Poultry Science®

Effects of fulvic acid on broiler performance, blood biochemistry, and intestinal microflora

Long Liu ,*,[1] Na Yang,*,[1] Yueji Chen,* Zhihao Xu,* Qingwei Zhang ,* Xiuxiu Miao ,* Yanan Zhao,* Geng Hu,* Liying Liu,† Zhi Song,‡ and Xianyao Li*,§,[2]

*College of Animal Science and Technology Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, Tai'an, Shandong, China; †College of Life Science, Shandong Agricultural University, Tai'an, Shandong 271018, China; ‡Shandong Agricultural Fertilizer Technology Co., Ltd., Feicheng, Shandong 271600, China; and §Key Laboratory of Efficient Utilization of Non-Grain Feed Resources (Co-Construction by Ministry and Province), Ministry of Agriculture and Rural Affairs, Tai'an 271018, China

ABSTRACT To study the effects of mineral fulvic acid (**FuA**) on broiler performance, slaughter performance, blood biochemistry index, antioxidant function, immune performance, and intestinal microflora, 360 Arbor Acres (**AA**) broiler chickens with similar body weights were randomly divided into 5 groups with 6 replicates in each group and 12 chickens in each replicate in the current study. Chickens in the control group (**C**) were fed with the basal diet, and chickens in the test groups (I, II, III, and IV) were fed with the diet supplemented with 0.05%, 0.1%, 0.2%, and 0.3% mineral FuA, respectively. The indicators were measured on the hatching day, d 21 and d 35. From the whole experimental period, FuA supplement significantly increased average body weight (**ABW**) ($P < 0.05$), average daily gain (**ADG**) of broilers ($P < 0.05$), and thymus weight ($P < 0.05$) in II and IV groups, but bascially reduced the pH value of thigh meat. FuA supplement significantly improved aspartate aminotransferase (**AST**) activity in the group III on d 35 ($P < 0.05$) and the serum levels of IgA and IgG on d 21 and d 35 ($P < 0.05$), but reduced glutathione peroxidase **(GSH-Px**) level on d 21 ($P < 0.05$) and malondialdehyde (**MDA**) level in serum on d 35 ($P < 0.05$). FuA supplement significantly affected the abundance of *Barnesiella, Lachnospiraceae, Alistipes, Lactobacillus*, and *Christensenellaceae* on genus level. Differences between group III and other groups were significant in the genera microflora composition on d 21 and d 35. Functional analysis showed that the cecum microbiota were mainly enriched in carbohydrate metabolism, amino acid metabolism, and energy metabolism. In conclusion, FuA may potentially have significant positive effects on the growth performance and immune function of AA chickens through the modulation of the gut microbiota, and the 0.1% FuA was the best in broiler diet based on the present study.

Key words: fulvic acid, broiler, growth performance, immune performance, cecal microbiota

2024 Poultry Science 103:103273
https://doi.org/10.1016/j.psj.2023.103273

INTRODUCTION

Mineral fulvic acid (**FuA**), formed by the decay of peat, weathered coal and other minerals, is a mixture of amorphous polymer compounds (Feng et al., 2020). Its characteristics are the abundance of biologically active molecules and small molecular weight (Alvarez-Puebla et al., 2006; Winkler and Ghosh, 2018). FuA, being brownish yellow, mainly consists of 5 elements: C, H, O, N, and S. It can be soluble in organic solvents such as acid, alkali, ethanol and acetone (Winkler and Ghosh, 2018; Gong et al., 2020). FuA holds a wide variety of reactive oxygen-containing functional groups, such as hydroxyl, phenolic hydroxyl, methoxy, carboxyl, carbonyl, and quinone groups (Gao et al., 2017), and contains heterocyclic structures such as benzene ring, thick ring, pyrrole, furan, and indole (Bai et al., 2015). Thus, FuA is difficult to separate from solution because of being a water-soluble high-polarity complex (Xiao et al., 2022). Currently, FuA is mainly extracted from weathered coal, lignite, and peat. The extraction methods include alkaline-acid solubilization, acetone sulfate, oxidative degradation, ion exchange resin, and electrodialysis (Zhang and Liu, 2014; He et al., 2017).

Received August 1, 2023.
Accepted November 10, 2023.

[1]Equal contribution.

[2]Corresponding author: xyli@sdau.edu.cn

In recent years, FuA has the effects on promoting the absorption of nutrients, resisting bacteria, diminishing inflammation, and improving immunity in livestock and poultry (Kocabağli et al., 2002; Jooné and Rensburg, 2004; Aeschbacher et al., 2012; Gao et al., 2017; Lieke et al., 2021). It is expected to become one of the new feed additives to replace antibiotics. Studies have shown that FuA could improve growth performance and meat quality in growing-finishing pigs and juvenile loaches (Bai et al., 2013; Chang et al., 2013; Gao et al., 2017), stimulate the immune response of rats and *Litopenaeus vannamei* (Vucskits et al., 2010; Gutiérrez-Dagnino et al., 2015), increase the shear force and intramuscular fat level of calf meat and decrease the pH of the meat (Leeuw et al., 2017; Mokotedi et al., 2018), and reduce the number of harmful pathogenic bacteria such as *E. coli* in the intestinal tract of calves (Tunc and Yoruk, 2017). FuA could also increase milk yield and reduce cholesterol levels in whole blood and serum in dairy goats (Degirmencioglu and Ozbilgin, 2013; Degirmencioglu, 2014), decrease the rate of carbohydrate degradation (Majewska et al., 2017) and also increase the number of rumen protozoa (Kara and Buda, 2016) in sheep. At present, there are few studies on the dosage and effect of mineral FuA in chicken production.

The current study aimed to explore the effects of mineral FuA on broiler production performance, blood biochemical indicators, immune performance and intestinal microflora, investigate the suitable supplemental amount of FuA in poultry feed and provide evidence for the application of FuA in poultry production.

MATERIALS AND METHODS

Ethical Statement

The study was approved by the Animal Care and Use Committee of Shandong Agricultural University (SDAUA-2022-188). All research procedures complied with the Regulations on the Administration of Laboratory Animals promulgated by National Science and Technology Commission of the People's Republic of China (Beijing).

Experimental Design

Three hundred sixty 0-day-old Arbor Acres (**AA**) broilers with similar body weights were obtained from Shandong Hekangyuan Group Co., Ltd. (Jinan, China), and were randomly divided into 5 groups with 6 replicates per group and 12 chickens per replicate. The mineral FuA (purity 50%, Ash content (silicate, sulfate) 30%, organic carbon content 7%, T-AOC 4.308 μmol Trolox/g, total phenolic acid content 2.681 mg/g), powdered and extracted from natural high quality humic acid, was provided by Shandong Agricultural Fertilizer Technology Co., Ltd. (Taian, China). Chickens in the control group (**C**) were fed with the basal diet, and chickens in the test groups (I, II, III, and IV) were fed with 0.05%, 0.1%, 0.2%, and 0.3% mineral FuA, respectively. The basal corn-soybean meal diet was purchased from Tai'an Golden Chicken Incubation Co., Ltd. (Taian, China). Composition and nutrients of basal diet were shown in Table 1. All chickens were free access to feed and water throughout the experimental period in an air-conditioned house. The temperature was kept at 35°C in the first week and gradually decreased by 1°C every 2 d until 25°C, with a humidity at 55% to 60%. Light was maintained at 24 h on d 1, 23 h at d 2 to 7, and gradually decreased to 20 h at d 8 to 35. Chickens were observed and recorded daily.

Table 1. Composition and nutrient level of basal diet (%).

Items	1−21 d of age	22−35 d of age
Corn	60	60
Soybean meal 43	28.8	25.28
Corn gluten meal 60	5.3	5.2
Salt	0.16	0.16
$NaHCO_3$	0.2	0.2
Limestone	1.3	1.2
$CaHPO_4$	0.75	0.65
Level 1 soybean oil	2.2	5.9
Vitamin premix	0.03	0.03
Trace element	0.2	0.2
50% Choline	0.1	0.1
Methionine	0.23	0.245
70% Lysine	0.58	0.646
98.5% Threonine	0.134	0.174
2,000 u phytase	0.02	0.02
Total	100	100
CP	20	19
ME (kcal/kg)	2980	3200
Ca	0.9	0.88
AP	0.45	0.40
Available lysine	1.15	1.1
Met+Cys	0.82	0.79
Threonine	0.75	0.74
Trp	0.175	0.17

Performance Measurement

Chickens body weight and feed were recorded at d 0, d 21 and d 35. Average body weight (**ABW**), average daily gain (**ADG**), average daily feed intake (**ADFI**), and ratio of feed/gain (**F/G**) were calculated.

On d 35, 2 chickens per replicate were randomly selected, weighed and euthanized by cervical dislocation. The weight of heart, liver, spleen, thymus and bursa of Fabricius were measured. The pH value of breast meat and thigh meat was measured. Slaughter performance was observed and calculated according to previous study (Liang et al., 2022).

Antioxidation and Immunology Ability

On d 21 and d 35, blood samples were collected from the wing veins of 2 chickens in each replicate, kept on ice for 20 min and centrifuged at 3,000 r/min for 10 min. Serum was collected in 1.5 mL tubes and stored at −80° C for further utilization. The concentration of aspartate aminotransferase (**AST**) and alanine aminotransferase (**ALT**) and the levels of IgA, IgG and IgM in serum were measured using an automatic biochemical analyzer

(7170A/7180, Hitachi, Beijing, China). The levels of total antioxidant capacity (**T-AOC**), glutathione peroxidase (**GSH-Px**), malondialdehyde (**MDA**), superoxide dismutase (**SOD**) and catalase (**CAT**) in serum were measured using the ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China).

DNA Extraction and PCR Amplification

Two chickens in each replicate, in total, 12 chickens in each group were selected for cecum contents collection on d 21 and d 35, respectively. Cecum contents were put in 1.5 mL tubes and stored in the −80°C. Genomic DNA was extracted from cecal contents using the QIAamp Fast DNA Stool Mini Kit (Qiagen, California). DNA concentration and purity were measured by NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Delaware) and DNA integrity was verified using a 1% agarose gel electrophoresis. Then qualified DNA samples were stored at −20°C for further analysis. V3 and V4 segments of the 16S rRNA were amplified using forward primers (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primers (5′-GGACTACHVGGGTWTCTA-3′). The PCR condition was as follows: 95°C for 3 min, followed by 27 cycles of 95°C 30 s, 55°C 30 s, and 72°C 45 s, with a final extension step at 72°C for 10 min.

16S rRNA Gene Sequencing and Data Analysis

The qualified amplicons were submitted to Promega (Beijing) Biotech Co., Ltd. (Beijing, China) to be quantified on QuantiFluor-ST Blue Fluorescence Quantification System. Miseq library was prepared by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) using TruSeqTM DNA Sample Prep Kit (llumina, San Diego, CA). Final valid data from sequencing results on Miseq platform (llumina, San Diego, CA) were obtained after quality control, trimming and mapping of high quality reads. The similar sequences were clustered into operational taxonomic units (**OTUs**) with a 97% identity threshold using UPARSE (http://www.drive5.com/uparse/) and the taxonomy was assigned against the Greengenes database (http://greengenes.secondgenome.com/). Alpha diversity was analyzed using Mothur (https://mothur.org/) and beta diversity was analyzed using QIIME2 (https://qiime2.org/) followed by principal coordinate analysis (**PCoA**) based on Bray-Curtis distance to study similarities and differences of the community composition among samples. Kruskal-Wallis sum-rank test was used to detect differences in cecal microorganisms. PICRUSt (http://picrust.github.io/picrust/) was used to standardize the OTUs' abundance, and then Clusters of Orthologous Groups (**COG**) and Kyoto encyclopedia of genes and genomes (**KEGG**) biological pathway enrichment analysis was performed.

Statistical Analysis

Data were preliminarily sorted by EXCEL 2010 and analyzed through 1-way ANOVA using SAS 9.2. Duncan's test was used to compare the means among the different groups. $P < 0.05$ was considered as significance.

RESULTS

Growth and Slaughter Performance

In d 1 to 21, ABW and ADG increased in a quadratic manner with the rising level of FuA supplement ($P < 0.05$), ABW in group II was 11.56% higher than those in the control group (C), ADG in group II was 12.59% higher than those in the control group (C) (Table 2). In d 22 to 35, ABW ($P < 0.05$) and ADG ($P < 0.05$) were significantly increased in group II compared with those in the control group (C), but F/G was decreased in the groups supplemented with FuA.

As shown in Table 3, FuA did not affect slaughter rate, full evisceration rate, half evisceration rate, abdominal fat rate and the pH value of breast meat ($P > 0.05$). Compared with those in the control group (C), chickens had a highly significant enhancement (+8.9%) in the breast muscle rate in group II ($P < 0.01$) and a significant decline in the leg muscle rate in I and III groups ($P < 0.05$). The pH value of thigh meat bascially decreased in a linear manner with the increased level of FuA ($P < 0.05$).

Table 2. Effects of dietary fulvic acid on growth performance of broilers (g/d).

Items		Dietary addition of fulvic acid					*P* value		
		C (0%)	I (0.05%)	II (0.1%)	III (0.2%)	IV (0.3%)	Treatments	Linear	Quadratic
1−21 d	ABW	562.95 ± 9.95^{b}	603.49 ± 11.84^{ab}	628.05 ± 12.05^{a}	599.87 ± 16.81^{ab}	610.28 ± 17.11^{a}	0.04	0.06	0.02
	ADG	27.17 ± 0.52^{b}	29.31 ± 0.63^{ab}	30.59 ± 0.64^{a}	29.11 ± 0.88^{ab}	29.66 ± 0.90^{a}	0.04	0.06	0.02
	ADFI	39.48 ± 0.64	40.29 ± 1.44	40.97 ± 0.47	39.77 ± 0.52	39.93 ± 1.41	0.89	0.91	0.72
	F/G	1.46 ± 0.03	1.37 ± 0.04	1.34 ± 0.02	1.37 ± 0.05	1.35 ± 0.03	0.16	0.05	0.05
22−35 d	ABW	1588.31 ± 54.27^{c}	1692.44 ± 22.50^{abc}	1807.67 ± 38.43^{a}	1642.45 ± 51.32^{bc}	1768.00 ± 37.52^{ab}	0.01	0.05	0.07
	ADG	68.47 ± 2.98^{b}	72.48 ± 1.74^{ab}	77.87 ± 2.30^{a}	68.83 ± 2.85^{b}	76.24 ± 2.26^{ab}	0.06	0.14	0.28
	ADFI	115.19 ± 6.79	111.30 ± 0.30	121.08 ± 2.01	109.71 ± 4.55	117.37 ± 2.78	0.41	0.81	0.96
	F/G	1.69 ± 0.12	1.54 ± 0.04	1.56 ± 0.03	1.60 ± 0.06	1.54 ± 0.01	0.48	0.21	0.33
1−35 d	ABW	1588.31 ± 54.27^{c}	1692.44 ± 22.50^{abc}	1807.67 ± 38.43^{a}	1642.45 ± 51.32^{bc}	1768.00 ± 37.52^{ab}	0.01	0.05	0.07
	ADG	44.04 ± 1.55^{c}	47.02 ± 0.64^{abc}	50.31 ± 1.10^{a}	45.59 ± 1.47^{bc}	49.18 ± 1.07^{ab}	0.01	0.05	0.07
	ADFI	75.13 ± 2.52	74.39 ± 1.59	79.58 ± 1.16	72.94 ± 3.67	76.69 ± 1.50	0.36	0.78	0.93
	F/G	1.71 ± 0.08	1.59 ± 0.05	1.58 ± 0.03	1.60 ± 0.05	1.56 ± 0.01	0.26	0.06	0.11

Abbreviations: ABW, average body weight; ADFI, average daily feed intake; ADG, average daily gain; F/G, ratio of feed/gain.
[a,b,c]Means that the difference is significant ($P < 0.05$) in peer data.

Table 3. Effects of dietary fulvic acid on slaughter performance of broilers (%).

Items	Dietary addition of fulvic acid					*P* value		
	C (0%)	I (0.05%)	II (0.1%)	III (0.2%)	IV (0.3%)	Treatments	Linear	Quadratic
Slaughter rate	89.37 ± 0.71	90.34 ± 0.39	89.23 ± 0.83	90.15 ± 0.34	90.07 ± 0.19	0.29	0.53	0.80
Half evisceration rate	82.56 ± 0.52	82.65 ± 0.77	83.92 ± 0.37	83.12 ± 0.72	84.27 ± 0.26	0.14	0.05	0.13
Full evisceration rate	70.43 ± 0.73	71.91 ± 0.36	71.02 ± 0.45	71.19 ± 0.40	71.18 ± 0.35	0.32	0.66	0.70
Abdominal fat rate	2.07 ± 0.16	2.41 ± 0.18	2.23 ± 0.15	2.07 ± 0.16	2.03 ± 0.14	0.42	0.58	0.27
Breast muscle rate	25.86 ± 0.52^{bc}	26.63 ± 0.36^{abc}	28.16 ± 0.57^{a}	25.45 ± 0.75^{c}	27.35 ± 0.52^{ab}	<0.01	0.48	0.56
Leg muscle rate	22.42 ± 0.36^{a}	20.61 ± 0.57^{b}	21.89 ± 0.34^{ab}	20.75 ± 0.49^{b}	21.87 ± 0.33^{ab}	0.02	0.53	0.80
Breast meat pH	6.39 ± 0.13	6.44 ± 0.06	6.12 ± 0.08	6.44 ± 0.12	5.94 ± 0.22	0.09	0.08	0.16
Thigh meat pH	7.14 ± 0.06^{a}	6.66 ± 0.08^{b}	6.79 ± 0.09^{b}	6.78 ± 0.08^{b}	6.75 ± 0.07^{b}	<0.01	0.02	<0.01

^{a,b,c}Means that the difference is significant ($P < 0.05$) in peer data.

Table 4. Effects of fulvic acid on organ index of broiler (%).

Items		Dietary addition of fulvic acid					*P* value		
		C (0%)	I (0.05%)	II (0.1%)	III (0.2%)	IV (0.3%)	Treatments	Linear	Quadratic
1–21 d	Cardiac index	0.61 ± 0.02	0.58 ± 0.01	0.53 ± 0.02	0.61 ± 0.02	0.58 ± 0.02	0.05	0.69	0.31
	Liver index	2.27 ± 0.06	2.25 ± 0.04	2.23 ± 0.05	2.22 ± 0.04	2.21 ± 0.06	0.96	0.41	0.71
	Spleen index	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.53	0.47	0.39
	Thymus index	0.28 ± 0.03	0.25 ± 0.01	0.26 ± 0.02	0.30 ± 0.03	0.28 ± 0.02	0.72	0.65	0.76
	Bursa index	0.28 ± 0.02	0.27 ± 0.02	0.28 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.77	0.27	0.48
22–35 d	Cardiac index	0.47 ± 0.02	0.42 ± 0.04	0.44 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.10	0.01	0.02
	Liver index	2.01 ± 0.02	1.88 ± 0.06	1.94 ± 0.05	2.03 ± 0.07	2.012 ± 0.07	0.43	0.43	0.43
	Spleen index	0.12 ± 0.02	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.19	0.93	0.05
	Thymus index	0.19 ± 0.02^{c}	0.22 ± 0.02^{bc}	0.29 ± 0.02^{a}	0.23 ± 0.02^{abc}	0.26 ± 0.02^{ab}	0.02	0.03	0.03
	Bursa index	0.25 ± 0.02^{a}	0.16 ± 0.02^{b}	0.21 ± 0.02^{ab}	0.21 ± 0.02^{ab}	0.23 ± 0.01^{a}	0.02	0.99	0.06

^{a,b,c}Means that the difference is significant ($P < 0.05$) in peer data.

As shown in Table 4, in d 1 to 21, FuA did not affect organ weight of liver, spleen, thymus and bursa of Fabricius ($P > 0.05$) but changed cardiac weight which was lower in group II than that in the control group (C) ($P > 0.05$). In d 22 to 35, bursa weight ($P < 0.05$) was significantly decreased in the group supplemented with FuA but thymus weight ($P < 0.05$) was increased in a linear manner with the rising level of FuA supplement ($P < 0.05$).

Serum Biochemical, Antioxidant, and Cytokine

No significant differences among 5 groups were observed in the serum AST activity on d 21 and the serum ALT activity on d 21 and d 35 (Table 5, $P > 0.05$). The serum AST activity was significantly higher in group III than that in the control group (C) (Table 5, $P < 0.05$) on d 35.

As shown in Table 6, the T-AOC level increased slightly, but GSH-Px activity decreased significantly ($P < 0.05$) in a linear manner with the increased level of FuA ($P < 0.05$) on d 21. Compared with those in the control group (C), the serum T-AOC level increased significantly ($P < 0.05$) but the SOD level was obviously decreased ($P > 0.05$) in group II on d 35. The CAT level in group III was significantly higher than those in the control group (C) on d 35 ($P < 0.01$). In d 22 to 35, the serum MDA level was gradually decreased ($P < 0.05$) in a linear manner with the increased level of FuA ($P < 0.05$).

The level of IgM improved slightly (Table 7, $P > 0.05$), but the levels of IgA and IgG in serum on d 21 and d 35 were significantly increased (Table 7, $P < 0.05$) in a linear manner with the increased level of FuA supplement (Table 7, $P < 0.05$).

Intestinal Microflora

OTUs detected in at least 1 sample from 1 group were counted into the number of OTUs in the group (Figure 1). On d 21, there were 663, 742, 715, 806, and 673 OTUs obtained and 4, 8, 7, 49 and 6 unique OTUs obtained in groups C, I, II, III and IV, respectively. On d

Table 5. Effects of dietary fulvic acid on serum biochemical indices of broilers.

Items		Dietary addition of fulvic acid					*P* value		
		C (0%)	I (0.05%)	II (0.1%)	III (0.2%)	IV (0.3%)	Treatments	Linear	Quadratic
1–21 d	AST (nmol/min/mL)	0.22 ± 0.04	0.25 ± 0.05	0.25 ± 0.04	0.18 ± 0.06	0.10 ± 0.01	0.13	0.04	0.03
	ALT (nmol/min/mL)	2.91 ± 0.28	3.01 ± 0.18	3.02 ± 0.18	2.82 ± 0.26	2.53 ± 0.21	0.53	0.18	0.20
22–35 d	AST (nmol/min/mL)	0.19 ± 0.04^{b}	0.17 ± 0.05^{b}	0.17 ± 0.03^{b}	0.35 ± 0.05^{a}	0.25 ± 0.03^{ab}	0.04	0.05	0.15
	ALT (nmol/min/mL)	2.48 ± 0.17	2.54 ± 0.25	2.01 ± 0.18	2.71 ± 0.16	2.33 ± 0.17	0.11	0.83	0.88

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.
^{a,b}Means that the difference is significant ($P < 0.05$) in peer data.

Table 6. Effects of fulvic acid on antioxidant indices of broilers.

Items		Dietary addition of fulvic acid					*P* value		
		C (0%)	I (0.05%)	II (0.1%)	III (0.2%)	IV (0.3%)	Treatments	Linear	Quadratic
1−21 d	T-AOC (μmol Trolox/mL)	0.43 ± 0.03	0.57 ± 0.03	0.50 ± 0.03	0.45 ± 0.03	0.48 ± 0.04	0.09	0.97	0.45
	GSH-Px (μmol/mL)	12.44 ± 0.75^{a}	10.66 ± 1.24^{ab}	9.68 ± 0.61^{b}	9.82 ± 0.60^{b}	9.25 ± 0.77^{b}	0.04	<0.01	0.01
	SOD (U/mL)	3.10 ± 0.32	2.38 ± 0.41	2.64 ± 0.28	3.23 ± 0.21	2.64 ± 0.32	0.31	0.83	0.87
	CAT (μmol/min/mL)	1.47 ± 0.30	0.95 ± 0.11	0.98 ± 0.16	0.86 ± 0.14	0.73 ± 0.11	0.06	0.01	0.02
	MDA (nmol/mL)	0.90 ± 0.06	0.92 ± 0.04	0.75 ± 0.04	0.83 ± 0.08	0.73 ± 0.10	0.28	0.08	0.21
22−35 d	T-AOC (μmol Trolox/mL)	0.42 ± 0.03^{bc}	0.48 ± 0.03^{ab}	0.52 ± 0.03^{a}	0.39 ± 0.02^{c}	0.44 ± 0.03^{abc}	0.02	0.54	0.22
	GSH-Px (μmol/mL)	9.59 ± 0.31	9.84 ± 0.91	8.00 ± 0.48	8.48 ± 0.45	8.87 ± 0.52	0.15	0.13	0.16
	SOD (U/mL)	2.27 ± 0.28	2.37 ± 0.21	1.48 ± 0.25	2.43 ± 0.19	2.06 ± 0.26	0.05	0.66	0.57
	CAT (μmol/min/mL)	0.60 ± 0.05^{b}	0.60 ± 0.05^{b}	0.54 ± 0.05^{b}	0.88 ± 0.10^{a}	0.50 ± 0.06^{b}	<0.01	0.98	0.31
	MDA (nmol/mL)	1.02 ± 0.15^{ab}	1.08 ± 0.11^{a}	0.88 ± 0.13^{ab}	0.71 ± 0.03^{b}	0.69 ± 0.05^{b}	0.04	<0.01	0.01

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

^{a,b,c}Means that the difference is significant ($P < 0.05$) in peer data.

Table 7. Effects of dietary fulvic acid on immune function of broilers.

Items		Dietary addition of fulvic acid					*P* value		
		C (0%)	I (0.05%)	II (0.1%)	III (0.2%)	IV (0.3%)	Treatments	Linear	Quadratic
1−21 d	IgA (ng/mL)	4878.89 ± 92.30^{b}	6933.12 ± 363.94^{a}	6913.85 ± 284.91^{a}	6805.93 ± 162.71^{a}	6775.10 ± 475.26^{a}	<0.01	<0.01	<0.01
	IgG (μg/mL)	62.64 ± 1.87^{b}	83.79 ± 3.05^{a}	82.88 ± 3.39^{b}	81.49 ± 3.90^{a}	80.94 ± 3.58^{b}	<0.01	0.01	<0.01
	IgM (ng/mL)	2825.54 ± 164.62	4029.94 ± 228.70	3971.31 ± 453.82	3744.11 ± 295.80	4069.03 ± 368.00	0.06	0.05	0.04
22−35 d	IgA (ng/mL)	4855.76 ± 188.72^{b}	6929.27 ± 390.83^{a}	7491.96 ± 410.25^{a}	7368.63 ± 382.49^{a}	6794.37 ± 471.33^{a}	<0.01	0.01	<0.01
	IgG (μg/mL)	67.89 ± 1.56^{bc}	60.83 ± 6.75^{c}	75.02 ± 3.06^{abc}	85.28 ± 4.65^{a}	81.84 ± 7.49^{ab}	0.02	<0.01	0.02
	IgM (ng/mL)	2908.60 ± 107.29	4115.45 ± 468.46	3998.18 ± 428.92	4330.43 ± 282.50	3949.32 ± 506.79	0.12	0.08	0.04

^{a,b,c}Means that the difference is significant ($P < 0.05$) in peer data.

35, there were 807, 819, 812, 754, and 844 OTUs obtained in groups C, I, II, III, and IV, respectively. There were 623, 704, 684, 712, and 635 OTUs that overlapped on d 21 and d 35 in groups C, I, II, III, and IV, respectively (Figure 1).

Cecal microbial community abundance among 5 groups across different levels of FuA was analyzed at the genus level. Genera *Bacteroides*, most dominant on d 21, was the most abundant in group IV (28.27%) but the least abundant in group III (7.64%). *Barnesiella* was more abundant in groups I, II, and III than that in the control group (C), and was the most abundant in group III (26.02%) (Figure 2). Genera *Bacteroides* and *Barnesiella* were most dominant on d 35. *Bacteroides* abundance up to 26.87% was higher in group III than that in other groups (Figure 3). *Barnesiella* abundance in groups C, I, II, III, and IV was 24.54%, 18.20%, 24.69%, 1.90%, and 19.79%, respectively (Figure 3). *Barnesiella* abundance increased in group C, I, II, and IV but decreased in group III compared to that on d 21. *Clostridia, Lachnospiraceae*, and *Ruminococcus* were more abundant in group III than those in other groups (Figure 3).

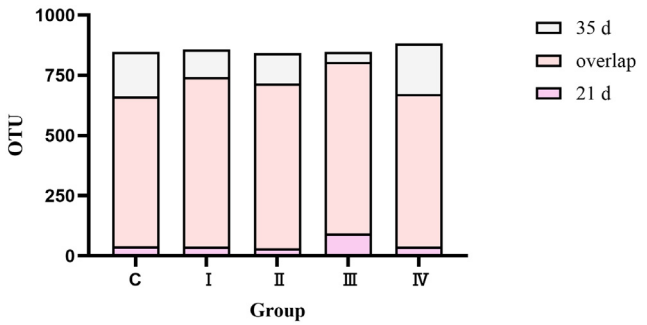

Figure 1. Number of OTUs in each group. Operational taxonomic units (OTUs) operational taxonomic units, d 21 and 35.

Alpha diversity metrics in all groups were compared (Figure 4). On d 21, Chao diversity was significantly lower in groups I (98.24) and IV (99.43) compared to the group C (112.63). Sobs diversity increased significantly in group III (107.40) than in other groups (both $P < 0.05$). On d 35, Chao diversity was significantly lower in group III (106.76) compared to the group C (126.33). Simpson diversity in group III (0.20) was significantly higher than in other groups, while Shannon diversity in group III (2.57) was significantly lower than at other groups (both $P < 0.05$). From d 21 to d 35, Chao and Sobs diversities increased significantly in groups C, I, II, and IV, and Shannon diversity was increased significantly in group I (both $P < 0.05$). Bray-Curtis was used for the PCoA (Figure 5). It was obvious that the samples in group III and the samples in other groups were far away on d 21 and d 35. The samples in group III basically gathered together on d 21 but was far away from each other on d 35.

Cecal microorganism among 5 groups across different levels of FuA was analyzed on the genus level. A significant difference ($P < 0.001$) among 5 groups in *Barnesiella* community abundance was observed across different time points. *Barnesiella* was more abundant in group III than that in other groups on d 21 (Figure 6), which was reversed on d 35 (Figure 7). Significant differences among 5 groups in community abundance of

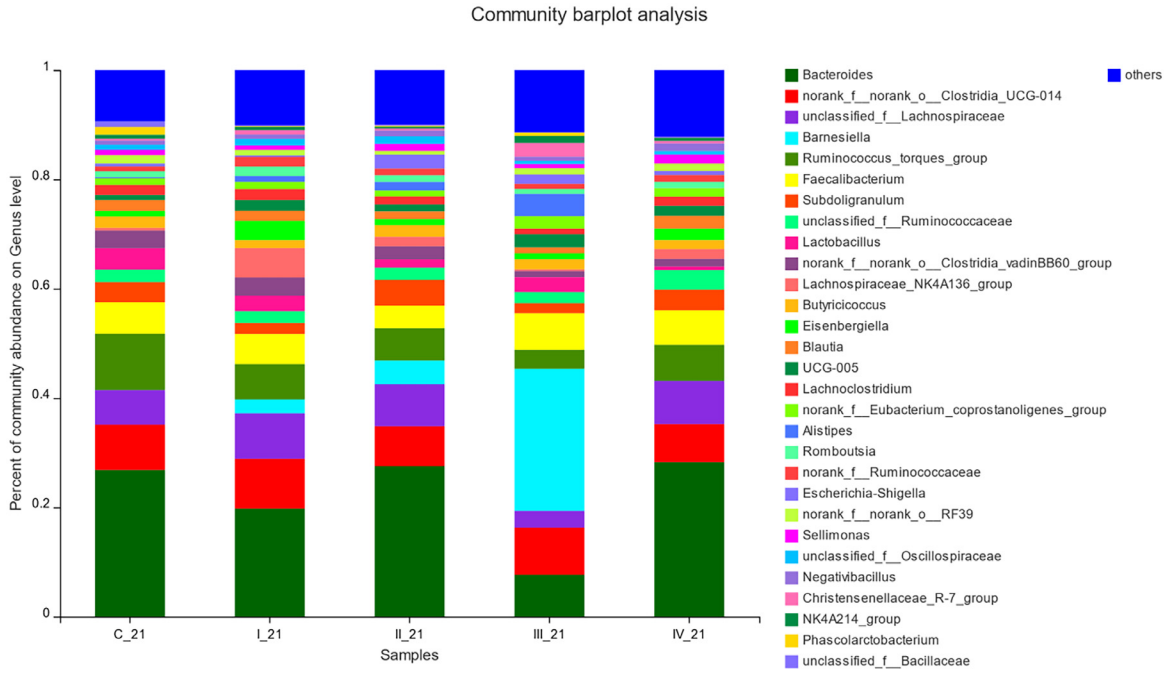

Figure 2. Genera cecal microbial community abundance (top 20) on d 21.

Lachnospiraceae (not annotated to a genus, $P < 0.05$), *Alistipes* ($P < 0.001$), *Lactobacillus* ($P < 0.05$), *Christensenellaceae* ($P < 0.001$) and *Eubacterium coprostanoligenes* ($P < 0.01$) were observed on d 35. *Lachnospiraceae* and *Eubacterium coprostanoligenes* were more abundant in group III than those in other groups. *Alistipes* and *Christensenellaceae* in group III were the least abundant among 5 groups. *Lactobacillus* was more abundant in group II than that in other groups (Figure 7).

The COG gene functions on d 21 and d 35 were mainly clustered in following categories: carbohydrate transport and metabolism (G), amino acid transport and metabolism (E), transcription (K), general function prediction only (R), replication, recombination and pair (L), cell wall/membrane/envelope biogenesis (M) and translation, ribosomal structure and biogenesis (J) (Figure 8). Cecal microbial gene functions (top 50) enriched KEGG pathways were divided into 5 categories in the secondary metabolic pathway (Figure 9). 1) The metabolism-related including global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, nucleotide metabolism, glycan biosynthesis and

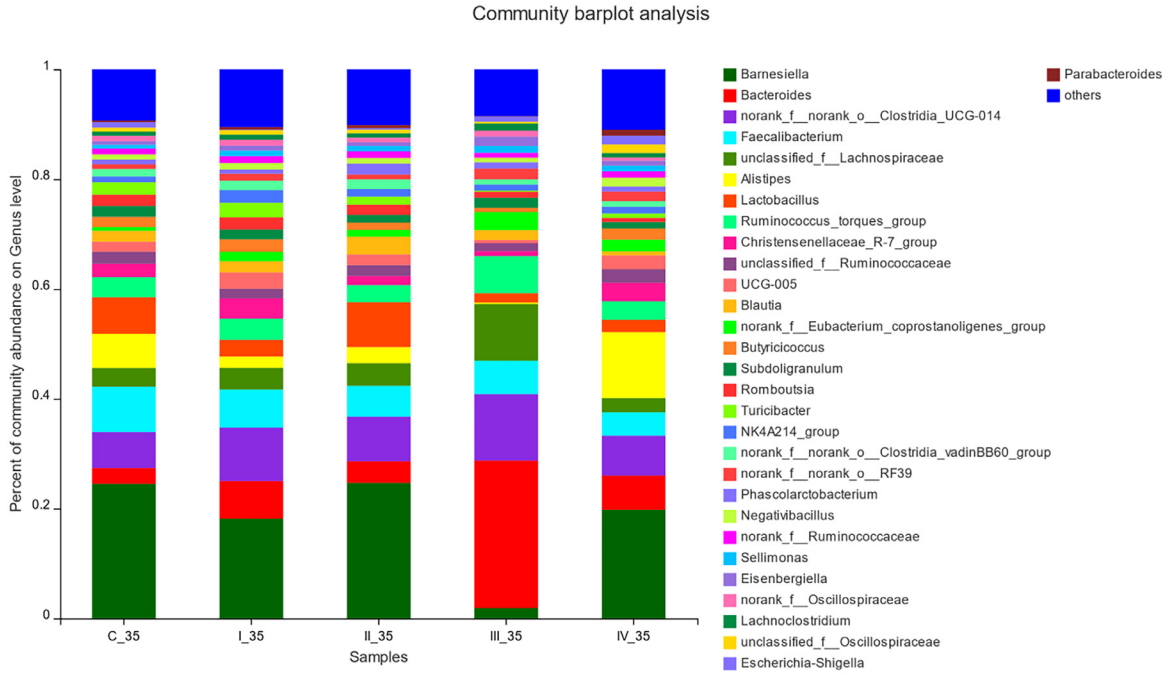

Figure 3. Genera cecal microbial community abundance (top 20) on d 35.

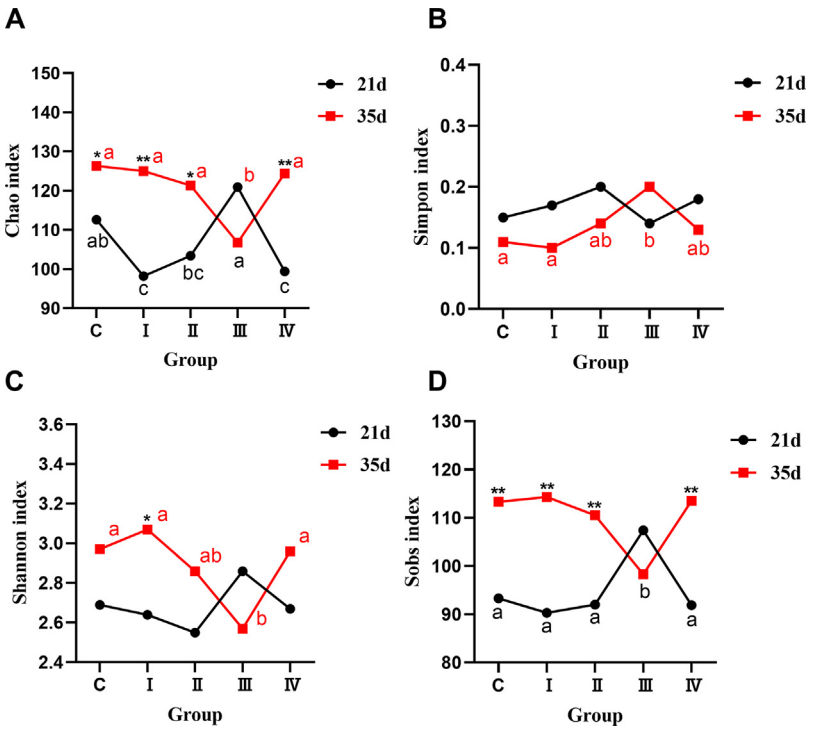

Figure 4. Microbial alpha diversity at different groups, d 21 and 35.(A) Chao index, d 21 and 35; (B) Simpson index, d 21 and 35; (C) Shannon index, d 21 and 35; (D) Sobs index, d 21 and 35. The different letters indicate that the difference is significant ($P < 0.05$), and * for $P < 0.05$, ** for $P < 0.01$.

metabolism, lipid metabolism, biosynthesis of other secondary metabolites, metabolism of terpenoids and polyketides, and xenobiotics biodegradation and metabolism. 2) The genetic information processing-related including replication and repair, folding, sorting and degradation, transcription, and translation. 3) The environmental information processing-related including membrane transport and signal transduction. 4) The cellular processes-related including cell growth and death, transport and catabolism, cellular community prokaryotes and cell motility. 5) Other pathways including endocrine and metabolic disease, cardiovascular disease, substance dependence, immune system, aging, etc.

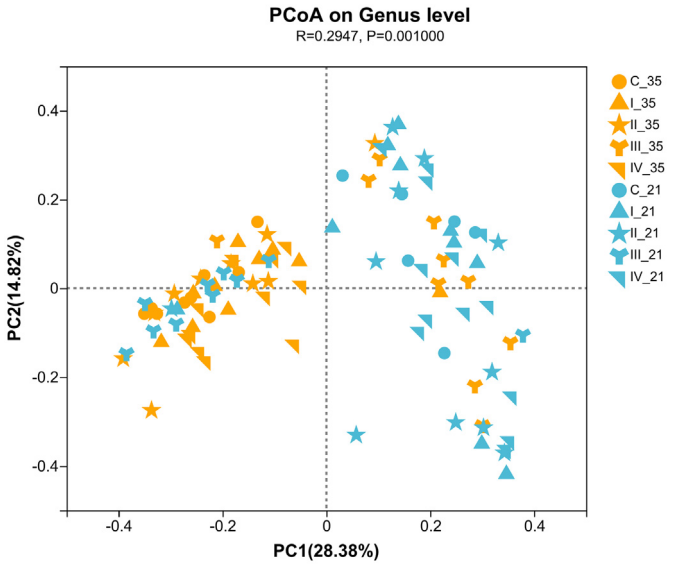

Figure 5. Principal coordinate analysis (PCoA) analysis of cecal microbial on the genus level, d 21 and 35.

DISCUSSION

Chicken is the number one production and consumption product in the world. Feed, as the main input item in broiler breeding, accounts for 75% of the production cost and plays a vital role in the broiler economy (Willems et al., 2013). FuA has small molecular weight, stable structure, and excellent acid and alkali resistance (Bai et al., 2013). FuA has low price, natural and environmental protection characteristics, and is in line with the current market consumption trend. In the livestock and poultry industry, FuA has been shown to reduce the number of harmful bacteria in the intestinal, promote nutrient absorption, and improve meat quality and growth performance (Chang et al., 2013; Tunc and Yoruk, 2017), consequently further improving the economic efficiency of farming. Therefore, it is of great significance to study and develop the functional value of FuA.

FuA addition increased ABW and ADG, and the best effect on weight gain and increased breast muscle rate with a supplement of 0.1% FuA were observed in the current study. Our findings were similar to other studies that FuA improved performance of broilers (Lu, 2010; Ozturk et al., 2010; Gong et al., 2011; Nagaraju et al., 2014; Popelka et al., 2015; Mao, 2019; Feng et al., 2022; Tang et al., 2023). Broilers fed 0.1% humate significantly improved performance (Nagaraju et al., 2014) and feed conversion ratio (Mevlut et al., 2004). This could be due to the increase in the activities of amylase, protease and lypase, which can effectively hydrolyze

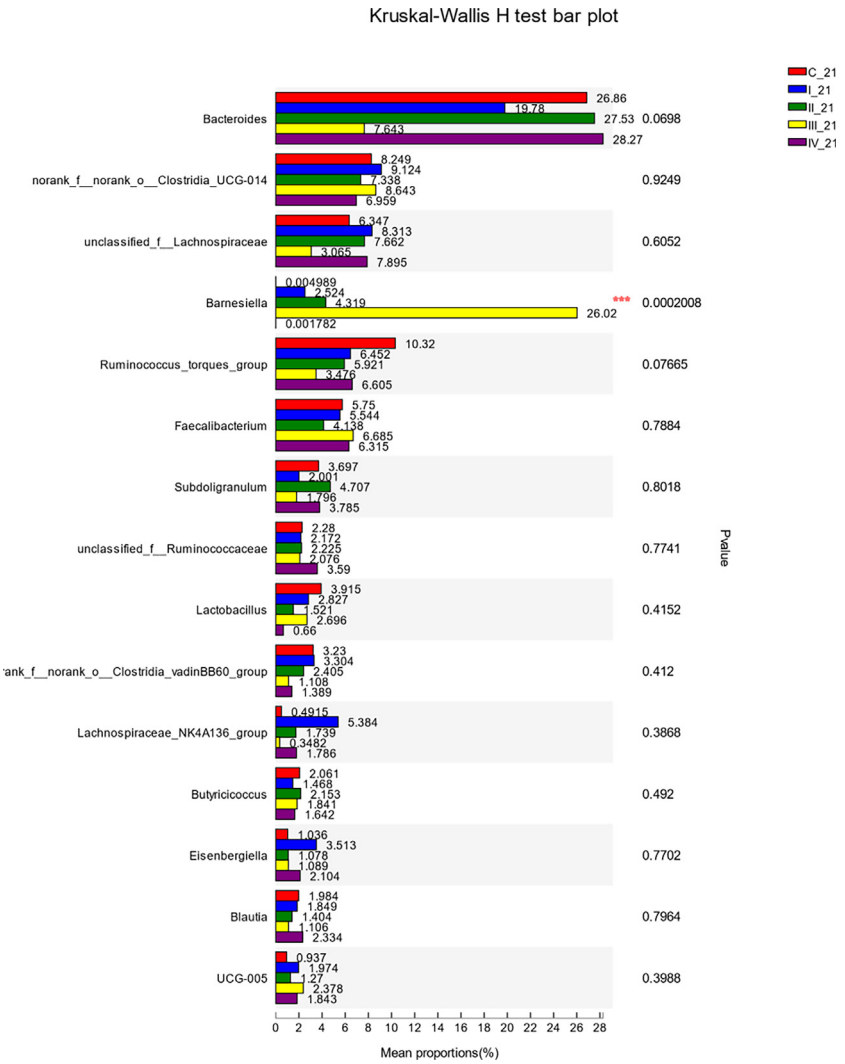

Figure 6. Genera cecal microorganism difference analysis on d 21. * means $P \leq 0.05$, ** means $P \leq 0.01$, *** means $P \leq 0.001$. Figure 7 is the same.

starch, protein and lipids into smaller molecules that can be more effectively absorbed by the intestine (Vucskits et al., 2010; Gao et al., 2017). Studies have shown that the feed form of the diet had a different structure of starch and protein fractions (Gracia et al., 2009; Zimonja and Svihus, 2009) and affected growth performance of brown egg-laying pullets (Saldaña et al., 2015). Mineral elements perform vital roles in the body (Branca and Ferrari; 2002) and are necessary for the growth and development. It could also be due to that FuA having a partially hydrophobic and hydrophilic structure and a variety of active oxygen-containing functional groups, is easily solubilized in the lipid bilayer of the membrane and can strongly chelate mineral ions and improve the utilization of mineral elements (Sanmanee and Areekijseree, 2010; Lu et al., 2019). According to Mao (2019), FuA administration also increased the content of polyunsaturated fatty acids being essential for the growth and development of the organism, thus being involved in the synthesis of lipids in the animal (Liang et al., 2023), increasing the enzyme activity in the digestive tract of broilers, and affecting the digestion and absorption of nutrients in animals.

FuA also has the ability to improve meat quality, it tended to reduce the pH value of breast meat and significantly reduced the thigh meat pH value in the current study. Our results were consistent with the findings that have shown the carcass pH value of broilers fed diets supplemented with humate was lowered (Esenbuğa et al., 2008; Ozturk et al., 2012; Semjon et al., 2020). But Ozturk et al. (2012) found no change in the pH value of thigh meat of broilers fed with humic acid. The phenomenon may be due to humus additives, and animal species. Studies have shown that 0.6 and 1 g kg^{-1} FuA administration increased the content of monounsaturated fatty acids and polyunsaturated fatty acids (Mao, 2019), which could cause oxidative deterioration of the meat and decrease the pH value. A higher muscle pH value is corresponding to darker meat and more myoglobin, whereas a lower muscle pH value is corresponding to lighter meat and less myoglobin (Holdstock et al., 2014; Gagaoua et al., 2018; Li et al., 2019). It was speculated that high levels of FuA has the undesirable effect of damaging meat quality.

The levels of SOD and GSH-Px were increased in juvenile broilers (Mao, 2019), Litopenaeus vannamei

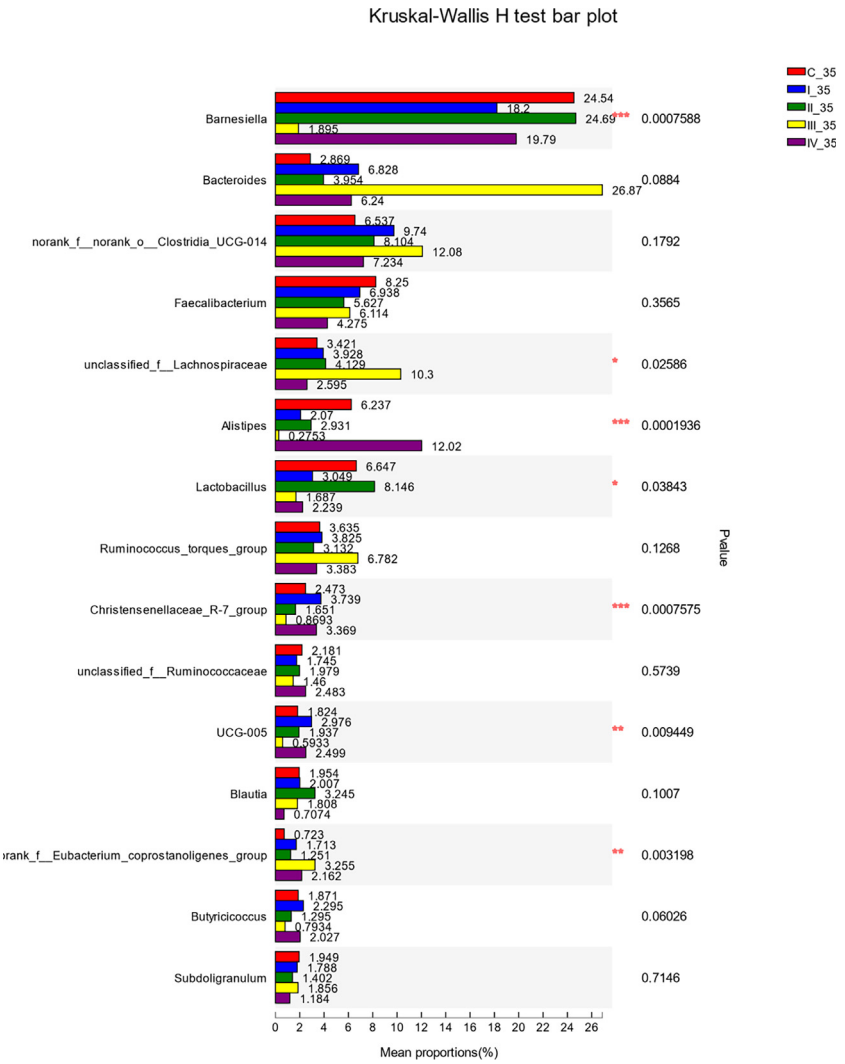

Figure 7. Genera cecal microorganism difference analysis on d 35.

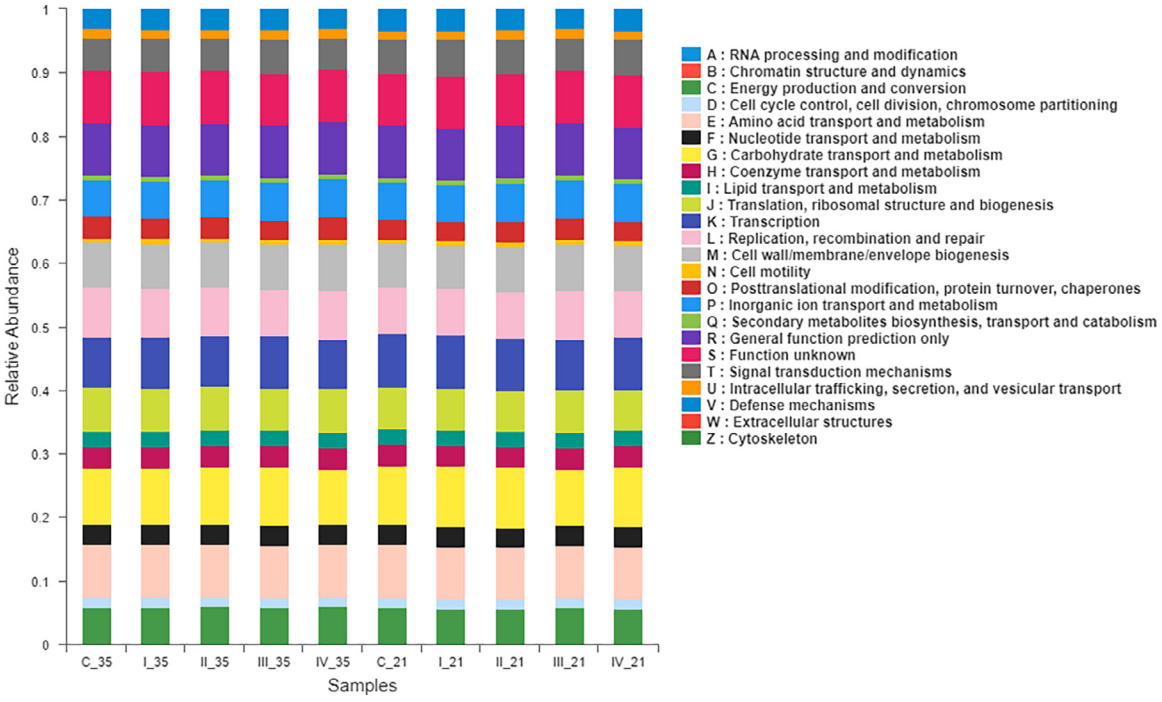

Figure 8. Clusters of orthologous groups (COG) secondary functional abundance of cecal microbial, d 21 and 35.

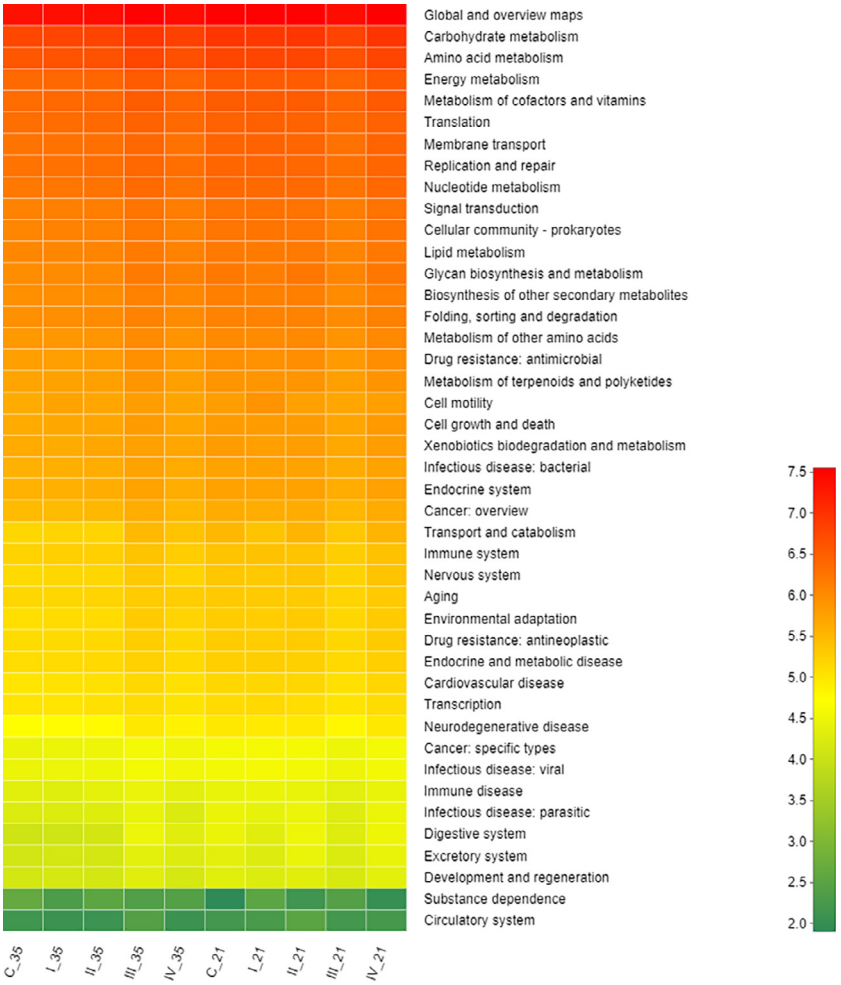

Figure 9. The secondary metabolic pathway of Kyoto encyclopedia of genes and genomes (KEGG) cecal microbes, d 21 and 35.

(Gutiérrez-Dagnino et al., 2015) and juvenile loach *Paramisgurnus dabryanus* (Sauvage) (Gao et al., 2017) fed with FuA. This result could be linked to the antioxidant activities of FuA (Rodríguez et al., 2011). The levels of IgA, IgG and IgM in serum of broilers were significantly increased with the increased level of FuA, which supported those of Mao (2019). This phenomenon could be attributed to the immunomodulatory activity of FuA (Wu et al., 2023) and the ability of humus to form compound sugars in body. This also could be caused increased thymus weight in d 22 to 35. According to Gao et al. (2022), dietary supplementation of Chinese medicine-probiotic compound microecological preparation increased the spleen and bursal index of broilers at 21 d of age. Bursa, thymus and spleen weights were increased in chickens offered sodium butyrate (Sikandar et al., 2017). This suggests that different from other preparations, FuA helps to promote the development of the thymus without effects on the function of other organs, and could be involved in humoral immunity through the thymus to improve resistance in livestock and poultry (Zhang, 2018).

Furthermore, body health can be reflected in blood biochemical indicators (Liu et al., 2018). Liver damage and protein metabolism status were indicated by AST and ALT in humans (Goodarzi et al., 2019). On d 35, the activities of AST and ALT in broilers supplemented with 0.3% FuA were higher than those in the control group (C). Rapeseed diets supplemented with a mixture of 1.5% potassium humic acid and enzymes significantly reduced the serum ALT activity in broiler chickens (Disetlhe et al., 2018). The opposite is true, which could be linked to the levels of potassium ions. Changes in potassium ions lead to changes in the permeability of cell membranes, which allows enzymes to leak into the bloodstream, causing changes in the activities of AST and ALT (Mao, 2019). Thus, further exploring the effect of FuA on ALT and AST to regulate liver and protein metabolism is needed.

Wealleans et al. (2017) found there were no effects of the probiotic alone on levels of any of the species of bacteria studied in the cecum in broilers. Changes in number of OTUs were shown from d 21 to d 35 in current study, which could be due to function of humic acid in reducing harmful bacteria such as *E. coli* (Bahadori et al., 2017). Earthworm (*Eisenia foetida*) meal with vermi-humus supplementation could be used as an organic alternative for antibiotics, according to Islam et al. (2005), which strongly supports this point. Therefore, adding FuA to the diet is more beneficial to regulate the intestinal flora than using the probiotic alone. Alpha diversity analysis corroborated those of Visscher et al.

(2019), who revealed a significant difference in cecal microbiota of piglets received humic acid at phylum and class levels. It was speculated FuA could regulate the abundance of intestinal microbial flora and affect the number of intestinal flora in animals. Differences in species showed that FuA at levels of 0.1% and 0.2% had a great effect on *Barnesiella* abundance in cecal microbiota on d 21 and d 35. The abundance of *Barnesiella* in group III was higher than that in group II on d 21, but the result was reversed on d 35. This phenomenon was consistent with the rapid intestinal development of broilers in d 22 to 35, suggesting the improved growth performance in group II could be related to the increased *Barnesiella* abundance. *Barnesiella*, one of the core microbiota in cecum of broilers, may contribute to the hydrolysis of starch and other macromolecular substances and form short-chain fatty acids to improve feed conversion ratio and promote growth, according to Pandit et al. (2018).

CONCLUSIONS

The data showed that dietary FuA supplementation increased ABW, ADG, and the breast muscle rate and improved performance with the best effect at 0.1% level. FuA increased the serum immunoglobulin level, raised thymus weight and enhanced the body immunity. Improvements in cecal microbial community abundance were observed in broilers fed with FuA, and the growth-promoting effect at 0.1% FuA may be related to the increased *Barnesiella* abundance. Taken together, implementation of FuA supplementation in the diet of broilers could be a beneficial method for their growth and development. The current state will provide a basis for the future application of FuA in poultry production.

ACKNOWLEDGMENTS

This work was supported by Shandong Modern Agricultural Industry and Technology System (SDAIT-11-02).

DISCLOSURES

The authors declare that they have no known conflicts of interest that could have appeared to influence the work reported in the present study.

REFERENCES

Aeschbacher, M., C. Graf, R. P. Schwarzenbach, and M. Sander. 2012. Antioxidant properties of humic substances. Environ. Sci. Technol. 46:4916–4925.

Alvarez-Puebla, R. A., C. Valenzuela-Calahorro, and J. J. Garrido. 2006. Theoretical study on fulvic acid structure, conformation and aggregation. A molecular modelling approach. Sci. Total Environ. 358:243–254.

Bahadori, Z., L. Esmaielzadeh, M. A. K. Torshizi, A. Seidavi, J. Olivares, S. Rojas, A. Z. M. Salem, and S. Lopez. 2017. The effect of earthworm (Eisenia foetida) meal with vermi-humus on growth performance, hematology, immunity, intestinal microbiota, carcass characteristics, and meat quality of broiler chickens. Livest. Sci. 202:74–81.

Bai, H. X., Q. F. Chang, B. M. Shi, and A. S. Shan. 2013. Effects of fulvic acid on growth performance and meat quality in growing-finishing pigs. Livest. Sci. 158:118–123.

Bai, Y., F. Wu, B. Xing, W. Meng, G. Shi, Y. Ma, and J. P. Giesy. 2015. Isolation and characterization of Chinese standard fulvic acid sub-fractions separated from forest soil by stepwise elution with pyrophosphate buffer. Sci. Rep.-UK 5:8723.

Branca, F., and M. Ferrari. 2002. Impact of micronutrient deficiencies on growth: the stunting syndrome. Ann. Nutr. Metab. 46:8–17.

Chang, Q. F., H. X. Bai, B. M. Shi, A. S. Shan, C. Y. Wei, C. Q. Yu, and B. S. Tong. 2013. Effects of dietary FA on the growth performance, serum biochemical indices, routine blood parameter and immunity of growing swine. Chin. J. Anim. Nutr. 25:1836–1842.

Degirmencioglu, T. 2014. Using humic acid in diets for dairy goats. Anim. Sci. Pap. Rep. 32:25–32.

Degirmencioglu, T., and S. Ozbilgin. 2013. Effect of administration of humic acid on somatic cell count and total bacteria in Saanen goats. Int. J. Vet. Sci. Med. 2:151–154.

Disetlhe, A. R. P., U. Marume, and V. Mlambo. 2018. Humic acid and enzymes inclusion in canola-based diets generate different responses in growth performance, protein utilization dynamics, and hemato-biochemical parameters in broiler chickens. Poult. Sci. 97:2745–2753.

Esenbuğa, N., M. Macit, M. Karaoglu, M. I. Aksu, and O. C. Bilgin. 2008. Effects of dietary humate supplementation to broilers on performance, slaughter, carcass and meat colour. J. Sci. Food Agric. 88:1201–1207.

Feng, P., Q. Li, H. Sun, J. Gao, X. Ye, Y. Tao, Y. Tian, and P. Wang. 2022. Effects of fulvic acid on growth performance, serum index, gut microbiota, and metabolites of Xianju yellow chicken. Front. Nutr. 9:963271.

Feng, P. S., Q. Q. Li, S. S. Jiang, and P. Wang. 2020. Advances in effect of fulvic acid on livestock and poultry production. Chin. J. Anim. Sci. 56:13–17.

Gagaoua, M., B. Picard, and V. Monteils. 2018. Associations among animal, carcass, muscle characteristics, and fresh meat color traits in Charolais cattle. Meat Sci. 140:145–156.

Gao, J., R. Wang, J. Liu, W. Wang, Y. Chen, and W. Cai. 2022. Effects of novel microecologics combined with traditional Chinese medicine and probiotics on growth performance and health of broilers. Poult. Sci. 101:101412.

Gao, Y., J. He, Z. He, Z. Li, B. Zhao, Y. Mu, J. Y. Lee, and Z. Chu. 2017. Effects of fulvic acid on growth performance and intestinal health of juvenile loach Paramisgurnus dabryanus (Sauvage). Fish Shellfish Immun. 62:47−56.

Gong, G., L. Xu, Y. Zhang, W. Liu, M. Wang, Y. Zhao, X. Yuan, and Y. Li. 2020. Extraction of fulvic acid from lignite and characterization of its functional groups. ACS Omega 5:27953–27961.

Gong, Z., Y. X. Cheng, M. M. Zhang, and L. L. Pan. 2011. Effects of humic-acid Na on slaughter performance and muscle quality of AA broilers. J. Anhui Sci. Technol. Univ. 25:5–8.

Goodarzi, R., K. Sabzian, F. Shishehbor, and A. Mansoori. 2019. Does turmeric/curcumin supplementation improve serum alanine aminotransferase and aspartate aminotransferase levels in patients with nonalcoholic fatty liver disease? A systematic review and meta-analysis of randomized controlled trials. Phytother. Res. 33:561–570.

Gracia, M. I., R. Lázaro, M. A. Latorre, P. Medel, M. J. Araníbar, E. Jiménez-Moreno, and G. G. Mateos. 2009. Influence of enzyme supplementation of diets and cooking−flaking of maize on digestive traits and growth performance of broilers from 1 to 21 days of age. Anim. Feed Sci. Technol. 150:303–315.

Gutiérrez-Dagnino, A., A. Luna-González, J. A. Fierro-Coronado, P. Álvarez-Ruíz, and R. Escamilla-Montes. 2015. Effect of inulin and fulvic acid on survival, growth, immune system, and WSSV prevalence in Litopenaeus vannamei. Lat. Am. J. Aquat. Res. 43:912–921.

He, W. Q., B. Li, and J. F. Song. 2017. Activation of humic acid from mineral. Phosphate Compound Fertilizer 32:3.

Holdstock, J., J. L. Aalhus, B. A. Uttaro, O. López-Campos, I. L. Larsen, and H. L. Bruce. 2014. The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. Meat Sci. 98:842–849.

Islam, K. M. S., A. Schuhmacher, and J. M. Gropp. 2005. Humic acid substances in animal agriculture. Pak. J. Nutr. 4:126–134, doi:10.3923/pjn.2005.126.134.
Jooné, G. K., and C. E. van Rensburg. 2004. An in vitro investigation of the anti-inflammatory properties of potassium humate. Inflammation 28:169–174.
Kara, U., and C. Buda. 2016. The effect of use of humic acid in some blood parameters and rumen protozoa in norduz lambs. J. Inst. Sci. Technol. 6:185.
Kocabağli, N., M. Alp, N. Acar, and R. Kahraman. 2002. The effects of dietary humate supplementation on broiler growth and carcass yield. Poult. Sci. 81:227–230.
Leeuw, K. J., N. P. Mokotedi, and U. Marume. 2017. Efficacy of potassium humate in feedlot rations on bull calve performance and carcass characteristics. 50th SASAS Congress, 18 to 21 September 2017.191 (Abstr.).
Li, J., C. Yang, H. Peng, H. Yin, Y. Wang, Y. Hu, C. Yu, X. Jiang, H. Du, Q. Li, and Y. Liu. 2019. Effects of slaughter age on muscle characteristics and meat quality traits of Da-Heng meat type birds. Anim. Basel 10:69.
Liang, F., L. Yan, Y. Li, Y. Jin, J. Zhang, H. Che, J. Diao, Y. Gao, Z. He, R. Sun, Y. He, and C. Zhou. 2022. Effect of season on slaughter performance, meat quality, muscle amino acid and fatty acid composition, and metabolism of pheasants (Phasianus colchicus). Anim. Sci. J. 93:e13735.
Liang, M. Q., W. Chen, Y. N. Zhang, S. Wang, and C. T. Zheng. 2023. Research progress on regulating effect of polyunsaturated fatty acids on lipid metabolism and reproductive performance of poultry. China Anim. Husband. Vet. Med. 10:4015–4024.
Lieke, T., C. E. W. Steinber, B. Pan, I. V. Perminova, T. Meinelt, K. Knopf, and W. Kloas. 2021. Phenol-rich fulvic acid as a water additive enhances growth, reduces stress, and stimulates the immune system of fish in aquaculture. Sci. Rep. UK 11:174.
Liu, Y., P. Zhao, M. Cheng, L. Yu, Z. Cheng, L. Fan, and C. Chen. 2018. AST to ALT ratio and arterial stiffness in non-fatty liver Japanese population:a secondary analysis based on a cross-sectional study. Lipids Health Dis. 17:275.
Lu, X. Y. 2010. Effects of biochemical fulvic acid on the performance of broilers. J. Eastern Liaoning Univ. (Nat. Sci.) 17:28–30.
Lu, Y., S. Hu, F. Liu, Y. Z. Liang, and Z. Q. Shi. 2019. Effects of humic acid and fulvic acid on the sequestration of copper and carbon during the iron oxide transformation. Chem. Eng. J. 383:123194.
Majewska, M. P., R. Miltko, J. Skomia, and B. Kowalik. 2017. Influence of humic acid supplemented to sheep diets on rumen enzymatic activity. Med. Weter 73:770–773.
Mao, Y. 2019. Modulation of the growth performance, meat composition, oxidative status, and immunity of broilers by dietary fulvic acids. Poult. Sci. 98:4509–4513.
Mevlut, K., M. Muhlis, E. Nurinisa, D. Hulya, and T. Leyla. 2004. Effect of supplemental humate at different levels on the growth performance, slaughter and carcass traits of broilers. Int. J. Poult. Sci. 3:406–410.
Mokotedi, N. P., K. J. Leeuw, U. Marume, and A. Hugo. 2018. Meat quality of weaner steers adapted to a diet containing potassium humate in the feedlot. S. Afr. J. Anim. Sci. 48:19.
Nagaraju, R., B. Reddy, R. Gloridoss, B. N. Suresh, and C. Ramesh. 2014. Effect of dietary supplementation of humic acids on performance of broilers. Indian J. Anim. Sci. 84:447–452.
Ozturk, E., N. Ocak, I. Coskun, S. Turhan, and G. Erener. 2010. Effects of humic substances supplementation provided through drinking water on performance, carcass traits and meat quality of broilers. J. Anim. Physiol. Anim. Nutr. 94:78–85.
Ozturk, E., N. Ocak, A. Turan, G. Erener, A. Altop, and S. Cankaya. 2012. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. J. Sci. Food Agric. 92:59–65.
Pandit, R. J., A. T. Hinsu, N. V. Patel, P. G. Koringa, S. J. Jakhesara, J. R. Thakkar, T. M. Shah, G. Limon, A. Psifidi, J. Guitian, D. A. Hume, F. M. Tomley, D. N. Rank, M. Raman, K. G. Tirumurugaan, D. P. Blake, and C. G. Joshi. 2018. Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16s rDNA amplicon sequencing. Microbiome 6:115.
Popelka, P., S. Marcincak, J. Nagy, J. Macanga, J. Vaskova, and I. Jad'uttova. 2015. Effect of supplementation of the diet with humic acids on growth performance and carcass yield of broilers. Folia Vet. 59:165–168.
Rodríguez, N. C., E. C. Urrutia, B. H. Gertrudis, J. P. Chaverri, and G. B. Mejía. 2011. Antioxidant activity of fulvic acid: a living matter-derived bioactive compound. J. Food Agric. Environ. 9:123–127. https://www.researchgate.net/publication/281760309.
Saldaña, B., P. Guzmán, L. Cámara, J. García, and G. G. Mateos. 2015. Feed form and energy concentration of the diet affect growth performance and digestive tract traits of brown-egg laying pullets from hatching to 17 weeks of age. Poult. Sci. 94:1879–1893.
Sanmanee, N., and M. Areekijseree. 2010. The effects of fulvic acid on copper bioavailability to porcine oviductal epithelial cells. Biol. Trace Elem. Res. 135:162–173.
Semjon, B., D. Marcinčáková, B. Koréneková, M. Bartkovský, J. Nagy, P. Turek, and S. Marcinčák. 2020. Multiple factorial analysis of physicochemical and organoleptic properties of breast and thigh meat of broilers fed a diet supplemented with humic substances. Poult. Sci. 99:1750–1760.
Sikandar, A., H. Zaneb, M. Younus, S. Masood, A. Aslam, F. Khattak, S. Ashraf, M. S. Yousaf, and H. Rehman. 2017. Effect of sodium butyrate on performance, immune status, microarchitecture of small intestinal mucosa and lymphoid organs in broiler chickens. Asian Austral. J. Anim. 30:690–699.
Tang, C., W. Kong, H. Wang, H. Liu, L. Shi, V. A. Uyanga, J. Zhao, X. Wang, H. Lin, and H. Jiao. 2023. Effects of fulvic acids on gut barrier, microbial composition, fecal ammonia emission, and growth performance in broiler chickens. J. Appl. Poult. Res. 32:100322.
Tunc, M. A., and M. A. Yoruk. 2017. Effects of humate and probiotic on the number of Escherichia coli, blood and antioxidant parameters in suckling period of calves. Asian J. Anim. Vet. Adv. 12:169–176.
Visscher, C., J. Hankel, A. Nies, B. Keller, E. Galvez, T. Strowig, C. Keller, and G. Breves. 2019. Performance, fermentation characteristics and composition of the microbiome in the digest of piglets kept on a feed with humic acid-rich peat. Front. Vet. Sci. 6:29.
Vucskits, A. V., I. Hullár, A. Bersényi, E. Andrásofszky, M. Kulcsár, and J. Szabó. 2010. Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J. Anim. Physiol. Anim. Nutr. 94:721–728.
Wealleans, A. L., M. C. Walsh, L. F. Romero, and V. Ravindran. 2017. Comparative effects of two multi-enzyme combinations and a Bacillus probiotic on growth performance, digestibility of energy and nutrients, disappearance of non-starch polysaccharides, and gut microflora in broiler chickens. Poult. Sci. 96:4287–4297.
Willems, O., S. Miller, and B. Wood. 2013. Aspects of selection for feed efficiency in meat producing poultry. Worlds Poult. Sci. J. 69:77–88.
Winkler, J., and S. Ghosh. 2018. Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes. J. Diabetes Res. 3:1–7.
Wu, C., A. Lyu, and S. Shan. 2023. Fulvic acid attenuates atopic dermatitis by downregulating CCL17/22. Molecules 28:3507.
Xiao, G., S. Liu, X. Yan, Y. Yang, Q. Qi, X. Feng, L. Gong, and H. Zhang. 2022. Effects of fulvic acid supplement on laying performance, biochemical indices, and gut microbiota of aged hens. Front. Vet. Sci. 9:953564.
Zhang, J. 2018. Modulation of growth performance and nonspecific immunity of red swamp crayfish Procambarus clarkia upon dietary fulvic acid supplementation. Fish Shellfish Immun. 83:158–161.
Zhang, M. F., and Y. X. Liu. 2014. Thinking and prospect of the method based on fulvic acid from weathered coal and lignite. China Chem. Trade 6:2.
Zimonja, O., and B. Svihus. 2009. Effects of processing of wheat or oats starch on physical pellet quality and nutritional value for broilers. Anim. Feed Sci. Technol. 149:287–297.