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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Effects of supplementation with *Angelica sinensis* Radix and its powder formulation on growth performance, serum antioxidant capacity, immune response and intestinal microflora of broiler chickens

J. Song, B. Ji, Y.N. Peng, Z. Ye, Z.Y. Wang*, T.W. Zhao and Y. Liang

Gansu Academy of Sciences, Institute of Biology, Key Laboratory of Microbial Resources Exploitation and Application of Gansu Province, Lanzhou 730000, China

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* Corresponding author:
e-mail: zhiye_wang@sina.com

ABSTRACT. The objective of this study was to characterise the effects of supplementation with *Angelica sinensis* Radix (ASR) powder and a preparation containing ASR powder (Danggui Buxue Decoction, DBD) on growth performance, antioxidant capacity, immune activity, and caecal bacterial microbiota in broiler chickens. A total of 180 one-day-old male Luhua broiler chickens were randomly assigned to three groups. Broiler chickens in the control group were fed a basal diet, the ASR group was administered a basal diet supplemented with 1% ASR powder, and the DBD group received a basal diet supplemented with 1% DBD powder. Results showed significant increases in serum glutathione peroxidase and catalase activities, as well as serum immunoglobulin A levels in both the ASR and DBD groups compared to the control group ($P < 0.05$). Additionally, the malondialdehyde content was significantly lower in the ASR group compared to other groups ($P < 0.05$). Supplementation with ASR and DBD also had notable effects on the composition of broiler intestinal microbiota, particularly influencing the abundance of Firmicutes and Bacteroidetes, as demonstrated by high-throughput sequencing analysis. These findings suggest that dietary ASR and DBD supplementation enhanced antioxidant capacity, immune status, and intestinal flora diversity in broiler chickens, indicating their potential as effective phytogenic feed additives.

Introduction

Broiler production is rapidly expanding to meet the increasing global demand for affordable and high-quality animal protein. However, intensive farming systems with high animal densities is likely to result in elevated stress levels and a higher incidence of infectious diseases, adversely affecting broilers' health (Clark et al., 2019; Bajagai et al., 2022). Therefore, it is essential to develop effective and natural additives to improve chickens' health and optimise output in intensive poultry production systems. Various feed additives such as probiotics, oligosaccharides, organic acids, enzymes, and phytogenics have been utilised to enhance poultry well-being and performance (Gadde et al., 2017; Salim et al., 2018; Abd El-Hack et al., 2022). Chinese herbal medicines have been demonstrated as efficient feed additives for improving poultry health and performance due to their potent therapeutic properties and lack of adverse side effects. Specifically, they have positively affected immune system function, intestinal health, antioxidant capacity as well as anti-inflammatory status in animals (Wang et al., 2021; Wu et al., 2023).

Angelica sinensis Radix (ASR) is the rhizome of *Angelica sinensis* (Oliv.) Diels, a perennial herbaceous plant belonging to the family Umbelliferae (Wei et al., 2016). Many scientists have investigated the biological characteristics of ASR, and their findings have demonstrated its antioxidant, immunomodulatory, hepatoprotective, and anti-inflammatory effects of this product (Chen et al., 2013b; Ma et al., 2015; Gu et al., 2019). The primary bioactive constituents of ASR include polysaccharides, organic acids, coumarins, flavonoids, and phthalides. Danggui Buxue Decoction (DBD), which consists of ASR and *Astragalus membranaceus* in a weight ratio of 1:5, is one of the best-known preparations derived from ASR. DBD is a traditional Chinese medicine prescription used to increase energy metabolism and stimulate circulation. Recent pharmacological studies have revealed that DBD exerts various pharmacological effects, including modulating immune function, increasing antioxidant activity, improving physical performance, influencing lymphocyte activity, promoting haematopoiesis and anti-inflammatory effects (Chang et al., 2020; Li et al., 2021).

Studies on the effects of ASR and its formulations have primarily focused on characterising its pro-angiogenic activity (Chen et al., 2022) and hepatoprotective effects (Wu et al., 2022a), as well as its potential for cancer adjuvant therapy in both humans and animals (Zhou, 2011). However, limited research has been conducted to evaluate the impact of ASR supplementation and its formulations on broiler chickens, including their influence on the regulation of intestinal microflora (Li et al., 2013). The composition of the intestinal microbial community, immune performance, and antioxidant capacity all play crucial roles in the growth of broiler chickens. In this study, we aimed to investigate the effects of dietary administration of ASR and its formulation on growth performance, antioxidant capacity, immune response, and ceacal microbiota composition in broiler chickens. The findings of our study will contribute to a better understanding of how ASR and DBD supplementation can improve broiler chicken production by elucidating the underlying mechanisms of their action. These insights can be utilised to optimise the effectiveness of ASR and DBD supplementation in poultry farming.

Material and methods

Experimental design and diets

Experimental procedures employed in this study were approved by the Animal Care and Use Committee of the Lanzhou University of Technology (LUT-2022-003). A total of 180 one-day-old male Luhua chickens (*Gallus gallus domesticus*) with comparable body weights were obtained from Lanzhou Zhengda Food Co., Ltd (Lanzhou, GS, China). Chickens were randomly allocated to three dietary groups, each consisting of 6 replicates with 10 broilers per replicate. The starter period encompassed days 1 to 21, while the finisher period covered days 22 to 42 of the chickens' lives. The three dietary treatments included control animals fed a basal diet (CK group), broilers fed a basal diet supplemented with 1% *Angelica sinensis* Radix powder (ASR group), and chickens administered a basal diet supplemented with 1% Danggui Buxue Decoction powder (DBD group). Both Chinese herb feed additives were pulverised and sieved through an 80 mm mesh to obtain fine powder, which was subsequently mixed and directly added to the basal diet. The broilers were housed in floor pens (100 cm × 150 cm × 60 cm) in an environmentally controlled room. The room temperature was maintained at 34 °C for 4 days and then gradually decreased by 1 °C every 2 days until it reached 24 °C. Throughout the experimental period, all broilers had unrestricted access to feed and clean water. Additionally, they were inoculated with the Newcastle disease vaccine and infectious bronchitis vaccine on days 7 and 21, respectively. The experimental diets were administered for a period of 42 days. Angelica sinensis Radix and *Astragalus membranaceus* (AM) were purchased from Minxian Huimin Pharmaceutical Co., Ltd (GS, China). The nutritional composition of the ASR and DBD is detailed in Table 1. The total polysaccharide contents of ASR and DBD were determined using ultraviolet spectrophotometry and the phenol-sulphuric acid method, and they amounted to 12.78% and 20.41%, respectively. In addition, the flavonoid content of ASR and DBD was determined at approximately 0.17% and 0.08%, respectively, using the aluminium chloride colorimetric assay with rutin as a standard,

Table 1. Nutritional composition of *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) powders

Composition, %	ASR	DBD
Dry matter	91.04	93.44
Crude protein	18.77	17.02
Crude fat	1.33	0.57
Crude fibre	5.76	19.07
Crude ash	5.67	3.00
Calcium	0.28	0.15
Phosphorus	0.41	0.30

following Soares' methodology (Soares et al., 2015). The basal diet was purchased from Beijing Keao Xieli Feed Co., Ltd (Beijing, China), and its composition and nutritional levels are listed in Table 2.

Table 2. Ingredient composition and nutrient analysis of the basal diet

Ingredients	Starter (days 0–21)	Finisher (days 21–42)
Corn	58.12	61.75
Soybean meal	29.15	26.45
Fish meal	5.00	3.51
Soybean oil	2.00	3.00
Premix[1]	5.00	5.00
Dicalcium phosphorus	0.47	0.29
Limestone	0.26	0.00
Calculated nutrient		
metabolizable energy, MJ/kg	12.02	12.49
crude protein	21.00	17.50
calcium	1.00	0.85
total phosphate	0.68	0.65
available phosphorus	0.50	0.42
lys	1.20	1.00
met	0.46	0.32

[1] provided per kg of diet: IU: vit. A 9875, vit. D_3 3000, vit. E 20, vit. K 3.25; mg: vit. B_{12} 0.025, vit. B_1 1.5, vit. B_2 5.0, vit. B_6 3.75, vit. H 0.032, folacin 1.25, niacin 12, pantothenic acid 12, manganese (Mn) 100, zinc (Zn) 80, iron (Fe) 80, copper (Cu) 8, iodine (I) 0.15, selenium (Se) 0.15

Growth performance

Body weight (BW) and feed intake were recorded on days 1, 21, and 42, following a 12-h period of feed withdrawal. Average daily gain (ADG) was calculated using the formula: (BWd − BW1) / day × 100%, and average daily feed intake (ADFI) was calculated using the following equation: (total feed weight − total residual feed weight) / day × 100%, and feed conversion ratio (FCR) was calculated according to ADG and ADFI.

Sample collection

On day 42 of the experiment, one broiler with a body weight close to the average was selected from each replicate. Blood samples were collected from pterygoid veins using vacuum blood collection tubes without anticoagulant, and subsequently centrifuged at 3000 *g* for 10 min at 4 °C to obtain serum. The separated serum was stored at −20 °C for biochemical analysis. After blood sample collection, the broilers were slaughtered by bleeding through the left jugular vein, and caecal content samples were collected in sterile 2 ml DNA- and RNA-free centrifuge tubes and stored at −80 °C.

Measurements of antioxidant indices

Serum levels of total antioxidant capacity (TAC), malondialdehyde (MDA), and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were quantified using commercially available kits from Jiancheng Bioengineering Institute, Nanjing, China; the assays used included A015-2-1 for TAC, A003-1-2 for MDA, A001-3 for SOD, A007-1-1 for CAT, and A005-1-2 for GSH-Px. All measurements were performed following the manufacturer's instructions.

Measurements of serum cytokine and immunoglobulin concentrations

The levels of IgG, IgM, IgA, interleukin 2 (IL-2), interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) in the serum were quantified using appropriate ELISA kits (H106-1-2, H109-1-2, H108-1-2, H003-1-1, H007-1-2 and H023-1-1, respectively) following the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, JS, China).

Faecal DNA extraction and 16S rRNA sequencing

Microbial DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified from genomic DNA template using the following primers: 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTWTCTAAT). Amplification products were purified using Agencourt AMPure XP Beads (Beckman Coulter Genomics, Danvers, MA, USA) according to the manufacturer's instructions, and quantified using a Qubit quantification system (Thermo Scientific, Wilmington, DE, USA). Amplicons were sequenced using an Illumina NovaSeq 6000 system by Biomarker Technologies Corporation (Beijing, China). Operational taxonomic units (OTUs) were clustered using UPARSE version 10. Each OTU sequence was compared against the Greengenes2 database (2022.10. https://docs.qiime2.org/2023.9/data-resources/) for classification at the phylum, class, order, family, genus, and/or species levels. Column accumulation diagrams displaying the relative abundances of individual species were generated based on the top 10 most abundant phyla and genera. Alpha and beta diversity indices were evaluated using QIIME2 software, while differential analysis of alpha diversity indices between groups

was performed using one-way ANOVA. Principal component analysis (PCA) and principal coordinate analysis (PCoA) plots were constructed using the R language toolset. Analyses of linear discriminant analysis effect size (LEfSe) with LDA log-score threshold set at 4.0 were conducted utilising the LEfSe tool to identify significant differences in microbiota composition between the groups at various taxonomic levels. Metagenomes and functional profiles of intestinal microbiota were analysed using PICRUSt2 software, whereas STAMP software was employed to identify differences in KEGG pathways between the groups. The significance of the difference in functional abundance between the groups was assessed using one-way ANOVA. Predictions of the intestinal microbiome phenotype were generated using the BugBase tool.

Data availability

The raw sequence data are publicly available through the BioProject Accession Number PRJNA1079044 at The National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database at https://www.ncbi.nlm.nih.gov/sra.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) implemented in SPSS statistical software (version 26.0; SPSS Inc., Chicago, IL, USA). The pen was defined as the experimental unit. Differences between means were compared using Tukey's multiple range test at a significance level of $P < 0.05$. All results are reported as means and pooled standard error of the means.

Results

Effects of ASR and DBD on growth performance

Growth performance, measured as ADG, ADFI, and FCR (Table 3), did not show any significant differences between the dietary treatments ($P > 0.05$) throughout the experimental period from 1 to 42 days of age. However, during the starter period from day 1 to 21, broilers supplemented with ASR and DBD demonstrated a significant increase in ADG by 10.87% and 15.98%, respectively, compared to the control group ($P < 0.05$). Additionally, supplementation with both ASR and DBD resulted in an increase in ADFI by 4.04% and 5.04%, respectively, accompanied by a decrease in FCR by 6.31% and 9.47%, respectively, compared to the control group; however, only the DBD treatment resulted in statistically significant effects ($P < 0.05$). No significant differences were observed for ADG, ADFI, or FCR among the three groups of broilers during the finisher period from day 22 to day 42.

Table 3. Effects of dietary supplementation with *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) powders on growth performance of broiler chickens

Items	Control	ASR	DBD	SEM	*P*-values
BW, g					
day 1	39.16	40.33	39.67	1.97	0.827
day 21	381.00^{a}	419.33^{b}	436.00^{b}	10.55	0.000
day 42	2303.00	2551.50	2344.16	130.90	0.173
ADG, g/bird/day					
days 1 to 21	16.27^{a}	18.04^{b}	18.87^{b}	0.51	0.000
days 22 to 42	91.52	101.53	90.86	6.19	0.230
days 1 to 42	53.90	59.78	54.86	3.11	0.176
ADFI, g/bird/D					
days 1 to 21	30.93	32.18	32.49	0.60	0.050
days 22 to 42	164.86	168.92	166.27	2.14	0.175
days 1 to 42	97.90^{a}	100.56^{b}	99.38^{ab}	1.01	0.058
FCR, g/g					
days 1 to 21	1.90^{a}	1.78^{ab}	1.72^{b}	0.06	0.030
days 22 to 42	1.84	1.66	1.85	0.12	0.299
days 1 to 42	1.84	1.68	1.82	0.10	0.276

control – broilers fed a basal diet, ASR – broilers fed a basal diet supplemented with 1% ASR, DBD – broilers fed a basal diet supplemented with 1% DBD. BW – body weight, ADG – average daily gain, ADFI – average daily feed intake, FCR – equal to ADFI/ADG, SEM – standard error of the mean, n = 6; ^{ab} – means within a row with no common superscript are significantly different at $P < 0.05$

Effects of ASR and DBD on serum antioxidant activity

Table 4 presents the effects of dietary supplementation with ASR and DBD on serum activity of SOD, CAT, and GSH-Px, as well as serum levels of TAC, and MDA in broiler chicks. No statistically significant differences were observed in serum activity of SOD, or MDA and TAC levels between the groups

Table 4. Effects of dietary supplementation with *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) powders on the antioxidant status of broiler chickens

Items	Control	ASR	DBD	SEM	*P*-values
SOD, U/ml	91.01	87.86	96.57	6.70	0.417
GSH-Px, U/l	365.42^{a}	434.11^{ab}	453.51^{b}	27.92	0.016
CAT, U/ml	41.26^{a}	53.72^{b}	52.57^{b}	2.47	0.000
TAC, U/ml	1.22	1.37	1.25	0.12	0.508
MDA, nmol/l	11.43	9.03	9.46	1.08	0.104

control – broilers fed a basal diet, ASR – broilers fed a basal diet supplemented with 1% ASR, DBD – broilers fed a basal diet supplemented with 1% DBD; SOD – superoxide dismutase, GSH-Px – glutathione peroxidase, CAT – catalase, TAC – total antioxidant capacity, MDA – malondialdehyde, SEM – standard error of the mean, n = 6; ^{ab} – means within the same row with different superscripts are significantly different ($P < 0.05$)

($P > 0.05$). However, compared to the control group, dietary supplementation with 1% ASR and 1% DBD significantly increased ($P < 0.05$) serum GSH-Px activity by 18.79% and 24.10%, respectively. Serum CAT activity in broilers fed diets containing 1% ASR and 1% DBD showed a significant increase ($P < 0.05$) by 30.19% and 27.41%, respectively, compared to those fed the control diet. In conclusion, our findings suggest that both additives ASR and DBD improved the antioxidant capacity in broiler chickens.

Effects of ASR and DBD on serum immune function

The effects of dietary ASR and DBD supplementation on the serum levels of IgM, IgG, IgA, IL-2, IL-6, and TNF-α in broiler chickens are presented in Table 5. Compared to the control group, the addition of ASR and DBD significantly increased serum IgA levels in broilers ($P < 0.05$). However, no significant differences ($P > 0.05$) were observed for the other measured immune parameters (IgM, IgG, IL-2, IL-6, TNF-α) between the three groups of the experimental broilers. These findings demonstrate that the administration of ASR and DBD in the diet positively influenced the immune capacity of broilers.

Table 5. Effects of dietary supplementation with *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) powders on the immune function of broiler chickens

Items	control	ASR	DBD	SEM	*P*-values
IgM, ng/ml	3717.88	4097.80	4003.68	356.37	0.549
IgG, μg/ml	79.34	85.47	78.52	5.92	0.486
IgA, ng/ml	7203.50^{a}	8859.46^{b}	8776.41^{b}	515.07	0.009
IL-2, ng/l	182.81	188.24	157.27	12.27	0.057
IL-6, ng/l	34.73	35.31	32.00	4.45	0.743
TNF-α, ng/l	79.77	76.34	76.46	4.37	0.717

control – broilers fed a basal diet, ASR – broilers fed a basal diet supplemented with 1% ASR, DBD – broilers fed a basal diet supplemented with 1% DBD, IgM – immunoglobulin M, IgG – immunoglobulin G, IgA – immunoglobulin A, IL-2 – interleukin 2, IL-6 – interleukin 6, TNF-α – tumour necrosis factor-α, SEM – standard error of the mean, n = 6; ^{ab} – means within the same row with different superscripts are significantly different ($P < 0.05$)

Effects of ASR and DBD on caecal microbiota diversity and structure in broilers

The intestinal microbiota is closely associated with the health and production performance of broiler chickens. Therefore, we analysed the caecal microbiota of chickens fed ASR and DBD using high-throughput 16S rRNA gene sequencing. This approach produced a total of 1436261 clean reads from 18 caecal samples in three groups. After denoising, removing chimeras, and filtering low-quality sequences, each sample contained an average of 48212 sequences. These sequences were further classified into 1431 operational taxonomic units (OTUs) based on a 97% identity threshold. Richness and diversity analyses of the caecal microbiota in the three groups are presented in Figure 1. The Chao1 index and rank abundance curve showed significantly higher values in both the ASR and DBD groups compared to control animals. Conversely, the Shannon diversity index assumed significantly lower values in the ASR group and DBD group, indicating that supplementation with ASR and DBD could increase microbial counts, while reducing its diversity in the intestines.

To further verify differences in species composition between samples from individual groups, principal component analysis (PCA) based on the EUCLIDEAN distance, and principal coordinate analysis (PCoA) based on the Bray-Curtis distance, were used to analyse beta diversity between the caecal microbiota of broilers fed the control diet or diets supplemented with ASR and its formulation. The caecal microbiota formed distinct, separated clusters in the three groups. Principal components PC1 and PC2 accounted for 87.49% and 5.94% of the variation, respectively (Figure 1D). The differences in the composition of the caecal microbiota community between the ASR and DBD groups were minor; however, they were more pronounced when compared to the control group. This finding suggested that the caecal microbiota differed significantly between the three groups and that supplementation with ASR and DBD could alter the microbial composition of the caecum of broiler chickens.

To identify the alterations in the broiler gut microbial communities induced by ASR and DBD, a comprehensive analysis of bacterial taxonomy was conducted at both the phylum and genus levels (Figure 2). The dietary intervention with ASR and DBD resulted in significant changes in the composition of the caecal microbiota. At the phylum level, Firmicutes emerged as the dominant group, followed by Bacteroidota, Desulfobacterota, and Proteobacteria. In comparison to the control group, there was an increase in the relative abundance of Bacteroidota and Desulfobacterota, while the number of Firmicutes and Proteobacteria showed a decrease in the caecal microbiota in the ASR and DBD groups. Regarding the genus level, *Bacteroides*, unclassified *Oscillospiraceae*, *Alistipes*, and unclassified *Lachnospiraceae* were identified as dominant taxonomic units in the broiler chicken caecal

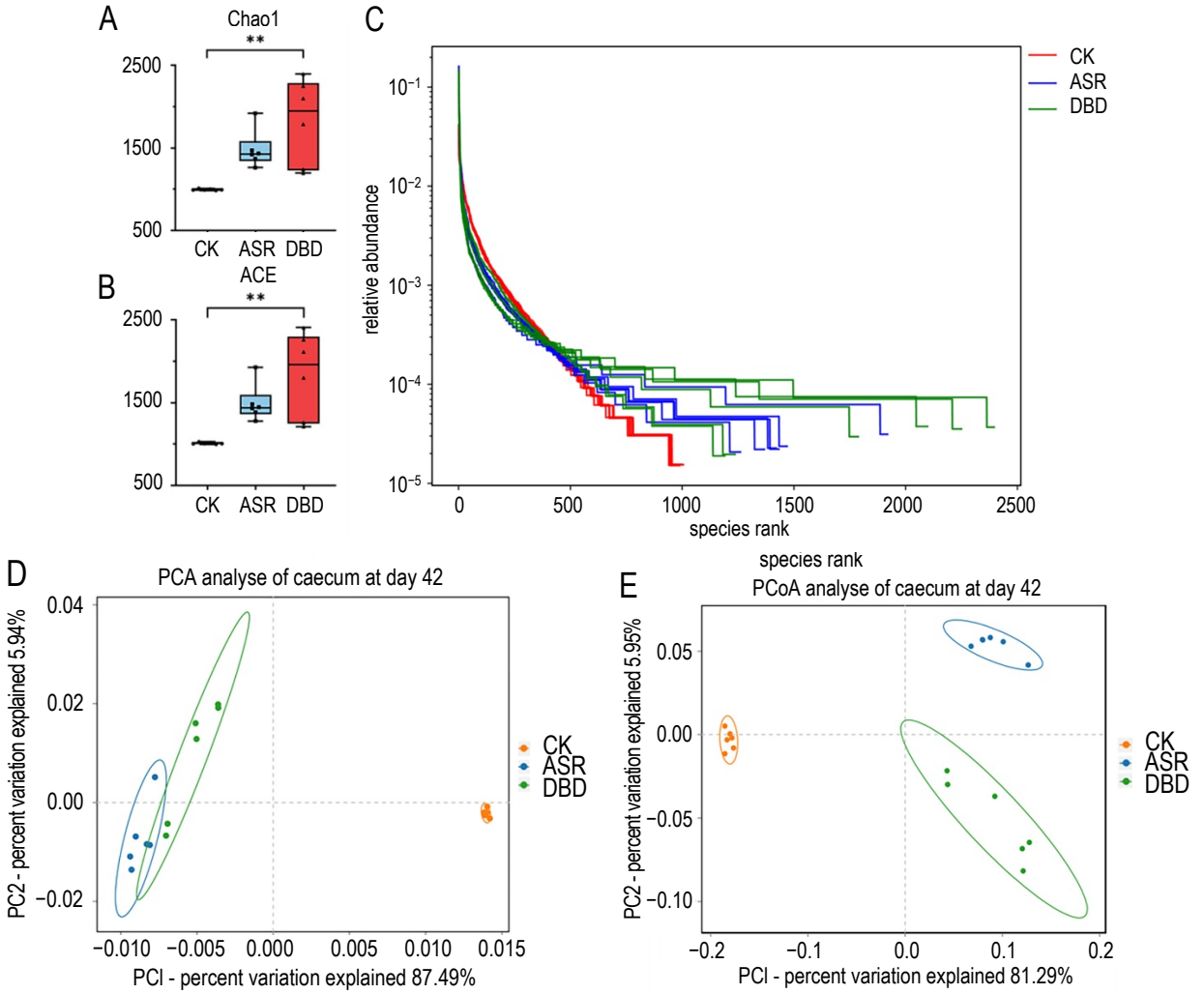

Figure 1. Differences in the diversity, richness, and structure of the bacterial community in the caecum of broilers fed with or without *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) powders. α diversity analysis of caecal microbiota in chicken. (A) Chao1 index, (B) ACE index, (C) Rank Abundance Curve (D) Principal components analysis (PCA) of the bacterial community structure in the CK, ASR and DBD group. Each symbol represents individual gut microbiota. (E) PCoA analysis showed significantly separated clusters between the CK, ASR and DBD groups

CK – basal diet, ASR – basal diet supplemented with ASR, DBD – basal diet supplemented with DBD

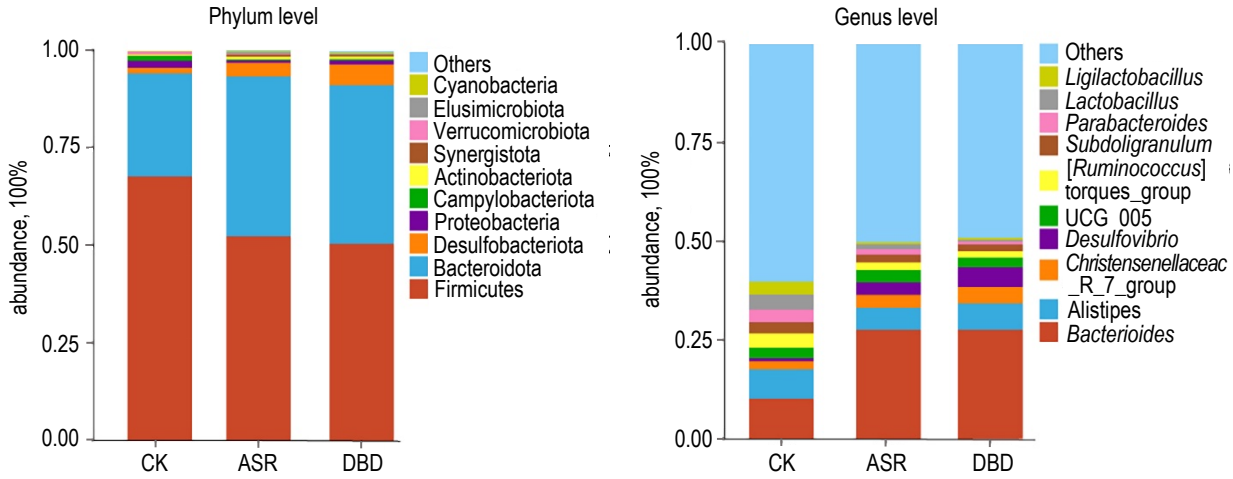

Figure 2. Changes in the microbial composition in the caecum of broilers fed with or without *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) powders. Microbial composition at the phylum and genus level; each bar represents the relative abundance of individual bacterial taxa in chickens' intestines

CK – basal diet, ASR – basal diet supplemented with 1% ASR, DBD – basal diet supplemented with 1% DBD

microbiota. It should be noted that compared to controls, there was an increase in the relative abundance of *Bacteroides*, unclassified *Oscillospiraceae*, *Christensenellaceae-R-7*, and *Desulfovibrio,* whereas *Alistipes*, unclassified *Ruminococcaceae*, unclassified *Clostridia-UCG-014*, and *Ruminococcus-torques* showed decreased abundance in the caecal microbiota of both the ASR and DBD groups. Furthermore, the count of unclassified *Lachnospiraceae* was higher in the DBD group than that observed in the ASR or control groups. In contrast, the abundance of *UCG-005* was higher in the ASR group compared to the DBD and control groups.

To directly observe the differential impact of ASR and DBD on the species composition of the caecal microbiota, we generated a heatmap illustrating the distribution and similarity of bacterial abundance between individual groups (Figure 3).

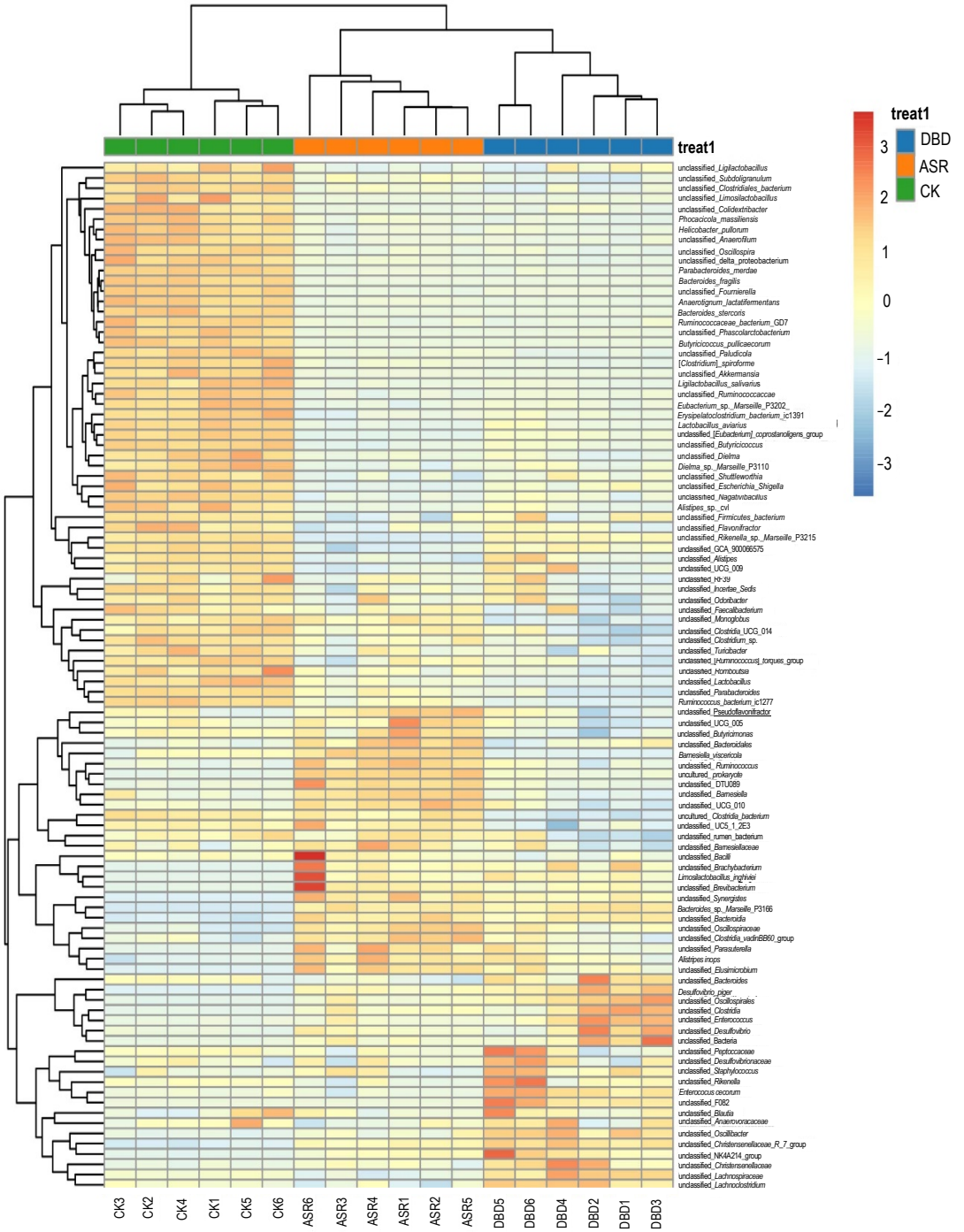

Figure 3. Significantly different bacterial taxa between the CK, *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) group, showing taxonomic abundance using cluster heatmap at level of species. Colour gradient (blue to red) represents relative richness (low to high)
CK – basal diet, ASR – basal diet supplemented with 1% ASR, DBD – basal diet supplemented with 1% DBD

To investigate the differences in the species composition of the gut microbiota in birds supplemented with ASR and DBD, a linear discriminant analysis was conducted of the effect size (LEfSe) to identify species-specific biomarkers (LDA score > 4). The LEfSe analysis revealed significant differences in microbial communities across different groups (Figure 4). The proportions of the genera *Lactobacillus*, unclassified *Ruminococcaceae*, *Ligilactobacillus*, *Parabacteroides*, and *Alistipes* were found to be higher in the caecal microbiota of the control group. On the other hand, the relative abun dance of unclassified *Oscillospiraceae*, uncultured *prokaryotes*, and *Barnesiella* genera were higher in the caecal microbiota in the ASR group. Additionally, the genera *Bacteroides*, *Desulfovibrio*, unclassified *Lachnospiraceae*, and *Christensenellaceae-R-7* were more abundant in the caecal microbiota of the DBD group.

The BugBase method provides organism-level predictions of biologically relevant microbiome phenotypes. Our analysis demonstrated that supplementation with ASR and DBD increased the relative abundance of Gram-negative bacteria, while reducing the relative abundance of potentially pathogenic bacteria (Figure 5).

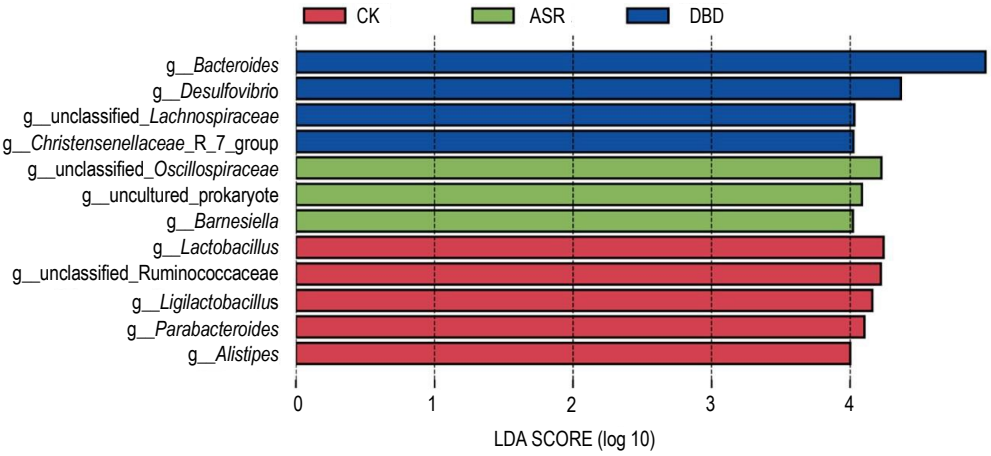

Figure 4. Significantly different bacterial taxa between the CK, *Angelica sinensis* Radix (ASR) and Danggui Buxue Decoction (DBD) group identified by linear discriminant analysis coupled with effect size (LEfSe) using default parameters

CK – basal diet, ASR – basal diet supplemented with 1% ASR, DBD – basal diet supplemented with 1% DBD

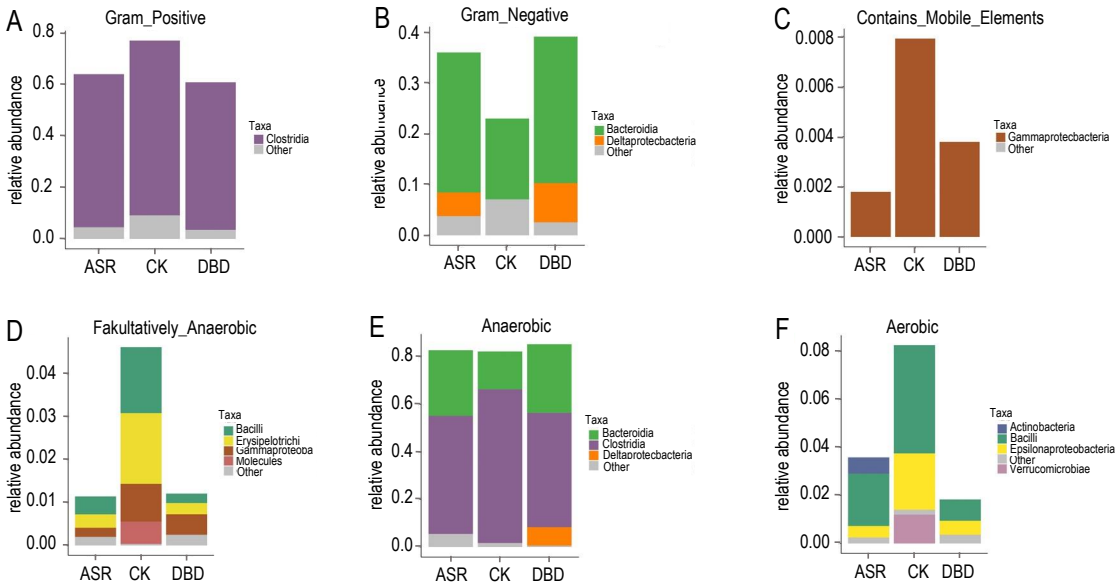

Figure 5. Prediction of 6 BugBase phenotypes: (A) Gram-positive, (B) Gram-negative, (C) potentially pathogenic, (D) aerobic, (E) anaerobic, (F) facultatively anaerobic

CK – basal diet, ASR – basal diet supplemented with 1% *Angelica sinensis* Radix, DBD – basal diet supplemented with 1% Danggui Buxue Decoction

Discussion

In the present study, supplementation with ASR or its formulation did not have a significant effect on growth performance parameters, including ADG, ADFI, and FCR, in the entire experimental period from 1 to 42 days of age. However, during the starter period from day 1 to day 21, broilers supplemented with DBD showed significantly higher ADG and ADFI values, accompanied by a significantly lower FCR compared to the other group. This improvement in growth performance during the starter period could be attributed to the presence of active metabolites in DBD, consistent with findings of Tian et al. (2023). Wu (2018), on the other hand, demonstrated that supplementing a basal diet with AM polysaccharides increased body weight in juvenile broilers by enhancing digestive enzyme activities. Both ASR and AM contain biologically active substances such as polyphenols and organic acids that can improve feed flavour and palatability, potentially contributing to improved growth performance during the starter period in broilers. It should be noted that DBD supplementation appears to benefit broiler chickens in the brood stage more than those in the rearing period, possibly due to the limited impact of low DBD concentrations on larger birds. Further studies are required to determine optimal levels of DBD supplementation.

Reactive oxygen species (ROS) are generated by living organisms as a result of normal cellular metabolic processes, and they play various physiological functions, including immune defence and maintenance of cellular homeostasis. However, when ROS levels are excessive, the cellular antioxidant defence system becomes insufficient to prevent their accumulation beyond acceptable limits. This can lead to damage to essential biomolecules within cells and disruption of the cellular redox balance, resulting in oxidative stress (Pisoschi et al., 2016). The latter state has been associated with metabolic disorders and organ degeneration, reduced metabolic efficiency, and the development of diseases in animals (Bhattacharyya et al., 2014; Lauridsen, 2019; Gulcin, 2020). Consumption of antioxidant substances has been shown to protect against damage caused by oxidative stress. A growing body of research has suggested that Chinese herbal supplements containing compounds such as flavonoids, polysaccharides, vitamin D, and vitamin C (Zhu et al., 2004; Chen et al., 2013a) exhibit potent antioxidant activities that can alleviate oxidative stress in animals (Jin et al., 2013; Yan et al., 2014; Gu et al., 2020; Gao et al., 2021). ASR is abundant in bioactive compounds, such as polysaccharides, ligustilide, or ferulic acid, which have been demonstrated to possess antioxidant properties (Wei et al., 2016; Fan et al., 2020; Nai et al., 2021). *In vitro* studies have shown that polysaccharide extracts from ASR can effectively reduce the accumulation of oxidants, while increasing the activities of GSH, SOD, and CAT (Zhuang et al., 2016; Du et al., 2023). Chang et al. (2020) reported that supplementation with DBD improved physical performance in swimming rats and mediated physiological adaptations. Therefore, we analysed the activity of endogenous antioxidant enzymes SOD, GSH-Px, CAT, and TAC, as well as the lipid peroxidation indicator MDA in broilers to evaluate the effects of dietary supplementation with ASR and DBD on antioxidant enzyme activities. In line with the results of Du et al. (2023), both ASR and DBD administration resulted in increased GSH-Px and CAT activity levels along with decreased serum MDA concentrations in broiler chickens. These results indicated that dietary supplementation with ASR and DBD improved the antioxidant capacity of broiler chickens.

Immunoglobulins are the main secretory products of the adaptive immune system, and play pivotal roles in animal immune function (Wang et al., 2019). IgA, IgG, and IgM represent the primary immunoglobulins synthesised by humoral immune cells in immune organs and tissues, serving as crucial indicators for assessing the health status of this system (Carsetti et al., 2004). IgA is indispensable for maintaining mucosal homeostasis, providing protection against antigens, and acting as an anti-inflammatory agent in the respiratory and gastrointestinal tracts (Mkaddem et al., 2014; Breedveld and van Egmond, 2019). Secretory IgA primarily regulates mucosal immunity by binding to pathogens to prevent their access to epithelial cells or by binding to specific receptors that subsequently trigger downstream immune responses such as cytokine release (Mantis et al., 2011). In the current study, supplementation with ASR and DBD significantly increased serum IgA levels in broilers. Pro- and anti-inflammatory cytokines play key roles in immune responses, with the latter alleviating inflammation and facilitating healing processes, and the former exacerbating inflammatory damage. However, no significant differences were observed in inflammationrelated parameters when ASR or DBD was administered with a basal diet, possibly because the experiment was conducted under normal conditions with regular levels of inflammatory cytokine expression.

The gut microbiota is a highly complex microbial community in the intestines that directly impacts the physiology, health, and productivity of animals (Wei et al., 2013). A growing number of studies has demonstrated the crucial role of microbial metabolism in nutrient digestion and absorption. Additionally, it regulates appetite and behaviour via neuronal signalling pathways connecting the gut microbiota to the brain (Gilbert et al., 2018). Microorganisms can also modulate immune system development and homeostasis by affecting pathogen defence and intestinal epithelium maturation. Additionally, the gut microbiota contributes to the occurrence and progression of diseases (Fan and Pedersen, 2021). Of all the components of the gastrointestinal tract, the caecum harbours a dense microbial population showing prolonged residence periods in this section of the broilers' digestive system (Oladeinde et al., 2019). The development of caecal microbiota shows some degree of conservation in broiler chickens (Kers et al., 2022). In the present study, a high-throughput 16S rRNA gene sequencing was employed to characterise the composition of the caecal microbiota in broiler chickens fed ASR and DBD diets. Both ASR and DBD treatments resulted in significant increases in microbial community richness in the caecum. Firmicutes and Bacteroidetes were identified as the predominant phyla in the caecal microbiota, comprising over 90% of the total microbiota in 42-day-old chickens, and this observation was consistent with previous findings by Rychlik et al. (Han et al., 2023; Lin et al., 2023; Liu et al., 2023). However, our results suggested that both ASR and DBD supplementation led to an increase in the relative abundance of Bacteroidetes, while reducing the number of Firmicutes within the caecal microbiota compared to the control group. Members belonging to Bacteroidetes are actively involved in polysaccharide fermentation and short-chain fatty acid (SCFA) production, which can contribute to animal health promotion. Additionally, Bacteroidetes have been shown to modulate host gut immune responses through the expression of secretory IgA (Cantarel et al., 2012; Wang et al., 2020). Our findings are in agreement with the results of a previous study, which demonstrated that supplementation with *Glycyrrhiza* polysaccharides reduced the ratio of Firmicutes to Bacteroidetes (Wu et al., 2022b). The polysaccharides present in ASR and DBD optimise the composition and abundance of microorganisms in the intestinal microbiota, which is closely associated with intestinal morphology, nutrient metabolism, and host health (Turnbaugh et al., 2006) The present results indicated that ASR and DBD increased the relative abundance of phyla such as Bacteroidetes, Desulfobacterota, Actinobacteriota, Synergistota, Elusimicrobiota, and Cyanobacteria. Additionally, they also raised the relative abundance of such genera as Bacteroides, unclassified *Oscillospiraceae*, *Christensenellaceae-R-7*, and *Desulfovibrio*. On the other hand, the relative abundance of phyla like Firmicutes, Proteobacteria, Campylobacterota, and Verrucomicrobiota decreased as a result of ASR and DBD treatment, alongside genera *Alistipes*, unclassified *Ruminococcaceae*, unclassified-Clostridia-UCG -014 and *Rominococcus*-torques in the caecal microbiota of broiler chickens. The relative abundance of the unclassified genus *Lachnospiraceae* was higher in the DBD group compared to other groups, while the genus UCG005 showed a higher relative abundance in the ASR group than in the remaining groups.

Bacteria of the genus *Oscillospira* have rarely been successfully cultured, but are commonly found in the gastrointestinal tract of healthy animals, including humans. This genus is believed to play a crucial role in maintaining the stability of the microbial community and promoting host health (Chen et al., 2020). The analysis of gut microbiome sequencing data revealed that *Oscillospira* constituted a substantial proportion of the human gut microbiome, and a strong correlation was shown between the abundance of *Oscillospira* and host health. The genus *Christensenellaceae* is commonly found in the gut microbiota of both animals and humans. Several studies have demonstrated that *Christensenellaceae* exhibits high heritability and is associated with human longevity and metabolic health (Kong et al., 2016; Waters and Ley, 2019). *Proteobacteria* have been frequently observed following antibiotic treatment or inflammation in animals and humans, and they are considered to be indicative of gut dysbiosis and epithelial dysfunction (Hollister et al., 2014; Litvak et al., 2017). *Alistipes*, on the other hand, is a potential opportunistic pathogen strongly associated with gastrointestinal disorders and host diseases in humans (Parker et al., 2020). Decreases in the relative abundance of *Proteobacteria* and *Alistipes* are indicators of gut microbiota health. Further, heatmap analysis revealed a significant impact of ASR and DBD supplementation on the gut microbiota, while the reported effects of Chinese herbal medicines on the metabolic caecal microbiota of broiler chickens varied. Overall, our findings suggest that ASR supplementation may improve intestinal ecology in broilers, promoting both their gut health and

systemic well-being. Further studies are required to elucidate how ASR and DBD supplementation affects the gut microbiota and interactions between the gut microbiota and the host.

Conclusions

In summary, our results demonstrate that supplementation with Angelica sinensis Radix and Danggui Buxue Decoction can effectively increase the antioxidant capacity, immune function, and composition of the caecal microbiota of broiler chickens. These results have significant implications for incorporating natural plant extracts as feed additives.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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