



DIETARY INCLUSION OF *ARTEMISIA ANNUA* IMPROVES ANTIOXIDANT PERFORMANCE, IMMUNOGLOBULIN PROTEIN LEVELS, LIPID PROFILE, CARCASS CHARACTERISTICS, MEAT QUALITY, AND HISTOMORPHOMETRIC FEATURES OF BROILER CHICKENS

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Abstract

Artemisia annua L. (*A. annua*), renowned for its medicinal properties, was investigated for its effects on broiler performance, biochemistry, antioxidant capacity, meat composition, and organ histology. A total of 90 one-day-old chicks were divided into four groups, each with three replicates. The chicks were fed either a basal diet or a basal diet supplemented with *A. annua* leaves meal at levels of 0.3%, 0.6%, or 0.9% over a period of 42 days. *A. annua* decreased mortality, final body weight, feed intake, and feed conversion ratio, but improved dressing percentages and liver/gizzard weights ($P < 0.05$). Serum analysis revealed reduced alanine/aspartate aminotransferase, cholesterol, triglycerides, and LDL, while total protein, albumin, globulin, high-density lipoprotein (HDL), and IgM levels increased ($P < 0.05$). Malondialdehyde (MDA) decreased, while total antioxidant capacity (TAC) and superoxide dismutase (SOD) increased ($P < 0.05$), confirming *A. annua*'s antioxidant activity. Meat analysis showed decreased moisture, fat, and fiber but increased protein content ($P < 0.05$). Sensory evaluation revealed improved taste, color, texture, and overall acceptability. Mild histopathological lesions were observed in *A. annua*-treated groups. Conclusively, *A. annua* in broiler diets has antioxidant, hypolipidemic, and immune-enhancing potential. Further studies are needed to evaluate its long-term safety and optimal dose.

Key words: broiler, *Artemisia annua*, meat quality, antioxidant, immunoglobulin, histopathology

In recent years, concerns over the widespread use of feed antibiotics have prompted numerous countries and organizations to develop various feeding strategies to eliminate their use and mitigate their adverse effects on animal, bird, and human health (Abd El-Hack et al., 2022; El-Ratel et al., 2023; El Basuini et al., 2023; Khat tab et al., 2021). Consequently, researchers have embarked on a quest for natural alternatives to antibiotics, particularly herbal extracts, oils, and powders (Alagawany et al., 2019; Yilmaz et al., 2015).

Artemisia, a genus belonging to the Asteraceae family, stands out for its remarkable diversity and extensive global distribution. Comprising over 500 species, this genus thrives in temperate regions across Asia, North America, and Europe, encompassing annual, perennial, and biennial herbs (Bora and Sharma, 2011). Among the diverse *Artemisia* species, *Artemisia annua* L. holds prominence due to its widely recognized medicinal properties and global presence (Hoseini et al., 2022; Khodakov and Kotikov, 2009). This species harbors a rich array of essential oil (ESO) components, including 1,8-cineole, camphor, and *Artemisia* ketone. These ESO components

are predominantly found in the leaves of *Artemisia annua* L., with concentrations ranging from 0.04 to 1.9% on a dry matter basis (Bilia et al., 2014; Tzenkova et al., 2010). Research by Xiao et al. (2022) has demonstrated the remarkable properties of these essential oils in maintaining a favorable microbiota balance, reducing protozoan populations, diminishing methane generation, and enhancing nitrogen uptake.

Artemisia annua leaf extract and powder supplementation in poultry feed have consistently demonstrated significant improvements in growth performance parameters, including body weight (BW) and feed conversion ratio (FCR) (Guo et al., 2023; Panaite et al., 2019). These positive effects are attributed to the plant's bioactive compounds, which promote nutrient absorption, enhance gut health, and stimulate growth hormones. *Artemisia* has garnered attention for its therapeutic properties, including anti-inflammatory, antihypertensive, antioxidant, and antibacterial activities (Gouveia and Castilho, 2013). Studies have shown that the plant's extracts can effectively reduce inflammation, lower blood pressure levels, protect against oxidative damage, combat infections, and lower cholesterol levels (Tao et

al., 2020). Additionally, *Artemisia* has demonstrated anti-malarial (De Donno et al., 2012) and anticancer (Lang et al., 2019) properties. *Artemisia* has also shown beneficial effects on meat quality and intestinal health. Wan et al. (2018) found that feeding broilers with dietary-treated *A. annua* through enzymatic treatment enhanced meat quality, antioxidant efficiency, and energy status. Moreover, Song et al. (2018) reported that supplementing broiler diets with 1 g/kg *A. annua* could effectively protect against intestinal damage and restore growth performance compromised by heat stress. The diverse therapeutic properties of *A. annua* are closely linked to its rich content of bioactive compounds, including polyphenols, flavonoids, and polysaccharides (Huo et al., 2020; Kontogianni et al., 2020; Wan et al., 2017). These compounds possess potent antioxidant, anti-inflammatory, antimicrobial, and immune-modulatory properties, contributing to the plant's therapeutic effects.

Extensive research has been conducted to evaluate the influence of *A. annua* on various broiler performance parameters. However, limited studies have explored its effects on the histopathological structure of internal organs and the histomorphometric features of intestinal tissues. To address this gap, the present study investigated the impact of incorporating *A. annua* into broiler diets at varying concentrations on broiler performance, carcass characteristics, biochemical blood indices, antioxidant status, meat quality and composition, intestinal morphology, and histopathological assessment of internal organs.

This study comprehensively evaluates *A. annua*'s effects on various aspects of broiler health and performance, providing valuable insights into its potential as a natural feed additive in the poultry industry.

Material and methods

Ethical approval

The study was conducted following the Declaration of Helsinki and approved by the Institutional Review Board of Agriculture College – Tanta University (No. AY 2019-2020/Session 6/2020.01.13).

Preparation of *Artemisia annua* leaf meal

The study utilized mature leaves of *A. annua* that were fresh, green, and undamaged. The leaves were dried in the shade to avoid direct contact to sunshine, with regular turning while they were being dried. Following a period of five days, the desiccated leaves underwent the process of being pulverized into an exceedingly fine powder, traversing a sieve with a mesh size of 0.15 mm. The leaf meal obtained was packed into polythene plastic bags and stored at ambient temperature until it was required. A 500-kg capacity vertical mixer was utilized to homogenize the feed treatments. Mixing commenced 15 minutes after the final ingredient was loaded. The micro-nutrients were pre-mixed, and the basal diet was supplemented with three quantities of *Artemisia annua* leaves meal (0.3%, 0.6%, and 0.9%).

Bird's management and experimental design

A total of 360 unsexed one-day-old Arbor Acres broiler chicks with an average body weight of 45 ± 3 g were purchased from Cairo Poultry Company. The chicks were randomly assigned to four equal experimental groups of 90 chicks each, with three replicates of 30 chicks per replicate. The first group received the basal diet and served as the control. The second, third, and fourth groups were fed the basal diet supplemented with 0.3%, 0.6%, and 0.9% *A. annua* leaves meal, respectively, based on the recommendations of previous studies (Coroian et al., 2022; Engberg et al., 2012). The management requirements for temperature, light, and ventilation were all fulfilled according to the Arbor Acres Broiler Management Guide, Aviagen, 2018 (https://aviagen.com/assets/Tech_Center/AA_Broiler/AA-BroilerHandbook2018-EN.pdf). The chicks were housed in deep litter partitions measuring 3×1 m. Brooding was provided using a gas heater, and the ambient temperature was maintained at 37°C for the first two days. Subsequently, the temperature gradually decreased by 3°C at four-day intervals until it reached 21°C. The birds had ad libitum access to feed and fresh water. The basal diet was prepared to meet the requirements of Arbor Acres broilers at different stages of growth, as shown in Table 1. During the experimental period, birds were exposed to six cycles of a 3-hour light/1-hour dark intermittent lighting program (Arbor Acres Broiler Management Guide, Aviagen, 2018).

Performance traits

All chicks were individually wing-banded at the beginning of the trial. Chicks were weighed individually, and their initial body weight (IBW) and final body weight (FBW) at six weeks of age were recorded to the nearest gram using a digital scale. Total weight gain (TWG) was calculated as the difference between initial and final body weight. Total feed intake (TFI) was determined daily by subtracting the remaining feed from the previous day from the feed offered to each replicate within each group. Feed conversion ratio (FCR) was also computed as the proportion of total feed intake to total weight gain. Mortality percentages were recorded on a replicate basis every day and totaled for the entire experimental period.

Carcass traits

At the end of the trial (42 days), nine birds from each group (3 birds from each replicate) were randomly selected for a slaughter test. After fasting for 12 hours, the birds were weighed and then slaughtered by severing their jugular veins in the morning. Then, they were scalded and defeathered once they had completely bled. The carcasses were eviscerated manually and weighed. The dressing percentage was calculated as the ratio of the weight of the eviscerated carcass to the live body weight. The liver, gizzard, heart, and spleen were weighed separately and expressed as a percentage of the live body weight.

Table 1. Physical and chemical composition of basal diet during experiment

Composition	Starter (0–10 days)	Grower (11–22 days)	Finisher (after 22 days)
Ingredients (100 g)			
Yellow corn	56.31	64.22	65.69
Soybean meal (44%)	33.6	25.2	24.7
Corn gluten meal	3.8	3.8	3.1
Vegetable oil	1.7	2.5	2.5
Dicalcium phosphate	1.5	1.3	1.2
Limestone	1.3	1.2	1.15
L-lysine	0.41	0.3	0.3
DL-methionine	0.36	0.31	0.29
Vitamin & mineral premix ¹	0.3	0.47	0.38
Sodium chloride	0.33	0.1	0.09
L-threonine	0.1	0.31	0.31
Sodium bicarbonate	0.09	0.1	0.1
Anticolesterdia	0.05	0.05	0.05
Antimycotoxin	0.05	0.05	0.05
Energy enzyme ²	0.05	0.05	0.05
Anticoccidia ³	0.03	0.03	0.03
Phytase enzyme ⁴	0.01	0.01	0.01
Ronozyme ProAct ⁵	0.01	0.01	0.01
Chemical analysis (%)			
ME (Kcal/Kg diet)	2,999.60	3142.13	3179.22
CP	22.02	19.02	18.05
CF	3.52	3.09	3.07
Linoleic acid	1.93	2.33	2.6
Lysine	1.35	1.2	1.11
Lysine Dig	1.24	1.1	1.02
Methionine	0.69	0.61	0.57
Methionine Dig	0.65	0.57	0.54
Methionine + Cystine	1.04	0.92	0.87
Methionine + Cystine Dig	0.93	0.82	0.77
Threonine	0.9	0.8	0.76
Threonine Dig	0.75	0.67	0.64
Calcium	0.95	0.84	0.81
Available phosphorus	0.48	0.43	0.4
Chloride	0.23	0.22	0.22
Sodium	0.17	0.16	0.16
Potassium	0.86	0.71	0.7

¹Purchased by AGRI-VIT 10th of Ramadan city, Egypt. Each 3 kg contains: Vitamin A 12,000,000 IU, Vitamin D₃ 2,000,000 IU, vitamin E 10,000 IU, vitamin K₃ 2000 mg, vitamin B₁ 1000 mg, vitamin B₂ 5000 mg, vitamin B₆ 1500 mg, vitamin B₁₂ 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 1000 mg, nicotinic acid 30,000 mg and mineral mixture are manganese 6000 mg, zinc 50,000 mg, iron 30,000 mg, copper 1000 mg, iodine 300 mg, selenium 100 mg, cobalt 1000 mg, carrier (CaCO₃) added to 3 kg.

²Energy enzyme: Hemicell.

³Diclacox, each 1 g contains Diclazuril 2 mg.

⁴Phytase enzyme: Avemix P5000, ⁽⁵⁾Ronozyme ProAct: protease enzyme.

Biochemical blood parameters

At the end of the experiment, nine blood samples were collected from each bird during slaughtering (three pooled samples from each replicate). The samples were divided into two portions. The first portion was collected in tubes containing 10% EDTA, an anticoagulant, to measure hematological parameters using the HBVET-1 automated hematology analyzer (Sinnova Medical Science and Technology Co., Ltd.) depending on the principle of electro-impedance, and photometric analysis. These parameters included red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count, platelet count, neutrophil percentage, lymphocyte percentage, monocyte percentage, eosinophil percentage, and basophil percentage.

The second portion of the blood sample was collected in tubes without anticoagulant and centrifuged at 3000 rpm for 15–30 minutes to separate the serum. The serum was collected and stored at –20°C until it was used to determine biochemical parameters. Serum total protein (TP) was measured using a colorimetric method according to Tietz (1995), employing specific diagnostic kits from MDSS GmbH (Hannover, Germany). Albumin (Alb) was determined similarly using the method described by Young (2001) and kits from the same manufacturer. Globulin (Glob) was calculated by subtracting the proportion of albumin from the total protein ratio. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), along with the concentrations of cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and triglycerides, were determined using a colorimetric method based on Friedman and Young (1997) with specific diagnostic kits from Bio-Systems S.A. (Spain). The albumin-to-globulin ratio (A/G ratio) was also recorded. Serum immune parameters, including IgM, IgG, and IgA, were measured in appropriately diluted samples using a sandwich enzyme-linked immunosorbent assay (ELISA) with microtiter plates and chicken-specific ELISA quantitation kits from Bethyl Laboratories Inc. (Montgomery, TX). The ELISA procedure followed the manufacturer's protocol, and absorbance was measured at 450 nm. IgM, IgG, and IgA concentrations were determined using standard curves constructed from respective Ig standards included in the assay microtiter plate, as described by Piquer et al. (1991).

Antioxidant parameters

Liver samples (250 mg) were homogenized with 1 ml of phosphate buffer saline (PBS) using a tissue homogenizer and centrifuged at 10,000 rpm for 15 minutes at 4°C in a refrigerated centrifuge. The supernatant was carefully aspirated and stored at –20°C in Eppendorf tubes until analysis. The levels of superoxide dismutase

(SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured using commercial kits (Bio-diagnostics, Giza, Egypt) following the manufacturer's instructions and the methodologies of Claiborne (1985), Koracevic et al. (2001), and Ohkawa and Yagi (1979), respectively.

Sensory evaluation parameters

Sensory evaluation of fresh chicken thigh and breast fillets samples was conducted immediately after preparation. Samples were grilled at 150°C for 5 minutes per side and subjected to organoleptic evaluation using the method described by Fathi et al. (2024, 2023). A panel of ten experienced staff members from the Meat and Fish Technology Research Department, Food Technology Research Institute, Agricultural Research Center (ARC), Egypt, evaluated the grilled samples. Panelists were asked to assess the taste, odor, color, texture, and overall acceptability of each sample using a nine-point hedonic scale as the grades range from "Dislike extremely" for point 1, to "Dislike very much" for point 2, "Dislike moderately" for point 3, "Dislike slightly" for point 4, "Neither like nor dislike" for point 5, "Like slightly" for point 6, "Like moderately" for point 7, "Like very much" for point 8, and finally "Like extremely" for point 9.

Chemical composition of meat

The chemical composition of the chicken flesh was determined according to the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2007). Dry matter (DM) content was determined by drying the samples in an oven at 105°C for 24 hours. Crude protein content was quantified using the micro-Kjeldahl method, with nitrogen content (N%) multiplied by 6.25. The analysis used a Kjeltac Auto Model 1030 analyzer from Tecator (Höganäs, Sweden). Crude fat content was determined by Soxhlet extraction using diethyl ether as the solvent at a temperature range of 40–60°C. Ash content was determined by incinerating the samples at 550°C for 12 hours.

Histopathological examination

Chicken liver, spleen, and small intestinal tissue samples were collected and fixed in 10% buffered formalin for 48 hours. Subsequently, the fixed specimens were processed using an automated tissue machine. Briefly, the specimens underwent a 30-minute wash in distilled water followed by dehydration in a graded series of alcohol (70%, 90%, and 100%). Next, the specimens were cleared in multiple changes of xylene, impregnated with molten paraffin wax, embedded, and blocked out. Sections of paraffin (4–5 µm) were stained using hematoxylin and eosin, bromophenol, periodic acid–Schiff (PAS), and Masson trichrome stains, as described by Suvana et al. (2018). An experienced pathologist conducted a thorough examination to assess the presence of circulatory disturbances, inflammation, degenerations, apoptosis, necrosis, and any other pathological alterations in the ex-

amined tissues. Representative microphotographs were captured using a digital camera (Leica EC3; Leica, Germany) mounted on a microscope (Leica DM500). The histomorphometric analysis was carried out quantitatively on images captured (×400) from intestinal (jejunum) sections obtained from control and treated groups. The parameters used for evaluation were (a) villi height (µm), (b) villi width (µm), (c) crypt depth (µm), and (d) muscular coat thickness (µm) of tunica muscularis layers. The evaluation was performed using computerized Image J software (Schneider et al., 2012). The mean values were estimated for fifteen intestinal villi per each test sample.

Statistical analysis

A general linear model (GLM) was implemented using SAS software version 9.4 to determine the presence of statistically significant differences between the treatments. Differences were considered statistically significant at a P-value less than 0.05. The results are presented as means accompanied by the pooled standard error of the mean (SEM). For the analysis of the collected data, the following statistical model was employed:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where:

Y_{ij} represents the response variable being measured,
 μ denotes the overall mean effect,
 α_i represents the effect of the i^{th} treatment,
 ε_{ij} represents the error term for the i^{th} observation.

Results

Performance parameters

Growth performance parameters of chickens fed diets supplemented with varying levels of *A. annua* are presented in Table 2. According to the Arbor Acres Broiler Management Guide, the Arbor Acre performance at 42 days, under standard farming conditions, is around 2900 g.

Our results indicate that birds consuming different levels of *A. annua* exhibited a significant dose-dependent decrease in final body weight and total feed intake, but significantly improved feed conversion ratio (FCR) and mortality percentage compared to the control group.

Carcass traits

The effects of varying levels of *A. annua* on broiler carcass parameters are presented in Table 3. Broilers fed diets supplemented with *A. annua* exhibited a significantly higher dressing percentage (DP) than the control group. Compared to all other groups, the highest DP ($P < 0.0001$) was observed in broilers fed the 0.9% *A. annua* diet. Additionally, liver and gizzard percentages were significantly increased ($P = 0.0005$) in experimental groups fed with 0.3% and 0.9% *A. annua* compared to other treated and control groups. Heart and spleen percentages showed no significant differences among treated and control birds.

Table 2. Growth performance of Arbor Acres broilers as affected by dietary additives of *A. annua*

Parameter	Control	<i>A. annua</i> (%)			SEM	P value
		0.3	0.6	0.9		
Initial body weight (g)	43.48	44.79	43.99	43.22	0.295	0.1131
Final body weight (g)	1950.98 a	1936.11 b	1925.97 b	1856.03 c	4.418	0.0001
Weight gain (g)	1907.66 a	1891.46 b	1882.02 c	1812.86 d	0.754	0.0001
Total feed intake (FI, g)	3430 a	3320 b	3270 c	3057 d	52.06	0.0001
Feed conversion ratio (FCR)	1.80 a	1.76 b	1.74 c	1.69 d	0.003	0.0001
Mortality (%)	8.33 a	4.00 b	2.33 c	0.67 d	0.160	0.0033

Means denoted by different small letters within the same row are statistically significant ($P < 0.05$, one-way ANOVA).

Table 3. Carcass characteristics of Arbor Acres broilers as affected by dietary additives of *A. annua*

Parameters*	Control	<i>A. annua</i> (%)			SEM	P value
		0.3	0.6	0.9		
Dressing percentage (DP)	80.66 c	81.73 b	81.63 b	84.37 a	0.294	0.0001
Liver (%)	2.36 b	2.80 a	2.49 b	3.15 a	0.277	0.0005
Gizzard (%)	1.99 b	2.49 a	2.24 b	2.44 a	0.308	0.0231
Heart (%)	0.51	0.47	0.52	0.56	0.065	0.2687
Spleen (%)	0.21	0.23	0.22	0.21	0.035	0.2596

Means denoted by different small letters within the same row are statistically significant ($P < 0.05$, one-way ANOVA).

*All parameters of carcass characteristics were expressed as percentage of live body weight.

Table 4. Hematological parameters of Arbor Acres broilers as affected by dietary additives of *A. annua*

Parameters	Control	<i>A. annua</i> (%)			SEM	P value
		0.3	0.6	0.9		
RBCs ($\times 10^6/\text{cmm}$)	3.18	3.34	2.87	4.3	0.521	0.2621
HB (g/dL)	9.34	9.78	8.43	9.11	0.608	0.4723
PCV (%)	27.02	28.22	24.35	28.84	1.552	0.2001
MCV (fL)	83.67	84.57	83.77	84.67	0.866	0.7794
MCH (Pg)	28.77	29.24	28.78	29.29	0.399	0.6734
MCHC (Pg%)	34.00	34.60	34.02	34.60	0.402	0.5511
Total leukocyte count $\times 10^3/\text{cmm}$	2.98 a	3.40 a	1.73 b	1.53 b	0.375	0.0025
Platelets count ($\times 10^3/\text{cmm}$)	1600.0	1662.2	1684.4	1746.7	120.01	0.8574
Neutrophil (%)	14.67	14.89	13.11	13.11	0.623	0.0871
Lymphocytes (%)	79.33	79.44	81.44	81.22	0.702	0.0725
Monocytes (%)	3.11 b	3.33 ab	3.54 ab	3.67 a	0.158	0.0449
Eosinophil (%)	2.67 a	2.33 ab	2.00 b	2.11 b	0.194	0.048
Basophils (%)	0.00	0.00	0.22	0.33	0.108	0.0905

Means denoted by different small letters within the same row are statistically significant ($P < 0.05$, one-way ANOVA).

Hematological parameters

Hematological parameters of Arbor Acres broilers affected by varying levels of dietary additives of *A. annua* are presented in Table 4. Most hematological parameters remained unaffected by the dietary inclusion of *A. annua*. However, total leukocyte and eosinophil counts significantly decreased ($P < 0.05$) at dietary levels of 0.6 and 0.9% *A. annua* compared to the control group. Conversely, broilers fed 0.9% *A. annua* exhibited a significant increase in monocyte count. These findings

suggest that *A. annua* is well tolerated at low doses of 0.3 and 0.6% without adverse effects on poultry health. However, at a higher dose of 0.9%, *A. annua* may suppress the cellular immune system of poultry, as evidenced by the decrease in total leukocyte count.

Regarding liver function parameters, ALT and AST levels exhibited a significant dose-dependent decrease in broilers fed *A. annua* compared to the control group. The TP, ALB, GL, and AL/GL ratios showed a dose-dependent increase in all treated groups compared with control broilers.

Table 6. The liver antioxidant parameters of Arbor Acres broilers as affected by dietary additives of *A. annua*

Parameters	Control	<i>A. annua</i> (%)			SEM	P value
		0.3	0.6	0.9		
MDA (NMOL/ML)	0.81 a	0.49 b	0.43 b	0.39 c	0.023	0.0001
TAC (Mm/l)	1.58 b	1.74 ab	1.85 a	1.85 a	0.082	0.0444
SOD (U/ml)	72.50 b	72.67 b	73.00 b	76.72 a	0.097	0.0177

Means denoted by different small letters within the same row are statistically significant ($P < 0.05$, one-way ANOVA).

Table 7. Proximate analysis of flesh of Arbor Acres broilers as affected by dietary additives of *A. annua*

Items	Control	<i>A. annua</i> (% ⁻¹ diet)			SEM	P value
		0.3	0.6	0.9		
Moisture (%)	77.747 a	76.897 b	74.017 c	71.667 d	0.226	0.0001
Crude protein (%)	15.177 d	17.060 c	18.530 b	20.720 a	0.093	0.0001
Crude fat (%)	2.697 a	1.927 ab	1.020 b	1.227 b	0.371	0.0464
Crude fiber (%)	0.367 a	0.210 b	0.153 b	0.127 b	0.024	0.0017
Ash (%)	1.440	1.517	1.990	2.283	0.248	0.1478
Available carbohydrates (%)	2.737 bc	2.240 c	4.293 a	3.980 ab	0.367	0.0209
Total carbohydrates (%)	2.947 bc	2.607 c	4.447 a	4.107 ab	0.365	0.0318

Means denoted by different small letters within the same row are statistically significant ($P < 0.05$, one-way ANOVA).

Table 8. Taste, color, odor, texture and general acceptance of Arbor Acres broilers as affected by dietary additives of *A. annua*

Items		Control	<i>A. annua</i> (% ⁻¹ diet)			SEM	P value
			0.5	0.6	0.9		
Drumstick (thigh)	Taste	7.08 b	7.25 b	7.79 ab	8.08 a	0.276	0.049
	Color	7.33	7.54	7.75	8.08	0.269	0.255
	Odor	7.50	7.54	7.83	7.92	0.255	0.579
	Texture	7.04 b	7.25 b	7.58 ab	8.08 a	0.261	0.039
	General acceptance	7.25	7.39	7.84	7.94	0.233	0.115
Breast	Taste	6.92 b	7.42 ab	8.00 a	8.04 a	0.251	0.008
	Color	7.17 b	7.42 ab	7.63 ab	7.96 a	0.234	0.049
	Odor	7.17	7.54	7.63	7.83	0.255	0.323
	Texture	6.97 b	7.21 ab	7.50 ab	7.83 a	0.238	0.046
	General acceptance	7.18 b	7.26 ab	7.50 ab	7.82 a	0.199	0.042

Means denoted by different small letters within the same row are statistically significant ($P < 0.05$, one-way ANOVA).

Concerning serum lipid profile, broilers fed diets supplemented with various levels of *A. annua* showed a significant dose-dependent decrease in cholesterol, triglycerides, and LDL. In contrast, HDL levels significantly increased compared with the control group. These results indicate a hypolipidemic effect of *A. annua* supplementation. Additionally, IgG showed a numerical, non-significant increase compared to control birds.

Antioxidant activities

The effects of *A. annua* supplementation on broiler antioxidant parameters are presented in Table 6. The results demonstrate that *A. annua* inclusion in broiler diets significantly decreased ($P = 0.0001$) MDA (malondialdehyde) levels compared to control birds. Broilers fed diets containing 0.6 and 0.9% *A. annua* exhibited the highest ($P < 0.05$) TAC (total antioxidant capacity) levels com-

pared to other experimental groups. Additionally, broilers fed diets containing 0.9% *A. annua* had the highest SOD (superoxide dismutase) levels compared to all other groups. These findings indicate the antioxidant effects of *A. annua*, particularly at inclusion levels of 0.06 and 0.9% in broiler diets.

Meat composition and sensory analysis

The chemical composition of broiler meat from Arbor Acres chickens fed diets with varying levels of *A. annua* is presented in Table 7. The data reveal a significant dose-dependent decrease in moisture, crude fat, and crude fiber percentages. In contrast, crude protein percentage increases significantly with increasing *A. annua* levels in the broiler diet. Available and total carbohydrate percentages were significantly increased in broilers fed 0.6% *A. annua*, while the lowest significant levels were

recorded for birds fed diets with 0.3% *A. annua*. The physical and sensory parameters of broiler meat samples are presented in Table 8. Regarding drumstick samples, broilers fed diets supplemented with 0.9% *A. annua* exhibited a significant enhancement in meat sample taste and texture compared to other treated and control birds. Additionally, taste, color, texture, and general acceptance of breast meat samples were significantly improved in broilers fed diets containing 0.9% *A. annua* compared to other groups.

Histopathological findings

Liver

Examination of serial sections from the livers of different experimental groups revealed nearly normal histo-

logical structures (Figure 1 A, B, H, D). The hepatic tissue comprised numerous lobules with indistinct boundaries due to poorly developed connective tissue septa, except in the vicinity of portal triads, which restricted the liver lobules. The hepatic parenchyma exhibited histologically normal, large, polygonal hepatocytes arranged in irregular cords or plates, each containing two rows of hepatocytes separated by hepatic sinusoids. The central vein displayed a circular or tortuous lumen lined by a single layer of endothelial cells surrounded by a scanty collagenous connective tissue. Mild biliary proliferative hyperplasia and associated inflammatory changes, primarily involving lymphocytes and plasma cells, were observed in some sections of G2 (Figure 1 C).

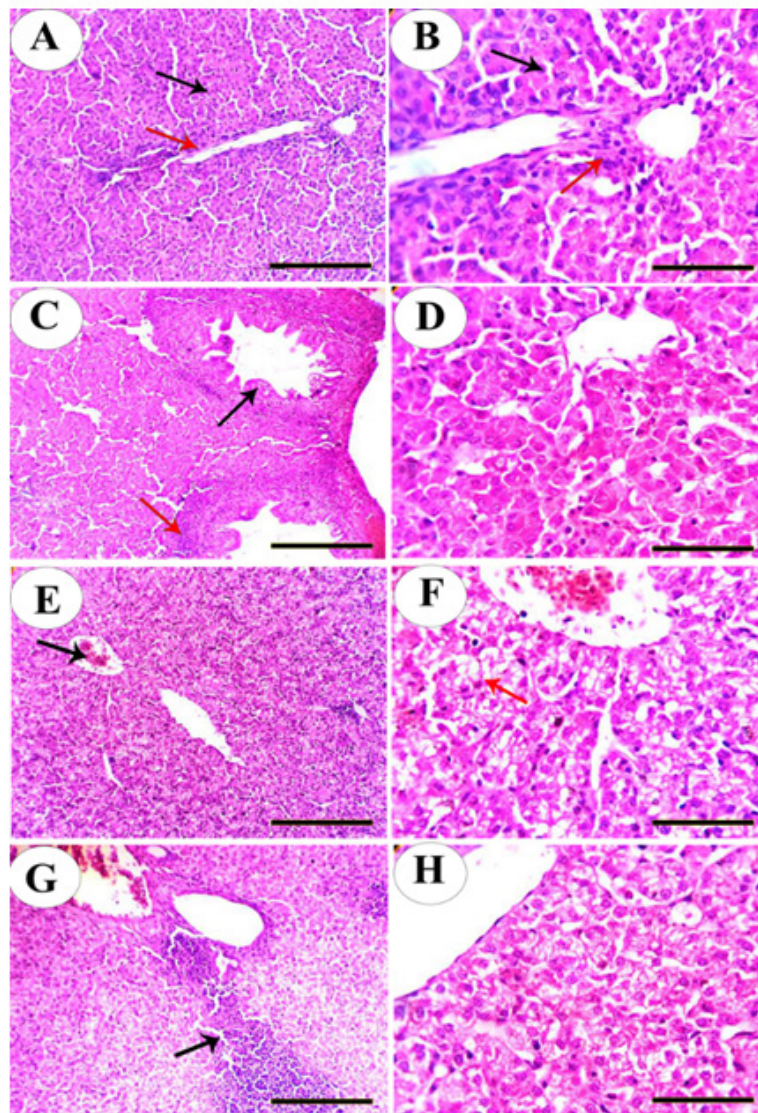


Figure 1. Representative photo-micrographs of liver sections from broiler chicken: (A, B) control (G1), (C, D) *A. annua*-treated (0.3% diet; G2), (E, F) *A. annua*-treated (0.6% diet; G3), and (G, H) *A. annua*-treated (0.9% diet; G2) showed: (A, B) Histo-morphological structures with keeping arrangement of the hepatic lobules, hepatic cellular masses (cords/plates) (black arrows), portal triad structures (red arrows), and sinusoids. (C, D) Normal histomorphology of hepatocytic plates (D) with mild biliary proliferative hyperplasia (black arrow) and associated inflammatory change, mostly of lymphocytes and plasma cells are seen in some sections (red arrows). (E, F) Histomorphological structures with keeping arrangement of the hepatic lobules, hepatic cellular masses, portal triad structures, and sinusoids, with mild congestion of central vein (black arrow) and occasional hepatocytic vacuolization (red arrow). (G, H) Normal histomorphology of the hepatic tissues, with portal-interstitial lympho-plasmacytic aggregations (black arrows). H&E; bar=200 μ m for A, C, E, and G; bar=50 μ m for B, D, F, and H

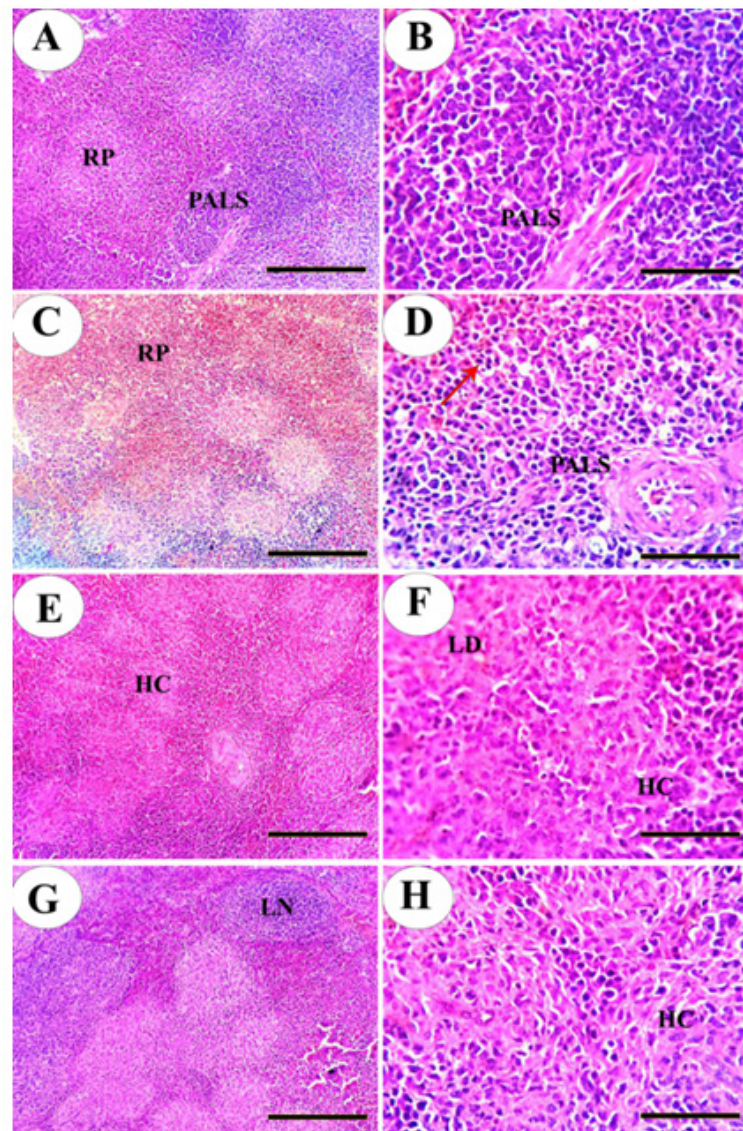


Figure 2. Representative photo-micrographs of spleen sections from broiler chicken: (A, B) control (G1), (C, D) *A. annua*-treated (0.3% diet; G2), (E, F) *A. annua*-treated (0.6% diet; G3), and (G, H) *A. annua*-treated (0.9% diet; G2) showed: (A, B) Normal histomorphologic structure of red pulp (RP) and white pulp including the periarteriolar lymphocyte sheaths (PALS) and lymph nodules (LN). Neither distinct marginal nor mantle zones are seen (C, D). Similar histomorphological structures in addition to mild histiocytosis (HC) and few inflammatory cells (heterophils) (red arrow). (E, F) Similar splenic histomorphological structures in addition to moderate histiocytosis (HC) and occasional lymphoid depletion (LD). (G, H) Normal splenic histomorphological structures beside moderate histiocytosis (HC). H&E; bar=200 μ m for A, C, E, and G; bar=50 μ m for B, D, F, and H

Additionally, mild congestion of the central vein (Figure 1 E) and occasional hepatocytic vacuolization (Figure 1 F) were evident in a few sections of G3. Furthermore, portal-interstitial lymphoplasmacytic aggregations were noted in some sections of G4 (Figure 1 G). However, no degenerative, necrotic, or apoptotic changes were detected in the experimental groups.

Spleen

Histopathological examination of serial sections from spleen tissues of different experimental groups revealed nearly normal histomorphological structures of the red pulp (RP) and white pulp, including the periarteriolar lymphocyte sheaths (PALS), peri-ellipsoid lymphocyte sheath (PELS), and lymph nodules (LN), without distinct marginal or mantle zones (Figure 2 A, B). However, a characteristic feature in all groups is the presence of mild to moderate histiocytosis in the red pulp. G2, G3, and G4 sections showed moderate red pulp histiocytosis (Figure 2 C-H).

Additionally, a few inflammatory cells (heterophils) were observed in some sections of G2, particularly infiltrating the red pulp (Figure 2 D). Mild occasional lymphoid depletion was reported in one section of G3 (Figure 2 F). However, no apoptotic or necrotic changes or architectural abnormalities were detected in any of the sections.

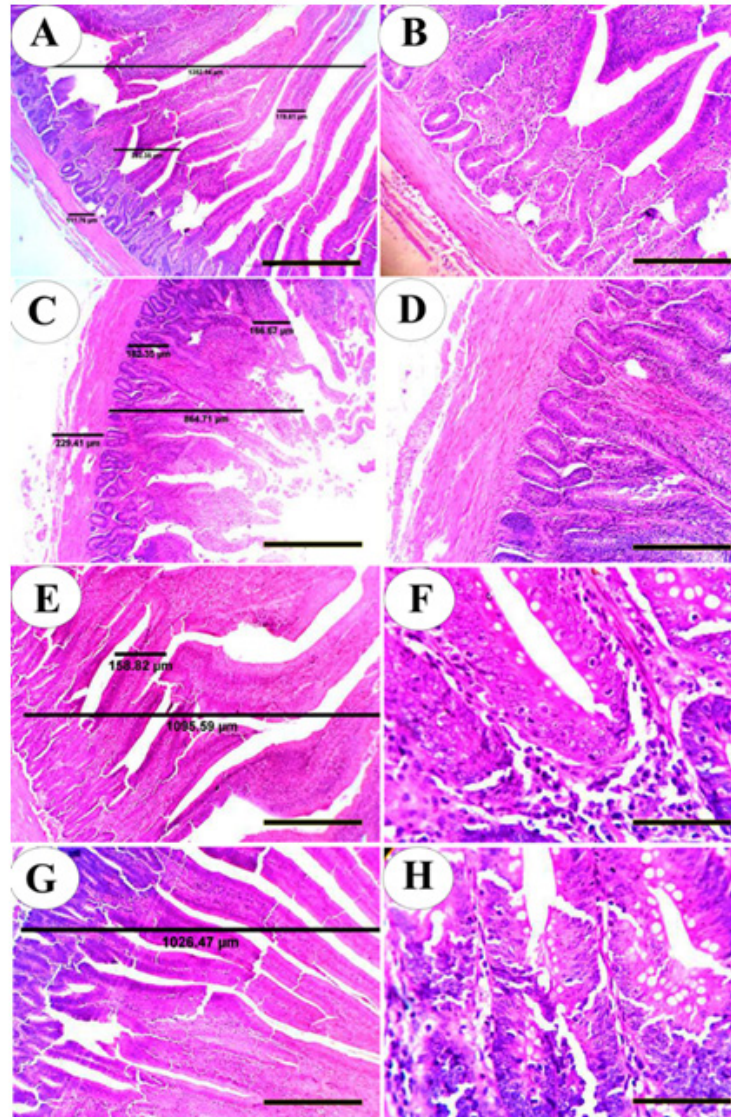


Figure 3. Representative photo-micrographs of small intestine (jejunum) sections from broiler chicken: (A, B) control (G1), (C, D) *A. annua*-treated (0.3% diet; G2), (E, F) *A. annua*-treated (0.6% diet; G3), and (G, H) *A. annua*-treated (0.9% diet; G2) showed: (A, B, C, D, E, G) Jejunum showing regular villous structures with secretory low mucus secreting absorptive epithelial lining. The lamina propria, submucosa, muscular coat and serosal covering are apparently normal. The intestinal glands are moderately active with focal proliferative epithelial linings. (F, H) The intestinal glands are strongly active; goblet cells are moderately pronounced and active. H&E; bar=200 µm for A, B, C, D, E and G; bar=50 µm for F and H

Small intestine

Examination of serial sections from small intestinal segments, most likely the jejunum, of different experimental groups revealed normal, healthy, and well-defined villous structures lined with secretory and low mucus-secreting absorptive epithelial cells. The lamina propria, submucosa, muscular coat, and serosal covering appeared normal (Figure 3 A–H). The intestinal glands exhibited moderate activity with focal proliferative epithelial linings in the different treatment groups. This reactivity was more pronounced in G3 (Figure 3 F) and G4 (Figure 3 H). No significant degeneration or necrotic changes were observed.

Regarding crypt height, a significant reduction ($P<0.0001$) was observed in G2 and G4 compared to the control group. However, a significant increase ($P<0.0001$)

in mean crypt height (µm) was reported in G2 compared to the control group. Furthermore, muscular coat thickness (µm) showed a significant increase ($P<0.0001$) in all treated groups compared to the control group, particularly in G1, followed by G2 and G3.

Discussion

During our 42-day study, the birds typically attained a weight of around 1950 g. This variation in weight can be attributed to small, uncontrollable environmental factors or the use of a basal diet with varying constituent profiles, resulting in differing nutrient levels. The growth performance may have been affected by alterations in

amino acid and fatty acid compositions of our basic diet elements, as well as varied energy consumption, when compared to standard catalog diets. Previous studies have revealed similar final weights of Arbor Acre chickens at a 42-day rearing period (Saki et al., 2010; Sunmola and Tuleun, 2023; Zhu La et al., 2023).

Our findings regarding FCR are align with those reported by Engberg et al. (2012), who observed a substantial decrease in final body weight and feed intake in broilers fed *A. annua* in a dose-dependent manner, along with a significant improvement in FCR compared to the control group. The reduction in body weight and feed intake may be attributed to significant amounts of non-soluble fiber and lignin, which adversely affect broiler performance. Additionally, the reduction in body weight and weight gain at the high inclusion of *A. annua* in the diet might have imparted a bitter taste on the feed and consequently reduced feed intake (Yeka and Harris, 2010).

Panaite et al. (2019) reported a non-significant impact of *A. annua* inclusion in the form of powder and essential oil on the growth of Cobb 500 broiler chickens. The obtained results partially agree with those of Khalaji et al. (2011), who found that Ross 308 chickens fed *Artemisia sieberi* leaves at a level of 0.1 g kg⁻¹ diet did not exhibit any significant changes in BW and FCR throughout the experimental period (1–42 days). Ulmer-Franco et al. (2010) reported a negative impact of *A. annua* on broiler chickens' initial weight and feed consumption from breeders fed *A. annua*. Coroian et al. (2022) experimented on broiler chickens to evaluate the effect of *A. annua* against coccidiosis. They found a significant improvement in FCR and a significant decrease in mortality rate compared to birds not supplemented with *A. annua*. The same authors reported a higher intestinal bacterial population compared to control birds. They concluded that the improved FCR, livability, and immunity of the treated birds may be due to

the increased intestinal microbiota, which enhances nutrient absorption and strengthens the immune system.

The findings of carcass traits are partially consistent with those of Saracila et al. (2018), who reported that the inclusion of *A. annua* powder and oils at various levels did not significantly impact different carcass parameters of broiler chickens. The increase in carcass traits, liver, and gizzard percentages may be attributed to the higher protein, carbohydrate, vitamin, and mineral contents associated with *A. annua* supplementation (Vargas-Sánchez et al., 2019).

Panaite et al. (2019) reported positive effects of *A. annua* on the health condition, performance, and development of broilers. Similarly, Sadr et al. (2022) demonstrated that *A. annua* supplementation enhances the overall health performance and condition of treated pigeons. The current findings partially align with those of Zvarivadza and Marume (2022), who observed that broilers fed 0.1% *Artemisia afra* essential oil exhibited the highest values for monocytes, neutrophils, and eosinophils compared to control birds. The effects of varying levels of *A. annua* supplementation on liver function, lipid profile, and immune parameters of broilers are presented in Table 5. Serum biochemical parameters are crucial determinants of bird physiology and are closely linked to the birds' nutrition, metabolism, and overall health status.

Our findings indicate improved liver function parameters due to adding *A. annua* to broiler diets. The observed improvements in TP, Alb, Gl, and AL/Gl ratio could be correlated with the birds' immune and health status due to herbal supplementation (Alghamdi et al., 2023; Liu et al., 2023). The present study's findings of reduced liver enzyme activity support the safety of the studied herbal plant at the recommended dosage. These results are consistent with those obtained by Ali et al. (2014) and Sohail et al. (2012), who reported decreased liver enzymatic activities following herbal supplementation.

Table 5. Serum blood biochemical parameters, liver function, lipid profile and immune parameters of broilers fed diets included with various *A. annua*

Parameters	Control	<i>A. annua</i> (%)			SEM	P value
		0.3	0.6	0.9		
ALT (U L ⁻¹)	2.715 a	2.596 b	2.485 c	2.120 d	0.014	0.0001
AST (U L ⁻¹)	315.83 a	299.17 b	276.50 c	254.17 d	1.911	0.0001
TP (g dL ⁻¹)	2.615 d	2.928 c	3.195 b	3.660 a	0.057	0.0001
Alb (g dL ⁻¹)	0.782 d	0.927 c	1.112 b	1.353 a	0.024	0.0001
Gl (g dL ⁻¹)	1.833 c	2.002 b	2.083 b	2.307 a	0.035	0.0001
Al/Gl ratio	0.427 d	0.462 c	0.532 b	0.588 a	0.005	0.0001
Cholesterol (mg/dl)	347.67 a	297.00 b	269.33 c	247.00 d	4.037	0.0001
Triglycerides (mg/dl)	231.83 a	210.67 b	194.33 bc	174.83 c	7.115	0.0002
HDL (mg/dl)	103.67 c	110.333 b	111.83 b	126.17 a	1.748	0.0001
LDL (mg/dl)	197.63 a	144.53 b	118.63 c	85.87 d	2.633	0.0001
VLDL (mg/dl)	46.37 a	42.13 b	38.87 bc	34.97 c	1.423	0.0002
IgM (μmol/L)	15.01 c	19.60 c	29.93 b	48.94 a	2.262	0.0001
IgG (μmol/L)	25.19	27.63	32.91	40.50	5.042	0.1823
IgA (μmol/L)	26.32	20.04	28.10	26.48	5.134	0.7001

Means denoted by different small letters within the same row are statistically significant (P<0.05, one-way ANOVA).

Results of lipid profile may be attributed to a lower de novo cholesterol synthesis, which has been shown to boost the expression of LDL cholesterol receptors on hepatocytes, resulting in a higher LDL uptake by the liver hepatocytes and ultimately lowering blood LDL concentrations. Therefore, therapeutic plants such as *A. annua* have been shown to possess cholesterol-lowering effects (Ghasemi et al., 2014). The current findings align with those obtained by Lee et al. (2020), who reported that broiler chicks fed *A. annua* powder had lower serum LDL-C, total lipid, triglyceride, and total cholesterol levels compared to the control and antibiotics-supplemented groups. Results revealed a significant increase in immune parameters, particularly IgM, in groups supplemented with *A. annua*.

For immunity data, our results confirm increased humoral immunity in broilers due to *A. annua* supplementation. Several studies have demonstrated that natural products can exert various actions on the immune system, such as enhancing macrophage activity (Dimov et al., 1992), promoting IL1 (Oršolić and Jazvinščak Jembrek, 2022), IL2, and IL4 production (Park et al., 2004). In this study, IgG and IgM levels were significantly increased, suggesting an improvement in humoral immunity. This improvement could be associated with a combination of these responses. This finding indicates stimulation of the immune system's B lymphocytes by these cytokines, which subsequently transform into plasma cells to generate antibodies (Taheri et al., 2005).

The antioxidant properties of *A. annua* are attributed to its natural antioxidant compounds, which have been shown to reduce lipid peroxidation and enhance oxidative stability in broilers (Baghban-Kanani et al., 2019). Additionally, the high phenolic and flavonoid content of *A. annua*, a characteristic of herbal plants, contributes to its antioxidant effects by scavenging and neutralizing free radicals (Abd El-Hack et al., 2023; Abdel-Moneim et al., 2020; Seidavi et al., 2022). The findings of the present study align with those of Guo et al. (2022), who found that broiler chicks fed *A. annua* L. aqueous extract exhibited a significant increase in CAT (catalase) and SOD activity in the ileum. TAC and GSH (glutathione) activity in the duodenum, accompanied by decreased MDA concentration in the ileum. Additionally, Arbor Acres broilers supplemented with 500–1500 mg/kg *A. annua* extract (AAE) demonstrated a significant increase in serum T-AOC, CAT, SOD, and GSH-Px (glutathione peroxidase) and a decrease in MDA levels (Guo et al., 2022).

The higher crude protein content in the meat may be linked to the higher crude protein content in *A. annua*. Guo et al. (2022) reported that *A. annua* supplementation in broiler diets significantly improved meat quality parameters. Ham et al. (2016) demonstrated that *A. annua* supplementation up to 0.1% enhanced the antioxidant activity of meat products such as sausages, improving their sensory properties, color, and flavor. The improvement in meat color and sensory parameters may be attributed to the oxidation status of myoglobin, a protein intimately linked to meat color (Yu et al., 2017). Meat color can be improved by supplements promoting antioxidant capacity, which may increase myoglobin concentration (Baowei et al., 2011). Meat tenderness typically correlated with myofibrillar protein (MIF) content and muscle fiber structure (Cai et al., 2018), is a factor that influences consumer acceptance of cooked meat (Joo et al., 2013). Shear force is a reliable measure of meat tenderness, with lower values indicating greater tenderness and a direct correlation with consumer acceptability (Ham et al., 2016). The present study found that *A. annua* supplementation significantly reduced shear force values, suggesting improved meat tenderness.

The mild lesions observed in the groups treated with *A. annua* may indicate potential intoxication; therefore, further research is warranted to investigate this possibility. The toxicological profile of *A. annua* plant material and extracts, including artemisinin, is not well established in poultry. However, previous studies on broiler chickens have explored this topic (Arab et al., 2009; Shahbazfar et al., 2011). Shahbazfar et al. (2011) reported histopathological evidence of hepatic and renal deterioration following long-term oral administration of artemisinin, regardless of dose (17 to 136 mg/kg feed). Additionally, they observed dose-dependent neuronal degeneration, even without clinical symptoms. Arab et al. (2009) reported bile retention in the liver and similar patterns in clinical and histological findings following single doses of 1250 mg/kg and 2500 mg/kg.

The immune system of chickens, encompassing both primary and secondary immunological organs, is a complex and intricate structure (Casteleyn et al., 2010) the so-called gut-associated lymphoid tissue (GALT). Among other factors, the composition of a bird's diet significantly impacts its immune function (Villagrán-de la Mora et al., 2020) line COBBAvian48 (free of growth-promoting antibiotics). This histiocytosis could be either a reactive change or an immune-enhancing stimulatory effect of *A. annua* (Emile et al., 2021).

Table 9. Histomorphometric parameters of intestine of Arbor Acres broilers as affected by dietary additives of *A. annua*

Items	Control	<i>A. annua</i> (% ¹ diet)			SEM	P value
		0.3	0.6	0.9		
Villi height (μm)	1310.60 a	862.84 d	1094.01 b	1037.16 c	10.424	0.0001
Villi width (μm)	118.47 c	148.62 b	157.49 b	199.17 a	8.299	0.0028
Crypt height (μm)	280.73 b	181.45 d	293.37 a	187.48 c	0.384	0.0001
Muscular coat thickness (μm)	110.82 d	227.61 a	204.20 b	160.34 c	0.573	0.0001

Means followed by different small letters in the same row are significantly different (P<0.05, one-way ANOVA)

The heterophils found in some sections of G2 could be a response to bacterial infections or various stressors (Maxwell and Robertson, 1998). Also, the mild occasional lymphoid depletion reported in one section of G3 could be attributed to the flavonoids and polysaccharides found in *A. annua*, which have been shown to enhance immunity by promoting macrophage phagocytic activity (Yang et al., 2021) which were, respectively, fed a basal diet (control).

The gut's health is recognized as crucial for the proper growth of animals, as it serves as the primary site for nutrient digestion and absorption (El Basuni et al., 2023; Khattab et al., 2021). No significant degeneration were observed, which could be attributed to *A. annua*'s ability to enhance intestinal immunity and antioxidant capacity (Song et al., 2018). Intestinal villi play a crucial role in nutrient absorption efficiency in birds. Kai (2021) stated that longer villi are typically associated with enhanced absorption efficiency and a healthy digestive tract. In this study, the histomorphometric analysis of intestinal parameters, as shown in Table 9, revealed a significant reduction ($P < 0.0001$) in villus height (μm) in all treated groups compared to the control group. The lowest reduction was observed in G1, followed by G3 and G2. Conversely, villus width (μm) showed a significant increase ($P < 0.0028$) in all treated groups compared to their counterparts in the control group. This increment was most pronounced in G3, followed by G2 and G4.

The findings of crypt height suggest that while *A. annua* did not induce significant intestinal histopathological lesions, it also did not improve intestinal morphology or intestinal absorption. Evidence suggests that deeper crypts and shorter villi can lead to poorer nutrient absorption, increased water and electrolyte secretion in the gastrointestinal tract, and worse performance (Ramlucken et al., 2020) following strict regulations around the use of antibiotic growth promoters (AGP). This suggestion aligns with the observed decrease in body weight of *A. annua*-supplemented broilers in this study. These results contrast those of He et al. (2022), who reported an improvement in intestinal histomorphology of juvenile largemouth bass. However, there is a lack of data regarding this effect on broiler intestinal histomorphology.

Conclusion

It is possible to conclude that adding *A. annua* to broiler feed at concentrations of 0.9% and 0.6 positively impacts liver function, immunoglobulin proteins, antioxidant status, lipid profiling, and meat quality indices. Many active substances found in *A. annua*, including sesquiterpenoids, flavonoids, coumarins, lipids, phenolics, purines, steroids, triterpenoids, aliphatics, and artemisinin, the plant's principal bioactive ingredient, may be primarily responsible for this encouraging result. More research is still required to clarify their potential toxicity and establish the ideal safe dose for broilers.

Author contributions

Conceptualization: TE-R, MEB, SE-D, MM and AET; Data curation: TE-R, AE and MM; Formal analysis: TE-R, AE, SE-D, MM and AET; Investigation: TE-R, AE, SE-D, MEAE and AET; Methodology: TE-R, MEB, AE, SE-D, MM, MEAE and AET; Resources: TE-R, MEB, AE, MEAE and AET; Software: TE-R, MEB, AE, SE-D, MEAE and AET; Validation: SE-D, MM, MEAE and AET; Writing – original draft: MEB, MEAE and AET; Writing – review and editing: MEB and AET. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Data are available with the request to the corresponding authors.

Conflicts of interest

The authors declare no conflicts of interest.

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