

Research Article

Effects of Aqueous Extract of *Artemisia annua* on Immune System, Hematological Characteristics, Intestinal Micro-flora, Growth Performances and Carcass Yield of Broilers

Chongsi Margaret Mary Momo* , **Noumbissi Marie Noel Bertine** ,
Bend Emma Fortune Magloire , **Mweugang Nguoupo Nathalie** ,
Jatsa Cyntia Dorimene , **Sambou Ngonseu Charlin No ð** , **Mi égon éEmile** ,
Tendonkeng Fernand 

Department of Animal Science, University of Dschang, Dschang, Cameroon

Abstract

Antibiotic residues are constantly being deposited in human organisms from the consumption of food of animal origin, leading to resistance and the health risks related to their use as growth promoters in animal feed and this has led to the ban of their use in animal feed by authorities. This restriction of their use has led to a resurgence of morbidity and mortality, a drop in growth performance, and consequently a decline in the economic profitability of farms making researchers to seek substitutes that play the same role without the same side effects. Plant-based products have been shown to possess properties that can play this role but their effects vary with form, dose, mode of administration and other factors. The present study was therefore designed to assess the effects of increasing levels of *A. annua* aqueous extract on growth performance, carcass and immune system organ characteristics, hematological parameters and intestinal microflora of broiler chickens. 288-day-old chicks were allocated to a completely randomized system comprising a base ration with no additives (T0-), a ration containing 0.1% antibiotic (T0+) and four rations with inclusions of 0.25, 0.5, 0.75 and 1 g aqueous extract of *A. annua*/Kg of feed with 3 replications each containing 16 chicks and treated till 49 days of age before sacrificing for data collection. The main results showed that the greatest live weight was obtained in T0.75-treatment birds with the least value in T0- at 49 days old with highest weight gain in T1. Feed conversion ratio was maximum in T0.25 and least in T1. T0.75 recorded significantly lower values for *Escherichia coli* while lactobacillus recorded values significantly ($p < 0.05$) lower in T0+ than the others. Granulocytes and platelets registered the greatest values in T1. Thus, aqueous extract of *A. annua* had greater beneficial effects in most characteristics at a rate of 1 g/kg feed, and may be used as natural source of feed additive instead of antibiotics in broilers.

Keywords

Broiler Chicken, *A. annua*, Growth Performance, Intestinal Microflora, Hematology

*Corresponding author: margareтчongsi@yahoo.fr (Chongsi Margaret Mary Momo)

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1. Introduction

Poultry farming has globally experienced a great rise (+4.3% per year) recently [1]. Most animals raised for meat production in the past were given antibiotic-supplemented feed for therapeutic or sub-therapeutic purposes [2] which lead to the accumulation of their residues in their products consumed by humans. The problem of transmission of antibiotic resistant bacteria to human through food chain and contamination of environment is another challenge of antibiotic usage in poultry production. Because of this constant deposit of antibiotic residues in human organisms from the consumption of food of animal origin, leading to resistance and the health risks related to their use as growth promoters, their use has been banned in animal feed by authorities. This restriction of their use has led to a resurgence of morbidity and mortality, a drop in growth performance, and consequently a decline in the economic profitability of farms [3].

This situation then sparked a rush towards the research of natural compounds derived from plants (phytobiotics) that can produce the same effects as antibiotics in animals without undesirable effects to humans and the environment [4]. Among these alternatives, plant-based products like spices, essential oils, and phyto-additives play a very important role [5, 6]. We equally have medicinal plants having preventive or curative properties that have to do with certain functional disorders or pathological conditions in humans and animals. At least one of their parts (leaf, stem, root, rhizome...) is used in order to prevent or treat diseases [7, 8]. These plants are rich in phyto-chemical compounds like alkaloids, flavonoids, steroids, terpenoids, artemisinin and quinones, which confer antibacterial, antioxidant, antiviral, antifungal, antiparasitic, immunomodulatory properties and digestive tract stimulating effects [9, 10, 7].

Among these medicinal plant extracts we have *Artemisia annua*, a traditional herb known for its pharmacological properties and now gaining attention as a potential natural feed additive in poultry production. *Artemisia annua*, commonly known as sweet wormwood, contains several active compounds that contribute to its effectiveness as an antimicrobial and health-promoting agent. The most notable among these is artemisinin, a sesquiterpene lactone with powerful antiparasitic and antimicrobial properties. In addition to artemisinin, *A. annua* also contains flavonoids, phenolic acids, tannins and essential oils, all of which exhibit antioxidant, anti-inflammatory, and immune-enhancing activities [11, 12]. These properties make *A. annua* a suitable and multifunctional alternative to antibiotics in poultry diets.

Therefore, these bioactive compounds in *Artemisia annua* could ensure the balance of the intestinal microflora, stimulate the immune system, and enhance growth performance if incorporated as additives in the feed of livestock, thus initiating the present work.

2. Materials and Methods

2.1. Animal Material

For this trial, a total number of 288 one-day-old Hubbard broiler chicks were purchased from the SKAB Company in Bafoussam with an average weight of 38.5 g were randomly distributed into 18 experimental units of 16 subjects each.

2.2. Housing and Feeding

Table 1. The Bromatological and Centesimal Composition of the Basal Diet.

Ingredients	Quantities for starters%	Quantities for finishing%
Maize	56.5	64
Wheat brand	2	2
Fish meals	2	2
Soya beans meals	23	16
Groundnut meals	7.5	7.5
oyster shell	1	1
Bones meals	1	1
Belgofox	1	1
Premix 5%	5	5
Palm oil	1	0.5
Total	100	100

Calculated nutrients composition

Metabolized energy (kcal/kg)	2965.91	3000
Crude proteins (%DM)	23.03	20.09
Fat	4.48	4.20
Cellulose (%)	4.71	4.87
Lysine (%)	1.31	1.10
Methionine (%)	0.45	0.41
Calcium (%)	1.24	1.60
Phosphorus (%)	0.53	0.51
Ca/P	2.32	3.16
E/protein	128.281	147.63

Ca: calcium; P: phosphorus; DM: dry matter; E: energy;

The startup took place in the chick-rearing building of the

Teaching and Research Farm at the University of Dschang. This building was first thoroughly washed and then disinfected. After a period of 2 weeks of sanitary emptying, each section was equipped with a 100-watt incandescent bulb serving as a heat source, a cone-shaped feeder for chicks with a capacity of 10 kg, and a 5-liter siphoid drinker. At 21 days old, the chicks were transferred to the finishing building made of 1.40 m high half-brick walls topped with small mesh fencing, and with a roof made of corrugated metal sheets. Each pen was equipped with a cone-shaped feeder that could hold 20 kg and a 10-liter siphoid drinker. The bedding in both the startup and finishing phases was made of previously treated white wood shavings. Throughout the experiment, the animals were given water *ad libitum*. A negative control base diet (containing no additives) was formulated for all animals in the trial (Table 1).

2.3. Prophylaxis

The animals were vaccinated against infectious bronchitis (H120®) and Newcastle disease (Hitchner B1®) on the 7th day with a booster on the 18th day, and against Gumboro disease (IBA Gumboro®) on the 14th day. Stress relief (T áracoli®) was provided in the drinking water during the first 3 days after the chicks were brought to the chick house. Vitamins (AMINTOTAL®) were also provided in the drinking water for 3 consecutive days each week. The same was done before, during, and after each handling (weighing, vaccinations, and transferring chicks from the chick house to the finishing building). A footbath was placed at the entrance of the breeding building, and the disinfectant made of bleach and cresol was renewed every two days. At both the start and the end, the water troughs were washed with soap and rinsed with clean water every morning throughout the study period.

2.4. Plant Material

The dried and healthy leaves of *Artemisia annua* were purchased from the APS association in Bagangté. They were ground into a fine powder using a mill and weighed using a sensitive scale with a precision of 1 g and a capacity of 7 kg. The powder was later on transported to the phytopathology laboratory where we proceeded to the production of the aqueous *Artemisia* extract. This extract was later on used as an additive in the feed of broilers during the experiment.

A portion of the aqueous extract of *Artemisia annua* was sent to the Laboratory of Natural Substance in the Department of Biochemistry at the University of Dschang for phytochemical screening based on the method described by Banso and Adeyemo [13] and Ngbede et al. [14] and the result are presented in Table 2.

Table 2. Result of the Phytochemical Test of the Aqueous Extract of *Artemisia annua*.

Constituents	Absence	Presence
Alkaloid	-	
Phenol		+
Flavonoid		+
Sterol		+
Triterpenoid		+
Tannins		+
Saponins		+
Anthocyanines		+
Anthroquinones		+

–: Absent; +: Present.

2.5. Experimental Design

The experiment was carried out for 7 weeks. The six experimental treatments with animals as earlier described were made up of:

T0–: Basal diet; T0+: Basal diet + doxycycline in drinking water; T0.25: 0.25% of *Artemisia* extract in feed; T0.5: 0.5% of *Artemisia* extract in feed; T0.75: 0.75% of *Artemisia* extract in feed; T 1: 1% of *Artemisia* extract in feed.

2.6. Studied Parameters and Data Collection

2.6.1. Growth Parameters

The data were collected every seven days on feed intake (FI), live weight (LW), and weight gain (WG), which allowed for the calculation of the feed conversion ratio (FCR).

(i). Feed Intake

The weekly feed intake was assessed by calculating the difference between the amounts served during the week and the leftovers.

Feed intake = food distributed during the week (g) – leftovers (g)

(ii). Live Weight and Weight Gain

At the beginning of the trial and every seven days thereafter, the animals in each experimental unit were weighed using an electronic scale accurate to the nearest gram and with a capacity of 7 kg. Weight gain was obtained by the difference between two consecutive weekly live weights.

Weight gain = final weight (g) – initial weight (g)

(iii). Feed Conversion Ratio (FCR)

The weekly feed conversion ratio was calculated by dividing the quantity of feed consumed by the weight gained (WG) during the same week.

$$FCR = \frac{\text{Total feed intake per week}}{\text{Weight gain per week}}$$

2.6.2. Carcass Characteristics

At 49 days of age, 8 chickens (4 males and 4 females) per treatment were randomly selected and subjected to a 24-hour fasting. They were then weighed, bled, plucked, and eviscerated. The carcass yield and the relative weight of the organs were calculated as carried out by Kana et al. (2014) [15] to evaluate the characteristics of the carcass. The dressed carcass was weighed and expressed as percentage of the live body weight using the formula below.

$$\text{Carcass yield (\%)} = \frac{\text{Dressed weight (g)}}{\text{Live body weight (g)}} \times 100$$

2.6.3. Relative Percentage of Organs or Parts (%)

From the broilers selected for carcass evaluation, the intestinal organs were carefully excised; then the weight measured using an electronic balance and length measured using measuring tape. The other organs, including the liver, pancreas, heart, gizzard and head were also removed and weighed. The relative percentage weight of organs was calculated as the ratio of the weight of the organ to the live body weight of the birds as follows:

$$\text{Percentage of organs (\%)} = \frac{\text{Weight of organs (g)}}{\text{Live body weight (g)}} \times 100$$

2.6.4. Measurement of Intestine

The length of the intestine was measured from the duodenal bulge to the cloaca using a tape measure, the weight with a high-precision scale (1g), and the density of the intestine calculated using the formula below.

$$\text{Density of the intestines} = \frac{\text{Average weight of the intestines (g)}}{\text{Average length of the intestines (cm)}}$$

2.6.5. Intestinal Microflora

Before the sacrifice of the chickens at 49 days of age, swabs were used to collect fecal matter from the cloaca, which was then transported immediately to the laboratory where the identification and quantification of lactic bacteria, *Escherichia coli*, and salmonella was carried out in the following respective and specific culture media: lactobacilli MRS AGAR, Mac Conkey AGAR, and SS AGAR. All homogenization, dilution solution preparation, and decimal dilution manipulations were performed aseptically to avoid contamination, and only packaged sterile materials (sterilized in an autoclave at 125 °C for 15

minutes) were used during these manipulations.

2.6.6. Hematological Characteristics

Blood samples collected in test tubes with anticoagulant were analyzed using a Veterinary Hematometer brand Uritit 300. These characteristics focused on red blood cells, hematocrit level, blood platelets, mean corpuscular volume, mean platelet volume, and mean corpuscular hemoglobin content.

2.7. Statistical Analysis

Data collected on all parameters were subjected to one way analysis of variance (GLM) and when differences existed between treatments, the means were separated by the LSD test at 5%. The statistical software used was SPSS 26.

3. Results

3.1. Effects of Aqueous Extract of *Artemisia annua* on Growth Characteristics of Broilers

Figures 1 to 4 show the weekly evolution of feed intake, live body weight, weight gain and feed conversion ratio of broilers fed with aqueous extract of *Artemisia annua*.

3.1.1. Feed Intake

Figure 1 illustrates the effects of aqueous extract of *Artemisia annua* on feed consumption. Feed intake increased from the 1st to the last week in all the groups. However, the subjects fed with the diet containing 0.25g of aqueous extract of *Artemisia annua* exhibited the highest feed consumption compared to the other treatments at the 7th week.

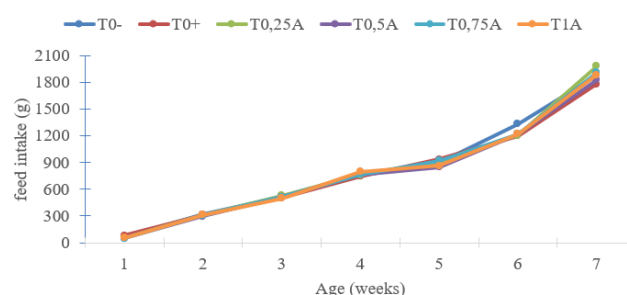


Figure 1. Effects of Aqueous Extract *Artemisia annua* on Evolution of Feed Intake in Broilers.

T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25A, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5A, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75A, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1A, control diet + 1 g aqueous extract of *Artemisia annua*.

3.1.2. Live Body Weight

The weekly body weight evolution curves (Figure 2) of the broilers increased similarly with time throughout the trial period. The live weight of broilers fed with feed containing 1 g (T1) of aqueous extract of *Artemisia annua* was higher than that of the other treatments.

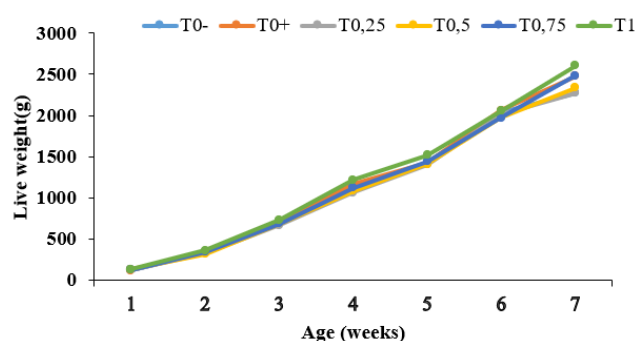


Figure 2. Weekly Evolution of the Live Weight of Broilers Receiving Aqueous Extract of *Artemisia annua* in Feed.

T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*.

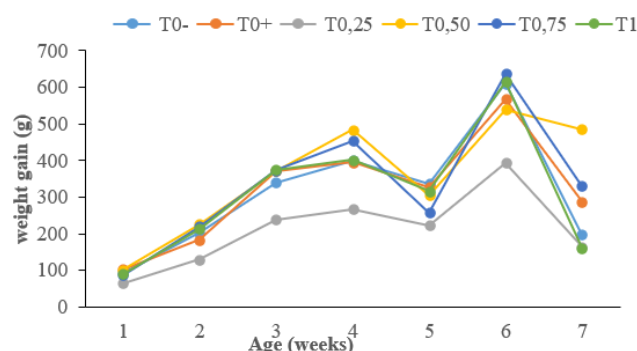


Figure 3. Weekly Evolution of the Weight Gain of Broiler Receiving Aqueous Extract of *Artemisia annua* in Feed.

T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*.

3.1.3. Weight Gain

The weekly evolution of the weight gain of broiler receiving aqueous extract of *Artemisia annua* in feed if put to evi-

dence in Figure 3. The weight gain of broilers increased simultaneously from the first to the fourth week for all treatments with broilers of the T0.50 having the highest weight gain. From the fourth to the fifth week, we observed a decrease of weight gain and it increased through the sixth before dropping down to the 7th week meanwhile T0.25 maintained the least weight gain throughout.

3.1.4. Feed Conversion Ratio (FCR)

Figure 4 shows the weekly evolution of feed conversion ratio of broilers fed with aqueous extract of *Artemisia annua*. The FCR of broilers receiving *A. annua* in feed increased simultaneously from the first to the fourth week after which we observed a rapid increase of the FCR of the positive control treatment (T0+) as compared to other treatments. From the fifth to the sixth week, we observed a decrease in the FCR in all treatments and a rapid increase during the seventh week with T0.25 showing the highest record.

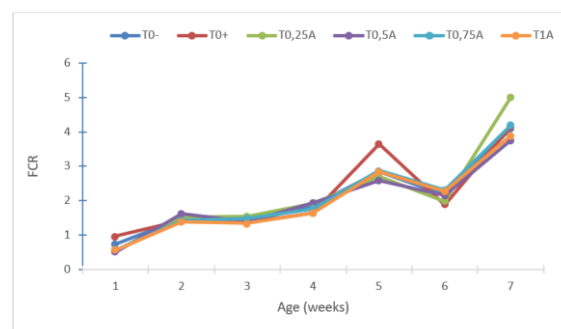


Figure 4. Effects of the Aqueous Extract of *Artemisia annua* on Evolution of Feed Conversion Ratio in Broilers.

T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*.

Table 3 summarizes the effects of the aqueous extract of *Artemisia annua* on cumulative growth characteristics in broilers.

Throughout the growth phase, no significant ($p > 0.05$) differences were observed among treatments for feed consumption of the broilers. The greatest live weight was obtained in T0.75-treatment birds with the least value in T0- at 49 days old though these values were generally comparable among the extract-treated groups and the controls, with a similar observation in weight gain. Feed conversion ratio was maximum in T0.25 and least in T1 though no significant difference was registered among the controls and extract-treated birds.

Table 3. Effects of Aqueous Extract of *A. annua* in Feed on Cumulative Growth Performances of Broilers.

Pe- riod (days)	Control treatments		Treatments				P
	T0-	T0+	T0.25	T0.5	T0.75	T1	
Feed intake (g)							
D21	901.85 ±10.93	899.76 ±25.29	883.95 ±15.90	863.77 ±24.49	870.85 ±17.73	866.25 ±21.75	0.13
D49	5808.39 ±83.56	5625.20 ±80.32	5871.25 ±39.95	5642.60 ±175.70	5739.58 ±157.31	5783.5 ±183.51	0.27
Live weight (g)							
D21	712.50 ±14.25	711.18 ±14.23	669.06 ±17.84	698.37 ±22.97	689.56 ±136.00	731.35 ±123.75	0.069
D49	2203.40 ±83.92 ^c	2389.34 ±181.92 ^{abc}	2212.14 ±135.56 ^c	2265.05 ±51.75 ^{bc}	2428.91 ±9.9 ^{ab}	2543.00 ±83.39 ^a	0.012
Weight gain (g)							
D21	678.68 ±19.23	677.27 ±14.45	635.54 ±18.48	655.84 ±122.72	652.58 ±135.12	697.56 ±22.33	0.65
D49	2170.17 ±84.13 ^c	2355.43 ±181.31 ^{abc}	2178.62 ±136.79 ^c	2231.53 ±51.75 ^{bc}	2394.94 ±10.71 ^{ab}	2509.21 ±181.31 ^b	0.012
Feed conversion ratio							
D21	1.32±0.03	1.32±0.06	1.39±0.05	1.31±0.07	1.33±0.07	1.24±0.06	0.20
D49	2.67 ±0.12 ^a	2.39 ±0.16 ^b	2.70 ±0.19 ^a	2.52 ±0.03 ^{ab}	2.39 ±0.02 ^b	2.31 ±0.07 ^b	0.004

^{a, b, c}, the means with the same letters on the same line are not significantly different ($P > 0.05$); D, day; T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*, P, probability.

3.2. Effects of Aqueous Extract of *Artemisia annua* on Carcass Yield and Relative Organs Weights

3.2.1. Carcass Yield

Table 4. Effects of Aqueous Extract of *A. annua* in Feed on Carcass Characteristics of Broilers.

Organs	Control treatments		Treatments				P
	T0-	T0+	T0.25	T0.5	T0.75	T1	
Carcass yield 1	85.61 ±6.24	92.88 ±16.67	90.83 ±7.26	85.65 ±1.77	88.68 ±2.01	84.60 ±3.1	0.256
Carcass yield 2	68.70 ±5.17 ^b	71.68 ±1.32 ^{abc}	74.44 ±5.59 ^a	70.18 ±1.32 ^{bc}	72.79 ±0.97 ^{ab}	68.86 ±3.38 ^{bc}	0.015
Head	2.13 ±0.44	2.16 ±0.36	2.44 ±0.56	2.20 ±0.56	2.35 ±0.51	2.11 ±0.18	0.503
Feet	3.73 ±0.65	3.94 ±1.20	3.97 ±0.61	3.73 ±0.42	3.83 ±0.53	3.72 ±0.31	0.953
Lungs	1.70 ±0.14 ^b	1.95 ±0.17 ^a	1.84 ±0.17 ^{ab}	1.90 ±0.17 ^{ab}	2.03 ±0.20 ^a	1.94 ±0.12 ^a	0.01
Heart	0.46 ±0.05 ^{ab}	0.45 ±0.06 ^{ab}	0.54 ±0.14 ^a	0.48 ±0.07 ^{ab}	0.43 ±0.13 ^b	0.48 ±0.05 ^{ab}	0.246
Abdominal fat	1.05 ±0.53 ^b	1.74 ±0.61 ^a	1.34 ±0.52 ^{ab}	1.27 ±0.40 ^{ab}	1.33 ±0.52 ^{ab}	1.50 ±0.67 ^{ab}	0.234

a, b, c, the means with the same letters on the same line are not significantly different ($P > 0.05$); T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*, P, probability.

The effects of aqueous extract of *Artemisia annua* on carcass characteristics is represented on Table 4. There was no significant ($p>0.05$) difference observed among the controls and those that received the extract in carcass yields 2, despite the fact that some groups had higher values than the others. Similar observations were made for relative weights of lungs, abdominal fats and heart.

3.2.2. Digestive Organs of Broilers

Table 5. Effects of Aqueous Extract of *A. annua* Diets on Digestive Organs of Broilers.

Organs	Control treatments		Treatments				P
	T0-	T0+	T0.25	T0.5	T0.75	T1	
Pancreas	1.67±0.20	1.62±0.17	1.59±0.15	1.58±0.15	1.63±0.23	1.76±0.19	0.42
Gizzard	1.67±0.40	1.62±0.24	1.76±0.23	1.58±0.15	1.59±0.35	1.59±0.15	0.762
Weight of the intestine	89.39±20.17	87.61±9.58	90.76±18.69	93.98±10.91	97.34±21.75	101.25±17.15	0.596
Length of the intestine	174.13±56.56	203.2±15.74	214.03±17.21	208.2±7.6	202.50±0.19	223.12±15.71	0.061
Density of the intestine	0.56±0.20	0.98±1.60	0.42±0.09	0.45±0.05	0.49±0.14	0.45±0.06	0.536

T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*, P, probability.

Table 5 represents the average weights of the digestive organs based on the rate of incorporation of *A. annua* in the ration. It was noticed that, there was no significant ($p>0.05$) difference among all treatments as far as all the digestive organs are concerned. Nevertheless, animals of the treatment T1 had the highest weight for organs such as pancreas (1.76±0.19),

weight and length of the intestine (101.25±17.15 and 223.12±15.71) and T0.25 the highest weight of the gizzard (1.76±0.23). The smallest weight of the pancreas and density of the intestines was obtained from the treatment T0.5 (1.58±0.15), with the smallest weight of the intestine under the treatment T0+ (174.13±56.56).

3.3. Effects of Aqueous Extract of *Artemisia annua* on Organs of the Immune System of Broilers

Table 6. Effects of Aqueous Extract of *A. annua* Diets on Organs of the Immune System of Broilers.

Organs	Control treatments		Treatments				P
	T0-	T0+	T0.25	T0.5	T0.75	T1	
Spleen's weight (g)	0.13±0.02	0.15±0.03	0.13±0.02	0.13±0.02	0.14±0.02	0.14±0.00	0.31
Volume spleen (g)	3.14±0.34 ^b	4.12±0.34 ^a	3.62±0.74 ^{ab}	3.50±0.53A ^{ab}	3.75±0.88 ^{ab}	4.00±0.75 ^a	0.04
Bursa's weight	0.047±0.009 ^b	0.046±0.007 ^b	0.054±0.003 ^{ab}	0.047±0.007 ^b	0.059±0.010 ^a	0.052±0.012 ^{ab}	0.03
Volume bursa	1.65±0.51	1.75±0.46	1.62±0.51	1.18±0.37	1.50±0.53A	1.62±0.51	0.29

^{a, b}, the means with the same letters on the same line are not significantly different ($P > 0.05$); T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*, P, probability.

The average weights and volumes of the organs of the immune system (Table 6) show no significant ($p>0.05$) difference among the controls and birds given the extract for the weight of the spleen and the volume of the bursa. Nevertheless, the volume of the spleen in the positive control treatment (T0+) and the treatment receiving 1 g aqueous extract of *A. annua* in feed significantly ($p<0.05$) higher than that of the negative control group (T0-).

3.4. Effects of Aqueous Extract of *Artemisia annua* on Hematological Characteristics of Broilers

3.4.1. Leucocytes Index of Broiler

Table 7 shows the effects of aqueous extract of *Artemisia annua* on leucocytes index of broilers. It appears that leucocytes had no significant ($p>0.05$) difference in all the groups no matter the characteristic considered, except in granulocytes. The concentration of granulocytes was significantly lower in T0.5 than the other groups but comparable to that of T0.25.

Table 7. Effects of Aqueous Extract of *Artemisia annua* on Feeding Regimes on Leucocytes Index of Broiler.

Characteristics	Controls		Treatments				P
	T0-	T0+	T0.25	T0.5	T0.75	T1	
WBC	147.27±14.94	151.67±13.62	147.57±10.55	145.27±12.42	151.62±4.38	146.47±6.77	0.9
RBC	2.33±0.15	2.51±0.25	2.48±0.17	2.35±0.26	2.43±0.17	2.40±0.11	0.7
HGB	11.17±1.06	11.52±1.24	11.82±1.04	11.30±1.53	11.65±0.78	11.32±0.47	0.9
HCT	32.60±2.50	33.80±3.59	34.82±2.56	33.05±3.77	33.85±2.18	33.15±0.64	0.8
GRAN	1.05±0.19 ^{ab}	1.22±0.20 ^a	0.80±0.14 ^{bc}	0.65±0.10 ^c	1.00±0.23 ^{ab}	1.30±0.45 ^a	0.01
MID (%)	11.20±1.16	11.88±1.27	10.62±1.13 ^B	9.90±1.92	11.35±0.91	11.10±0.55	0.3
LYM	87.75±2.73	86.85±3.76	88.57±1.26	89.42±3.03	87.40±1.51	87.50±0.35	0.7
MCV	140.10±3.54	134.80±3.57	140.47±5.29	142.90±7.55	139.37±1.18	137.62±4.47	0.2
MCH	47.87±1.51	45.85±0.75	47.57±2.34	49.35±3.92	47.85±1.12	47.35±2.14	0.4
MCHC	34.20±0.92	34.10±1.38	33.87±0.50	34.55±0.69	34.35±0.78	34.45±0.99	0.9
RDW-SD	81.90±4.84	77.42±3.33	84.32±8.63	79.42±6.83	79.12±3.63	79.30±3.23	0.5
RDW-CV	11.62±1.25	11.57±0.65	12.25±1.96	10.93±0.41	11.10±0.60	11.85±1.58	0.6

^{a, b, c}, the means with the same letters on the same line are not significantly different ($P > 0.05$); T0-, control diet without additives; T0+, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*; P, probability; WBC, White Blood Cells; RBC, Red Blood Cell; HGB, Hemoglobin; HCT, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean Hemoglobin Concentration; GRAN, Granulocytes, LYM, Lymphocytes, MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; RDW, Red-cell Distribution Width.

3.4.2. Platelet Index

The effects of aqueous extract of *Artemisia annua* on platelet index of broiler is represented by Table 8.

It was observed for PDW that, the treatment receiving 1%

(T1) was comparable to the controls (T0- and T0+). However, PDW count was significantly lower in the treatments receiving 0.25, 0.5, and 0.75% than in T0- but comparable to the positive control treatment (T0+). T0- and T0.25 recorded significantly ($p<0.05$) lower platelet counts than the other birds and the greatest count was obtained in T1.

Table 8. Effects of Aqueous Extract of *Artemisia annua* on Feeding Regimes on Platelet Index of Broiler.

Characteristics	Control		Treatments				P
	T0 ⁻	T0 ⁺	T0.25	T0.5	T0.75	T1	
PLT	26.33 ±4.49 ^d	45.66 ±3.09 ^b	28.66 ±1.69 ^d	37.33 ±3.09 ^c	46.75 ±8.05 ^{ab}	54.00 ±6.53 ^a	0.00
MPV	6.15 ±1.16	5.62 ±0.26	5.80 ±0.82	5.52 ±0.46	5.65 ±0.26	5.97 ±0.84	0.82
PDW	7.92 ±0.95 ^a	7.12 ±0.81 ^{ab}	6.06 ±0.18 ^b	6.25 ±1.17 ^b	6.02 ±0.35 ^b	7.70 ±0.66 ^a	0.00
PCT	0.03 ±0.01	0.03 ±0.01	0.02 ±0.00	0.03 ±0.01	0.02 ±0.00	0.04 ±0.01	0.28
P-LCR	9.95 ±3.25	8.40 ±2.54	9.53 ±2.36	8.37 ±2.38	8.70 ±1.66	10.10 ±2.50	0.84

^{a, b, c, d}, the means with the same letters on the same line are not significantly different ($P > 0.05$); T0⁻, control diet without additives; T0⁺, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*; P, probability, PLT, Platelet; MPV, Mean Platelet Volume; PDW, Platelet Distribution Width; PCT, Plateletcrit.

3.5. Effects of Aqueous Extract of *Artemisia annua* on the Intestinal Microflora of Broiler

Table 9 is a brief summary of the effects of aqueous extract

of *Artemisia annua* on the intestinal microflora of broilers. T0.75 recorded significantly lower values for *Escherichia coli* in comparison to the other groups. Nevertheless, the other extract-treated birds registered values for *E. coli* which are similar to those of the controls. On the other hand, lactobacillus recorded values significantly lower in T0⁺ than the others, with T0.5, T0.75 and T1 having similar values with T0⁻.

Table 9. Effects of Aqueous of *Artemisia annua* Extract on Feeding Regimes on the Intestinal Microflora of Broiler.

Parameters	Control		Treatments				P
	T0 ⁻	T0 ⁺	T0.25	T0.5	T0.75	T1	
<i>E. coli</i>	4.96 ±0.49 ^b	5.55 ±0.34 ^{ab}	6.03 ±0.42 ^a	5.20 ±0.52 ^b	4.84 ±0.35 ^c	5.82 ±0.26 ^{ab}	0.00
Salmonella	3.38 ±0.27	3.31 ±0.34	3.43 ±0.13	3.81 ±0.39	2.76 ±0.31	3.99 ±0.23	0.05
Lactobacillus	3.36 ±0.35 ^b	2.85 ±0.32 ^c	3.90 ±0.27 ^a	3.70 ±0.24 ^{ab}	3.44 ±0.38 ^{ab}	3.48 ±0.16 ^{ab}	

a, b, c, the means with the same letters on the same line are not significantly different ($P > 0.05$); T0⁻, control diet without additives; T0⁺, control diet + 1 g doxycycline/kg of feed; T0.25, control diet + 0.25 g aqueous extract of *Artemisia annua*; T0.5, control diet + 0.50 g aqueous extract of *Artemisia annua*; T0.75, control diet + 0.75 g aqueous extract of *Artemisia annua*; T1, control diet + 1 g aqueous extract of *Artemisia annua*; P, probability.

4. Discussion

The present study revealed that, the inclusion of the aqueous extract of *A. annua* at different levels in the diet of broiler had no significant effect on cumulative feed intake, irrespective of the period. This could be explained by the absence of alkaloids and the poor quantity of tannins (1.23 mg/g) in the aqueous extract of *A. annua* that did not impair palatability, allowing adequate intake. This result corroborates that of [16],

who reported that incorporating 2% and 4% *D. glomerata* in feed respectively had no significant effect on feed consumption during the starter phase. This result equally agrees with that of [17] who reported that the administration of *Tetrapleura tetraoptera* powder through cold water, hot water, and food does not induce any significant effect on the feed consumption of broiler chickens. On the other hand, [18] reported a significant increase in feed intake of broiler chickens that received garlic and ginger powder and infusion in their diet. Birds consume less of the feed that has a bitter taste, followed

by salty, sour, and sweet, according to [19]. This also contradicts that of [20] who noted that feed intake in broiler significantly decreases with the incorporation of neem leaf powder (4%) into the feed which could be explained by the presence of solanine (1.3 mg/g), a triterpenoid found in neem that can inhibit feed consumption in animals by reducing their appetite [21].

Throughout the trial, the animals receiving aqueous extract of *A. annua* at 0.75% and 1% showed higher live weight and weight gain than those of the control treatments. This could be explained by the presence of the active principles present in the *A. annua* powder, thus allowing for effective action. Also, the diets containing the aqueous extract of *A. annua* registered a significant increase in the live weight and weight gain of broiler at the finishing phase. In disagreement with this, it was demonstrated by [22] that putting 2g/kg of the powder of turmeric rhizome in feed of broiler resulted in non-significant difference in live weight and weight gain. The increase in weight gain and live weight observed in the present study could be attributed to the anti-inflammatory and antioxidant activities of the phenolic compounds found in these plants. According to [23], substances with antioxidant properties could reduce reactive oxygen species that attack the membranes of animal cells, thereby increasing the thickness of the cell membrane and the weight of the animal.

Animals fed with rations supplemented with 0.25 of extract of *A. annua* in feed recorded lower feed conversion ratio (FCR) compared to the control treatment, which had higher FCR than the rest of the treatments. These results are similar to those of [24], who reported a decrease in the FCR of broiler chickens receiving varying levels of garlic in their feed. These results may be due to the presence of tannins at the end of the extraction process and after analysis had a concentration of 1.23 mg/g, by their ability to complex proteins, sugars, and minerals, may have reduced the availability of certain substrates essential for bacterial growth and consequently improved feed efficiency. This would involve improving weight performance and consequently that of the FCR.

There was no significant ($p>0.05$) difference observed generally among the controls and those that received the extract in carcass yields 2, despite the fact that some groups had higher values than the others. Similar observations were made for relative weights of lungs, abdominal fats and heart. These results are in agreement with those of [4], who recorded no significant effect from the incorporation of increasing rates of neem oil on carcass characteristics and the relative weight of organs compared to antibiotics and the diet without additives. However, effects of incorporating *A. annua* at increasing rates in feed on the digestive organs of broiler showed no significant differences between treatments for the various studied characteristics.

The production of *E. coli* and lactobacillus decreases with the increase of the aqueous extract in feed, that's from the treatment receiving 0.25g to 0.75g of the extract. This de-

crease in lactobacillus and the decrease in pathogenic microbes (*E. coli*) in the digestive tract of chickens could explain their poor health conditions encountered by the end of the trial, which was due to seasonal change and heavy rain falls. Furthermore, the addition of *A. annua*, regardless of the rate in the feed, did not significantly affect the number of pathogenic microbes (salmonella) with the animals consuming feed with 1g/kg of *A. annua* having the highest production of salmonella (3.99 ± 0.23). Similar results were reported by [25] with the addition of 1.5% turmeric rhizome powder in the feed of broiler. It disagrees with that of [26], who reported that the number of lactobacilli significantly increased in the digestive tract with the inclusion of methylamine both in the feed and in acidified or non-acidified water compared to the controls.

The incorporation of increasing rates of aqueous extract of *A. annua* had no significant effect on the hematological parameters of broiler. However, the increasing rates of the extract tend to increase the levels of hematocrits, blood platelet, lymphocytes, red-cells distribution width and hemoglobin compared to the control treatments. Also, the increasing rates of the extract tend to decrease the levels of platelet distribution width, mean platelet distribution volume, and granulocytes compared to the control treatments. These results are similar with those of [18] who recorded an increase in the number of hemoglobin in broiler chickens receiving aqueous extracts of ginger. They are equally consistent with those of [27] who recorded no significant difference in hemoglobin (HGB) levels in chickens subjected to different garlic incorporation rates. However, this is in contradiction with that of [28], who registered in broiler, that the hemoglobin level (7.75 mg/dl) and hematocrit level (30.16%) increase when aqueous garlic extracts (20 ml/L) are given in drinking water.

5. Conclusions

In conclusion, aqueous extract of *A. annua* has a very beneficial effect on growth performance at a rate of 1g/kg of feed for most considered characteristics like the highest weight and the least value of feed conversion ratio. The extract of *A. annua* in feed modulated the intestinal microflora in favor of a pathogenic bacteria (*E. coli*) and to the detriment of beneficial bacteria (Lactobacillus) and pathogenic bacteria (salmonella). Aqueous extract of *A. annua* had no negative effect on the hematological parameters. Thus, aqueous extract of *A. annua* had greater beneficial effects in most characteristics at a rate of 1 g/kg feed, and may be used as natural source of feed additive in broilers instead of antibiotics since values were not generally significantly different from those of birds given antibiotic showing no harmful consequences.

6. Recommendations

Looking at the findings of this work, it will be interesting to carry out further studies on the effects of aqueous extract of *A.*

annua in drinking water since the effects of products vary with doses, form as well as mode of administration. It will equally be interesting to evaluate their effects on the quality of meat.

Abbreviations

FCR	Feed Conversion Ratio
FI	Feed Intake
LW	Live Weight
WG	Weight Gain
Ca	Calcium
P	Phosphorus
DM	Dry Matter
E	Energy
T0+	Control Diet + 1 G Doxycycline/Kg of Feed
T0.25	Control Diet + 0.25 g Aqueous Extract of <i>Artemisia annua</i>
T0.5	Control Diet + 0.50 g Aqueous Extract of <i>Artemisia annua</i>
T0.75	Control Diet + 0.75 g Aqueous Extract of <i>Artemisia annua</i>
T1	Control Diet + 1 g Aqueous Extract of <i>Artemisia annua</i>
P	Probability
WBC	White Blood Cells
RBC	Red Blood Cell
HGB	Hemoglobin
HTC	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Hemoglobin Concentration
GRAN	Granulocytes
LYM	Lymphocytes
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
RDW	Red-cell Distribution Width

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Author Contributions

Chongsi Margaret Mary Momo: Conceptualization, Funding acquisition, Validation, Writing – original draft, Writing – review & editing

Noumbissi Marie Noel Bertine: Formal Analysis, Investigation, Methodology, Writing – review & editing

Bend Emma Fortune Magloire: Formal Analysis, Methodology, Writing – review & editing

Mweugang Nguoupo Nathalie: Methodology, Validation, Writing – review & editing

Jatsa Cyntia Dorimene: Conceptualization, Funding acquisition, Methodology, Writing – original draft

Sambou Ngonseu Charlin Noël: Conceptualization, Data curation, Funding acquisition, Methodology

Miéguoué Emile: Supervision, Validation, Writing – original draft

Tendonkeng Fernand: Conceptualization, Supervision, Writing – original draft, Methodology

Conflicts of Interest

The authors declare no conflicts of interest.

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