood TCHO and TG. The considerable drop in blood TCHO seen in broiler hens fed diets supplemented with probiotics may be due to decreased gastrointestinal tract TCHO absorption and synthesis caused by probiotic supplementation, as described by Mohan et al. (1995, 1996). Additionally, it was hypothesized that *Lactobacillus acidophillus* lowers blood TCHO by deconjugating bile salts in the colon and preventing them from serving as precursors for cholesterol synthesis (Abdulrahim et al., 1996). Some investigations have shown that dietary supplementation with *Clostridium butyricum* has impacts on the intestinal microbiota in addition to the effects on the intestinal mucosa (Liu X et al., 2018).

In this study, the cecal contents of 21-d-old broilers were sequenced using bacterial 16S rDNA gene amplicon sequencing. Although there were no significant differences between the *A. aeriphila* and control groups in terms of alpha diversity indices, the beta diversity results showed a separation, indicating that there were some differences in the microbial communities between the *A. aeriphila* and control groups. According to the phylum-level classification, Firmicutes dominated both groupings. Relative to the control group, the *A. aeriphila* group had a greater Firmicutes content. The ability to absorb nutrients has been associated with Firmicutes and Bacteroides in digesta. Greater

nutritional absorption may result from having more Firmicutes in the population labelled by Adalsteinsdottir et al. (2018), whereas an increase in Bacteroides could be the opposite, in line with our findings. Further examination of the microbiota at the genus level showed that the *A. aeriphila* group had greater relative concentrations of *Butyricicoccus*, *Lactobacillus*, *Barnisiella*, and *Phascolarctobacterium* than the control group. *Butyricicoccus* and *Phascolarctobacterium* are butyrate-producing bacteria known to have anti-inflammatory properties and promote intestinal epithelial integrity (Louis and Flint, 2009). The increased abundance of *Butyricicoccus* in the *A. aeriphila* group might contribute to improved gut health and immune function in broilers. *Lactobacillus* are probiotic bacteria that inhibit the growth of harmful bacteria, regulate mucosal immunity, and stimulate the production of antimicrobial substances (Hill et al., 2014), which might contribute to improving growth performance and immunity in broilers. *Barnisiella* has been reported to produce SCFAs and improve intestinal health (Vital et al., 2014). SCFAs can modulate immune function, improve gut barrier function, and provide energy for intestinal cells. In conclusion, oral supplementation with *A. aeriphila* led to alterations in the microbial community composition, with an increased abundance of specific taxa, suggesting a potential positive impact on the growth and immunity of broilers. These bacteria may contribute to improved nutrient absorption, metabolism, and immune function by promoting a balanced gut microbiota, producing SCFAs, and inhibiting the growth of harmful bacteria.

5 Conclusions

In summary, oral administration of *A. aeriphila* contributes to improving the growth performance, serum antioxidant capacity, immunological functions, and gut health of broilers. In particular, a blooming of Firmicutes, particularly from the *Anaerotruncus* genus, was observed in the gut microbiota of broilers. Our research may support the application of *A. aeriphila* in poultry production to improve the growth performance and gut health of broilers.

Data availability statement

All data generated or analyzed during this study can be made available by the corresponding author upon reasonable request.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 31925037). We thank Yan lab's members for helpful discussion and critical reading of the manuscript.

Author contributions

Muhammad Zahid FAROOQ and Xinkai WANG performed the experimental research and data analysis, wrote and edited the manuscript. Xianghua YAN contributed to the study design, data analysis, writing and editing of the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Xianghua YAN is an editorial board member for *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)* and was not involved in the editorial review or the decision to publish this article. Muhammad Zahid FAROOQ, Xinkai WANG, and Xianghua YAN declare that they have no conflict of interest.

All experimental procedures involving chickens were approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University (approval number: HZAUCH-2019-014).

References

Abdulrahim SM, Haddadin MSY, Hashlamoun EAR, et al., 1996. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. *Br Poultry Sci*, 37(2):341-346. https://doi.org/10.1080/00071669608417865

Adalsteinsdottir SA, Magnusdottir OK, Halldorsson TI, et al., 2018. Towards an individualized nutrition treatment: role of the gastrointestinal microbiome in the interplay between diet and obesity. *Curr Obes Rep*, 7(4):289-293. https://doi.org/10.1007/s13679-018-0321-z

Alaqaby ARA, Glaskovich AA, Kapitonova EA, et al., 2014. Study the effect of using probiotic (vetlactoflorum) on some of biochemical and immunological parameters of broiler chickens. *Basrah J Vet Res*, 13(1):166-179. https://doi.org/10.33762/bvetr.2014.88137

Bader J, Albin A, Stahl U, 2012. Spore-forming bacteria and their utilisation as probiotics. *Benefic Microbes*, 3(1):67-75. https://doi.org/10.3920/BM2011.0039

Bai SP, Wu AM, Ding XM, et al., 2013. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poult Sci*, 92(3):663-670. https://doi.org/10.3382/ps.2012-02813

Beckman BR, Larsen DA, Moriyama S, et al., 1998. Insulin-like growth factor-I and environmental modulation of

growth during smoltification of spring chinook salmon (*Oncorhynchus tshawytscha*). *Gen Comp Endocrinol*, 109(3): 325-335.
https://doi.org/10.1006/gcen.1997.7036

Bian XF, Wallstrom G, Davis A, et al., 2016. Immunoproteomic profiling of antiviral antibodies in new-onset type 1 diabetes using protein arrays. *Diabetes*, 65(1):285-296.
https://doi.org/10.2337/db15-0179

Birbrair A, Zhang T, Wang ZM, et al., 2013. Role of pericytes in skeletal muscle regeneration and fat accumulation. *Stem Cells Dev*, 22(16):2298-2314.
https://doi.org/10.1089/scd.2012.0647

Buntyn JO, Schmidt TB, Nisbet DJ, et al., 2016. The role of direct-fed microbials in conventional livestock production. *Annu Rev Anim Biosci*, 4:335-355.
https://doi.org/10.1146/annurev-animal-022114-111123

Cao GT, Dai B, Wang KL, et al., 2019. *Bacillus licheniformis*, a potential probiotic, inhibits obesity by modulating colonic microflora in C57BL/6J mice model. *J Appl Microbiol*, 127(3):880-888.
https://doi.org/10.1111/jam.14352

Caporaso JG, Kuczynski J, Stombaugh J, et al., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*, 7(5):335-336.
https://doi.org/10.1038/nmeth.f.303

Chen YC, Yu YH, 2020. *Bacillus licheniformis*-fermented products improve growth performance and the fecal microbiota community in broilers. *Poult Sci*, 99(3):1432-1443.
https://doi.org/10.1016/j.psj.2019.10.061

Cheng GY, Hao HH, Xie SY, et al., 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Front Microbiol*, 5:217.
https://doi.org/10.3389/fmicb.2014.00217

Das HK, Medhi AK, Islam M, 2005. Effect of probiotics on certain blood parameters and carcass characteristics of broiler chicken. *Indian J Poult Sci*, 40(1):83-86.
https://doi.org/10.21608/epsj.2022.225224

de Boever S, Vangestel C, de Backer P, et al., 2008. Identification and validation of housekeeping genes as internal control for gene expression in an intravenous LPS inflammation model in chickens. *Vet Immunol Immunopathol*, 122(3-4): 312-317.
https://doi.org/10.1016/j.vetimm.2007.12.002

Djouvinov D, Boicheva S, Simeonova T, et al., 2005. Effect of feeding Lactina® probiotic on performance, some blood parameters and caecal microflora of mule ducklings. *Trakia J Sci*, 3(2):22-28.

Edgar RC, 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*, 10(10): 996-998.
https://doi.org/10.1038/nmeth.2604

Edgar RC, Haas BJ, Clemente JC, et al., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27(16):2194-2200.
https://doi.org/10.1093/bioinformatics/btr381

Ferrario C, Milani C, Mancabelli L, et al., 2016. Modulation of the *eps*-ome transcription of *Bifidobacteria* through simulation of human intestinal environment. *FEMS Microbiol Ecol*, 92(4):fiw056.
https://doi.org/10.1093/femsec/fiw056

García-Cayuela T, Korany AM, Bustos I, et al., 2014. Adhesion abilities of dairy *Lactobacillus plantarum* strains showing an aggregation phenotype. *Food Res Int*, 57:44-50.
https://doi.org/10.1016/j.foodres.2014.01.010

Hill C, Guarner F, Reid G, et al., 2014. Expert consensus document: the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*, 11(8):506-514.
https://doi.org/10.1038/nrgastro.2014.66

Hirayama H, Suzuki Y, Abe M, et al., 2011. *Methylothermus subterraneus* sp. Nov., a moderately thermophilic methanotroph isolated from a terrestrial subsurface hot aquifer. *Int J Syst Evol Microbiol*, 61(Pt 11):2646-2653.
https://doi.org/10.1099/ijs.0.028092-0

Khalique A, Zeng D, Shoaib M, et al., 2020. Probiotics mitigating subclinical necrotic enteritis (SNE) as potential alternatives to antibiotics in poultry. *AMB Express*, 10:50.
https://doi.org/10.1186/s13568-020-00989-6

Khan S, Roberts J, Wu SB, 2017. Reference gene selection for gene expression study in shell gland and spleen of laying hens challenged with infectious bronchitis virus. *Sci Rep*, 7:14271.
https://doi.org/10.1038/s41598-017-14693-2

Lammers A, Wieland WH, Kruijt L, et al., 2010. Successive immunoglobulin and cytokine expression in the small intestine of juvenile chicken. *Dev Comp Immunol*, 34(12): 1254-1262.
https://doi.org/10.1016/j.dci.2010.07.001

Lin LH, Ke FR, Zhan TT, et al., 2014. Effects of *Bacillus coagulans* on performance, serum biochemical indices and antioxidant function of yellow broilers. *Chin J Anim Nutr*, 26(12):3806-3813 (in Chinese).
https://doi.org/10.3969/j.issn.1006-267x.2014.12.034

Liu JB, Yan HL, Zhang Y, et al., 2020. Effects of stale maize on growth performance, immunity, intestinal morphology and antioxidant capacity in broilers. *Asian-Australas J Anim Sci*, 33(4):605-614.
https://doi.org/10.5713/ajas.19.0224

Liu SQ, Wang LY, Liu GH, et al., 2018. Leucine alters immunoglobulin a secretion and inflammatory cytokine expression induced by lipopolysaccharide via the nuclear factor-κB pathway in intestine of chicken embryos. *Animal*, 12(9): 1903-1911.
https://doi.org/10.1017/S1751731117003342

Liu X, Li SR, Wang ZY, et al., 2018. Effects of dietary lipids and clostridium butyricum on chicken volatile flavour compounds. *Indian J Anim Res*, 52(8):1167-1173.
https://doi.org/10.18805/ijar.v0iOF.6998

Liu XL, Yan H, Lv L, et al., 2012. Growth performance and meat quality of broiler chickens supplemented with *Bacillus licheniformis* in drinking water. *Asian-Australas J Anim Sci*, 25(5):682-689.
https://doi.org/10.5713/ajas.2011.11334

Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the

$2^{-\Delta\Delta C_T}$ method. *Methods*, 25(4):402-408. https://doi.org/10.1006/meth.2001.1262

Livingston ML, Cowieson AJ, Crespo R, et al., 2020. Effect of broiler genetics, age, and gender on performance and blood chemistry. *Heliyon*, 6(7):e04400. https://doi.org/10.1016/j.heliyon.2020.e04400

Lokapirnasari WP, al Arif A, Soeharsono S, et al., 2019. Improves in external and internal egg quality of Japanese quail (*Coturnix coturnix japonica*) by giving lactic acid bacteria as alternative antibiotic growth promoter. *Iran J Microbiol*, 11(5):406-411. https://doi.org/10.18502/ijm.v11i5.1959

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

Magoč T, Salzberg SL, 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21):2957-2963. https://doi.org/10.1093/bioinformatics/btr507

Massacci FR, Lovito C, Tofani S, et al., 2019. Dietary *Saccharomyces cerevisiae boulardii* CNCM I-1079 positively affects performance and intestinal ecosystem in broilers during a *Campylobacter jejuni* infection. *Microorganisms*, 7(12):596. https://doi.org/10.3390/microorganisms7120596

Mehdi Y, Létourneau-Montminy MP, Gaucher ML, et al., 2018. Use of antibiotics in broiler production: global impacts and alternatives. *Anim Nutr*, 4(2):170-178. https://doi.org/10.1016/j.aninu.2018.03.002

Michaelidou K, Tzovaras A, Missitzis I, et al., 2013. The expression of the CEACAM19 gene, a novel member of the CEA family, is associated with breast cancer progression. *Int J Oncol*, 42(5):1770-1777. https://doi.org/10.3892/ijo.2013.1860

Mohan B, Kadirvel R, Bhaskaran M, et al., 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *Br Poultry Sci*, 36(5): 799-803. https://doi.org/10.1080/00071669508417824

Mohan B, Kadirvel R, Natarajan A, et al., 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. *Br Poultry Sci*, 37(2):395-401. https://doi.org/10.1080/00071669608417870

Panda AK, Raju MVLN, Rama RSV, et al., 2005. The influence of supplementation of *Lactobacillus sporogenes* on the performance of broilers. *Indian J Anim Nutr*, 22(1):37-40. https://doi.org/10.2141/jpsa.43.235

Panda AK, Rao SVR, Raju MVLN, et al., 2006. Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. *J Poult Sci*, 43(3):235-240. https://doi.org/10.2141/jpsa.43.235

Russell DA, Ross RP, Fitzgerald GF, et al., 2011. Metabolic activities and probiotic potential of bifidobacteria. *Int J Food Microbiol*, 149(1):88-105. https://doi.org/10.1016/j.ijfoodmicro.2011.06.003

Scanes CG, 2009. Perspectives on the endocrinology of poultry growth and metabolism. *Gen Comp Endocrinol*, 163(1-2): 24-32. https://doi.org/10.1016/j.ygcen.2009.04.013

Surai PF, Kochish II, Fisinin VI, 2017. Antioxidant systems in poultry biology: nutritional modulation of vitagenes. *Eur Poult Sci*, 81:1-21. https://doi.org/10.1399/eps.2017.214

Thapa BR, Walia A, 2007. Liver function tests and their interpretation. *Indian J Pediatr*, 74(7):663-671. https://doi.org/10.1007/s12098-007-0118-7

Vital M, Howe AC, Tiedje JM, 2014. Revealing the bacterial butyrate synthesis pathways by analyzing (meta) genomic data. *mBio*, 5(2):e00889-14. https://doi.org/10.1128/mBio.00889-14

Wang X., Hu Y., Zhu X, et al., 2023. Bacteroides-derived isovaleric acid enhances mucosal immunity by facilitating intestinal IgA response in broilers. *J Animal Sci Biotechnol*, 14:4. https://doi.org/10.1186/s40104-022-00807-y

Zheng JS, Wittouck S, Salvetti E, et al., 2020. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int J Syst Evol Microbiol*, 70(4):2782-2858. https://doi.org/10.1099/ijsem.0.004107