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Effects of *Artemisia annua* L. Water Extract on Growth Performance and Intestinal Related Indicators in Broilers

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Artemisia annua L. is a natural herb with a variety of bioactive substances, which can play a variety of biological functions such as anti-inflammatory, antioxidant, antibacterial and antiviral, and can be used as a potential feed additive. The purpose of this study was to investigate the effects of different doses of *Artemisia annua* L. water extract (AAWE) on growth performance and intestinal related indicators in broilers. A total of 200 one-day-old Arbor Acre broilers were selected and randomly divided into five treatment groups, with five replicates in each group and eight birds per replicate. The control group was fed a basal diet, whereas the other groups were fed a basal diet supplemented with 0.5, 1.0, 1.5, or 2.0 g/kg AAWE. On d 21, with the increase in AAWE dose, final body weight and feed efficiency showed a quadratic increase effect, whereas feed intake showed a linear reduction effect; however, the apparent metabolic rate of dry matter, crude protein, and ether extract increased quadratically on d 42. In addition, the activity of duodenal chymotrypsin and trypsin, and of jejunal lipase quadratically increased, whereas the intestine crypt depth linearly decreased on d 42. The number of total anaerobic bacteria increased quadratically, whereas the number of *Escherichia coli* decreased quadratically. The number of *Lactobacillus* increased linearly, whereas H_2S emission linearly decreased on d 21; moreover, NH_3 emission (24 h) quadratically decreased on d 42. In conclusion, AAWE promoted the growth performance and intestinal related indicators of broilers.

Key words: apparent metabolic rate, *Artemisia annua* L. water extract, digestive enzyme activity, growth performance, harmful gas, intestinal flora

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Introduction

With the rise of antibiotic-resistant bacteria and associated public health concerns, the European Union has changed its attitude toward the use of antibiotics in animals, banning them completely in animal feed since 2006. As a consequence, the development and utilization of green, safe, and pollution-free antibiotic substitutes has become a research and application hotspot in the feed industry. Potential antibiotic substitutes include natural phytogenic additives, which can improve growth and have received extensive attention when considering the safety of animal food origins[1,2].

Recently, Chinese herb extracts, especially those of *Artemisia annua* L. (*A. annua*), have been the focus of research attention since Youyou Tu first isolated artemisinin from *A. annua*, a popular traditional Chinese herb that is generally regarded as a natural source of therapeutic agents[3]. *A. annua* contains numerous bioactive substances, such as essential oils, sesquiterpenoids, phenolics, flavonoids, coumarins, and steroids, as well as amino acids, vitamins, and mineral elements[4,5], which support its use as a potential plant-derived feed additive for animals. Previous studies have confirmed that *A. annua* has multiple beneficial effects. The use of *A. annua* for treating fever and malaria has been reported[6,7]. The antihypertensive, antibacterial, anti-inflammatory, and antioxidant activities and nutritional characteristics of *A. annua* have also been investigated[4,8]. In particular, it has been reported that *A. annua* could improve the growth performance and intestinal microflora of broilers[9]. Moreover, dietary supplementation of 1 g/kg enzymatically treated *A. annua* improved the growth performance and alleviated the intestinal damage of broilers caused by heat stress[10]. This suggests that further extraction of biologically active components of *A. annua* would

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yield more meaningful effects. Consistent with this, it was found that the water extract of *A. annua* has strong immune, antioxidant (lesser mulberry snout moth (*Glyphodes pyloalis*)), antilipidemic (rats), antibacterial, and antiviral activity (human cervical cancer cells)[11–13]. Notably, using water as a solvent to extract bioactive ingredients results in high yield and high content of each bioactive ingredient[14], with relatively low cost. However, there have been few reports of the effects of *A. annua* water extract on broilers. Therefore, in this study we aimed to investigate the effects of *A. annua* water extract (AAWE) on growth performance, digestive enzyme activity, intestinal morphology, and the number of cecum microorganisms in broilers, along with harmful gas production from broiler manure. Our findings will provide a theoretical basis for the scientific application of *A. annua* in broiler production.

Materials and Methods

Preparation of AAWE

The plant *Artemisia annua* L. (*A. annua*) used in this study was harvested from Hohhot, China. It was identified by experts from the College of Grassland and Resources and Environment of Inner Mongolia Agricultural University as *Artemisia annua*, it should be in italics L. The aboveground part of *A. annua* was mowed, placed in the shade to dry, and then soaked in distilled water at 80°C for 6 h to obtain the filtrate, which was concentrated and freeze-dried into powder for future use.

Animal Research Ethics Statement

The experiment was carried out in a poultry research facility located in Inner Mongolia Agricultural University, Hohhot, China. The animal experiment was conducted after the approval by the Experimental Animal Welfare and Ethics Committee of Inner Mongolia Agricultural University and were performed following the national standard Guideline for Ethical Review of Animal Welfare (GB/T 35892-2018).

Animals, Diets, and Experimental Design

Two hundred healthy 1-day-old Arbor Acre broilers (purchased from a commercial hatchery in Hohhot, China) were selected and randomly divided into five treatment groups; each treatment group had five replicates, with eight chickens per replicate. In the control group, broilers were fed a basal diet that was formulated to meet the nutrient requirements suggested by Feeding Standard of Chicken, China (NY/T 33-2004) Chinese Ministry of Agriculture (Table 1). The other experimental groups were fed the basal diet supplemented with 0.5, 1.0, 1.5, and 2.0 g/kg AAWE. The experiment lasted for 42 days, divided into the starter period (d 1to21) and the finisher period (d 22 to 42). During the experimental period, broilers were free to intake and drink water, and the conditions of all experiment groups were consistent. The lighting scheme: 23 lights (L):1 darkness (D) (d 0 to 3), 10 L:14 D (d 4 to 21), 14 L:10 D (d 22 to 28), 18 L:6 D (d 29 to 35), and 23 L:1 D (d 36 to 42). The temperature of the experimental room was set at 32 to 34°C for the first 3 days and then gradually reduced by 3°C every week, and reached a final temperature of 21°C. The relative humidity was maintained at about 55 ± 5%.All birds were reared in stainless-steel wire cages. Each treatment was randomly divided into five equal replicates, with eight chickens/cage (150×50×50 cm). The health status of all broilers was observed daily. Routine immunization of broilers was performed; the specific immunization procedures were as follows: on d 5, broilers were inoculated with dual live vaccines against Newcastle disease and infectious bronchitis; on d 10, the inactivated vaccine against Newcastle disease was administered; on d 14, broilers were inoculated with the live infectious bursal disease vaccine; on d 20, Newcastle disease and infectious bronchitis dual live vaccines were administered; and on d 28, birds were inoculated with live infectious bursal disease vaccine.

Table 1. **Composition and nutrient levels of the basal diet (as-fed basis), %**

Item	1 to 21 d (age)	22 to 42 d (age)
Ingredients		
Corn	52.50	58.80
Soybean meal	40.00	33.80
Soybean oil	3.00	3.00
Dicalcium phosphate	1.90	1.80
Limestone	1.08	1.22
Salt	0.37	0.37
Lysine	0.05	0.03
Methionine	0.19	0.07
Premix[a]	0.80	0.80
Choline	0.11	0.11
Total	100.00	100.00
Nutrient levels[b]		
Metabolic energy (MJ/kg)	12.42	12.62
Crude protein	21.77	19.65
Calcium	1.00	1.02
Available phosphorus	0.44	0.42
Lysine	1.34	1.15
Methionine	0.55	0.40
Cystine	0.40	0.36

[a]Premix provided the following per kilogram of diet: vitamin A 9000 IU, vitamin D_3 3000 IU, vitamin E 26 mg, vitamin K_3 1.20 mg, vitamin B_1 3.00 mg, vitamin B_2 8.00 mg, vitamin B_6 4.40 mg, vitamin B_{12} 0.012 mg, nicotinic acid 45 mg, folic acid 0.75 mg, biotin 0.20 mg, calcium pantothenate 15 mg, Fe 100 mg, Cu 10 mg, Zn 108 mg, Mn 120 mg, I 1.5 mg, Se 0.35 mg.
[b]Crude protein was a measured value, whereas other values were calculated.

Growth Performance and Apparent Nutrient Metabolic Rate

The broilers were weighed on d 1, 21, and 42, body weight (BW) and feed intake (FI) were recorded accurately, and then the feed efficiency (FE) was calculated. During d 19 to 21 and d 40 to 42 of the experiment, feces from each replicate group were collected, and the fecal weight and feed intake of each group were recorded after continuous fecal collection for three days. The apparent nutrient retention was measured via the total feces collection method, calculating the apparent metabolic rate of feed dry

matter (DM), crude protein (CP), crude fat (ether extract, EE), calcium, and phosphorus[15].

Intestinal Digestive Enzyme Activity

On d 21 and d 42, one chicken was randomly selected from each replicate group and euthanized. The abdominal cavity was opened, the intestinal tract was removed, and then the duodenum, jejunum, ileum, and cecum were separated. The chyme in different intestinal tracts was extruded and stored in a centrifuge tube at −20°C for further analysis, which was conducted according to the following procedure. A Coomassie brilliant blue assay (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China) was used to determine the protein content in the homogenate in accordance with manufacturer instructions.

First, 0.5 g of chyme sample was put into a centrifuge tube, and then 4.5 mL of cold 0.9% sodium chloride (medical saline) was added to the centrifuge tube according to the ratio of l:9, and homogenized on ice with a handheld homogenizer (FA6/10, FLUKO, Shanghai, China).

After full homogenization, the tube was centrifuged at 4°C for 15 min (3000×*g*). After centrifugation, the supernatant solution was divided into several parts and stored at −20°C for the determination of enzyme activity. Chymotrypsin, trypsin, lipase, and amylase were all determined using specific kits provided by Nanjing Jiancheng Bioengineering Institute. The activity of chymotrypsin, trypsin, lipase, and amylase in intestinal chyme was expressed as activity unit per milligram of chyme protein (unit/mg protein).

Intestinal Morphology

A small fragment of the intestinal (duodenum, jejunum, and ileum) tissues were preserved in 10% formalin and embedded in paraffin, then sliced into 7 μm thick sections using a rotary microtome (YD-1508R Rotary Slicer, Yidi Medical Equipment Factory, Jinhua, Zhejiang, China), and stained with hematoxylin and eosin. The villus height (VH) and crypt depth (CD) of 10 intact villi were measured, and the average values of each tissue were calculated according to the description of Qiao *et al*. (2022) [16], following photography under 100× magnification using a light microscope (Olympus SZX10, Tokyo, Japan). The VH was measured from the top of the villi to the villus–crypt junction; CD was taken as the depth from this junction to the base of the crypt. The ratio of villus height to crypt depth (VH/CD) was calculated from the obtained VH and CD values.

Cecum Microorganisms

To evaluate cecum microorganisms, 0.5 g of cecum chyme was collected and placed into a centrifuge tube, then 4.5 mL of cold normal saline was added to the centrifuge tube according to the ratio of l:9, and diluted at different concentrations and cultivated with an appropriate concentration in selective media for total anaerobic bacteria, total aerobic bacteria, *Escherichia coli*, *Bifidobacterium*, and *Lactobacillus*. Colony counting of the aforementioned strains was carried out by referring to the method described by Xing *et al*. (2020)[17]. The results were expressed as log base 10 colony-forming unit (CFU) per gram of cecal contents. The CFU from total anaerobic bacteria, total aerobic bacteria, *E. coli*, *Bifidobacterium*, and *Lactobacillus* were determined using a colony counter (XK97-A, Hangzhou Qiwei Instrument Co., Ltd., China).

Harmful Gas Emissions

On d 21 and 42, fresh feces were collected and accurately weighed, and 100 g of feces were quickly put into 600 mL plastic bottles to allow natural aerobic and anaerobic fermentation at room temperature (around 25°C). When the aerobic treatment group was fermented for 24, 48, and 72 h, the gas in the bottle was collected with a 100 mL syringe to determine the NH_3 content at each time point. The same method was used to determine H_2S emission at 72 h of fermentation in the anaerobic treatment group.

Statistical Analysis

SAS statistical software (SAS Institute, Cary, NC, USA) was used for regression analysis. $P < 0.05$ was considered significant. Regression analysis was used to determine the dose-dependent effect of all variables with the increase of AAWE supplementation (linear and quadratic).

Results

Growth Performance and Nutrient Apparent Metabolic Rate

As shown in Table 2, during the starter period, with the increase of AAWE dose, final body weight and FE showed a quadratic increase effect ($P = 0.027$, $P = 0.002$, respectively), whereas FI showed a linear reduction effect ($P = 0.01$). During the whole experimental period, with the increase of AAWE dose, FI showed a linear decreasing trend ($P = 0.083$).

As described in Table 3, with the increase of AAWE dose, the apparent metabolic rate of CP increased linearly during the starter period ($P = 0.004$); the apparent metabolic rate of DM, CP, and EE increased quadratically during the finisher period ($P = 0.05$, $P = 0.005$, $P = 0.027$, respectively).

Digestive Enzyme Activity

As described in Table 4, the activity of duodenal chymotrypsin and trypsin, and of jejunal lipase showed a linear increase effect with the increase of AAWE dose on d 42 ($P = 0.039$, $P = 0.002$, $P = 0.021$, respectively).

Intestinal Morphology

As indicated in Table 5, on d 21, with the increase of AAWE dose, the CD in the duodenum and jejunum exhibited a quadratic reduction effect ($P = 0.003$, $P = 0.005$, respectively), and that in the ileum showed a linear reduction effect ($P = 0.036$). Moreover, the CD in the small intestine exhibited a linear reduction effect with the increase of AAWE dose on d 42 ($P = 0.001$, $P = 0.045$, $P < 0.001$, respectively). Representative images of the villi and crypt associated with each treatment are shown in Fig. 1 and Fig. 2.

Cecal Microflora Count

As shown in Table 6, during the starter period, the number of total anaerobic bacteria increased quadratically ($P = 0.041$), whereas the number of *E. coli* decreased quadratically ($P = 0.001$). During the finisher period, the number of total aerobic bacteria tended to decrease quadratically ($P = 0.05$), the number

Table 2. **Effect of AAWE on growth performance in broilers**

Item	AAWE supplemental level, g/kg					SEM	R^2		*P*-value[a]	
	0	0.5	1	1.5	2		Linear	Quadratic	Linear	Quadratic
IBW, g/bird	40.15	40.03	40.2	40.13	40.2	0.18	-	-	0.71	0.911
FBW, g/bird										
d 21	751.8	756	756.7	778.6	685.4	5.98	0.244	0.677	0.095	0.027
d 42	2275	2322	2245	2351	2211	43.19	-	-	0.538	0.492
FI, g/bird										
d 1 to 21	965.3	954.6	931	957.7	905	10.79	0.568	0.601	0.01	0.032
d 22 to 42	2946	2904	2819	2921	2862	53.75	-	-	0.451	0.568
d 1 to 42	3911	3859	3825	3884	3691	66.73	0.583	-	0.083	0.2
FE, g/g										
d 1 to 21	0.753	0.75	0.75	0.76	0.707	0.01	0.322	0.703	0.016	0.002
d 22 to 42	0.531	0.544	0.532	0.54	0.539	0.01	-	-	0.432	0.687
d 1 to 42	0.587	0.587	0.584	0.591	0.585	0.01	-	-	0.988	0.97

[a]The probability value of $P < 0.05$ was considered to be statistically significant.
AAWE, *Artemisia annua* L. water extract; IBW, initial body weight; FBW, final body weight; FI, feed intake; FE, feed efficiency; R^2, correlation coefficient; SEM: standard error of the mean.

Table 3. **Effect of AAWE on the apparent nutrient metabolic rate in broilers, (%)**

Item	AAWE supplementation level, g/kg					SEM	R^2		*P*-value[a]	
	0	0.5	1	1.5	2		Linear	Quadratic	Linear	Quadratic
d 21										
DM	58.85	61.32	63.18	61.19	64.24	1.62	0.661	-	0.08	0.19
CP	61.89	61.99	66.99	64.62	67.22	0.99	0.661	0.676	0.004	0.013
EE	64.29	65	68.29	64.78	65.48	2.13	-	-	0.871	0.71
Ca	28.3	30.65	31.92	27.83	35.62	4.45	-	-	0.395	0.679
P	54.4	57	57.5	57.97	56.76	1.98	-	-	0.312	0.391
d 42										
DM	61.05	63.87	64.36	62.37	61.07	0.99	-	0.887	0.87	0.05
CP	55.82	65.58	67.52	61.58	60	1.52	-	0.802	0.743	0.005
EE	79.9	83.77	82.6	77.21	75.15	1.85	0.497	0.853	0.04	0.027
Ca	29.37	36.05	36.91	32.01	36.73	2.57	-	-	0.247	0.251
P	35.98	37.45	41.45	37.43	37.86	2.83	-	-	0.382	0.29

[a]The probability value of $P < 0.05$ was considered to be statistically significant.
AAWE, *Artemisia annua* L. water extract; DM, dry matter; CP, crude protein; EE, ether extract; Ca, calcium; P, phosphorus; R^2, correlation coefficient; SEM: standard error of the mean.

of *E. coli* decreased quadratically, ($P = 0.029$), and the number of *Lactobacillus* increased linearly ($P = 0.022$).

Harmful Gas Emissions

Table 7 shows the effect of dietary AAWE on the harmful gas emissions from broiler feces. During the starter period, with the increase of AAWE, NH_3 emission (72 h) tended to decrease quadratically ($P = 0.077$); H_2S emission also decreased linearly ($P = 0.019$). Moreover, NH_3 emission (24 h) showed a quadratic decrease during the finisher period ($P = 0.001$).

Discussion

In broiler production, improving growth performance by enhancing the utilization efficiency of nutrients is an important strategy. Some studies have reported that the BW,FE, and survival rate of broilers were increased by adding Chinese herbal active substances into the feed[18,19]. Similarly, in the current study we found that supplements with AAWE had an improving effect on the growth performance and nutrient apparent metabolic rate of broilers. In particular, dietary inclusion AAWE quadratically increased the final BW and FE during the starter period, with the metabolic rate of CP showing a quadratic increase effect with the increase of AAWE dose. Furthermore, previous research also suggested that the improvement in growth performance was coordinate with the improvement of FI and nutrient absorption efficiency[20,21]. Notably, various plant extracts have similar effects. Wan *et al*. (2016) reported that *A. annua* leaves and en-

Table 4. **Effects of AAWE on the intestinal digestive enzyme activities of broilers (U/g prot.)**

Item	AAWE supplementation level, g/kg					SEM	R^2		*P*-value[a]	
	0	0.5	1	1.5	2		Linear	Quadratic	Linear	Quadratic
d 21										
α-Amylase										
Duodenum	0.18	0.23	0.24	0.18	0.18	0.04	-	-	0.862	0.766
Jejunum	0.22	0.29	0.42	0.4	0.34	0.1	-	-	0.295	0.316
Ileum	0.38	0.41	0.53	0.36	0.36	0.06	-	-	0.201	0.335
Chymotrypsin										
Duodenum	62.37	64.71	94.57	92.41	72.05	13.06	-	-	0.475	0.308
Jejunum	29.11	58.81	62.95	32.7	39.41	8.54	-	-	0.902	0.176
Ileum	15.06	16.94	15.06	13.44	14.9	1.54	-	-	0.482	0.788
Trypsin										
Duodenum	9729	12031	12649	12896	10886	1979	-	-	0.555	0.462
Jejunum	18254	22289	22952	25109	21784	3112	-	-	0.288	0.31
Ileum	22403	23958	23664	28374	21319	2155	-	-	0.73	0.412
Lipase										
Duodenum	271.9	297.1	380.9	191	178	84.88	-	-	0.37	0.48
Jejunum	439.8	510.7	563.4	509.7	702.9	92.1	-	-	0.14	0.33
Ileum	419	532.3	506.2	382	450	77.94	-	-	0.71	0.69
d 42										
α-Amylase										
Duodenum	0.2	0.17	0.2	0.22	0.24	0.02	-	-	0.566	0.85
Jejunum	0.4	0.6	1.02	0.77	0.8	0.21	-	-	0.183	0.203
Ileum	0.33	0.24	0.43	0.29	0.39	0.07	-	-	0.775	0.812
Chymotrypsin										
Duodenum	26.77	22.69	27.8	33.39	37.07	3.43	0.759	0.9	0.039	0.082
Jejunum	26.05	40.1	24.51	26.73	29.41	5.19	-	-	0.749	0.934
Ileum	19.45	20.32	27.9	32.6	28.39	3.31	0.712	0.808	0.03	0.063
Trypsin										
Duodenum	6762	8024	9863	11339	10982	912	0.901	0.962	0.002	0.008
Jejunum	20398	26988	28336	34339	27814	5917	-	-	0.221	0.283
Ileum	15742	16854	18880	20147	19072	1967	-	-	0.132	0.271
Lipase										
Duodenum	198.7	216.3	243.2	241.8	232.2	57.08	-	-	0.65	0.86
Jejunum	463.6	467.3	481	700.5	911.6	108.1	0.81	0.985	0.021	0.035
Ileum	189.1	183.1	236	184.8	163.7	34.55	-	-	0.969	0.736

[a]The probability value of $P < 0.05$ was considered to be statistically significant.
AAWE, *Artemisia annua* L. water extract; R^2, correlation coefficient; SEM: standard error of the mean.

zymatically treated *A. annua* positively affected growth performance in broilers[22]. moreover, feeding *Forsythia suspensa* extract significantly improved growth performance including average daily gain and FE[23]. In parallel with growth performance results during the starter period, dietary supplementation with AAWE increased the apparent metabolic rate of DM and CP. The majority of our data showed that the addition of AAWE could improve the apparent metabolic rate of broilers. The results of improving the digestibility of AAWE were consistent with those of Sørensen *et al*. (2011), who reported the effects of supplements of plant extracts from *Yucca shidigera*, *Quillaja saponaria,* and a combination effect on diet digestibility in piglets and sows[24]. The effects of AAWE might be due to its content of hydrophilic bioactive substances such as phenols (quinic acid, caffeic acid, luteolin, quercetin, rutin, apigenin, isorhamnetin, kaempferol, mearnsetin, artemetin, casticin, chrysosplenetin, chrysoprenol D, cirsilineol, and eupatorine), along with soluble polysaccharide, the water-soluble derivative SM905, and the water-soluble artemisinin analog SM934, with concomitant enhancement of metabolic, anti-tumor, anti-microbial and immunomodulatory

Table 5. **Effects of AAWE on the intestinal morphology of broilers**

Item	AAWE supplemental level, g/kg					SEM	R^2		*P*-value[a]	
	0	0.5	1	1.5	2		Linear	Quadratic	Linear	Quadratic
d 21										
Villus height, μm										
Duodenum	894.7	920.7	920.1	921	898.8	33.6	-	-	0.87	0.799
Jejunum	609.6	621.4	623.5	618.1	612.9	37.68	-	-	0.981	0.966
Ileum	357.9	378.3	384.5	368.5	379.9	20.23	-	-	0.7	0.853
Crypt depth, μm										
Duodenum	48.15	43.04	42.77	42.51	43.71	0.96	0.403	0.992	0.04	0.003
Jejunum	35.16	32.65	32.37	32.02	32.48	0.63	0.561	0.946	0.019	0.005
Ileum	24.2	22.61	23.76	22.9	22.2	0.42	0.506	-	0.036	0.116
VCR										
Duodenum	17.73	20.67	18.82	20.51	17.63	0.9	-	-	0.911	0.155
Jejunum	18.34	17.73	17.18	18.61	19.11	1.28	-	-	0.549	0.571
Ileum	14.45	15.16	18.71	15.88	17.81	1.24	-	-	0.1	0.2
d 42										
Villus height, μm										
Duodenum	770.8	824.7	855.1	875.3	820.6	35.55	-	-	0.31	0.152
Jejunum	624.1	667.4	677.2	662.9	642.3	23.57	-	-	0.637	0.224
Ileum	439	470.6	467.6	477.4	447	12.8	-	-	0.768	0.409
Crypt depth, μm										
Duodenum	36.61	34.64	34.72	30.96	32.43	0.86	0.749	0.783	0.001	0.004
Jejunum	31.93	31.24	31.41	30.41	30.85	0.44	0.677	-	0.045	0.106
Ileum	22.55	20.88	20.75	21	19.34	0.37	0.765	0.767	<.001	<.001
VCR										
Duodenum	23.48	24.06	24.92	26.04	25.45	1.27	-	-	0.13	0.301
Jejunum	19.72	21.19	23.13	19.65	20.09	1.01	-	-	0.823	0.19
Ileum	20.18	23.89	22.41	20.41	23.93	0.89	-	-	0.273	0.546

[a]The probability value of $P < 0.05$ was considered to be statistically significant.
AAWE, *Artemisia annua* L. water extract; VCR, villus height-to-crypt depth ratio; R^2, correlation coefficient; SEM: standard error of the mean.

properties[5], thereby promoting the growth of broilers. In addition, artemisinin and flavonoids in the hot water extract of *A. annua* may also play a role[25]. Furthermore, we observed that the final BW and FE of the 2.0 g/kg group were lower. Similar results were reported by Wan *et al.* (2017), who studied the effects of dietary supplementation of enzymatically treated *A. annua* (0, 0.5, 1.0, and 1.5 g/kg) on growth performance and meat quality in broilers, and found that the values of the 1.5 g/kg group were lower than those of other groups[26]. This outcome might be related to the palatability of *A. annua*; a dose-dependent effect of AAWE may also contribute[22]. Specifically, when the level of AAWE supplementation is 1.5 g/kg, the active ingredient of AAWE can play an optimal role. However, it has also been reported that adding plant extracts to the diet had little effect on nutrient digestibility, which might be due to different extraction methods and experimental protocols[24]. Nevertheless, the findings of the present and previous studies show that AAWE can indeed promote growth. We can therefore speculate that the bioactive substances contained in AAWE were beneficial to the growth of poultry. Otherwise, the biological function of AAWE might also depend on its active components.

Generally speaking, feed digestion in poultry can be divided into physical, chemical, and microbial action. The increased digestibility observed in this study thus derives from these factors. One possible mechanism is that chemical factors such as digestive enzymes play a decisive role. The digestive tract contains various digestive enzymes, whose main function is to hydrolyze the ingested large molecular nutrients into absorbable small molecular nutrients so that the body can fully absorb and utilize them. The current study showed that the increase in digestive enzyme activity coincided with the increase in apparent nutrient metabolic rate. *A. annua* extracts in contact with animals can stimulate digestive enzyme activity and improve digestibility[10,27]. The results of this and other studies show that *A. annua* extracts mainly induce animal growth by promoting feed intake and improving nutrient digestibility and absorption. In particular, our observed results of increased digestive enzyme activity could underlie the AAWE-mediated digestibility effects.

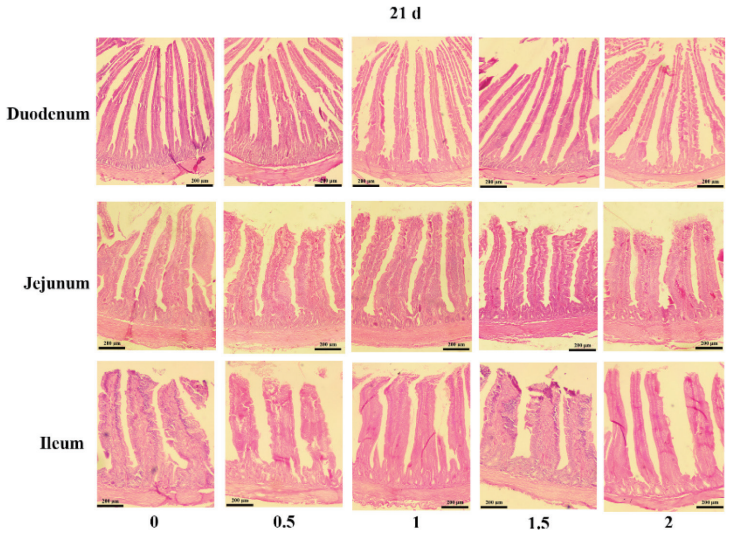

Fig. 1. **Histomorphology of the duodenum, jejunum, and ileum in broilers at d 21.** Representative images illustrating the effects of supplementation levels of 0, 0.5, 1, 1.5, and 2.0 g/kg are shown.

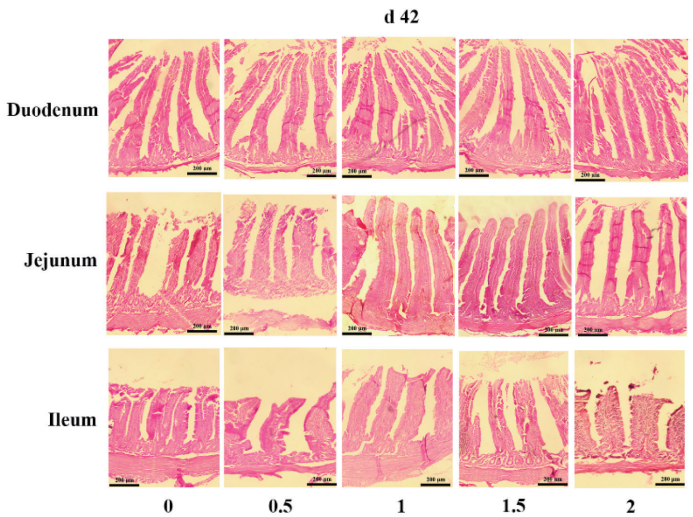

Fig. 2. **Histomorphology of the duodenum, jejunum, and ileum in broilers at d 42.** Representative images illustrating the effects of supplementation levels of 0, 0.5, 1, 1.5, and 2.0 g/kg are shown.

Furthermore, physical factors such as VH, CD, and microbial factors such as the number of bacteria in the intestinal flora also influence digestibility. In the current study, the good results on growth performance might be related to the intestinal flora. Our data showed that dietary supplementation with AAWE quadratically decreased the number of *E. coli* and linearly increased the number of *Lactobacillus*, which led to a change in intestinal flora balance. Numerous reports have demonstrated the influence of plant extracts on intestinal microorganisms. Balasubramanian *et al*. (2021) reported that plant extracts linearly increased the number of beneficial *Lactobacilli*[28]. It has also been previously reported that intestinal bacteria directly affected the metabolism of the intestinal tract and therefore have a variety of effects on the growth performance of broilers[29]. Supplementation with probiotics (especially *Bacillus* spp.) contributes to the stabilization of intestinal microbiota and relieves growth retardation by improving BWs through a higher GH/IGF-1 ratio[30]. The diversity of antibacterial mechanisms of plant extracts depends on their

Table 6. **Effect of AAWE on cecum microbiota in broilers (log CFU/g)**

Item	AAWE supplemental level, g/kg					SEM	R^2		*P*-value[a]	
	0	0.5	1	1.5	2		Linear	Quadratic	Linear	Quadratic
d 21										
Total anaerobic bacteria	8.2	8.27	8.42	8.03	7.71	0.19	0.505	0.927	0.071	0.041
Total aerobic bacteria	8.04	8.08	7.83	7.86	7.61	0.16	0.825	-	0.073	0.192
Escherichia coli	6.34	5.41	5.35	5.64	5.41	0.19	0.391	0.713	0.024	0.001
Bifidobacterium	8.25	8.32	8.37	8.29	7.97	0.15	-	-	0.326	0.306
Lactobacillus	8.31	8.41	8.55	8.4	8.55	0.15	-	-	0.471	0.739
d 42										
Total anaerobic bacteria	8.92	9.4	9.27	9.15	8.84	0.17	-	0.876	0.673	0.024
Total aerobic bacteria	9.46	9.22	9.14	8.96	9.35	0.15	-	0.803	0.268	0.05
Escherichia coli	6.62	6.38	6.29	5.9	6.24	0.21	0.566	0.761	0.075	0.029
Bifidobacterium	8.42	8.59	8.53	8.86	8.81	0.21	-	-	0.133	0.322
Lactobacillus	8.36	8.56	9.08	9.03	8.93	0.22	0.65	0.895	0.022	0.038

[a]The probability value of $P < 0.05$ was considered to be statistically significant.
AAWE, *Artemisia annua* L. water extract; R^2, correlation coefficient; SEM: standard error of the mean.

Table 7. **Effects of AAWE on the harmful gas emission from feces of broilers (mg/m^3)**

Item	AAWE supplemental level, g/kg					SEM	R^2		*P*-value[a]	
	0	0.5	1	1.5	2		Linear	Quadratic	Linear	Quadratic
d 21										
NH_3, 24 h	142.3	133.8	175.6	129.4	125.2	23.97	-	-	0.616	0.605
NH_3, 48 h	327.3	286.4	211.6	286.9	254.3	23.08	-	-	0.14	0.117
NH_3, 72 h	434.1	325.1	298.4	355.3	381.3	36.14	-	0.866	0.662	0.077
H_2S, 72 h	704.6	729.2	604.5	329.3	520	70.7	0.564	0.587	0.019	0.045
d 42										
NH_3, 24 h	130.2	87	63.7	86.5	81.2	10.05	0.403	0.844	0.031	0.001
NH_3, 48 h	106.1	129	98.1	108.4	141.9	20.49	-	-	0.442	0.543
NH_3, 72 h	191.9	192.9	216.3	250.8	194.7	33.89	-	-	0.578	0.653
H_2S, 72 h	916.7	811.1	911.1	941.7	889.6	87.39	-	-	0.86	0.976

[a]The probability value of $P < 0.05$ was considered to be statistically significant.
AAWE, *Artemisia annua* L. water extract; R^2, correlation coefficient; SEM: standard error of the mean.

species and active components[31]. For example, the antimicrobial activity of licorice is attributed to the bioactivity of alkaloids, saponins, flavonoids, tannin, glycosides, and phenols[32,33]. Hence, its antimicrobial activity might be comparable to the rich bioactive substances in *A. annua*, such as monoterpenes, sesquiterpenes, and phenolic compounds, which have strong antibacterial, antifungal, antiplasmodial, antihelminthic, and antiviral activity[5]. The active components in plant extracts serve mainly to destroy the phospholipid structure in the cell membrane, thereby disrupting the cell structure and causing cell death[34,35]. In turn, bacterial metabolites can affect pancreatic secretion, which increases the activity of digestive enzymes. Such effects may also explain the observation that the modification of gut flora can promote growth.

Many factors affect the homeostasis of intestinal microorganisms, including the modulation of animal intestinal microorganisms by nutritional means. Plant extracts are widely used toward this end, with their effective bioactive components generally including polyphenols, polysaccharides, terpenes, alkaloids, and flavonoids[36–38]. Notably, *A. annua* contains substantial quantities of sesquiterpenoids, artemisinin, flavonoids, coumarins, ethers, volatile oil, and other components[25,39]. Moreover, Tao *et al*. (2020) found that AAWE had antibacterial and antiviral activities, which might account for the decrease of harmful bacteria in broilers[12]. Consistent with this, in the present study, dietary AAWE significantly reduced the *E. coli* population in the cecum of birds but did not affect the *Lactobacillus* population. Previous research has further suggested that extracts of *A. annua* exhibited important antimicrobial activity against bacteria, yeasts, dermatophytes, and aspergillums[40]. One potential mechanism is illustrated by the finding that the antibacterial activity of plant extract (thyme essential oil) affects the invasive ability of bacte-

ria by changing the protein structure of the bacterial outer membrane[41].

In addition, fecal noxious gases such as NH_3 and H_2S constitute the major elements of air pollution in modern animal production. To reduce the emissions of NH_3 and H_2S, it is necessary to take measures to decrease the emission of malodorous gas. The digestibility of the feed directly affects the emission of the animal odor. Owing to the short digestive tract of poultry, nutrients cannot be fully absorbed and utilized, resulting in more nutrients being excreted from the body, the fermentation of feces, and the discharge of harmful gases, which affects the health of animals and humans and damages the environment[28,42]. In the present study, the addition of AAWE reduced the emission of harmful gases from feces. With the increase of AAWE dose, NH_3 emission showed a quadratic reduction effect. The lower emission of NH_3 in the current study was determined by the level of nitrogen metabolism in the body, which depended on the digestive products of proteins. In the present study, the increase in the apparent metabolism rate of CP was consistent with the decrease in NH_3 emission. Another possible mechanism is that a related pathway had the effect of inhibiting urease, inhibiting the activity of urease in the intestinal tract or countering the activity of microorganisms, thereby suppressing the decomposition of uric acid and urea-containing nitrogen compounds, and reducing the production of NH_3 in the gastrointestinal tract[43]. This mechanism is also consistent with changes in the flora status observed in the present study. However, the specific mechanisms need to be further studied. Reda *et al*. (2021) reported that supplementing licorice powder in the diet of quail decreased cecum *E. coli*[44]. In turn, we found that dietary inclusion of AAWE linearly decreased H_2S emission. Thus, altering intestinal microflora might be the main cause underlying the reduction in H_2S emission from feces observed in the current study; however, additional research is needed to confirm this model.

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Author Contributions

Shiwei Guo and Binlin Shi conceptualized the study. Jiaxin Ma and Shiwei Guo curated the data. Shiwei Guo and Jiaxin Ma conducted formal analysis of the data. Shiwei Guo and Lulu Shi developed the methodology. Shiwei Guo and Linghui Zhang developed the software. Sumei Yan validated the findings. Yuanqing Xu and Xiao Jin performed the investigations. Shiwei Guo and Yuanyuan Xing wrote the original manuscript draft. Shiwei Guo, Jiaxin Ma, and Binlin Shi reviewed and edited the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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