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ORIGINAL RESEARCH PAPER

Growth performance, haemato-biochemical indices of broiler chicken fed *Aristolochia indica* as a phytogenic feed additive

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Abstract: This experiment was carried out to examine the growth performance and some heamato-biochemical indices of broiler chicken fed *Aristolochia indica* (ALM) as a phytogenic feed additive. A total of 500 – 1-day old (Vencobb 450) with initial body weight of 48.0 ± 0.02 g were randomly divided to 5 groups with 5 replicates for each group. Basal diet (a corn-soya meal based diet) was sufficient in all nutrients (NRC, 1994). Birds in group 1 was fed basal diet without *A. indica* leaf meal, those in group 2, 3, 4 and 5 were fed basal diet with supplemented with *A. indica* leaf meal at 100 g, 200 g, 300 g and 400 g per 100 kg^{-1} respectively. Fresh clean water and feed were offered ad libitum. Results on phyto-constituents of Aristolochia indica leaf meal showed that it contained flavonoids (996.05 mg/100 g^{-1}), tannins (609.7 mg/100 g^{-1}), phenols (1341.8 mg/100 g^{-1}), steroids (206.17 mg/100 g^{-1}), alkaloids (151.8 mg/100g) and saponins (91.21 mg/100g). Average daily weight gain, average daily feed intake and feed conversion ratio were higher in group 4 and 5, intermediate in group 3 and lower in group 1 and 2 ($P < 0.05$). Mortality of 2.20 % was recorded in group 1 while none was recorded in the other treatment ($P < 0.05$). Pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, lymphocytes and monocyte values were significantly ($P < 0.05$) different among the groups except for basophil values ($P > 0.05$). Total protein, albumin/globulin ratio, urea, creatinine, glucose and cholesterol were influenced ($P < 0.05$) by the treatment except for alkaline phosphatase, alanine transaminase and aspartate transaminase values ($P > 0.05$). In conclusion, ALM can be fed up to 400 g in the diet of broilers without compromising their performance and health status.
Keywords: *Aristolochia indica*, broilers, hematology, serum, performance, food safety.

1. Introduction

The topic of antibiotic growth promoters and their potential replacement continues to be of great interest to poultry producers with a wide range of options now available for those who either prevented from using antibiotic growth promoter's by local regulation, or prefer to use alternative (Liz, 2020; Christine, 2021). According to the WHO (2015), about 600 million (or nearly one in ten) people become ill after consuming contaminated food with antibiotics.

The use of medicinal plant (phytogenics) has been regarded as natural alternative to antibiotics because they contain phytochemicals which are generally regarded as safe (Hashemi; Davoodi, 2010; Shittu; Alagbe, 2019). Phytochemicals used as poultry feed additives can improve animal's health and performance because of their anti-microbial, anti-stress (Wang et al., 1998) and antioxidant properties (Valenzuela, 1995), and their ability to modulate gut microbiota (Hashemi et al., 2009) and enhance immune responses (Chowdhury et al., 2018).

According to Menezes Filho (2019), the genus *Aristolochia* consists of approximately 400 to 550 species distributed in the central, tropical and

temperate regions. In general, *Aristolochia* species are climbing, decumbent, erect or prostrate herbaceous plants with rhizomes or tubers. The genus is divided into four subgenera: *Isotrema*, *Endotheca*, *Pararistolochia* and *Aristolochia*.

Aristolochia indica is a perennial plant belonging to the family Aristolochiaceae. The plant is widely distributed in tropical, sub-tropical and Mediterranean regions (Abhijit; Jitendra, 2011). It has a woody root stock and glabrous leaves with undulate margins (Das et al., 2010; Vaghasiya; Chanda, 2007). According Kamaraj et al. (2010), plants used as traditional medicine possess phytochemicals such as oxygenated terpenes, tannins, alkaloids, aldehydes and cardiac glycosides, which are responsible for the plant's biological properties.

The roots of *Aristolochia indica* contains cephradione, aristololide, stigmastenones, 2-hydroxyl-1-methoxy 4H dibenzo, ishwarone, β-silosterol-β-D-glucoside (Abhijit; Jitendra, 2011). Both the bark and the leaves are combined for the treatment of snake bite and scorpion stings (Hemlata et al., 2011). The roots are used for the treatment of cholera, leprosy, ulcers, gonorrhoea, toothache, stomachache, dysentery and joint pains (Hemlata et al., 2011).

Abhijit & Jitendra (2011) investigated the antimicrobial potency of *A. indica*. Their results demonstrated potent antimicrobial activity of Aristolochia indica against both Gram-positive and Gram-negative bacteria. *Shigella flexneri*, *Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Listeria monocytogenes* and *Bacillus cereus* were all found to be susceptible to leaf, root and stem bark extracts of *A. indica*.

Previous studies have shown that phytochemicals used in poultry feed have many functions including being antioxidant, anti-estrogenic, anti-inflammatory, immunomodulatory, anti-carcinogenic, hepato-protective, hypolipidemic amongst others (Laparra; Sanz, 2010). They can also enhance the growth of beneficial bacteria and suppressing the growth of pathogenic bacteria (Cencic; Chingwaru, 2010).

Thus, they reward the host by shaping gut microbiota in a beneficial way (Laparra; Sanz, 2010), stimulation of healing intestinal mucosa damage (Asfar et al., 2003); decrease in colonic mucosa DNA oxidative damage (Dolara et al., 2005); alteration in colon microbiota; and regulation of metabolic, immunological, and adaptive gene expression (López-Oliva et al., 2010).

Notable researchers (Oloruntola et al., 2016; Alagbe, 2021; Esonu et al., 2002; Odunsi et al., 2002; Muritala et al., 2022) have recorded positive results when medicinal plants were supplemented in the diets of broiler chickens. However, there is a dearth of information on the utilization of *A. indica* in broiler production.

Therefore, this study will help to remove the use of antibiotics as performance enhancers and promote food safety.

2. Material and Methods

2.1. Experimental location, ethical statement and preparation of Aristolochia indica leaf meal

The study was carried out at the Poultry section of Sumitra Research Institute, Gujarat, India which is located between 23º 13' N and 72º 41' E West coast, India. Experimental procedures and management was carried out according to the guidelines of the ethic committee of Sumitra Research Institute, India (RR/00A211C).

Matured and fresh leaves of *A. indica* was harvested from the Research farm of Sumitra Research Institute, Gujarat, India and sent to the Department of Crop Science for authentication and a voucher specimen number (AA/SM/008C) was assigned to it. Leaves were rinsed twice in a clean plastic bucket and air dried under a shade for 11 days until a constant weight was achieved. Dried *A. indica* leaf were grinded using an electric blender and packed into labeled polythene bags and transferred into the laboratory for phytochemical analysis.

2.2 Quantification of phyto-constituents in Aristolochia indica leaf meal was carried out according to the procedures stated by Alagbe (2024).

2.2.1 Reagents/chemicals required for analysis

Sodium hydroxide, sodium bicarbonate, *Folin-Ciocalteau's* reagent, aluminum chloride, sodium nitrate, sulphuric acid, bromocresol solution, ferric ammonium sulphate, amyl alcohol and ammonium thiocyanate solution.

2.2.2 Kits required

Test tubes, beaker, conical flask, water bath, thermometer and conical flask.

2.3. Total flavonoids analysis

Total flavonoid was estimated by adding 0.1 mL of aluminum chloride and 0.2 mL of 5 percent sodium nitrite, 2.0 grams of *A. indica* leaf meal was added to 3 mL of 1 M sodium hydroxide. The mixture was thoroughly mixed and incubated for 10 minutes at room temperature. Immediately, 10 mL of distilled water were added to the final volume. Using a UV-*Vis* spectrophotometer, the reaction mixture's absorbance at 510 nm was evaluated in comparison to a blank.

2.4. Quantification of tannins and phenolic compounds in Aristolochia indica leaf meal

2.0 g of *A. indica* leaf meal was added to 1.5 mL of *Folin-Ciocalteau* reagent in a conical flask, the mixture was covered for 5 min and kept under room temperature followed by the addition of 1.0 mL sodium bicarbonate, 10 mL distilled water and stirred before it was introduced to spectrophotometer at an optical density of 760 nm and 660 nm to determine the tannin and phenolic concentrations respectively.

2.5. Quantification of terpenoids and saponin contents in Aristolochia indica leaf meal

2.0 g of *A. indica* leaf meal was added was added to 0.8 mL nitric oxide mixed together in a test tube, the mixture was incubated for 10 minutes followed by the addition of 0.5 mL of sodium hydroxide. UV-*Vis* Spectrophotometer was adjusted at an optical density of 480 nm and 607nm to determine the concentrations of terpenoids and saponins respectively.

2.6. Quantification of steroids in Aristolochia indica leaf meal

Analysis of steroids was carried using anion exchange technique. Briefly, 2.0 g of *A. indica* leaf meal was diluted with 1.0 mL of distilled water followed by the addition of 0.5 mL ferric ammonium sulphate. The mixture was thoroughly mixed in a test tube, covered and cooled at room temperature for 10 min. UV-*Vis* Spectrophotometer was adjusted at an optical density of 480 nm to determine its concentration.

2.7. Determination of alkaloid concentration in Aristolochia indica leaf meal

2.0 g of *A. indica* leaf meal was diluted with 2 mL of phosphate buffer and 1.5 mL of bromocresol green solution were mixed together in a test tube covered and allowed to stay for 30 min before it was injected into UV-*Vis* spectrophotometer and adjusted at an optical density of 400 nm.

2.8. Management of birds and experimental design

Specially constructed battery cages measuring 200 cm by 160 cm by 120 cm (length/width/height) placed in a semi-closed pens equipped with nipple drinkers and aluminum cone feeders were properly disinfected fourteen days before the commencement of the trial. A total of 500 – 1-day old (Vencobb 450) with initial body weight of 48.0 ± 0.02 g were randomly divided to 5 groups with 5 replicates for each group. Basal diet (a corn-soya meal based diet) was sufficient in all nutrients (NRC, 1994).

Experimental diet was formulated in two phases: starter phase (0 to 21 days) and grower (22 to 42 days). Birds in group 1 was fed basal diet without *A. indica* leaf meal, those in group 2, 3, 4 and 5 were fed basal diet with supplemented with *A. indica* leaf meal at 100 g, 200 g, 300 g and 400 g per 100 kg^{-1} respectively. Fresh clean water and feed were offered ad libitum. Feed intake was estimated by subtracting left over from the feed served.

Weight gain was calculated by subtracting the initial weight of birds from the final weight expressed in gram. Average daily weight gain was calculated by dividing weight gain by the experimental period (42 days) expressed in gram. Similarly, average daily feed intake was calculated by dividing the total feed intake by the experimental period (42 days) expressed in gram. Dividing the average daily feed intake by the average weight gain equals feed conversion ratio.

2.9. Proximate analysis of basal diet

Basal diet was analyzed in the laboratory using Cole-Parmer portable NIR feed analyzer (Model: 59824-00) adjusted at a minimum and maximum wavelength of 893 nm and 1045 nm and scan time less than 1 min before the results were displayed via the visual display unit of the computer.

2.10 Blood analysis (haematology and serum biochemical indices)

At the end of the trial, 4 mL of blood was collected from the wing web of 15 randomly selected birds per group. Blood samples for haematology (2 mL) was collected into a sterile labeled bottles with anticoagulant (ethylene diamine tetraacetic acid) and kept in an ice pack before it was taken to the laboratory while the remaining 2 mL for serum analysis was transferred into sample bottles without anticoagulant.

Haematological parameters were analyzed using 5-part Auto haematology analyzer (Model: BK-6310) which work based on the principle of triangle laser scatter, flow cytometry method, 3D scatter gram analysis, impedence method for red blood cell count. For efficiency in performance, white blood cell and haemoglobin count are adjusted at a linearity range of 300 × $109/L^{-1}$ and 250 × $109/L^{-1}$ respectively.

Serum biochemical indices were analyzed using 600T/H automatic chemistry analyzer (Model: BK-600). For optimum accuracy the kit was adjusted to a sample and reagent volume of 70 µL and 350 µL, absorbance (3.5 Abs), reagent cooling (2-8 ℃), temperature control (37 ± 0.1 ℃) and wavelength > 340 nm before results are generated via the monitor of the computer attached to the kit.

2.11 Statistical analysis

The results were analyzed statistically using Statistical Package for Social Science (SPSS for Windows (IBM), version 23). The data were analyzed by using one-way ANOVA and subsequent *Duncan's* multiple range test to

determine the differences between the treatments. Results are expressed as means ± SEM. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

Table 1. Ingredient and chemical composition of basal diet from 0 – 42 days.

	0 to 21 days (Starter phase)	**22 to 42 days (Grower phase)**
Ingredients	Inclusion rate (kg^{-1})	Inclusion rate (kg^{-1})
Corn	52.00	53.00
Soya bean meal	37.00	34.10
Bone meal	5.00	6.00
Limestone	2.00	2.80
Lysine	0.20	0.20
Methionine	0.20	0.25
[a]Mineral/Vitamin premix	0.25	0.25
Salt	0.35	0.40
Palm oil	3.00	3.00
Total	100.0	100.0
Calculated analysis		
Metabolizable energy (kcal/kg)	2900.9	3200.7
Crude protein	22.85	20.96
Crude fibre	3.72	5.02
Ether extract	4.87	4.92
Calcium	1.32	1.51
Phosphorus	0.31	0.40
Methionine + Cysteine	0.52	0.61
Determined analysis		
Metabolizable energy (kcal/kg)	2980.5	3187.7
Crude protein	23.44	21.44
Crude fibre	3.39	5.72
Ether extract	5.00	5.11
Calcium	1.40	1.53
Phosphorus	0.33	0.49
Methionine + Cysteine	0.61	0.65

Note: [a]Vitamins premix each 2.5 kg contain Vit. A 12000000 IU; Vit. D3 2000000 IU; Vit. E 10000 mg; Vit. K3 2000 mg; Vit. B1 1000 mg; Vit. B2 5000 mg; Vit. B6 1500 mg; Vit. B12 10 mg; Biotin 50 mg; Pantothenic acid 10000 mg; Nicotinic acid 30000 mg; Folic acid 1000 mg; Choline chloride 250000 mg; Mineral premix contains: Mn 60000 mg; Zn 50000 mg; Fe 30000 mg; Cu 10000 mg; I 1000 mg; Se 100 mg; Co 100 mg and complete to 3.0 kg by calcium carbonate. Source: Author, 2023.

3. Results

As presented in Table 2, quantification of phyto-constituents in *A. indica* leaf meal. Phenolic compound had the highest concentration (1341 mg/100 g^{-1}) followed by flavonoids (996 mg/100 g^{-1}), terpenoids (806 mg/100 g^{-1}), tannins (609 mg/100 g^{-1}), steroids (206 mg/100 g^{-1}), alkaloids (151 mg/100 g^{-1}) and saponins (91 mg/100 g^{-1}).

Table 2. Phyto-constituents in *Aristolochia indica* leaf

meal.

Indices	Concentrations (mg/100 g^{-1})
Flavonoids	996.05
Tannins	609.7
Phenols	1341.8
Terpenoids	806.43
Steroids	206.17
Alkaloids	151.8
Saponins	91.21

Source: Author, 2023.

Performance traits of broiler chicken fed diet supplemented with *A. indica* leaf meal (ALM) is presented in (Table 3). In the starter phase (0-21 days), average daily weight gain of birds fed diet supplemented in 0 g ALM (group 1), 100 g ALM/100 kg^{-1} (group 2) were similar ($P > 0.05$) to those fed 200 g ALT/100 kg^{-1} (group 3) but significantly ($P < 0.05$) lower than those fed 300 g ALM/100 kg^{-1} (group 4) and 400 g ALM/100 kg^{-1} (group 5). Average daily feed intake were higher in group 4 and 5, intermediate in group 2, 3 and lower in group 1 ($P < 0.05$). 1.2 % mortality was recorded in group 1 while none was recorded in the other groups ($P < 0.05$). Feed conversion ratio value ranged from 1.38 to 1.53.

In the grower phase (22-42 days), average daily weight gain value which ranged from 59.31 to 85.76 g was higher in group 4 (300 g ALM) and 5 (400 g ALM) relative to the other groups ($P < 0.05$). Average daily feed intake of birds supplemented diets with 300 g ALM (group 4) were similar to those fed 400 g ALM (group 5) but significantly ($P < 0.05$) higher than those fed 100 g ALT (group 2) were similar to those fed 200 g ALM (group 3). Feed conversion ratio value ranged from 1.34 to 1.76. Conversely, in the overall production cycle (0-42 days), average daily weight gain, average daily feed intake and feed conversion ratio of birds fed diets supplemented with ALM in group 4 were similar ($P > 0.05$) to those in group 5 but significantly ($P < 0.05$) higher than the other groups.

Table 3. Performance traits of broiler chicken fed diet supplemented with *Aristolochia indica* leaf meal.

Phase: 0–21 day (Starter)	**[1]G1**	**[2]G2**	**[3]G3**	**[4]G4**	**[5]G5**	**P-value**
Index						
Number of birds	100	100	100	100	100	-
[6]IW (g/bird)	48.02 ± 0.00	48.01± 0.01	48.00 ± 0.01	48.01 ± 0.01	47.98 ± 0.01	0.35
[7]FW (g/bird)	578.22 ± 18.09^{b}	581.11 ±16.88^{b}	588.8 ± 18.40^{b}	638.11 ±16.72^{a}	642.08 ± 18.02^{a}	2.81
[8]WG (g/bird)	530.2 ± 11.56^{b}	533.1 ± 10.92^{b}	540.8 ± 12.90^{b}	590.1 ± 11.25^{a}	594.1± 10.47^{a}	1.92
[9]ADWG (g/bird)	25.25 ± 0.62^{b}	25.39 ± 0.51^{b}	25.75 ± 0.92^{b}	28.10 ± 0.82^{a}	28.29 ± 0.90^{a}	0.17
[10]FI (g/bird)	812 ± 19.13^{c}	819 ± 19.08^{b}	820 ± 18.60^{b}	825 ± 19.06^{a}	825 ± 19.00^{a}	2.95
[11]ADFI (g/bird)	38.67 ± 3.44^{c}	38.90 ± 2.96^{b}	39.05 ± 3.11^{b}	39.29 ± 3.07^{a}	39.29 ± 2.88^{a}	0.20
[12]FCR	1.53 ± 0.01^{a}	1.53 ± 0.09^{a}	1.51 ± 0.00^{b}	1.39 ± 0.04c	1.38 ± 0.01^{c}	0.01
Mortality (%)	1.20 ± 0.04	-	-	-	-	0.01
22–42 day (Grower)						
WG (g/bird)	1245.6 ± 21.10^{c}	1300.8 ± 20.97^{c}	1400.34 ± 21.00^{b}	1780.1± 22.18^{a}	1800.9 ± 22.06^{a}	4.31
ADWG (g/bird)	59.31 ± 2.66^{c}	61.94 ± 2.50^{c}	66.68 ± 3.61^{b}	84.77 ± 2.60^{a}	85.76 ± 2.82^{a}	0.25
FI (g/bird)	2140.9 ± 15.1^{c}	2300.1 ± 14.2^{b}	2300.8 ± 15.6^{b}	2400.1 ± 14.2^{a}	2400.9 ± 13.7^{a}	5.60
ADFI (g/bird)	101.95 ± 5.00^{c}	109.52 ± 4.91^{b}	109.56 ± 5.02^{b}	114.29 ± 5.06^{a}	114.32 ± 5.00^{a}	0.22
FCR	1.71 ± 0.06^{a}	1.76 ± 0.02^{a}	1.64 ± 0.03^{b}	1.35 ± 0.02^{a}	1.34 ± 0.01^{a}	0.01
Mortality (%)	1.00 ± 0.01^{a}	-	-	-	-	
Overall production						

(0–42 days)						
FW (g/bird)	1823.82 ± 23.6^{c}	1881.91 ± 21.0^{c}	1989.14 ± 23.6^{b}	2418.2 ± 20.1^{a}	2442.98 ± 21.1^{a}	0.27
WG (g/bird)	1775.8 ± 11.1^{c}	1833.9 ± 10.5^{c}	1941.14 ± 12.5^{b}	2370.2 ± 10.7^{a}	2395 ± 11.6^{a}	0.35
ADWG (g/bird)	42.28 ± 2.14^{c}	43.66 ± 2.06^{c}	46.22 ± 2.71^{b}	56.43 ± 2.56^{a}	57.02 ± 2.00^{a}	0.02
FI (g/bird)	2952.9 ± 31.4^{c}	3117.1 ± 30.5^{b}	3120.8 ± 34.5^{b}	3225.1 ± 35.0^{a}	3225.9 ± 33.1^{a}	0.41
ADFI (g/bird)	70.31 ± 2.17^{c}	74.22 ± 1.96^{b}	74.30 ± 2.03^{b}	76.79 ± 2.05^{a}	76.81 ± 2.10^{a}	0.03
FCR	1.66 ± 0.09^{c}	1.65 ± 0.04^{c}	1.61 ± 0.02^{b}	1.35 ± 0.01^{a}	1.35 ± 0.03^{a}	0.01
Mortality (%)	2.20 ± 0.08^{a}	-	-	-	-	0.01

Note: $^{a, b, c}$ Means within the same row with different superscripts differ significantly ($P < 0.05$); [1]basal diet supplemented with no ALM; [2] 100g ALM per 100 kg^{-1} basal diet; [3] 200 g ALM per 100 kg^{-1} basal diet; [4] 300 g ALM per 100 kg^{-1} basal diet; [5] 400 g ALM per 100 kg^{-1} basal diet; [6] Initial body weight; [7] final body weight; [8] weight gain; [9] Average daily weight gain; [10] feed intake; [11] Average daily feed intake; [12] feed conversion ratio. Source: Author, 2023.

Haematological parameters of broiler chicken fed diet supplemented with *A. indica* leaf meal is presented in (Table 3). Pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and white blood cell of birds fed diets supplemented with ALM at 100 g (group 2) were similar ($P > 0.05$) to those fed diet without ALM (group 1). Similarly, birds fed 300 g ALM (group 4) were also similar ($P > 0.05$) to those in group 5 (400 g ALM). However, those in group 4 and 5 values were significantly higher ($P < 0.05$) than the other groups. Lymphocytes and monocytes values were higher in group 4 and 5 compared to the other groups. Values for basophils were not influenced ($P > 0.05$) by the treatments.

Table 3. Haematological parameters of broiler chicken fed diet supplemented with *Aristolochia indica* leaf meal.

Index	**[1]G1**	**[2]G2**	**[3]G3**	**[4]G4**	**[5]G5**	**P-value**
[6]PCV (%)	31.07 ± 0.09^{c}	31.66 ± 0.03^{c}	34.72 ± 0.98^{b}	36.84 ± 0.95^{a}	36.88 ± 0.91^{a}	0.06
[7]RBC (×10^{6}/L)	2.16 ± 1.12^{c}	2.13 ± 1.07^{c}	2.33 ± 1.52^{b}	2.89 ± 1.88^{a}	2.98 ± 1.06^{a}	0.02
[8]HB (g/dL)	9.97 ± 0.12^{c}	10.42 ± 0.18^{b}	10.71 ± 0.20^{b}	12.06 ± 0.15^{a}	12.12 ± 0.23^{a}	0.13
[9]MCH (pg)	38.06 ± 1.08^{c}	42.05 ± 1.17^{b}	42.79 ± 1.94^{b}	46.95 ± 1.06^{a}	47.01 ± 1.13^{a}	0.22
[10]MCV (fl)	90.68 ± 0.66^{c}	93.17 ± 0.61^{b}	99.81 ± 0.77^{b}	107.1 ± 0.91^{a}	109.3 ± 0.88^{a}	0.57
[11]MCHC (g/dL)	55.60 ± 0.10^{c}	60.33 ± 0.11^{b}	61.13 ± 0.12^{b}	68.04 ± 0.03^{a}	68.11 ± 0.05^{a}	0.87
[12]WBC (×10^{9}/L)	9.33 ± 0.88^{c}	10.81 ± 0.76^{b}	10.96 ± 0.85^{b}	12.10 ± 0.81^{a}	12.44 ± 0.92^{a}	0.03
[13] LYM (%)	2.00 ± 0.03^{b}	2.13 ± 0.06^{b}	2.15 ± 0.01^{b}	3.30 ± 0.02^{a}	3.51 ± 0.05^{a}	0.01
[14]MON (%)	2.10 ± 0.05^{b}	2.25 ± 0.09^{b}	2.86 ± 0.04^{b}	2.92 ± 0.12^{a}	2.95 ± 0.19^{a}	0.12
[16]BAS	3.17 ± 0.16	3.08 ± 0.12	3.11 ± 0.17	3.18 ± 0.22	3.25 ± 0.82	0.01

Note: $^{a, b, c}$ Means within the same row with different superscripts differ significantly ($P < 0.05$); [1] basal diet supplemented with no ALM; [2] 100 g ALM per 100 kg^{-1} basal diet; [3] 200 g ALM per 100 kg^{-1} basal diet; [4] 300 g ALM per 100 kg^{-1} basal diet; [5] 400 g ALM per 100 kg^{-1} basal diet. Source: Author, 2023.

As presented in Table 4, serum biochemical indices of broiler chicken fed diet supplemented with Aristolochia indica leaf meal. Total protein values of birds fed diet supplemented with 0 g ALM (group 1) and 100 g ALM (group 2) were similar to those given 200 g ALM (group 3) but significantly lower ($P < 0.05$) than those in group 4 (300 g ALM) and group 5 (400 g ALM). Albumin/globulin ratio and glucose values were higher ($P < 0.05$) in group 4 and 5 relatives to the other groups. Conversely, cholesterol concentration was higher ($P < 0.05$) in group 1, intermediate in group 2 and lower in group 3, 4 and 5. Urea, creatinine, alkaline phosphatase, alanine transaminase and aspartate transaminase values were similar ($P > 0.05$) across the group.

Table 4. Serum biochemical indices of broiler chicken fed diet supplemented with *Aristolochia indica* leaf meal.

Index	[1]G1	[2]G2	[3]G3	[4]G4	[5]G5	P-value
Total protein (g/dL)	3.90 ± 0.12[b]	3.91 ± 0.06[b]	3.93 ± 0.09[b]	4.31 ± 0.15[a]	4.50 ± 0.11[a]	0.02
Albumin (g/dL)	2.00 ± 0.02[b]	2.10 ± 0.01[b]	2.3 ± 0.01[b]	2.87 ± 0.03[a]	2.90 ± 0.02[a]	0.01
Globulin (g/dL)	1.90 ± 0.01	1.81 ± 0.01	1.63 ± 0.01	1.44 ± 0.01	2.00 ± 0.01	0.01
A/G ratio	1.05 ± 0.02[c]	1.16 ± 0.08[c]	1.41 ± 0.10[b]	1.99 ± 0.12[a]	1.45 ± 0.19[a]	0.02
Urea (mg/dL)	4.38 ± 0.00	4.21 ± 0.00	4.10 ± 0.00	4.28 ± 0.00	4.19 ± 0.01	0.11
Creatinine (mg/dL)	0.76 ± 0.17	0.72 ± 0.19	0.74 ± 0.10	0.70 ± 0.16	0.75 ± 0.12	0.02
Glucose (mg/dL)	100.7 ± 3.21[d]	118.4 ± 4.11[c]	120.9 ± 2.91[b]	123.2 ± 3.11[a]	123.5 ± 2.91[a]	2.05
Cholesterol (mg/dL)	86.11 ± 0.12[a]	81.22 ± 0.17[b]	78.00 ± 0.92[c]	70.80 ± 0.07[c]	70.61 ± 0.12[c]	1.02
[6]ALP (U/L)	72.88 ± 4.08	70.91 ± 5.01	72.88 ± 4.72	72.88 ± 5.51	72.88 ± 5.07	0.35
[7]ALT (U/L)	102.1 ± 9.05	100.9 ± 9.93	106.4 ± 10.1	105.5 ± 10.9	109.3 ± 11.1	0.09
[8]AST (U/L)	56.90 ± 3.44	54.12 ± 3.09	56.71 ± 3.66	56.09 ± 2.00	56.18 ± 2.03	0.02

Note: [a, b, c] Means within the same row with different superscripts differ significantly ($P < 0.05$); [1] basal diet supplemented with no ALM; [2] 100 g ALM per 100 kg^{-1} basal diet; [3] 200 g ALM per 100 kg^{-1} basal diet; [4] 300 g ALM per 100 kg^{-1} basal diet; [5] 400 g ALM per 100 kg^{-1} basal diet; [6] Alkaline phosphatases; [7] Alanine transaminase; [8] Aspartate transaminase. Source: Author, 2023.

4. Discussion

Several phytocompounds from *A. indica* are attributed to important biological activities (Menezes Filho, 2019; Padhy, 2021; Dey et al., 2021). These phyto-constituents has several pharmacological or therapeutic properties (Singh et al., 2022). They are also generally recognized as safe, effective and environmentally friendly which make them good candidates to be used as feed additives in poultry production in comparison with antibiotics (Hashemi et al., 2008; Afnan, 2018).

The biological mechanism of action of phyto-constituents or phytochemicals depends on their chemical structure (Hashemi; Davoodi, 2010; Shittu et al., 2021). Phytochemicals used in animals have many functions including being antioxidant, anti-estrogenic, anti-inflammatory, immunomodulatory and anti-carcinogenic (Laparra; Sanz, 2010; Musa et al., 2021). For instance, saponins belong to a group of phytochemicals that serves as one of the major defense systems for plants against microbial, fungal, and insect attack (Nden, 2019). They can be found in most plant species although the amount and the concentration of saponins in plants varies from species to species (González-Lamothe et al., 2009). Saponins act by forming complexes with sterols or polysaccharides within the microbial cell membrane so destroying the cytoplasmic membrane integrity (Nden, 2019).

They also possess anti-diarrheal properties (Daniel; Alagbe, 2023). Phenolic compounds have been reported to have anti-inflammatory, antioxidant and antibacterial properties (Adewale et al., 2021; Alagbe et al., 2022). Terpenoids is reported to have antimicrobial, antidiarrheal and anti-cancer activity (Hashemi et al., 2008a, 2009b). Alkaloids have also been reported to exhibit anti-malarial, analgesics, anti-hypertensive, anti-diabetic and anti-inflammatory activities (Ayushi et al., 2021). Flavonoids have anti-inflammatory, antioxidant, anti-cancer and hypolipidemic properties (Musa et al., 2021). They can also aid to scavenge free radicals thus preventing infections (Alagbe et al., 2024). However, the result obtained in this experiment is in agreement with the findings of Abhijit & Jitendra (2011).

The result suggests that phytochemicals in ALM can improve animal's health and performance because of their anti-microbial, anti-stress (Wang et al., 1998) and antioxidant properties (Valenzuela, 1995), and their ability to modulate gut microbiota (Hashemi et al., 2009) and enhance immune responses (Chowdhury et al., 2018).

The efficiency of these phytochemicals is determined by intrinsic and extrinsic factors such as animal's nutrition and health, type of diet and environment (Giannenas et al., 2003). Supplementation of ALM at 300 g (group 4) and 400 g (group 5) can effectively inhibit the activities of pathogenic organisms, improve palatability and promote the activities of intestinal enzymes, thus positively influencing the digestion and absorption of nutrients, reduce the digesta transit time and the increase feed conversion efficiency in birds.

This outcome agrees with the findings of Kanduri et al. (2013) and Dharma et al. (2014) when natural growth promoters were supplemented in the diet of broilers. Similar observation was made by Oloruntola et al. (2020) who reported that pawpaw leaf and seed meals exerts growth promoting effect on broiler chickens. The result on the overall mortality rate reveals that supplementing

ALM can increase the survivability of birds due to the presence of phyto-constituents or bioactive compounds in them. This explains why mortality was recorded only among birds in group 1 with no ALM. This result is in consonance with the report of Daniel & Alagbe (2023) when pawpaw seed oil was supplemented in the diet of broilers.

The pack cell volume, red blood cell and haemoglobin values were within the normal ranges (23.0-35.0%), 2.00-8.00 ($\times 106/L^{-1}$) and 7.00-13.00 g/dL^{-1} reported by Akpabio & Offiong (2014). Decrease in pack cell volume suggests the presence of anaemia or mineral and vitamin deficiency (Jain, 1986). Low red blood cell and haemoglobin concentrations is an indication of bone marrow disorder, kidney failure and iron deficiency (Alagbe, 2020). Haemoglobins are conjugated protein that transports oxygen from lungs to tissues and carbon dioxide from tissues to the lungs (Omokore; Alagbe, 2019).

Improved pack cell volume, red blood cell and heamoglobin concentration among birds in group 4 (300 g ALM) and group 5 (400 g ALM) suggests the efficient distribution of oxygen and other nutrients in the body of birds (Muritala et al., 2022). Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations values obtained in this study were within the established range (90.00-112.0 fl), 30.00-50.00 pg and 40.00-60.00 g/dL^{-1} reported in Merck Veterinary Manual (2001). Values recorded were lower than those reported cited by Jain (1986), this variation can be attributed to age of birds, breeds, nutrition and management procedures for the animals (Omokore; Alagbe, 2019).

White blood cells are important part of the body's immune defense system that are involved in protecting the body against both infectious diseases and foreign invaders through the production of antibodies (Thrall, 2007; Jain, 1993). White blood cell values recorded in this experiment was within the normal range 7.00-18.00 ($\times 109/L^{-1}$) reported by Islam et al. (2004). The fundamental role of lymphocytes and monocytes is to perform immune-modulatory activities in animals (Alagbe, 2024). Basophil counts were similar ($P > 0.05$) across the group and values were within the normal range (2.00–5.00 %) reported by Jain (1986); Bounous and Stedman (2000).

Total protein values recorded in this study were within the normal range 2.7-6.0 g/dL^{-1} cited by Biwas et al. (2011). Albumin/globulin ratio gives an insight to nutritional status and immune status of animals (Musa et al., 2021). It can also be used in the diagnosis of certain health conditions viz kidney disease and other chronic infections (Adewale et al., 2021; Omokore; Alagbe, 2019). Low Albumin/globulin ratio suggests malnutrition, pancreatitis and other chronic diseases (liver and liver infections) (Kaneko, 1997). Urea, creatinine and glucose levels were within the normal range (1.2-5.00 mg/dL^{-1}), 0.02-1.00 mg/dL^{-1} and 91.20-200.0 mg/dL^{-1} cited by Café et al. (2012). The results on urea and creatinine values suggests that the kidney and liver of birds were functioning well (Shittu et al., 2021). High glucose level in the serum can be triggered during period of stress due to nutrition or starvation, poor management and housing (Alagbe, 2018). High cholesterol is the major cause of heart disease or cardio vascular infections (Muritala et al., 2022; Alagbe et al., 2022).

Alkaline phosphatase, alanine transaminase and aspartate transaminase values were within the normal range (60.0-80.0 U/L^{-1}), 51.00-133.9 (U/L) and 30.0-65.0 (U/L) cited by Oloruntola et al. (2021) who fed *Irvingia gabonensis* and *Ocimum gratissimum* leaf powder. However, values were lower than those reported by Alagbe et al. (2020) who recorded ALP (31.0-50.80 U/L^{-1}); ALT (40.9-71.0 U/L^{-1}) for broiler chicken fed *Albizia lebbeck* stem bark aqueous extract. Elevation in ALT and AST values suggests liver damage and muscle damage in birds (Muritala et al., 2022).

5. Conclusion

In conclusion, Aristolochia indica leaf meal contains several phyto-constituents (alkaloids, flavonoids, tannins, terpenoids, saponins, phenols and steroids) at different concentrations. These compounds are effective, non-toxic and environmentally friendly and can express wide range of biological activities including antioxidant, anti-inflammatory, anti-helminthic, hepato-protective, immune-stimulatory, antifungal amongst others. They can be fed to broilers up to 400 g without compromising their health status and growth.

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Author's Contributions

John Olujimi Alagbe: study design, practical testing, data collection, writing, corrections, submission and publication.

Ethics

Experimental procedures and management was carried out according to the guidelines of the ethic committee of Sumitra Research Institute, India (RR/00A211C).