
Research article

***Artemisia annua* as a dietary additive: Effects on productivity and selected blood parameters in broiler chickens**

Voemesse Kokou^{1,2}, Kin'tche Robert¹, Paulinus Essen^{1,*} and Attivi Komi¹ and Kulo Essosimna³

¹ Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, PMB 1515, Lomé, Togo

² Togolese Institute of Agricultural Research (ITRA-Togo), Agoè Cacavéli, PMB 1163, Lomé, Togo

³ Agricultural High School, University of Lomé, PMB 1515, Lomé, Togo

***Correspondence:** Email: essenpaulinus93@gmail.com; Tel: +234(0)8131907537; Fax: +22898906435.

Abstract: Background: One of the major challenges facing the poultry industry today is the prevalence of diseases like coccidiosis, which have resulted in substantial economic losses. **Objective:** We aimed to evaluate the effects of dietary supplementation with varying levels of *Artemisia annua* on growth performance, blood parameters, and anticoccidial activity in broiler chickens under farm conditions. **Methods:** A total of 600 unisex Sasso day-old chicks were randomly allocated to five dietary treatment groups (T_0 , T_{am}^+ , T_1 , $T_{1.75}$, $T_{2.5}$), each comprising five replicates of 24 chicks. Beginning on day 21 of rearing, daily coprological analysis was conducted to monitor the presence of coccidial oocysts. By day 28, oocyst presence was confirmed. Following this, treatments T_1 , $T_{1.75}$, and $T_{2.5}$ were administered basal diets supplemented with 1.0%, 1.75%, and 2.5% *Artemisia annua* leaf powder (AaLP), respectively. Treatment T_0 served as the negative control (unmedicated), while T_{am}^+ was designated the positive control, receiving amprolium as a standard anticoccidial agent. **Results:** Overall, no significant differences were observed in average feed intake across the treatment groups throughout the trial. However, dietary supplementation with AaLP significantly increased body weight (BW) and body weight gain (BWG) ($P < 0.001$) in the treated groups. The feed conversion ratio (FCR) also showed a significant improvement ($P < 0.05$) at the 12th week and overall trial period. AaLP supplementation significantly reduced oocyst counts and mortality rate ($P < 0.05$) among the treated birds. Hematocrit values were significantly elevated ($P < 0.05$) at 8th and 12th weeks in the AaLP-treated groups, while no significant differences ($P > 0.05$) were noted in serum levels of Aspartate

Aminotransferase (ASAT) and Alanine Aminotransferase (ALAT). **Conclusion:** AaLP positively influenced gut health, as evidenced by reduced oocyst shedding and lower mortality rates. Overall, *A. annua* supplementation enhanced zootechnical performance without inducing adverse effects on blood parameters.

Keywords: Sasso broiler chickens; coccidiosis; *Artemisia annua* leaf meal; oocyst shedding; blood indices; growth performance

1. Introduction

The global population continues to rise, intensifying the demand for animal protein sources such as poultry meat and eggs [1,2]. To meet this growing need, researchers and poultry producers are actively exploring strategies to enhance productivity. However, intensive poultry production systems characterized by large-scale rearing and high stocking densities have led to increased stress and disease prevalence among birds [3].

Among the most prevalent diseases in such systems is avian coccidiosis, a parasitic infection caused by intracellular protozoa of the genus *Eimeria*. This disease poses significant economic challenges. *Eimeria* infections compromise intestinal integrity by reducing villus height and increasing crypt depth, thereby impairing nutrient absorption and digestion. The physiological effects include increased morbidity, mortality, poor feed efficiency, reduced weight gain, and decreased egg production [4–6].

To combat coccidiosis, poultry farmers employ various strategies such as vaccination, improved management practices, and synthetic anticoccidial drugs [7,8]. While these methods have shown efficacy, challenges persist, including the emergence of drug-resistant *Eimeria* strains, accumulation of harmful residues in poultry products, and increasing consumer demand for organic alternatives. These concerns have spurred interest in natural remedies, particularly the use of medicinal plants and herbs as dietary additives [2,9].

In this context, phytobiotics have emerged as promising alternatives to conventional treatments. Studies have demonstrated that herbs, spices, and plant extracts traditionally used in human medicine can offer health benefits in poultry [10]. For example, the researchers in [11] reported that extracts from *Azadirachta indica* leaves, *Combretum micranthum* seeds, *Carica papaya* seeds, *Sarcocephalus latifolius* roots, and *Vernonia amygdalina* leaves were effective in vitro against *Eimeria* oocyst sporulation and sporozoite viability. Similarly, *Cinnamomum verum* supplementation has been shown to moderately reduce coccidiosis and improve production efficiency in broilers [12].

Among these medicinal plants, *Artemisia annua* stands out for its antiparasitic properties. Widely used in human medicine to treat malaria, *A. annua* and its active compound artemisinin have shown potential therapeutic effects against chicken coccidiosis. Moreover, studies have demonstrated that dietary inclusion of *A. annua* significantly reduces oocyst shedding and intestinal lesions while enhancing productive performance in broilers [13,14].

Despite its therapeutic potential, few researchers have explored the effects of *A. annua* on blood

parameters in poultry. Therefore, we aimed to evaluate the anticoccidial efficacy of different levels of *A. annua* supplementation on growth performance, oocyst counts, and blood parameters in broiler chickens. We hypothesized that dietary inclusion of *A. annua* would enhance broiler performance and reduce oocyst counts, positioning it as a natural alternative to synthetic coccidiostats. The findings confirm that we achieved our aim, showing that *A. annua* supplementation improved performance, blood profiles, and reduced oocyst shedding in broilers. The experiment lasted 84 days.

2. Materials and methods

2.1. Experimental location

The research was conducted at the Experimental Unit of the Teaching and Research Farm, Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, in the Maritime Region, Lomé, Togo. The site is at a latitude of 6.13° North and a longitude of 1.22° East, with an altitude of approximately 64 meters above sea level. It lies within the tropical savanna (Aw) agro-ecological zone of southeastern Togo. The region is characterized by an average annual rainfall of about 900 to 1,400 mm, distributed over 70 to 100 rainy days per year. Relative humidity during the rainy season typically ranges from 70% to 84%, while environmental temperatures vary between 23.4°C and 32.3°C throughout the year [15].

2.1.1. Sourcing and processing of test ingredient (*Artemisia annua*)

Fresh leaves of *A. annua*, including their petioles, were harvested from the farm field of the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, in the Maritime Region of Lomé, Togo. The plant material was identified using the *Flora of West Tropical Africa* (Hutchinson and Dalziel, 1954) and authenticated by a plant taxonomist from the Department of Agronomy, University of Lomé. A voucher specimen number (AD/AHS-UL-Aa1975-088) was deposited in the University's herbarium for future reference.

The harvested leaves were air-dried under controlled conditions at 16°C for 7 days to ensure gradual and uniform moisture reduction. During the drying period, the leaves were stirred daily to promote consistent dehydration. Once fully dried, the leaves were ground using a hammer mill and stored in sealed bags for use in experimental feed formulation. The processing protocol for the test ingredient was adapted from the method described in [16].

2.1.2. Experimental design and management

A total of 600 unisex day-old broiler chicks of the Sasso strain, with an average initial body weight of 35–45 g, were used for this study. The birds were reared on a deep litter system throughout the trial period, maintained at a stocking density of 25 chicks per square meter to facilitate natural exposure to infection. For the first 3 weeks, all chicks were brooded together on a farm known to have a history of coccidiosis, enabling natural infection through environmental exposure.

Beginning on day 21, coprological analysis was conducted daily to monitor the presence of

Eimeria oocysts. This infection strategy was designed to mimic typical transmission pathways observed under field conditions. Upon confirmation of oocyst presence at day 28 (week 4), the infected birds were weighed and randomly assigned 5 dietary treatments in a randomized design (CRD). Each treatment group consisted of 5 replicates, with 24 chicks per replicate. The treatment groups were as follows:

- **T₀ (Negative Control):** Basal diet without medication.
- **T_{am}⁺ (Positive Control):** Basal diet supplemented with amprolium, administered according to the manufacturer's posology.
- **T₁:** Basal diet containing 1.0% *Artemisia annua* leaf powder (AaLP).
- **T_{1.75}:** Basal diet containing 1.75% AaLP.
- **T_{2.5}:** Basal diet containing 2.5% AaLP.

Feed and clean drinking water were provided ad libitum. All necessary vaccinations and routine medications were administered as required. Standard management practices were strictly followed throughout the trial. The AaLP-supplemented diets were administered for 7 consecutive days beginning on day 28, and then every 14 days until day 84. During this period (days 28 to 84), *A. annua* was applied 3 times: during the 4th–5th week, 7th–8th week, and 10th–11th week of the study. The chemical composition of the experimental diets is presented in Table 1.

Table 1. Chemical composition of experimental straight diets for Sasso broiler chickens containing varying levels of *Artemisia annua* leaf powder (AaLP).

Ingredients	Inclusion (%)
Maize	51.00
Soybean meal	27.00
Palm Kernal Cake	7.00
Wheat brand	5.00
Fish meal	4.00
Brewer's dried grains	3.00
Blood meal	2.50
Common salt (Nacl)	0.25
Broiler premix	0.25
Total	100.00
Calculated values	
Crude Protein (%)	22.49
Metabolizable Energy (Kcal/Kg)	2898
Crude fibre (%)	4.92
Lysine total (%)	1.40
Methionine total (%)	0.40
Calcium (%)	0.34
Phosphorus (%)	0.47

Note: Vitamin and trace mineral declaration 0.25kg of premix contains: Vitamin A: 2400000 IU, vitamin D: 1000000 IU, vitamin E: 16000 IU, vitamin K: 800mg, vitamin B6: 1000 mg, vitamin B12: 6mg, Niacin: 8000mg, folic acid: 400mg, pantothenic acid: 3000mg, Biotin: 40mg, antioxidant: 3000mg, Cobalt: 80mg, copper: 2000mg, Iodine: 400mg, iron: 1200mg, Manganese: 1800mg, Selenium: 60mg, and Zinc: 14000mg

2.1.3. Data collection

Production parameter analysis: Feed intake (FI) was recorded daily by subtracting the residual feed weight from the offered feed weight. Additionally, mortality was recorded daily and was used to calculate weekly mortality rate % (MR %). Broiler chickens were weighed at the start of the trial (4th week), and then weekly until the end, and the BWG was assessed individually. The FCR for each treatment was calculated using the data obtained from FI and BWG using the formula $FCR = FI (g)/BWG (g)$. Oocyst analysis: Oocyst quantification was performed using the McMaster technique; approximately 2 g of fecal samples were collected from each experimental group and subjected to flotation analysis using saturated sodium chloride solution (specific gravity: 1.18–1.20) to detect *Eimeria* spp. oocysts. Samples that tested positive were further analyzed using the McMaster counting method to determine the oocyst load, expressed as oocysts per gram of feces (OPG). Blood sample collection and analysis (Hematological and serum biochemical evaluation): At 4 weeks of age and subsequently at 4-week intervals, approximately 1.5 mL of whole blood was collected in the morning from the wing or jugular vein of well-restrained birds (15 birds per treatment; 3 birds per replicate). Samples were drawn using sterile needles and transferred into blood collection tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant. Hematocrit (packed cell volume (PCV)) was determined using microhematocrit capillary tubes filled with blood and centrifuged at 10,000–12,000 rpm for 5 minutes using a microhematocrit centrifuge. The PCV was read using a standard hematocrit reader. On day 84 (week 12), an additional blood sample was collected from the same birds into sterile tubes without an anticoagulant. The samples were allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 10 minutes using a tabletop centrifuge to separate the serum. The serum was stored at -20°C until analysis. Serum concentrations of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined using an enzymatic colorimetric method following the manufacturer's protocol (BIOLABO). This method was based on the formation of a colored product, where the intensity of coloration was directly proportional to the concentration of the enzyme. To validate the assay, a standard was included, and optical density was measured at 340 nm using a BA-88A Mindray Auto-biochemical analyzer.

2.1.4. Ethics approval of research

All experimental protocols were reviewed and approved by the Ethics Committee of the Regional Centre of Excellence in Poultry Science (CERSA) at the University of Lomé, Lomé Togo, a branch of the National Ethics Committee for the control and supervision of experiments on animals.

2.2. Statistical analysis

All data generated during the study were subjected to a one-way analysis of variance (ANOVA) using GraphPad Prism version 5.0. Where significant differences were detected, Tukey's Multiple Range Test was employed to separate treatment means within the same statistical package. Results were considered statistically significant at a confidence level of $P < 0.05$. Prior to analysis, data were checked for normality and homogeneity of variances to ensure compliance with ANOVA assumptions. All values were expressed as mean $\pm$ standard error of the mean (SEM). The one-way statistical model is as follows: $Y_{ij} = \mu + T_i + e_{ij}$

where

Y_{ij} = Single observation

μ = Over all mean

T_i = Treatment effect

e_{ij} = Random error.

3. Results

3.1. Effect of AaLP on feed intake

Table 2 shows the feed intake (FI) result of broiler chickens administered varying levels of AaLP as a dietary additive. During the first four weeks of treatment, no significant differences ($P > 0.05$) in FI were observed among the treatment groups. However, from the 9th week onward, FI values differed significantly ($P < 0.05$) across treatments.

Table 2. Effect of *Artemisia annua* leaf powder (AaLP) on feed intake (g) of sasso broiler chickens.

Age (weeks)	Treatments					Sign.
	T_0	T_{am}^+	T_1	$T_{1.75}$	$T_{2.5}$	
4	41.83	41.83	41.83	41.83	41.83	NS
5	68.52 $\pm$ 1.90	68.49 $\pm$ 1.53	69.49 $\pm$ 53	70.73 $\pm$ 1.00	69.36 $\pm$ 1.53	NS
7	73.39 $\pm$ 2.36	73.82 $\pm$ 3.37	72.82 $\pm$ 3.37	74.38 $\pm$ 3.15	73.33 $\pm$ 3.61	NS
6	80.57 $\pm$ 0.44	78.58 $\pm$ 3.03	79.22 $\pm$ 1.42	83.32 $\pm$ 2.51	84.21 $\pm$ 1.00	NS
8	96.92 $\pm$ 0.42	95.54 $\pm$ 1.19	96.21 $\pm$ 2.69	99.22 $\pm$ 1.91	96.98 $\pm$ 2.15	NS
9	102.45 $\pm$ 2.41 ^b	110.54 $\pm$ 1.38 ^a	108.35 $\pm$ 3.16 ^a	108.40 $\pm$ 2.05 ^a	106.89 $\pm$ 2.14 ^a	*
10	122.78 $\pm$ 0.00 ^a	119.53 $\pm$ 4.41 ^{ab}	123.33 $\pm$ 2.35 ^a	115.62 $\pm$ 1.47 ^b	116.11 $\pm$ 1.06 ^b	***
11	135.07 $\pm$ 2.01 ^a	127.48 $\pm$ 1.50 ^b	129.93 $\pm$ 3.42 ^b	126.14 $\pm$ 1.35 ^b	127.95 $\pm$ 1.66 ^b	***
12	130.09 $\pm$ 1.90 ^b	134.23 $\pm$ 1.39 ^{ab}	133.66 $\pm$ 3.96 ^{ab}	138.01 $\pm$ 2.23 ^a	137.71 $\pm$ 1.88 ^a	*
4-12	101.22 $\pm$ 1.20	101.02 $\pm$ 1.31	101.62 $\pm$ 1.96	101.97 $\pm$ 1.22	101.56 $\pm$ 1.38	NS

Note : Means within the row having different lower case letters (a, b, c) differ significantly from one another (* $P < 0.05$; *** $P < 0.001$). T_0 (0%) = Negative control (unmedicated treatment); T_{am}^+ = Positive control (treatment with amprolium); and T_1 , $T_{1.75}$ and $T_{2.5}$ contained 1.0%, 1.75%, and 2.5% of AaLP in basal diet, respectively.

At week 9, birds in the treated groups (T_{am}^+ – $T_{2.5}$) exhibited higher FI compared to the negative control group (T_0). In weeks 10 and 11, FI values for treatments T_{am} , T_1 , and $T_{2.5}$ were statistically similar but lower than those recorded for T_0 . By week 12, FI in the negative control group (T_0) had significantly declined relative to the other treatment groups.

During weeks 9 to 12 of the trial (days 56–84), statistically significant differences in feed intake were observed between groups, while overall averages remained comparable.

3.2. Effect of AaLP on body weight

Figure 1 illustrates the weekly body weight (BW) of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. Between weeks 4 and 6, BW increased steadily across all groups, with no significant differences ($P > 0.05$) observed among treatments.

However, from week 7 onward, a treatment-dependent linear increase in BW was evident, following the order: T_0 (Control) < T_1 (1.0% AaLP) < $T_{1.75}$ (1.75% AaLP) < T_{am}^+ (Amprolium) < $T_{2.5}$ (2.5% AaLP).

By week 12, birds in treatments T_{am}^+ and $T_{2.5}$ recorded the highest BW, which was significantly greater ($P < 0.05$) than those in other groups.

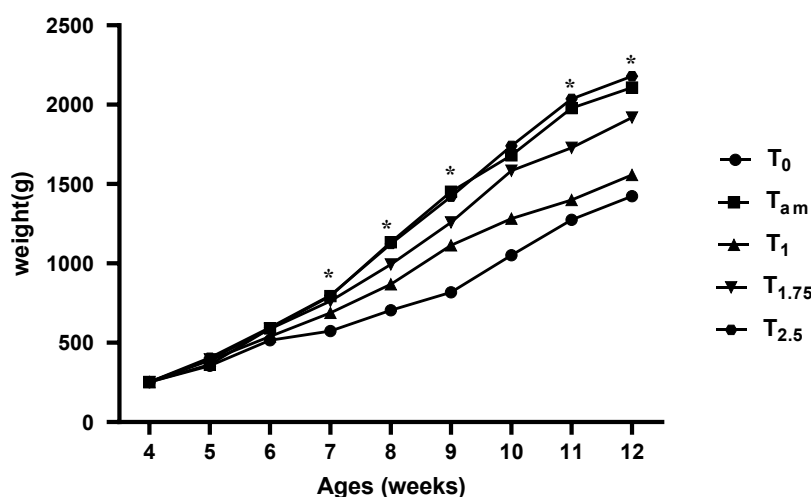


Figure 1. Body weight progression according to treatments and age (* $P < 0.05$).

3.3. Effect of AaLP on body weight gain

Table 3 present the body weight gain (BWG) results of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. During the initial two weeks of treatment (weeks 5–6), no significant differences ($P > 0.05$) in BWG were observed among the treatment groups.

However, from week 7 onward, BWG values differed significantly ($P < 0.05$) across treatments. At week 7, all treated groups recorded significantly higher BWG compared to the negative control group T_0 (0%). Specifically, the highest BWG values were observed in the following treatments at each week: Week 7: $T_{2.5}$ ($P < 0.05$); Week 8: T_{am}^+ ($P < 0.01$); Week 9: $T_{1.75}$ ($P < 0.001$); Week 10: $T_{2.5}$ ($P < 0.01$); Week 11: $T_{2.5}$ ($P < 0.001$); and Week 12: T_2 ($P < 0.001$). Overall, throughout the trial period,

average BWG values were comparable between T_{am}^+ and $T_{2.5}$, both of which were significantly higher ($P<0.001$) than the negative control group.

Table 3. Effect of *Artemisia annua* leaf powder (AaLP) on body weight gain (g) of sasso broiler chickens.

Age (weeks)	Treatments					Sign.
	T_0 (0%)	T_{am}^+	T_1	$T_{1.75}$	$T_{2.5}$	
5	16.29±0.56	15.89±1.43	16.56±0.87	17.17±1.46	17.08±0.69	NS
6	22.81±0.99	21.77±0.64	21.77±0.64	21.32±2.58	22.49±2.02	NS
7	24.85±1.20 ^b	27.75±0.53 ^a	27.08±0.81 ^a	27.59±2.78 ^a	31.18±8.56 ^a	*
8	32.61±2.43 ^b	40.36±2.49 ^a	40.03±2.74 ^a	37.11±0.99 ^a	37.98±1.83 ^a	**
9	30.14±1.03 ^c	35.40±2.05 ^b	40.54±1.38 ^a	41.66±2.84 ^a	38.38±1.86 ^{ab}	***
10	26.81±1.36 ^c	36.85±2.66 ^a	31.49±2.58 ^b	34.80±2.49 ^{ab}	37.64±2.36 ^a	**
11	28.77±1.37 ^c	34.05±1.52 ^b	35.89±2.88 ^b	35.25±2.41 ^b	42.42±1.35 ^a	***
12	25.26±1.10 ^c	37.59±0.84 ^a	23.49±2.75 ^c	26.88±0.78 ^c	32.31±2.86 ^b	***
5-12	25.94±1.12 ^c	31.20±0.64 ^a	29.60±1.57 ^b	30.22±0.18 ^b	32.43±1.36 ^a	***

Note: Means within the row having different lower case letters (a, b, c) differ significantly from one another (* $P<0.05$; ** $P<0.01$; *** $P<0.001$). T_0 (0%) = Negative control (unmedicated treatment); T_{am}^+ = Positive control (treatment with amprolium); and T_1 , $T_{1.75}$, and $T_{2.5}$ contained 1.0%, 1.75%, and 2.5% of AaLP in basal diet, respectively.

Table 4. Effect of *Artemisia annua* leaf powder (AaLP) on the feed conversion ratio (FCR) of sasso broiler chickens.

Age (weeks)	Treatments					Sign,
	T_0	T_{am}^+	T_1	$T_{1.75}$	$T_{2.5}$	
5	2.48±0.71	2.33±0.51	2.43±0.51	2.64±1.09	3.26±0.51	NS
6	2.6±0.36	3.29±0.51	3.29±0.51	3.65±0.48	3.15±0.49	NS
7	3.33±0.57	2.78±0.20	2.58±0.20	2.45±0.91	2.64±0.83	NS
8	2.99±0.33	2.19±0.27	2.33±0.27	2.11±0.52	2.28±0.69	NS
9	3.73±0.74	3.05±0.44	3.04±0.75	2.75±0.60	2.15±0.43	NS
10	3.48±0.63	3.14±0.25	3.02±0.22	3.10±0.39	3.06±0.47	NS
11	3.94±0.91	2.08±0.89	2.58±0.66	2.25±0.63	2.02±0.31	NS
12	3.51±0.60 ^a	2.08±0.23 ^c	2.28±0.37 ^b	2.24±0.56 ^b	2.11±0.34 ^c	*
5-12	3.29±0.60 ^a	2.21±0.23 ^c	2.39±0.37 ^b	2.45±0.56 ^b	2.28±0.34 ^c	*

Note: Means within the row having different lower case letters (a, b, c) differ significantly from one another (* $P<0.05$). T_0 (0%) = Negative control (unmedicated treatment); T_{am}^+ = Positive control (treatment with amprolium); and T_1 , $T_{1.75}$ and $T_{2.5}$ contained 1.0%, 1.75%, and 2.5% of

AaLP the in basal diet, respectively.

3.4. Effect of AaLP on feed conversion ratio

Table 4 presents the feed conversion ratio (FCR) of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. From weeks 5 to 11, FCR values were statistically comparable across all treatment groups ($P>0.05$), indicating similar feed efficiency during this period.

However, at week 12, a significant difference ($P<0.05$) was observed, with the negative control group (T_0) recording the highest FCR value, reflecting poorer feed efficiency. In contrast, birds in the amprolium-treated group (T_{am}^+) and those fed 2.5% AaLP ($T_{2.5}$) consistently showed the lowest FCR values, indicating superior feed utilization.

Over the trial period, treatments T_{am}^+ and $T_{2.5}$ (2.5% AaLP) maintained significantly lower FCR values ($P<0.05$) compared to other groups.

3.5. Effect of AaLP on mortality rate

Figure 2 illustrates the mortality rate (MR) of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. No significant differences ($P>0.05$) in MR were observed among the groups treated with AaLP or amprolium. However, a numerical trend was evident, with mortality rates following the order: $T_{am}^+ < T_{2.5} < T_{1.75} < T_1 < T_0$.

All treated groups, whether with amprolium or AaLP, exhibited significantly lower mortality rates ($P<0.05$) compared to the negative control group (T_0), indicating the effectiveness of both interventions in reducing bird mortality.

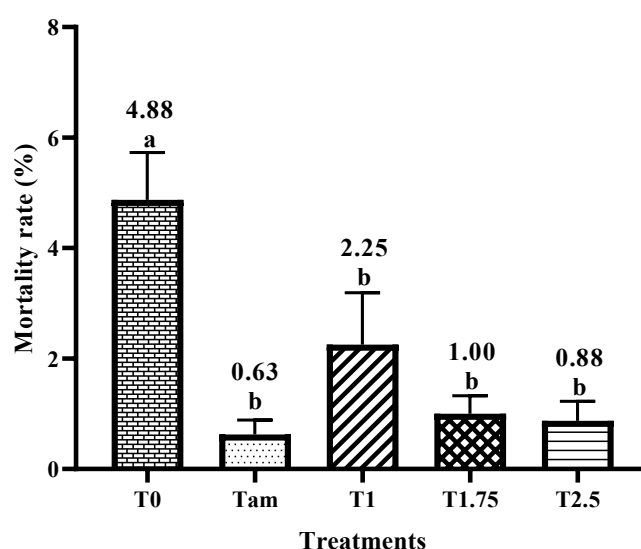


Figure 2. Mortality rate analysis according to the treatments and age. Different letters indicate statistically significant differences ($P<0.05$).

3.6. Effect of AaLP on oocysts counts

Figure 3 illustrates the oocyst count (OC) of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. Between weeks 5 and 7, oocyst counts decreased significantly ($P<0.001$) in all treated groups compared to the standard control (T_{am}^+). From week 7 onward, oocyst levels remained relatively stable within the treated groups and continued to be significantly lower ($P<0.001$) than those observed in the control group.

From weeks 10 to 12, the oocyst count followed a consistent numerical trend across treatments, ranked as follows: $T_{am}^+ < T_{2.5} < T_{1.75} < T_1 < T_0$.

These results suggest that amprolium and higher inclusion levels of AaLP were effective in reducing *Eimeria* spp. oocyst shedding in broiler chickens.

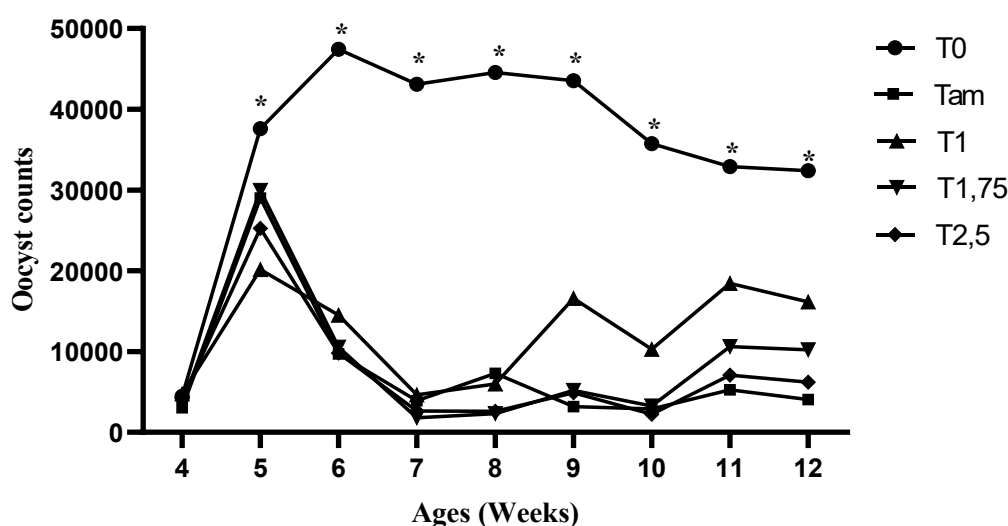


Figure 3. Oocyst count (g) Analysis according to age and treatments. Significant differences are indicated by * $P<0.05$ and *** $P<0.001$.

3.7. Effect of AaLP on hematocrit

Table 5 presents the hematocrit (PCV) values of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. At the beginning of the trial, no significant differences ($P>0.05$) were observed in PCV among the treatment groups.

However, by days 56 and 84 (corresponding to the 8th and 12th weeks), significant differences emerged ($P<0.05$), with birds in the negative control group (T_0) consistently exhibiting the lowest hematocrit values ($P<0.001$) compared to the treated groups. These findings suggest that dietary inclusion of AaLP positively influenced hematocrit levels in broiler chickens over time.

Table 5. Effect of *Artemisia annua* leaf powder (AaLP) on hematocrit (PCV) of sasso broiler chickens.

Age (week)	Treatments					Sign.
	T ₁ ⁻	T _{am} ⁺	T ₁	T _{1.75}	T _{2.5}	
4	39.25±8.27	39.33±4.52	35.50±3.42	37.50±5.71	38.08±3.65	NS
8	32.17±2.59 ^c	35.67±4.01 ^a	33.80±3.06 ^{bc}	36.17±0.83 ^a	35.17±1.27 ^{ab}	**
12	26.50±3.55 ^c	35.33±2.60 ^a	32.17±3.71 ^b	34.25±2.34 ^{ab}	36.75±2.83 ^a	***

Note: Means within the row having different lower case letters (a, b, c) differ significantly from one another (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). T₀ (0%) = Negative control (unmedicated treatment); T_{am}⁺ = Positive control (treatment with amprolium); and T₁, T_{1.75}, and T_{2.5} contained 1.0%, 1.75%, and 2.5% of AaLP in basal diet, respectively.

3.8. Effect of AaLP on cytotoxicity markers

Table 6 presents the serum cytotoxicity marker values of broiler chickens fed diets containing graded levels of AaLP as a dietary additive. At day 84 (corresponding to week 12), no statistically significant differences ($P > 0.05$) were observed among the 5 treatment groups in terms of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) concentrations, indicating that dietary inclusion of AaLP had no adverse effect on liver function.

Table 6. Effect of *Artemisia annua* Leaf Powder (AaLP) on Serum Cytotoxicity Markers in Sasso Broiler Chickens at Day 84 (Week 12).

Parameters	Treatments					Sign
	T ₁ ⁻	T _{am} ⁺	T ₁	T _{1.75}	T _{2.5}	
ASAT	26.40±0.62	26.62±1.11	26.37±1.71	27.18±0.86	27.21±0.91	NS
ALAT	17.82±2.32	17.83±3.31	17.74±2.25	17.91±0.48	18.38±2.79	NS

Note : Means within the row having different lower case letters (a, b, c) differ significantly from one another (* $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$). T₀ (0%) = Negative control (unmedicated treatment); T_{am}⁺ = Positive control (treatment with amprolium); and T₁, T_{1.75}, and T_{2.5} contained 1.0%, 1.75%, and 2.5% of AaLP in basal diet, respectively.

4. Discussion

Although significant differences in feed consumption were observed among treatment groups between weeks 9 and 12, the overall mean feed intake throughout the trial period did not differ significantly across treatments. This indicates that the inclusion of AaLP did not negatively affect diet palatability. The absence or relatively low levels of anti-nutritional phytochemicals such as tannins and saponins within the safety thresholds recommended for rabbits and poultry [2,17] may have contributed to this outcome.

These results align with the findings of the researchers in [18], who reported no significant differences in feed intake among broiler chickens fed varying concentrations of *A. annua*. Similarly, the researchers in [12] observed no significant changes in feed intake when broiler chickens infected with *Eimeria tenella* were administered diets containing the traditional medicinal plant *C. verum*.

The feed intake values among the treated groups ranged from 5349.79 to 5362.83 g, indicating that certain phytogenic additives may influence feed consumption depending on their composition and dosage.

Broiler chickens supplemented with AaLP exhibited a marked increase in body weight and body weight gain throughout the trial period. By week 12, birds in the group receiving 2.5% AaLP ($T_{2.5}$) showed similar BW and BWG values to those treated with the synthetic coccidiostat amprolium (T_{am}^+), indicating comparable growth-promoting effects. These findings align with those reported by the researchers in [19,20], who supplemented broiler diets with *A. annua* (0.5% and 1%) and neem leaf powder (2.0, 4.0, 6.0, and 8.0 g/kg) over 35-day and 28-day periods, respectively. Their studies showed significant improvements in body weight, weight gain, feed intake, and feed efficiency, reinforcing the growth-promoting potential of medicinal plant-based additives in poultry production.

Coccidiosis is known to impair nutrient absorption due to intestinal swelling, epithelial damage, and villi destruction, leading to hemorrhage and reduced feed efficiency [21]. In this study, birds in the untreated control group (T_0) exhibited the poorest performance, likely due to high oocyst counts and severe intestinal damage. The resulting epithelial disruption would have impaired nutrient uptake and triggered increased intestinal cell turnover, elevating nutrient demands for tissue repair. This mechanism may explain the higher feed conversion ratio (FCR) observed in T_0 despite similar feed intake across groups.

A lower FCR value indicates higher efficiency [22]. Throughout the trial, birds in T_{am}^+ and $T_{2.5}$ maintained significantly lower FCR values compared to the negative control group, reflecting improved feed efficiency. These results suggest that synthetic and phytogenic interventions enhanced nutrient utilization and growth performance in broiler chickens. This observation aligns with the reports of [23], who observed improved feed efficiency in layers supplemented with *A. annua*, and those in [24,25], who documented improved feed efficiency in broilers supplemented with plant-derived feed additives.

A consistent decline in oocyst counts was observed in broiler chickens treated with AaLP and amprolium, particularly between weeks 5 to 12 of the trial. This reduction was accompanied by lower mortality rates in treated groups, further confirming the anticoccidial efficacy of AaLP. In contrast, the untreated control group (T_0) showed the highest oocyst burden and mortality, underscoring the protective role of both treatments. Our findings of this study are consistent with those of [13], who reported reduced oocyst counts following supplementation with alcoholic extracts of *A. annua*. Similarly, [26] observed improved zootechnical performance and gut microbiota health in broilers fed *A. annua*. These findings reinforce the potential of AaLP as a natural alternative to synthetic anticoccidials.

This observation aligns also with other studies. The researchers in [27] demonstrated that supplementation with *C. verum* and *Rumex nervosus* significantly reduced caecal lesions, oocyst shedding, and mortality in *E. tenella*-infected broilers. Similarly, the researchers in [28] reported that a blend of medicinal plant extracts, including *Punica granatum*, *Bidens pilosa*, *Plantago asiatica*, *Artemisia argyi*, and *Camellia sinensis*, effectively inhibited *Eimeria* invasion, lowered fecal oocyst

counts, and improved survival rates. Moreover, the researchers in [29] found that *Yucca schidigera* supplementation led to reduced oocyst counts and mortality in broilers challenged with *Eimeria* spp., alongside improved growth performance.

The effectiveness of synthetic anticoccidials is attributed to their ability to disrupt parasite metabolism, block ion transport across membranes, and alter osmotic balance [30]. Similarly, the antimicrobial activity of *A. annua* is linked to its rich phytochemical profile, including flavonoids, tannins, saponins, and artemisinin [31,32]. These compounds inhibit oocyst sporulation [33,34], induce host cell apoptosis, and suppress inflammatory responses [35]. In this study, birds fed AaLP showed reduced fecal oocyst shedding, improved intestinal health, and enhanced nutrient uptake; mechanisms that likely contributed to their comparable BW and FCR to those treated with synthetic drugs.

Blood analysis is a well-established method for evaluating nutritional status, health, and disease conditions in animals. It is particularly useful in studies involving medicinal plant supplementation [36]. In this study, blood samples were analyzed to assess the impact of dietary treatments on the physiological profile of broiler chickens.

Hematological evaluation revealed a significant reduction in hematocrit levels in the untreated control group (T_0), likely due to blood loss into the gastrointestinal tract caused by severe coccidial infection. This finding is consistent with the observations of [37], who associated erythrocyte depletion with intestinal bleeding, and with the observations of [38], who described the destructive impact of *E. tenella*'s second-generation schizonts on cecal tissues, including excessive damage, hemorrhage, and disruption of the mucosa and muscularis layers.

Liver function was assessed by measuring plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), enzymes typically released into the bloodstream following hepatic tissue damage [39]. No significant differences in ALT and AST levels were observed among the treatment groups, indicating that AaLP supplementation did not compromise liver integrity. These results support the safety of AaLP as a dietary additive in broiler nutrition.

5. Conclusions

The results of this study demonstrate that dietary supplementation with AaLP, particularly at a 2.5% inclusion level, significantly enhances the growth performance, feed efficiency, and gut health status of broiler chickens. Birds in the AaLP-treated groups exhibited improved body weight (BW) and body weight gain (BWG), with values comparable to those observed in the amprolium-treated group, especially from week 7 onward.

Mortality rates and oocyst counts were significantly reduced in all treated groups compared to the control, confirming the anticoccidial efficacy of AaLP. The consistent decline in oocyst shedding and improved survival rates highlight the protective role of AaLP against *Eimeria* spp. infection.

Hematocrit values were positively influenced by AaLP supplementation, suggesting improved physiological resilience in treated birds. Furthermore, the absence of significant differences in serum ALT and AST levels across treatments indicated that AaLP has no adverse effects on liver function, affirming its safety as a dietary additive.

Overall, the findings support the use of AaLP as a natural and effective alternative to synthetic

coccidiostats in broiler production, offering zootechnical and health-related benefits without compromising organ integrity and contributing to sustainable and antibiotic-free poultry farming.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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