Rovedar
JWPS
Journal of World's Poultry Science. 2023; 2(4): 39-46.
DOI: 10.58803/jwps.v2i4.20
http://jwps.rovedar.com/

Research Article

Effects of Graded Levels of *Typha angustifolia* Rhizomes on Intestinal Microflora, Immune System, and Growth Performance of Broiler Chickens

Camile Kondo Nyembo[1], Herve Tchoffo[2,*], Margaret Momo Chongsi[2], Pascaline Azine Ciza[3], Innocent Murhula Amani[1], Leslie Tsopingni Tieubou[2], and Raphaël Jean Kana[2]

[1] Department of Animal Sciences and Plant Technology, Faculty of Agronomic Sciences, La Sapientia Catholic University of Goma, Democratic Republic of Congo
[2] Department of Animal Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon
[3] Department of Animal Sciences, Faculty of Agricultural and Environmental Sciences, Evangelical University in Africa, Democratic Republic of Congo

* ***Corresponding author:*** Herve Tchoffo, Department of Animal Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon. Email: tchoffo.herv@yahoo.fr

ARTICLE INFO

Article History:
Received: 02/10/2023
Revised: 03/11/2023
Accepted: 10/11/2023
Published: 25/12/2023

check for updates

Keywords:
Broilers
Feed digestibility
Growth performance Immunity
Microbial flora
Phyto-additive
Typha angustifolia

ABSTRACT

Introduction: The ability of growth-promoting antibiotics to accumulate in livestock products such as meat, eggs, and milk, and the antibiotic resistance conferred on bacteria has prompted researchers to turn to phyto-additives. The present study was designed to evaluate the potential of *Typha angustifolia* rhizome powder as an alternative to antibiotic growth promoters in broiler chicken.
Materials and methods: For this purpose, 512 one-day-old chickens of the Cobb500 strain were randomly distributed in a completely randomized design of 8 treatments of 16 chicks replicated four times. Experimental diets involved adding 1 g of Doxycycline® (0+), and 1, 2, 4, 6, 8, and 10 g of *Typha angustifolia* per kilogram of feed to a control diet, respectively.
Results: The main results revealed that feed intake was not significantly affected by the different treatments. Over the study period, supplementing chicken with 6 g of *T. angustifolia*/kg increased live weight and weight gain by about 6.72% and 6.82%, respectively, compared to the negative control. Similarly, this phyto-additive, at the rate of 6 g/kg, decreased the chicken feed conversion ratio by 12.83% compared to the control without additives. The 6 g of *Typha angustifolia*/kg of feed induced a significant increase in the weight, length, and density of the intestine, compared to the negative control diet. This phyto-additive, whatever the rate, significantly increased the number of lactic acid bacteria, and the digestibility of crude protein and dietary fiber compared to the negative control. Apart from the serum concentration of total cholesterol and high-density cholesterol, which increased significantly with 1 g of *Typha angustifolia* compared to the negative control, all the haemato-biochemical parameters were not significantly affected by this phyto-additive, whatever the the inclusion rate.
Conclusion: In the condition of the present study, it was concluded that 6 g of *Typha angustifolia*/kg of feed can be used as a substitute for antibiotics growth promoters in animal feed.

1. Introduction

Due to resistance and health risks associated with the use of antibiotics as growth activators, their use has been banned in animal feed, with the consequent resurgence of morbidity and mortality, and reduced growth performance, leading to a decline in the economic profitability of farms[1]. Faced to this situation, the search for alternatives to antibiotics that have the potential to improve animal performance has been intensified. Among these alternatives, products of plant origin such as spices, essential oils, and phyto-additive medicinal plants occupy a very important place[2-6].

The medicinal plants used in pharmacopoeia have

▶ *Cite this paper as*: Nyembo CK, Tchoffo H, Chongsi MM, Ciza PA, Amani IM, Tieubou LT, Kana RJ. Effects of Graded Levels of *Typha angustifolia* Rhizomes on Intestinal Microflora, Immune System, and Growth Performance of Broiler Chicken. Journal of World's Poultry Science. 2023; 2(4): 39-46. DOI: 10.58803/jwps.v2i4.20

preventive or curative properties with regard to certain functional disorders or pathological states in humans and animals[7]. Rich in phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, and quinones, these plants exhibit antibacterial, antioxidant, antiviral, antifungal, antiparasitic, immunomodulatory properties, and stimulatory effects on the digestive tract[7-11]. One of the medicinal plants classified as a phyto-additive is *Typha angustifolia* (*T. angustifolia*)[11]. The rhizomes of this plant contain active ingredients, such as flavonoids, phenols, sterols, and triterpenoids, providing antimicrobial, antiparasitic, immunomodulatory properties, and stimulatory effects on the digestive tract[7,11,12]. Varghese et al.[12] reported antibacterial activities of *Typha angustifolius* extracts on *Escherichia coli* and *Staphylococcus aureus*. Bashige et al.[7] noted antimicrobial activities of *Typha angustifolia* rhizome powder on *Staphylococcus aureus*, *Candida albican,* and *Salmonella typhi*. According to Nyembo et al.[11] the powder from the rhizomes of *T. angustifolia* at the rate of 2 g/kg as an additive in the diet of chicken improves growth performance, increases the number of lactic acid bacteria and the digestibility of crude protein, and reinforces the defense system. In this regard, supplementing broilers with the optimum rate of *T. angustifolia* could yield more significant improvements in growth performance and enhance their defense mechanisms against pathogens. The present study aimed to evaluate the effects of adding *T. angustifolia* rhizome powder into the diet at the optimum rate on the growth performance, intestinal microbial flora, feed digestibility, immune system response, and the haemato-biochemical profile of broiler chickens.

2. Materials and Methods

2.1. 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 with respect to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2. Study zone

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

2.3. Phyto-additive

Typha angustifolia was collected at the vegetative stage near the Natural Science Research Center (CRSN) of Lwiro, 30 km from the city of Bukavu in the Democratic Republic of Congo. The plant's identity was confirmed at the CRSN Lwiro herbarium. Following the harvest, the rhizomes were meticulously separated from other plant components, subsequently undergoing a drying process in shaded open air. The dried rhizomes were then ground using a Nima brand mill, and the resulting powder was stored in hermetically sealed boxes, designated for use as a feed additive. The phytochemical analysis of the powder of the rhizomes of this plant according to the standard methods described by Harborne revealed the presence of phenols, flavonoids, sterols, and triterpenoids[13].

2.4. Chickens and prophylaxis

A total of 512 one-day-old chickens of Cobb500 strain weighing 38 g were randomly allocated in a completely randomized design to eight treatments with four replicates of 16 chicks each, including eight males and eight females. As soon as the chickens arrived at the brooder house, an anti-stress (5 g of INTROVITA+WS® in 2 liters of water) was administered for the first 3 days. Then, they were vaccinated against infectious bronchitis (H52, Holland) and Newcastle disease (Hitchner B1®, Holland) on day 7 and against Gumboro's disease (CEVAC® TRANSMUNE IBD, Holland) on day 10 with a booster of all vaccines on day 18. An anti-stress (5 g in 2 liters of water) was administered through drinking water before and after each weighing and vaccination of the chicks.

2.5. Experimental diets

A control diet without additives (Table 1) was Formulated.

Table 1. Composition of experimental diets in the starter and grower phases of broiler chickens

Ingredients (%)	Starter phase (1 -21 days)	Grower phase (22-49 days)
Maize	60	67
Cotton seed meal	5	5
Soya bean meal 49	22	15
Fish meal	5	5
Wheat bran	2	2
Oyster shell	1	1
CMAV 5%*	5	5
Total	100	100
Analyzed chemical composition		
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 (%)	23.01	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
Cellulose (%)	2.43	2.61

*CMAV 5%: Mineral Nitrogen and Vitamin Complex: Crude protein: 40%, Calcium: 8%, Phosphorus: 2.05%, Lysine: 3.3%, Methionine: 2.40%, Metabolizable energy: 2078 kcal/k, 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, Vit B12: 4mg, Iron: 8000 mg, Cu: 2000 mg, Zn: 10,000 mg, Se: 20 mg, Mn: 14,000 mg, CP: Crude protein, ME: Metabolizable energy; CC: Crude cellulose; DM: Dry matter

Subsequently, seven additional diets were prepared by adding 1 g of Doxycycline® (0+) along with 1, 2, 4, 6, 8, and 10 g of *T. angustifolia* rhizome powder/kg of food. The experiment lasted 49 days.

2.6. Data collection and studied parameters

Throughout the study period, data on feed intake and live weight were collected weekly, while feed conversion ratio was calculated by dividing feed intake by the weight gain of the corresponding week. At 49 days of age, 10 chickens (5 males and 5 females) were randomly selected by 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 the density of the intestine was calculated by dividing the weight of the intestine by its length.

At the beginning of the trial and subsequently every 7 days, the chickens in each experimental unit were weighed using a precision (1 g) electronic balance. Feed conversion ratio (FCR) was calculated according to the following Formula 1:

$$\text{Feed conversion ratio} = \frac{\text{Amount of feed consumed (g)}}{\text{Weight gain (g)}}$$

Formula 1

2.7. Feed digestibility and microbial flora

The apparent digestive utilization coefficients (aDUC) of feed components were evaluated on six chickens (three males and three females) per treatment for 3 consecutive days. The chickens were transferred to digestibility cages and subjected to a 3-day adaptation period. After 3 days of adaptation, the tarpaulins were placed under cages to facilitate the collection of feces from each repetition. The feed was weighed before being distributed to the chickens, and the refusals were collected and weighed every day for 3 consecutive days. After collection, the samples of feces were dried in an oven at 60°C until a constant weight was obtained to determine the content of dry matter (DM) and organic matter (OM), according to the process described by A.O.A.C[14]. Dietary fibers (NDF) were determined using the method of Van Soest et al.[15] and crude protein by the Kjedhal method.

At 49 days of age, samples of feces were taken from the cloaca of four chickens per treatment (two males and two females), using swabs, then transported immediately to the laboratory for the quantification of lactic acid bacteria, *Escherichia coli,* and *salmonella*. The quantification was conducted on lactobacilli MRS AGAR (Acumedia®, India) for lactic acid bacteria, MacConkey AGAR (Liofilchem® Diagnostic, India) for *Escherichia coli*, and SS AGAR (Liofilchem® Diagnostic, India) for *Salmonella*, each referencing ISO standards (ISO 9001, ISO 610028, and ISO 610042, respectively).

The inoculum was prepared by decimal dilutions, which consisted of 9 ml of physiological water in tubes numbered at their base by the sample type and the dilution number. Then, the swab carrying the sample was introduced into the first tube. The latter was stirred to homogenize the solution (S1), then 1 ml of S1 was taken using a micropipette and introduced into the second tube to complete the solution to 10 ml, the dilution was thus obtained 10^{-2}. After homogenization of 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 each was introduced into a Petri dish[16].

2.8. Immune system and hemato-biochemical profiles

During the evaluation of the carcass, the lymphoid organs (bursa of Fabricius and spleen) of six chickens/treatment were removed, weighed, and their indices were calculated according to Stice[17] as following Formula 2:

$$\text{Organ index} = \frac{\text{Organ weight (g)}}{\text{Fasting live weight (g)}} \times 100$$

Formula 2

In the next step, 5 ml of blood from the sacrificed chickens. This blood was divided into test tubes with anticoagulant for the quantification of white blood cells, red blood cells, hemoglobin, hematocrits, blood platelets and mean corpuscular volume using the Urtit 3000 plus haematimeter kit (manufactured in China) and in tubes without anticoagulants for the quantification of Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), urea, creatinine, triglycerides, total cholesterol, Hight density lipoproteins-cholesterol, and low-density lipoproteins cholesterol using the commercial kit (Chronolab®, manufactured in Barcelona, Spain). The quantification of immune cells, including granulocytes, lymphocytes, and proteins of the immune system, such as albumin and globulins, was carried out according to the instructions of the Urtit 3000 plus kit (manufactured in China).

2.9. Statistical analysis

All data collected was subjected to a one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate the means at the 5% significance level. The Statistical Package for Social Sciences (SPSS version 20) was used for the analyses.

3. Results

3.1. Growth performance

Regardless of the study period, feed intake remained unaffected by the increasing levels of *T. angustifolia* in the diet ($p > 0.05$). During the starter phase (1-21 days), different treatments induced comparable live weight gain ($p > 0.05$), compared to controls. Over the entire study period, supplementation of the chicken diet with 6 g of *T. angustifolia*/kg increased weight gain by 204.79 g compared to the negative control ($p < 0.05$). Similarly, this phyto-additive, at the inclusion rate of 6 g/kg, decreased ($p < 0.05$) the chicken feed conversion ratio by 12.83% compared to the control without additive (Table 2).

Table 2. Effects of adding *T. angustifolia* rhizome to the diet on the growth performance of broiler chickens

Period (days)	Control		Incorporation rate (g/kg)						P-value
	0-	0+	1	2	4	6	8	10	
Feed intake (g)									
1-21	1135.20 ± 46.34	1080.95 ± 26.59	1116.78 ± 46.37	1024.28 ± 37.61	1116.78 ± 66.50	1088.98 ± 57.13	1095.43 ± 33.66	1059.81 ± 51.44	0.169
22-49	4749.00 ± 86.80	4636.55 ± 96.55	4768.06 ± 95.62	4686.72 ± 138.03	4570.35 ± 89.44	4565.75 ± 99.08	4717.41 ± 99.99	4618.90 ± 179.55	0.295
1-49	5884.2 ± 133.14	5717.5 ± 202.66	5884.85 ± 141.9	5711.69 ± 124.20	5677.18±151.92	5654.76 ± 147.20	5812.54 ± 133.66	5678.40 ± 230.95	0.112
Live weight (g)									
1-21	524.75 ± 55.90	603.51 ± 61.02	562.11 ± 33.96	600.20 ± 56.72	552.77 ± 56.32	579.65 ± 50.46	599.27 ± 62.90	577.45 ± 54.71	0.166
1-49	2841.00 ±119.24[c]	3109.69 ± 85.28[a]	2895.72 ± 114.29[bc]	2982.20 ± 104.18[abc]	2894.40±70.32[bc]	3045.79 ± 62.97[ab]	3013.23 ± 63.31[abc]	2813.87 ± 168.30[c]	0.032
Weight gain (g)									
1-21	485.17 ± 73.83	560.60 ± 56.84	522.54 ± 32.94	557.29 ± 56.72	509.86 ± 56.32	536.74 ± 34.12	556.36 ± 73.78	534.54 ± 44.71	0.223
22-49	1791.50 ± 104.62	1902.66 ± 71.99	1764.82 ± 82.21	1781.80 ± 179.32	1788.85 ± 170.39	1886.49 ± 71.24	1814.69 ± 63.11	1658.98 ± 138.96	0.337
1-49	2798.09 ±119.24[c]	3066.78 ± 85.28[a]	2852.81 ± 114.29[bc]	2939.29 ± 104.18[abc]	2851.49 ± 70.32[bc]	3002.88 ± 62.97[ab]	2970.32 ± 63.31[abc]	2770.96 ± 168.30[c]	0.032
Feed conversion ratio									
1-21	2.16 ± 0.05	1.83 ± 0.07	2.14 ± 0.12	2.03 ± 0.05	2.17 ± 0.27	1.97 ± 0.04	2.01 ± 0.07	2.12 ± 0.05	0.190
22-49	2.66 ± 0.18	2.44 ± 0.17	2.71 ± 0.17	2.65 ± 0.28	2.57 ± 0.29	2.42 ± 0.14	2.60 ± 0.13	2.80 ± 0.27	0.427
1-49	2.11 ± 0.09[a]	1.85 ± 0.12[b]	2.06 ± 0.08[a]	1.97 ± 0.08[ab]	1.99 ± 0.06[ab]	1.87 ± 0.08[b]	1.95 ± 0.05[ab]	2.08 ± 0.14[a]	0.023

[a, b, c] means same letter on the same line are not significantly different (p > 0.05); 0-: Diet without additive, 0+: 0- + 1g of Doxycycline®/kg of ration, 1: 1g/kg of feed, 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8g/kg of feed, 10: 10g/kg of feed

Table 3. Effects of adding *T. angustifolia* rhizome to the diet on the carcass yield and the digestive organs of broiler chickens

Carcass characteristics (% LW)	Control		Incorporation rate (g/kg)						P-value
	0-	0+	1	2	4	6	8	10	
Carcass yield Head	79.59 ± 1.72	83.23±1.85	80.31 ± 2.99	81.89 ± 1.05	81.70 ± 2.54	81.64 ± 1.72	82.03 ± 3.27	80.11± 5.19	0.093
Legs	2.11 ± 0.27	2.13 ± 0.26	2.27 ± 0.30	2.13 ± 0.23	2.17 ± 0.18	2.27 ± 0.39	2.26 ± 0.26	2.42 ± 0.41	0.288
Liver	3.56 ± 0.56	3.37 ± 0.49	3.62 ± 0.68	3.35 ± 0.46	3.41 ± 0.61	3.39 ± 0.63	3.60 ± 0.80	4.14 ± 0.63	0.106
Heart	1.76 ± 0.22	1.66 ± 0.20	1.85 ± 0.22	1.61 ± 0.16	1.75 ± 0.35	1.62 ± 0.31	1.86 ± 0.34	1.80 ± 0.24	0.245
Abdominale fat	0.49 ± 0.04	0.50 ± 0.10	0.48 ± 0.06	0.48 ± 0.06	0.49 ± 0.07	0.52 ± 0.08	0.50 ± 0.10	0.56 ± 0.09	0.318
Gizzard weight (% LW)	1.76 ± 0.67	1.78 ± 0.46	1.78 ± 0.45	1.63 ± 0.38	1.55 ± 0.41	1.68 ± 0.33	1.61 ± 0.43	1.87 ± 0.39	0.768
Pancreas weight (% LW)	1.62 ± 0.35	1.40 ± 0.22	1.47 ± 0.25	1.39 ± 0.20	1.40 ± 0.13	1.38 ± 0.14	1.53 ± 0.18	1.44 ± 0.17	0.188
Carcass yield	0.18 ± 0.04	0.19 ± 0.03	0.19 ± 0.03	0.22 ± 0.04	0.20 ± 0.04	0.20 ± 0.05	0.20 ± 0.05	0.22 ± 0.05	0.394
Weight of intestine (g)	92.30±11.33[b]	108.80±8.42[a]	108.80±20.71[a]	92.90±10.15[b]	112.40±8.33[a]	120.40±11.16[a]	118.40±14.52[a]	121.5±10.49[a]	0.001
Length of intestine (cm)	236±12.96[cd]	250.40±18.24[ab]	221.00±13.91[e]	229.70±17.07[de]	261.70±12.97[a]	250.80±11.13[a]	264.80±11.50[a]	259.8±13.54[ab]	0.001
Density of intestine (g/cm)	0.39 ± 0.04[c]	0.44±0.04[abc]	0.50 ± 0.11[a]	0.40 ± 0.05[c]	0.43 ± 0.04[bc]	0.48 ± 0.08[a]	0.45 ± 0.05[abc]	0.47±0.06[ab]	0.001

[a, b, c, d, e] means with the same letter on the same line are not significantly different (p > 0.05); 0-: Diet without additive, 0+: 0- +1g of Doxycycline®/kg of ration, 1: 1g/kg of feed; 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8 g/kg of feed, 10: 10 g/kg of feed, LW: Live weight

Table 4. Effects of adding *T. angustifolia* rhizome to the diet on the intestinal microbial flora of broiler chicken

Number of bacteria (Log_{10} UFC)	Control		Incorporation rate (g/kg)						P-value
	0-	0+	1	2	4	6	8	10	
Escherichia coli	2.79 ± 0.56	1.92 ± 0.52	2.64 ± 0.19	2.38±0.33	2.47±0.31	2.07 ± 0.61	2.67 ± 0.58	2.39 ± 0.37	0.092
Salmonella	2.51 ± 0.29	2.27 ± 0.13	2.46 ± 0.20	2.50±0.51	2.26±0.21	2.41 ± 0.54	2.40 ± 0.21	2.69 ± 0.56	0.632
Lactobacilli	1.53 ± 0.48[b]	2.63 ± 0.26[a]	2.78 ± 0.18[a]	2.68±0.19[a]	2.70±0.41[a]	2.45 ± 0.37[a]	2.48 ± 0.27[a]	2.50 ± 0.10[a]	0.001

[a, b] means with the same letter on the same line are not significantly different (p > 0.05); 0-: Diet without additive, 0+: 0- + 1g of Doxycycline®/kg of diet, 1: 1g/kg of feed, 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8g/kg of feed, 10: 10g/kg of feed

Table 5. Effects of adding *T. angustifolia* rhizome to the diet on the broiler chicken immune system indices

Parameters	Control		Incorporation rate (g/kg)						P-value
	0-	0+	1	2	4	6	8	10	
Spleen weight (%LW)	0.09 ± 0.02	0.09 ± 0.01	0.07±0.01	0.10±0.05	0.11±0.01	0.14±0.03	0.09 ± 0.03	0.10 ± .03	0.097
Spleen volume (ml)	4 ± 0.82	4.33 ± 0.58	3.75±0.50	5.00±1.15	4.50±1.00	5.50±0.58	4.00 ± 0.82	4.25 ±0.50	0.075
BF weight (%LW)	0.13 ± 0.04	0.08 ± 0.03	0.10 ± 0.04	0.12 ± 0.03	0.10 ±0.02	0.09 ± 0.03	0.13 ± 0.01	0.12 ±0.04	0.294
BF volume (ml)	5.00 ± 0.82	4.00 ± 1.00	5.00 ± 1.15	5.75 ± 0.96	5.00 ±0.82	4.50 ± 1.00	5.25 ± 0.60	5.25±1.26	0.429
Granulocyte level (%)	3.12 ± 0.21	2.96 ± 0.21	3.23 ± 0.23	3.57 ± 0.65	3.22±0.40	3.04 ± 0.21	3.23 ± 0.23	3.57±0.65	0.477
Lymphocyte level (%)	80.50 ± 1.80	84.4 ± 3.16	80.37 ± 0.99	79.13 ± 6.73	80.77±3.93	84.23 ± 1.80	80.37 ± 0.99	79.13±6.73	0.581
Globulines level (g/dL)	1.11 ± 0.56	1.44 ± 0.74	1.31 ± 0.81	1.97 ± 0.56	1.66 ±0.79	1.79 ± 0.71	1.45 ± 1.02	1.62 ±0.82	0.423

0-: Diet without additive; 0-: Diet without additive, 0+: 0- + 1g of Doxycycline®/kg of diet, 1: 1g/kg of feed, 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8g/kg of feed, 10: 10g/kg of feed, BF: Burse of Fabricius; LW: Live weight.

Carcass characteristics, gizzard, and pancreas weight were not significantly affected (p > 0.05) by the different treatments. Treatments induced a significant increase (p < 0.05) in the weight, length, and density of the intestine for about 23.34%, 5.9%, and 18.75%, respectively, compared to the negative control diet (Table 3).

3.2. Microbial flora

The *Escherichia coli* and *Salmonella* count, and immune system indices were insignificantly affected (p > 0.05) by the increasing incorporation rate of *T. angustifolia* in the diet (Tables 4 and 5) with the reference to controls. Supplementing broilers with this phyto-additive, whatever the rate, increased significantly the lactic bacteria count in the intestine of the chicken, compared to the diet without additive (p < 0.05).

3.3. Immune system and feed digestibility

Table 6 reports that immune system indices were not affected by the incorporation of increasing levels of *T. angustifolia* rhizome powder into the diet.

The apparent digestibility of dry matter (aDUC MS) and organic matter (aDUC MO) were not significantly affected by the powder from the rhizomes of *T. angustifolia* regardless of the supplementation amount, compared to controls (p > 0.05). This phyto-additive significantly increased the digestibility across varying supplementation rates, demonstrating its efficacy in enhancing both crude protein and dietary fiber digestion when compared to the negative control (p < 0.05).

3.4. Haemato-biochemical parameter

Apart from the serum concentration of total cholesterol and HDL-cholesterol, which increased significantly with 1 g of *T. angustifolia* in the diet, compared to the negative control (p < 0.05), all the haemato-biochemical parameters were not significantly affected by the incorporation of this phyto-additive in the diet (p > 0.05; Tables 7 and 8).

Table 6. Effects of graded levels of *T. angustifolia* rhizome in the diet on the apparent digestibility of the feed components in Cobb 500 chickens

AD (%)	Control		Incorporation rate (g/kg)						P-value
	0-	0+	1	2	4	6	8	10	
ADDM	78.60 ± 2.08	78.59 ± 1.71	78.56 ± 3.97	78.90 ± 3.05	79.18 ± 0.63	79.77±1.93	80.48±1.85	81.27 ± 0.61	0.155
ADOM	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
ADCP	85.13 ± 4.5[b]	92.64 ± 1.6[a]	93.77 ± 2.3[a]	95.79 ± 2.0[a]	94.41 ± 1.9[a]	93.19 ± 3.1[a]	93.87 ± 1.9[a]	92.68±1.67[a]	0.000
ADNDF	78.64 ± 6.2[b]	84.34 ± 2.0[a]	84.57 ± 4.4[a]	89.24 ± 2.1[a]	85.94 ± 1.7[a]	88.91 ± 0.4[a]	88.35 ± 2.0[a]	85.84 ± 2.87[a]	0.019

[a, b] means with the same letter on the same line are not significantly different (p > 0.05); 0-: Diet without additive, 0+: 0- + 1g of Doxycycline®/kg of diet, 1: 1g/kg of feed, 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8g/kg of feed, 10: 10g/kg of feed, AD: Apparent digestibility, DM: Dry matter, OM: Organic matter, CP: Crude protein, NDF: Neutral detergent fiber

Table 7. Variation of hematological characteristics of broiler chickens according to the incorporation rate of *T. angustifolia* rhizome in the diet

Blood parameters	Control		Incorporation rate (g/kg)						P-value
	0-	0+	1	2	4	6	8	10	
WBC (10^9/ul)	174.40 ± 7.46	171.17 ± 9.79	192.03±8.07	174.27±9.11	180.20 ± 6.87	171.07±7.65	192.03 ± 8.07	174.27±18.19	0.083
GR (10^{12}/ul)	3.35 ± 0.42	3.22 ± 0.65	3.35 ± 0.42	3.22 ± 0.65	3.23 ± 0.23	3.57 ± 0.65	3.22 ± 0.40	3.04 ± 0.21	0.843
RBC (10^{12}/ul)	17.13 ± 1.40	13.70 ± 2.19	17.47 ± 1.92	17.50 ± 3.69	16.90 ± 3.12	14.40 ± 1.55	17.47 ± 2.92	17.50 ± 3.69	0.359
HGB (g/dl)	38.60 ± 2.57	36.03 ± 2.54	40.67 ± 2.84	43.33 ± 8.39	40.37 ± 4.79	37.00 ± 2.52	40.67 ± 2.84	43.33 ± 8.39	0.557
HCT (%)	1.00 ± 0.50	1.33 ± 0.58	2.00 ± 0.73	2.67 ± 0.54	1.33 ± 0.58	1.07 ± 0.05	2.00 0 .67	2.67 ± 0.87	0.648
PLT (10^9/ul)	124.00 ± 1.40	122. ± 1.93	115.87±9.85	121.27±1.83	112.40 ± 12.38	121.70±0.85	112.53 ± 15.20	121.27±1.83	0.456

0-: Diet without additive, 0+: 0- + 1g of Doxycycline®/kg of diet, 1: 1g/kg of feed, 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8g/kg of feed, 10: 10g/kg of feed; WBC: White blood cells, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, PLT: Blood platelets, MCV: Mean corpuscular volume

Table 8. Variation of biochemical parameters according to the incorporation rate of *T. angustifolia* rhizome in the broiler chicken feed

Parameters	Control		Incorporation rate (g/kg)						P value
	0-	0+	1	2	4	6	8	10	
AST (UI/L)	124..55 ± 24.92	124.41 ± 18.56	105.97±16.51	100.90 ± 25.98	104.54 ± 32.11	118.98±9.83	113.09±20.14	121.51±18.41	0.136
ALT (UI/L)	36.05 ± 5.74	36.99 ± 8.29	26.77 ± 8.97	31.72 ± 9.05	32.61 ± 9.09	32.15 ± 8.40	29.97 ± 8.09	33.37 ± 9.55	0.209
Urea (mg/dL	6.88 ± 1.51	7.39 ± 2.25	8.97 ± 3.62	7.37 ± 1.20	7.38 ± 2.04	7.57 ± 1.17	6.74 ± 2.18	6.31 ± 1.87	0.367
Creatinine (mg/dL)	1.26 ± 0.21	1.31 ± 0.22	1.15 ± 0.13	1.22 ± 0.16	1.28 ± 0.15	1.47 ± 0.48	1.35 ± 0.38	1.53 ± 0.52	0.292
Total prot (g/dL)	4.73 ± 0.89	4.97 ± 1.12	4.48 ± 0.72	5.28 ± 1.00	4.76 ± 1.08	4.96 ± 1.18	5.11 ± 1.23	4.62 ± 0.61	0.787
Alb (g/dL)	3.62 ± 0.83	3.53 ± 0.68	3.17 ± 0.28	3.31 ± 0.65	3.10 ± 0.58	3.18 ± 0.69	3.66 ± 0.64	3.01 ± 0.37	0.253
Tryg (mg/dL)	99.68 ± 30.81	109.09 ± 21.41	110.90 ±38.24	103.31 ± 18.72	91.97 ± 20.26	126.34 ± 39.44	144.09 ± 69.69	123.52±31.66	0.142
Choltot (mg/dL)	150.94 ± 12.99^{b}	156.57 ± 19.00^{b}	179.54 ± 20.62^{a}	173.50 ± 27.87^{ab}	172.93 ± 20.10^{ab}	174.05 ± 18.61^{ab}	174.13 ± 18.10^{ab}	173.81±12.09^{ab}	0.002
Chol-HDL(mg/dL)	114.36 ± 10.81^{bc}	120.60 ±28.67^{bc}	155.33 ± 17.64^{a}	128.50 ± 23.64^{b}	111.27 ± 30.19^{bc}	110.35 ± 11.49^{bc}	103.42 ± 21.57^{c}	70.98±16.69^{d}	0.001
Chol LDL (mg/dL)	34.14 ± 7.63	32.91 ± 5.07	36.65 ± 8.57	40.59 ± 10.92	45.64 ± 9.28	44.55 ± 10.79	36.90 ± 8.70	46.26 ± 9.65	0.266

$^{a.\ b.\ c.\ d}$ means same letter on the same line are not significantly different ($p > 0.05$); 0-: Diet without additive, 0+: 0- + 1g of Doxycycline®/kg of diet, 1: 1g/kg of feed, 2: 2g/kg of feed, 4: 4g/kg of feed, 6: 6g/kg of feed, 8: 8g/kg of feed, 10: 10g/kg of feed, ALT: Alanine-amino-transferase; AST: Aspartate amino transferase, Chol: Cholesterol, Cho HDL: High-density cholesterol, Chol LDL: Low-density cholesterol, Prot: Proteins, Tryg: Tryglyceridestot

4. Discussion

Supplementing broilers with *T. angustifolia* rhizome powder, whatever the rate, had no significant effect on feed intake. These results corroborate the findings of Zhang et al.[18], reporting that dietary supplementation of ginger rhizome powder at the rate of 5 g/kg, did not significantly affect chicken feed intake. On the contrary, Durrani et al.[19] reported significant drops in broiler chickens' feed intake with neem leaf powder as a feed additive. The results could be partly explained by variability in study conditions regarding products tested, animals used, diets, or husbandry conditions applied. The very origin of these phyto-additives may contribute to explaining this variability.

The incorporation of 6 g of *T. angustifolia*/kg of feed increases the live weight and weight gain of chickens compared to the negative control. These results agree with those of Shihab et al.[20], who reported that supplementing the diet with 2 g neem leaf powder/kg feed significantly increased live weight and weight gain in chickens. On the contrary, Rahmatnejad et al.[21] concluded that adding 2 g/kg of turmeric rhizome powder to chicken feed has no significant effects on live weight and weight gain. The weight gain improvement recorded in the present study could be attributed to the anti-inflammatory and antioxidant activities of the phenolic compounds in *T. angustifolia* rhizomes. According to Humphrey and Klasing, these compounds reduce inflammatory reactions, which have a high energy cost for the animal, to the detriment of its growth[22]. For Tchoffo et al.[23], substances with antioxidant properties could reduce reactive oxygen species (free radicals) that attack animal cell membranes, and hence, they increase cell membrane thickness and cell weight in animals. The increase in live weight and weight gain could also be linked to the increase in the number of 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,* through producing bacteriocins and hydrogen peroxides[24,25]. They compete with pathogenic bacteria for nutrients and the occupation of binding sites on the intestinal mucosa[26]. This increase in the number of lactic acid bacteria in the digestive tract of chickens could explain their good health and the improvement in growth observed. The present results are in agreement with the findings of Rahimian et al.[27] who recorded a significant increase in lactobacilli in the digestive tract of chickens with the incorporation of black pepper powder in the diet.

The supplementation of feed by the powder of the rhizomes of *T. angustifolia* at the rate of 6 g/kg, significantly lowered the feed conversion ratio compared to the negative control. This decrease is the consequence of the significant increase in weight gain in chickens fed this phyto-additive at this precise dose. Similar results were reported by Ouedraogo et al.[28] with the addition of 1.5% turmeric rhizome powder in the broiler diet. Similarly, Herawati et al.[29] reported a decrease in broilers' feed conversion ratio with the incorporation of 2 g of ginger rhizome powder in the feed.

Although carcass yields tended to increase with the inclusion of additives in the diet, no significant differences were recorded between animal groups with respect to carcass characteristics and digestive organs. This tendency to increase carcass yield is linked to the increase in live weight and weight gain in chickens fed diets, containing this phyto-additive. The present results are in agreement with those of Ouedraogo et al.[28], who reported that the incorporation of turmeric rhizome powder at the rate of 1.5% has no significant effects on carcass characteristics in broilers. On the contrary, Nouzarian et al.[30] reported that supplementing broilers with turmeric rhizome powder at levels of 3.3, 6.6, and 10 g/kg significantly reduces the weight of abdominal fat.

Supplementing broilers with *T. angustifolia* at any rate significantly increased the digestibility of crude protein and dietary fiber, compared to the negative control. The increase in digestibility of feed components could be due to the phenolic compounds, flavonoids, terpenoids, and sterols contained in this phyto-additive. These would have stimulated the secretion of digestive enzymes, which would have improved the digestibility of these feed components, thus increasing the availability of nutrients for absorption and growth in chickens. Phenolic compounds increase the villus/crypt ratio of the intestine[31], which would indirectly increase the surface area for absorption of nutrients, thus improving their absorption and the growth of animals. The present results differ from those of Brenes and Roura, who reported that supplementing chicken with increasing levels (15, 30, and 60 g/kg) of grape seed extract had no significant effects on crude protein digestibility[32].

Apart from the serum concentration of total cholesterol and HDL-cholesterol, which increased significantly with the dietary supplementation of 1 g of *T. angustifolia* compared to the negative control, all the hematological and biochemical parameters studied were not significantly affected by *T. angustifolia*, whatever the rate of incorporation. The non-variation of the hematological parameters studied in the present study could insinuate that the incorporation rates of this phyto-additive did not exceed the harmful threshold for the health of chickens. The haemato-biochemical parameters are physiological, pathological, and nutritional indices, allowing appreciation of the state of an organism. Any change that has occurred in the constituent elements of the blood, compared to normal values, constitutes an important index for the interpretation of the physiological or metabolic state of the animal, but also and above all of the quality of the feed[33,34]. The present results contradict those of Kana et al.[35] who reported that *Dichostachys glomerata* bark powder in broiler chicken feed causes a significant decrease and increase in serum alanine-amino-transferase and aspartate aminotransferase, respectively.

5. Conclusion

In the condition of the present study, 6 g of *Typha angustifolia* can be used as an alternative to antibiotic growth promoters to balance microflora and improve intestinal health, to improve feed digestibility and growth performance in chickens with no side effects on animals and humans. It would be advisable to extract, isolate and quantify the main bioactive compounds present in this phytoadditive, and to assess their individual effects on the growth performance of broilers.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

Camile Kondo Nyembo, Hervé Tchoffo, and Raphaël Jean Kana conceived, designed the research, and reviewed the manuscript. Margaret Momo Chongsi, Pascaline Azine Ciza, Innocent Murhula Amani, and Leslie Tsopingni Tieubou collected the data, carried out data analysis, and wrote the manuscript. All authors read and approved the final manuscript.

Funding

The authors declare that no financial help was received for this work.

Availability of data and materials

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

Ethical considerations

All authors have reviewed this work for ethical problems, such as plagiarism, consent for publication, misconduct, data manipulation and/or deceit, and duplication of work.

Acknowledgments

The authors are grateful to the Faculty of Agronomy and Agricultural Sciences for providing an ideal framework for data collection.

References

1. Chardon H, and Brugere H. Uses of antibiotics in livestock and meat sectors. Notebooks health safety animal health. Meat Information Center. France: Pôle Industriel Toul Europe; 2014. Available at: http://www.gds03.fr/pages/CIV.pdf
2. Onu PN. Evaluation of two herbal spices as feed additives for finisher broilers. Biotechnol Anim Husb. 2010; 26(5-6): 383-392. DOI: 10.2298/BAH1006383O
3. Krishan G, and Narang A. Use of essential oils in poultry nutrition: A new approach. J Adv Vet Anim Res. 2014; 1(4): 156-162. DOI: 10.5455/javar.2014.a36
4. Mafouo SV, Kana JR, Raphaël J, and Kissel ND. Preservative effects of Neem oil (*Azadirachta indica*) on farm-mixed poultry feed. Int J Livest Res. 2019a; 9(3): 49-61. DOI: 10.5455/ijlr.20181027101318
5. Mafouo SV, Kana JR, and Nguepi DK. Effects of gradel levels of Azadirachta indica seed oil on growth performance and biochemical profiles of broiler chikens. Anim Nutr Prod Res. 2019b; 5(3): 442-450. DOI: 10.1002/vms3.162
6. Romario KTT, Ngouana RT, Kana ADS, Ciza PA, Donfack M, Mouchilli M, et al. Effects of neem oil (*Azadirachta indica*) on feed digestibility, growth performance and gut microbiota of broilers chikens. Sci J of Anim Sci. 2022; 10(6): 767-775.
7. Bashige V, Bakari A, Okusa P, Kalonda E, and Lumbu J. Phytochemical screening and antimicrobial activity of six edible rhizomes used in traditional medicine in Lubumbashi (DRC). Int J Biol Chem Sci. 2020;

14(4) : 1367-1380. DOI: 10.4314/ijbcs.v14i4.16

8. Dieumou FE, Teguia A, Kuiate JR, Tamokou JD, Fonge NB, and Dongmo MC. Effects of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) essential oils on growth performance and gut microbial population of broiler chickens. Livest Res Rural Dev. 2009; 21(8): 131. Available at: https://www.lrrd.cipav.org.co/lrrd21/8/dieu21131.htm
9. Ruiz-Navajas Y, Viuda-Martos M, Sendr E, Perez-Avarez JF, and Fernández-López J. *In vitro* antibacterial and antioxidant Properties of chitosan edible films incorporated with Thymus moroderi or Thymus piperella essential oils. Food Control. 2013; 30(2): 386-392. DOI: 10.1016/j.foodcont.2012.07.052
10. Shalukoma C, Bocreat J, Duez P, Pongombo C, and Visser M. Medicinal plants of the mountainous region of kahuzi-biega in the Democratic Republic of Congo: Use, accessibility and consensus of traditional healers. Bois Forets Tropiques. 2015; 326(326): 43-55. DOI: 10.19182/bft2015.326.a31282
11. Nyembo CK, Pascaline CA, Hervé T, Innocent AM, Gilchrist TD, Maël TF, et al. Effects of *Cyperus alternifolius, Echinochloa pyramidalis, Typha angustifolia* and *Imperata cylindrica* on growth performance, feed digestibility, gut microbiota, heamato-biochemical and immunity parameters in broiler chikens. J World's Poult Res. 2023; 13(2): 268-279. DOI: 10.36380/jwpr.2023.30
12. Varghese A, Gavani U, Abraham S, Parambi DGT, Sathianarayanan, and Jose A. Phytochemical screening and antimicrobial investigation of Typha angustifolia LINN. Int J Chem Sci. 2009; 7(3): 1905-1910. Available at: https://www.cabdirect.org/cabdirect/abstract/20103017329
13. Harborne JB. Methods of plant analysis. Phytochemical methods. Dordrecht, Netherlands: Springer; 1973. p. 1-32. DOI: 10.1007/978-94-009-5921-7_1
14. Association of official analytical chemists (AOAC). Official methods of analysis. 15th ed. Washington, DC, USA: 1990.
15. Van-Soest PJ, Robertson JB, and Lewis BA. Methods for dietary fiber neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991; 74(10): 3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-2
16. Association française de normalisation (AFNOR). Water tests, determination of inhibition of photobacterium, phosphoreum luminescence. National standards and national normative documents, NT90-320 experimental standard. 1991. Available at: https://www.boutique.afnor.org/fr-fr/norme/t90320/essais-des-eauxdetermination-de-linhibition-de-la-luminescence-dephotobac/fa024059/77210
17. Stice E, Telch CL, and Rizvi SL. Development and validation of the eating disorder diagnostic scale: A brief self-report measure of anorexia, bulimia, and binge-eating disorder. Psychol Assess. 2000. 12(2): 123-131. DOI: 10.1037/1040-3590.12.2.123
18. Zhang GF, Yang YZ, Wang B, Yang WR, Jiang SZ, and Gai GS. Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. Poult Sci. 2009; 88(10): 2159-2166. DOI: 10.3382/ps.2009-00165
19. Durrani FR, Sultan A, Jan M, Chand N, and Durrani Z. Immunomodulatory and growth promoting effects of neem (*Azadirachta indica*) leaves infusion in broiler chicks. Sarhad J Agric. 2008; 24(4): 655-659. Available at : https://www.aup.edu.pk/sj_pdf/IMMUNOMODULATORY%20AND%20GROWTH%20PROMOTING.pdf
20. Shihab IM, AL-Zuhari MT, Abdullah SM, and Mutar SS. Impact of supplementation Neem powder (*Azadirachta indica*) to diet broiler in immunological, physiological and productive traits. Adv Environ Biol. 2017; 11(3): 44-51. DOI: http://www.aensiweb.net/AENSIWEB/aeb/aeb/2017/March/44-51.pdf
21. Rahmatnejad E, Roshanferk H, Ashayerizadeh O, Mamooee M, and Ashayerizadeh A. Evaluation the effect of several non-antibiotic additives on growth performance of broiler chikens. J Anim Vet Adv. 2009; 8(8): 1670-1673. DOI: https://medwelljournals.com/abstract/?doi=javaa.2009.1670.1673
22. Humphrey BD, and Klasing KC. Modulation of nutrient metabolism and homeostasis by the immune system. Worlds Poult Sci J. 2004; 60(1): 90-100. DOI: 10.1079/WPS20037
23. Tchoffo Herve T, Raphaël KJ, Ferdinand N, Victor Herman N, Willy Marvel NM, et al. Effects of ginger (*Zingiber officinale*) essential oil on growth and laying performances, serum metabolites, and egg yolk antioxidant and cholesterol status in laying Japanese quail. J Vet Med. 2019 ; 2019: 7857504. DOI: 10.1155/2019/7857504
24. Grajek W, Olejnik A, and Sip A. Probiotics, prebiotics and antioxidants as functional foods. Biochim Pol. 2005; 52(3): 665-671. DOI: 10.18388/abp.2005_3428
25. Elaroussi MA, Mohamed MS, El-Barkouki EM, Abdou AM, and Hatab MH. Ochratoxicosis in broiler chickens: Functional and histological changes in target organs. Int J Poult Sci. 2008; 7(5): 414-422. DOI: 10.3923/ijps.2008.414.422
26. Fooks LJ, and Gibson GR. Probiotics as modulators of the gut flora. Br J Nutr. 2002; 88(S1): 39-49. DOI: 10.1079/BJN2002628
27. Rahimian Y, Faghani M, Davoodi SM, Rafiee A, Davoodpoor, and Nezhad MHG. Potential use of protexin probiotic and black pepper powder on Cobb 500 broiler chicks. Azarian J Agric. 2016; 3(6): 129-134. Available at: https://www.cabdirect.org/cabdirect/abstract/20173103282
28. Ouedraogo B, Nikiema ZS, Sanou J, and Zoundi SJ. Study of the supplementation of turmeric powder as a growth promoting phytobiotic in chicken feed in Burkina Faso. Int J Biol Chem Sci. 2021; 15(5): 16820-16833. Available at: https://www.ajol.info/index.php/ijbcs/article/view/220225
29. Herawati. The effect of feeding red ginger as phytobiotic on body weight gain, feed conversion and internal organs condition of broiler. Int J Poult Sci. 2010; 9: 963-967. DOI: 10.3923/ijps.2010.963.967
30. Nouzarian R, Tabeidian SA, Toghyani M, and Ghalamkari G. Effect of turmeric powder on performance, carcass traits, humoral immune responses, and serum metabolites in broiler chikens. J Anim Feed Sci. 2011; 20: 389-400. DOI: 10.22358/jafs/66194/2011
31. Huarachi-Olivera R, Duenas-Gonza A, Yapo-Patricia U, and Margiht V. Bioelectrogenesis with microbial fuel cells (MFCs) using the microalga chlorella vulgaris and bacteria communities. Electron J Biotechnol. 2018; 31: 34-43. DOI: 10.1016/j.ejbt.2017.10.013
32. Brenes A, and Roura E. Essential oils in poultry nutrition: Main effects and modes of action. Anim Feed Sci Technol. 2010; 158(1-2): 1-14. DOI: 10.1016/j.anifeedsci.2010.03.007
33. Isaac LJ, Abah G, Akpan B, and Ekaette IU. Haematological properties of different bredds and sexes of rabbits. Proceeding of the 18th Annual conference of Animal Science Associated of Nigeria. 2013; p. 24-27.
34. Etim NN, Williams ED, Akpabio U, and Offiong EEA. Haematological parameters and factors affecting their values. Agric Sci. 2014; 2(1): 37-47. DOI: 10.12735/as.v2i1p37
35. Kana JR, Mube KH, Ngouana TR, Tsafong F, Komguep R, Yangoue A, et al. Effect of dietary mimosa small bell (*Dichostachys glomerata*) fruit supplement as alternative to antibiotic growth promoter for broiler chicken. J World's Poult Res. 2017; 7(2): 27-34. Available at: https://jwpr.science-line.com/attachments/article/40/J%20World%20Poult%20Res%207(1)%2027-34,%202017.pdf