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Research Article

Effects of Graded Levels of *Cyperus Alternifolius* Rhizome Powder on Feed Digestibility, Growth Performance, Intestinal Microbial Flora, Serum Metabolites, and Immune Responses in Broiler Chickens

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ABSTRACT

Introduction: The use of antibiotics as growth promoters in animal husbandry has been banned due to concerns that antibiotic residues can accumulate in animal tissues and lead to bacterial resistance. The present study aimed to explore the effects of varying amounts of *Cyperus alternifolius* (*C. alternifolius*) rhizome powder on feed digestibility, growth performance, intestinal microbial flora, hemato-biochemical parameters, and immune responses in broiler chickens as an alternative to antibiotics.
Materials and methods: An experiment was conducted at the Application and Research Farm of the University of Dschang (Cameroon) over 49 days. A total of 512 day-old broiler chickens, including 256 males and 256 females, were randomly assigned to eight treatment groups, with four replicates of 16 chickens each. The treatment groups included a negative control group which administered a basal diet without additive (NC), a basal diet containing 1 g of doxycycline/kg of feed as the positive control group (PC), and six diets containing the powder of *C. alternifolius* rhizome as a phyto-additive, at the dose of 1 g of *C. alternifolius*/kg of feed (T1), 2 g (T2), 4 g (T4), 6 g (T6), 8 g (T8) and 10 g (T10). The growth performance, carcass characteristics, microbial flora, feed digestibility, the immune system, and hematological and biochemical parameters were evaluated.
Results: Live weight and weight gain increased by approximately 7.02% and 7.11%, respectively, in T4 compared with NC during the finisher phase. Feed conversion ratio was significantly reduced in T4 by approximately 11.05% compared to NC, but was comparable to that of PC, T6, and T8. The number of lactobacilli and crude protein digestibility increased significantly in all treatment groups compared to NC. Furthermore, in T4, the number of *Escherichia coli* significantly decreased by 40.9% compared to NC, T1, T8, and T10. Besides the notable increase in total protein level in T4 compared with other groups, the hemato-biochemical parameters of the chickens showed no significant differences across groups.
Conclusion: Cyperus *alternifolius* at a dose of 4 g/kg of feed demonstrated potential as an alternative to growth-promoting antibiotics, with diminished adverse effects on broiler chicken health.

1. Introduction

Antibiotics used as growth promoters have helped to preserve the balance of microbial flora and, as a result, optimize growth performance in poultry farming[1]. Despite the high efficacy of antibiotics used as growth promoters, antibiotics are prohibited in animal feed due to the accumulation of residues in livestock products and the development of antibiotic resistance in animals[2]. The prohibition on the use of antibiotics as additives has

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resulted in adverse economic consequences for the poultry industry, following the unexpected emergence of disease within flocks, leading to reduced growth and increased mortality rates[3]. Following the ban on antibiotics as feed additives, problems such as slowed growth and increased mortality on livestock farms led to the use of other growth promoters, such as probiotics, prebiotics, and phytobiotics, for their benefits for digestive health, immune support, and production performance. Among these phyto-additive growth activators, *Cyperus alternifolius* (*C. alternifolius*) and *Typha angustifolia* (*T. angustifolia*) were found to be effective[4].

Cyperus alternifolius has rhizomes rich in bioactive compounds, such as flavonoids, phenols, sterols, and triterpenoids. These elements have antimicrobial, antiparasitic, immunomodulatory, and digestive system-stimulating properties[3,4]. According to Bashige et al.[5], *C.* alternifolius rhizome powder has demonstrated *in vitro* antimicrobial activity against pathogens such as *Neisseria meningitidis*, *Staphylococcus aureus*, *Candida albicans*, and *Salmonella typhi*. Incorporating the optimal level of *C. alternifolius* rhizome powder into the diet may improve the growth performance of broiler chickens and strengthen their defenses against pathogenic bacteria, thereby boosting the economic profitability of the poultry sector as a whole. *Cyperus alternifolius* is a readily available plant used as an antimicrobial phyto-additive in broiler feed, reducing production costs compared to commercial antibiotics[4]. Adding the ideal amount of *C. alternifolius* rhizome powder to the diet may enhance the chicken's ability to grow and strengthen its defenses against harmful microorganisms[4]. The present study aimed to evaluate the effects of incorporating *C. alternifolius* rhizome powder into the feed on growth performance, intestinal microbial flora, feed component digestibility, immune system indices, and hemato-biochemical parameters in broiler chickens.

2. Materials and Methods

2.1. Ethical approval

The present study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were handled humanely under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Goma, Democratic Republic of the Congo.

2.2. Study area

The current investigation was conducted from May to July 2022 at the University of Dschang's Application and Research Farm in Cameroon. This farm is located at an average elevation of 1420 meters and at 5°26' North latitude, 10°26' East longitude. Average annual precipitation is 2000 mm, average temperature is about 21°C, average relative humidity is 76.8%, and average yearly insolation is 1873 hours.

2.3. Cyperus alternifolius collection and phytochemical screening

Cyperus alternifolius was collected at the vegetative stage in the vicinity of the Center for Research in Natural Sciences (CRSN) of Lwiro, 30 km from the city of Bukavu in the Democratic Republic of Congo. The rhizomes were harvested, separated from the other parts of the plant, and then dried in both shade and open air before being processed using a grinder (Nima, China). The powder obtained (4500 g) was then stored in hermetically sealed boxes for use as a phytochemical additive. The powder from the rhizomes of *C. alternifolius* was subjected to phytochemical analysis according to the established procedures described by Harborne[6]. This analysis revealed the presence of triterpenoids, sterols, flavonoids, and phenols in *C. alternifolius*.

2.4. Animals

A total of 512 day-old Cobb500 broiler chickens, weighing an average of 48 g, were randomly divided into eight treatments, each with four replicates of 16 chickens (eight males and eight females). Upon arrival at the brooder, the chickens received an anti-stress treatment consisting of 5 g of Introvit A+ WS (Interchemie werken De Adelaar BV, Holland) in 2 liters of water, as recommended by the manufacturer's guidelines, for the first three days. An anti-stress treatment was administered to the chickens via the drinking water before and after each weighing and vaccination. Then, the chickens were vaccinated against infectious bronchitis (H52, MSD Animal Health, Holland) and Newcastle disease (Hitchner B1®, Holland) on the seventh day, against Gumboro's disease (CEVAC® TRANSMUNE IBD, Holland) on the tenth day, and received a booster of all vaccines on day 18. The vaccines were administered through the drinking water.

2.5. Experimental design

The chemical composition of the experimental diets is presented in Table 1. The feed formulations were created to meet the chickens' nutritional needs, aligning with those outlined by NRC[7]. The ingredients were obtained from the local market, including corn, soybean meal, fish meal, bone meal, cottonseed meal, wheat bran, mineral nitrogen, and vitamin complex (CMAV) 5%.

The treatment groups included a negative control group, which was fed the basal diet without any additive (NC), and a positive control group containing 1 g of doxycycline® (Holand, Interchemie werken De Adelaar BV, Holland) per kg of feed (PC). Other treatment groups included 1g of *C. alternifolius* rhizomes powder per kg feed (T1), 2 g of *C. alternifolius* rhizomes powder per kg feed (T2), 4 g of *C. alternifolius* rhizomes powder per kg feed (T4), 6 g of *C. alternifolius* rhizomes powder per kg feed (T6), 8 g of *C. alternifolius* rhizomes powder per kg feed (T8), and 10 g of *C. alternifolius* rhizomes powder per kg feed (T10)[7].

Table 1. Composition of experimental diets in the starter and grower-finisher phases in Cobb 500 chickens

Ingredients (%)	Starter phase	Grower-finisher phase
Maize	60	67
Cottonseed cake	5	5
Soya bean meal 49%*	22	15
Fish meal	5	5
Wheat bran	2	2
Shell	1	1
CMAV 5%**	5	5
Total	100	100
Analyzed chemical composition		
Metabolizable energy (kcal/kg)	2960.13	3063.15
Crude protein (% DM)	22.63	19.55
Crude cellulose (% DM)	3.15	3.25
Calculated chemical composition		
Metabolizable energy (kcal/kg)	2977	3108
Crude protein (%)	23.09	20.3
Energy /protein	129.4	153.1
Calcium (%)	1.05	1.03
Phosphorus (%)	0.6	0.6
Calcium/Phosphorus	1.75	1.72
Lysine (%)	1.4	1.2
Methionine (%)	0.5	0.45
Lysine/Methionine	2.8	2.7
Cellulose (%)	2.43	2.61

**CMAV 5% referes to mineral nitrogen and vitamin complex: 40% Crude protein, 8% Calcium, 2.05% Phosphorus, 3.3% Lysine, 2.40% Methionine, 2078 kcal/k Metabolizable energy, 3,000,000 IU Vit A, 600,000 IU Vit D3, 4,000 mg Vit E, 500 mg Vit K, 200 mg Vit B1, 1000 mg VitB2, 4000 mg Vit B6, 4 mg Vit B12, 8000 mg Iron, 2000 mg Cu, 10,000 mg Zn, 20 mg Se, 14,000 mg Mn, DM: Dry matter. *Soya bean meal 49%: Contains 49% protein.

2.6. Growth performance

Weekly, feed intake and live weight were evaluated throughout the study period, and the feed conversion ratio was computed as the ratio of feed intake to weight gain[8]. At 49 days of age, 10 chickens (five males and five females) were randomly selected from each treatment, fasted for 24 hours, and then slaughtered for carcass evaluation. Carcass yield and relative organ weight were calculated. The length of the intestine was measured using a measuring tape, and its density was calculated by dividing the weight of the intestine by its length[5,8].

2.7. Feed digestibility

For three consecutive days, six chickens, three males and three females per treatment, were selected to assess the apparent digestive utilization coefficients (ADUC) of meal components. After being moved to digestibility cages, the chickens underwent a three-day acclimatization period. To facilitate the collection of feces from each, the tarps were positioned beneath the cages after the three days of adaptation. Before the meal was given to the chickens, it was weighed, and for three days, the refusals were gathered and weighed daily. Following the procedure outlined by AOAC[9], the fecal samples were dried in an oven (FB1300-FB1400/Geneq, Canada) at 60°C until they reached a constant weight to determine the amount of dry matter (DM) and organic matter (OM). To measure dietary fiber (DF), the method of Van Soest et al.[10] was used, and the Kjeldahl method[11] was applied to determine crude protein (CP). The ADCU for DM, OM, CP, and neutral dietary fiber (NDF) was computed for the experimental meals.

2.8. Microbial flora

At 49 days of age, samples of feces were collected from the ceca of four chickens per treatment (two males and two females) were used to identify and quantify lactic acid bacteria, *Escherichia coli* (*E. coli*), and *Salmonella* spp. on specific culture media. MacConkey agar for *E. coli* isolation and Salmonella-Shigella agar (Medexia BV, headquartered in Lokeren, Belgium) for *Salmonella* spp. Isolation was used. For lactic acid bacteria, Man-Rogosa-Sharpe agar (Acumedia®, India, ISO 9001), utilized according to the guidelines of de Man et al.[12].

The bacterial inoculum was prepared using decimal dilutions. A total of 9 mL of physiological water was dispensed into tubes numbered from S1 to S8 at the base, according to the dilution number and the sample type. The sample-carrying swab was then inserted into the first tube. After agitating the latter to make the solution (S1) homogeneous, 1 mL of S1 was extracted with a micropipette and added to the second tube to bring the solution's volume to 10 mL. This produced the dilution. Stirring the 10^{-2} solution (S1) ensured homogeneity. A micropipette was then used to transfer 1 mL of S1 into the second tube, increasing the volume to 10 mL. As a result, the dilution was 10^{-2}. Following the homogenization of this solution, the process was repeated up to the 10^{-8} dilution. Subsequently, 1 mL aliquots from the 10^{-6} and 10^{-8} dilutions of each sample were plated onto agar in Petri dishes for bacterial enumeration and analysis[13].

2.9. Immune system and hemato-biochemical profiles

The lymphoid organs (spleen and bursa of Fabricius) of the six chickens per treatment were removed during carcass evaluation, weighed, and their indices were calculated using the following formula[4].

$$\text{Organe index} = \frac{\text{Weight of organ (g)}}{\text{Live weight when fasting (g)}} \times 100$$

Red blood cells ($\times 10^{12}$/L), mean cell volumes (fL), hemoglobin (g/dL), white blood cells ($\times 10^{9}$/L), and blood platelets ($\times 10^{9}$/L) were measured using the URIT-3000Plus hematometer kit (YSENMED, China). Additionally, in tubes without anticoagulants, alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), urea (mg/dL), creatinine (mg/dL), triglycerides (mg/dL), total cholesterol (mg/dL), high-density lipoprotein (HDL, mg/dL), and light-density lipoprotein (LDL, mg/dL) using commercial kits (Chronolab®, Barcelona, Spain). Following the guidelines provided by the URIT-3000Plus kit (China), the quantification of immune cells of granulocytes (%), lymphocytes (%), and immune system proteins such as albumin and globulins (g/dL) were measured.

2.10. Statistical analysis

All data collected were subjected to one-way analysis of variance (ANOVA). For significant differences between the means of the treatments, Duncan's test was applied to separate the means at the 5% significance level and presented using the standard deviation (SD). The SPSS version 20.0 software was used for the present analyses.

3. Results

3.1. Growth performance

All treatments, regardless of the study period, had no significant effect on the chickens' feed intake ($p > 0.05$; Table 2). Furthermore, during the finisher phase (22-49 days), T4 induced a significant increase in live weight by 7.02% and a weight gain of approximately 7.12% compared to the NC group, which had an average live weight of 2841 g and a weight gain of 2,798.09 g ($p < 0.05$). Furthermore, live weight and weight gain were not significantly different between the T4 and PC groups ($p > 0.05$). The weight gain in T4 (3012.57 ± 44.13 g) was significantly higher than in T1 (2842.29 ± 35.97 g), the NC (2798.09 ± 119.24 g), and T10 (2809.91 ± 71.30 g; $p < 0.05$). Throughout the study period (1 to 49 days), the consumption index decreased significantly by 11.05% in T4 compared to the NC group (2.11 ± 0.09; $p < 0.05$). However, the consumption index in T4 (1.90 ± 0.04) was comparable to that of the PC group (1.85 ± 0.12; $p > 0.05$). Feed conversion ratio significantly decreased in T4 (1.90 ± 0.04), compared to T1 (2.05 ± 0.03) and T10 (2.08 ± 0.10; $p < 0.05$).

3.2. Carcass characteristics

The characteristics of the carcass, head, legs, liver, heart, abdominal fat, the weight of the gizzard, and the pancreas were not significantly affected by different treatments across all groups ($p > 0.05$). In T4, there was a significant increase in intestinal weight (10.54%), intestinal length (22.7%), and intestinal density (13.33%) compared to the NC group ($p < 0.05$; Table 3). The intestinal length in T4 (263.80 ± 14.99 cm) was significantly higher than that of the NC (236.00 ± 12.96 cm), T1 (225.00 ± 11.46 cm), T2 (230.70 ± 16.92 cm), T6 (236.80 ± 10.69 cm), T8 (233.00 ± 15.99 cm), and T10 (248.40 ± 19.29 cm; $p < 0.05$), but insignificant compared to the PC group. The weight of the intestine in the T4 (119.40 ± 10.43 g) was significantly higher than that of the NC group (92.30 ± 11.33g), T1 (93.80 ± 11.89 g), T6 (87.40 ± 11.06 g), T8 (92.30 ± 14.82 g), and T10 (102.40 ± 7.20 g; $p < 0.05$), but insignificant compared to the PC group (108.80 ± 8.42 g) and T2 (114.90 ± 17.17 g; $p > 0.05$).

Table 2. Growth performance of broiler chickens fed different levels of *Cyperus alternifolius* in 49 days

Period (days)	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
Feed intake (g)									
1-21	1135.20 ± 46.34	1080.95 ± 26.59	1148.41 ± 35.12	1094.97 ± 43.51	1095.53 ± 35.24	1077.36 ± 42.17	1121.85 ± 21.40	1117.76 ± 69.85	0.359
22-49	4749.00 ± 86.80	4636.55 ± 96.55	4677.07 ± 132.2	4697.60 ± 81.16	4660.72 ± 98.46	4674.18 ± 148.53	4715.57 ± 153.55	4729.37 ± 174.94	0.930
1-49	5884.2 ± 133.14	5717.5 ± 202.66	5825.48 ± 166.07	5792.56 ± 183.90	5756.31 ± 164.78	5643.14 ± 161.84	5837.41 ± 185.82	5847.13 ± 244.79	0.478
Live weight (g)									
1-21	524.75 ± 55.90	603.51 ± 61.02	571.56 ± 46.59	561.27 ± 44.91	592.73 ± 32.36	591.86 ± 46.61	557.96 ± 52.91	544.08 ± 62.69	0.274
1-49	2841.00 ± 119.24^{d}	3109.69 ± 85.28^{a}	2885.20 ± 135.97^{cd}	2946.96 ± 74.80^{bcd}	3055.48 ± 44.13^{ab}	2993.22 ± 39.40^{abc}	2979.49 ± 94.78^{abcd}	2852.82 ± 71.30^{cd}	0.004
Weight gain (g)									
1-21	485.17 ± 73.83	560.60 ± 56.84	528.65 ± 46.59	518.36 ± 44.91	546.49 ± 57.72	548.95 ± 55.95	515.05 ± 52.91	501.17 ± 62.69	0.319
22-49	1791.50 ± 104.62	1902.66 ± 71.99	1742.09 ± 61.57	1824.43 ± 67.47	1876.68 ± 72.10	1812.83 ± 57.82	1863.56 ± 139.76	1764.65 ± 84.31	0.196
1-49	2798.09 ± 119.24^{d}	3066.78 ± 85.28^{a}	2842.29 ± 35.97^{cd}	2904.05 ± 74.80^{bcd}	3012.57 ± 44.13^{ab}	2950.31 ± 39.40^{abc}	2936.58 ± 94.78^{abcd}	2809.91 ± 71.30^{cd}	0.004
Feed conversion ratio									
1-21	2.16 ± 0.05	1.83 ± 0.07	2.17 ± 0.05	2.10 ± 0.15	1.94 ± 0.04	1.96 ± 0.04	2.19 ± 0.22	2.26 ± 0.38	0.077
22-49	2.66 ± 0.18	2.44 ± 0.17	2.69 ± 0.10	2.58 ± 0.05	2.48 ± 0.07	2.58 ± 0.07	2.54 ± 0.18	2.68 ± 0.05	0.195
1-49	2.11 ± 0.09^{a}	1.85 ± 0.12^{d}	2.05 ± 0.03^{ab}	2.00 ± 0.05^{abc}	1.90 ± 0.04^{cd}	1.95 ± 0.03^{bcd}	1.99 ± 0.08^{abc}	2.08 ± 0.10^{ab}	0.008

Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed. $^{a, b, c, \text{and } d}$ Means with the same superscript letters on the same row are not significantly different ($p > 0.05$). The data are presented as mean ± SD.

Table 3. Carcass characteristics and relative organ weights of broiler chickens fed different levels of *Cyperus alternifolius* in 49 days

Characteristics (LBW %)	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
Carcass (LBW %)	79.59 ± 1.72	83.23 ± 1.85	80.39 ± 4.01	82.10 ± 5.06	79.69 ± 4.41	79.61 ± 5.87	77.36 ± 6.87	78.93 ± 2.85	0.122
Head (LBW %)	2.11 ± 0.27	2.13 ± 0.26	2.25 ± 0.23	2.12 ± 0.22	2.17 ± 0.20	2.32 ± 0.35	2.14 ± 0.33	2.30 ± 0.42	0.578
Legs (LBW %)	3.56 ± 0.56	3.37 ± 0.49	3.67 ± 0.58	3.33 ± 0.57	3.42 ± 0.60	3.46 ± 0.62	3.57 ± 0.61	3.71 ± 0.68	0.787
Liver (LBW %)	1.76 ± 0.22	1.66 ± 0.20	1.8000 ± 0.22	1.58 ± 0.16	1.72 ± 0.36	1.70 ± 0.21	1.80 ± 0.21	1.81 ± 0.21	0.125
Heart (LBW %)	0.49 ± 0.04	0.50 ± 0.10	0.47 ± 0.06	0.51 ± 0.13	0.51 ± 0.09	0.54 ± 0.11	0.48 ± 0.07	0.46 ± 0.11	0.611
Abdominal fat (LBW %)	1.76 ± 0.67	1.78 ± 0.46	1.75 ± 0.44	1.63 ± 0.32	1.55 ± 0.46	1.84 ± 0.29	1.73 ± 0.58	1.94 ± 0.37	0.694
Gizzard weight (% LW)	1.62 ± 0.35	1.40 ± 0.22	1.40 ± 0.16	1.45 ± 0.25	1.47 ± 0.10	1.36 ± 0.17	1.32 ± 0.23	1.36 ± 0.14	0.080
Pancreas weight (% LW)	0.18 ± 0.04	0.19 ± 0.03	0.18 ± 0.04	0.19 ± 0.03	0.18 ± 0.04	0.21 ± 0.04	0.20 ± 0.03	0.20 ± 0.04	0.634
Intestine lenght (cm)	236 ± 12.96^{bc}	250.40 ± 18.24^{ab}	225.00 ± 11.46^{c}	230.70 ± 16.92^{c}	263.80 ± 14.99^{a}	236.80 ± 10.69^{bc}	233.00 ± 15.99^{c}	248.40 ± 19.29^{b}	0.000
Intestine weight (g)	92.30 ± 11.33^{cd}	108.80 ± 8.42^{ab}	93.80 ± 11.89^{cd}	114.90 ± 17.17^{ab}	119.40 ± 10.43^{a}	87.40 ± 11.06^{d}	92.30 ± 14.82^{cd}	102.40 ± 7.20^{bc}	0.000
Intestine density (g/cm)	0.39 ± 0.03^{bc}	0.44 ± 0.04^{bc}	0.42 ± 0.05^{bc}	0.50 ± 0.05^{a}	0.45 ± 0.02^{a}	0.37 ± 0.06^{c}	0.40 ± 0.06^{bc}	0.41 ± 0.04^{bc}	0.001

LBW: Live body weight, LW: Live weight. Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed. $^{a, b, c, and d}$ Means with the same superscript letters on the same row are not significantly different (p > 0.05). The data are presented as mean ± SD.

3.3. Microbial flora

Chickens in T4 exhibited a significant decrease (40.9%) in the number of *E. coli* in their ceca compared to those in the NC group, T1, T8, and T10 (p < 0.05). However, T4 recorded a similar number of *E. coli* compared to the PC and T2 groups (p > 0.05). The different groups indicated no significant differences in *Salmonella* spp. prevalence (p > 0.05). In addition, PC group, T1, T2, T4, T6, T8, and T10 indicated a significant increase in the number of intestinal lactic acid bacteria compared to the NC group (p < 0.05; Table 4).

Table 4. Number of intestinal microorganisms in 49-day-old broiler chickens fed different levels of *Cyperus alternifolius*

Number of bacteria (Log $_{10}$ UFC)	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
Escherichia coli	2.79 ± 0.56^{a}	1.92 ± 0.52^{b}	2.74 ± 0.32^{a}	2.40 ± 0.45^{ab}	1.98 ± 0.23^{b}	2.47 ± 0.41^{ab}	3.00 ± 0.11^{a}	2.98 ± 0.26^{a}	0.002
Salmonella	2.51 ± 0.29	2.27 ± 0.13	2.49 ± 0.32	2.25 ± 0.39	2.53 ± 0.30	2.77 ± 0.36	2.69 ± 0.44	2.39 ± 0.27	0.288
Lactobacilli	1.53 ± 0.48^{b}	2.63 ± 0.26^{a}	2.27 ± 0.26^{a}	2.64 ± 0.39^{a}	2.60 ± 0.38^{a}	2.56 ± 0.24^{a}	2.40 ± 0.34^{a}	2.27 ± 0.11^{a}	0.001

Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed. $^{a \text{ and } b}$ Means with the same superscript letters on the same row are not significantly different (p > 0.05). The data are presented as mean ± SD.

3.4. Immune system

In all groups, immune system indices (weight and volume of the spleen and bursa of Fabricius, granulocyte and lymphocyte counts) were not significantly affected by the different treatments (p > 0.05), except for the globulin levels, which increased significantly in T4 (p < 0.05). This indicator of the defense system, globulin, increased significantly in the T4 compared to the NC group by 51.53%, by 37.12% compared to the PC group, and by 34.06% compared to the T10 (p < 0.05; Table 5).

Table 5. Immune system parameters of broiler chickens fed different levels of *Cyperus alternifolius* in 49 days

Parameters	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
Spleen weight (% PV)	0.09 ± 0.02	0.09 ± 0.01	0.13 ± 0.02	0.11 ± 0.03	0.10 ± 0.03	0.07 ± 0.01	0.10 ± 0.03	0.10 ± 0.02	0.063
Spleen volume (ml)	4.40 ± 0.92	4.33 ± 0.58	5.00 ± 0.8	5.00 ± 0.82	4.60 ± 1.00	4.50 ± 0.58	4.55 ± 0.96	4.59 ± 0.58	0.050
BF weight (% PV)	0.13 ± 0.04	0.08 ± 0.03	0.11 ± 0.02	0.09 ± 0.03	0.09 ± 0.01	0.11 ± 0.03	0.13 ± 0.02	0.08 ± 0.02	0.103
BF volume (ml)	5.00 ± 0.82	4.00 ± 1.00	4.50 ± 1.00	4.55 ± 1.00	4.25 ± 0.50	4.75 ± 0.96	5.25 ± 0.50	4.00 ± 1.15	0.471
Granulocyte level (%)	3.12 ± 0.21	2.96 ± 0.21	3.11 ± 0.37	3.53 ± 0.04	3.30 ± 0.72	3.64 ± 0.30	3.02 ± 0.09	3.16 ± 0.32	0.231
Lymphocyte level (%)	80.50 ± 1.80	84.40 ± 3.16	81.83 ± 3.55	78.47 ± 1.84	79.97± 5.24	76.50 ± 2.65	82.30 ± 1.08	82.50 ± 2.72	0.106
Globulin level (g/dL)	1.11 ± 0.56^{c}	1.44 ± 0.74^{bc}	1.63 ± 0.63^{abc}	1.98 ± 0.46^{ab}	2.29 ± 0.74^{a}	1.78 ± 0.62^{abc}	1.60 ± 0.60^{abc}	1.51 ± 0.56^{bc}	0.021

BF: Bursa of Fabricius. Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed $^{a, b, c,}$ Means with the same superscript letters on the same row are not significantly different (p > 0.05). The data are presented as mean ± SD.

3.5. Feed digestibility

The digestibility of DM, OM, and NDF was not significantly affected by the increasing levels of rhizome powder of *C. alternifolius* in the feed across all groups (p > 0.05). Furthermore, the digestibility of CP increased significantly in T1 (92.31 ± 2.7), T2 (91.94 ± 1.8), T4 (92.61

± 0.2), T6 (90.22 ± 1.5), T8 (92.34 ± 1.7), T10 (92.40 ± 1.23) and in the positive control group (92.64 ± 1.6) compared to the negative control group (85.13 ± 4.5; $p < 0.05$; Table 6).

Table 6. Effects of incorporating *Cyperus alternifolius* in the diets on the digestibility of the components of the feed in broiler chickens

ADUC (%)	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
ADUC of dry matter	78.60 ± 2.08	78.59 ± 1.71	75.83 ± 4.28	76.88 ± 5.37	79.53 ± 1.13	77.04 ± 4.23	80.46 ± 3.19	79.77 ± 1.67	0.655
ADUC of organic matter	81.67 ± 1.60	81.93 ± 1.65	79.20 ± 4.08	81.77 ± 4.06	81.42 ± 1.14	80.51 ± 3.67	84.38 ± 2.41	82.82 ± 1.49	0.525
ADUC of crude protein	85.13 ± 4.5[b]	92.64 ± 1.6[a]	92.31 ± 2.7[a]	91.94 ± 1.8[a]	92.61 ± 0.2[a]	90.22 ± 1.5[a]	92.34 ± 1.7[a]	92.40 ± 1.23[a]	0.012
ADUC of neutral dietary fiber	78.64 ± 6.2	84.04 ± 2.0	85.15 ± 4.14	86.53 ± 2.40	87.26 ± 1.53	85.85 ± 2.61	84.53 ± 2.2	87.74 ± 0.66	0.066

ADUC: Apparent digestive utilization coefficient, NDF: Neutral dietary fiber. Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed. [a and b] Means with the same superscript letters on the same row are not significantly different ($p > 0.05$). The data are presented as mean ± SD.

3.6. Hematological and biochemical parameters

Total protein levels increased significantly in chickens in T4 (6.33 ± 1.00 g/dL) compared to chickens in NC group (54.73 ± 0.89 g/dL), PC group (4.97 ± 1.12 g/dL), T1 (4.48 ± 0.78 g/dL), T6 (4.72 ± 0.75 g/dL), T8 (4.82 ± 0.80 g/dL), and T10 (4.60 ± 0.54 g/dL; $p < 0.05$). All hematological parameters including white blood cell count, red blood cell count, platelet count, hemoglobin concentration, mean corpuscular volume, and hematocrit and biochemical parameters (AST, ALT, urea, creatinine, albumin, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol) were not significantly affected by the different treatments in all groups ($p > 0.05$; Tables 7, and 8).

Table 7. Effects of increasing levels of *Cyperus alternifolius* on hematological parameters in 49-day-old broiler chickens

Parameters	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
WBC (10^9/L)	174.40 ± 7.46	171.17 ± 9.79	190.40 ± 12.58	172.50 ± 24.27	160.77±15.72	168.27 ± 16.32	182.17 ± 9.24	167.77 ± 11.36	0.336
RBC (10^{12}/L)	3.35 ± 0.42	3.22 ± 0.65	3.44 ± 0.35	3.53 ± 0.04	3.30 ± 0.72	3.64 ± 0.30	3.02 ± 0.09	3.16 ± 0.32	0.679
HGB (g/dL)	17.13 ± 1.40	13.70 ± 2.19	16.17 ± 2.93	18.67 ± 1.93	17.63 ± 4.11	20.30 ± 1.80	16.70 ± 2.34	15.90 ± 2.02	0.117
HCT (%)	38.60 ± 2.57	36.03 ± 2.54	38.33 ± 4.77	44.70 ± 2.75	40.80 ± 7.51	45.67 ± 3.07	37.17 ± 1.52	36.40 ± 5.38	0.065
PLT (10^9/L)	1.00 ± 0.50	1.33 ± 0.58	2.33 ± 0.31	1.00 ± 0.06	1.67 ± 0.15	1.00 ± 0.04	1.33 ± 0.58	2.00 ± 0.98	0.653
MCV (fL)	124.00 ± 1.40	122.00 ± 1.93	123.53 ± 1.00	126.67 ± 1.93	124.37 ± 4.32	125.60 ± 3.04	123.07 ± 1.86	121.87 ± 3.93	0.378

WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, PLT: Platelets, MCV: Mean corpuscular volume. Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed. The data are presented as mean ± SD.

Table 8. Effects of increasing levels of *Cyperus alternifolius* on biochemical parameters in 49-day-old broiler chickens

Parameters	Treatment groups								P value
	Negative control	Positive control	T1	T2	T4	T6	T8	T10	
AST (U/L)	124.55 ± 24.92	124.41 ± 18.56	121.73 ± 19.80	112.25 ± 22.36	120.33 ± 24.36	103.89 ± 32.69	124.12 ± 20.59	106.51 ± 25.63	0.377
ALT (U/L)	36.05 ± 5.74	36.99 ± 8.29	25.87 ± 7.91	34.34 ± 8.11	32.52 ± 6.94	35.25 ± 7.11	27.27 ± 8.68	36.02 ± 9.37	0.219
Urea (mg/dL)	6.88 ± 1.51	7.39 ± 2.25	6.55 ± 1.48	8.26 ± 2.81	6.68 ± 1.00	7.79 ± 2.31	6.77 ± 2.63	7.87 ± 2.95	0.769
Creatinine (mg/dL)	1.26 ± 0.21	1.31 ± 0.22	1.42 ± 0.15	1.40 ± 0.52	1.18 ± 0.08	1.22 ± 0.16	1.19 ± 0.08	1.29 ± 0.26	0.444
Total protein (g/dL)	4.73 ± 0.89[bc]	4.97 ± 1.12[bc]	4.48 ± 0.78[c]	5.65 ± 0.98[ab]	6.33 ± 1.00[a]	4.72 ± 0.75[bc]	4.82 ± 0.80[bc]	4.60 ± 0.54[c]	0.001
Albumin (g/dL)	3.62 ± 0.83	3.53 ± 0.68	2.85 ± 0.50	3.17 ± 0.63	3.42 ± 0.90	2.94 ± 0.75	3.22 ± 0.54	3.09 ± 0.29	0.251
tryglyceride (mg/dL)	99.68 ± 30.81	109.09 ± 21.41	100.00 ± 20.17	125.61 ± 46.90	100.20 ± 27.97	102.27 ± 14.11	88.82 ± 20.62	103.77 ± 20.67	0.121
Total cholesterol (mg/dL)	150.94 ± 12.99	156.57 ± 19.00	151.14 ± 26.37	140.40 ± 32.08	144.60 ± 21.84	159.42 ± 28.71	147.97 ± 28.33	152.79 ± 15.39	0.808
HDL (mg/dL)	114.36 ± 10.81	120.60 ± 28.67	110.39 ± 21.16	98.45 ± 23.87	119.33 ± 21.42	122.13 ± 20.97	103.33 ± 26.23	131.79 ± 18.23	0.052
LDL (mg/dL)	34.14 ± 7.63	32.91 ± 5.07	28.25 ± 5.79	30.63 ± 6.59	26.98 ± 5.14	29.58 ± 6.54	31.16 ± 5.03	30.25 ± 8.58	0.131

Negative control: Basal diet without additive, Positive control: Basal diet with 1 g of doxycycline/kg, T1: 1 g of *C. alternifolius* /kg of feed, T2: 2 g of *C. alternifolius*/kg of feed, T4: 4 g of *C. alternifolius* /kg of feed, T6: 6 g of *C. alternifolius*/kg of feed, T8: 8 g of *C. alternifolius*/kg of feed, T10: 10 g *C. alternifolius*/kg of feed. ALT: Alanine-amino-transferase; AST: Aspartate aminotransferase, HDL: High-density cholesterol, LDL: Low-density cholesterol. [a, b, and c] Means with the same superscript letters on the same row are not significantly different ($p > 0.05$). The data are presented as mean ± SD.

4. Discussion

During the present study, the feed intake was not affected by increasing rates of *C. alternifolius* incorporation into the diet. These results corroborate the findings of Nyembo et al.[4], who found no substantial effects of *C. alternifolius* rhizome powder at a dose of 2 g/kg of feed on the feed intake in broiler chickens. The present results indicated an increase in live weight and weight gain in broiler chickens during the finisher phase when 4 g of *C. alternifolius*/kg of feed was incorporated. The increase in weight gain and live weight recorded in the present study could be attributed to bioactive compounds, such as phenols, flavonoids, terpenoids, and sterols, present in *C. alternifolius*[4]. These results partially align with Shihab et al.[14], who observed a numerical increase in live weight and

weight gain when neem leaf powder (2 g/kg feed) was added to the broiler chicken diet, attributable to the neem bioactive compounds.

The decrease in FCR observed in the present study could be the result of the notable increase in weight gain induced by the consumption of feed containing *C. alternifolius* powder at a dose of 4 g/kg as a phyto-additive, which was in disagreement with the findings of Ouedraogo et al.[15] who reported a decrease in the consumption index by using of a phyto-additive (Turmeric) at a rate of 1.5%.

The increase in beneficial bacteria in the digestive tract and elevated blood globulin levels indicated enhanced immune function against pathogenic microbes, such as *E. coli* and *Salmonella*, thereby promoting animal health and growth. The results of the present study are consistent with those of Nyembo et al.[4], who reported that incorporating *C. alternifolius* and *T. angustifolia* into broiler feed as an additive at 2 g/kg notably increases the number of lactic acid bacteria in the digestive tract of broiler chickens. The positive health and improved growth performance observed in chickens during the present study may be explained by a decrease in the number of colonies of harmful bacteria in the digestive tract, as well as a decrease in morbidity. The present findings contradicted those of Nyembo et al.[4], who reported that using the same phytobiotic (*Cyperus alternifolius*) at 2 g/kg feed did not affect *E. coli* levels in the digestive tract of broiler chickens. Differences in the incorporation rate of *C. alternifolius* across the studies could explain the divergent results. In the present study, the dose of 4 g/kg appears to have released a large amount of antimicrobial substances, which would have led to the elimination of a substantial number of harmful microbes, such as *E. coli*, thus reducing their numbers in the digestive tract of broiler chickens.

Globulins or immunoglobulins are immune proteins or antibodies secreted by B lymphocytes (B-cell dependent) and plasma cells in response to either microbial (virus and bacteria) and parasitic infections or food allergies[15]. The increase in globulin levels observed in the present study might be attributable to food allergies or other exogenous factors induced by ingestion of *C. alternifolius* at doses of 2 and 4g/kg.

Regardless of the incorporation rate in the feed, *C. alternifolius* substantially improved CP digestibility. The phenolic compounds, flavonoids, terpenoids, or sterols present in the *C. alternifolius* additive may have increased protein digestibility by stimulating the release of proteases that hydrolyze dietary proteins. These proteases would then have induced an increase in the amount of amino acids available and their susceptibility to intestinal absorption. Once these proteins were absorbed in the form of amino acids, they were used for muscle growth in chickens.

The increase in intestinal length, weight, and density suggested an increase in the absorption surface area for digested nutrients in the digestive tract, with a direct impact on the growth of the hen. These results corroborate those of Nyembo et al.[4], who reported that the use of *C. alternifolius* and *T. angustifolia* rhizome powder at a rate of 2 g/kg improved CP digestibility in broiler chickens. Phenolic compounds indirectly increase the absorption surface (Length and width of the villi) of nutrients, thus improving their utilization for the benefit and growth of chickens. The current results are in contradiction with those of Chamorro et al.[16], who recorded a decrease in the digestibility of CP with the incorporation of 5 g/kg of grape seed extract in broiler feed. The differences in results between these two studies could be due to differences in the phytobiotics used and their incorporation rates. This contrast highlighted that beneficial phytobiotic action observed in *C. alternifolius* can shift to anti-nutritive outcomes of high-dose grape seed extract, depending on the polyphenol profile and concentration, emphasizing the need for tailored dosage optimization in feed formulation. Additionally, Brenes and Roura[17] highlighted that the performance and digestibility responses to plant-derived feed additives in poultry are highly variable. This aligns with observations that certain additives, such as grape seed extract at elevated inclusion rates, may not improve protein digestibility.

The hematological parameters studied were not significantly affected by the *C. alternifolius* rhizome powder, regardless of the incorporation rate. The lack of variation in the hematological parameters investigated in the present study could suggest that the incorporation rate of this phyto-additive did not exceed a threshold that would be harmful to the health of the chickens. Any change in blood components compared to normal values is an important indicator for interpreting the animal's physiological or metabolic state, but also, and especially, the quality of its feed[18]. As noted by Etim et al.[18], blood constituents change in relation to health and dietary conditions, and stable values are therefore indicative of a non-detrimental feed quality.

All other biochemical parameters, including AST, ALT, urea, creatinine, triglycerides, HDL cholesterol, and LDL cholesterol, were not affected by the use of *C. alternifolius* in the diet. In contrast, Kana et al.[4] reported that *Dichostachys glomerata (D. glomerata)* fruit altered liver enzyme profiles, decreasing ALT and increasing AST, which may indicate a hepatotropic or hepatotoxic influence distinct from the primarily digestive action of *C. alternifolius*. These studies underscore that plant additives can exert fundamentally different physiological effects, such as *C. alternifolius*, which may primarily improve protein metabolism, whereas others, such as *D. glomerata*, may directly modulate hepatic function or induce mild metabolic stress. The different effects of *C. alternifolius* and *D. glomerata* fruit on broiler blood biochemistry highlighted the source-specific bioactivity of phytogenic feed additives. In the present study, supplementation with *C. alternifolius* at 4 g/kg increased serum total protein, likely a direct consequence of its enhancement of CP digestibility and amino acid availability, without altering liver enzymes (AST, ALT), renal markers (urea, creatinine), or lipid profiles. This pattern suggests a nutritive, growth-supporting role without hepatic or renal stress.

5. Conclusion

Supplementing broilers' diet with *Cyperus alternifolius*

rhizome powder at the rate of 4 g/kg improved crude protein digestibility without affecting the digestibility of dry matter, organic matter, or dietary fiber. Feed digestibility stimulated the multiplication of lactobacilli in the chicken's digestive tract, increased live weight and weight gain, and increased the globulin content. *Cyperus alternifolius* at a dose of 4 g/kg can therefore be used as a substitute for synthetic antibiotics in animal feed. Future studies would benefit from extracting, isolating, and quantifying the bioactive compounds present in this plant-based additive, and from investigating their effects on gut flora, feed component digestibility, the immune system, and growth performance in broiler chickens.

Declarations

Competing interests

The authors declared that they have no competing interests.

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Authors' contributions

Nyembo Kondo Camile, Hervé Tchoffo, and Raphaël Jean Kana conceived and designed the study and reviewed the manuscript. Innocent Murhula Amani, Basubi Muke Matthieu, Manga Tchomba, Balume Kayani Isaac, Kasereka Patrick, Zamani Ngike, and Niyorugira Sebigunda Jackson collected the data, carried out data analysis, and wrote the manuscript. All authors read and approved the final edition of the manuscript.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical considerations

All authors have reviewed the present study for ethical problems, such as plagiarism, consent for publication, misconduct, data manipulation and/or deceit, and duplication of study. The authors confirmed that they have not used AI during writing and preparing the manuscript.

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