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Effects of dietary *Artemisia annua* supplementation on growth performance, antioxidant capacity, immune function, and gut microbiota of geese

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ABSTRACT This experiment aimed to study the effect of 1% *Artemisia annua* added to the diet on growth performance, antioxidant capacity, immunity and intestinal morphology, and gut microbiota of geese. Seventy-two 35-day-old male geese (**Zi goose**) with similar body weight were selected and randomly divided into 2 groups. Each treatment group of 36 geese was divided into 6 subgroups, each having 6 male geese. The experiment lasted for 21 d. Control group (**CON**) was fed a basal diet and the experimental group (**AAL**) was fed a basal diet + 1% *Artemisia annua*. BW, ADG, and ADFI of the AAL group increased ($p < 0.05$) and the FCR decreased ($p < 0.05$) compared with the CON group. The addition of *Artemisia annua* to the diet increased catalase (**CAT**), glutathione peroxidase (**GSH-px**), and superoxide dismutase (**SOD**) enzyme activities, increased total antioxidant capacity (**T-AOC**), and decreased malondialdehyde (**MDA**) content in serum and jejunum of geese ($p < 0.05$). Meanwhile, serum IgA, IgG, IgM, and lysozyme (**LZM**), increased at different time points in the AAL group compared to the CON group ($p < 0.05$), and decrease in the content of interferon-γ (**IFN-γ**) , IL-6 ($p < 0.05$), but no effect on complement C3 and C4. Morphological observation of the small intestine showed that the jejunal crypt depth was decreased in the AAL group ($p < 0.05$) while elevating the jejunal villus height/crypt depth ($p < 0.05$). 16S rRNA sequencing results showed the *Artemisia annua* increased the diversity of cecum microbiota, increasing the relative abundance of *Bacteroides, Fecalibacterium,* and *Parapre-votella*. In conclusion, the addition of 1% *Artemisia annua* to the diet could improve the growth performance, antioxidant and immune ability of geese, as well as improve the development of the jejunum intestinal tract of geese, and change the structure of the cecum microbiota, which had a positive effect on the growth and development of geese. *Artemisia annua* can be further developed as a feed additive.

Key words: goose, antioxidant activity, immunity, intestinal microbiota

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INTRODUCTION

In the current process of intensive poultry farming, multiple causes trigger problems such as oxidative stress in the intestinal tract, disorders in the intestinal system, and intestinal subhealth in poultry, such as pathogenic bacteria, nutritional supply, and feeding management. These problems lead to reduced poultry performance, lower quality of livestock products, lower feed utilization, and economic losses. A healthy gut is essential in the growth and production of poultry. A healthy intestinal system improves the digestibility and absorption of feed nutrients, reduces nutrient wastage, and improves poultry productivity while reducing the emission of harmful gases during poultry production. Intestinal barriers mainly include mechanical, immune, and microbial barriers, which interact with each other to protect the health of the gut (Gou et al., 2022). The integrity of the intestinal mucosal tissue morphology and the diversity of the intestinal microflora are essential for maintaining intestinal health in poultry, and they prevent bacteria and viruses in the intestinal system from entering the bloodstream and then the organism (Martens et al., 2018). At the same time, the gut also has important immune functions and is an essential component of the body's immune defense (Ding et al., 2021). Poultry gut health is the result of a combination of host immunity, and microbial and environmental factors (Kalia et al.,

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2022). When the composition of intestinal micro-organisms changes, the intestinal mucosal barrier is damaged, and the immune function is reduced, which will affect the intestinal health of poultry. With the current intensive poultry farming model and more and more countries issuing policies to ban antibiotics one after another, how to maintain a healthy intestinal system in poultry has become a problem that needs to be solved.

Artemisia annua (***Artemisia annua L.***) is a commonly used traditional Chinese medicine from a wide range of sources. Studies have shown that *Artemisia annua* contains a variety of constituents including sesquiterpenes, diterpenes, flavonoids, coumarins, and volatile oils (Septembre-Malaterre et al., 2020). studies have found that *Artemisia annua* has a variety of effects, including direct antibacterial, antiparasitic, antiviral, antioxidant, and immune-enhancing properties (Baggieri et al., 2023; Shinyuy et al., 2023). It has been reported that the addition of *Artemisia annua* extract to diets can reduce serum malondialdehyde levels in mice and protect against galactose-induced oxidative stress in mice (Kim et al., 2014). In addition, the addition of *Artemisia annua* to the diet can also improve the growth performance of largemouth bass and regulate the structure of gut microbiota (He et al., 2022). *Artemisia annua* is effective in promoting livestock growth, improving feed conversion, enhancing immune levels, and preventing the occurrence of diseases, and can be used as a good alternative to antibiotics (Coroian et al., 2022). Despite the multiple biological activities of *Artemisia annua*, there are fewer relevant reports for geese. In this experiment, the effects of *Artemisia annua* on growth performance, antioxidants, immunocompetence, and gut microbiota of geese were investigated by adding 1% *Artemisia annua* to the diet. The research method and results of this experiment can provide a theoretical basis for the application of *Artemisia annua* in goose production.

MATERIAL AND METHODS

Ethics Approval

This study protocol was approved by the Ethics Committee for the Use and Care of Animals at Heilongjiang Bayi Agricultural University (Daqing, China).

Experimental Material

According to the experimental design, 1% *Artemisia annua* was added to the basal diet. *Artemisia annua* (Artemisic acid 46.16 mg/g, Scopoletin 5.76 mg/g, Arteannuin 126.18 mg/g and Artemisinin 372.45 mg/g) was supplied by Shandong Luxi Veterinary Pharmaceuticals Co. Ltd (Shandong, China).

Experimental Design and Geese Management

Seventy-two healthy 35-day-old male Zi geese (Daqing, China) were randomly divided into 2 groups (n = 36 /group). Each treatment group of 36 geese was divided into 6 subgroups, each having 6 male geese. The control group was fed a basal diet (Table 1). The AAL group was fed a basal diet supplemented with 1% *Artemisia annua*. The experiment lasted for 21 d and the 6 replicate geese of the subgroup were kept in a house with a feeding area (length (**L**) × width (**W**): 15 × 10 m), a windowless feeding house, and a floor feeding system. The average high temperature during the experimental period was 26°C, the average low temperature was 18°C, and the average humidity was 63%. Daily natural light exposure. The geese were fed once a day at 9:00 am and once a day at 5:30 pm, and water was freely available during this period. In addition, all geese were vaccinated against avian influenza and goose paramyxovirus.

Table 1. Composition and nutrient levels of basal diets (air-dry basis) %.

Ingredients	Content	Nutrient levels	Content
Corn	61.50	ME/(MJ/Kg)[2]	10.92
Soybean meal	25.00	Crude protein	16.02
Rice bran	7.00	Crude fibre	5.60
Wheat bran	3.00	Calcium	0.72
NaCl	0.30	Phosphorus	0.54
Premix[1]	1.00	Lysine	0.80
DL-Met	0.10	Methionine	0.33
$CaHPO_4$	1.10		
Mountain flour	1.00		

[1]The premix provides per kg of concentrate: Zn 60 mg, Cu 50 mg, Mn 80 mg, Fe 50 mg, I 1.6 mg, Se 12 mg, vitamin A 30.000 IU, vitamin E 100 mg, vitamin D3 100000 IU.

[2]ME was a calculated value, while other values were quantified through measurement.

Sample Collection and Growth Performance Measurement

Body weight (**BW**) was measured before morning feeding on d 1, 7, 14, and 21 of the experiment. Daily feed consumption was recorded to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**). The electronic balance, with a 0.01 g accuracy (Shanghai Liang Ping Instruments Co., Ltd), was used for these measurements. Serum samples were isolated from the blood samples of 6 geese per group and stored at −20°C for subsequent testing. After slaughter, samples of the duodenum, jejunum, ileum, and cecum contents were collected from 6 geese in each group for detection of intestinal morphology and high-throughput sequencing of gut microbiota.

Measurement of Antioxidant Capacity Index

Jejunal tissue was mixed with physiological saline at a ratio of 1:9 (w/v) and homogenized in an ice bath. The supernatant was collected by centrifugation at 1,000 × *g* for 15 min at 4°C. Subsequently, the serum and jejunum samples were immediately tested for antioxidant indicators, including catalase (**CAT**), glutathione peroxidase

(**GSH-px**), superoxide dismutase (**SOD**), total antioxidant capacity (**T-AOC**), and malondialdehyde (**MDA**), using a kit from Suzhou Grace Biotechnology Co., Ltd, following the manufacturer's instructions.

Measurement of Serum Immunity Indices

Serum immune parameters, such as total IgA, IgG, IgM, complement 3, complement 4, IL-6, IFN-γ, and LZM (lysozyme), were analyzed with commercial kits according to the instructions provided by the manufacturer (Shanghai Enzyme-linked Biotechnology Co., Ltd.).

Histology

The frozen sections of the duodenum, jejunum, and ileum collected on the 21st d of the experiment were reheated and dried. They were then fixed with 4% paraformaldehyde for 15 min, differentiated with 75% alcohol, restained with hematoxylin for 3 to 5 min, and stained with hydrochloric acid. The sections were rapidly differentiated and turned blue with ammonia solution. Subsequently, the intestinal tissue sections were observed and photographed using a Nikon optical microscope with a magnification of 10 × 20. Image Pro Plus 6.0 software was used to analyze the villus length, crypt depth, and villus length/crypt depth ratio of the duodenum, jejunum, and ileum.

High-Throughput Sequencing Analysis of the Gut Microbiota of the Cecum

The extraction of total genome DNA from cecal contents samples was performed using the CTAB/SDS method. The DNA concentration and purity were evaluated using a NanoDrop 2,000 UV-vis spectrophotometer and verified through a 1% agarose gel. Subsequently, the DNA was diluted to a concentration of 1 μg/μL with sterile water. The V3 + V4 region of the bacterial 16S rRNA gene was amplified using 341F (5′-CCT AYG GGR BGC ASC AG −3′) and 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′) primers. The amplification was performed on cecal contents samples. All the libraries were constructed using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, SanDiego, CA) and quantified using Qubit. Subsequently, the sequencing was conducted on the Illumina NovaSeq platform. These assembled reads were processed and taxonomy assigned using QIIME2 (v.2021.2). Using the denoise-paired method, amplicon sequence variants (**ASV**) were determined with DADA2. The correlation between gut microbiota and immune and oxidative stress indicators was analyzed using RStudio software and represented as a Spearman correlation heatmap.

Statistical Analysis

SPSS 23 was used to perform a general linear model analysis on growth performance, with initial body weight as a covariate. Statistical analysis on unpaired 2-tailed t-tests for antioxidant and immune indicators was conducted using GraphPad Prism 9.5.1 software. Mean values were used to express all results. The reliability of experimental data was indicated by the standard error of the mean (SEM). Differences were considered significant for $P < 0.05$ and highly significant for $P < 0.01$.

Table 2. Effect of adding 1% *Artemisia annua* to the diet on the growth performance of geese.

	Groups[1]			
Item	CON	AAL	SEM[2]	*P*-value
BW(kg)				
1 d	1.47	1.47	0.03	1.00
7 d	1.89	2.55	0.02	<0.01
14 d	2.66	2.72	0.03	0.21
21 d	2.78	3.56	0.04	<0.01
ADG(g)				
1 to 7 d	60.71	154.28	12.79	<0.01
7 to 14 d	109.28	23.57	10.48	<0.01
14 to 21 d	16.42	120.00	12.51	<0.01
1 to 21 d	62.14	99.28	4.71	<0.01
ADFI(g)				
1 to 7 d	235.20	235.20	6.23	1.00
7 to 14 d	303.20	408.00	4.58	<0.01
14 to 21 d	425.60	434.40	3.37	0.21
1 to 21 d	321.33	359.20	2.43	<0.01
FCR				
1 to 21 d	5.27	3.66	0.12	<0.01

Abbreviations: BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

[1]CON: control group; AAL: 1% *Artemisia annua* group added to the diet.

[2]SEM=standard error of the mean.

RESULTS

Growth Performance

Table 2 presents the impact of incorporating *Artemisia annua* into the diet of geese on various parameters including body weight, average daily feed intake, average daily gain, and feed conversion ratio. Initially, there were no significant differences in body weight among the groups. However, after the feeding period, the geese in the AAL group exhibited a significant increase in body weight on the 7th and 21st d of the experiment compared to the CON group ($p < 0.05$). Furthermore, the AAL group demonstrated significantly higher average daily gain (**ADG**) than the CON group at each feeding period ($p < 0.01$). Additionally, the AAL group exhibited significantly higher average daily feed intake (**ADFI**) than the CON group during the periods of 7 to 14 d and 1 to 21 d ($p < 0.01$). Notably, the feed conversion ratio (**FCR**) of the AAL group was significantly lower than that of the CON group ($p < 0.05$).

Indicators of Antioxidant Capacity of the Jejunum

The effect of *Artemisia annua* on the antioxidant capacity of the jejunum of geese is shown in Figure 1. There was a tendency for the antioxidant capacity of

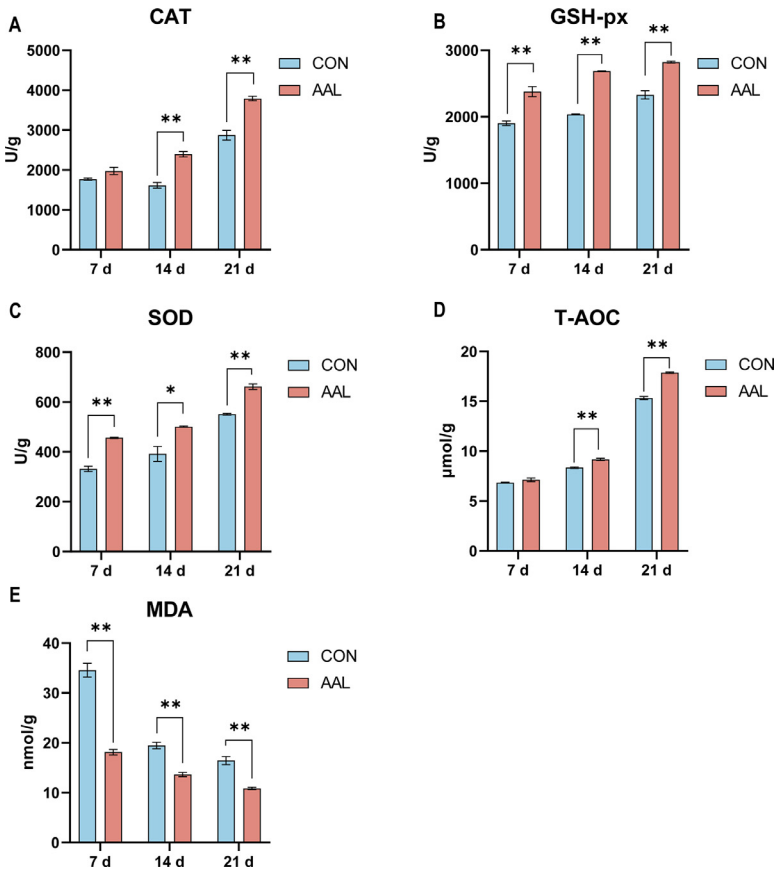

Figure 1. Effect of Artemisia annua on the antioxidant capacity of the jejunum of geese. (A) Jejunal catalase (CAT) activity. (B) Jejunal glutathione peroxidase (GSH-px) activity. (C) Jejunal superoxide dismutase (SOD) activity. (D) Total antioxidant capacity (T-AOC) of jejunum. (E) Jejunal malondialdehyde (**MDA**) content. CON = control group; AAL = 1% *Artemisia annua* group added to the diet. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CON.

both groups, CON and AAL, to increase with the age of the day. As shown in Figure 1A, jejunum CAT activity increased significantly in the AAL group at 14 d and 21 d of the experiment in comparison with the CON group ($p < 0.01$). As shown in Figure 1B, GSH-px was significantly increased in the AAL group at experimental 7 d, 14 d, and 21 d compared with the CON group ($p < 0.01$). In terms of SOD activity, the AAL group showed a highly significant increase ($P < 0.01$) in SOD activity at 7 d and 21 d and a significant increase ($p < 0.05$) at 14 d of the experiment compared with the CON group (Figure 1C). As shown in Figure 1D, T-AOC was significantly higher in the AAL group than in the CON group at 14 d and 21 d ($p < 0.01$). As shown in Figure 1E, MDA content was significantly reduced in the AAL group compared with the CON group at 7, 14, and 21 d ($p < 0.01$).

Indicators of Serum Antioxidant Capacity

The effect of *Artemisia annua* on the serum antioxidant capacity of geese is shown in Figure 2. Similarly, there was an increasing trend in the antioxidant capacity of both CON and AAL groups with the increase in the number of experimental d. As shown in Figure 2A, the serum CAT activity of the AAL group was highly significant ($P < 0.01$) at 7 d, 14 d, and 21 d of the experiment compared to the CON group. As shown in Figure 2B, GSH-px activity in the AAL group was significantly lower than that in the CON group at 7 d of the experiment ($p < 0.01$), but at 14 d and 21 d of the experiment, GSH-px in the AAL group was significantly increased compared with the CON group ($p < 0.01$). In terms of SOD activity, the AAL group showed significant ($p < 0.01$) increases in SOD activity at 7, 14, and 21 d of the experiment compared to the CON group (Figure 2C). In terms of T-AOC, as shown in Figure 2D. the T-AOC of the AAL group was significantly higher than that of the CON group at 7, 14, and 21 d ($p < 0.01$). As shown in Figure 2E, it was significantly lower in the AAL group at 7, 14, and 21 d compared with the CON group.

Serum Immunological Index

The effect of *Artemisia annua* addition on the immune performance of geese was assessed by measuring the levels of immunoglobulins IgA, IgG, IgM, complement 3, complement 4, lysozyme (**LZM**), and cytokines IFN-γ and IL-6 in serum samples at 7, 14, and 21 d of the experiment (Figure 3A-H). Compared to the CON group, the AAL group showed a significant increase in IgA levels at 7 d ($p < 0.01$) and a significant increase at 14 d ($p < 0.05$) (Figure 3A). Serum IgG levels were significantly increased in the AAL group at 7 and 21 d

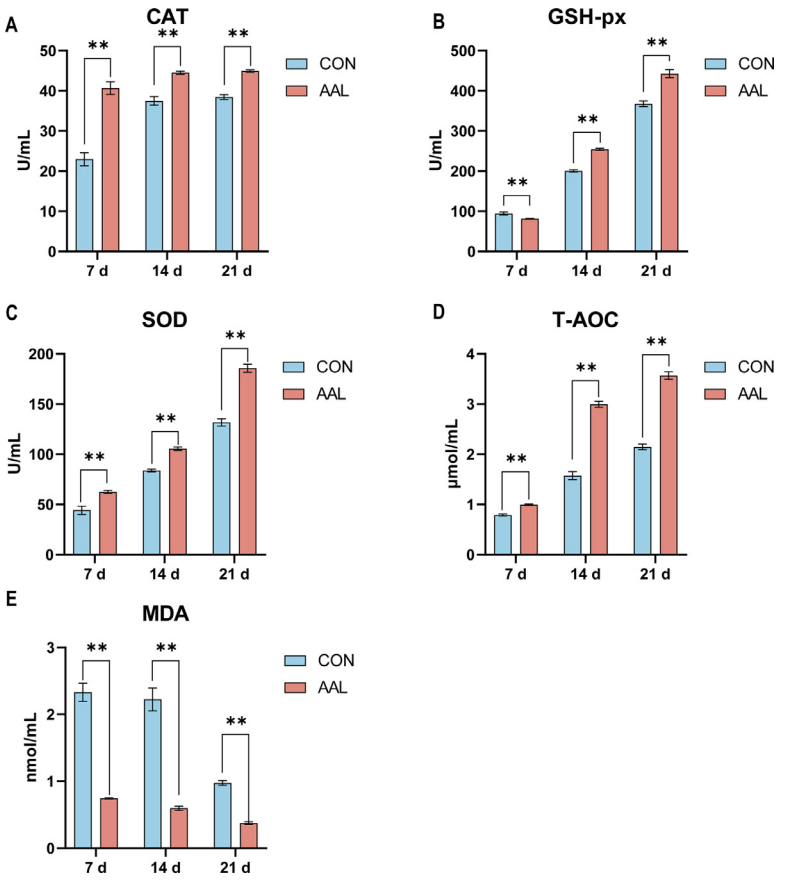

Figure 2. Effect of *Artemisia annua* on the antioxidant capacity of the serum of geese. (A) Serum catalase (CAT) activity. (B) Serum glutathione peroxidase (**GSH-px**) activity. (C) Serum superoxide dismutase (**SOD**) activity. (D) Total antioxidant capacity (**T-AOC**) of serum. (E) Serum malondialdehyde (**MDA**) content. CON = control group; AAL = 1% *Artemisia annua* group added to the diet. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CON.

compared to the CON group ($p < 0.05$) (Figure 3B). The addition of AAL to the diet significantly increased the serum IgM level at 7 d (Figure 3C). There was no significant difference in C3 and C4 levels between the 2 groups at all periods ($p > 0.05$) (Figures 3D and 3E). The level of lysozyme in the AAL group was significantly ($p < 0.01$) higher than that in the CON group at 7 d, but there was no significant difference at 14 and 21 d (Figure 3F). Compared to the CON group, the AAL group showed significantly lower levels of IFN-γ at 14 d ($p < 0.05$) and lower levels at 21 d ($p < 0.01$) (Figure 3G). IL-6 levels were significantly lower in the

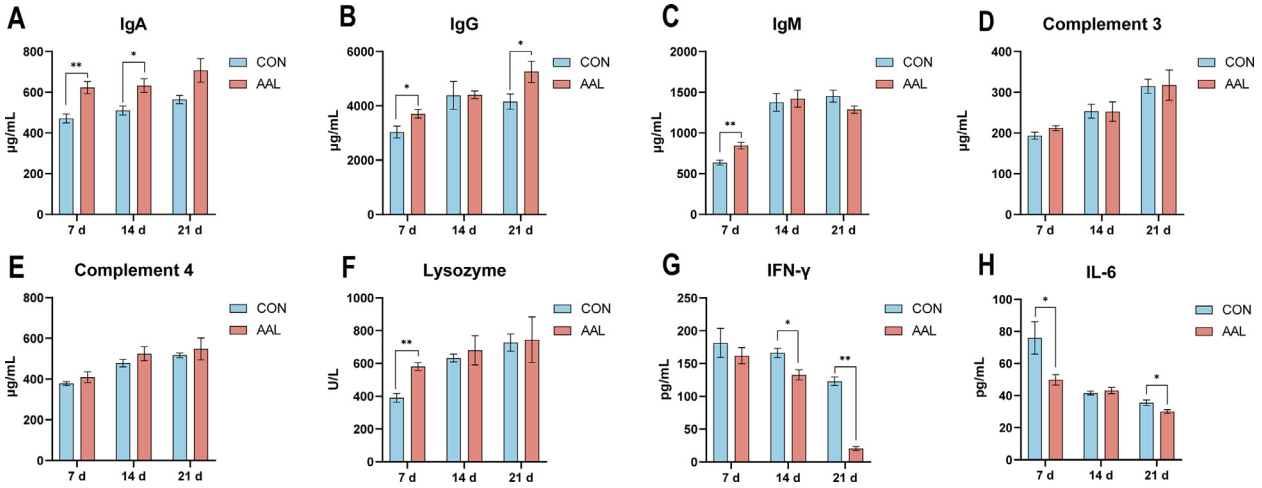

Figure 3. Effect of *Artemisia annua* on the immune function of geese. (A) Serum IgA level. (B) Serum IgG level. (C) Serum IgM level. (D) Serum levels of complement C3. (E) Serum levels of complement C4. (F) Serum Lysozyme Levels. (G) Serum levels of IFN-γ. (H) Serum levels of IL-6. CON = control group;AAL = 1% *Artemisia annua* group added to the diet. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CON.

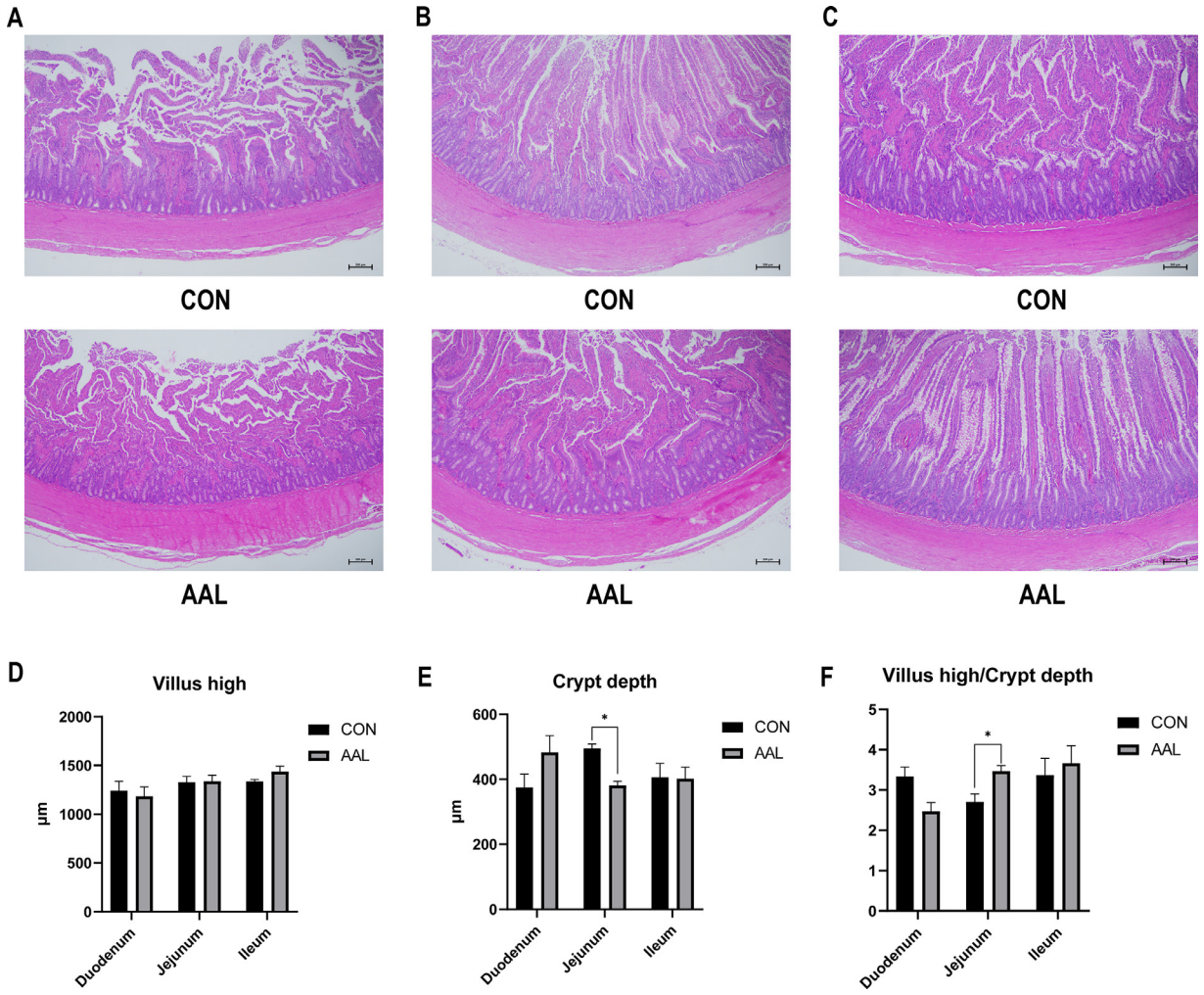

Figure 4. HE staining to analyze the morphology of the small intestine. (A) Slices of duodenum. (B) Slices of jejunum. (C) Slices of ileum. (D) villus high. (E) crypt depth. (F) Villus height/crypt depth. CON = control group;AAL = 1% *Artemisia annua* group added to the diet. $^*P < 0.05$ compared with the CON.

AAL group than in the CON group at 7 and 21 d ($p < 0.05$) (Figure 3H).

Morphology of the Small Intestine

The results of HE (Hematoxylin Eosin staining) of intestinal tissues on the 21st d of the experiment are shown in Figure 4. Figures 4A−4C depicts the morphology of the duodenum, jejunum, and ileum after HE staining. The height of villi in the intestinal tissues is presented in Figure 4D, and there was no significant difference between the CON and AAL groups ($p > 0.05$). Figure 4E displays the crypt depth of each intestinal tissue, revealing that the jejunal crypt depth was significantly lower in the AAL group compared to the CON group ($p < 0.05$) after 21 d of the experiment. At the same time, there was no significant change in the depth of duodenal and ileal crypts between the 2 groups ($p > 0.05$). The jejunal villas high/crypt depth ratio in the AAL group was significantly higher than that in the CON group ($p < 0.05$), and there was no significant difference in the duodenal and ileum villas high/crypt depth ratio between the 2 groups ($P > 0.05$) (Figure 4F).

16S rDNA Sequencing Data Statistics

To further investigate the effect of dietary *Artemisia annua* supplementation on the intestinal microbiota of geese, we performed amplicon sequencing on samples of goose cecum contents collected on the 21st d of the experiment. As shown in the results of Figure 5A, there were 4438 core ASVs in the CON group and 6614 core ASVs in the AAL group, of which, 819 ASVs were common to both groups in terms of ASV composition. In addition, both the sparse and rank abundance curves in Figures 5B−5D that can reflect the sequencing depth and uniformity indicate that the sequencing depth is sufficient.

Alpha Diversity Analysis

To evaluate the impact of dietary *Artemisia annua* addition on the bacterial diversity of the goose cecum, we employed the Alpha index to assess the changes in the diversity of the intestinal microbiota, as depicted in Figure 6. The Good_coverage index was used to gauge the library coverage of each sample, with the lowest index of 98.28% and the highest index of 99.91% in the 2

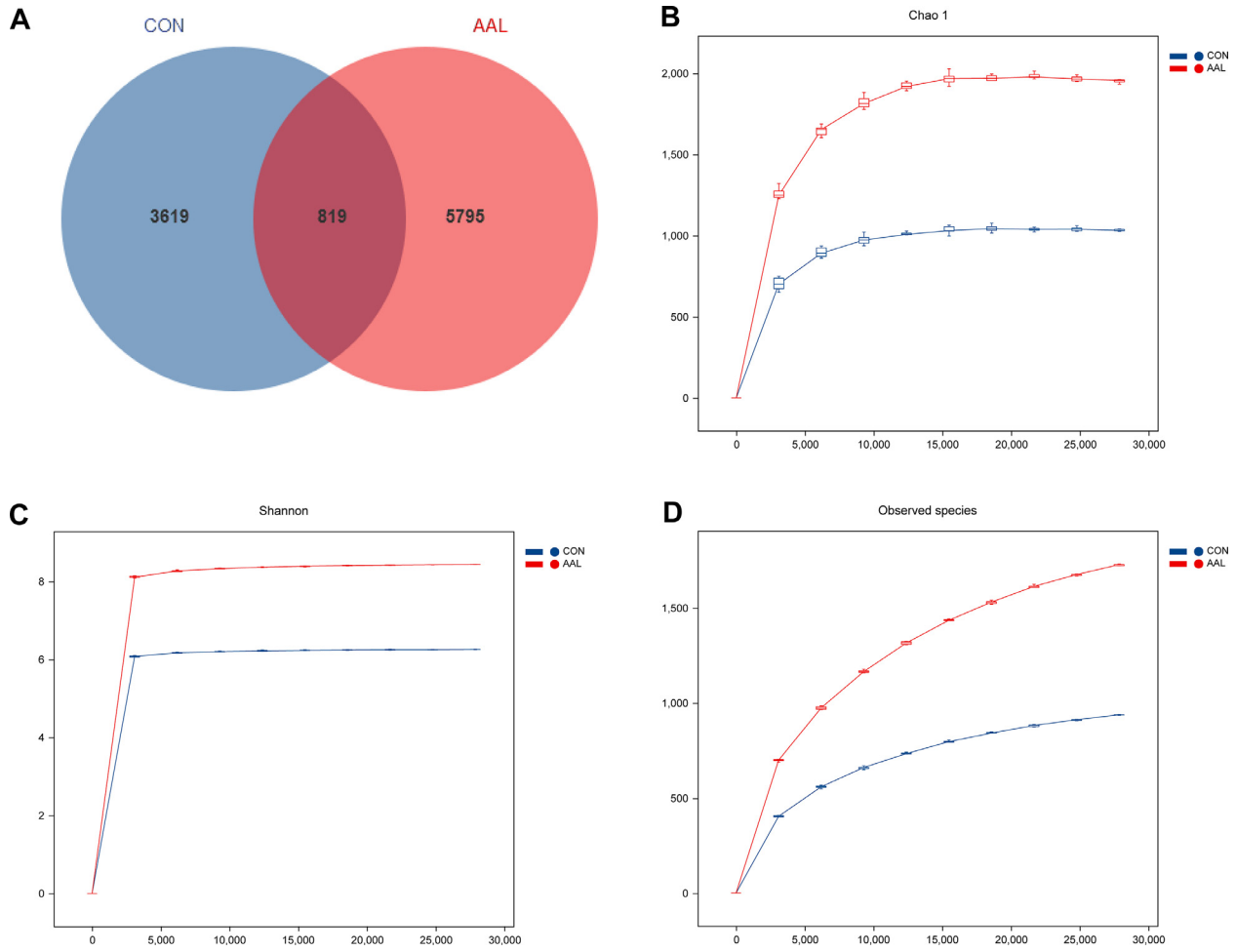

Figure 5. Quality assessment of cecal colony sequencing data and ASV counts. (A) Venn diagram. (B) Dilution curve of Chao1. (C) Dilution curve of Shannon. (D) Dilution curve of Observed_species. CON = control group; AAL = 1% *Artemisia annua* group added to the diet.

groups indicating that this sequencing method detected nearly all bacterial phenotypes in the cecum. The Chao 1 and Observed_species indices were utilized to measure the differences in species richness between the 2 groups. Both indices were significantly higher in the AAL group compared to the CON group ($p < 0.05$), suggesting that the AAL group harbored a greater number of bacteria. The diversity of the samples was assessed using the Shannon and Simpson indices, where higher values indicated greater species diversity. The Shannon index was significantly higher ($p < 0.01$) and the Simpson index was higher ($p < 0.05$) in the AAL group compared to the CON group. Additionally, the Pielou_e index was employed to evaluate the similarity between the 2 groups of microbiota, and the AAL group exhibited a significantly higher Pielou_e index than the CON group ($p < 0.01$). These findings suggest that *Artemisia annua* supplementation can significantly increase the abundance, diversity, and homogeneity of the intestinal microbiota.

Beta Diversity Analysis

As depicted in Figure 7A, the multidimensional species data were downscaled to assess the disparities in bacterial community composition between the 2 groups using principal coordinate analysis (PCoA) based on the bray_curtis distance algorithm. The PCoA results revealed that the samples from the CON group and the AAL group were distinctly separated from each other, indicating a significant difference in community composition between the 2 groups. Additionally, the NMDS analysis, which is a crucial indicator of sample differences, demonstrated that the Stress value was less than 0.2, suggesting a noticeable distinction between the samples. Specifically, the Stress value of 0.0355 (<0.2) in Figure 7B indicated a substantial difference in community composition between the CON group and the AAL group.

Species Composition Analysis

Based on the analysis of the taxonomic composition of dominant bacteria, it is evident that there were changes in the dominant bacteria at both the microbial phylum and genus levels in the AAL group compared to the CON group. Figure 8A illustrates that in the CON group, the bacteria with a relative abundance greater than 1% were Firmicutes (44.7%), Bacteroidetes (20.88%), Proteobacteria (16.16%), and Actinobacteria (5.16%). In contrast, the AAL group had Firmicutes (40.27%), Bacteroidetes (47.46%), Proteobacteria

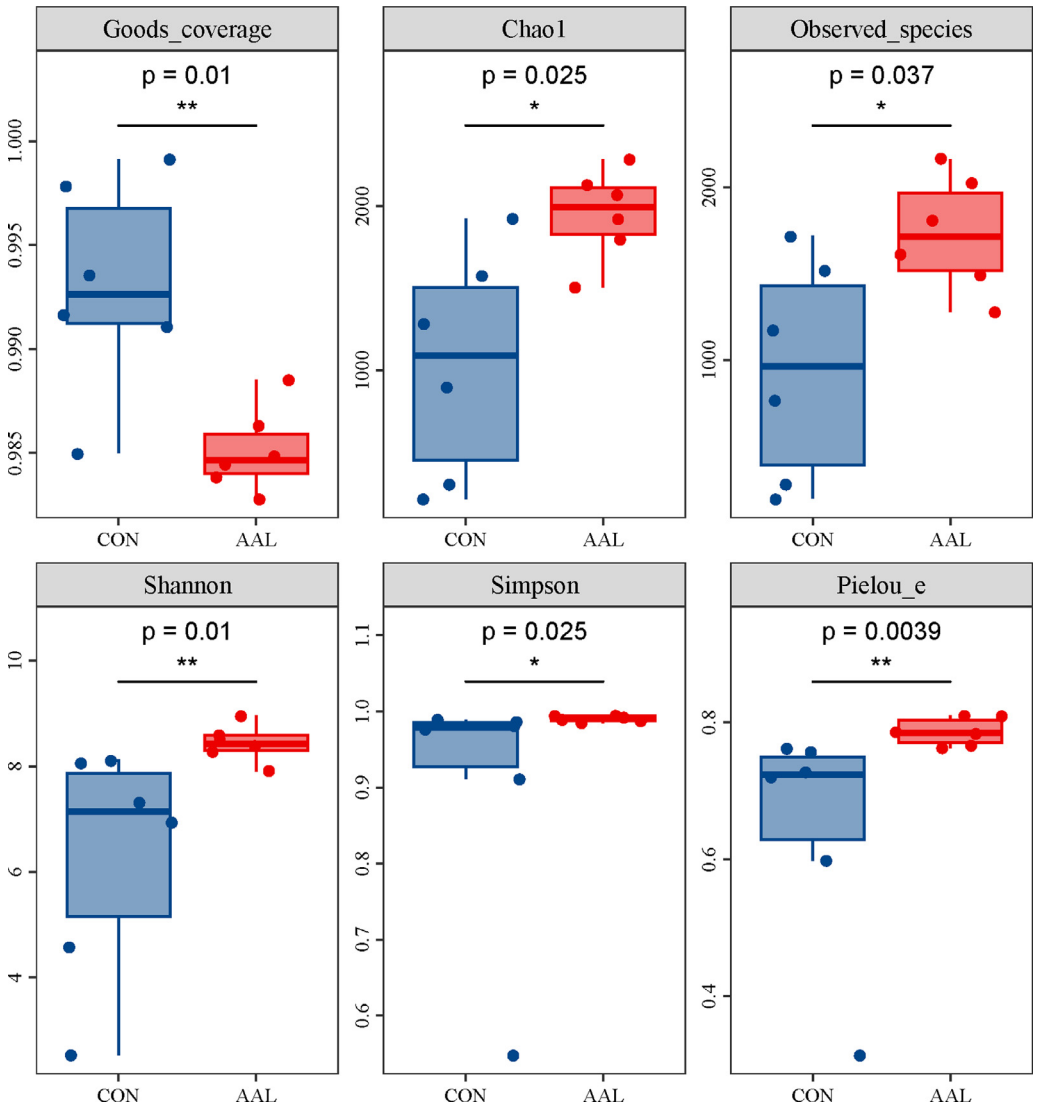

Figure 6. Alpha Diversity Index. CON = control group; AAL = 1% *Artemisia annua* group added to the diet.

(4.61%), and Actinobacteria (5.16%) as the bacteria with a relative abundance greater than 1%. As depicted in Figure 8B, the AAL group exhibited changes in the top 10 species at the genus level compared to the CON group. Specifically, the relative abundance of *Bacteroides* increased from 14.15% to 19.72%, while *Shigella* decreased from 12.09% to 0.14%. Additionally, *Subdoligranulum, Oscillospira, Peptococcus*, and *Ruminococcus* showed decreased relative abundances, whereas *Prevotella, Fecalibacterium, Paraprevotella*, and *Desulfovibrio* exhibited increased relative abundances. Notably, statistical analysis revealed that both the structure and abundance of the intestinal microbiota were altered in the 2 groups. At the phylum level (Figure 8C), the AAL group significantly increased the relative abundance of Bacteroidetes ($p < 0.05$). Furthermore, at the genus level (Figure 8D), *Fecalibacterium* and *Paraprevotella* exhibited significantly higher relative abundances in the AAL group ($p < 0.05$). The LEfSe analysis (Figure 8E) indicated that the AAL group was enriched in *Paraprevotella, Fecalibacterium, Ruminococcus, and Parabacteroides*. while the CON group was enriched in *Lactobacillus* and *Saccharopolyspora*.

Correlation of Gut Microbiota With Antioxidant and Immune Indicators

According to Figure 9A, the top 10 bacteria at the phylum level showed a correlation with antioxidant indicators and immune indicators. The correlation analysis revealed that *Bacteroides* had a positive correlation with jejunal CAT, jejunal T-AOC, and serum CAT. Spirochaetes showed a positive correlation with SOD-jejunum. In terms of immune indicators, *Bacteroides* had a negative correlation with IFN-γ, Deferribacteres had a positive correlation with IL-6, Firmicutes had a positive correlation with IgM, and Fusobacteria had a positive correlation with IFN-γ and IL-6. Moving to the genus level, Figure 9B presents the correlation analysis between bacterial abundance and antioxidant indicators. *Fecalibacterium* showed a positive correlation with jejunal CAT, jejunal SOD, jejunal T-AOC, serum CAT, serum GSH-px, serum SOD, and serum T-AOC. Simultaneously, *Fecalibacterium* had a negative correlation with jejunal MDA and serum MDA. *Paraprevotella* showed a positive correlation with jejunal CAT, jejunal SOD, jejunal T-AOC, serum CAT, serum GSH-px,

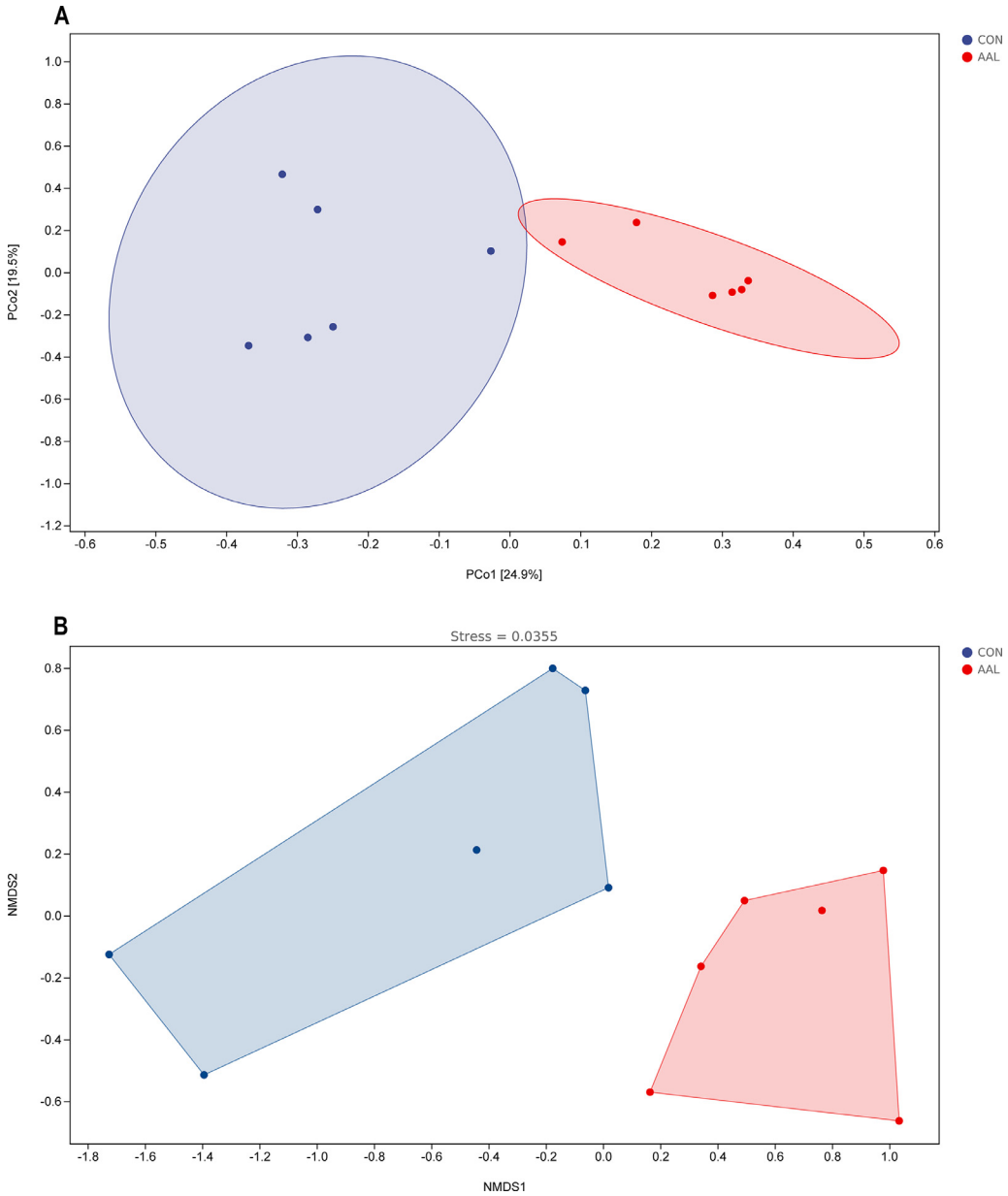

Figure 7. Analysis of the beta diversity index. (A) Principal coordinate analysis (PCoA) plot of the bacterial community based on unweighted UniFrac distances. (B) Beta diversity analysis based on NMDS. CON = control group; AAL = 1% *Artemisia annua* group added to the diet.

serum T-AOC, and a negative correlation with serum MDA. Regarding immune indicators, *Fecalibacterium* had a positive correlation with IgA and a negative correlation with IFN-γ. *Paraprevotelal* had a negative correlation with IFN-γ. *Subdoligranulum* and *Ruminococcus* showed a positive correlation with IgM.

DISCUSSION

The ban on antibiotic growth promoters has stimulated the development of natural herbs as an ideal strategy for maintaining gut health in today's intensive poultry industry. A variety of organic acids and polyphenols contained in *Artemisia annua* increase the secretion of enzymes in the stomach, improve the activity of digestive enzymes, and promote gastric motility and digestion of food, thus enhancing appetite and feed intake and promoting weight gain in young animals, and it is useful in improving poultry performance and altering the intestinal microbiota of poultry. Body weight gain is one of the basic indicators of growth of an organism. Previous studies have found that the addition of 2 g/kg of enzymatically digested *Artemisia annua* improved growth performance and reduced diarrhea in weaned piglets (Niu et al., 2020). In addition, supplementation with ethanolic extract of *Artemisia annua* improved productivity and treated coccidiosis in rabbits (Watsop et al., 2022). In the present experiment, it was found that the addition of 1% *Artemisia annua* to the diet significantly increased the average body weight, average daily gain, and average daily feed intake, and decreased the FCR of geese.

Under normal physiological conditions, the organism can maintain a dynamic balance between the production and elimination of endogenous ROS. The antioxidant capacity of the organism can reflect its resistance to

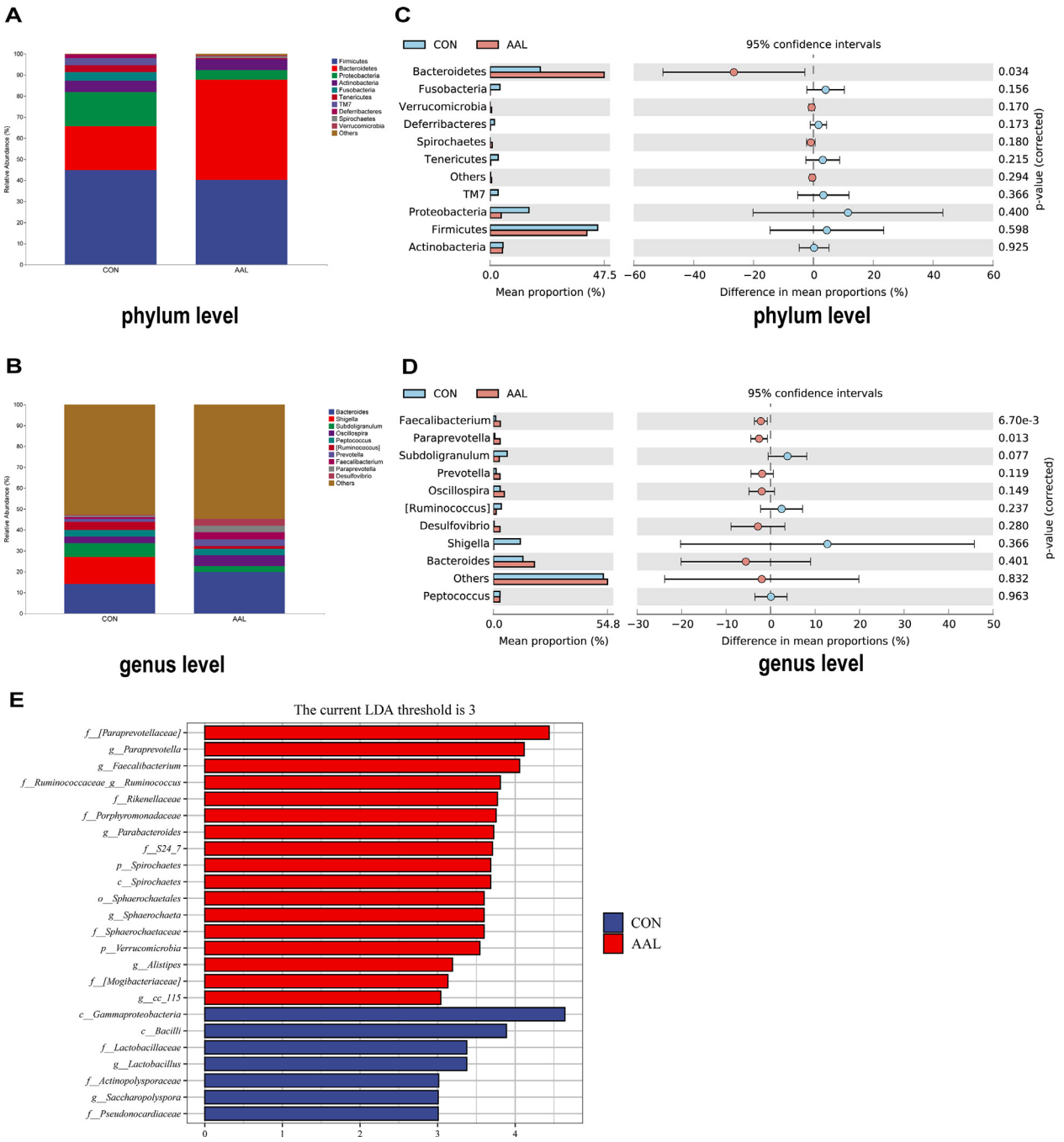

Figure 8. Structure of the intestinal microbiota of the cecum. (A) Bar plots at the phylum level. (B) Bar plots at the genus level. (C) Differential bacterial analysis at the phylum level. (D) Differential bacterial analysis at the genus level. (E) LEfSe histogram showing the LDA scores (>3.0) computed for features at the ASV level. CON = control group; AAL = 1% *Artemisia annua* group added to the diet.

endogenous oxidative damage. The key is to maintain the body's antioxidant defense system, which includes antioxidant enzymes such as CAT, GSH-px, and SOD. These enzymes can effectively convert ROS into H_2O and O_2, thus protecting the organism from ROS-induced oxidative damage (Zhang et al., 2022). Total antioxidant capacity (**T-AOC**) can reflect the capacity of the body's non-enzymatic antioxidant defense system. When the cell membrane is damaged, the unsaturated fatty acids in the cell membrane phospholipids undergo lipid peroxidation to produce malondialdehyde (**MDA**). Various studies have found that a large number of flavonoids and phenolic compounds contained in *Artemisia annua* have certain antioxidant activity and can improve the antioxidant capacity of the body (Yang et al., 2009; Song et al., 2016). It has been found that the addition of aqueous extract of *Artemisia annua* to the diet can improve the antioxidant capacity of broiler chickens through the Nrf2 signaling pathway in the small intestinal mucosa of broiler chickens, significantly increase the activities of CAT, GSH-px, and SOD enzymes in the small intestinal enteric tissues, increase the T-AOC, and significantly reduce the content of MDA in the enteric tissues (Guo et al., 2022). In addition, Song et al. found that the addition of 1 g/kg of enzymatically digested *Artemisia annua* to the diet

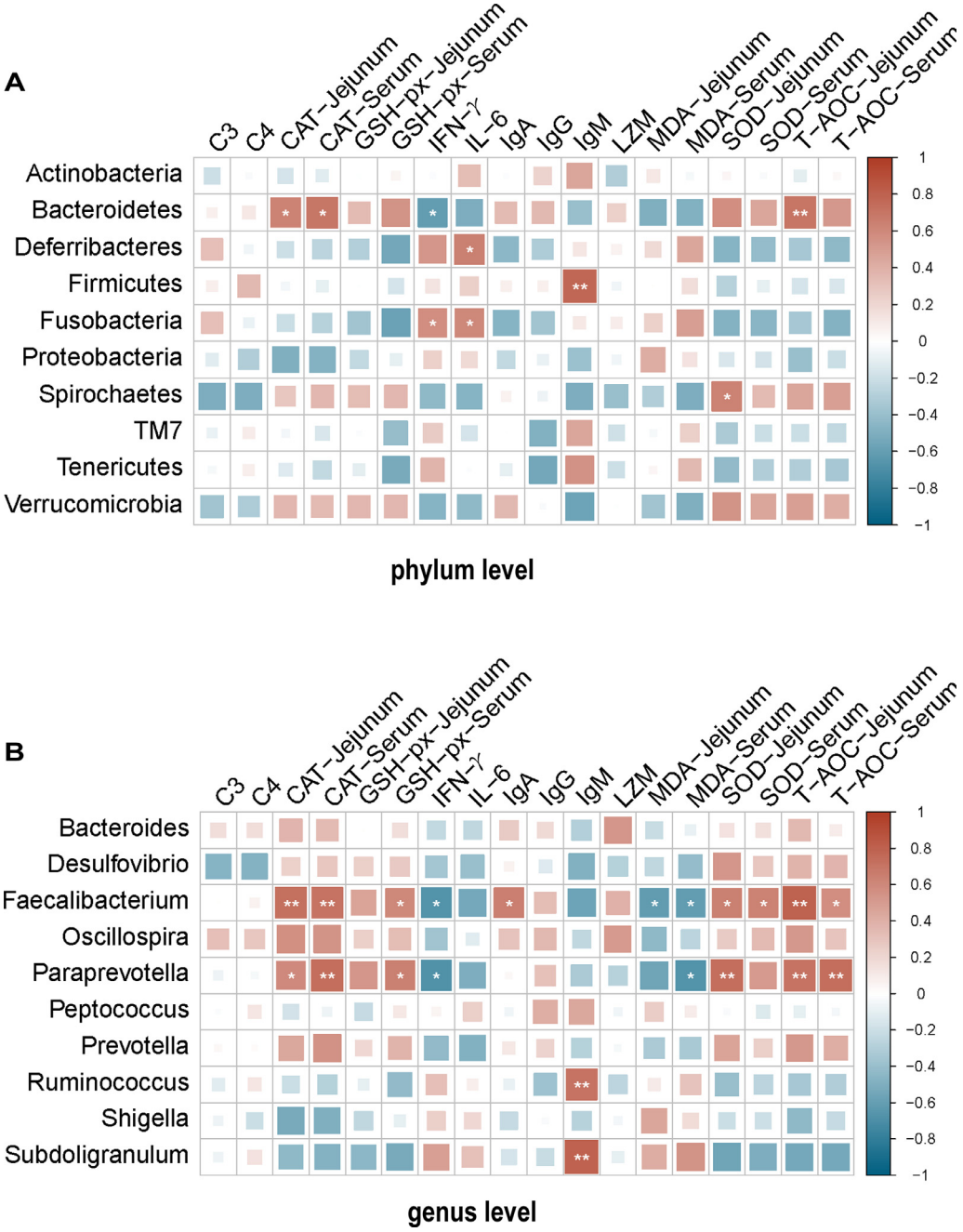

Figure 9. Spearman correlation analysis. (A) Spearman correlation analysis of differences in intestinal antioxidant indices, serum antioxidant indices, serum immune indices, and gut microbiota (phylum level). (B) Spearman correlation analysis of differences in intestinal antioxidant indices, serum antioxidant indices, serum immune indices, and gut microbiota (genus level). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the CON.

reduced MDA content and increased mRNA expression of GSH-px enzyme in the intestinal tract of heat-stressed broilers (Song et al., 2018). In this experiment, we found that *Artemisia annua* can significantly increase the activities of CAT, GSH-px, and SOD in serum and jejunum tissues. Interestingly, despite the decrease in serum GSH-px activity in the pre-experimental period, it significantly increased again in the late experimental period to elevate which indicates that *Artemisia annua* can reduce the effects of oxidants on the cells of the body; meanwhile, *Artemisia annua* can significantly increase the content of T-AOC, which indicates that *Artemisia annua* can increase the activities of antioxidant enzymes as well as enhance the metabolism of free radicals in the organism; the content of MDA in the AAL group was significantly lower than that of CON group, which indicates that *Artemisia annua* can alleviate the effects of oxidative stress on the cell membranes of the organism. The increase in antioxidant ability may be one of the reasons for the improvement in growth performance. Taken together, these results suggest that the addition of *Artemisia annua* to the diet may improve the antioxidant capacity of geese.

Immunoglobulins are proteins produced by plasma cells with antibody activity, mainly including IgG and IgM. In serum, IgG is the main component of immunoglobulins, accounting for about 75% of the total content. It inhibits the multiplication of bacteria and viruses.

The results of the present experiment are similar to the results of Sani L et al. who found that the addition of *Artemisia annua* methanolic extract to the diet increased the IgG content in broiler serum (Gholamrezaie Sani et al., 2013). In this study, it was found that immunoglobulins IgA, IgG, and IgM were significantly higher in the AAL group than in the CON group at 7d of the experiment, and IgG content was significantly higher in the AAL group than in the CON group at the time of experiment carried out up to 21d. In terms of lysozyme, lysozyme is widely present in the tissues of the organism. During bacterial infections, intestinal Pan cells secrete lysozyme, which destroys the structure of bacterial cell walls, thus restricting bacterial invasion (Bel et al., 2017). Existing studies have found that the addition of ethanolic extract of *Artemisia annua* to the diet significantly increased the level of lysozyme in the serum of Nile tilapia attacked by Aeromonas hydrophila (Soares et al., 2020). In the present study, it was found that on the 7th d of the experiment, lysozyme enzyme activity was highly significantly higher in the AAL group as compared to the CON group, suggesting that *Artemisia annua* can increase the lysozyme activity in geese during the pre-feeding period.

Cytokines are biologically active small molecule proteins that play important roles such as transmitting intercellular information and regulating cellular immunity. Cytokines such as IL-6 and IFN-γ can also affect the integrity of the intestinal barrier (Capaldo and Nusrat, 2009). It was found that IL-6 increases endothelial cell permeability by altering the ultrastructure of the tight junction protein, ZO-1 (Desai et al., 2002).IL-6 contributes to bacterial infections, viral infections, colitis, and many other inflammatory diseases (Kopf et al., 1994). Artemisinin and flavonoids in *Artemisia annua* inhibit the secretion of cytokines such as IL-6. Kim et al (Kim et al., 2015) found that *Artemisia annua* extracts significantly reduced IL-6 levels in the LPS-treated group when co-cultured with mouse macrophage Raw246.7. In addition, Song et al (Song et al., 2022) found that ethanol extract of *Artemisia annua* could down-regulate the expression level of IL-6 by decreasing CD36 expression and inhibiting the NF-κB cell pathway in bovine mammary epithelial cells. In the present study, the addition of *Artemisia annua* to the diet was found to reduce the serum levels of IL-6 in geese. IFN-γ, which can act as a Th1 pro-inflammatory cytokine, can decrease the levels of ZO-1 and the tight junction protein Occludin, which can increase the paracellular permeability (Youakim and Ahdieh, 1999). Available studies have shown that the addition of enzyme-treated *Artemisia annua* to the diet reduced IFN-γ mRNA expression in the jejunum and ileum of broilers (Song et al., 2017). In this study, we found that the serum IFN-γ in the AAL group was significantly lower than that in the CON group. In conclusion, the addition of 1% *Artemisia annua* to the diet of lion white geese could increase the immunoglobulin content in the serum of lion white geese, increase the lysozyme activity, and decrease the levels of IL-6 and IFN-γ cytokines in the serum. The results showed that *Artemisia annua* had a certain enhancement effect on the immunocompetence of geese.

Intestinal villi are elongated projections located on the mucosa of the small intestine that serve to increase the absorption surface area. By increasing the height of the villi, the intestines can provide a greater surface area for absorption, thus enhancing the ability to absorb nutrients. Microvilli cover the villi, further increasing the absorptive surface area. Crypts are depressed structures located beneath the intestinal mucosa that correspond to the villi. The height of the villi and the depth of the crypts together form a complex structure of the intestinal mucosa, optimizing the nutrient absorption process, with the function of maintaining the stability of the intestinal mucosa and barrier function to promote the digestion and absorption of nutrients. The villus height/crypt depth is an important indicator of hair nutrient absorption (Zou et al., 2019). In this study, it was found that the addition of *Artemisia annua* to the diet significantly decreased the jejunal crypt depth in the AAL group and increased the jejunal villus height over the crypt depth value. As the main place of nutrient absorption, *Artemisia annua* promotes the development of jejunal morphology in geese, which may be one of the reasons for the improvement of growth performance in the AAL group.

Various studies have long found that a normal and stable microbiota system in the organism's intestinal tract is essential for maintaining intestinal health. It has been found that intestinal microbiota can improve the digestive capacity of poultry through the synthesis of vitamins and other substances, which in turn affects the growth performance of poultry to improve the digestive efficiency of feed. In addition to improving growth performance, it has been found that altering the composition and structure of intestinal microbiota can increase the body's antioxidants and influence the regulation of the body's immune system. For the structure and composition of intestinal microbiota results. Existing studies have found that when the diversity of microbiome groups increases can increase the resistance to colonization and limit the pathogens that may cause disease in the intestine never improving the body's ability to defend against pathogens (Spragge et al., 2023), the present study found that the diversity and abundance of intestinal microbiota of cecum in the AAL group was significantly increased. *Artemisia annua* may improve growth performance by altering the diversity of cecum intestinal microbiota in geese to enhance the body's ability to resist disease.

In terms of species composition structure, at the phylum level, the AAL group significantly increased the relative abundance of *Bacteroides* compared with the CON group, which was previously found to promote nutrient absorption and metabolism by the body through participation in body glycolysis (Sun et al., 2023). At the same time, various members of *Bacteroides* can reduce the production of lipopolysaccharides by intestinal microorganisms (Yoshida et al., 2018), effectively inhibiting the occurrence of intestinal pro-inflammatory response and

producing host-absorbable short-chain fatty acids and organic acids as the main source of host energy to improve the growth performance of the host (Porter et al., 2018). Outer membrane vesicles produced by *Bacteroides* also contribute to the growth and stabilization of the intestinal microbiota by other bacteria (Miquel et al., 2013). growth to maintain the homeostasis of the intestinal microbiota. At the genus level, the AAL group significantly increased the relative abundance of *Fecalibacterium* and *Paraprevotella*, and the genus *Fecalibacterium* plays an important role in the regulation of the immune system, intestinal barrier protection, and microbiota regulation (Miquel et al., 2013). In the gut butyrate is the main source of energy for colonocytes and *Fecalibacterium* ferments glucose to produce butyrate (Miquel et al., 2013; Martin-Gallausiaux et al., 2021). Increased abundance of *Fecalibacterium* promotes the growth and development of colonocytes. *Paraprevotella* is an important cornerstone in the maintenance of intestinal microbiota, and it has been found that *Paraprevotella* colonization protects IgA from degradation by trypsin, which contributes to the maintenance of the body's intestinal homeostasis and the prevention of pathogenic bacterial infections (Li et al., 2022). Correlation analysis revealed that *Fecalibacterium* showed a significant positive correlation with antioxidant indicators, it was found. O2 and ROS levels during intestinal inflammation may pose a challenge to *Fecalibacterium*, and one of the features associated with IBD is a significant decrease in *Fecalibacterium* abundance in the gut (Cao et al., 2014). So the increased antioxidant capacity of the organism may be responsible for the increased abundance of *Fecalibacterium. Fecalibacterium* is also involved in immune homeostasis, significantly reducing the levels of IL-6, and IFN-γ cytokines when used to treat Caco-2 cells (Rabiei et al., 2019). This explains the negative association of *Fecalibacterium* with IL-6, IFN-γ. In conclusion, the addition of *Artemisia annua* to the diet can change the structure of the intestinal microbiota and the relative abundance of bacteria in the cecum of geese.

CONCLUSIONS

In conclusion, the addition of 1% *Artemisia annua* to the diet can improve the growth performance of geese by increasing their antioxidant and immune capacity and improving the jejunal morphology and the structure of the cecum bacteria.

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DISCLOSURES

All authors disclosed no relevant relationships.

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