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Effects of *Cyperus alternifolius, Echinochloa pyramidalis, Typha angustifolia,* and *Imperata cylindrica* on Growth Performance, Feed Digestibility, Gut Microbiota, Haemato-biochemical and Immunity Parameters in Broiler Chickens

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ABSTRACT

The rhizomes of *Cyperus (C.) alternifolius, Echinochloa (E.) pyramidalis, Typha (T.) angustifolia,* and *Imperata (I.) cylindrica* are rich in secondary metabolites and have diverse pharmacological activities. The present study was designed to evaluate the effects of dietary *C. alternifolius*, *E. pyramidalis, T. angustifolia,* and *I. cylindrical* rhizomes on the performance of broiler chickens. A total of 384 day-old chicks were randomly assigned to six treatment groups (each treatment replicated four times). The first group received a basal diet (negative control), and the second group received a basal diet with 1 gr of antibiotic (Doxicycline, positive control). Other groups received a basal diet with 2 gr of each phyto-additives/kg feed. The results revealed that treatments had no significant effects on feed intake and carcass yield in chickens. The *C. alternifolius* and *T. angustifolia* significantly increased live weight and weight gain, and decreased feed conversion ratio, compared to negative control. The addition of *C. alternifolius, T. angustifolia,* and *I. cylindrica* to broilers' diet significantly increased the apparent digestibility of dry matter and crude protein, compared to the negative control. Compared to the negative control, the lactic acid bacteria count significantly increased with the incorporation of *T. angustifolia* and *I. cylindrica*. The granulocytes count and globulins concentration were not affected by the different treatments. However, the lymphocyte count was significantly decreased with the diet containing *E. pyramidalis* compared to the negative and positive controls, and the diets containing *C. alternifolius* and *T. angustifolia.* The spleen and bursa weights and volumes significantly increased in all groups of chickens fed on phyto-additives, compared to the negative control. Except for haematocrit, which significantly increased with *C. alternifolius and T. angustifolia* in the treatments compared to the negative control, the feed additives did not significantly affect the hematological parameters. Compared to the negative control, *T. angustifolia* and *I. cylindrica* significantly increased HDL-cholesterol concentration in broiler chickens' serum, while all treatment groups were comparable for all the other biochemical parameters. Incorporating 2 g of *C. alternifolius* and *T. angustifolia* in broiler chickens' feed improves feed digestibility, enhances the population of lactic acid bacteria in the gut, and causes subsequent improvement in growth performance.

Keywords: Broiler chicken, Digestibility, Growth performance, Gut microbiota, Immunity, Phyto-additive

INTRODUCTION

The use of antibiotic growth promoters in poultry nutrition has been banned due to concerns about the accumulation of their residues in animal tissues and pathogens' resistance (Mirzaei et al., 2022). This ban could lead to

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increased morbidity and mortality, reduced growth performance, and decreased economic profitability for farmers (Chardon and Brugere, 2014). As an alternative solution, the use of medicinal plants with positive effects on the digestive tract health, immune system, and growth performance has been encouraged (Onu, 2010).

Medicinal plants used in traditional pharmacopoeia have preventive and curative properties in human and animal health (Shalukoma et al., 2015; Bashige et al., 2020). These plants are rich in phytochemicals compounds, such as alkaloids, flavonoids, steroids, terpenoids, and quinones possessing antibacterial, antioxidant, antiviral, antimycotic, antiparasitic, immune-modulatory properties and digestive tract stimulatory effects (Dieumou et al., 2009; Ruiz-Navajas et al., 2013). Several studies have reported the improvement of growth performance in poultry through modulation of gut flora, reduction of pathogenic bacteria counts, stimulation of digestive secretions, improvement of feed components digestibility, absorption of nutrients, and stimulation of the immune system (Malekizadeh et al., 2012; Gong et al., 2014; Kana et al., 2017). Some plants commonly used in the Democratic Republic of Congo with pharmacological properties include *Cyperus (C.) alternifolius, Echinochloa (E.) pyramidalis, Typha (T.) angustifolia* and *Imperata (I.) cylindrica*.

These plant rhizomes are rich in secondary metabolites, including alkaloids, terpenoids, steroids, flavonoids, and tannins with antimicrobial and antiparasitic potential (Varghese et al., 2009; Lalthanpuii et al., 2015; Bashige et al., 2020). According to Varghese et al. (2009), *T. angustifolius* extracts have antibacterial effects on *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) and antifungal effects on *Aspergillus flavus*. Lalthanpuii et al. (2015) noted antibacterial and antiparasitic effects of *I. cylindrica* rhizome powder against Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and Gram-positive bacteria of *Bacillus subtilis.* However, the extract of this plant is effective against intestinal parasites, such as the tapeworm *Raillietina echinobothrida* and the roundworm *Ascaridia galli*. Bashige et al. (2020) reported antimicrobial activities of *Cyperus alternifolius, E. pyramidalis, T. angustifolia,* and *I. cylindrica* rhizome powder on *Neisseria meningitidis, S. aureus, Candida albican, Streptococcus pneumoniae, Salmonella typhi, E. coli* and *Trichophyton rubrum.* These phyto-additives are available and considered in several African countries as herbs. They contain bioactive compounds that can balance the gut microbiota, improve digestion and nutrient absorption, stimulate the immune system, and enhance growth performance if administered as feed additives to livestock. The main objective of the present study was to evaluate the individual effects of powdered rhizomes of *C. alternifolius, E. pyramidalis, T. angustifolia* and *I. cylindrica* as feed additives on growth performance, feed digestibility, gut microbiota, haemato-biochemical and immunity parameters in broiler chickens.

MATERIAL AND METHODS

Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Study area

The study was conducted at the Teaching and Research Farm of the University of Dschang, Cameroon, from February to April 2022. This farm is located at 5°26' North latitude, 10°26' East longitude at an average altitude of 1420 m. The average temperature was around 21°C, the average rainfall was 2000 mm, the average annual insolation was 1873 hours, and the average relative humidity was 76.8%.

Phyto-additives

The *C. alternifolius, E. pyramidalis, T. angustifolia* and *I. cylindrica* were harvested in the vicinity of the Centre de Recherche en Sciences Naturelles (CRSN) in Lwiro, 30 Km from the town of Bukavu in the Democratic Republic of Congo. The identification of the plants used as phyto-additives was confirmed at the CRSN/Lwiro herbarium. The rhizomes were separated from other parts of the plant, open-air dried in the shade, then grind (using a Nima mill brand, China) and the different powders obtained (1 kg each plant) were kept in hermetically sealed boxes and later used as feed additives. Phytochemical analysis of the powdered rhizomes of each plant was carried out in order to identify the bioactive compounds present (Table 1). The determination of the classes of compounds present in the different extracts was made according to the standard methods described by Harborne (1973). Flavonoids were determined by the Shinoda test, 0.1 g of extract was dissolved in 3 ml of methanol. The mixture was treated with 0.05 g of magnesium chips and 3 drops of concentrated HCl. Flavonoids were identified by the presence of stains (orange for flavones, red for

xanthones and pink for flavonols). Alkaloids were determined by the Meyer test, 0.1 g of extract was placed in a test tube in the presence of 3 ml of an aqueous hydrochloric acid solution (50% V/V). The mixture was treated with 3 drops of Meyer's reagent. The formation of a white or yellowish precipitate indicated the presence of alkaloids. Triterpenes and steroids were determined by the Liebermann-Burchard test. Then, 0.1 g of extract was dissolved in 3 ml of chloroform, 3 ml of acetic anhydride was added, and the mixture was cooled in ice for 3 minutes. Finally, one drop of concentrated sulfuric acid was added. The presence of triterpenes was confirmed by the appearance of a purplish red color and that of steroids by the successive appearance of blue, green, red or orange colors. Anthraquinones, 0.1 g of extract was dissolved in a 4 ml mixture of ether-chloroform (1:1 v/v). The solution was treated with 4 ml of 10% (W/V) sodium hydroxide. The quinones were identified by the presence of red coloration. For phenols, 0.1 g of extract was dissolved in 3 ml of ethanol. The mixture was treated with 3 drops of 10% (v/v) iron III chloride. The appearance of the violet-blue or greenish coloration indicated the presence of phenols. For tannins, 0.1 g of extract was boiled for 5 minutes in a tube containing 5 ml of water. The mixture was treated with 5 ml of 2% NaCl (W/V) and 5 ml of 1% gelatine (W/V) after cooling. The appearance of a precipitate confirmed the presence of tannins.

Table 1. Phytochemical composition of phyto-additives

Plant	Alkaloids	Phenols	Flavonoids	Sterols	Triterpenoids	Tannins	Anthraquinones
Cyperus alternifolius	-	+	+	+	+	-	-
Echinochloa pyramidalis	-	+	+	+	+	-	-
Typha angustifolia	-	+	+	+	+	-	-
Imperata cylindrica	-	+	+	+	+	-	-

Present: +; Absent: -

Chickens

A total of 384 one-day-old Cobb 500 chicks were randomly assigned to 6 experimental groups (treatments) following a completely randomized design. Each treatment had 4 replicates of 16 chicks (8 males and 8 females). As soon as the chicks arrived, multivitamins and minerals (5 g in 2 liters of water, INTROVITA+WS, Holland) were administered through drinking water for the first 3 days. They were then vaccinated against infectious bronchitis (H52, Holland) and Newcastle (Hitchner B1, Holland) disease on day 7 and against Gumboro (CEVAC^{R} TRANSMUNE IBD, Holland) disease on day 10 with a booster on day 18. Multivitamins and minerals (5 g in 2 liters of water) were administered through drinking water after each weighting session and vaccination of the chicks.

Experimental diets

Chicks in the control group received basal diet (Table 2), the positive control group received a basal diet with 1 g of Doxycycline® in /kg of diet (dry matter [DM]), and other groups received a basal diet supplemented with 2 g of each plant in /kg of diet (DM).

Table 2. Composition of the experimental diet at the starter and finisher phases

Ingredients (%)	Starter	Finisher
Maize	60	67
Cotton meal	5	5
Soybean meal	22	15
Fish meal	5	5
Wheat bran	2	2
Oyster shell meal	1	1
Premix 5%*	5	5
Total	100	100
Chemical composition		
Metabolizable energy (kcal/kg)	2977	3108
Crude Protein (%)	23.01	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
Crude cellulose (%)	2.43	2.61

Premix 5%*: Vit A: 3 000 000 IU, Vit D3:600 000 IU, Vit E: 4 000 mg, Vit K: 500 mg, Vit B1: 200 mg, VitB2: 1000 mg, Vit B6: 4000 mg, Vitamin B12: 4 mg, Iron: 8000 mg, Cu: 2000 mg, Zn: 10 000 mg, Se: 20 mg, Mn: 14000 mg.

Data collection

Growth performance, feed digestibility, and microbial flora

Throughout the study period (1-49 days), growth parameters (feed intake, weight gain, live weight, and feed conversion ratio [FCR]) were collected weekly. At the end of the study, 5 female and 5 male chickens were randomly selected from each group. They were then fasted for 24 hours to evacuate all digestive tract contents, weighed, plucked, and eviscerated without anesthesia.

Carcass yield and relative weights of organs were calculated. Moreover, intestine length was measured using a measuring tape, and intestine density was calculated by dividing the intestine length by the intestine weight.

The apparent digestive utilization coefficients (aDUC) of feed components were evaluated for 6 broiler chickens , including 3 males and 3 females, per treatment for 3 consecutive days. The 6 chicks per treatment were kept in the digestibility cages, and tarps were placed underneath the cages after 3 days of adaptation period to collect faces from each replicate. Feed was weighed prior to feeding. Afterward, faces and feed refusals were collected and weighed daily for 3 days. Fecal samples were oven-dried at 60°C to constant weight for proximate analysis of DM and organic matter (OM) in accordance with AOAC (1990) processes. Neutral Detergent Fiber (NDF) was determined by Van Soest et al. (1991) method, and crude protein (CP) by the Kjedhal method. The apparent digestive utilization coefficients (aDUC) of DM, OM, CP, and NDF of the experimental diets were calculated.

At the end of the trial (49 days), fecal samples were collected from the cloaca of four chickens per treatment (two males and two females), using cloacal swabs and immediately used for the identification and quantification of lactic acid bacteria, *E. coli* and *Salmonella* in their respective specific culture media, for determining of Lactic acid bacteria, the culture medium used was lactobacilli M.R.S AGAR produced by Acumedia® (India) and ISO 9001 reference. The final pH was 7.5 ± 0.2 at 25°C. The preparation procedure consisted of dissolving 70 g of this medium in 1 liter of distilled water in an Erlenmeyer flask, then heating with frequent stirring until complete dissolution. This medium was autoclaved at 121°C for 15 minutes.

For *E. coli*, the culture medium used was Mac Conkey Agar manufactured by Liofilchem® (India, dignostic and reference ISO 610028). The final pH was 7.1 ± 0.2 at 25°C. The preparation procedure consisted of pouring 51.5 g of the suspension into 1 liter of distilled water, then heating the mixture until completely dissolved. Finally, it was autoclaved at 121°C for 15 minutes.

For *Salmonella*, the culture medium used was SS AGAR of reference ISO 610042 and produced by Liofilchem® (India) dignostic. The final pH was 7 ± 0.2 at 25°C. The preparation procedure consisted of pouring 52 g of the suspension into 1 liter of distilled water, then boiling until complete dissolution without autoclaving according to the manufacturer's prescription. The inoculum was prepared by decimal dilutions, which consisted of placing 9 ml of physiological water in tubes numbered at the base by the type of sample and the dilution number. The swab bearing the sample was then introduced into the first tube. The latter was shaken in order to homogenize the solution (S1), then 1 ml of S1 was taken with a micropipette and introduced into the second tube to complete the solution to 10 ml, thus obtaining the 10-2 dilution. After homogenizing this solution, the procedure was carried out up to the 10-8 dilution. 1 ml of the 10-6 and 10-8 dilutions of each sample was taken and introduced into a petri dish each (Afnor, 1991).

The previously prepared solution of each culture medium (MRS Agar, SS Agar, and Mac Conkey Agar) was introduced each time just after the introduction of the inoculum into the petri dish and homogenized.

Immune system and haemato-biochemical parameters

During carcass evaluation, lymphoid organs (bursa of Fabricius and spleen) of 6 chickens/treatment were collected and weighed. Their indices were calculated by the ratio of organ weight (g)/fasting live weight (g) multiplied by 100 according to Stice (2000). In the next step, 5 ml of blood samples were collected in tubes containing anticoagulant for the quantification of white blood cells, red blood cells, haemoglobin, haematocrit, blood platelets, mean cell volume, and packed cell volume (PCV) using Urtit 3000 plus haematimater (China) and blood without anticoagulant was used to measure alanine aminotransferase (ALT), aspartate aminotransferase, Urea, Creatinin, Trygliceride, Total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL)-Cholesterol according to kit manufacturers' instructions (Chronolab®, Barcelona, Spain). With regard to the immune system status, the immune cells quantified including granulocytes and lymphocytes, and the immune system proteins including

albumin and globulins were investigated according to the instruction of the Urtit 3000 Plus kit (China).

Statistical analysis

The statistical software Statistical Package for Social Sciences (SPSS version 20.0) was used for the analyses. All collected data were submitted to a one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate significant level at $p < 0.05$. The normality of data was tested by the Shapiro-Wilk test.

RESULTS

Growth performance

Table 3 summarizes the effects of different treatments on growth performance in broiler chickens. The different additives did not significantly affect feed intake in any of the study periods. During the starter phase (1-21 days), the addition of different additives in the diets did not have significant effects on the live weight and weight gain of chickens. Over the entire study period (1-49 days), live weight and weight gain recorded in broilers fed diets supplemented with *C. alternifolius*, *T. angustifolia*, and *I. cylindrica* were comparable to the result recorded with antibiotic, but significantly higher than the negative control diet ($p < 0.05$). During the finisher phase and throughout the study period, the addition of *C. alternifolius*, *T. angustifolia*, and *I. cylindrica* in feed significantly lowered FCR, compared to the control group ($p < 0.05$).

Feed digestibility

The apparent digestive utilization coefficients (aDUC) of DM induced by the supplementation of the diet with *T. angustifolia*, *I. cylindrical*, and antibiotic were significantly higher than that of the negative control diet ($p < 0.05$). The aDUC of CP significantly increased with *C. alternifolius* and *T. angustifolia* compared to that of negative control ($p < 0.05$, Table 4). The aDUC of organic matter and neutral detergent fiber were not significantly affected by the inclusion of *C. alternifolius*, *T. angustifolia*, and *I. cylindrical* in the diets.

Carcass characteristics

The different treatments did not significantly affect relative organ weights and carcass yields (Table 5). However, carcass yields tended to increase by supplementing diets with different additives.

Microbial flora

Table 6 shows that incorporating the different phyto-additives in the diet has no significant effect on *E. coli* and *Salmonella counts* in the gut of broiler chickens ($p > 0.05$). However, *E. coli* and Salmonella counts tended to decrease with the dietary supplementation of different phyto-additives compared to the negative control ($p > 0.05$). Lactobacilli counts in the gut of chickens significantly increased with the incorporation of *C. alternifolius, T. angustifolia, and antibiotic*, compared to the number recorded with the control diet ($p < 0.05$).

Effects of in-feed additive on the immune system

As can be seen in Table 7, the weights and volumes of spleen and bursa of Fabricius increased significantly with the incorporation of phyto-additives in the diet compared to the negative control ($p < 0.05$). With the exception of chickens fed on *I. cylindrical*, supplementing broiler's feed with *E. pyramidalis* rhizome significantly decreased blood lymphocytes, compared to other treatments ($p < 0.05$). Meanwhile, the phyto-supplements did not significantly affect granulocyte count, and the number of globulins in the blood of broiler chickens, compared to the controls ($p > 0.05$).

Hematological parameters

The supplementation of broiler's feed with rhizomes of *C. alternifolius* and *T. angustifolia* significantly increased blood hematocrit level, compared to the negative control ($p < 0.05$). Nevertheless, blood hematocrit level of chickens fed on diets supplemented with these two phyto-additives was comparable to that of chickens fed on an antibiotic as supplement. On the other hand, the phyto-additives did not significantly affect the other hematological parameters regardless of their type ($p > 0.05$, Table 8).

Biochemical parameters

All treatments induced comparable biochemical parameters values ($p > 0.05$), except HDL-cholesterol level which increased significantly with the incorporation of *T. angustifolia* and *I. cylindrica* in the diet, compared to the negative control and *Cyperusalternifolius* supplemented diet ($p < 0.05$, Table 9). However, the analysis of variance revealed no significant influence of phyto-additives on ALT, total cholesterol and LDL-cholesterol relative to controls. Inversely, total protein, albumin, and creatinine levels showed a slight increase with the addition of these phyto-additives in broiler fed, compared to the negative control ($p > 0.05$).

Table 3. Growth performances of broiler chickens fed with phyto-additives for 49 days

Study period (days)	Controls		Phyto-additives (2 g/kg feed)				p
	0-	0+	Ca	Ep	Ta	Ic	
Feed intake (g)							
01-21	1232.27 ± 71.49	1184.72 ± 52.05	1203.41 ± 84.46	1214.04 ± 66.85	1185.12 ± 94.02	1225.21 ± 75.29	0.231
22-49	5173.82 ± 98.23	5119.82 ± 73.19	5083.28 ± 84.15	5224.37 ± 94.18	5134.41 ± 118.88	5213.76 ± 85.74	0.088
01-49	6406.09 ± 102.66	6304.54 ± 97.95	6286.69 ± 97.03	6438.41±167.97	6319.53 ± 172.46	6438.97 ± 145.67	0.384
Live body weight (g)							
01-21	632.91 ± 53.52	646.48 ± 80.64	648.50 ± 77.44	627.15 ± 88.76	641.36 ± 60.87	653.97 ± 52.30	0.145
01-49	2785.41 ± 98.53^{c}	3044.51 ± 109.36^{a}	2941.72 ± 77.44^{ab}	2887.93 ± 131.96^{bc}	3034.46 ± 109.71^{a}	2935.86 ± 99.43^{ab}	0.004
Weight gain (g)							
01-21	594.48 ± 63.52	608.05 ± 70.64	610.07 ± 77.44	588.72 ± 98.76	602.93 ± 90.87	615.54 ± 62.30	0.145
22-49	2152.50 ± 107.08^{c}	2398.03 ± 129.71^{a}	2293.22 ± 88.82^{ab}	2260.78 ± 126.07^{bc}	2393.10 ± 106.71^{a}	2281.89 ± 95.21^{ab}	0.004
01-49	2746.98 ± 148.53^{c}	3006.08 ± 149.36^{a}	2903.29 ± 188.71^{ab}	2849.50 ± 131.96^{ab}	2996.03 ± 159.71^{a}	2897.43 ± 119.43^{ab}	0.004
Feed conversion ratio							
01-21	2.07 ± 0.05	1.95 ± 0.07	1.97 ± 0.06	2.06 ± 0.04	1.97 ± 0.11	1.99 ± 0.02	0.053
22-49	2.40 ± 0.03^{a}	2.14 ± 0.02^{c}	2.22 ± 0.10^{bc}	2.32 ± 0.11^{ab}	2.15 ± 0.02^{c}	2.29 ± 0.09^{b}	0.001
01-49	2.33 ± 0.05^{a}	2.09 ± 0.03^{d}	2.16 ± 0.07^{cd}	2.26 ± 0.06^{ab}	2.11 ± 0.03^{d}	2.22 ± 0.03^{bc}	0.001

^{a,b,c}: Means the same letter on the same row is not significantly different ($p > 0.05$); 0-: Diet without additive (R0-), 0+: 1 g Doxycycline®/kg diet, Ca: R0- + 2 g Cyperus alternifolius/kg diet, Ep: R0- + 2 g Echinochloa pyramidalis/kg diet, Ta: R0- + 2 g Typha angustifolia/kg diet, Ic: R0- + 2 g Imperata cylindrica/kg diet, p: P-value

Table 4. Effects of phyto-additives on apparent digestive utilization coefficients of feed components in broiler chickens

aDUC (%)	Controls		Phyto-additives (2 g/kg feed)				p
	0-	0+	Ca	Ep	Ta	Ic	
DM aDUC	92.45±0.94^{b}	95.89±1.90^{a}	95.65±2.61^{a}	93.95±2.84^{ab}	96.25±1.46^{a}	95.71±2.49^{a}	0.026
OM aDUC	94.24±2.75	95.47±2.74	95.73±2.61	93.86±3.25	94.61±4.23	95.34±3.66	0.274
CP aDUC	88.92±0.83^{b}	92.17±1.42^{a}	93.77±2.58^{a}	88.00±2.7^{b}	93.74±2.22^{a}	91.35±3.98^{ab}	0.001
NDF aDUC	92.91±3.05	94.24±3.25	93.65±2.47	93.16±1.44	93.79±2.72	93.88±2.18	0.957

^{a,b,c}: Means the same letter on the same row is not significantly different ($p > 0.05$); 0-: Ration without additive (R0-); 0+ : 1 g Doxycycline®/kg ration; Ca: R0- + 2 g Cyperus *alternifolius*/kg ration; Ep: R0- + 2 g *Echinochloa pyramidalis*/kg ration; Ta: R0- + 2 g *Typha angustifolia*/kg ration; Ic : R0- + 2 g *Imperata cylindrica*/kg ration; p: P-value; aDUC: Apparent digestive utilisation coefficient; DM: Dry matter; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fiber

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Table 5. The carcass yields and relative weights of broiler chickens' organs with respect to phyto-additives at the age of 49 days

Caracteristics (% LBW)	Control		Phyto-additives (2 g/kg feed)				p
	0-	0+	Ca	Ep	Ta	Ic	
Carcasse yeild	75.16 ± 2.89	80.10 ± 3.42	78.37 ± 2.80	76.08 ± 4.46	77.74 ± 3.42	77.69 ± 5.32	0.084
Head	2.08 ± 0.15	2.07 ± 0.27	2.19 ± 0.20	2.17 ± 0.18	2.07 ± 0.17	2.26 ± 0.37	0.357
Legs	3.28 ± 0.33	3.22 ± 0.49	3.52 ± 0.59	3.41 ± 0.53	3.26 ± 0.57	3.37 ± 0.62	0.815
Liver	1.74 ± 0.27	1.71 ± 0.36	1.82 ± 0.22	1.62 ± 0.17	1.66 ± 0.32	1.60 ± 0.25	0.497
Heart	0.47 ± 0.05	0.41 ± 0.07	0.46 ± 0.06	0.49 ± 0.06	0.46 ± 0.07	0.50 ± 0.10	0.087
Abdominal fat	1.63 ± 0.65	1.64 ± 0.54	1.69 ± 0.43	1.56 ± 0.47	1.44 ± 0.45	1.71 ± 0.36	0.856

[a,b,c]: Means the same letter on the same row is not significantly different (p > 0.05); LBW: Live Body Weight, 0-: Diet without additive (R0-), 0+: 1 g Doxycycline®/kg diet, Ca: R0- + 2 g Cyperus alternifolius/kg diet, Ep: R0- + 2 g Echinochloa pyramidalis/kg diet, Ta: R0- + 2 g Typha angustifolia/kg diet, Ic: R0- + 2 g Imperata cylindrica/kg diet, p: P-value

Table 6. The gut microbiota counts (Log_{10} CFU) with respect to the phyto-additives in broiler chickens at 49 days old

Bacteria count (Log_{10}CFU)	Control		Phyto-additives (2 g/kg feed)				p
	0-	0+	Ca	Ep	Ta	Ic	
Escherichia coli	2.25 ± 0.52	1.07 ± 0.33	1.34 ± 0.62	1.85 ± 0.36	1.56 ± 0.84	1.26 ± 0.26	0.056
Salmonella	2.11 ± 0.76	1.22 ± 0.22	1.49 ± 0.43	1.56 ± 0.78	1.27 ± 0.39	1.45 ± 0.50	0.279
Lactobacilli	0.85 ± 0.21^{b}	2.68 ± 0.96^{a}	1.79 ± 0.82^{ab}	0.86 ± 0.30^{b}	2.43 ± 0.67^{a}	1.79 ± 0.82^{ab}	0.001

[a,b,c]: Means the same letter on the same row is not significantly different (p > 0.05); LBW: Live Body Weight, 0-: Diet without additive (R0-), 0+: 1 g Doxycycline®/kg diet, Ca: R0- + 2 g Cyperus alternifolius/kg diet, Ep: R0- + 2 g Echinochloa pyramidalis/kg diet, Ta: R0- + 2 g Typha angustifolia/kg diet, Ic: R0- + 2 g Imperata cylindrica/kg diet, p: P-value

Table 7. The immune system parameters of 49 days old broiler chickens with respect to phyto-additives

Parameters	Control		Phyto-additives (2 g/kg feed)				p
	0-	0+	Ca	Ep	Ta	Ic	
Spleen weight (%LBW)	0.11 ± 0.05^{b}	0.24 ± 0.10^{a}	0.22 ± 0.05^{a}	0.23 ± 0.05^{a}	0.21 ± 0.05^{a}	0.20 ± 0.07^{a}	0.001
Spleen volume (ml)	3.60 ± 1.35^{b}	6.50 ± 0.97^{a}	6.30 ± 1.34^{a}	6.30 ± 1.25^{a}	6.60 ± 1.58^{a}	6.30 ± 1.70^{a}	0.001
BF weight (%LBW)	0.13 ± 0.02^{b}	0.23 ± 0.05^{a}	0.21 ± 0.05^{a}	0.22 ± 0.05^{a}	0.20 ± 0.05^{a}	0.20 ± 0.06^{a}	0.001
BF volume (ml)	3.10 ± 0.88^{b}	5.30 ± 1.16^{a}	5.60 ± 1.16^{a}	3.40 ± 0.84^{b}	5.20 ± 1.40^{a}	5.30 ± 1.42^{a}	0.001
Granulocytes (%)	3.30 ± 0.86	2.48 ± 0.80	2.80 ± 0.78	3.08 ± 0.62	3.10 ± 0.94	2.72 ± 0.71	0.519
Lymphocytes (%)	83.28 ± 3.1^{a}	79.32 ± 4.11^{ab}	79.73 ± 3.86^{ab}	74.07 ± 3.99^{c}	79.42 ± 3.06^{ab}	76.83 ± 4.77^{bc}	0.008
Globulins (g/dL)	3.43 ± 0.85	3.90 ± 1.00	3.73 ± 0.81	3.58 ± 0.99	3.94 ± 1.10	3.85 ± 0.71	0.352

[a,b,c]: Means the same letter on the same row is not significantly different (p > 0.05); 0-: Diet without additive (R0-), 0+: 1 g Doxycycline®/kg diet, Ca: R0- + 2 g Cyperus alternifolius/kg diet, Ep: R0- + 2 g Echinochloa pyramidalis/kg diet, Ta: R0- + 2 g Typha angustifolia/kg diet, Ic : R0- + 2 g Imperata cylindrica/kg diet, p: P-value, BF: Bursa of Fabricius, LBW: Live Body Weight

Table 8. Effects of phyto-additives on hematological parameters in 49 days old broiler chickens

Parameters	Control		Phyto-additives (2 g/kg feed)				p
	0-	0+	Ca	Ep	Ta	Ic	
WBC (10^9/ul)	177.78 ± 17.67	151.78 ± 24.06	159.52 ± 12.11	158.88 ± 22.89	153.15 ± 19.24	155.63 ± 21.62	0.271
RBC (10^{12}/ul)	3.04 ± 0.21	3.49 ± 0.23	3.47 ± 0.53	3.26 ± 0.42	3.14 ± 0.75	3.63 ± 0.62	0.302
HGB (g/dl)	13.45 ± 2.83	15.25 ± 3.84	15.97 ± 4.19	13.62 ± 3.34	15.23 ± 4.44	14.50 ± 3.32	0.116
HCT (%)	37.32 ± 2.68^{b}	41.85 ± 4.03^{ab}	43.23 ± 5.78^{a}	36.95 ± 3.53^{b}	43.67 ± 5.63^{a}	40.85 ± 3.9^{ab}	0.046
PLT (10^9/ul)	1.17 ± 0.41	2.33 ± 1.51	2.00 ± 0.89	1.67 ± 0.82	2.50 ± 1.38	2.67 ± 1.86	0.316
MCV (f/L)	123.00 ± 1.86	125.10 ± 2.20	124.98 ± 3.41	122.47 ± 2.83	123.57 ± 2.78	123.72 ± 2.34	0.449

[a,b,c]: Means the same letter on the same row is not significantly different (p > 0.05); 0-: Diet without additive (R0-), 0+: 1 g Doxycycline®/kg diet, Ca: R0- + 2 g Cyperus alternifolius/kg diet, Ep: R0- + 2 g Echinochloa pyramidalis/kg diet, Ta: R0- + 2 g Typha angustifolia/kg diet, Ic: R0- + 2 g Imperata cylindrica/kg diet; p: P-value, WBC: white blood cells, RBC: red blood cells, HGB: Haemoglobin, HCT: Haematocrit, PLT: Blood platelets, MCV: Mean cell volume

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Table 9. Effects of phyto-additives on biochemical parameters of 49 days old broiler chickens

Parameters	Control			Phyto-additives (2 g/kg feed)			p
	0-	0+	Ca	Ep	Ta	Ic	
AST (UI/L)	177.62 ± 24.56	184.00 ± 56.58	209.96 ± 68.14	152.01 ± 24.22	195.61 ± 35.34	197.94 ± 65.09	0.264
ALT (UI/L)	55.50 ± 10.04	66.38 ± 16.55	43.88 ± 6.29	48.00 ± 11.48	44.63 ± 6.84	46.38 ± 10.81	0.072
Urea (mg/dL)	3.46 ± 0.50	3.53 ± 0.70	3.44 ± 0.56	3.41 ± 0.86	3.79 ± 0.66	3.77 ± 0.62	0.748
Creatinine (mg/dL)	1.67 ± 0.38	1.91 ± 0.51	1.63 ± 0.39	1.68 ± 0.45	1.87 ± 0.40	1.76 ± 0.56	0.764
Total Protein (g/dL)	5.62 ± 0.38	6.40 ± 1.16	7.06 ± 1.16	6.09 ± 0.99	6.90 ± 1.59	6.94 ± 1.09	0.090
Albumin (g/dL)	3.90 ± 0.41	4.44 ± 0.67	4.42 ± 0.86	4.35 ± 1.10	4.59 ± 1.06	4.80 ± 1.24	0.535
Tryg (mg/dL)	83.22 ± 4.88	81.16 ± 6.11	83.10 ± 10.12	75.00 ± 4.54	84.36 ± 5.89	83.56 ± 6.48	0.071
Total Chol (mg/dL)	158.50 ± 8.52	147.88 ± 12.71	142.13 ± 12.92	143.75 ± 17.28	151.25 ± 16.12	149.38 ± 12.43	0.218
HDL- Chol (mg/dL)	76.28 ± 7.34^{b}	88.78 ± 10.66^{a}	76.87 ± 6.67^{b}	82.86 ± 13.39^{ab}	88.54 ± 10.66^{a}	88.93 ± 9.45^{a}	0.030
LDL-Chol (mg/dL)	67.13 ± 11.32	68.25 ± 14.69	62.24 ± 16.11	69.03 ± 9.62	62.53 ± 22.43	53.64 ± 12.39	0.338

^{a,b,c}: Means the same letter on the same row is not significantly different (p > 0.05); 0-: Diet without additive (R0-), 0+: 1 g Doxycycline®/kg diet, Ca: R0- + 2 g Cyperus alternifolius/kg diet; Ep: R0- + 2 g Echinochloa pyramidalis/kg diet, Ta: R0- + 2 g Typha angustifolia/kg diet, Ic: R0- + 2 g Imperata cylindrica/kg diet, p: P-value, AST: Aspartate amino transferase, ALT: Alanine amino transferase, Tryg: Tryglyceride, Total Chol: Total cholesterol, HDL-Chol: HDL-cholesterol, LDL-Chol: LDL- cholesterol

DISCUSSION

The addition of-phyto-additives in the broiler chicken feed had no significant effects on feed intake compared to the controls throughout the study period. This result is in contradiction with the findings of Durrani et al. (2008), who reported that feed intake in broilers decreased significantly with the incorporation of neem leaf powder (4%) in the drinking water. This decrease could be explained by the presence of salannin (1.3 mg/g), a triterpenoid contained in neem leaves that is able to inhibit feed intake in chickens by reducing their appetite (Singh, 2015). The variability of the results between these two studies could be explained by the difference between the phyto-additives used, the incorporation rates, the diets and the rearing conditions.

Throughout the study period, a significant increase in live weight and weight gain was observed in broilers fed diets supplemented with *C. alternifolius*, *T. angustifolia*, and *I. cylindrical*. Similar results were obtained by Zainali et al. (2009), who noted that supplementation of broiler feed with turmeric rhizome powder at a dose of 10g/kg significantly increased live weight and weight gain in broilers. In contrast, Rahmatnejad et al. (2009) reported that the addition of 2g/kg turmeric rhizome powder to broiler feed did not significantly affect live weight and weight gain in broilers. The increases in weight gain and live weight observed in the present study could be attributed to the anti-inflammatory and antioxidant activities induced by the phenolic compounds contained in these plants. According to Humphrey and Klasing (2004), the use of anti-inflammatory drugs could reduce inflammation which has an energetic cost for the animal to the detriment of its growth. Tchoffo et al. (2019) reported that substances with antioxidant properties could reduce the reactive oxygen species that attack the animal cell membrane and consequently increase the cell membrane thickness and animal weight.

The increase in live weight and weight gain could be linked to increased lactic acid bacteria in the digestive tract of chickens recorded in the present study. Lactobacilli regulate the intestinal flora by selectively eliminating pathogenic bacteria such as *Escherichia coli* and *Salmonella* by producing bacteriocins and hydrogen peroxides (Elaroussi et al., 2008). They compete with pathogenic bacteria for nutrients and occupation of attachment sites on the intestinal mucosa (Fooks and Gibson, 2002). This increase in the number of lactic acid bacteria and the decrease in pathogenic microbes in the digestive tract of chickens could explain their good health and improvement in growth.

The incorporation of *C. alternifolius*, *T. angustifolia*, and *I. cylindrica* in the diet significantly decreased feed conversion ratio in broilers. This decrease should be the result of an increase in weight gain in chickens fed these phyto-additives. The decrease in feed conversion in this study would be the logical consequence of a significant increase in weight gain and the trend of decreasing feed intake in chickens induced by diets containing this phyto-additive. Supplementing the broiler's diet with powdered rhizome of *T. angustifolia* and *I. cylindrical*, induced a significant increase in DM

digestibility compared to the negative control. Meanwhile, CP digestibility increased significantly with the incorporation of *C. alternifolius* and *T. angustifolia* in the diet, compared to the negative control. The increase in DM and CP digestibility induced by these phyto-additives could be due to the phenolic compounds, flavonoids, terpenoids, and sterols contained in these plants, which stimulated the secretion of enzymes that improve the digestibility of feed components, thus increasing their availability in the digestive tract and their susceptibility to be utilized by the chickens. Phenolic compounds increase the villi/crypt ratio in the gut, which would indirectly increase the surface area for nutrient absorption, thus improving their uptake, and consequently the growth performance of the animals (Kothadia et al., 2018). The results of the present study are contrary to those of Brenes and Roura (2010) reported that the incorporation of doses of grape seed extract (15, 30, and 60 g/kg) in the diet of broilers had no significant effect on protein digestibility. The digestibility of OM and fiber was not affected by the different treatments. This could be explained by the fact that the bioactive compounds in these different plants did not effectively stimulate the production of enzymes responsible for cutting the bonds of these feed components.

Although carcass yield tends to increase with the inclusion of these additives in the diet, no significant differences were recorded among the treatment groups. The increase in carcass yield is associated with an increase in live weight and weight gain in chickens fed these phyto-additives. In contrast, Nouzarian et al. (2011) reported that supplementation of broiler diets with turmeric rhizome powder (3.3, 6.6, and 10g/kg) significantly reduces abdominal fat weight compared to control. The result of the present work is in agreement with those of Ouedraogo et al. (2021), who noted that the incorporation of turmeric rhizome powder at a rate of 1.5% had no significant effects on carcass characteristics in broilers.

The incorporation of the different additives in feed did not have a significant effect on *E. coli* and *Salmonella* counts in the digestive tract of chickens. On the other hand, the addition of *C. alternifolius* and *T. angustifolia* in feed induced a significant increase in lactic acid bacteria counts in the broiler chickens' gut. This could be due to the antibacterial properties induced by the phenolic compounds and terpenoids contained in these plants, which significantly reduced the proliferation of pathogenic bacteria in the digestive tract, thus favoring the multiplication and colonization of the digestive tract by lactic acid bacteria. The latter plays the role of regulator for the intestinal flora by eliminating pathogenic bacteria, such as *Salmonella* spp. and *E. coli* through the production of bacteriocins and hydrogen peroxides (Elaroussi et al., 2008). They compete with pathogenic bacteria for nutrients and occupation of attachment sites on the intestinal mucosa. This increase in lactic acid bacteria count in the digestive tract of chickens explains the good health and improved growth performance observed.

The weights and volumes of the spleen and bursa of Fabricius significantly increased with the incorporation of the phyto-additives in the diet. The increase in weights and volumes of immune organs in chickens suggested that they participated in the increased production of immune cells by these organs, thereby increasing the immunological defense capacity in these animals. The increase in the defense capacity of chickens against external aggression induced by secondary metabolites of these phyto-additives would explain the good health status observed in chickens and the improvement in their growth performance. Supplementing feed with rhizome of *E. pyramidalis* significantly decreased Lymphocyte count in the blood of chickens. This decrease suggests that chickens are more susceptible to infection. This could explain the decrease in growth performance observed in chickens fed diet supplemented with this rhizome compared to other phyto-additives. However, the incorporation of supplements in feed did not significantly affect the granulocyte count and the number of globulins in the blood of broilers. These results are contradictory to those of Hassan and Awad (2017), who concluded that the addition of 5g thyme powder/kg feed, significantly increases the serum concentration of white blood cells and globulins in broiler chickens.

With the exception of the hematocrit level, which increased significantly with the incorporation of *C. alternifolius* and *T. angustifolia* in feed, all other hematological parameters studied were not affected by the different treatments. On the other hand, Toghyani et al. (2010) found that the inclusion of thyme powder in the broiler diet had no significant effect on hemoglobin levels, white blood cells, and red blood cell counts. The increase in hematocrit levels in the blood of chickens on diets containing *C. alternifolius* and *T. angustifolia* rhizome powder in the present work reflects good oxygen and nutrient transport in the blood, leading to accelerated growth of chickens.

The addition of different phyto-additives in the broiler's feed did not have significant effects on biochemical parameters, except HDL-cholesterol level, which significantly increased with the incorporation of *T. angustifolia* and *I. cylindrica* in the diet. Ali et al. (2007)

indicated that the supplementation of broiler feed with thyme powder could decrease HDL and total cholesterol levels in blood serum.

In the present study, the increase in high-density lipoproteins responsible for transporting cholesterol did not use in the target cells to the liver for their elimination, suggesting a considerable decrease in the risk of cardiovascular diseases, sometimes caused by excessive deposition of cholesterol in the arteries by low-density lipoproteins, leading to the sudden death of chickens. The results of the present work are similar to those of Oleforuh-Okoleh et al. (2015), who concluded that the incorporation of 50 ml aqueous extract of ginger, garlic, and ginger-garlic mixture per liter of drinking water has no significant effect on the serum urea concentration.

CONCLUSION

Supplementing feed with powdered rhizomes of *C. alternifolius* and *T. angustifia* at 2g/kg of feed improves feed digestibility, growth performance, and the immunity system, and increases lactic acid bacteria count in broiler's gut. *E. pyramidalis* and *I. cylindrica* induced poor weight gain and high feed conversion ratio, compared to the results obtained with the antibiotic (positive control). It would be useful to extract, isolate and quantify the major bioactive compounds present in each phyto-additive studied and assess their individual effects on the growth performance of broilers.

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Authors' contributions

Nyembo Camile, Kana Jean Raphael and Ciza Pascaline conceived, designed the research and wrote the manuscript. Tchoffo Hervé, Amani Innocent, Tchakounte Mael, Tindo Romario, and Tabounda Evariste collected the data, carried out data analysis, and wrote the manuscript. Tchoun Gilchrist and Edie Wilfried revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflict of interest.

Ethical consideration

The authors have ensured that this article respects the ethical issues of the journal, such as plagiarism, fabrication and/or falsification of data, permissions to publish and duplicate publication.

Availability of data and materials

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

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